Compendium of
Animal Reproduction

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PARTNERS IN REPRODUCTION

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Preface

The animals that are our patients make our world a much better place in which to live and work. They enrich our lives and serve us in so many different capacities. They allow us to go beyond our own abilities and technologies to help and comfort the disabled. They expand our ability to work and do things we could not do without them. They give us enjoyment, companionship, transportation, and power. They provide sport, food, clothing, and many other by-products, from life-saving medicines to cosmetics and everything in between.

Without reproduction there would be no production. Animal reproduction is an essential element in the continued interaction between humans and animals.

It is with great pleasure that I present to you the 11th edition of the *Compendium of Animal Reproduction.*

The objective of this book is to update and inspire those interested in the management of reproduction in domestic animals and to provide usable solutions to challenges in the everyday life of veterinarians and their clients.

It would have been impossible to accomplish this new edition without the help of colleagues who devoted much time and effort to this project. I would like to express my extreme gratitude to my colleagues Dr. Linda Horspool, Dr. Marc-Antoine Driancourt, Dr. Miquel Collell, Dr. Brad Thacker, Dr. Todd Bilby, and to Dr. J. A. (Lulu) Skidmore from the Camel Reproduction Centre in Dubai, United Arab Emirates, for their help in editing the content.

Reproduction in domestic animals is very complex and dynamic. I hope that you find the Compendium useful and stimulating. This book only scratches the surface of reproduction in domestic animals. There is so much more to know and to explore. Have a great adventure in this fascinating area.

Andrew L. Skidmore, Editor, 11th edition

ISBN 90-801886-6-2
Publisher: Intervet International bv

060200.12.09
11th revised edition, 2015

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1.1 Introduction

Reproductive performance in breeding females is the key to economic performance. The three prerequisites to reach this target are well known:

- A maximum proportion of females in a herd should display cyclic ovarian activity at the time of breeding. While this is straightforward in species in which seasonal or postpartum anoestrus does not occur, this may be more challenging in herds where cycling and noncycling females are mixed, without any easy way to identify these two subpopulations.

- There should be close synchrony between insemination and ovulation. Such synchrony is very easily obtained in the few species (eg, rabbits, cats, camelids) where ovulation is induced by mating. In other species, the occurrence of oestrus helps to get some synchrony between mating and ovulation. However, in some species, such as horses, which display long oestrus periods with ovulation at the end of oestrus, the beginning of oestrus is a poor indicator of the optimal time for mating.

- Use sperm of high fertilising ability for insemination or highly fertile males with high libido and in numbers suitable for the numbers of females to be mated.

This chapter presents a summary of the mechanisms controlling reproduction and reviews the processes involved in:

- Follicular growth, maturation, and ovulation
- Seasonal anoestrus
- Postpartum anoestrus
- Quality of sperm

Species-specific features as well as manipulation of these processes are presented in the chapters dedicated to each species.

1.2 Endocrine, paracrine, and autocrine regulation of reproduction; regulatory loops and feedback mechanisms

1.2.1 Definitions

There are three types of hormonal regulation – endocrine, paracrine, and autocrine. All three types of regulation are modulated by feedback loops. Feedback loops are very well understood for all endocrine mechanisms (see below). Feedback loops may be negative, when they slow down the process initiated by the initial action, or positive, when they increase the initial action. The intracellular mechanisms involved in these different regulatory loops are numerous and complex and are outside the scope of this review.

a. Endocrine regulation (Figure 1)

In endocrine regulation, the hormone is synthesised in an endocrine gland and released into the bloodstream, which transports it to its target organ, often distant from the source. The endocrine control of gonadal function by the hypothalamic-pituitary axis through the release of follicle stimulating hormone (FSH) and luteinizing hormone (LH) by the pituitary is a good example of an endocrine control mechanism. Usually, there are regulatory feedback loops that maintain the balance between stimulation and inhibition.

Interestingly, recent research has demonstrated that there are a number of endocrine regulatory mechanisms that involve organs outside of the classic hypothalamic-pituitary-gonadal axis, which are also involved in the control of gonadal function. Just to name a few, insulin (produced by the pancreas), leptin (produced by fat), and ghrelin (produced by the stomach) have all been shown to modulate gonadal function (Figure 1).

b. Paracrine regulation (Figure 1)

Paracrine regulation is said to occur when two neighboring tissues interact. Such interactions are well documented in the ovary (cross-talk between the granulosa and theca cells and between the oocyte and the granulosa cells) as well as in the testicle (cross-talk between Leydig and Sertoli cells and between Sertoli cells and germ cells). Another example of paracrine regulation is the cross-talk between the large and small luteal cells, not only to maximise progesterone synthesis but also during the regression of the corpus luteum.

c. Autocrine regulation (Figure 1)

Autocrine regulation involves the action of compounds produced by a specific tissue on the same tissue. A good example of autocrine regulation is the role of oestradiol (produced by granulosa cells) in the differentiation process (measured, for example, by the presence of LH receptors) of these same cells.

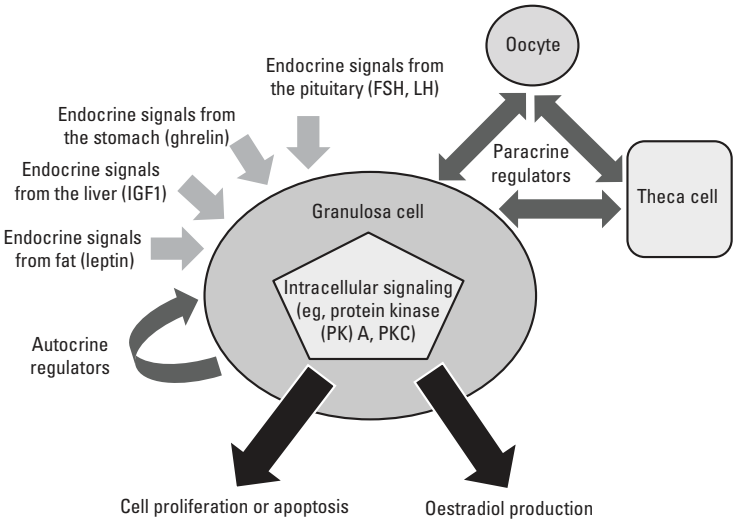

Figure 1 Endocrine, paracrine, and autocrine regulation and the interactions between the oocyte and its somatic (granulosa and theca) cells modulating granulosa cell proliferation, apoptosis, and differentiation

1.2.2 Regulation of reproduction in the female

a. The hypothalamic-pituitary axis and follicle function

Gonadotropin-releasing hormone (GnRH), a ten-amino acid peptide (decapeptide), is released into the hypothalamic-hypophyseal portal system and transported to the anterior lobe of the pituitary, its target organ, where it acts on specific cells to stimulate the synthesis and release of the gonadotropins FSH and LH. As GnRH is secreted in a pulsatile way (ie, rapid bursts separated by a quiescent period) by GnRH neurons, it is not surprising that LH secretion by the pituitary is also pulsatile. In contrast, the pulsatile nature of FSH secretion is usually less obvious. It is the amplitude and frequency of GnRH pulses that convey the endocrine signals to the pituitary-ovarian axis. Both internal factors (through feedback loops) and external factors (eg, photoperiod, pheromones, nutrition, and metabolic status) exert their primary effect on reproduction through the modulation of pulsatile secretion of GnRH by the hypothalamus. This ensures that the target organ is always exposed to efficient hormonal stimuli. Indeed, constant stimulation by high concentrations of GnRH results in desensitisation of the target cells in the pituitary. This is most probably caused by a decrease in the number of GnRH receptors on the cell membrane of the target cells.

The pituitary gonadotropins, FSH and LH, belong to the superfamily of glycoprotein hormones. They have two different subunits, alpha and beta, which are noncovalently associated. The two hormones are not secreted synchronously in vivo since they are regulated independently. GnRH is of major importance in controlling the secretion of LH. It acts by triggering both the release and the biosynthesis of LH in order to replenish stores of it in the pituitary. The LH content of the pituitary of most mammalian species is up to ten times higher than that of FSH. In contrast, FSH synthesis is mainly modulated by various gonadal factors (eg, oestradiol and members of the inhibin family ie, inhibin, activin, and follistatin), although GnRH is also involved. The pituitary stores of FSH are low, and its secretion mirrors the rate and extent of its biosynthesis.

At the ovarian level, FSH has two main roles. The first is to sustain growth of recruited follicles (see Section 1.3.1) until the gonadotropin dependence is transferred to LH, usually around the time of selection of the dominant follicle. The second is the induction of aromatase in the granulosa cells (see Section 1.3.2). Aromatase is the enzyme that converts androgens into oestrogens. Its successful induction is a prerequisite for further maturation of the dominant follicle. The granulosa cells of the dominant follicle also produce inhibin, which acts by negative feedback on FSH release from the pituitary. This negative feedback loop prevents hyperstimulation of the ovary by FSH.

In the theca interna, LH stimulates the synthesis of androstenedione from cholesterol and progestagens (progestins). Androstenedione is converted into testosterone, which is transferred to the granulosa cells to be converted into oestradiol-17β by aromatase (see Section 1.3.2). Oestradiol, when its concentrations exceed a certain threshold, exerts positive feedback on the hypothalamus to induce the LH surge that triggers ovulation. The time interval between the LH surge and ovulation is very consistent within a species but quite variable between species. For example, it is around 40 hours in swine and horses but only 24-28 hours in cattle and sheep. An additional effect of oestradiol is the induction of the signs of oestrus. Oestrus can be described as the behavioural and physical signs that signal to other animals that the female is in the fertile phase of its cycle and will allow mating.

Interestingly, androgens and oestrogens as well as members of the inhibin family are involved in paracrine and autocrine regulation that modulates endocrine signaling in the ovary. An example of paracrine interaction is detailed in Section 1.3.2.

b. The hypothalamic-pituitary axis and corpus luteum function

Changes in progesterone concentrations after ovulation follow the same pattern in all species. Progesterone is produced by the corpus luteum, and concentrations start to rise in the days following ovulation and steadily increase until around day 6 postovulation. Progesterone concentrations then plateau for 7 to 12 days, depending on the duration of the luteal phase of the species concerned. Progesterone concentrations during this period are species specific, ranging from around 5 ng/mL in sheep to about 40 ng/mL in swine. Following the initiation of luteolysis (see Section 1.2.2c), progesterone concentrations quickly decline to very low levels (below 0.5 ng/mL), allowing a new follicular phase to start.

Progesterone is the hormone responsible for the maintenance of pregnancy. It is produced and secreted jointly by large and small luteal cells. Large luteal cells are derived from granulosa cells and have a low sensitivity to LH and a high sensitivity to prostaglandins. Small luteal cells are derived from theca interna cells and are highly sensitive to LH. LH alone, or together with prolactin (in some species of rodent), is the key hormone supporting the formation of the corpus luteum and the initiation of progesterone production. Progesterone acts on several targets. Firstly, it prepares the oviduct and endometrium to accommodate the freshly fertilised, young embryo (oviduct) and later the developing embryo when it enters the uterus at the blastocyst stage. Secondly, by exerting negative feedback, it slows down GnRH release at the level of the hypothalamus and reduces the concentrations of LH available to support terminal follicular growth, thereby preventing return to oestrus and new ovulation(s).

In pregnant females, interferon tau production by the developing embryo (in cattle) or oestrogen production by the multiple embryos (in swine) acts to maintain the corpus luteum, thereby allowing pregnancy initiation (see Section 1.2.2c).

c. Interactions between the uterus, embryo, and corpus luteum in the control of luteolysis

Prostaglandin (PG) $F_{2\alpha}$ initiates the regression of the corpus luteum, known as luteolysis. The luteolytic signal is increased pulsatile secretion of $PGF_{2\alpha}$. Uterine venous $PGF_{2\alpha}$ concentrations begin to increase on days 11-13 in sheep, days 13-14 in swine, and days 16-17 postoestrus in cattle (reviewed by Weems et al., 2006). The mechanism by which PG induce luteolysis has not been completely elucidated, but it involves a reduction of the blood supply to the corpus luteum by vasoconstriction, as well as direct inhibition of luteal steroidogenesis coupled to increased cell death (apoptosis) of luteal cells. It is generally assumed that functional luteolysis (ie, a drop in progesterone production) precedes morphological luteolysis (ie, a reduction in size leading to a corpus albicans). The primary site for the initiation of luteolysis is the large luteal cells of the aging corpus luteum. Oxytocin produced in the corpus luteum is believed to be the first signal triggering luteolysis. Binding of oxytocin to its receptor in the uterine endometrium of nonpregnant cattle and sheep stimulates the pulsatile secretion of $PGF_{2\alpha}$. Oestrogens increase expression of uterine oxytocin receptors, while progesterone has the opposite effect. This is why it is possible to postpone luteolysis by preventing the growth of large oestrogen-active follicles (See Luteolysis, Section 2.1.4c). During the initiation of pregnancy in cattle, luteolysis is prevented through increased interferon tau production by the embryo before pulsatile $PGF_{2\alpha}$ secretion is initiated. In pregnant swine, luteolysis is stopped by embryonic oestradiol production that diverts $PGF_{2\alpha}$ away from the ovarian circulation, thus preventing it from reaching the ovaries.

Figure 2 summarises the interactions between the different levels of the hypothalamic-pituitary-ovarian-uterine axis involved in the control of reproduction and endocrine mediators.

Figure 3 presents an overview of the changes in gonadotropin and steroid hormone (progesterone and oestradiol) concentrations during the bovine oestrous cycle.

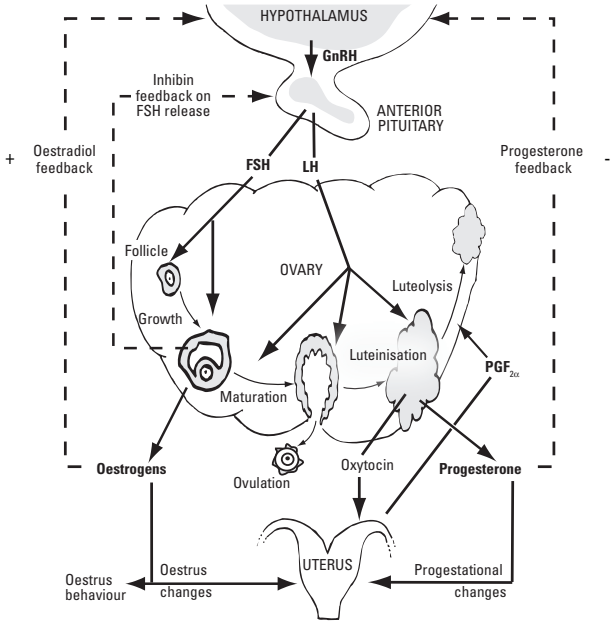

Figure 2 The hypothalamic-pituitary-ovarian-uterine axis and the endocrine regulators of follicular growth, corpus luteum formation, and luteolysis

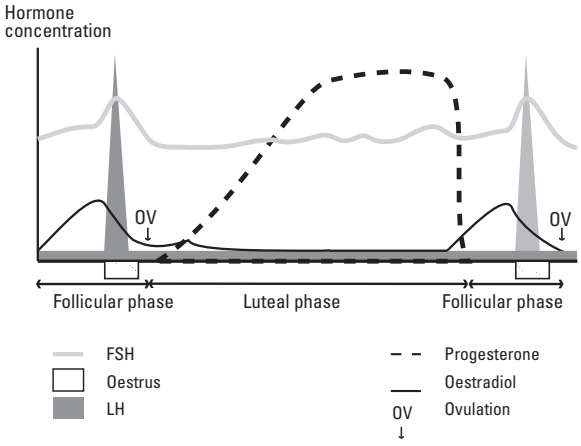

Figure 3 Schematic profile of the changes in gonadotropin (FSH and LH) and steroid hormone concentrations (progesterone and oestradiol) during the bovine oestrous cycle

1.2.3 Regulation of reproduction in the male

In males, the testicles or testes produce sperm and male steroid hormones (mainly androgens). This requires interactions between the two constitutive compartments of the testis, the seminiferous tubules, in which germ cells and Sertoli cells are located, and interstitial tissue, which includes Leydig cells. In rodents, the volume of interstitial tissue (Leydig cells) does not exceed 5% of the total testicular volume; however, this proportion reaches 10% in sheep and is far higher in swine and horses. Seminiferous tubules therefore represent 60% (swine, horses) to 90% (rodents) of the testicular volume. The time needed for production of a spermatid from a quiescent (A0) spermatagonium is species specific and ranges from 35 days (mice) to 41 days (swine), 45 days (sheep), and 54 days (cattle). Daily sperm production by both testicles (in billions of spermatozoa) averages 5.2 (horses), 7.5 (cattle), and 16.2 (swine), but the daily sperm production per gram of testicular tissue appears quite consistent across species (at around 12-20 million/g).

In the seminiferous tubules, the germ cells divide by mitosis, generating several generations of spermatogonia, and initiate meiosis when they reach the spermatocyte stage. They are released into the lumen of the seminiferous tubules when they become spermatids. All steps of germ cell proliferation and maturation occur with the different generations of germ cells in close proximity to the Sertoli cells that line the basal membrane of the seminiferous tubules. Sertoli cells also produce regulatory proteins, such as inhibin, that reduce FSH concentrations by exerting negative feedback on the pituitary.

In the interstitial tissue, Leydig cells actively produce the testicular androgens. In addition, the testis also produces limited amounts of oestradiol.

Control of reproduction in the male involves the same types of regulation (ie, endocrine, paracrine, and autocrine) as in females, and the endocrine regulatory loops are generally very similar to those described in Section 1.2.2.

In the prepubertal animal, FSH stimulates proliferation of Sertoli cells, with their final number reached at puberty. Around puberty, FSH is responsible for the maturation of the Sertoli cells, resulting in increased inhibin and androgen-binding protein (ABP) production. The pubertal increase in pulsatile LH secretion stimulates androgen production by the Leydig cells, followed by its possible aromatisation to oestradiol in Sertoli cells. In sheep, the endocrine control of germ-cell proliferation is well understood (Courot and Ortavant, 1981) and involves the actions of FSH, LH, and androgens at specific steps of the mitotic and meiotic processes. For example, differentiation of A0 to A1 spermatogonia is controlled by LH, while transition from A1 to intermediate spermatogonia is controlled by testosterone. The last spermatogonial divisions, changing intermediate spermatogonia into primary spermatocytes, are under the control of FSH. Once the leptotene stage is reached, the prophase of meiosis and spermatogenesis is controlled by androgens. As germ cells display oestradiol receptors, this steroid hormone is also likely to modulate the actions of FSH, LH, and androgens.

Endocrine regulation also involves positive and negative feedback regulatory loops. Inhibin produced by Sertoli cells acts by negative feedback on FSH. Androgens produced by Leydig cells act by negative feedback on LH secretion. There are also numerous paracrine and autocrine regulatory mechanisms controlling the function of the testis. Examples of paracrine regulation include the stimulatory effects on Sertoli cells of growth factors (such as insulin-like growth factor (IGF) 1) produced by the Leydig cells and by the germinal epithelium at specific stages and stimulating (epidermal growth factor (EGF)) or inhibiting Sertoli cell production of inhibin. Autocrine regulation also actively modulates testicular function. A good example of this is the local stimulatory effect of IGF-1 in the amplification of the Leydig cell response to LH and in sustaining the stimulatory effects of LH on several steps of testicular steroidogenesis.

Figure 4 shows a schematic representation of the endocrine, paracrine, and autocrine regulation involved in the control of testicular function.

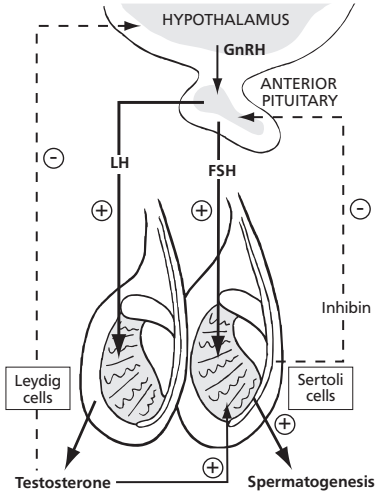

Figure 4 Endocrine, paracrine, and autocrine regulation involved in testis function (sperm and testosterone production)

1.3 Regulation of follicular growth, maturation, and ovulation

1.3.1 Endocrine and autocrine regulation of follicular growth

Large follicles (seen using ultrasonography or on the surface of ovaries collected at slaughter) are the tip of a large iceberg. The ovary of most farm animals (ie, cattle, sheep, goats, and swine) contains a large store of tiny primordial follicles (around 50,000 to 100,000) that are formed during foetal life. The size of this follicular store is large enough to ensure ovulation throughout the reproductive life of the female; there is no equivalent to menopause (the end of menstruation) in animals. The growth process from the primordial follicle (measuring about 0.04 mm (40 microns) in diameter) to the preovulatory stage lasts around 3-5 months. The mechanisms involved in the control of follicular growth between 0.04 mm and 1 mm in diameter are not fully understood (Scaramuzzi et al., 2011). In contrast, terminal follicular growth has been extensively studied in all species using ultrasonography. Terminal follicular growth starts when follicles become acutely dependent on gonadotropin support (ie, 2 mm in sheep and swine, 4 mm in cattle, and around 10 mm in horses). During terminal follicular growth, recruitment of a cohort of gonadotropin-dependent follicles is followed a few days later by

the selection of the dominant follicle (Figure 5). This dominant follicle will continue growing and matures until it produces enough oestradiol to trigger oestrus and ovulation. The other follicles from the cohort will regress and there is apoptosis of their somatic cells (Figure 5). It is noteworthy that a single ultrasound scan does not allow growing, potentially dominant follicles to be distinguished from regressing, apoptotic follicles. Repeated daily ultrasound scans are needed. Several studies in experimental paradigms when LH and/or FSH concentrations were manipulated (cattle: Gong et al., 1996; Crowe et al., 2001; sheep: Picton et al., 1991; swine: Driancourt et al., 1995) have shown that it is around the time of selection that follicles transfer their gonadotropin dependence from FSH to LH. In all models, large follicles rely on the consecutive exposure to FSH and then LH to grow to preovulatory size (Figure 6). The steps that are FSH- or LH-dependent in cattle, sheep, swine, and horses are presented in Figure 6. While the range of follicular diameter needing FSH or LH appears to be species specific, it is interesting to note that the FSH/LH sequence is common to all species (Figure 6). This is why hormones with both FSH and LH activity (such as pregnant mare serum gonadotropin (PMSG, also called equine chorionic gonadotropin, eCG) are potent stimulators of follicular growth in all species.

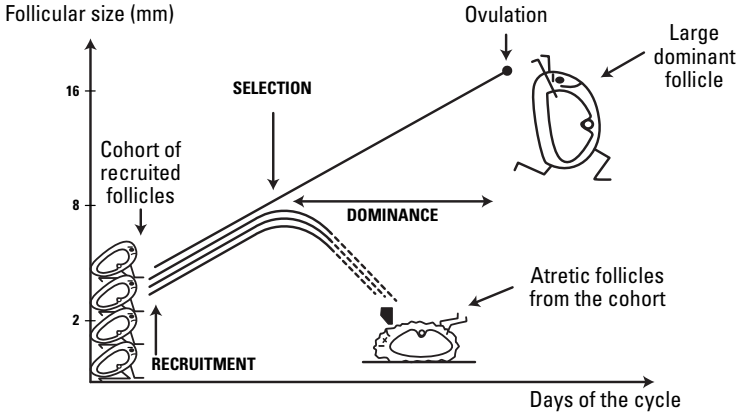

Figure 5 Main events occurring during terminal follicular growth (after Driancourt, 2001). In species with a single ovulation (cattle, horses), recruitment of several follicles is followed by selection of a single follicle that becomes dominant and fully matures and ovulates. All other recruited follicles regress by apoptosis. In multiovulatory species (swine), the same events occur, but the number of recruited and dominant follicles is higher

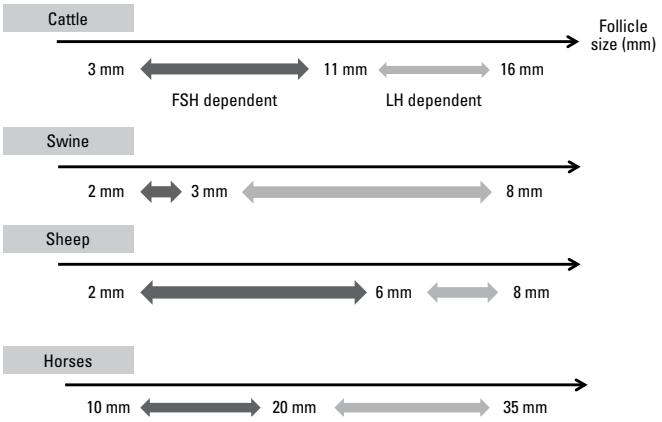

Figure 6 Control of terminal follicular growth during the gonadotropin-dependent phase in cattle (top), swine, sheep, and horses (bottom). The size at which follicles become acutely gonadotropin dependent (left), at which they transfer their dependence from FSH to LH (center), and at which ovulation occurs (right) are indicated below each line

Once the dominant follicle has emerged and has become LH-dependent, differentiation of a maximal population of LH receptors on the granulosa cells is a prerequisite for the dominant follicle to continue growth and maturation and finally ovulate, following the endogenous LH surge. Oestradiol, produced by the granulosa cells of the young dominant follicle acts, in synergy with FSH, on the granulosa cells to increase the expression of the genes coding for the LH receptor. This is a typical autocrine regulation loop, during which a specific cell layer, through one of its secretory products, modulates its own differentiation.

1.3.2 Endocrine, paracrine, and autocrine regulation of follicular steroidogenesis

Follicular steroidogenesis works according to the "two cells, two gonadotropins" concept (reviewed by Driancourt, 2001). More specifically, oestradiol production by the dominant follicle is the result of collaboration between the theca interna cells that produce androgens and granulosa cells that convert androgens into oestradiol (via aromatase). Androgen production is stimulated by LH, following its binding to the LH receptors present in this

cell layer. FSH acts on the granulosa cell layer via FSH receptors and triggers the expression of the gene coding for aromatase. Oestradiol production is an excellent example of the cooperation between the two layers of somatic cells within the follicle (Figure 7). However, the two cells, two gonadotropins model appears to be an oversimplification of the mechanisms at work. Indeed, compounds such as inhibin, produced by granulosa cells, appear to exert paracrine stimulatory effects on the LH-stimulated androgen production by theca cells (Figure 7). Finally, there is also an autocrine amplification loop involving the intrafollicular IGF-1 system that maximises the stimulatory effects of FSH on aromatase expression. Briefly, the increased follicular response to FSH, which occurs as the future dominant follicle grows, results in decreased amounts of two IGF-binding proteins (BP). IGFBP2 expression in granulosa cells is reduced, while expression of a protease triggering proteolysis of IGFBP4 is increased. The net result is increased bioavailability of IGF-1 that synergises with FSH to maximally increase aromatase (Figure 7). The combination of maximally stimulated androgen production by theca cells and maximally stimulated aromatase activity in granulosa cells is responsible for the sharp rise in oestradiol concentrations observed during the late follicular phase.

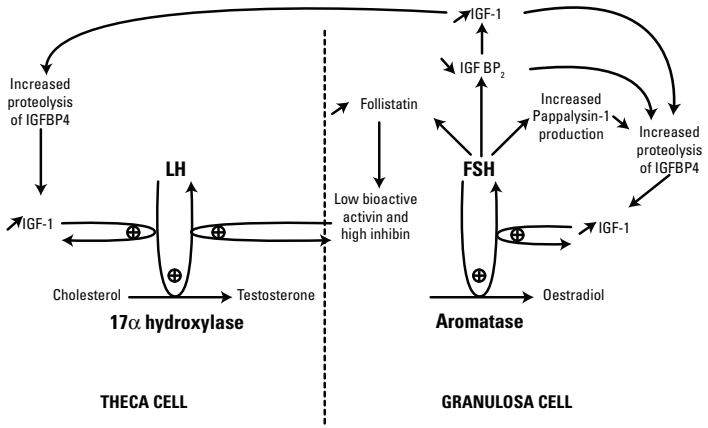

Figure 7 Mechanisms involved in the development of an oestrogen-active dominant follicle (after Driancourt, 2001)

1.3.3 Endocrine, paracrine, and autocrine regulation of oocyte function

There are two prerequisites for an oocyte to be fertilised. It needs to become competent to undergo nuclear maturation and secondly to have completed cytoplasmic maturation.

Nuclear maturation is the process whereby the oocyte nuclear material completes meiosis, moving from the fourth stage of the prophase of meiosis (diplotene stage) to the metaphase II stage. This normally occurs when the preovulatory follicle containing the oocyte is exposed to the LH surge. In vitro the ability of oocytes removed from their follicles to resume meiosis increases with the increasing size of the follicle. It reaches 100% for oocytes obtained from 2-3 mm (cattle) and 1-2 mm (sheep) diameter follicles. Oocytes originating from smaller follicles may resume meiosis in vitro but commonly fail to complete it properly, generally remaining at the metaphase I stage. Nuclear maturation is controlled by the balance between stimulatory signals, such as the LH surge, and inhibitory ones produced by the granulosa cells surrounding the oocyte (the cumulus cells) or by the theca cells. The exact paracrine mediators produced by the somatic cells of the follicle modulating nuclear maturation of the oocyte have not been fully clarified but may include purines, such as hypoxanthine, produced by theca cells. Purines act by maintaining high cyclic adenosine monophosphate (cAMP) concentrations within the oocyte.

Cytoplasmic maturation is the process where the oocyte stores a number of messenger ribonucleic acids (mRNAs) and proteins needed for survival and the first rounds of cleavage following fertilisation (before activation of the embryonic genome). Organelles are also widely redistributed within the oocyte during this process. In all species, full cytoplasmic maturation is acquired gradually and progresses in synchrony with follicular development. In cattle, oocytes that are enclosed in follicles around 4-6 mm in diameter are thought to have completed cytoplasmic maturation. Proper cytoplasmic maturation of the oocyte, such as occurs during terminal follicular growth in vivo, is associated with a high rate of embryonic development (around 60% blastocyst development rate) following in vitro fertilisation (IVF) followed by culture (IVC). In contrast, the culture conditions applied during in vitro

maturation (IVM) partly interfere with the quality of cytoplasmic maturation, markedly reducing the blastocyst production rate (to around 30% in good IVM conditions). It is likely that cytoplasmic maturation of the oocyte is controlled mainly by paracrine regulators (including cAMP) transferred from the cumulus cells to the oocyte via gap junctions (Gilchrist and Thompson, 2007).

1.4 Regulatory mechanisms involved in seasonality

1.4.1 A few facts about seasonality

In temperate latitudes, recurrent, seasonal changes in temperature, climate, and food availability influence reproductive activity. One of the common features of most wild and some domesticated species is the development of a reproductive pattern favoring birth at an optimal time of year, usually spring, which allows the newborn to grow under optimal conditions of climate and food availability.

This means that periods of sexual activity (oestrus) alternate with periods of sexual inactivity (anoestrus). Among domesticated species, sheep, goats, and horses display the strongest seasonality. In sheep, sexual activity begins as the day length becomes shorter (short-day breeders). In horses, sexual activity starts when day length increases (long-day breeders). In both species, young are born in the spring, when the environmental conditions are optimal for their growth and survival.

All species that display seasonal anoestrus may display either "shallow" or "deep" anoestrus, a feature that is typical of a breed, season, and nutritional status. Shallow anoestrus is characterised by a limited reduction in GnRH secretion, with the hypothalamus still generating infrequent LH pulses that may partly support terminal follicular growth but fail to support follicular maturation. This explains why sheep in shallow anoestrus may be induced to ovulate within 24 hours of exposure to a sexually active male. In contrast, deep anoestrus is characterised by a profound inhibition of the pulsatile secretion of GnRH, resulting in very low pulses of LH that prevent terminal follicular growth and maturation. In such females, ovulation cannot be

induced by exposure to a sexually active male, but administration of an exogenous gonadotropin (ie, PMSG) induces the growth of preovulatory follicles.

It is obvious that females that display deep anoestrus will transition through periods of shallow anoestrus when entering anoestrus and moving toward the breeding season. It is also noteworthy that shallow anoestrus may change to deep anoestrus in underfed females or females that display postpartum anoestrus and give birth outside of the breeding season (eg, sheep lambing in April-May).

1.4.2 The cascade blocking reproductive function in seasonal anoestrus (Figure 8)

Seasonality of reproduction is linked to the duration of daylight and darkness. There is general agreement that the eye is the window that reads the daylight/darkness information. The downstream mediator of this information is melatonin produced by the pineal gland. High concentrations of melatonin are observed during darkness, while its concentrations are very low during daylight. As days become shorter, the exposure to melatonin increases. Secondly, treatment of anoestrus sheep and goats with melatonin implants can induce the resumption of oestrous cycles. Until recently, the links between melatonin and the hypothalamic centers responsible for GnRH secretion were unknown, as melatonin receptors had not been demonstrated on GnRH neurons. Two other types of neurons, kiss and RF-amide-related peptide-3 (RFRP3) neurons, are now believed to be the ones that form a bridge between melatonin and GnRH release. Receptors for kisspeptin (kiSS-1-derived peptide receptor or GPR 54) and gonadotropin-inhibitory hormone (GnIH) (RFRP3 receptors or GPR 147) have been detected on GnRH neurons. Kiss appears to increase the pulsatile secretion of GnRH, while RFRP3 has the opposite effect. During the breeding season of sheep, expression of kiss and GPR 54 in the hypothalamus increases, while the number of connections between RFRP3 and GnRH neurons drops.

At the initiation of anoestrus, the balance between kiss and RFRP3 activities shifts and a strong activity of RFRP3 neurons reduces the pulsatile nature of GnRH secretion. Clear support for this hypothesis has been provided by the demonstration that an infusion of kiss successfully induced ovulation in all treated sheep (Caraty et al., 2007).

While such a balance perfectly explains seasonality in sheep, it does not appear to be valid for mares, where kiss treatment during seasonal anoestrus does not stimulate follicular growth and ovulation.

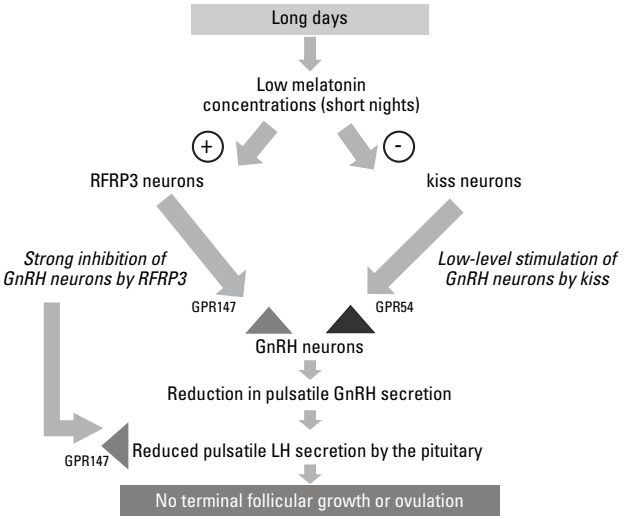

Figure 8 Mechanisms explaining why long days cause seasonal anoestrus in sheep

1.5 Regulatory mechanisms involved in postpartum anoestrus

1.5.1 A few facts about postpartum anoestrus

Postpartum anoestrus commonly occurs in the weeks following parturition. Most farm animal species (cattle, sheep, goats, and swine) do not display oestrus or ovulate for a variable period after parturition, during which milk production is maximized and young suckled following the development of a strong bond between the dam and its offspring. The purpose of this is to

optimise the survival of the newborn, and initiation of a new pregnancy has a lower priority. There are two exceptions to this: horses return to oestrus in the 2 weeks following parturition, and rabbits can be successfully mated on the day of parturition.

As in seasonal anoestrus, interactions between the female and its environment modulate the occurrence and depth of postpartum anoestrus. In dairy cattle, the duration of postpartum anoestrus is increased when the depth and/or duration of the period of negative energy balance is increased. This is a period when the nutrients ingested do not compensate for the energy requirements of milk production. In beef cattle and ewes, in which the udder is stimulated repeatedly by suckling throughout the day, the duration and/or depth of postpartum anoestrus is longer and/or deeper than in dairy cattle. This also applies to swine, where oestrus and ovulation are generally prevented before weaning. In all species that raise their offspring, maternal bonding between the dam and its offspring also interferes with the resumption of cyclic reproductive activity. Initiation of this bonding for the first time in primiparous females contributes to the increased length and/or depth of postpartum anoestrus in these animals.

1.5.2 The cascade blocking reproductive function during postpartum anoestrus (Figure 9)

During the postpartum period, nutritional, metabolic, and behavioural factors affect reproductive function at multiple levels of the hypothalamic-pituitary-ovarian axis. For example, in cattle, negative energy balance has been shown to reduce the frequency of GnRH pulses produced by the hypothalamus. This results in a reduced frequency of LH pulses available to support terminal follicular growth. In addition, negative energy balance appears to reduce circulating concentrations of IGF-1, thereby limiting its positive effects on follicular steroidogenesis. The maximisation of androgen production by thecal cells, which is a result of synergy between LH and IGF-1, fails to occur. Furthermore, oestradiol production from granulosa cells is limited by lack of induction of aromatase because the low IGF-1 concentrations do not synergise with FSH. Finally, the high nonesterified fatty acid (NEFA) concentrations produced by mobilisation of body reserves reduce granulosa cell proliferation and limit terminal follicular growth. Hence, during the period of negative energy balance, the blunted growth of

follicles, together with their incomplete maturation, explain why there is no oestradiol surge and therefore no LH surge followed by ovulation. Additional details on the nutrition-reproduction interface have been reviewed by Scaramuzzi et al. (2011).

1.6 Quality of sperm

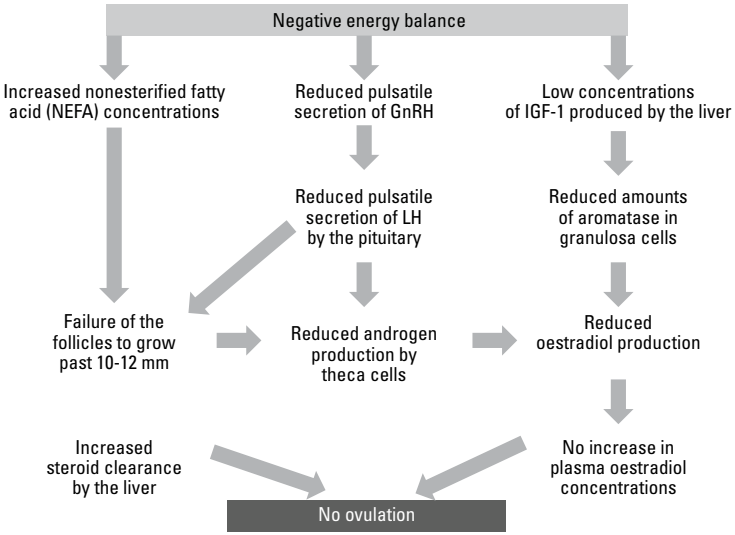

Figure 9 Mechanisms explaining why negative energy balance triggers postpartum anoestrus in cattle

Sperm quality has the ability to strongly modulate reproductive performance. This is why semen quality is regularly checked in most of the males used for natural mating. In addition, the semen collected for use in artificial insemination (AI) is carefully assessed before being released for use in the field. However, the identification of the key factors modulating sperm quality and use of this information to optimise reproductive performance are not easy tasks. Indeed, in species where there is a single ovulation, such as cattle and horses, sperm quality is defined by an all-or-nothing response (ie, pregnant or nonpregnant) that leaves little room to relate this information to in vitro markers of sperm quality. In contrast, in species with multiple

ovulations, such as swine, where a wide range of litter sizes may be obtained, it may be easier to identify useful markers of sperm quality. The section that follows focuses on swine.

1.6.1 Features of sperm that may be related to its fertilising ability

There are six groups of features and associated technologies that provide relevant information on the potential quality of a semen sample.

- The most obvious feature, which has been measured since the early years of AI, is the proportion of live and morphologically normal spermatozoa. This can easily be assessed under the microscope. This approach is mainly used to identify semen samples that would be unfit for use in the field.

- The ability of sperm to swim and move forward. While, in the past, this was evaluated under the microscope, this is now done using computer-assisted technologies (CASA) (Amman and Waberski, 2014). This is now the most widely used approach to assess semen samples for release for use in the field. However, it must be clear that this technology, while allowing discarding of semen samples of limited quality, does not predict the fertilising ability of the semen. Indeed, motility parameters only explain 9%-10% of the variation in the fertility of swine (Broekhuisje et al., 2012).

- The ability to undergo capacitation when exposed to suitable environments. Capacitation of sperm is a prerequisite for successful fertilisation. The response of sperm to in vitro capacitating agents can be monitored under the microscope. This parameter is only useful for the identification of semen samples that display a poor capacitation response.

- The ability of sperm to bind to zona pellucida (ZP) proteins. This is an obvious test system, as semen that is unable to bind to ZP proteins is unfit for use. However, monitoring this requires access to a source of ZP along with conditions similar to those used for in vitro fertilisation (IVF). In addition, the value of this test may be limited, as there is not a consistent relationship between the test results and fertility.

- A further refinement of in vitro tests is to assess and monitor fertilisation and development rates following IVF and in vitro culture (IVC). Sperm penetration into the oocyte, decondensation of the sperm nucleus, fertilisation, and embryo cleavage may be evaluated. This test has value in that decondensation of the sperm nucleus explains between 12% and 17% of the variation in fertility in vivo (Foxcroft et al., 2008). Although this is certainly the most informative system, it requires a proper IVF/IVC setting to generate relevant information.

- Recently, research has focused on the identification of specific proteins in seminal plasma that may be markers of the fertilising ability of semen. A few proteins have been found to be consistently present in the semen of swine with low fertility (Dyck et al., 2011). These findings need to be confirmed in larger and more diverse populations.

While all such tests may provide useful information, it must be remembered that fertility is a multifactorial trait generated by a heterologous sperm population resulting from several spermatogenic waves and variable durations of storage in the epididymis. It should therefore not be surprising that it has proven difficult to characterise the fertility of sperm using a single in vitro test. In addition, insemination with very large numbers of spermatozoa (eg, 3 billion in swine) may not allow the identification of males with the least fertile semen.

1.6.2 Sperm biotechnologies and fertility

While describing the different semen biotechnologies is out of the scope of this chapter, it is worth remembering that for a specific semen sample, the steps needed to store the semen (cooling or freezing) or split the sperm population into X- and Y-bearing spermatozoa (semen sexing) may strongly alter its fertilising features. For example, it is well known that:

- Use of sexed semen in cattle is commonly associated with a 10%-15% drop in fertility compared to control animals inseminated with the same semen not submitted to the sexing procedure.

- In swine, unless the insemination-to-ovulation interval is no more than 4 hours, the use of frozen, thawed semen results in a drop in both farrowing rate and prolificacy. The window of opportunity for obtaining high reproductive performance using frozen, thawed semen is therefore much narrower than with fresh semen.

- Semen from specific horses, which is normally fertile when fresh semen is used immediately following collection, does not withstand conservation under cooled conditions or the freeze-thaw cycle.

1.7 Further reading

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Without reproduction, there is no production. Reproduction is not only essential but is very complex and dynamic. There are many factors that can impact the final outcome. The fishbone diagram below in Figure 1 illustrates many of the factors that can influence pregnancy outcomes.

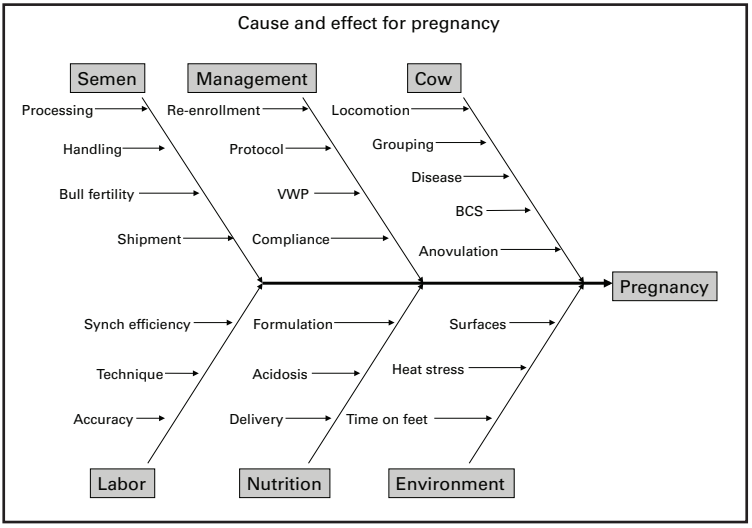

Figure 1. Adapted from ABS Cause and Effect Chart for Pregnancy Production.

The scope of this chapter is not to cover all the different factors but a general overview of management and health factors that influence reproduction effiency in cattle.

2.1 Physiology

2.1.1 Nutritional influence

Numerous studies in dairy herds have clearly shown that a marked increase in milk production during early lactation increases the incidence of various reproductive problems (Grohn et al, 1994; Macmillan et al., 1996; Poso et al., 1996). The genetic capacities for high levels of milk production in dairy cattle have been associated with a gradual decline in fertility.

The inability to meet the high energy requirements for both maintenance and production in high yielding cows leads to a negative energy balance, particularly during the first few weeks after calving.

It has been well documented that overconditioned cows at calving often have a reduced appetite and develop greater negative energy balance than normal herd mates. Overconditioned cows exhibit more extensive mobilisation of body fat leading to hepatic lipidosis (Rukkwamsuk et al., 1998), which is associated with impaired fertility.

Energy balance during the first three weeks of lactation is highly correlated with the interval between calving and first ovulation (Butler et al., 2000). Low energy availability during the first few weeks of lactation impairs LH secretion, but it also reduces the responsiveness of the ovary to LH stimulation (Jolly et al., 1995; Butler, 2000).

2.1.2 Transition cow management

The transition period is traditionally defined as the periparturient period from about 3 weeks before calving to 3 weeks after calving. This is a time of tremendous change metabolically and physiologically. The cow must transition from a pregnant and nonlactating state to a nonpregnant and lactating state in a very short time. Because of these changes that are happening, the cow is very vulnerable to both metabolic and infectious disease. Any small perturbation will disrupt these Olympic-class metabolic athletes. Even though the event of insemination may still be months away, what happens in this time period can have significant impact on fertility.

2.1.3 Endocrine environment in high-yielding dairy cows

In a trial reported by Lopez et al (2004), the duration of oestrus was correlated positively with peak oestradiol concentrations and correlated negatively with milk production. Wiltbank et al (2006) suggested that high levels of milk production lead to reduced circulating oestradiol concentrations, resulting in the decreased duration and intensity of oestrus. See Figure 2.

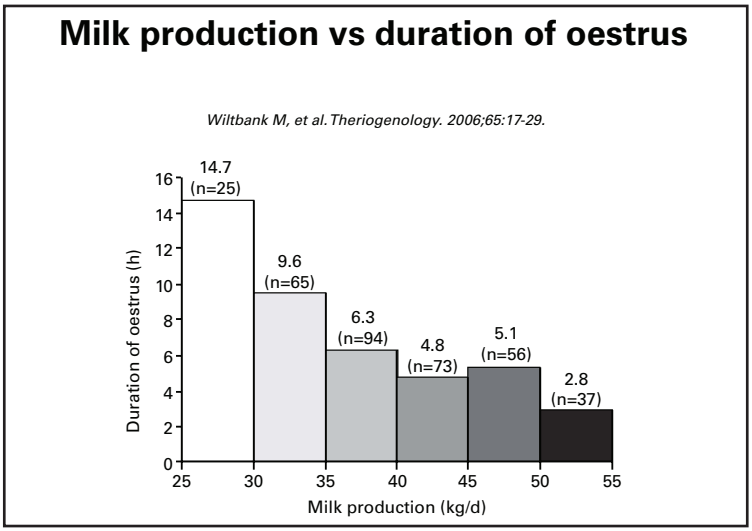

Figure 2. Relationship between level of milk production and duration of oestrus. Analysis included all single ovulations (n=350) except first postpartum ovulations. Average milk production is for the 10 days before oestrus; from Wiltbank et al. (2006).

High-yielding cows may exhibit a different endocrine profile than non-lactating or lower yielding cows due to their high metabolic rate. Cows producing more milk develop larger follicles, but with lower circulating oestradiol concentrations (Lopez et al., 2004). They also have a greater volume of luteal tissue with reduced circulating progesterone concentrations. As milk production increases in lactating dairy cows, metabolism of steroid hormones increases.

Wiltbank et al (2006) proposed that some of the reproductive changes in lactating dairy cows are caused by a dramatic increase in steroid metabolism due to the enhanced feed intake and blood flow through the liver. In lactating dairy cows, a continuously high plane of nutrition leads to chronically elevated hepatic blood flow and approximately double the rate of metabolism of steroid hormones as compared to nonlactating peers of similar size and age. Results of recent trials indicate that even with a similar level of hormone production, the level of circulating concentrations of steroid hormones is lower during lactation (Sangsritavong et al., 2002; Wiltbank et al., 2006).

In addition to lower oestradiol concentrations at the start of oestrus, there is also likely to be a more rapid reduction in circulating oestradiol after LH surge due to the increased metabolism of this steroid. This would result in a shorter duration of oestrus in high-yielding cows. Elevated steroid metabolism due to high milk production levels can also have a more profound detrimental effect on fertility. The preovulatory follicle and oocyte may be exposed to an extended period of elevated LH pulses, which in turn may lead to ovulation of an overstimulated or prematurely activated oocyte and thus to reduced fertility. Also the reduced rate of rise of progesterone following ovulation can reduce fertility because of the poorer survival of embryos.

Follicular growth and development are most probably directly influenced by altered insulin, insulin-like growth factor I (IGF-I), leptin and levels of NEFAs in high yielding cows. Since insulin stimulates follicular growth, maturation, and steroidogenesis locally, its reduced concentrations postpartum are linked with impaired follicular development.

It is important to recognise that the effects of negative energy balance can still be observed even after cows begin to recover in body condition. Research workers have postulated the existence of a carryover effect of the adverse metabolic conditions on the health of the preovulatory follicle during the early postpartum period 2-3 months later. Such follicles may be less capable of producing adequate amounts of oestradiol, contain an oocyte of lower quality, and, following ovulation, form a corpus luteum of reduced steroidogenic capacity.

2.1.4 Physiology of the oestrous cycle

The sexual cycle of the cow is generally independent of the season of the year. Oestrus or 'heat' is observed every 21 days on average, with a range of 18-24 days. The beginning of oestrus is taken as day zero of the cycle. It is of relatively short duration, lasting on average 18 hours, with a range of 4-24 hours. Ovulation takes place about 24-32 hours after the onset of oestrus. Fertilisation of the ovum takes place in the oviduct. The blastocyst arrives in the uterus at around day 5. Pregnancy lasts for 279-290 days. The interval from calving to first ovulation varies greatly depending on the breed of cow, nutrition, milk yield, season, and the presence of a sucking calf. First

ovulation after calving is frequently not accompanied by oestrus behaviour and is known as a 'silent heat'.

a. Follicular growth

Follicular growth and development in ruminants is characterised by two or three consecutive follicular waves per oestrous cycle. Ultrasonography has provided information about the stages of follicular growth and follicle selection. Each wave involves the recruitment of a cohort of follicles from the total ovarian follicular stock and selection of a dominant follicle that continues to grow and mature to the preovulatory stage, while the others undergo atresia. Three distinct stages can be distinguished in follicular development: recruitment, selection, and dominance phases. See Figure 3.

Each wave consists of the simultaneous recruitment of three to six follicles to grow larger than 4-5 mm in diameter. Within a few days of the start of a wave, one follicle emerges as dominant. The dominant follicle continues to grow and differentiate, whereas its sister follicles stop growing and regress. The dominant follicle of the first wave in two-wave cycles, and of the first and second waves in three-wave cycles, regresses. When the dominant follicle reaches a size of approximately 10 mm, it is capable of being stimulated to ovulate at any time, as it continues to grow until it becomes atretic.

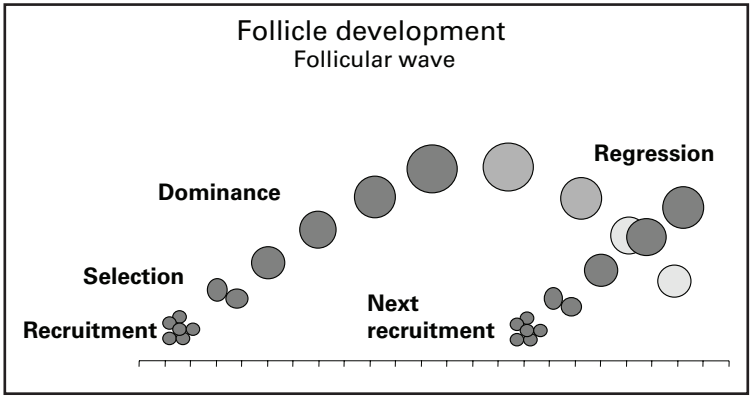

Figure 3. Follicle wave.

i. Follicle recruitment

In cattle and other species, follicular waves are preceded or accompanied by a small rise in FSH. See Figure 4. All follicles growing as a cohort contain specific receptors for FSH and depend on this gonadotrophin for their growth. At this stage, the growing follicles do not have a sufficient population of LH receptors to respond to an LH-like stimulation, which is why this stage of growth is sometimes called FSH-dependent.

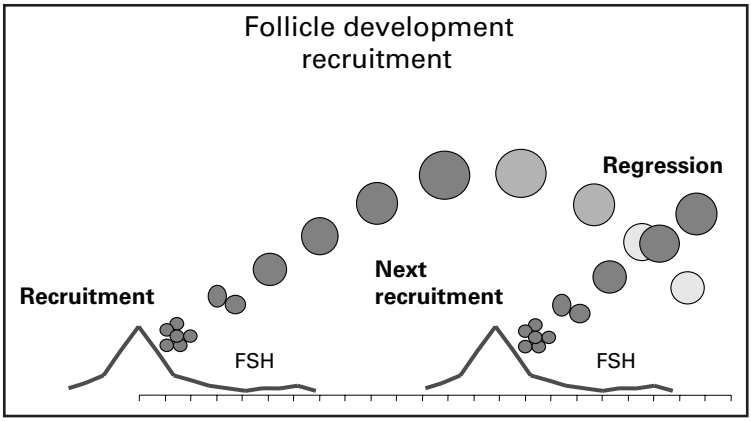

Figure 4. Follicle recruitment.

ii. Follicle deviation (dominant follicle selection)

For reasons not yet totally understood, only one dominant follicle deviates or is selected from the cohort recruited by the small rise in FSH. A defining characteristic of the dominant follicle appears to be its greater capacity for oestradiol production. Secretion of oestradiol, and perhaps androgen, by the dominant follicle is associated with the cessation of the rise in FSH, and then its maintenance at basal levels (Ginther et al., 2000 a, b). The future dominant follicle acquires LH receptors that allow it to continue to grow in the environment of low FSH and increasing LH levels. See Figure 5. This is confirmed by the enhanced binding ability of the granulosa cells of the newly selected dominant follicle.

By indirectly lowering the FSH level, the dominant follicle decreases the support crucial for the subordinate follicles while at the same time

benefiting from low FSH and increasing LH levels. At the time of deviation, the dominant follicle can maintain follicular cell proliferation and enhance oestradiol production in spite of declining FSH stimulation. It is possible that the dominant follicle increased or maintained high FSH-receptor mRNA expression and FSH-binding, allowing it to pass the 8.5 mm diameter threshold (Mihm and Evans, 2008).

Recently, important information has emerged about the role of other modulators such as growth factors, inhibin, and insulin in the differentiation and selection of the dominant follicle (Fortune et al., 2001; Mihm et al., 2003). Somatotropin causes an increase in the synthesis of insulin-like growth factor I (IGF-I) and its principal carrier protein along with its binding protein. It is now well accepted that the ovary is an important site of both IGF-I gene expression and reception. Most of the components of the IGF system are expressed in bovine follicles.

A recent review published by Silva et al (2009) listed the following processes in which IGF-I is involved in cattle: stimulation of growth in primary and secondary follicles; oestradiol production by secondary and antral follicles; proliferation of granulosa cells in antral follicles; oocyte viability and maturation; increase in the sensitivity of follicles to gonadotrophins; follicle dominance; and multiple ovulations.

Since IGF-I has been demonstrated to enhance the FSH-induced granulosa cell differentiation, and especially LH receptor acquisition, increased free IGF-I concentrations in the selected future dominant follicle may be responsible for its developmental advantage.

Progesterone levels are very important at this time of deviation or dominance selection. Lower circulating concentrations of progesterone during development of the preovulatory wave in cows lead to an increased number of selected follicles and multiple ovulations in higher producing cows (Lopez et al., 2005). See Figure 6. Because both codominant follicles deviate from subordinate follicles and acquire ovulatory capacity, they are responsive to ovulatory stimuli leading to the occurrence of multiple ovulations. If oocytes from such ovulations are fertilised and pregnancy is maintained until parturition, dizygotic twins will occur.

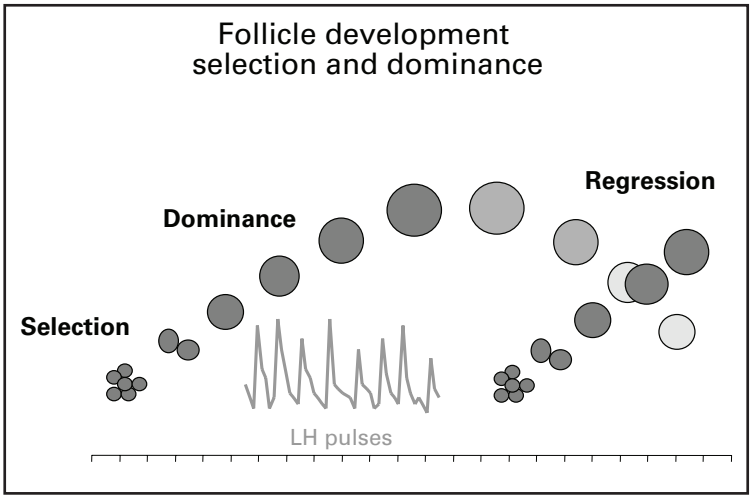

Figure 5. Follicle selection and dominance.

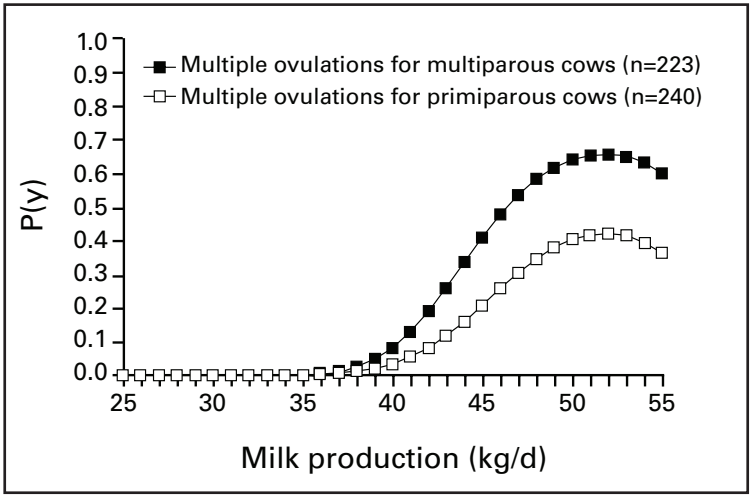

Figure 6. The relationship between the probability (y) of multiple ovulation and level of milk production during the 14 d preceding oestrus.

iii. Dominance

Following its selection, the growth, oestrogen activity, and life span of the dominant follicle are controlled by the LH pulse pattern. Therefore, any changes in GnRH, and thus LH, release pattern will have a profound effect on the continued growth of the dominant follicle and its ovulation. It is now well known that the increased frequency of LH pulses seen following progestagen treatments will prolong the period of dominance from 2-7 days to more than 14 days, which affects the fertility of the oocyte (Diskin et al., 2002). Nutritional, environmental, and even infectious factors that directly and indirectly affect the GnRH/LH pattern in cattle will have a considerable effect on the fate of the dominant follicle, and consequently on ovulation and fertility.

b. Formation and function of the corpus luteum

A very distinct structure is formed in place of an ovulated follicle called the corpus luteum. The corpus luteum is created by a process called luteinisation. It involves both structural and functional changes in the granulosa and theca cells of the ovulated follicle, which lead to the establishment of a transient secretory structure.

There are at least two types of cells with steroidogenic properties in the bovine corpus luteum: large and small luteal cells. The large luteal cells are formed from the granulosa cells and the small luteal cells from the thecal cells of the follicle after ovulation. It is important to remember that the corpus luteum consists not only of steroidogenic luteal cells but also of a multitude of other cell types, such as vascular endothelial cells, various immune cells, and fibroblasts.

The formation of the corpus luteum is directly stimulated by the action of LH. However, there is clear evidence that steroidal and protein hormones, growth factors, eicosanoids, and cytokines play important roles in the establishment of a functional corpus luteum (Berisha and Schams, 2005).

In cattle and other domestic animals, it is the luteinising hormone (LH) released from the pituitary gland that acts as the most potent regulator of the synthesis and secretion of progesterone by the corpus luteum (Skarzynski et al., 2008). LH stimulates the production of progesterone in small luteal

cells via a specific LH receptor. Intraluteal progesterone concentrations have recently been demonstrated to be one of the most important factors supporting the maintenance of the function of the corpus luteum.

The newly formed corpus luteum is resistant to the action of exogenous $PGF_{2\alpha}$ until day 4-6 in cattle. It also seems that the sensitivity of the corpus luteum to extragonadal $PGF_{2\alpha}$ increases progressively towards the end of the luteal phase.

c. Luteolysis

In animals that have not become pregnant, regression of the corpus luteum, the process called luteolysis, is essential for sustained cyclicity and allows for the development of a new dominant follicle, giving the cow yet another chance to conceive.

Luteolysis starts between days 16 and 17 postoestrus in the cow. It is initiated by prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) release from the uterus during the late luteal phase. Uterine venous $PGF_{2\alpha}$ increases initially on day 16-17 postoestrus in cows.

The luteolytic cascade can be summarised as follows: Oxytocin produced by the corpus luteum binds to specific oxytocin receptors in the endometrium (which have been induced by limited amounts of oestradiol produced in late luteal phase follicles). Release of $PGF_{2\alpha}$ is stimulated from the endometrial cells. Prostaglandin is released into the uterine vein and reaches the ovary as previously described via the ovarian artery, and causes regression of the corpus luteum.

A rapid functional regression of the corpus luteum is characterised by a decrease in progesterone production, followed by a structural regression phase. The mechanism of the luteolytic action of $PGF_{2\alpha}$ has not yet been completely elucidated, but two major mechanisms have been suggested:

i. Reduction of the blood flow in the corpus luteum

A rapid decrease in luteal blood flow has been proposed recently as one of the main luteolytic actions of $PGF_{2\alpha}$. The histological changes that take place during luteolysis include hypertrophy and hyperplasia of cells

in the arteriolar wall, accumulation of elastic fibres in the media, mucoid degeneration of the intima, protrusion of some endothelial cells into the capillary lumen, and the formation of adherent junctions across them, resulting in a decrease in the vascular diameter. Also, it has been recently demonstrated that an injection of a luteolytic dose of prostaglandin $F_{2\alpha}$ analogue in the mid-luteal phase increases the intraluteal production of vasoactive substances, which play an important part in the luteolytic cascade. It was shown that the reduction in luteal blood supply at 8 hours after prostaglandin injection was coincident with the onset of structural luteolysis, which was reflected in the initial significant decrease in CL volume.

ii. Direct action on luteal cells

A direct action of prostaglandin on the luteal cells, resulting from both the decrease in cAMP synthesis normally produced in response to LH and the inhibition of the steroidogenic action of cAMP. These effects would be further amplified by a reduction in the number of LH receptors. During the structural phase of luteolysis, the luteal cells undergo a process of apoptosis (so-called 'programmed death').

This thesis is further supported by the results of a study that showed that a prostaglandin-induced reduction in plasma progesterone concentrations occurs before a detectable decrease in both the volume of the CL and the luteal blood flow.

2.2 Economic aspects

Reproductive performance directly affects farm profitability through milk production per cow per day, increased cow maintenance costs, number of calves weaned, number of replacements available, and culling opportunities.

2.2.1 Losses in milk yield

Longer intervals between calvings result in extended lactations. The extended days in lactation are at lower and lower milk yields the further away it is from calving. Dry periods may also be extended, resulting in excessively long periods of no milk yield. Good fertility is critical to maintain sustainable milk yields.

2.2.2 Maintenance costs

Every cow has maintenance costs associated with survival. It is the costs to maintain body functions when no millk is being produced. Maintenance costs are fixed costs and independent of milk yield. The lower the milk yield, the higher the proportion of total nutrient and energy costs relative to the amount of milk being produced. In other words, higher milk yields will dilute fixed or maintenance costs over more units of milk. Longer intervals between calvings from lower fertility result in higher fixed or maintenance costs per unit of milk produced, decreasing profitability.

2.2.3 Number of calves born

Longer intervals between calvings result in lower numbers of calves that will be born in any given year. This will impact the number of calves available for replacements or the number of calves that can be marketed in a given year. This leads to increased production costs and lower returns.

2.2.4 Culling

High reproductive efficiency provides great opportunity to cull less productive cows for reasons other then reproduction. Remember that without reproduction there can be no production. If all the cows are pregnant, then economic opportunities are much greater.

The losses caused by premature culling due to infertility depend on the age and the production level of the cow culled. These losses represent the missed future income from that cow, the cost to replace her, and the lost genetic potential. The losses are maximal for a high-yielding cow in her second lactation and, thereafter, decrease with age and lower production level (Dijkhuizen et al., 1991).

Additional lost opportunity comes from culling a productive nonfertile cow and keeping a less productive but fertile cow.

A high annual replacement rate will shift the age of the herd towards an increasing proportion of first lactation cows. Above a certain threshold, this is unfavourable with respect to both milk production and reproductive efficiency.

First lactation cows tend to have the greatest frequency of postpartum reproductive disorders that impact both milk yield and fertility. They also have not generated enough income to pay for their development or purchase.

2.3 Herd fertility management

There are many technologies that have been developed and many more will be developed to improve reproduction efficiency. A combination of technologies can be used by herd managers, veterinarians, and advisors. These technologies can be grouped into general categories in a cycle that keeps a cow productive for the duration of her productive life. Figure 7 illustrates the reproductive technology cycle utilised in managing reproduction. A portfolio of technologies can be implemented on a farm to match available resources, housing and handling facilities, goals, values, and management styles. It is not uncommon for different technologies to be lumped together in a casual manner with little oversight, creating great inefficiencies. The portfolio of technologies must also match the management structure and resources on the farm.

The degree of compliance to the portfolio of technologies employed on a given farm will do more to deterimine reproductive efficiency than any given technology by itself.

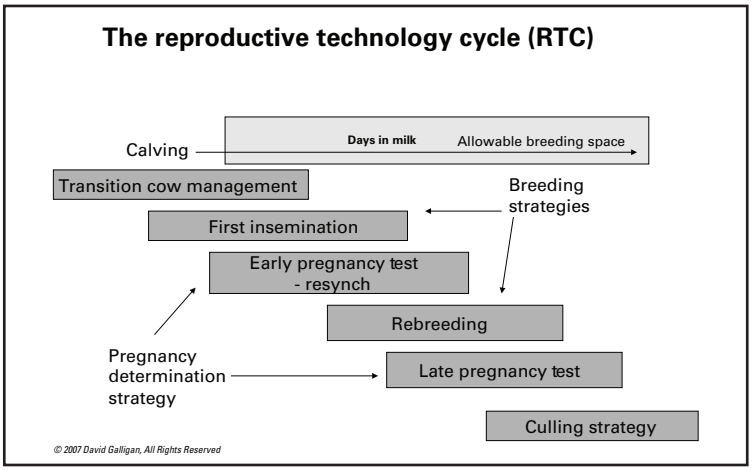

Figure 7. Reproductive technology cycle.

Allowable breeding space is that period between when the decision has been made to begin breeding and the point in time in which it is more profitable to replace the cow with a better investment (replacement heifer) than it is to continue trying to get her pregnant. This is unique to each individual cow and herd because it is dependent on many economic factors that are unique to that cow and that farm. Typically, many herds will manage for the exceptions.

For the optimal production of both milk and calves, the target is generally for every cow in the herd to produce a live healthy calf each year. Numerous studies have documented that cows that fail to become pregnant during the allowable breeding space are an expensive proposition (Groenendaal et al., 2004; De Vries, 2006).

2.3.1 Evaluation of fertility

Data collection for evaluation is very simple and easy. Everyone collects the same data. From calving dates, every breeding date, pregnancy confirmation, and mating start dates, every major reproduction indice can be calculated. Adding additional information, such as lactation number, allows the data to be parsed and a more granular analysis. It is the evaluation that is very complex and confusing because of many issues with the data.

The data issues that must be taken into consideration when evaluating reproduction are variation, momentum, lag, and bias. There is variation in everything in life. Data on reproduction is no different. Averages can be very deceiving and cover up a lot of issues. As someone once said, the average of an elephant and a mouse make a nice cow. There are many ways the data can be parsed to look at distributions of data and not just averages.

Momentum in the data is when history hides current performance. In other words, it takes a lot of change across time to get a parameter to change. The parameter is not very sensitive to time. The profile of a dairy herd with year-round calving would have the distribution and type of data available at any one point in time found in Table 1.

Time postcalving	Type of data
< 60 days	No data
60 to 120 days	First insemination Some repeat insemination Some pregnancy confirmation
120 to 180 days	Limited first insemination Repeat insemination 30%-50% pregnancy confirmation
180 to 365 days	85% pregnancy confirmation Dry cows Problem breeders
>365 days	Problem breeders Peers or calving cohorts have recalved Skews seasonal data

Table 1. Type of reproduction data available by days in milk.

There is tremendous momentum in calculating average days open or calving interval for the herd. The data from the cows greater than 180 days since calving is not very current. It has very little reflection on what is happening today on the farm and will not change until those cows recalve or are culled. It would require a monumental change for it to be reflected in average days open or average calving interval.

This does not mean that average days open or average calving interval is a bad number. It just means one needs to understand the momentum behind the number and what it represents. It is not very sensitive to change and current conditions on the farm. Many people will parse the data and only look at those cows within a certain time period. This decreases the momentum it takes to change the number but introduces a bias into the evaluation.

For seasonal calving herds, 6 week-in-calf rates or pregnancy rates for the season is a parameter with lots of momentum because of the lag between the event (conception) and when it is diagnosed. Once you know the 6 week-in-calf rate or pregnancy rate for the breeding season, the "train has already left the station." There is nothing you can do to change it until next year.

Bias is introduced when the data is parsed to reflect a certain group of animals. Days open only in pregnant cows is valuable information but is only for those cows that are pregnant and ignores those that are not confirmed pregnant. The reverse is also true – days open only in cows not confirmed pregnant does not reflect the entire picture. Many times the data is parsed and biased to try and find where opportunities are hiding. It is not bad but must be recognised.

Lag is the period of time between when an event occurs and when the outcome is known. In the case of reproduction, the event is conception. It can be a few weeks to many months before the outcome of that event is known. This makes for some real challenges in monitoring what is happening right now so that management intervention can be implemented to correct a less than optimal outcome.

The farm manager requires information that is actionable – information that is current, accurate, and sensitive to change. The farm manager requires parameters that can monitor what is happening today and reflect change for what happens tomorrow or the next day. The bottom line is the number of cows that get pregnant every day, week, or month.

There are too many parameters that can be evaluated to list all of them. It is best to use a trusted advisor that knows and understands farm management. Every advisor has his or her favorite parameter(s) to look at that he or she understands very well. It is not the intent of this chapter to provide a comprehensive list of reproduction parameters, definitions, nuances, and interpretations. The role of the advisor is to "mine" the data, identify opportunities, and provide direction – "doing the right things," while the farm manager's role is to get the work done – "doing things right." In doing so, the farm manager must monitor actionable parameters and plan on how to get the work done.

Oftentimes, parameters that are used to evaluate reproduction efficiency are good to monitor and help identify areas of opportunity but lack the actionable information that a farm manager needs to make decisions today. Because of the limitations of the data, evaluation of fertility is often after the fact.

The list in Table 2 is a proposed list of information that a farm manager needs for current day-to-day management.

Parameter	Target
Percent of cows that conceived 30-60 days ago, what percent were less than 150 DIM* at conception	>80%
List of cows >150 DIM* and not confirmed pregnant	Make action plan for cows on list
List of problem breeders	Make action plan for cows on list
Percent of herd that conceived 30-60 days ago	7.5%
Abortions (>60 days postinsemination)	<5%

*DIM = days in milk

Table 2. List of parameters and targets recommended for monthly monitoring providing day-to-day management information in a year round continuous calving herd.

In a seasonal calving herd with a very short breeding window, there is a real challenge to get good management information that is current and actionable because of lag in the data. Most of the evaluation is done after the fact and helps to analyse what happened and how to make it better the next season. The list in Table 3 is a proposed list of information that a farm manager needs for current day-to-day management in a seasonal calving herd. There are plenty of other measures to evaluate reproduction efficiency in a seasonal calving herd, but they are all after the fact and there is no opportunity to intervene until the next year. It is a very intense time. Most farm managers are very good at meeting 3 week submission targets. It is after 3 weeks that it becomes difficult to measure and monitor progress until well after the "train has left the station." This is all because of the lag in detecting cows that are not pregnant and not showing oestrus. Conception rates become success limiting as much as any other parameter.

Parameter	Target
3 week insemination submission rate	>90%
List of cows not inseminated and beyond 3 weeks from mating start date	Make action plan for cows on list
6 week in calf rate	>80%

Table 3. List of parameters and targets for seasonal calving herds.

2.3.2 Transition cow management

Many postpartum diseases are multifactorial and do not occur in isolation. They are interrelated. For example, many infectious diseases occur as secondary illness to primary metabolic conditions. Transition diseases have both short-term and long-term impacts on cow health. One of the long-term impacts is reproductive performance.

Reproductive performance is primarily associated with uterine diseases but can also be impacted by energy balance and lameness. In a meta-analysis, links were found between clinical ketosis, dystocia, and retained placenta with increase in days to first service and lower conception rates at first service (Fourichon et al., 2000).

Negative energy balance occurs at the beginning of lactation when nutrient demand is greater than nutrient intake. Prolonged periods of negative energy balance will impact the onset of first ovulation and impact fertility through reduced levels of progesterone. Lower progesterone levels will alter uterine function, early embryonic development, and oocyte quality (Butler, 2003).

Critical to achieving optimal performance is a good monitoring system. Monitoring consists of the regular observation and recording of activities, events, and yields. A recent publication by Overton (2014) suggests that tranisiton metrics can typically be classified as either leading or lagging indicators. A lagging indicator is one that follows the event of interest, whereas a leading indicator predicts future events and usually changes ahead of the event in question. For example, postpartum disease events are lagging indicators of poor nutritional, housing, metabolic, and/or general management of cows during the transition period. Stocking density of the far-off dry and close-up pens as well as the total amount of energy consumed during the far-off dry period could be viewed as leading indicators.

Most monitoring programs have focused, traditionally, on the evaluation of postpartum disease outcomes (lagging indicators), but more emphasis should be placed on monitoring of leading indicators to prevent disease rather than simply waiting to respond to increased disease incidence.

Table 4 below contains suggested key monitors for the transition period (Overton, 2014).

Transition group	Key monitors	Targets
Far-off dry group	Distribution of days dry for all cows that calved in the previous month	85%-95% within 14 days of dry period length goal
	Dry matter intake	Depends on breed/body size
	Housing and comfort	Depends on housing type
Close-up dry group	Days in close-up pen	Average – 23-24 days 90% >10 days
	Dry matter intake	Depends on breed/body size
	Stocking density/bunk space	>76 cm/cow
	Housing and comfort	Depends on housing type
Recently calved group	Dry matter intake	Depends on breed/body size
	Stocking density	>76 cm/cow
	Clinical disease	
	Energy status, herd level – BHBA at 3-9 days in milk	<15% cows >1,200 µmol/L (sample size 15-20)
	Energy status, herd level – milk fat:protein ratio at 10-40 days in milk	<40% cows with ratio ≥1.4
	Milk yield 15-30 days in milk	Depends on breed/ management system
	Cow comfort/time budget	Never restrained more than 35-45 minutes
	Body condition score loss in first 30 days postcalving	<0.75 body condition score on 1 to 5 scale

Table 4. Suggested key monitors and targets for the transition period.

2.3.3 Breeding strategies

There are many factors that can interfere with a cow becoming pregnant, but the primary cause is a management disease that results in a semen deficiency in cows. The cows are not getting inseminated or exposed to fertile bulls in a way that allows the cows to express their true potential. The strategy employed by management to expose cows to semen either through artificial insemination or natural service is essential for the productivity of any cattle farm.

2.3.4 First insemination strategies

Getting semen into cows for the first time in a timely manner is the most critical factor and has the greatest influence on overall reproductive performance, more than any other factor associated with reproduction in cattle. How efficient a farm is at getting semen into cows for the first time is a good indicator of how well management is controlling reproduction on the farm.

First insemination strategies are all about getting semen into cows and are a function of oestrus detection or ovulation induction to create the opportunity for pregnancy to occur. It is recognised that fertilisation is important, but fertilisation is impossible without semen being deposited in the cow.

For herds that calve all year round, the following measures in Table 4 are recommended to evaluate and monitor first insemination strategies. It is not intended that a manager would need to look at each parameter listed in the table. Several of them are measuring the same outcome in different ways. The shorter the time period used in the calculation, the more meaningful the information. In other words, monthly estimates are much more current and meaningful than rolling annual averages. But there still needs to be a sufficient number of animals in the estimate to make it meaningful.

Parameter	Target
First service insemination efficiency	>90%
DIM when 80% bred for first time	<80 DIM
Percent of cows inseminated by 80 DIM	>80%
Percent of cows beyond VWP+20 days and not bred	<20%
List of cows >80 DIM and not inseminated	Make action plan for cows on list

DIM = days in milk; VWP = voluntary wait period.

Table 5. Recommended measures to evaluate and monitor first insemination strategies.

First service insemination efficiency is estimated as the percent of cows that actually get bred in the window VWP+20 days out of the cows at risk for being bred for the first time in the same window. It is essentially a measure of oestrus detection to first service.

Voluntary wait period is a management decision as to the interval from calving to the start of breeding cows. It can be estimated by determining at what DIM when 5% of the cows have been bred for the first time. In general, cows are not bred before 40 DIM because the uterus is not ready to sustain a pregnancy and conception results are not very good. The ideal voluntary wait period is a balance between breeding as soon as possible after calving, uterine, and ovarian health, and good conception results. For every 10 day increase in voluntary wait period there must be a corresponding increase in conception of at least 6-8 percentage points. At the same time, for every 10 day decrease in voluntary wait period, there must be a corresponding decrease in interbreeding intervals or increase in aggressive management to identify nonpregnant cows and get them inseminated again.

A seasonal calving herd presents a much more challenging set of circumstances to manage and get good actionable information during the breeding season. This is because of the inherent challenges with data lag. A very useful number is the 3 week insemination or submission rate. This is the percent of cows that get bred between the mating start date (MSD) and 3 weeks later. It is actionable because those cows that are not bred in this window can be identified easily and intervention made to get semen into them. See Table 5. Everything else is measuring the outcome after the "train has left the station" – it is too late to intervene. This makes seasonal calving particularly challenging to manage; good management or control of reproduction is much more critical than in a year-round calving herd.

Parameter	Target
3-week insemination submission rate	>90%
List of cows not inseminated and beyond 3 weeks from MSD	Make action plan for cows on list

MSD = mating start date.

Table 6. Suggested measures in seasonal calving herd to evaluate and monitor first insemination strategies.

2.3.5 Oestrus detection

Oestrus detection is a very critical component of first service insemination efficiency; identifying those cows that do not conceive to first service to get semen into them again is equally critical. Insufficient and/or inaccurate oestrus detection leads to delayed insemination, reduced conception rates, and thus, extended calving intervals.

Oestrus is the complex of physiological and behavioural signs occurring just before ovulation. The length of oestrus varies from 4 to 24 hours. The signs of oestrus are: standing when mounted; swollen vulva; hyperaemic vaginal mucosa; clear and elastic mucous vaginal discharge; ruffled tailhead, possibly with minor skin lesions; restlessness; group formation; chin rubbing; flehmen; licking, pushing, fighting, mounting other animals; lordosis; and possibly reduced feed intake and/or milk yield. The standing reflex (standing when mounted) is a truly reliable indication of oestrus. The cow is then said to be in 'standing heat.'

a. Heat mount detectors

There are many different types of heat mount detectors on the market. All are applied just in front of the tail head on the mid-line of the cow's back. A 'triggered' detector indicates that the animal has been mounted. All are considered to be aids in heat detection and can only supplement visual observation. Each technology presents its unique advantages and disadvantages.

Perhaps the most sophisticated mount detector comprises a pressure-sensitive battery-operated radio transmitter. When activated, the transmitter emits a radio signal, which is picked up by a receiver. The signal is then digitised and stored on a computer with the date, time, duration of each mount, and the cow's identity.

Tail paint is a simple and cost-effective heat detection aid when managed properly. A strip of brightly coloured paint (20 cm long and 5 cm wide) is applied to the midline area in front of the tail head. When the cow is mounted during standing heat the paint is rubbed off by mounting cows. Depending on the type of paint being applied, it may need to be refreshed daily. At a minimum, each individual cow needs to be observed every day.

b. Teasers

Teaser animals, ie, vasectomised bulls or testosterone-treated cull cows, will mount a cow in heat. It still requires someone to observe the standing heat. They may be equipped with a chin ball marker to mark cows that they are mounting so that actual observation of standing heat is not required. Aggressive behaviour, and the development of favouritism (ignoring nonfavorite cows in heat), are disadvantages of this system. Vasectomised bulls may also be vectors of venereal diseases.

c. Pedometers

When a cow is in heat, there is a large spike in activity relative to when it is not in oestrus. By measuring the number of steps using pedometers, a cow in oestrus can be identified. There is a lot of cow-to-cow variation. Comparisons can only be made for an individual cow against her baseline level of activity. Initial investment in the technology can be quite high, but it can be a highly efficient and accurate method of oestrus detection as long as the cows are cycling.

d. TV surveillance

This method involves camera surveillance and recording of the behaviour of cows in a confined area. It requires careful evaluation of a day's recordings and relies on the subjective interpretation of the animals' behaviour.

e. Electrical resistance of vaginal mucus – Draminsky method

Changes in the electrical resistance of vaginal mucus are measured with a so-called Draminsky apparatus equipped with an intravaginal probe. The method requires good records of results in individual animals from previous heats and at least two readings from the current heat to be reliable. A single reading may be misleading (standard values are provided, but there is considerable individual variation).

2.3.6 Oestrus or ovulation control

The oestrous cycle can be regulated pharmacologically to induce or control the time of oestrus and ovulation. There are essentially 3 main approaches to pharmacological oestrus management.

1. Shorten the luteal phase using single or multiple administrations of $PGF_{2\alpha}$
2. Prolong or induce the luteal phase using progesterone administration
3. Control follicular dynamics using administrations of $PGF_{2\alpha}$ and GnRH in a specified sequence. Other hormones such as progesterone, oestrogen, and pregnant mare serum gonadotrophin (PMSG) or equine serum gonadotrophin (eCG) may be added to the protocol to counter specific challenges

Protocols that do not control follicle development or ovulation require oestrus detection. Fixed time insemination can be used when ovulation is induced as part of the protocol. The spectrum of technology and pharmacological intervention ranges from only oestrus detection with or without pharmacological intervention to total fixed time insemination with absolutely no oestrus detection and total reliance on pharmacological intervention. Both extremes or somewhere in between can be very successful and result in high levels of fertility. Each requires different levels of resources and management capabilities. There are no quick fixes. Whatever protocol is chosen to implement it, attention to detail, consistency, and compliance to that protocol are most essential to achieving high levels of fertility.

a. Planning and cost-effectiveness of oestrus management programs

There are many different technologies available to use and several factors to be considered when making a decision about what technology to implement to achieve high levels of reproductive efficiency.

Prior reproductive performance is very important when assessing which system is likely to be of best advantage to a given farm. The type of animals, age structure of the herd, and the level of productivity should be carefully analysed. Human resources, the level of education/skills of the farm staff, and their work environment must also be taken into account. This is especially important if a complex regime is to be implemented, as compliance in the accuracy and timing of the administration of products will have a decisive effect on the efficacy of the program.

It should be always borne in mind that the costs of any oestrus management program must be weighed not only against the possible improvement of pregnancy rates and reduction in days open, but also against the reduced labour costs of oestrus detection. Visual heat detection is more labour

intensive, and thus more cost sensitive than a synchronisation program. The cost-effectiveness should be calculated for each individual herd when assessing the advantages of introducing systematic breeding programs.

It is very difficult to predict how a given protocol will work on any respective farm because of the many factors that influence getting cows pregnant. A useful way to compare protocols is to put them in a table and make broad comparisons based on what research is available and the experience of others. The major categories to consider are costs, labor required to manage and perform the protocol, and what kind of results might be expected relative to other alternatives. An example of such a table is Table 7.

Protocol	Cost	Labor	Results
Presynch 14, Heat Detect, Cosynch 72	Low	Mod	Low
Presynch 14, Heat Detect, Ovsynch 48	Low	Mod	Low
Presynch 14, Heat Detect, Ovsynch 56	Low	Mod	Low
Presynch 14 Cosynch 72	Mod	Low	Mod
Presynch 14 Ovsynch 48	Mod	Low	Mod
Presynch 14 Ovsynch 56	Mod	Low	Mod
Presynch 12 Ovsynch 56	Mod	Mod	Hi
G6G	Hi	Hi	Hi
Double Ovsynch	Hi	Hi	Hi

Table 7. Protocol comparison.

Certain aspects of oestrus management need to be highlighted separately for beef and dairy herds.

i. Beef cattle

Beef herds are often managed extensively and on a group basis. Oestrus detection is, therefore, a much less intensive activity and less accurate than in dairy herds. The presence of a sucking calf and seasonal influences can depress or block cyclical activity in beef cattle. For these reasons, many beef cows do not show signs of oestrus during the 40-60 days postpartum, when they should be inseminated.

Most beef herds are restricted to a specific breeding period. Cows that have not resumed ovarian activity in time, and fail to conceive, will generally be culled.

In beef herds, AI has several advantages over natural service:
- Fewer bulls need to be maintained
- Higher quality semen can be used from progeny tested bulls, increasing the genetic value of the herd

In beef herds, oestrus detection is often the limiting factor for the successful use of AI. Oestrus control and synchronisation can offer a solution. The use of a progestagen/PMSG system at the start of the natural breeding period stimulates and synchronises ovarian activity.

The advantages of such a system are considerable:
- Close supervision during the shortened calving period, which reduces calf losses due to dystocia
- If weaned on a fixed date, calves will be older and heavier by sale time
- A short calving period will improve herd fertility for the following season
- Calves can be sold in batches of similar age and of consistent quality, which increases their value
- It enables and/or facilitates the use of AI and allows more rational semen management

ii. Dairy cattle

In dairy herds that practice year-round calving, cows must be managed individually and more intensively than beef cattle. With a target of one calf per cow per year, the interval between calving and conception is limited to about 85 days during which involution of the uterus must take place, ovarian activity must be resumed, and oestrus detected.

Oestrus control is used in dairy cattle for the following indications:
- To induce oestrus and ovulation in cows with postpartum anoestrus in order to shorten the interval between calving and first insemination
- To synchronise donor and recipient cows for embryo transfer
- To synchronise oestrus in groups of animals to improve oestrus detection or to reduce the time required for oestrus detection
- To control a herd's calving period

For many dairy farms, the failure to efficiently observe oestrus efficiently significantly limits their reproductive performance. Increased levels of production, coupled with increased herd size, have influenced the way in which dairy farms manage reproduction, resulting in the stimulation of the development of oestrus synchronisation programs that allow artificial insemination at predetermined times, without the need for oestrus detection.

For a more in-depth economic analysis see De Vries (2006) and Olynk et al (2007, 2008).

b. Methods of oestrus control

Due to changing requirements of the ovarian follicles for gonadotrophin sup port during their development, it is difficult to develop one simple exogenous hormone treatment to stimulate the predictable emergence of a new wave, irrespective of the stage of the follicle wave at time of treatment.

All pharmacological methods of oestrus management should be regarded as useful tools to increase the efficiency of breeding and to improve breeding organisation or correct some organisational deficiency. In some cases, oestrus management systems can be used as a treatment for certain reproductive disorders such as 'silent heat,' cystic ovarian disease, or delayed ovulation.

Pharmacological methods for oestrus management should never be perceived as replacing proper nutrition and appropriate management of breeding cattle.

In cattle with active ovaries, the oestrous cycle can be manipulated in three ways:

- By the use of prostaglandins, to induce early regression of the corpus luteum
- By the sequential use of prostaglandins and GnRH analogues to obtain synchronised follicular development
- By the use of progestagens that act as an 'artificial' corpus luteum

In cattle that do not have active ovaries, protocols should be employed that mimic an 'artificial' corpus luteum and allow for the induction of follicular growth and ovulation. Such protocols are progestagen-based systems and

are usually combined with GnRH and/or PMSG/eCG.

i. Prostaglandins

Between day 6 and day 16 of the oestrus cycle, an injection of prostaglandin (PG or $PGF_{2\alpha}$) will induce regression of the corpus luteum, ending the luteal phase. The animal will come into oestrus and ovulate in 2-6 days. Prostaglandins will not change or influence follicle development. They will only work on the corpus luteum, and the presence of a corpus lutem is required for a response. The fertility at the induced oestrus is similar to that of a natural oestrus.

Despite rapid luteolysis, the interval to onset of oestrus after treatment with $PGF_{2\alpha}$ is variable and dependent on the stage of the animal's follicular development when treated (Figure 8). Animals with a functional dominant follicle are in oestrus within 2-3 days because the dominant follicle ovulates at the time of induced luteolysis (Figure 9). However, animals at the predominance phase of the wave will require 2-4 days to form a dominant follicle and hence have a longer and more variable interval

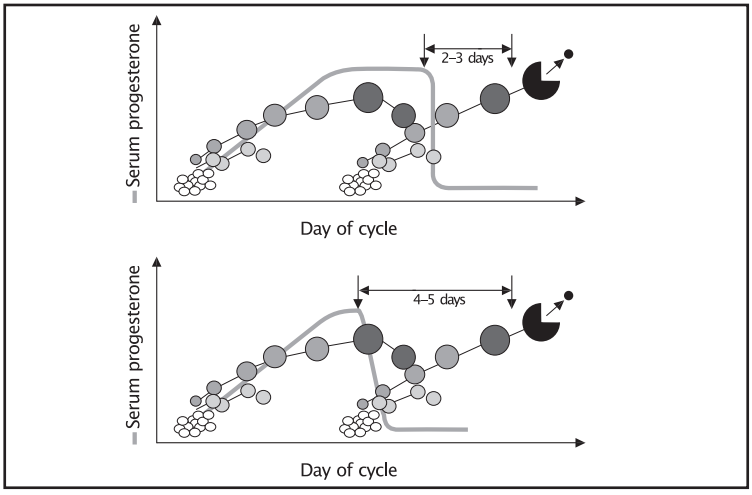

Figure 8. Interval from PGF injection to ovulation in cattle.

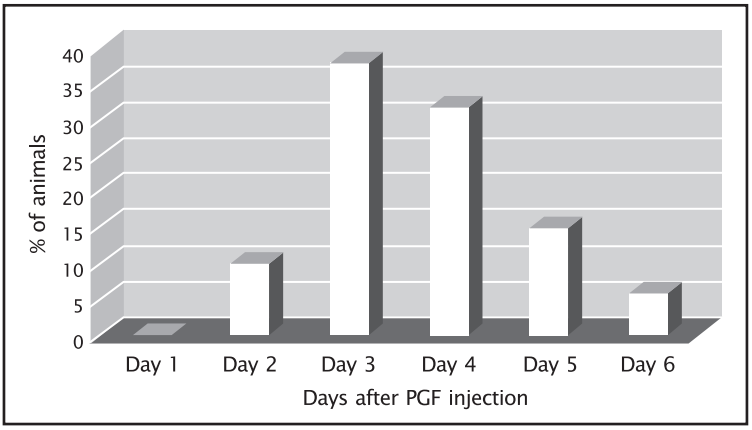

Figure 9. Distribution of oestrus in cows treated with PGF.

to the onset of oestrus. For this reason fixed time insemination does not work in prostaglandin-only protocols.

Insemination at an observed oestrus will give the best conception rates and is recommended. If oestrus is not detected after a single injection, a second injection can be given 11-14 days later to increase the number of animals with synchronised oestrus and decrease the interval between opportunities to inseminate.

Prostaglandins can be used in several different ways for oestrus control, depending on the intentions of the herdsman, the type of animal, and the conditions on the farm. An overview adapted from Cavalieri et al (2006) outlines the most frequently used systems (Figure 10).

Targeted breeding was developed to improve reproductive efficiency in large dairy herds (Nebel and Jobst, 1998). In this system, cows are systematically treated on the same day of the week, to facilitate treatment, oestrus detection, and insemination during specific days of the week. Animals receive a prostaglandin injection at 14-day intervals and are inseminated at observed oestrus. Cows not detected in oestrus after a third prostaglandin treatment are inseminated at a fixed time, 72-80 hours after the last $PGF_{2\alpha}$ injection.

Protocols using sequential PGF treatment were demonstrated to have a beneficial effect in herds with a high prevalence of postpartum uterine infections. Prostaglandins promote uterine contractions and are postulated to have a positive effect on the activity of endometrial immune cells.

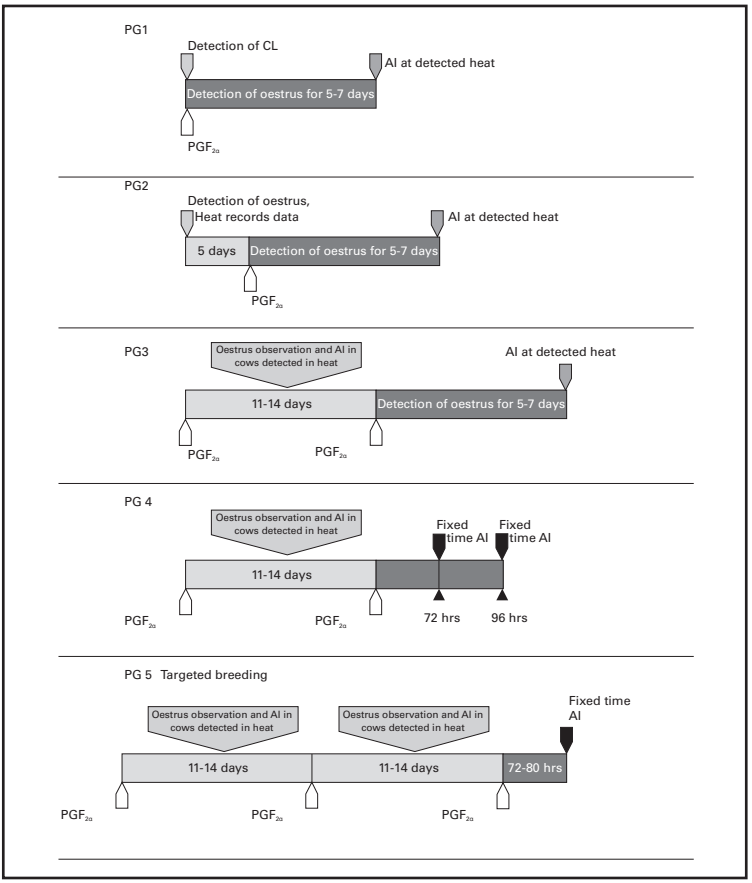

Figure 10. Various systems of oestrus management with prostaglandins.

ii. Application in beef cows

Due to a high incidence of postpartum anoestrus in beef cows, prostaglandins are not considered to be the method of choice for oestrus management in beef cattle. Should this method be used, it is essential to ensure that the cows are cycling and in appropriate body condition.

iii. Prostaglandins and GnRH analogues

Ovsynch (Figure 11) is primarily indicated for dairy cows and involves two injections of a GnRH analogue separated by a single administration of $PGF_{2\alpha}$ (Pursley et al., 1995).

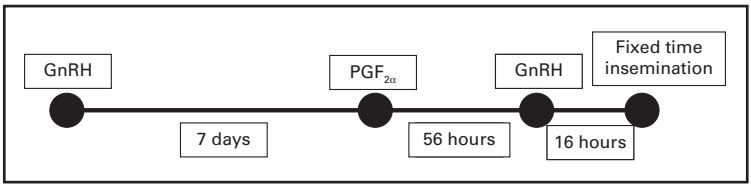

Figure 11. The Ovsynch protocol.

The first administration of GnRH is given at a random stage of the oestrous cycle and causes ovulation of any follicle greater than 10 mm in diameter in about 85% of cows (Pursley et al., 1995). The initiation of a new follicular wave is then triggered. The administration of prostaglandin causes regression of all active corpus luteum on the ovary as the dominant follicle from the induced new follicular wave matures. This follicle will be ovulated with the second administration of GnRH. See Figure 12.

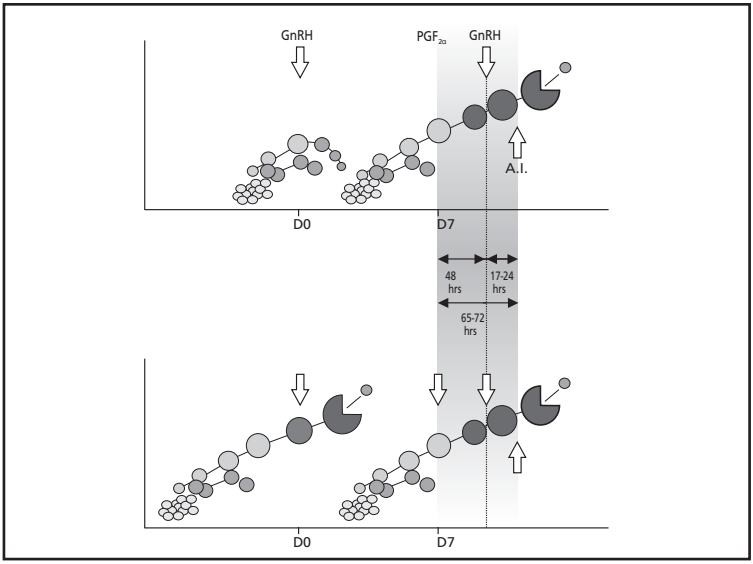

Figure 12. Follicular dynamics in cows treated with the Ovsynch protocol.

Ovulation is tightly synchronised and occurs approximately 26-32 hours after the second GnRH injection. Fixed timed insemination at 17-24 hours after GnRH should result in successful conception (Peters et al., 1999). By controlling follicle development in this manner, ovulation can be synchronised and fixed time insemination utilised to assist in reproduction success.

Ovsynch facilitates the precise scheduling of the first postpartum AI, while improving reproductive performance during the early postpartum period, eliminating the need for oestrus detection. Coleman et al (1991) and Twagiramungu et al (1992) reported that the fertility rate of cows synchronised with GnRH and $PGF_{2\alpha}$ varied between 35% and 65% and was similar to that of control animals inseminated at a first observed oestrus.

iv. Efficacy of the Ovsynch protocol

The ability of GnRH-$PGF_{2\alpha}$-based protocols to synchronise oestrus and ovulation effectively is dependent on the stage of follicular development at the time of the initial GnRH injection. Fertility obtained with the Ovsynch protocol is greatest when cows ovulate to the first GnRH injection.

Several studies conducted during the past few years compared pregnancy rates obtained with the use of the Ovsynch protocol and other oestrus management programmes, such as the use of prostaglandins (Pursley et al., 1997; de la Sota et al., 1998; Keister et al., 1998; Stevenson et al., 1999, 2000; Cartmill, 2001), progestagens (Gaery et al., 1998; Williams et al., 2002), various Ovsynch program modifications (Bartolome et al., 2002; Pancarci et al., 2002), and natural breeding (Cordoba and Fricke, 2001).

A meta-analysis performed by Rabiee et al (2005) compared the results reported in numerous trials with the use of the Ovsynch protocol, natural breeding, single, double, or triple prostaglandin injection, Select Synch, Heat Synch, and modified Ovsynch. These authors concluded that pregnancy rates for the Ovsynch program did not differ significantly from those obtained with natural breeding. The likelihood of conception and pregnancy did not differ significantly between the Ovsynch group and cows treated with prostaglandins. Comparison of the probability of

pregnancy in cows treated with Ovsynch, Heat Synch, and Select Synch did not differ significantly.

Vasconcelos et al (1999) evaluated the influence of the day of the oestrous cycle on which Ovsynch is initiated and resulting pregnancy rates in lactating dairy cows. See Table 8.

Day of oestrous cycle	1st GnRH injection ovulation	2nd GnRH injection ovulation
1-4	23%	94%
5-9	96%	89%
10-16	54%	85%
17-21	77%	81%
Overall	64%	87%

Table 8. Efficacy of oestrus induction in Ovsynch protocol initiated on different days of the oestrous cycle. (Vasconcelos et al., 1999).

Conception rates are greatest when the Ovsynch protocol is initiated between days 5 and 9 of the oestrous cycle. Monitoring of the cow's oestrous cycle to select the most promising time to initiate the Ovsynch protocol is impractical. It would require daily monitoring and mapping of the structures on the ovaries with ultrasound. It is impossible to distinguish an atretic follicle from a viable dominant follicle with less than daily ultrasound scanning or a minimum of every other day scanning. Diagnosing the age of corpus luteum is also not very precise.

For this reason many different presynch protocols have been developed to move cows of unknown follicle or corpus luteum development status into the optimum window of opportunity for greatest response to the first GnRH administered in the Ovsynch protocol. This will increase the likelihood of conception with fixed time insemination at the termination of the Ovsynch protocol.

A presynchronisation protocol prior to implementation of the Ovsynch protocol was developed by giving two injections of $PGF_{2\alpha}$, 14 days apart, with the second injection given 12 days prior to the first GnRH of the Ovsynch protocol. The Presynch Ovsynch protocol increased pregnancy

rates by 18% (25% to 43%) in lactating cyclic cows as reported by Moreira et al (2001).

It should be emphasised that prostaglandin-based presynchronisation protocols can increase the efficacy of insemination after the final Ovsynch only in cyclic cows, as only these animals are capable of responding to prostaglandin.

Postpartum presynchronisation with GnRH can also be undertaken at 7 days before the actual Ovsynch protocol. This approach also has the advantage of being potentially effective in both cyclic and anoestrus cows (Thompson et al., 1999; Stevenson et al., 2000) and this has also been demonstrated to be advantageous in heifers (Stevenson et al., 2008).

The combination of prostaglandin and GnRH as a presynchronisation treatment preceding the classical Ovsynch or Cosynch protocol was also tried with variable success in improvement in pregnancy rates to the final Ovsynch AI (DeJarnette et al., 2003). See Figure 13 for examples of published Presynch/Ovsynch protocols.

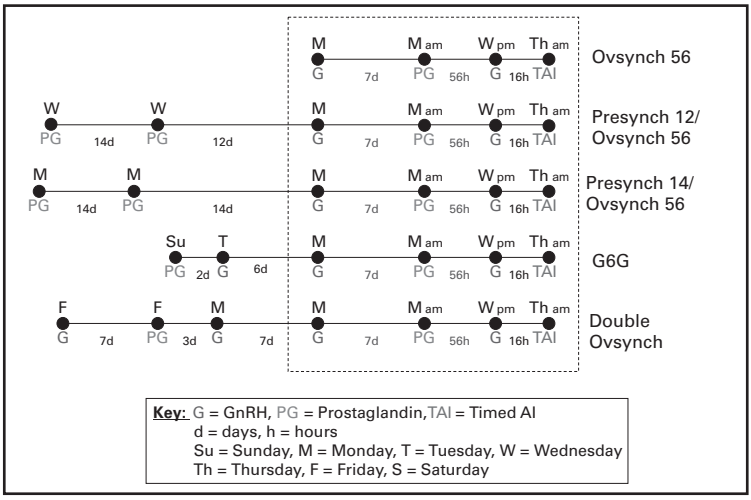

Figure 13. Examples of published Presynch/Ovsynch protocols.

v. Modifications of the Ovsynch protocol

One of the simplest modifications of the classical Ovsynch system is the so-called Cosynch protocol, the difference being that both the second injection of GnRH and AI are performed at the same time, ie, 72 hours after the treatment with prostaglandin (Portaluppi and Stevenson, 2005). Research using the Cosynch protocol has focused on a 72 hour interval between prostaglandin injection and GnRH+AI. Conflicting results have been reported when compared to Ovsynch (Portaluppi and Stevenson, 2005; Brusveen et al., 2008). Certainly with Cosynch, there is a reduction in animal handling and all the potential confounding factors associated with animal handling that can significantly influence the results (DeJarnette et al., 2003). A stochastic analysis published by Olynk et al (2009) indicated that risk-averse managers are willing to incur additional labour costs of using Ovsynch, thus avoiding the potential risk of a reduction in conception rate associated with Cosynch.

A Heat Synch protocol can be used where the second GnRH injection of Ovsynch is replaced with oestradiol (Geary et al., 2000; Stevenson et al., 2004). Enthusiasts of this system indicate that oestradiol synchronises the ovulation of the dominant follicle more tightly and increases the behavioural expression of oestrus in treated cows. This system is limited geographically to those countries where it is legal to use oestrogens in food-producing animals.

Injections of human chorionic gonadotropin (hCG) or implants containing a potent GnRH agonist, deslorelin, have also been tried, to replace the second GnRH injection in the Ovsynch protocol to induce ovulation. The use of hCG does not give comparable results because of the much longer half life and variable timing to ovulation that does not coincide well with fixed time insemination. A protocol with deslorelin resulted in prolonged interovulatory intervals (Bartolome et al., 2004) due to the desensitisation of the hypothalamus (Padula et al., 2002; 2005) and reduced pregnancy rates when a higher dose of deslorelin was used (Santos et al., 2004).

vi. Progestagens

Progestagen treatments mimic the luteal phase of the cycle. To obtain a normally fertile oestrus, the duration of treatment varies between 5 and 14 days.

One of the advantages of progestagen-based treatments is that they are capable of initiating oestrous cycles in anoestrus cows. In noncyclic cows, the progestagen sensitises the hypothalamo-pituitary-gonadal axis and mimics a normal corpus luteum. The administration of PMSG, when the progestagen is removed, further stimulates follicular maturation and ovulation. The success rate of progestagen-based methods in the treatment of anoestrus can be variable (50%-70%), depending on the postpartum interval at the time of treatment, the body condition of the cow, and other underlying causes of anoestrus. Nonetheless, progestagen based systems should be seen as the method of choice in managing anoestrus in beef cows.

Treatment of anovulatory or anoestrus cows with low doses of progestagens for 6-8 days rarely induces the formation of persistent dominant follicles in cows that are cycling without the presence of a functional corpus luteum (McDougal et al., 2004). Cerri et al. (2009) found that treatment of high-yielding cycling Holstein cows with a progesterone-releasing intravaginal device resulted in subluteal concentrations of progesterone and no effect on fertility. But in anoestrus cows, this treatment increased the induction of oestrous cycles.

A feature of current progestagen-based systems in countries where oestrogens can legally be used is the administration of oestradiol at the start of the treatment to:

- Shorten the life span of the corpus luteum
- Terminate the existing follicular wave
- Induce the emergence of a new follicular wave

All progesterone/progestagen-releasing systems create subluteal levels of progesterone in the circulation of treated cows. These levels are sufficient to create a negative feedback and prevent a preovulatory LH surge, ovulation, and oestrus. They are not able to block the LH release completely, and a small pulsatile secretion is maintained, allowing the

persistence of a dominant follicle that may be present on the ovary at the start of treatment.

It has been shown that when the duration of dominance of the ovulatory follicle exceeds 4 days (persistent dominant follicle), there is a progressive decline in fertility that has been attributed to a reduction in oocyte competence and an increase in embryonic loss (Figure 14; Diskin et al., 2002).

Exogenous oestradiol, administered with progesterone, suppresses the formation of, or decreases the diameter of, the dominant follicle due to suppression of FSH and perhaps LH. When follicle selection has occurred, oestradiol treatment results in a decrease in dominant follicle diameter at the same time, initiating the emergence of the next follicular wave (Figure 15).

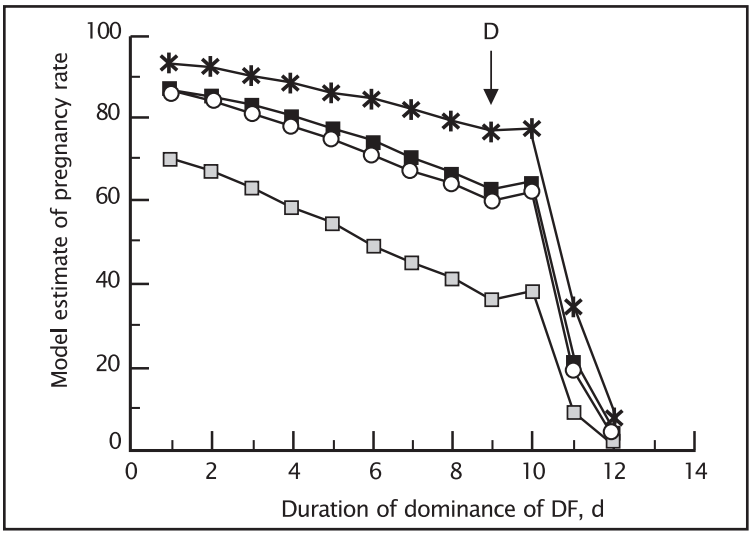

Figure 14. Estimation of pregnancy rate as duration of dominance of the preovulatory follicle increases. Data from 4 different experiments, where D = day of change in dominance. (Diskin et al., 2002).

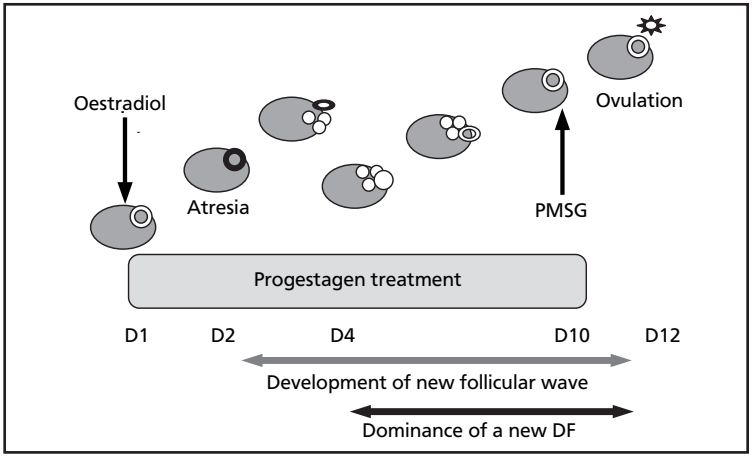

Figure 15. Follicular dynamics in cows treated with oestradiol at the start of progestagen-based oestrus synchronisation program.

The use of oestradiol at the beginning of a progesterone synchronisation treatment does not always guarantee that corpus luteum regression is complete in all animals at the time of, or 24 hours after, progesterone withdrawal, even when the duration of treatment is extended to 12 days. Consequently, it is highly recommended that $PGF_{2\alpha}$ be administered at, or before, progesterone withdrawal to ensure corpus luteum regression in all cows.

In recent studies, systems have been proposed in which the injection of oestradiol was replaced by GnRH administration at the start of the treatment (Thompson et al., 1999; Stevenson et al., 2000; Garcia et al., 2004). The mechanism of action used in systems combining GnRH and progestagens is slightly different to that of the oestradiol-progestagen-based ones, because GnRH induces ovulation of the dominant follicle and the creation of an additional corpus luteum (Figure 16) (Cavalieri et al., 2006).

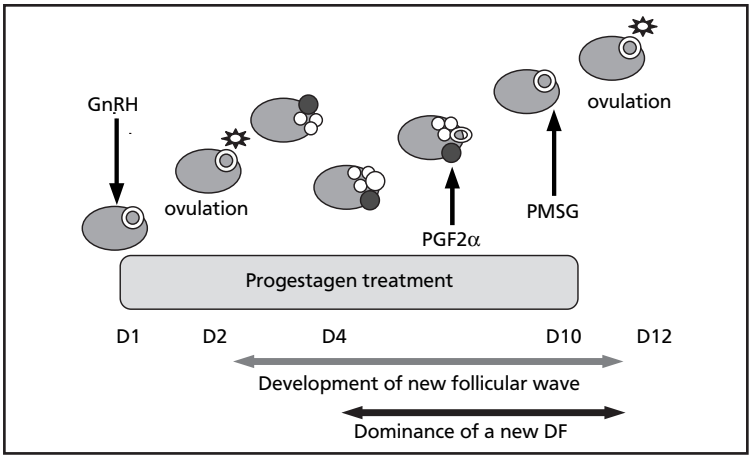

Figure 16. Follicular dynamics in cows treated with GnRH at the start of progestagen-based oestrus synchronisation program.

If this system is to be adopted, there are certain important aspects to be considered. By replacing oestradiol with GnRH, additional benefits, such as stimulation of follicular growth (especially important in anovulatory cows) and the creation of an additional corpus luteum, if a follicle capable of ovulating is present, can be expected. It is necessary to administer $PGF_{2\alpha}$ to all treated animals, preferably 48 hours before the removal of the progesterone source.

The supplementation of progesterone during the development of the follicular wave and the future ovulatory follicle should have an obvious beneficial effect on the quality of the oocyte and also reduce the occurrence of shortened luteal phases after insemination when used in the treatment of anoestrus cows. Melendez et al (2006) showed that cows not previously detected in oestrus and subjected to the Ovsynch program with supplemental progesterone had higher pregnancy rates and concentrations of progesterone after AI than those subjected to the Ovsynch protocol, which received no additional progesterone. The results of such a protocol are highly dependent on the prevalence of anovulation, anoestrus, and heat detection before submission to treatment.

vii. Replacement heifers

Replacement heifers do not respond to synchronisation the same as cows. There are two main issues with respect to breeding virgin replacement heifers. One issue is if they have reached puberty and if the ovaries are cycling. Age and weight influence the onset of puberty. Heifers should attain 60 percent of their mature weight before breeding (Johnson, et al., 2014). There can be significant differences within breed as well as across breeds on mature weight. Mature cow sizes also change with time with the changes in genetics. It is important to know the mature size of the parents to estimate when puberty might occur.

A good alternative is to score reproductive tracts 50 to 60 days before breeding. This will provide a much clearer picture of the physiological maturity of the reproductive tract in the heifer. It also provides time to make nutritional adjustments to improve physiologic maturity before breeding. A tract score of 3 or greater (1=infantile to 5=mature and ovary with corpus luteum) in more than 50% of the heifers 50 to 60 days before breeding will improve success to a synchronisation protocol (Johnson, et al., 2014). Protocols that include progesterone will induce some heifers to cycle.

The other issue is that a large majority of virgin heifers have three waves of follicle development within an oestrus cycle. This results in the timing of treatments in protocols developed for cows (such as Ovsynch) and is not optimal for a response that will improve fertility.

Because virgin heifers are not under the same metabolic demands that lactating cows experience, they tend to be much more demonstrative in the expression of oestrus. They are easier to detect in oestrus and have much higher conception rates. This makes it much easier to use heat detection in finding the heifers to breed artificially.

All recommended timed insemination synchronisation protocols in virgin heifers include a source of progesterone. See Figure 17 for examples of recommended synchronisation protocols (Johnson et al., 2014, DCRC, 2013).

2.3.7 Rebreeding strategies

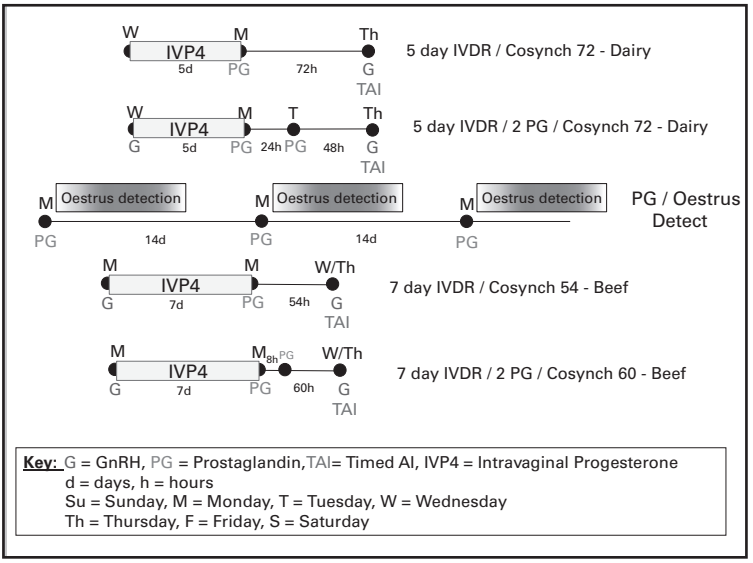

Figure 17. Heifer synchronisation protocols.

Not every cow will conceive at first breeding. It is critical for overall reproduction success to have a good strategy on how to identify those cows that do not conceive at first breeding and how to get them inseminated again. This is essential for all types of cattle operations with breeding females. The strategy can range from using natural service by letting bulls do everything to diagnostic protocols for nonpregnant cow identification coupled with pharmacology to control follicle development and fixed time insemination.

The first and most critical component of a rebreeding stategy is how and when to identify the cows that need to be bred again. An underlying element that must be factored into every strategy is early embryonic loss and how it will impact nonpregnant cow detection and rebreeding strategies. Early embryonic loss will be discussed further in the section on Nonpregnancy determination strategies and reproductive disorders.

Timeliness of nonpregnant cow detection is always a challenge. There is significant lag in the time between conception and time to detection of conception. New technologies are constantly being developed to shorten this lag time. Even if oestrus detection was 100%, there is a lag in time between events. This lag time can get very expensive, depending on the many economic factors previously discussed. If the interbreeding interval is extended beyond reasonable limits, it can become very costly to the dairy.

Oestrus detection is always an option and can be a very good option if managed and executed correctly in a timely manner. There can also be many challenges with cows that do not show oestrus or just in the detection of oestrus. For this reason, many protocols have been developed and researched to use pharmacology to control follicle development.

Many have reported lower fertility to fixed time inseminations after a resynchronisation protocol as compared to fertility to fixed time insemination after a first service synchronisation protocol (Galvao et al., 2007; Silva et al., 2009; Thompson et al., 2010). The poor fertility of resynchronised cattle is related to progesterone concentrations at initiation of the resynchronisation protocol. Several have reported that 15% to 26% of cows submitted for resynchronisation protocols do not have a corpus luteum or have low progesterone concentrations (Fricke et al., 2003; Sterry et al., 2006; Silva et al., 2009). Follicle development in the low progesterone environment is associated with reduced embryo quality (Rivera et al., 2011) and lower fertility (Follman et al., 1990; Wiltbank et al., 2012). Because of this, many different protocols have been developed and tested to try and improve fertility to resynchronisation. See Figure 18 for examples of some protocols.

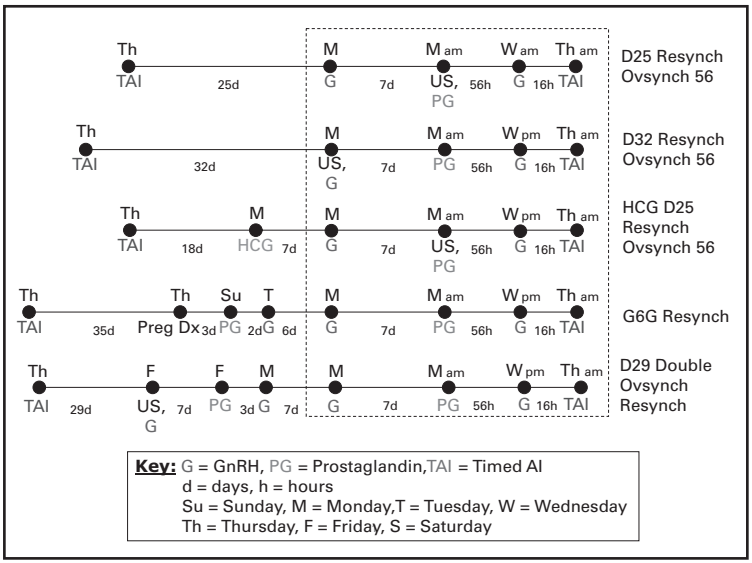

Figure 18. Resynch protocols.

Lopes et al (2013) hypothesised that the ideal time to initiate resynchronisation is between 28 and 32 days after a previous insemination. They reported that presynchronisation with GnRH at day 32 or day 39 postinsemination and 7 days before the start of a resynch protocol (Ovsynch) improved pregnancy per insemination (P/AI) from 33.8% to 38.9%. There was no difference between starting on day 32 and starting on day 39.

An earlier report by Fricke et al, 2003, that compared 19 day, 28 day, and 33 day intervals from insemination to initiation of resynch found that it was optimal to start at day 33 after previous insemination. Even though higher fertility was observed at 28 day nonpregnancy exams at 19 day and 28 day initiation intervals, the lower embryonic loss at day 33 initiation was enough to offset the apparent higher fertility at the shorter intervals.

The optimal resynchronisation protocol and strategy will not be the same for every herd.

2.3.8 Nonpregnancy determination strategies

Not all cows get pregnant on the first breeding. Pregnancy determination and heat detection define how quickly cows that fail to conceive are identified and re-inseminated. Early pregnancy determination is a key factor in defining the reproductive success and economic efficiency of a dairy herd. It must be coordinated into the overall breeding program in a timely manner to maximise the economic value of testing.

There are many technologies available to determine pregnancy status and many more yet to be developed. They range from waiting to see who calves next season to ultrasound and testing of body fluids for pregnancy indicators. A brief review of the current technologies can be found in the next section. Each technology differs in terms of cost, skill requirements, and interval from breeding to diagnosis and management. Each methodology has its own attributes of testing, such as sensitivity and specificity, to indicate accuracy.

The value of any test is related to the subsequent decisions that management can make as a consequence of information from the test. The sooner a cow is known to not be pregnant the sooner she can be bred again. This will reduce the accumulated days open on a proportion of cows and has real economic value (Galligan et al., 2009).

Retesting of animals diagnosed as pregnant is important in finding animals that were either incorrectly diagnosed as pregnant or have lost their pregnancy due to early embryonic death. A potential economic loss can occur if incorrectly diagnosed cows are allowed to accumulate additional days open before retesting and rebreeding occurs. The value of retesting depends on the underlying pattern of early embryonic loses and the accuracy of the test. The earlier that testing is done (higher underlying early embryonic losses) the more important the interval to retesting itself.

Our knowledge of early embryonic loss is dependent on when the initial testing is done and the interval to retesting. The rate of loss is independent of the test. Our knowledge of the magnitude of the loss is dependent on when the initial testing was done. A test done sooner will potentially declare a larger number of cows pregnant (not false positives)

and embryonic death rates may appear greater, depending on the interval to retest. The loss would have occurred anyway, without the test, but as a consequence of earlier testing, we now have knowledge of it. This is controlled by timely retesting of pregnant cows.

The whole purpose of pregnancy testing is to detect nonpregnant cows that can be re-inseminated sooner than without the test. Early pregnancy testing is an important tool to ensure timely rebreeding of cows failing to conceive. Testing must match the breeding strategy implemented on the farm. Potential costs associated with early nonpregnant cow testing and subsequent early embryonic losses can be controlled by retesting.

2.3.9 Pregnancy diagnostic technology

a. Nonreturn to oestrus

If a cow is not observed in oestrus at around 3 weeks after service or insemination, she is generally assumed to be in calf. Even if oestrus detection is good, not all of these cows will be pregnant. On the other hand, up to 7% of pregnant cows will show some signs of oestrus during pregnancy. Insemination of these animals may result in embryonic death.

b. Rectal palpation

Early pregnancy diagnosis (1-3 months) is based on a combination of the following: asymmetry of the uterine horns, lesser tone of the pregnant horn and fluctuant contents in the pregnant horn (later both horns), a palpable corpus luteum on the ovary on the same side as the pregnant horn, membrane slip, and appreciation of an amniotic vesicle. In the later stages of pregnancy (>3 months), the cervix is located anterior to the pelvic rim and the uterus cannot be easily retracted. The uterus is flaccid and placentomes, and sometimes the foetus, are palpable. The median uterine artery increases in diameter and fremitus can be detected. See Table 9.

Stage of pregnancy	Membrane slip	Amniotic Vesicle	Foetus	Placentomes	Fremitus A. uterine media	
					Ipsilateral	Contralateral
30 days	±	+				
45 days	+	+				
60 days	+	+				
75 days	+	+		+		
90 days	+		+	+		
105 days			+	+	+	
4 months			+	+	+	
5 months			+	+	+	+
6 months				+	+	+
7 months			+	+	+	+

Table 9. Positive signs of pregnancy at rectal palpation.

The advantage of rectal palpation is that it provides an immediate answer. It requires minimum equipment and personnel if good handling facilities are available. A skilled practitioner can detect pregnancy in cattle as early as day 35 postbreeding.

Common reasons for errors in rectal palpation include failure to retract the uterus, abnormal uterine contents (pyometra or mucometra), and incorrect breeding dates. There is contradictory information regarding the possible risk to the embryo/foetus of palpation per rectum. Early or inappropriate palpation of the amniotic vesicle may damage the embryo and cause embryonic mortality. A recent publication by Romano et al (2007) indicated that pregnancy diagnosis by rectal palpation between days 34 and 41 of pregnancy, using the so-called 'membrane slip' technique, did not affect embryonic/foetal viability.

Regardless of the advantages of rectal palpation, pregnancy diagnosis by this method provides no information about foetal development (deformities, vitality, gender, etc).

c. Progesterone assay

The progesterone secreted by a functional corpus luteum between 18 and 24 days after service or insemination is an early indication of pregnancy. It can be assayed in milk or plasma. The optimum time for the assay is 24 days after service or AI, eliminating the problem of long oestrus intervals that might lead to a false positive diagnosis.

The sensitivity (ie, accuracy in detecting pregnancy) of the cow-side milk progesterone (EIA) test was 93.1% in a study by Pieterse et al (1989). However, its specificity (ie, accuracy in detecting nonpregnancy) was only 39.3%, which meant that there were a rather large number of animals diagnosed as pregnant that were, in fact, not pregnant. The most common reasons for error are pyometra/persistent corpus luteum, short oestrus intervals, cystic ovarian disease (luteal cysts), and the incorrect handling of the samples and test kit, as well as early embryonic mortality.

This indicates that relying on a single progesterone measurement is unsatisfactory, and pregnancy must be confirmed by another technology or a series of samples. If a series of samples are collected between days 21 and 24, the accuracy of early diagnosis of nonpregnancy approaches 95% to 100%. Milk progesterone tests could be used for the early identification of cows that have not conceived, allowing for their re-introduction into the breeding program.

d. Ultrasound examination

The use of transrectal ultrasonography to assess pregnancy status early in gestation is among the most practical applications of ultrasound for dairy cattle reproduction. Table 10 contains a list of days to first detection of ultrasonographically identifiable characteristics of the bovine conceptus. Early identification of nonpregnant cows following natural or artificial insemination improves reproductive efficiency and pregnancy rate by reducing the interval between AI services and increasing AI service rate. Real-time (B-mode) ultrasound is a reliable and relatively simple method of diagnosing pregnancy. Under most farm conditions, pregnancy diagnosis can be rapidly and accurately diagnosed using ultrasound as early as 26 days post-AI.

Characteristic	First day detected	
	Mean	Range
Embryo proper	20.3	19 to 24
Heartbeat	20.9	19 to 24
Allantois	23.2	22 to 25
Spinal cord	29.1	26 to 33
Forelimb buds	29.1	28 to 31
Amnion	29.5	28 to 33
Eye orbit	30.2	29 to 33
Hindlimb buds	31.2	30 to 33
Placentomes	35.2	33 to 38
Split hooves	44.6	42 to 49
Foetal movement	44.8	42 to 50
Ribs	52.8	51 to 55
Adapted from Curran et al., 1986.		

Table 10. Day of first detection of ultrasonographically identifiable characterstics of the bovine conceptus.

Using ultrasound scanning techniques, an accuracy of over 99% can be achieved, enabling fertility problems to be identified rapidly. Generally, two factors affect the speed at which ultrasound examinations can be conducted on a dairy farm: operator proficiency and the availability and restraint of animals. When both factors are optimised, the speed of ultrasonography can approach that of rectal palpation. The main advantage of scanning is that it can give an accurate diagnosis earlier than rectal palpation.

Diagnosis of pregnancy using high resolution ultrasound days 18 to 21 postinsemination was made based on CL blood flow, CL size, and uterine echotexture. Accuracy of pregnancy diagnosis was highest on day 21, with sensitivity and specificity being 97.6% and 97.5%, respectively (Scully et al., 2014).

Because pregnancy can be identified earlier using ultrasound than by rectal palpation, the detection of early embryonic losses are often higher. Of cows diagnosed pregnant at 28 days after AI, 10% to 16% experience early embryonic loss by 56 days (Mee et al., 1994; Vasconcelos et al., 1997). Therefore, cows diagnosed pregnant at 28 days after AI, using ultrasound, should be submitted to a subsequent examination at 45 to 60 days postbreeding (Vasconcelos et al., 1997).

e. Early pregnancy diagnosis based on the detection of pregnancy-specific molecules

Techniques have been developed for early pregnanacy diagnosis in cattle based on the detection of pregnancy-specific proteins.

i. PAGs

Pregnancy-associated glycoproteins (PAGs) are known under a variety of names, including pregnancy-specific protein B (PSPB). They constitute a large family of glycoproteins expressed in the outer epithelial cell layer (chorion/trophectoderm) of the placenta of eutherian species. The PAG molecules belong to a group of proteolytic enzymes known as aspartic proteinases (AP). Several closely related PAG molecules have been identified between early blastocyst development and parturition (Sousa et al., 2006). Pregnancy-specific protein-B was the first such protein to be identified in cattle (Butler et al., 1982) and was later found to have the same N-terminal AA sequence as pregnancy-associated glycoprotein (Xie et al., 1991; Lynch et al., 1992). Both pregnancy-specific protein-B and PAG have subsequently been reclassified as boPAG-1.

Mean PAG concentrations in cattle increase from 15 to 35 days of gestation. However, the variation between cows in serum PAG levels limits their use as a reliable indicator of pregnancy until about 26 to 30 days of gestation (Humblot, 2001).

There are several different diagnostic approaches employing either radioimmunoassay (RIA) (Haugejorden et al., 2006; Lopez-Gatius et al., 2007; Ayad et al., 2009) or variations of the enzyme-linked immunosorbent assay (ELISA) (Green et al., 2005; Silva et al., 2007; Friedrich and Holtz, 2009). Several laboratories at universities or scientific institutes use the

PAG-based assays for pregnancy diagnosis for experimental purposes, and some offer the tests to practitioners on a commercial basis.

An ELISA test for PAGs (BioPRYN™, BioTracking, Moscow, ID, USA: IDEXX Bovine Pregnancy Test, IDEXX Milk Pregnancy Test, Westbrook, Maine, USA) is available in many countries. The assay is performed on serum or milk samples by licensed laboratories and is recommended for pregnancy detection from day 28 after insemination. Because PAG molecules persist for a long time in the circulation after calving, only cows served more than 60 days after calving should be included in any PAG-based assays.

PAGs also appear in milk. IDEXX has developed a milk sample-based PAG ELISA test (IDEXX Milk Pregnancy Test, Westbrook, Maine, USA) to confirm pregnancy from 28 days postbreeding. The sensitivity is reported to be 98.7% and the specificity to be 94.4%. The overall agreement between the milk-based and serum-based IDEXX ELISA tests were 98.4% (IDEXX data).

IDEXX also markets a visual pregnancy test for serum samples. It is an ELISA for PAGs that does not require special equipment. The tests are accurate and convenient complements to routine palpation and ultrasound examinations.

ii. ECF/EPF

Early pregnancy factor (EPF) was first identified in pregnant mice (Morton et al., 1987) and later in sheep and cattle (Nancarrow et al., 1981) by using the rosette inhibition test. Early pregnancy factor was demonstrated to have specific growth regulatory and immunomodulatory properties and is required for the successful establishment of pregnancy and the proliferation of both normal and neoplastic cells in vivo and in vitro (Cavanagh, 1996). Significant differences in rosette inhibition titer were observed between pregnant and nonpregnant cows on days 13 to 16 and 25 post-AI (Sakonju et al., 1993), suggesting that measurement of EPF activity could be used as a method of early pregnancy diagnosis. Several publications have reported conflicting results from trials assessing the accuracy of ECF/EPF-based laboratory tests, mainly indicating the low specificity of the assays when used in the early postbreeding period (Cordoba et al).

f. Identification of twin pregnancies

Cows carrying twins can be accurately identified with transrectal ultrasonography. When conducting an early examination to identify twins, the entire length of both uterine horns must be carefully scanned to ensure that an embryo is not missed. Depending on the type of cow (dairy, beef, or dual purpose), several management scenarios can be considered once twins have been identified, including abortion and rebreeding, or continued management until parturition. Although approaches to twin pregnancies are limited under field conditions, identifying cows carrying twins is always beneficial, so that extra care can be provided at calving.

g. Identification of foetal gender

Transrectal ultrasound can be used to determine the sex of bovine foetuses by evaluating the morphology and location of the genital tubercle; it is reliable and accurate from day 55 to 60 of gestation (Fricke, 2002). A much greater level of proficiency and experience is required for determining the sex of the foetus than that required for early pregnancy diagnosis or examination of ovarian structures.

Although it has its attractions, foetal sex determination should only be included in reproduction management if the information generated is used for management decisions.

2.3.10 Improvement of conception rate at and after AI

Pharmacological attempts to improve fertility have concentrated on synchronisation of oestrus and ovulation through control of follicle development and prevention of early embryonic loss through increasing progesterone concentrations or prevention of precocious luteolysis.

a. Support of luteal function

Several attempts have been made in high-yielding cows to prevent early embryonic loss after insemination or embryo transfer and in those exposed to heat stress.

Ovulation of early luteal-phase follicles leads to the creation of accessory corpora lutea, increasing progesterone concentration, which has been

associated with higher pregnancy rates (Butler et al., 1996; Lamming et al., 1989; Mann et al., 1995, 2001; Lopez-Gatius et al., 2006). On day 5 of the oestrous cycle, granulosa cells of the dominant follicle contain LH receptors, so that hCG will induce ovulation and the formation of an accessory corpus luteum. Administration of hCG 5 days after AI has the potential to increase progesterone secretion during early pregnancy. The positive effect of hCG on conception rates is mediated by reducing early embryonic losses. Most of the benefit of hCG treatment was observed in lactating dairy cows that were losing body condition during the breeding period. Since high-yielding cows have a greater metabolism of progesterone (Wiltbank et al., 2006), they are more likely to be responsive to hCG treatment.

Santos et al (2001) administered hCG on day 5 after AI in high-yielding dairy cows and noted that the treatment induced the formation of accessory corpora lutea, enhanced plasma progesterone concentrations, and improved conception rates when evaluated on days 28, 45, and 90 after insemination. The effect was especially pronounced in cows that were losing body condition in the month following AI. Similarly, Breuel et al (1989), Sianangama et al (1992), and Rajamahedran and Sianangama (1992) reported a significant increase in pregnancy rates with the administration of hCG at 7 days post-AI. Keneda et al (1981) and Kerbler et al (1997) achieved improvements in pregnancy rate with the administration of hCG at a dose of 1500 IU after AI.

Small et al (2002) evaluated the influence of hCG (2500 IU/cow) administration on day 7 in embryo transfer recipients and inseminated cows. They found that treatment with hCG 7 days after AI improved timed-AI pregnancy rates in cows carrying twins and first-calving heifers. The authors postulated that treatment with hCG at 7 days post-AI may be used to improve pregnancy rates in metabolically stressed cows and first-calving heifers.

Nishigai et al (2002) administered hCG 6 days after oestrus in embryo transfer recipients. The trial results showed that the administration of hCG (1500 IU/cow) 6 days after oestrus improved the pregnancy rate for non-surgical frozen embryo transfer 7 days after oestrus.

A study reported by Chagas e Silva et al (2008) evaluated the influence of hCG administration on the day of embryo transfer in high-yielding dairy

cows in a highly discriminative setup (the transfer of bisected embryos). The treatment with 1500 IU of hCG enhanced the survival of low-viability embryos (half-embryos) and increased progesterone concentrations associated with the formation of an additional corpus luteum.

It is important to recognise that the use of GnRH is associated with a shorter duration of LH exposure, with the induction of an accessory corpus luteum that is less responsive to LH and a substantially lower increase in plasma progesterone concentration during the subsequent luteal phase. This has been attributed to formation of a corpus luteum after hCG administration having greater steroidogenic capacity (Schmitt et al., 1996a, b). Human chorionic gonadotrophin treatment in sheep and cows has been linked to elevated numbers of large luteal cells and a concomitant reduction in the number of small luteal cells, accompanied by increased plasma progesterone concentrations.

Although the rationale for the administration of GnRH and hCG on the day of embryo transfer is the same, few studies have reported positive results in terms of improvements in pregnancy rates in embryo recipients after treatment with GnRH. Ellington et al (1991) evaluated the effect of administration of buserelin at the time of embryo transfer and at 4-7 days after transfer but found no significant improvement in pregnancy rates in comparison with the untreated controls.

b. Prevention of precocious luteolysis

The effect of GnRH treatment in mid-cycle (usually 11-14 days post-insemination) on embryo survival and the resulting pregnancy rate has been studied. GnRH treatment aims to enhance embryo survival by suppressing the luteolytic mechanism that ensues if there is no maternal recognition of pregnancy. Depending on the stage of follicular development, treatment with GnRH analogues during the luteal phase causes luteinisation or ovulation of the existing responsive luteal-phase follicles, which continue to grow after the ovulation of the dominant follicle of the previous cycle. Thus, not only is progesterone secretion increased, but oestradiol concentrations are also reduced as follicular turnover reduces oestradiol production. It results in a failure to upregulate the oxytocin receptors and hence blocks $PGF_{2\alpha}$ secretion. This thesis was confirmed in a study reported by Matsui et al (2008), who found that oestradiol secretion by a dominant follicle from the

first follicular wave after insemination had a negative effect on conception rate.

Mann et al (1995) concluded that GnRH attenuated the luteolytic signal, allowing embryos more time to develop their antiluteolytic ability. Depending on the stage of follicular development, treatment with GnRH analogues during the luteal phase causes advanced atresia, luteinisation, or ovulation followed by luteinisation of the responding follicle. Administration of GnRH between 11 and 13 days after service produced a marked increase in pregnancy rates (MacMillan et al., 1986; Mee et al., 1990; Peters et al., 1992; Stevenson et al., 1990; Ryan et al., 1994).

Peters (2000) summarised the results of various studies analysing the effects of GnRH injections between days 11 and 13 of the oestrous cycle on pregnancy rates in cows and noted a wide variation in both respect to the experimental design and the degree of improvement in pregnancy rates obtained. This analysis suggested that in certain circumstances GnRH treatment after insemination may produce significant benefits. This thesis was further supported in a study reported by Sterry et al (2006), who found that treatment with GnRH 5 days after fixed-time insemination in dairy cows improved the pregnancy rate per insemination for noncycling, but not for cycling, cows.

Lopez-Gatius et al (2006) demonstrated that GnRH treatment at the time of insemination, and 12 days later, increases the conception rate in high-yielding dairy cows during the warm season. Similar beneficial effects of post-insemination treatment with GnRH were found by Bech-Sabat et al (2009) in certain target groups of cows.

Two experiments reported by Franco et al (2006a,b) failed to show any significant improvement in pregnancy rate in cows treated with GnRH after AI or embryo transfer. These results highlight the fact that treatment with GnRH post-insemination may be of considerable value in certain groups of cows, but, due to the complex aetiology of embryonic loss in cattle, it will not always bring consistent improvement in pregnancy rates.

Several recent publications have reported on the use of nonsteroidal anti-inflammatory drugs (NSAIDs) such as flunixin meglumine during the

post-insemination period in dairy cows. This approach is based on the ability of NSAIDs to interfere with the synthesis of various pro-inflammatory molecules from arachidonic acid, including prostaglandins. Thus, the precocious synthesis of $PGF_{2\alpha}$ can be inhibited in cows with delayed embryonic development that produce inadequate amounts of IFN-τ.

Guzeloglu et al (2007) described an increase in pregnancy rate in heifers treated with flunixin meglumine on days 15 and 16 post-insemination, as compared with untreated animals. Similarly, Merril et al (2007) found that treatment of inseminated cows with flunixin meglumine increased pregnancy rates, irrespective of whether they were subjected to transportation stress. By contrast, the administration of flunixin meglumine 11-16 days post-AI in beef cows did not result in improvement in pregnancy rate (Lucacin, 2008).

Ketoprofen used in the same experimental design by Guzeloglu et al (2008) did not bring any significant improvement in pregnancy rates as compared to an untreated control group. If confirmed in further trials, this approach may become particularly attractive in situations in which precocious luteolysis is thought to be the main reason for early embryonic loss. At present, such treatment should be considered cautiously, as the controlled studies are limited and registration of products containing NSAIDs generally does not include the treatment of dairy cows after AI for improving fertility.

2.4 Factors affecting fertility

2.4.1 Factors affecting fertility

In dairy cattle, fertilisation rates are similar in lactating and nonlactating cows, averaging 76.2% (ranging from 55.3% to 87.8%) and 78.1% (ranging from 58.0% to 98.0%), respectively (Santos et al., 2004). In beef cattle, the rate averages 75.0%, with a range from 60% to 100%.

Humblot (2001) showed that fertilisation failure and early embryonic loss were responsible for 20%-45% of pregnancy failures, late embryonic loss for 8%-17.5%, and late abortion for 1%-4%.

Factors contributing to losses after insemination can be grouped as follows:
1. Factors contributing to fertilisation failure:

a. Unfavourable endocrine environment causing impaired follicular growth and poor oocyte quality
- Heat stress
- Negative energy balance
- Infection with bovine viral diarrhoea virus (BVDV) and infectious bovine rhinotracheitis virus (IBRV)

b. Ovulation delay and/or failure
- Heat stress
- Negative energy balance

c. Factors affecting the quality of spermatozoa
- Factors affecting spermatogenesis: infections with BVDV, IBRV, *Brucella* spp., heat stress, fever
- Factors affecting the survival of sperm before deposition in the female reproductive tract: semen preservation technique, semen management

2. Factors affecting early embryonic development, pregnancy recognition, and implantation

a. Impaired early luteal function
- High metabolic rate in dairy cows
- Infections with BVDV and IBRV
- Lack of progesterone priming in the first postanoestrus cycles
- Luteotoxic factors causing precocious luteolysis:

- Mycotoxins
- Bacterial toxins associated with mastitis

b. Impaired function of endometrium and unfavourable uterine environment
- Increased levels of plasma urea nitrogen
- Subclinical endometritis

3. Factors causing late embryonic/foetal death

a. Infectious factors directly detrimental to the foetus or impairing the function of the placenta
- Viral infections: BVDV, IBRV
- Bacterial infections: *Brucella* spp., *Chlamydia* spp.
- Protozoan infections: *Neospora caninum*, *Trichomonas* spp.

b. Noninfectious factors directly detrimental to the foetus
- Or impairing the function of the placenta
- Mycotoxins
- Certain substances such as: PVP, lead, etc

2.4.2 Timing of insemination

Fertilisation of the ovum occurs in the oviduct at the junction of the isthmus and ampulla. The life span of the ovum is around 12-18 hours and its viability decreases with time. About 8 hours after service, sufficient numbers of spermatozoa have reached the isthmus of the oviduct. Capacitation of the spermatozoa is required for fertilisation and is characterised by their hypermotility and completed acrosome reaction. The optimal time at which insemination should take place relative to ovulation depends mainly on the fertile life span of spermatozoa and on the viable life span of the oocyte in the female reproductive tract.

Spermatozoa have a limited life span; therefore, if insemination takes place too early, the sperm cells will die before they can fertilise the ovum. Conversely, when insemination is delayed too long, the ovum will have lost its capacity to be fertilised.

Ovulation normally occurs between 24 and 32 hours from the beginning of oestrus (Pursley et al., 1995). The optimum time for insemination is, therefore, towards the end of oestrus (see Figure 19).

Because of the limited life span of both ovum and sperm, there is a 'window' of about 12 hours during which optimal conception rates are achieved. A study by Roelofs et al (2006) showed that the interval between insemination and ovulation with a high probability of fertilisation is quite long (between 36 and 12 hours before ovulation). But the interval in which the fertilised oocyte has a high probability of developing into a good embryo is shorter (24-12 hours before ovulation).

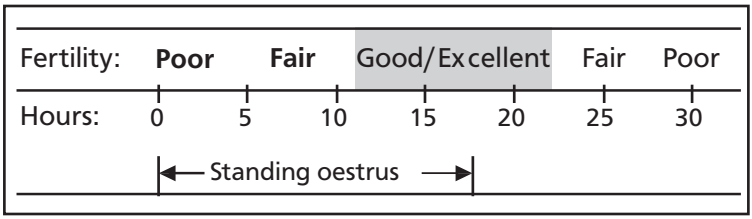

Figure 19. Optimum time of insemination in relation to oestrus.

For practical purposes, it is best to use the AM/PM rule: all cows seen in oestrus during the morning are inseminated during the afternoon. Cows still in heat the next morning are re-inseminated. Cows observed in oestrus during the afternoon or evening are inseminated the following morning. This represents, in fact, a compromise between ensuring the best chance of fertilisation and obtaining an embryo of optimal quality and developmental potential (Figure 20). In other words, early insemination gives good prospects for an embryo of high quality, but at a lower fertilisation rate (decreased sperm survival due to "waiting" for the oocyte). On the other hand, late insemination makes a high fertilisation rate possible (a lot of fresh sperm) but due to aging of the oocyte that ovulated much earlier, there is a risk of low embryo quality (Saacke, 2008).

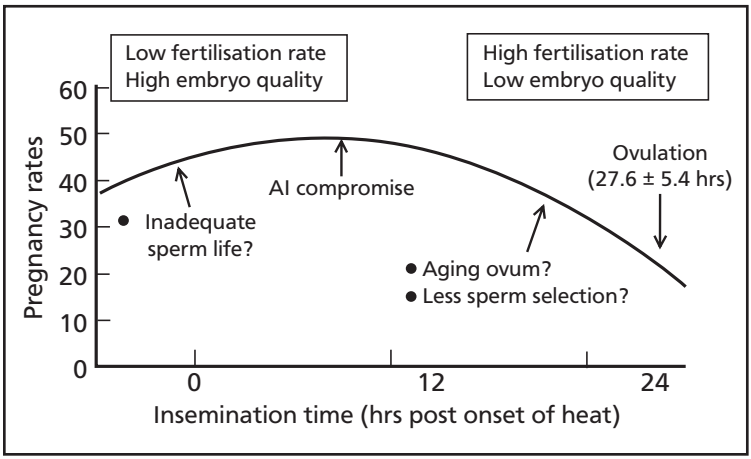

Figure 20. Calculated pregnancy in relation to the insemination time (adapted from Saacke, 2008).

The same principles apply to when GnRH is used to induce ovulation. Insemination at the time of or shortly after administration of GnRH results in lower availability of capacitated sperm for fertilisation. Simply saying, everyone arrived too early. The sperm are old and tired by the time the oocyte arrives. But excellent embryo quality results, since the oocyte is fresh and young.

Inseminations greater than 24 hours after the administration of GnRH are also characterised by lower numbers of capacitated sperm, but for a different reason. The sperm haven't had enough time to capacitate and be capable of fertilisation. The sperm are young and immature. The embryo is also of lower quality. The oocyte is old and aging. Figure 21 illustrates the effect of timing of insemination relative to ovulation induction with GnRH on pregnancy rate.

Insemination is best done 12-16 hours after the administration of GnRH and before ovulation (Pursley et al., 1998).

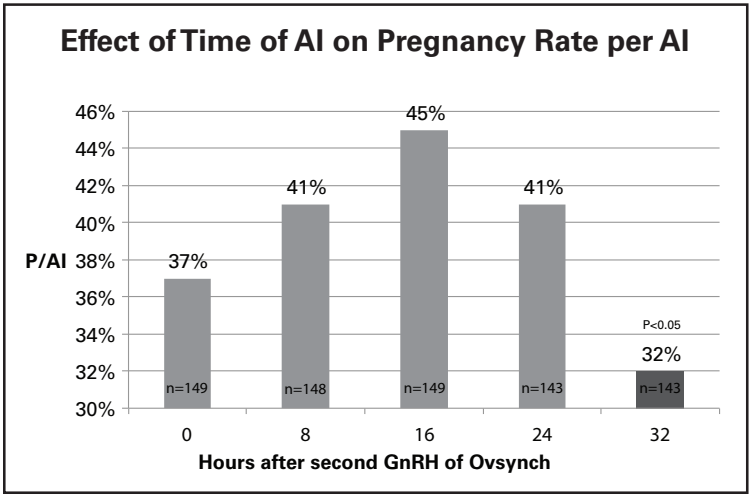

Figure 21. Effect of time of AI on pregnancy rate per AI (adapted from Pursley et al., 1998).

2.4.3 Delayed ovulation

Variations in the duration of oestrus, and problems with oestrus detection, may lead to the inappropriate timing of insemination and poor success rates. On the other hand, in high-yielders, both delayed ovulation and follicular atresia can contribute to the failure of conception. They are responsible for a high proportion of the so-called 'asymptomatic' failures to conceive observed.

Ovulation takes place about 30 hours after the onset of oestrus. Various factors can, however, influence the actual time of ovulation in relation to the oestradiol peak (maximal oestrus signs). Compromised luteal function due to metabolic deficiencies and excessive metabolic rate, or the effects of high ambient temperature (heat stress), can lead to a delay in ovulation.

Prolonged follicular dominance is associated with compromised oocyte competence and increased embryonic loss (Diskin et al., 2004). In a recent study published by Bloch et al (2006), the endocrine profile of cows with different oestrus-to-ovulation intervals was investigated. The results indicated an association between preovulatory reduced oestradiol concentrations, a lower delayed preovulatory LH surge, and an extended oestrus-to-ovulation interval (Figure 22).

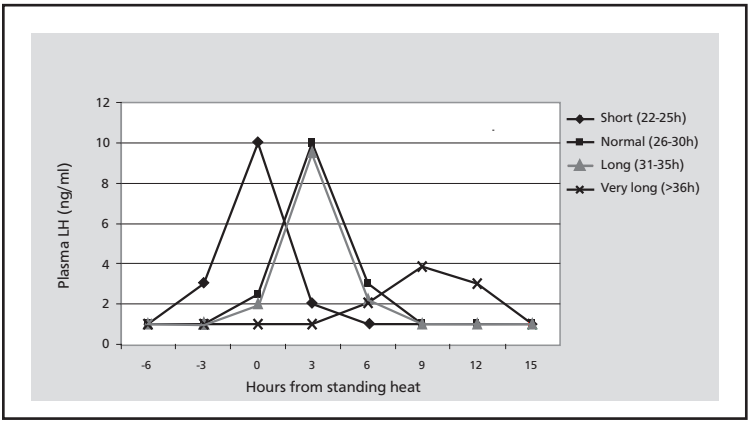

Figure 22. Concentrations of plasma preovulatory LH surges in cows with various oestrus-to-ovulation intervals (Adapted from Bloch et al., 2006).

This study also reported lower postovulation progesterone concentration during the mid-luteal phase in animals with a long or very long oestrus-to-ovulation interval as compared to those with a short or normal interval. This provides important clues about the possible contribution of delayed ovulation and prolonged oestrus-to-ovulation interval to early embryonic losses caused by an inadequate early progesterone pattern.

One of the methods of obtaining satisfactory conception rates is to ensure that ovulation occurs within 7-18 hours of AI. One possible method is by the administration of GnRH around the time of service. Depending on the size and maturity of the dominant follicle, ovulation usually occurs within 24-32 hours of GnRH injection, which is similar to the time between the onset of oestrus and ovulation (Pursley et al., 1995).

It is postulated that the administration of GnRH analogues at the time of insemination may modify the function or characteristics of preovulatory ovarian follicles and the secretory capacity of the developing corpus luteum (Mee et al., 1993). Results reported by these authors suggest that GnRH may serve to enhance or alter theca-lutein or granulosa-lutein differentiation in the preovulatory or postovulatory follicle or developing corpus luteum and may act on the developing corpus luteum to promote the conversion of small to large luteal cells, thereby increasing progesterone secretion.

a. Results of treatment

Rosenberger et al (1991) evaluated the effect of GnRH injection during oestrus on plasma LH and conception, in relation to the timing of the treatment and insemination. In groups suffering from low conception rates following the first postpartum AI, treatment with GnRH improved insemination results. It was suggested that GnRH treatment could reduce the variation in the timing of ovulation or prevent ovulation failure. Several earlier studies demonstrated that treatment with GnRH at the time of insemination in repeat breeders improved pregnancy rates (Stevenson et al., 1988, 1989; Lee et al., 1983; Phatak et al., 1986; Kharche et al., 2007).

The study by Morgan and Lean (1993) presented an extensive analysis of the possible effect of treatment with GnRH at the time of insemination on conception rate in cattle. The article compared results from numerous previous studies in which GnRH or GnRH analogues had been used at AI and submitted them to meta-analysis. There was a significant increase in the likelihood of pregnancy in cows treated with a GnRH analogue at the first postpartum insemination, at the second service after calving, and in repeat breeder cows treated at the time of insemination. Repeat breeders responded better to the treatment than the other groups, which supports

the hypothesis that a proportion of repeat breeders have failed to conceive beforehand because of a failure in the timing or the magnitude of GnRH, LH, or FSH surge at oestrus.

Heuwieser et al (1994), in a large study involving 2,437 dairy cows, analysed the relationship between the administration of GnRH, body condition score, and fertility. Conception rate improved when GnRH was administered at the first breeding postpartum in cows with a body condition score below 3.0, regardless of their parity.

A study reported by Kaim et al (2003) involving the administration of buserelin indicated that the use of GnRH eliminated differences in conception rates for cows inseminated early or late relative to the onset of oestrus and increased the efficacy of insemination in cows suffering from postpartum reproductive disorders. The authors of this study concluded that the administration of GnRH at the onset of oestrus increased LH surges, prevented delayed ovulation, and may have increased subsequent progesterone concentrations. Treatment with buserelin in this trial increased conception in primiparous cows during the summer and in cows with lower body condition scores.

2.4.4 Inadequate uterine environment

Other factors that limit fertility in the dairy herd include the accumulation of toxic concentrations of urea and nitrogen in cows fed high levels of crude protein in the face of an inadequate energy supply. As the amino acids are degraded, concentration of both ammonia and urea increase in circulation. This, in turn, is believed to lead to unfavourable changes in the pH of the endometrium, which can impair implantation.

It has been postulated that increased concentrations of nitrogen and urea, in both the blood stream and the endometrial fluid, might affect the embryo's viability and its capacity for further development. The greatest changes in the uterine environment occur during the mid-luteal phase, which is a critical

period for early embryo development that ultimately determines long-term embryo survival. Recent work by Rhoads et al (2006) revealed that high plasma urea nitrogen concentrations in lactating dairy cows reduce the viability of the embryo through effects exerted on the oocyte and embryo before its recovery from the uterus 7 days after insemination.

There is limited information, in cattle, about the possible effect of subclinical endometritis and the irreversible morphological changes in the endometrium caused by a prolonged inflammatory process on the success of implantation. The data available in mares, however (see chapter on equine reproduction), clearly indicate that such changes can have a negative effect on the recognition of pregnancy and impair the implantation process, leading to early embryonic losses.

2.4.5 Importance of early luteal function in pregnancy recognition and maintenance

It has been established for many years that the concentration of progesterone during early pregnancy has a marked effect on the outcome of insemination. The formation of a functional corpus luteum and the post-ovulatory rise in progesterone are of critical importance for the developing embryo (Robinson et al., 2008).

Peripheral progesterone concentrations begin to increase by about day 4 after ovulation and reach maximal levels by day 8-10. It is this rapid decline in oestradiol concentrations and the subsequent rise in progesterone that ensures the timely control of both oviductal and endometrial functions supporting the survival and development of the embryo. Progesterone receptor levels are maximal in both endometrial glands and the sub-epithelial stroma from day 4 to 10 postovulation. These endometrial glands synthesise, secrete, and transport a complex of amino acids, glucose, transport proteins, and growth factors called histotroph. Histotroph is an essential source of nutrients and regulatory molecules for the developing blastocyst in the pre-attachment period.

Numerous studies have revealed lower concentrations of progesterone in milk (Lamming et al., 1989; Mann et al., 1995) and plasma (Mann et al., 1995, 1996; Buttler et al., 1996; Mann et al., 2001) in cows that fail to maintain pregnancy (Figure 23).

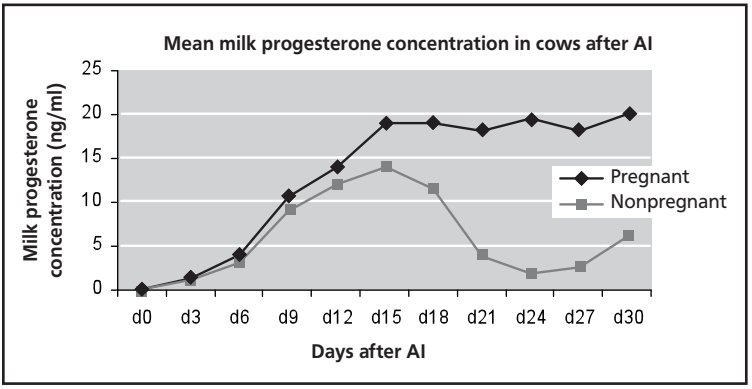

Figure 23. Progesterone profiles after AI in cows confirmed later as pregnant or nonpregnant (Adapted from Mann et al., 1999).

Embryos from cows with higher progesterone concentrations showed more advanced development as early as 5 days postovulation (Green et al., 2005).

It is well established that fertility and milk production are negatively associated in dairy cows. Lopez et al (2005) indicated that high-yielding cows have lower circulating concentrations of progesterone than low-yielding cows, which may possibly be associated with their higher metabolic rate and, consequently, higher rate of progesterone catabolism (Wiltbank et al., 2006).

Several studies of pregnancy recognition and maintenance in cattle have revealed that these two groups of factors are closely connected, as sufficient developmental potential of the embryo is a prerequisite for continued luteal function in cattle. In the study by Mann et al (2001), it was demonstrated that the degree of embryo development was closely related to the maternal progesterone environment. In this study, even a single day's delay in the postovulatory rise in progesterone concentrations was shown to reduce significantly the subsequent development of the embryo.

Poor progesterone support of the developing embryo clearly can affect its ability to synthesise and secrete interferon-τ, a widely recognised embryonic signal for the maternal recognition of pregnancy in ruminants (Mann et al., 1999).

The elongation of the blastocyst initiates interferon-τ production, which is detectable in uterine flushes from day 12 to day 25 postovulation. Cows with poorly developed embryos on day 16 after the first insemination that produced little or no interferon-τ exhibited a delayed increase in progesterone concentration after ovulation and had a lower luteal phase plateau than did cows with well-developed embryos.

2.5 Heat stress

Heat stress can occur over a wide combination of solar radiation levels, ambient temperatures, and relative humidity. Milk production decline and reproduction losses during the summer substantially impact the economic potential of dairy farms. Heat stress can be further aggravated by increased metabolic heat production, due to greater production levels and dry matter intake. Strategies should be initiated to lessen the severity of heat stress on both reproduction and milk production to improve cow performance and farm profitability.

a. Oestrous activity, hormone function, and follicular development
Heat stress reduces the length and intensity of oestrus. For example, in summer, motor activity and other manifestations of oestrus are reduced (Hansen and Arechiga, 1999) and incidence of anestrous and silent ovulations are increased (Gwazdauskas et al., 1981). Nebel et al (1997) reported that Holsteins in oestrus during the summer had 4.5 mounts vs 8.6 mounts per oestrus for those in winter. One possible reason for the reduction in expression of oestrus observed during heat stress is from reduction in physical activity, as a response to limit heat production.

On a commercial dairy in Florida, undetected oestrous events were estimated at 76% to 82% during June through September, compared to 44% to 65% during October through May (Thatcher and Collier, 1986). Another possible reason for reduced oestrous expression is from suppressed endocrine hormones such as luteinising hormone and oestradiol, important

for follicle growth and triggering oestrous behaviour (Rensis and Scaramuzzi, 2003). Seasonal studies report lower steroid concentrations in the follicular fluid obtained from large follicles during the hot season, associated with reduced viability of granulosa cells and impaired aromatase activity (Badinga et al., 1993; Wolfenson et al., 1995). In a study by Wolfenson et al (1997), androstenedione production by thecal cells was reduced and low oestradiol concentrations were observed in follicular fluid collected from dominant follicles during autumn. Authors concluded alteration of steroidogenic capacity induced by heat stress carries over to the final stages of follicle development. In addition, Roth et al (2000) observed decreased oestradiol and androstenedione production from granulosa and thecal cells obtained from follicles 3 to 4 weeks after acute heat stress. In a similar study, low concentrations of oestradiol were observed in the follicular fluid of cows during summer, which increased throughout autumn (Roth et al., 2004).

Heat stress impairs follicle selection and increases the length of follicular waves, thus reducing the quality of oocytes and modulating follicular steroidogenesis (Roth et al., 2001a). Summer heat stress has been shown to increase the number of subordinate follicles, while reducing the degree of dominance of the dominant follicle and decreasing inhibin and oestrogen levels (Wolfenson et al., 1995; Wilson et al., 1998). In another experiment, Wolfenson et al (1995) detected a tendency for reduction in plasma inhibin concentrations in heat stress in lactating dairy cows, and Paltra et al. (1997) observed similar results for cyclic water buffaloes. Subsequently, exposure of lactating dairy cows to heat stress during an entire oestrous cycle induced a 50% increase in the number of large (>10 mm) follicles during the first follicular wave (Wolfenson et al., 1995). Also, a similar result was observed in heat-stressed heifers during days 17-21 of the oestrous cycle (Wilson et al., 1998). This may account for the increase in twinning rate following insemination of cows during the summer, in that increases in the number of large follicles occur in summer compared with winter months (Ryan and Boland, 1991). Summer heat stress reduces follicular dominance, allowing more than one dominant follicle to develop; this explains the increased twinning seen in summer months. As explained earlier, the follicle destined to ovulate emerges 40-50 days prior to ovulation. Therefore, heat stress occurring at any time during this period can compromise follicular growth and steroidogenic capacity. In addition, either due to direct actions of elevated temperature or alterations of follicular function, the oocyte has

potential to be compromised. Further investigation is warranted to ascertain heat stress effects on the endocrine system and subsequent follicle and oocyte growth.

b. Oocytes, fertilisation, and early-developing embryos

During summer, heat stress reduces pregnancy and conception rates, which can carry over into the fall months (Wolfenson et al., 2000). Presently, it is not known at what stage in follicular development that heat stress damages the ovarian follicle and/or oocyte. This may be an important area for future investigation, since the negative effects of heat stress on the ovary are comparable to negative effects on the testis, in which a time lag of 40-50 days is required before completion of the spermatogenic cycle, leading to the production and ejaculation of new sperm that were not damaged by heat stress. A comparable time lag for recovery in the female ovary/oocyte most likely accounts for a considerable portion of the delay in restoration of fertility seen well into the fall (Roth et al., 2001b). Oocytes obtained from dairy cows during the summer heat stress period had reduced developmental competence in vitro (Rocha et al., 1998). Rutledge et al (1999) also reported a decrease in the number of Holstein oocytes that developed to the blastocyst stage during July and August compared to cooler months. In both of these studies, fertilisation rate was not affected by season, but the lower development following fertilisation during summer was indicative of oocyte damage. In contrast, Sartori et al (2002) showed a significant reduction in the summer for fertilisation rate, embryo quality, and nuclei/embryo in lactating cows versus nulliparous heifers. When superovulated donor heifers were exposed to heat stress for 16 hours, beginning at the onset of oestrus, there was no effect on fertilisation rate. However, there were a reduced number of normal embryos recovered on day 7 after oestrus (Putney et al., 1988a). This illustrates that a brief heat stress can still affect oocyte competence within the preovulatory follicle. In addition, exposure of cultured oocytes to elevated temperatures during maturation decreased cleavage rate and the proportion of oocytes that became blastocysts (Edwards and Hansen, 1997). Effects of heat stress on the developing and ovulated oocyte could significantly impact growth and quality of the subsequent embryo, contributing to the increased amount of embryo loss observed in lactating dairy cattle.

Heat stress can also affect the early-developing embryo. When heat stress was applied from day 1 to 7 after oestrus, there was a reduction in quality

and development of embryos flushed from the reproductive tract on day 7 after oestrus (Putney et al., 1989). In addition, embryos collected from superovulated donor cows in summer months were less able to develop in culture than embryos collected from superovulated cows during fall, winter, and spring months (Monty and Racowsky, 1987). Drost et al (1999) demonstrated that transfer of in vivo produced embryos from cows in thermoneutral conditions increased pregnancy rate in heat stress recipient cows compared to that of heat stress cows subjected to AI. Embryos appear to have developmental stages in which they are more susceptible to the deleterious effects of heat stress, as shown in vitro. In vitro heat stress at the 2- to 4-cell stage caused a larger reduction in embryo cell number than heat stress at the morula stage (Paula-Lopes and Hansen, 2002). An earlier study also observed that heat stress caused a greater reduction in embryo development when applied at the 2-cell stage than the morula stage (Edwards and Hansen, 1997) or at day 3 following fertilisation than at day 4 (Ju et al., 1999). Utilising techniques (ie, embryo transfer) to bypass the critical stage at which embryos are most sensitive to heat stress can dramatically improve fertility, as discussed in later sections.

c. Latter stages of embryo development

Not only can heat stress affect the oocyte and early embryo, it can also reduce embryo growth up to day 17, which is a critical time point for embryo production of interferon-τ. Adequate amounts of interferon-τ are critical for reducing pulsatile secretion of prostaglandin $F_{2\alpha}$, thus blocking CL regression and maintaining pregnancy. Biggers et al (1987) indicated that heat stress reduced weights of embryos recovered on day 17 from beef cows. This reduction in embryo size was associated with reduced interferon-τ available to inhibit prostaglandin $F_{2\alpha}$ pulsatile secretion, which causes CL regression. Putney et al (1988b) incubated embryos and endometrial explants obtained on day 17 of pregnancy at thermoneutral (39°C, 24 hours) or heat stress (39°C, 6 hours; 43°C, 18 hours) temperatures. The heat stress conditions decreased protein synthesis and secretion of interferon-τ by 71% in embryos; however, endometrial secretion of prostaglandin $F_{2\alpha}$ and embryo secretion of prostaglandin E_2 increased in response to heat stress by 72%. Wolfenson et al (1993) observed that secretion of prostaglandin $F_{2\alpha}$ was increased in vivo when heifers were exposed to high ambient temperatures. Collectively, these studies demonstrate that both the embryo and uterine environment can be disrupted due to heat stress, inhibiting the embryo's

ability to secrete interferon-τ (signal to block CL regression) and maintain pregnancy and (or) manipulating production of important proteins from the uterine lining.

A reduction in the amount of growth factors, due to an increased level of milk production and (or) decline in nutritional status due to heat stress, may reduce the amount of necessary embryotrophic growth factors. Secretion of embryotrophic growth factors into the uterine lumen may be controlled by nutritional status of the cow, since embryo transfer pregnancy rates were reduced in recipients with low BCS (Mapletoft et al., 1986). Plasma concentrations of insulin, insulin-like growth factor-1, and glucose are decreased in summer compared to winter months; this is most likely due to low DMI and increased negative energy balance. This reduction in important growth factors and nutrients for reproduction hampers the embryo's ability for normal growth and production of interferon-τ. Bilby et al (2006a) reported that supplementing lactating dairy cows with recombinant growth hormone at the time of AI and 11 days later increased growth factors, conceptus lengths, interferon-τ production, and pregnancy rates in lactating dairy cows compared to cows without bST supplementation. Possibly increasing availability of important growth factors during heat stress may improve embryo growth and survival. This strategy, combined with feeding by-pass fats enriched in EPA and DHA, may benefit reproductive performance during summer heat stress (Bilby et al., 2006 a,b,c). However, when using techniques such as improved nutrition or a pharmaceutical such as bST to improve embryo viability and growth in vivo, the inherent increase is shifted towards enhancing milk production at the expense of the reproductive organ, in turn, possibly masking the true benefits on fertility. Further studies are warranted to develop tools to target hormonal or nutrient delivery to the reproductive organ in order to improve fertility without losing the additional nutrients and hormones towards increased milk production.

Embryo loss is another important factor that affects fertility and is increased during heat stress. Dairy cows conceiving with singletons or twins are 3.7 and 5.4 times more likely to lose their embryo, respectively, during the hot vs cool season (Lopez-Gatius et al., 2006). In addition, the likelihood of pregnancy loss has been shown to increase by a factor of 1.05 for each unit increase in mean maximum temperature-humidity index (THI) from days 21-30 of gestation. Pregnancy losses with a maximum THI of 55,

55-59, 60-64, 65-69, and >69 were 0, 1, 2, 8, and 12%, respectively (Garcia Ispierto et al., 2006). Interestingly, the maximum THI at which embryo loss dramatically increases is from 60-64 to 65-69. This is much lower than the 72 THI threshold once thought to be the threshold at which cows become heat stressed. This provides additional evidence that reproductive failure occurs at much lower temperatures than once expected.

d. Uterine environment and immune function

The reproductive organ can be compromised during heat stress, providing a suboptimal uterine environment for fertilisation, embryo growth, and implantation. Heat stress further reduces available nutrients and hormones by causing redistribution of blood flow from the visceral organs to the periphery, resulting in decreased availability of nutrients and hormones, ultimately compromising uterine function. Increases in uterine blood flow caused by injection of oestradiol-17β were reduced in cows not exposed to shade in summer, compared with those receiving shade (Thatcher and Collier, 1986). Also, as mentioned earlier, prostaglandin production is increased and embryo growth and interferon-τ produced by the embryo are reduced due to heat shock exposure. A culmination of reduced blood flow (which provides the essential nutrients for embryo development) and increased prostaglandin production will severely inhibit embryo survival during summer months.

The effect heat stress has on immune function has not been evaluated in detail, especially in agriculturally important species. However, the incidence of some health problems certainly appears to increase during the summer months, as increased rates of mastitis, retained placenta, metritis, and ketosis have been reported (Collier et al., 1982). Several epidemiological studies reveal a reduction in fertility for cows affected by disorders of the reproductive tract, mammary gland, feet, and metabolic diseases such as ketosis, milk fever, and left-displaced abomasums. Retained placenta, metritis, and ovarian cysts are risk factors for conception. Cows had lower conception rates of 14% with retained placenta, 15% with metritis, and 21% for those with ovarian cysts (Grohn and Rajala-Schultz, 2000). Mastitis also significantly reduces fertility in lactating dairy cattle (Hansen et al., 2004). In addition, general *stress* enhances glucocorticoid levels, which reduces neutrophil function. Therefore, heat stress-induced increases in cortisol levels may partially explain the negative effects heat stress has on health.

An additional cause of compromised immune function may be negative energy balance. Negative energy balance in early lactation is associated with a variety of health and reproductive issues (Drackley, 1999). The heat-stressed cow also enters negative energy balance and thus (probably not surprisingly) experiences many of the same health problems and reduced reproductive parameters as transitioning cows. The calculated negative energy balance during heat stress (approx. -5 Mcal/day) is not as severe as in early lactation (ie, approx. d 7:approx. -15 Mcal/day), but it almost certainly is not a coincidence that both situations have increased rates of similar disorders.

e. Placental and foetal development

When heat stress is imposed the last 2-3 months of pregnancy, there are clear effects on placental function and endocrine parameters. Prepartum heat stress may decrease thyroid hormones and placental oestrogen levels while increasing nonesterified fatty acid concentrations in blood, all of which can alter growth of the udder and placenta, nutrients delivered to the unborn calf, and subsequent milk production (Collier et al., 1982). Collier et al (1982) also reported that dairy cows experiencing heat stress during late gestation had calves with lower birth weights and produced less milk than cows not exposed to heat stress. This was associated with a reduction in circulating thyroxine, prolactin, growth hormone, and glucocorticoid concentrations. Other researchers have suggested that cooling prepartum cows may increase birth weights, improve colostrum quality, decrease calving-related health disorders, and increase subsequent milk production (Avendano-Reyes et al. 2006; Wolfenson et al., 1988). Feed intake and metabolic rate are adversely affected by heat stress during the immediate prepartum period, and this may adversely affect the ability of the dairy cow to ramp up production postpartum.

2.5.1 Modifications to reproduction programs

a. Natural service fertility

A majority of dairy producers still utilise natural service as a component of their reproductive program, further accentuating the effects of heat stress on reproductive performance in dairy cattle. Heat stress significantly impairs natural service sires by effects on spermatogenesis and reduced

libido. Semen quality decreases when bulls are continually exposed to ambient temperatures of 30ºC for 5 weeks or 37.5ºC for 2 weeks despite no apparent effect on libido. Heat stress decreases sperm concentration, lowers sperm motility, and increases percentage of morphologically abnormal sperm in an ejaculate. After a period of heat stress, semen quality does not return to normal for approximately 2 months because of the length of the spermatic cycle, adding to the carryover effect of heat stress on reproduction. However, the use of frozen-thawed conventional semen and AI bypasses effects of heat stress on male fertility. For example, many dairy producers in the USA use AI for a set number of breedings (ie, 3 AI breedings) and then move the cow to a corral with natural breeding; however, it may be advantageous to continue AI for several more breedings to bypass the deleterious effects described above, during, and for a 2-month period after heat stress. Also, the use of natural mating will improve genetic progress and decrease the chance of cows contracting a disease introduced by natural mating.

b. Timed artificial insemination

The use of fixed timed AI (TAI) to avoid the deleterious effects of reduced oestrus detection has been well documented. Utilising some type of TAI protocol (ie, Ovsynch, Cosynch72, or Ovsynch56), either coupled with or without oestrus detection, can improve fertility during the summer. Past studies conducted in Florida, during the summer months, observed an increase in number of cows pregnant at 90 days (Arechiga et al., 1998) or 120 days postpartum (De la Sota et al., 1998) than cows inseminated at observed oestrus, even though conception rate at first service was not different (Arechiga et al., 1998). The positive effects of the first service TAI during heat stress were consistent for the course of a year, with fewer cows being culled (12.9% vs 22%) and additional cows conceiving (87% vs 78%) if TAI was utilised for first service vs AI at detected oestrus (De la Sota et al., 1998). These results concluded that using TAI during heat stress decreased days open, interval from calving to first breeding, and services per conception vs insemination at detected oestrus (De la Sota et al., 1998). Subsequently, Jordan et al (2003) observed two different first-service TAI programs over the course of 11 months, and the effect of season on first insemination was not significant. Other studies have also reported more consistent pregnancy

rates through the summer when a synchronisation program was used compared with AI at detected oestrus (Burke et al., 1996; Britt and Gaska, 1998). Although TAI ensures cows are inseminated by a certain day in milk and can bypass reduced oestrus detection seen during heat stress, these programs will not overcome the negative impacts of heat stress on oocyte maturation and embryo development.

c. Use of GnRH or hCG on or after oestrus

Ovulation failure and undetected ovulations increase during heat stress (Gwazdauskas et al., 1981; Thatcher and Collier, 1986). One possible way to circumvent the lack of ovulation and possibly improve fertility in the summer is through an injection of GnRH at oestrus. Ullah et al (1996) injected GnRH into lactating dairy cows at detected oestrus during late summer in Mississippi and increased conception rate from 18% to 29%. In agreement with this study, lactating dairy cows were injected with GnRH at the first signs of standing oestrus during the summer and autumn months in Israel, and conception rates increased compared to untreated controls (41% to 56%, respectively; Kaim et al., 2003). Interestingly, a study conducted during the summer in Spain increased conception rates only when GnRH was injected at the time of AI and 12 days later (35.4%) compared to injecting only at TAI (30.8%) or only 12 days after TAI (20.6%; Lopez-Gatius et al., 2006). Authors concluded that although double treatment with GnRH was lower, strong benefits were also registered following a single GnRH treatment at insemination. In addition, treatment did not affect twin pregnancy rates yet increased the incidence of an additional corpus luteum.

Progesterone production by the CL is critical for establishment and maintenance of pregnancy. In a recent review, Wolfenson et al (2000) concluded that chronic heat stress reduces progesterone concentrations; however, progesterone concentrations may be increased after an acute heat stress. Several studies have shown that progesterone concentrations can be elevated by inducing an accessory CL with the use of GnRH or hCG from 5-14 days after AI. When injecting either hCG or a GnRH agonist on day 5 of the oestrous cycle in lactating dairy cows during summer, formation of an accessory CL and elevated progesterone occurred (Schmitt et al., 1996). Nonetheless, conception rates were not improved during heat stress.

Further studies are warranted to ascertain when hormonal manipulation should be utilised post-TAI, which hormonal product to use, and at what degree of heat stress is hormonal administration post-TAI needed.

d. Embryo manipulation and transfer

Embryo transfer can significantly improve pregnancy rates during the summer months (Drost et al., 1999). Embryo transfers can bypass the period (ie, before day 7) in which the embryo is more susceptible to heat stress. In a recent study, in vitro-produced embryos with sex-sorted semen were either vitrified or remained fresh and transferred after a timed embryo transfer program into lactating dairy cows during summer vs conventional AI (Stewart et al., 2011). Conception risk was doubled with fresh embryos (39%) and no difference was found between vitrified embryos (27%) compared with conventional AI (21%). When calving rate and gender was evaluated, fresh embryos remained superior (27.5%) compared with vitrified (17.1%) or conventional AI (14.6%) and number of heifers born was increased in both fresh and vitrified embryo groups vs conventional AI (88% and 84% vs 50%; Stewart et al., 2011). Improvements need to be in made in the in vitro embryo production techniques, embryo freezing, timed embryo transfer, and lowering cost of commercially available embryos before this becomes a feasible solution. Logistical implementation on a large scale with the need of a skilled technician will also slow commercial adoption.

Hormonal treatments of embryos in vitro for improved posttransfer survival during summer have been investigated. Past studies have shown improved development of the embryo to the blastocyst stage when bovine embryos were stimulated with IGF-I (Moreira et al., 2002a, b; Block et al., 2003). The IGF-I has proven beneficial in not only stimulating embryo development, but in protecting embryos from deleterious effects of thermal stress. Jousan and Hansen (2004) conducted a series of studies utilising in vitro fertilised (IVF) bovine embryos cultured with or without IGF-I. For the first experiment, day 5 embryos (≥16 cells) were exposed to either a thermal neutral environment (38.5°C for 24 hours) or heat stress environment (41°C for 9 hours followed by 38.5°C for 15 hours). Heat stress reduced the total cell number at 24 hours after initiation of heat stress and elevated the number of apoptotic cells within the embryo. However, IGF-I blocked the reduction in cell number and reduced the percent of cells within the embryo that were

apoptotic. In addition, the second experiment utilised similar treatments but evaluated embryos at day 8, and similar results were obtained. This series of experiments, and others, illustrate that IGF-I can enhance embryo survival during thermal stress in vitro.

Since IGF-I appears to have thermal protective properties in vitro, studies were designed to investigate whether transferring embryos cultured with IGF-I into recipient cows during heat stress would improve pregnancy rates (Block et al., 2003). Lactating Holstein, heat-stressed cows (n = 260) were synchronised with a TAI protocol and received an IVF-produced embryo cultured with or without IGF-I (100 ng/mL) on day 7. A single embryo was transferred to all recipients (n = 210) with a palpable CL. Transfer of IGF-I-treated embryos increased pregnancy rate at day 53, tended to increase pregnancy rate at day 81, and improved calving rates (Block et al., 2003). This proved that transferring IVF-produced embryos cultured with IGF-I can improve pregnancy rates in recipient, heat-stressed dairy cows.

Although the results from these studies confirm that IGF-I affects the embryo in vitro to improve viability during thermal stress, the question remains if elevated peripheral IGF-I in vivo can stimulate pregnancy rates during summer. Additional studies were conducted in Florida utilising recombinant bovine somatotropin (rbST) to stimulate IGF-I production and possibly improve fertility during summer (Jousan et al., 2007). Lactating dairy cows (n = 276) were synchronised and received sequential injections of rbST treatments beginning at approximately 60 DIM or no treatment with rbST. Pregnancy rates (day 45-80) did not differ between control and rbST treated cows for first (15.2% vs 16.7%) or second-service TAI (17.2% vs 14.8%). However, plasma concentrations of IGF-I, milk yield, and rectal and vaginal temperatures were greater for rbST treated cows with a reduction in body condition score (Jousan et al., 2007).

Since elevated body temperature compromises fertility in lactating dairy cows (eg, a 0.5°C increase in uterine temperature on the day of insemination resulted in a 12.8% decrease in fertility; Gwazdauskas et al., 1973), it is possible that rbST treatment protected the developing embryo from the elevated body temperature associated with rbST, ultimately maintaining similar pregnancy rates.

Manipulation of embryos in vitro and (or) development of hormonal therapies for use in vivo may allow for improvements in summer fertility.

Improved cooling is still the most profitable and effective way to improve both milk production and reproduction during the summer months. Reproductive programs can be modified through hormonal manipulations, embryo transfer, and continued AI to bypass critical time points of which heat stress appears to be most detrimental. Implementing aggressive reproductive programs during the summer can help improve reproductive performance but will not eliminate reproductive shortfalls. Research is still warranted in developing novel approaches to improve the already low fertility of lactating dairy cows during heat stress.

2.6 Reproductive disorders

Infertility can be a serious problem, especially in high-yielding dairy cows. During the postpartum period, there must be a rapid and uneventful involution of the uterus and early resumption of normal ovarian activity. At the same time, the cow is being asked to produce large amounts of milk. To achieve and maintain good herd fertility requires prevention and early diagnosis with proper treatment when necessary.

The reproductive problems of the individual cow can be divided into the following groups:

- Retained placenta
- Uterine infections
- Anoestrus
- Cystic ovarian disease (COD)
- Embryonic mortality
- Repeat breeders
- Abortion

All of these will be discussed in the following sections, starting with the physiological aspects of the postpartum period.

2.6.1 Physiological aspects of the postpartum period

a. Uterine involution

It generally takes 3 weeks for the uterus to return to its normal nonpregnant size. The time required for complete physiological involution (including regeneration of the epithelium of the endometrium) varies from 40 to 50 days.

Endogenous levels of prostaglandin $F_{2\alpha}$ metabolites are elevated during the first 7 to 23 days after calving, which supports rapid uterine involution.

Uterine involution involves physical shrinkage, necrosis and shedding of caruncles, and the regeneration of the endometrium. Following the loss of the allantochorion, necrosis of the uterine caruncles takes place, which are normally separated and shed by 12 days after parturition. The separation of the caruncles contributes significantly to the rapid reduction in weight of the involuting postpartum uterus, from 13 kg at parturition to about 1 kg 3 weeks later. Lochia is formed from the caruncles and the remains of foetal fluids and blood from the ruptured umbilicus, leading to a noticeable loss of fluid and tissue debris during the first 7 to 10 days after calving. The volume can vary from 500 mL in primipara to 1000-2000 mL in pluripara.

Although the correlation between uterine involution and ovarian activity in the early postpartum period has not yet been completely elucidated, there is strong evidence that such a correlation exists and that it can influence subsequent fertility. The early resumption of normal ovarian activity is known to hasten uterine involution. Moreover, the marked increase in uterine tone and reduction in size of the uterus from day 10 to 14 postpartum, which occurs in normal cows, usually coincides with the onset of the first oestrus and oestrogen production. At the same time, oestrogens are known to exert a beneficial effect on the uterine defence mechanisms and contraction of uterine smooth muscle fibers (Hussain, 1989).

The influence of uterine involution on the resumption of ovarian activity is mainly based on massive postpartum release of $PGF_{2\alpha}$ by the endometrium (Kindahl et al., 1992). It was concluded that in cows with a normal puerperium, and in those in which the duration of the postpartum release of prostaglandin is extended, uterine involution was completed more quickly, and first ovulation (followed by a luteal phase of normal duration) occurred earlier. In cows with abnormal puerperium, characterised by prolonged uterine involution, the resumption of ovarian activity was markedly delayed.

b. Ovarian activity

It has been clearly shown that during the postpartum anovulatory period, a distinct pattern of follicular activity can be observed in most cows. Their ovaries are characterised by several small to medium-sized follicles, leading to the recruitment of a first dominant follicle within a considerably short time after parturition (Opsomer et al., 1996). However, the interval between calving and first ovulation in commercial cattle herds varies greatly, depending on the breed, nutrition, milk yield, season, and the presence of a sucking calf.

In milking dairy cows, medium follicles are detectable by day 5 postpartum, with the first dominant follicle ovulating between days 15 and 27 post-partum. The majority of dairy cows should have resumed cyclic activity by day 40 postpartum. Many are not observed in oestrus.

In suckled beef cows, first ovulation occurs later, with considerable variation both within and between herds. Short cycles (luteal phase <10 days) are frequently found during the postpartum period. Medium follicles are present by day 5 to 7 postpartum, while the dominant follicles are detectable by day 10 to 21 postpartum. These dominant follicles fail to undergo final maturation and ovulation, due to the absence of appropriate LH pulses, and become atretic. The absence of LH pulses in the early postcalving period is associated with the depletion of LH stores in the anterior pituitary gland and is independent of suckling (Yavas and Walton, 2000). After the replenishment of LH stores between day 15 and 30 postcalving, the absence of LH pulses becomes suckling dependent. The stimuli generated by suckling suppress pulsatile LH release by inhibiting GnRH secretion from the hypothalamus. Ovarian oestrogens modulate this inhibitory effect. Suckling increases the

sensitivity of the hypothalamus to the negative feedback effect of ovarian oestrogens, suppressing LH release from the pituitary gland (Yavas and Walton, 2000). Pulsatile LH release recovers around day 25 to 32 postpartum and cows start cycling again between day 29 and 67 postpartum.

2.6.2 Complications of the postpartum period

Slow recovery of reproductive competence during the postpartum period is a major limitation to the success of subsequent reproduction management programmes.

a. Retained placenta

The release of foetal membranes (placenta) postpartum is a physiological process that involves the loss of foeto-maternal adherence, combined with contractions of the myometrium. The separation of foetal membranes is based on a complex immunological process. Maternal immunological recognition of foetal MHC (major histocompatibility complex) class I proteins expressed by trophoblast cells triggers an immune/inflammatory response that contributes to placental separation at parturition (Davies et al., 2004).

Normally the placenta is expelled within 6-8 hours of calving. A placenta that has not been shed by 24 hours after calving is often referred to as 'retained' placenta or retained foetal membranes. The incidence of retained placenta varies from 4.0% to 16.1%, but can be much higher in problem herds.

Failure of placental detachment appears to be largely due to an inability of the immune system to successfully degrade the placentomes at the end of pregnancy. Davies et al (2004) presented evidence from various published works that indicate impaired function of the immune processes leading to the failure of timely separation of foetal membranes in cattle. Bovine placentomes from cows with normal placental separation contain a chemotactic factor for leukocytes, which is lacking in placentomes from cows with retained placenta. Leukocytes and neutrophils of cows with retained placenta are less reactive to chemotactic stimuli than in cows with normal placental separation.

It is important to recognise that the lack of uterine contractility to expel the placenta plays little or no role in the occurrence of retained foetal membranes (Eiler, 1997); cows with retained placenta have normal to increased uterine activity in the days after calving (Frazer, 2005).

There is a clear association between cows' metabolic status and their ability to expel their foetal membranes. Cows in a more profoundly negative energy balance prepartum are 80% more likely to have retained placenta, and those with lower circulating vitamin E are also at greater risk of this condition (LeBlanc et al., 2004; LeBlanc, 2008).

Retention of the foetal membranes is a common disorder that has a detrimental effect on the reproductive efficiency of the cows, predisposing them to uterine infections later in the postpartum period and affecting the resumption of ovarian activity after calving.

It is estimated that pregnancy rate in affected cows is reduced by approximately 15% compared with unaffected cows, but it is likely that impaired reproductive performance only occurs if placental retention leads to the development of metritis or endometritis.

Although it has been established that several genetic, nutritional, immunological, and pathological factors influence the separation of the bovine placenta, the aetiology of retained placenta is not fully understood.

The manual removal of the placenta can traumatise the uterus and delay the return to normal reproductive status (Bolinder et al., 1988). It appears to be better to allow the placenta to separate of its own accord or to withdraw it gently from the uterus 7-10 days postcalving.

The aim of therapy should be to prevent the adverse effects of postpartum endometritis. Local therapy with various forms of intrauterine antibiotics is well established, yet brings limited benefits. The results of some trials indicate that treatment of retained foetal membranes with parenteral antibiotics, but without intrauterine manipulation and treatment, can be as effective as conventional treatment, including detachment and local antibiotic treatment (Drilrich et al., 2001). Several studies indicate that approximately 50%-80% of cows with untreated, retained placenta have a

temperature >39.5ºC on at least 1 day within 10 days of parturition (Drillich et al., 2003, 2006). In a study of febrile cows (Drilrich et al., 2006), neither intrauterine antibiotics nor manual removal of foetal membranes, alone or in combination, reduced the percentage of cows needing therapy. Nor did local treatment improve reproductive parameters in the current lactation when compared with systemic antibiotic treatment alone. Systemic treatment alone was effective, assessed on elevated rectal temperature, and reduced the use of antibiotics compared with therapies that included intrauterine antibiosis.

One of the pharmacological approaches to the prevention and treatment of retained foetal membranes is the administration of prostaglandins immediately after calving (Stevens et al., 1995). The efficacy of this approach is difficult to assess because of the scarcity of controlled trials. Drugs that increase uterine motility–oxytocin, ergot derivatives, calcium–have shown, at best, a limited benefit.

The reduced incidence of placental retention when vitamin E and selenium were administered, alone or in combination, suggests that oxidative stress has a part to play in the aetiology of the disorder (Campbell et al., 1998; Gupta et al., 2005). Prevention remains limited to general guidance on hygiene at calving, adequate nutrition (Ca, Se, Vit E, etc), and the control of infectious disease.

Whichever therapeutic approach is selected, the body temperature and clinical appearance of cows with retained placenta must be monitored, as typically 25%-50% of affected cows develop metritis.

b. Uterine infections

Uterine bacterial infections are important because they disrupt not only the function of the uterus, but also the ovary and the higher control centres in the hypothalamus and pituitary. For the veterinary practitioner, accurate diagnosis and adequate treatment of uterine disease is a key component of all reproduction management programmes.

Typically, 25%-40% of animals have clinical metritis in the first 2 weeks after calving, which persists in up to 20% of animals as clinical endometritis. Numerous factors, either directly associated with the function of the

reproductive tract and the general health of the cow or indirectly with management conditions, are known to predispose cows to uterine infections (Table 11).

Risk factors for the establishment of uterine bacterial disease in cattle
Uterine damage - Stillbirth, twins, dystocia, Caesarean section operation - Retained placenta - Delayed uterine involution
Metabolic conditions - Milk fever, ketosis and left displaced abomasum
Balance between pathogenicity and immunity - Disruption of neutrophil function - Type of bacterial flora in the uterine lumen - Progesterone or glucocorticoid administration; early formation of corpus luteum - Level of hygiene of the environment, cows or calving boxes may be less important

Table 11. Risk factors for the establishment of uterine bacterial disease in cattle. Adapted from Sheldon and Dobson (2004).

i. Definition

For many years, both research scientists and veterinary practitioners have identified a need for a clear set of definitions to describe various uterine conditions. One of the most popular classifications separated acute endometritis (vaginal discharge, enlarged uterus and clinical disease) occurring up to 14 days postpartum from subacute-chronic endometritis (limited vaginal discharge, absence of clinical signs) occurring after 14 days postpartum.

Recently, Sheldon et al (2006; 2008) proposed clear clinical definitions that allowed most important uterine problems to be described and differentiated.

ii. Puerperal metritis

Puerperal metritis is an acute systemic disease caused by a bacterial infection of the uterus, taking place usually within the first 10 days postpartum. Clinical signs include a fetid, brown, watery uterine discharge and usually pyrexia. In severe cases, reduced milk yield, dullness, inappetance, elevated heart rate, and apparent dehydratation may also be present.

Puerperal metritis is often associated with retained placenta, dystocia, stillbirth, or twin pregnancy. It is proposed that animals with an abnormally enlarged uterus and a purulent uterine discharge detectable in the vagina within the first 21 days postpartum, but not clinically ill, should be classified as having clinical endometritis.

iii. Clinical endometritis

Clinical endometritis is characterised by the presence of purulent (>50% pus) or mucopurulent (approx. 50% pus and 50% mucus) uterine exudate in the vagina, 21 or more days postpartum, not accompanied by systemic signs.

iv. Subclinical endometritis

Subclinical endometritis is characterised by endometrial inflammation of the uterus; the condition is usually determined by cytology, in the absence of purulent material in the vagina. A cow with subclinical endometritis is defined by the presence of >18% neutrophils in uterine cytology samples collected 21-33 days postpartum or >10% neutrophils at 34-47 days, in the absence of clinical endometritis.

The prevalence of subclinical endometritis reported in various studies ranges from 19% to 90%, depending on the diagnostic method employed and the timing of the postpartum examination. Affected cows show a significant reduction in their reproductive performance (Lincke et al., 2007).

v. Immunological aspects of uterine diseases

Bacteria from the environment contaminate the uterine lumen of most postpartum cows. The elimination of this contamination is dependent on uterine involution, regeneration of the endometrium, and the uterine defence mechanisms.

The immune system of the cow's uterus is active during pregnancy, playing an important role in maintaining pregnancy, supporting foetal growth, and preventing infection.

The innate defence system is principally responsible for combating bacterial contamination of the uterus by a range of anatomical, physiological, phagocytic, and inflammatory mechanisms. Neutrophils are the earliest and most important phagocytic cell to be recruited from the peripheral circulation to the uterine lumen in the case of bacterial infection.

Hormonal changes and alterations in immune responsiveness during the periparturient period have been associated with increased susceptibility to infections of the uterus, udder, and other tissues. Following normal delivery, the phagocytic capacity of bovine neutrophils in the peripheral circulation remains high throughout the period around parturition, but the bactericidal capacity and oxidative burst activity of neutrophils is slightly impaired during parturition itself (Singh et al., 2008). These activities are enhanced one week after parturition, which favours the spontaneous resolution of uterine infections.

During the prepartum period, there is a rise in cortisol levels, which leads to peripheral leucocytosis. This is followed by peripheral leucopaenia during the first week postpartum, which is thought to be due to the migration of neutrophils towards the uterine lumen immediately after calving.

There is now compelling evidence that both metritis and endometritis are associated with reduced feed intake, a more pronounced negative energy balance, and reduced immune function, and these differences are measurable from 2 weeks before calving, ie, 3-7 weeks before the conditions are diagnosed (Le Blanc, 2008).

Zerbe et al (2000) demonstrated that metabolic disease and, especially, an increased blood level of liver triacilgycerols were associated with reduced cytotoxic activity in neutrophils obtained from both the general circulation and the uterine wall, most probably predisposing them to uterine disease.

vi. Role of progesterone, oestradiol, and prostaglandins

The postpartum endocrine environment has a profound effect on the uterine immune response. It is generally accepted that a high

progesterone environment suppresses cervical mucus production, myometrial contractility, uterine gland secretion, and the phagocytic activity of uterine neutrophils. It has been shown and summarised by Lewis (2003) that luteal phase concentrations of progesterone suppress the immune response, making the uterus more susceptible to bacterial infection. As Lewis concludes from the numerous trials reported, susceptibility to uterine infections is associated with increased progesterone concentrations, reduced $PGF_{2\alpha}$ production, and reduced lymphocyte proliferation in vitro.

Uterine immune function appears to improve under the influence of oestrogens. It is unclear, however, whether oestradiol causes an absolute increase in phagocytic and bactericidal activity of the endometrial immune cells, or whether the observed enhancements are simply relative to the situation that occurs under progesterone dominance (Le Blanc, 2008). Sheldon et al (2004) found that administration of oestradiol into the lumen of the previously gravid uterine horn did not enhance the elimination of postpartum uterine bacterial infection.

The physiological role of $PGF_{2\alpha}$ in the first month postpartum is unclear, but it might help to promote uterine contractility and so contribute to uterine involution. From the immunological point of view, $PGF_{2\alpha}$ is pro-inflammatory, stimulating the production of cytokines that enhance phagocytosis and lymphocyte function. If produced in significant concentrations, $PGF_{2\alpha}$ can enhance uterine immune defences by mitigating the immunosuppressive effects of progesterone.

vii. Bacteriology of uterine infections

Acute endometritis is characterised by the presence of coliforms, gram-negative anaerobes, *Arcanobacterium pyogenes,* and other bacteria (including *Peptostreptococci*), each with a similar frequency.

Endotoxins and lipo-polysaccharides, excreted by coliform bacteria, are among the most important virulence factors leading to complications in cases of dystocia and retained placenta in cattle. These endotoxins have a direct cytotoxic effect that probably favours the establishment of infections with *A. pyogenes*. Zerbe et al (2001) showed that prolonged contact

between fragments or solubles of *E. coli* and *A. pyogenes* and bovine neutrophils resulted in a functional depression of these cells. A similar effect was obtained with neutrophils exposed to uterine secretions from cows infected with *A. pyogenes* and *E. coli* (Zerbe et al., 2002). It can be concluded that a high level of contamination of the endometrium with *E. coli* during the early postpartum period has a negative effect on the function of endometrial immune defence mechanisms and facilitates the persistence of uterine infections.

In cows with subacute/chronic endometritis, the bacteria most commonly isolated from the uterus are *Arcanobacterium pyogenes* and gram-negative anaerobes. This opportunistic, gram-positive facultative anaerobe is commonly present in mixed culture with a wide variety of organisms, but most often with the anaerobes *Fusobacterium necrophorum* and *Prevotella melaninogenicus*, *E. coli,* or *Streptococcus* spp.

There appears to be a synergism between *Arcanobacterium pyogenes* and gram-negative anaerobes. *Bacteroides melanogenicus* and *B. fragilis* produce and release certain substances that can impair the phagocytosis of bacteria by immune cells. *F. necrophorum* has been shown to produce leucotoxins, which exert their cytotoxic effect on phagocytic immune cells. *A. pyogenes* is capable of releasing growth-factor-like substances, stimulating multiplication of *F. necrophorum*.

viii. Effects of uterine health on fertility

The negative influence of uterine bacterial infections is associated with both the presence of the bacteria and their toxins and also the damage caused by the inflammatory process taking place in response to the infection. The presence of *A. pyogenes* or anaerobic bacteria lead to reduced fertility. It is extremely important to realise that endometritis causes infertility at the time of infection and subfertility even after the successful resolution of the disease. It is estimated that in cows with endometritis, the conception rate is approximately 20% lower, and calving interval 30 days longer, resulting in 3% more animals culled for reasons of reproductive failure (LeBlanc et al., 2002).

The subfertility associated with uterine infections also involves disruption of ovarian function. Opsomer et al (2000) suggested that uterine damage disrupts the luteolytic mechanism causing the prolonged luteal phase. These epidemiological studies also indicated that uterine infection leads to delayed ovulation. Moreover, Sheldon et al (2002) showed that ovarian function is disturbed in cattle with greater bacterial contamination after parturition.

As well as the effects on fertility, uterine infections contribute to lower milk yields, particularly if associated with retained placenta (Esslemont and Kossaibati 2002; Sheldon et al., 2004).

Data on the prevalence of endometritis in dairy herds vary, ranging from 7.5%-8.9% to over 40% (Gilbert et al., 2006). Later research by these authors found the prevalence of cytologically diagnosed endometritis to be 37%-74% between 40 and 60 days postpartum. Regardless of the mechanisms underlying the subfertility caused by uterine infections, it is important for veterinary practitioners to diagnose and treat uterine disease promptly and effectively.

ix. Diagnosis of uterine infections

Generally speaking, the diagnostic protocol for assessing uterine health in cattle follows that of a detailed clinical examination, supported by additional laboratory investigation of samples collected during this process (Table 12). Data regarding the animal's reproductive history is of considerable value both in assessing likely causative factors and duration of the disease process. It also helps in deciding on treatment options (eg, distinguishing between cyclic and noncyclic cows).

Phase of diagnostic process	Information obtained
Reproductive history	Time elapsed since calving Service history since calving Recent treatments (general and/or focused on reproductive tract)
General clinical examination	General appearance of the animal: posture, alertness, mobility Presence of vaginal discharge, presence of dried exudate in the perineal region and legs Position of the tail (elevated tail base, held to one side) Body temperature
Detailed clinical examination of the reproductive tract	Palpation of vaginal walls and visual examination of the vaginal discharge: presence of discharge in the vagina, presence of lesions in the vaginal walls. Character of the vaginal discharge: colour, smell, consistency Rectal palpation: position and size of the cervix and the uterus, uterine tone, presence of fluid in the uterine cavity, presence of corpus luteum and other structures on the ovary Vaginoscopy: external cervical orifice: degree of closure, presence, character and volume of discharge; vaginal mucosa Transrectal ultrasound examination of the reproductive tract: Uterus: position, size, thickness of the uterine wall, presence of fluid in the uterine lumen Ovaries: presence of corpus luteum, other ovarian structures
Laboratory tests	Cytology: evaluation of the presence and type of cells in the endometrial smear (percentage of PMNs) Microbiological culture of uterine discharge: type of bacteria and their sensitivity to antibiotics Endometrial biopsy: histological evaluation of biopsy samples of endometrium: presence and degree of inflammation, degree of morphological changes in endometrium.

Table 12. Diagnostic steps in the assessment of uterine health in cattle.

Diagnosis of metritis within the first 10 days postpartum is relatively easy. It is associated with pyrexia, fetid pus within the uterine lumen and vagina, and discharging from the vulva, with delayed uterine involution. The presence of abundant red-brown lochia is normal in the first 2 weeks postpartum. Additionally, fever of 1-2 days' duration is common in the first week after calving and is not well correlated with uterine infection (Sheldon et al., 2004a).

Rectal palpation allows for a general estimation of size, contents, and position of the uterus. Detection of fluid accumulation is possible if considerable amounts of exudate have accumulated in the uterine lumen, but no information can be gained about the character of this fluid or the condition of the uterine wall.

The use of transrectal ultrasonography allows a more objective measurement of the diameter of the uterine horns and cervix, and the appreciation of the presence of mucus and pus within the uterine lumen.

Vaginoscopy can be performed using autoclavable plastic, metal, or disposable foil-lined cardboard vaginoscopes, which allow inspection of the contents of the vagina. It is a rapid and simple technique, but because a positive diagnosis is based on the presence of cervical exudate, it tends to underestimate the proportion of cows with uterine pathology. Vaginoscopy is a far superior diagnostic technique to transrectal palpation of the uterus for the detection of endometritis but is underutilised by veterinary practitioners, mainly because they overestimate the time and effort needed and the cost of the equipment.

The definitive diagnosis of endometritis is made on the basis of the histological examination of endometrial biopsy samples, which are also useful for assessing subsequent fertility (Bonnet et al., 1993). However, this technique is costly, time-consuming, and not easily accessible under field conditions. Moreover, there is some evidence that collecting biopsy samples can have a negative effect on fertility.

Cytology of the uterine contents provides very valuable information, allowing the diagnosis of subclinical cases (Gilbert et al., 2004;

Kasimanickam et al., 2004; Sheldon et al., 2004b, 2006, 2008). Polymorphonuclearneutrophils (PMNs) are the predominant inflammatory cell type found in accumulations of intrauterine fluid, and the determination of the relative proportion of PMNs has been shown to be predictive of reproductive performance in the postpartum cow.

The samples can be collected either using a protected cotton swab inserted into the uterus, by uterine lavage, or with a small device called a cytobrush.

A recent study by Barlund et al (2008) compared the accuracy of endometritis diagnosis by vaginoscopy, ultrasonographic assessment of uterine fluid volume, ultrasonographic assessment of endometrial thickness, endometrial cytology collected by cytobrush, and endometrial cytology following uterine lavage. The cytological examination of samples collected by the cytobrush was found to be the most reliable method of diagnosing endometritis in cattle.

Neither of these methods is widely used in the field, and the diagnosis of uterine disease usually depends entirely on clinical examination.

The most accurate method for the diagnosis of endometritis in clinical conditions is examination of the vagina for the presence of pus. The use of vaginoscopy is, therefore, highly recommended or, alternatively, the vagina can be explored manually, withdrawing the cervical mucus for examination. The advantage of the latter method is that it is cheap, quick, and allows vaginal lacerations and the odour of any vaginal discharge to be detected (Sheldon et al., 2006). The procedure involves cleaning of the vulva using a dry paper towel and the insertion of a clean, lubricated, and gloved hand through the vulva into the vagina followed by the withdrawal of the mucoid contents of the vagina for examination. Manual vaginal examination does not cause uterine bacterial contamination, provoke an acute-phase protein response, or affect uterine horn diameter.

Also, a new device called Metricheck (Metricheck®, Simcro, New Zealand), which consists of a stainless steel rod with a rubber hemisphere, can be used to retrieve the vaginal contents.

Some important points should be taken into account when using this simple and very effective device:

- The tool should be cleaned and disinfected before use in each animal
- The perineum must be cleaned to avoid the introduction of faecal material into the vagina
- The animals need to be restrained to ensure the security and comfort of both animal and operator

The assessment of endometritis is made on the basis of the uterine status and the characteristics of the vaginal mucus. A mucus scoring system is widely adopted to indicate the degree of the inflammation process (Table 13).

Description	Score
Mucus character	
Clear or translucent mucus	0
Clear or translucent mucus containing flecks of white pus	1
< 50 mL exudate containing < 50% white or cream pus	2
> 50 mL exudate containing > 50% white, cream, or bloody pus	3
Mucus odour	
No unpleasant odour	0
Fetid odour	3

Table 13. Clinical endometritis score (Sheldon and Dobson, 2004). The vaginal mucus is scored for character and odour according to the following descriptions. The sum of the two scores gives the endometritis score.

x. General guidelines in the treatment of uterine infections in cattle

In the therapy of acute puerperal metritis, there are three main objectives to be addressed: elimination of the bacterial infection, treatment of the general clinical signs of toxaemia, and supportive measures aimed at sustaining the animal's homeostasis and minimising the damage caused by the inflammatory process and toxaemia. Antibacterial treatment should employ antibiotics with a broad spectrum of activity (especially against *E. coli*). Intrauterine products can be used, but the fact that many affected cows have an elevated body temperature prompts practitioners to select parenteral antibiosis. Moreover, as affected cattle have moderate to severe illness, there is usually little dispute that cows with metritis require systemic antibiotic treatment. Injectable preparations of cephalosporins such as ceftiofur are currently in common use (Sheldon et al., 2004d; Drillich et al., 2006).

Animal welfare and evidence of a destructive nature of the inflammatory process to the endometrium motivates many practitioners to add non-steroidal anti-inflammatory drugs (NSAIDs) to their standard treatment of acute metritis. Although there is, so far, little evidence that this practice has any beneficial effect on reproductive efficiency, reduction in body temperature and improved clinical appearance have been demonstrated (Drillich et al., 2007). Whenever a decision is made to include NSAIDs in the therapy of metritis, it should be borne in mind that these products inhibit the production of endogenous prostaglandins from arachidonic acid and can thus lead to impaired uterine involution. In such cases, exogenous $PGF_{2\alpha}$ should always be administered.

The possible benefits of the routine inclusion of prostaglandins in the treatment of uterine infections in the early postpartum period remain disputed. There is little evidence to support the use of $PGF_{2\alpha}$ before 3 weeks postpartum (LeBlanc, 2008), though many practitioners report highly satisfactory results.

The antibiotic chosen to treat chronic endometritis must be able to eliminate the bacterial infection whilst remaining active in the anaerobic uterine environment. What is more, it should lead to minimal drug residues in milk or meat.

Treated cows have been shown to eliminate the uterine bacterial contamination, leading to improved reproductive performance (Kasimanickam et al., 2005; LeBlanc et al., 2002; McDougall et al., 2001).

In a study in New Zealand, reported by McDougall et al (2001), intrauterine treatment improved reproductive performance of dairy cattle, especially those with a history of retained placenta, stillbirth, or a vulval discharge.

One of the important features of this product is its ability to provide adequate concentrations of the active, cephapirin, not only in the uterine lumen but also in the endometrium (Table 14). This allows for an effective elimination of the bacteria from endometrial crypts.

Hours after administration of Metricure®	4h	8h	24h	72h
Concentration of cephapirin in endometrium (mcg/g)	9.62 (>38MIC)	23.08 (>92 MIC)	4.9 (>19 MIC)	0.8 (>3MIC)
Concentration of cephapirin in plasma (mcg/g)	0.06	0.02	< 0.01	< 0.01
MIC90 for *A. pyogenes* = 0.25 mcg/mL Detection limit – 0.01 mcg/mL				

Table 14. Concentrations of cephapirin in endometrium and plasma at 4, 8, 24, and 72 hours after administration (Data source: Intervet product file).

Antibiotic treatments that have led to the rapid and effective elimination of uterine infections with *A. pyogenes* have been associated with improved reproductive performance in treated cows. Cephapirin was found to be very effective against the major uterine pathogens involved in endometritis in cattle (Sheldon et al., 2004d).

It is extremely important to ensure that any intrauterine treatment used for the treatment of uterine infection has no irritatant effect and does not impair the function of the endometrial defence mechanisms. Few antibacterial substances have actually been examined in controlled studies in this respect. Cephapirin, at clinical dose rates, has been demonstrated to have no negative effect on neutrophil function or on their ability to eliminate the bacteria (Dosogne et al., 1998).

Sometimes the chronic/subacute condition is only spotted when small flecks of pus are detected in the vaginal mucus or on the end of an insemination pipette. It is not uncommon for these flecks to appear in the vaginal mucus some 2-3 hours after AI, because the manual examination of the uterus and cervix allows small amounts of exudate to find their way out of the uterine lumen. In such cases, the cow may still be inseminated and given intra-uterine treatment the day after AI. The embryo will remain protected in the oviduct, only arriving in the treated uterus on around day 5.

When treating uterine infections during the later postpartum period (>21 days postcalving) the strategy should be based on the severity of clinical signs and ovarian activity (Figure 24).

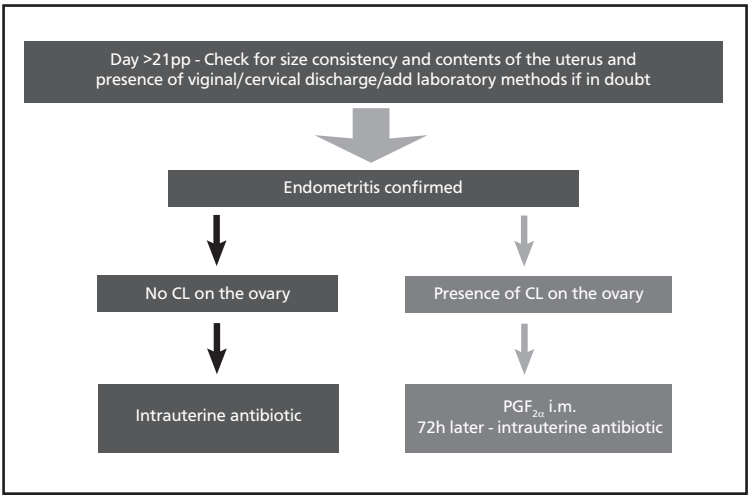

Figure 24. Proposed decision tree in the therapy of endometritis in cattle.

In cases of endometritis in which a corpus luteum is present, the treatment of choice is a combination of prostaglandin injection and intrauterine antibiotics. The induced luteolysis eliminates the immunosuppressive effect of progesterone and improves uterine tonicity. The intrauterine administration of broad spectrum antibiotics not only eliminates the bacterial contamination responsible for the inflammatory process, but also avoids some bacteria remaining in the uterine lumen and multiplying during the next luteal phase, with a consequent recrudescence of the endometritis (Lewis, 2004).

xi. Routine use of prostaglandins in treatment and prevention of uterine disorders

Prostaglandins have been used for decades as a treatment of both metritis and endometritis, as well as a form of prophylaxis when administered routinely postpartum. As is well known, exogenous $PGF_{2\alpha}$ induces luteolysis, which reduces circulating progesterone levels, eliminating

its immunosuppressive effect and permitting the uterus to rid itself of infections (Murray et al., 1990; Lewis, 1997; Heuwieser et al., 2000). The results of clinical trials of $PGF_{2\alpha}$ for the treatment of clinical endometritis in the absence of an active corpus luteum are inconsistent (Sheldon and Noakes, 1998; LeBlanc et al., 2002; Mejia Lacau-Mengido, 2005). Lewis (2004) suggests, however, that even the administration of prostaglandins in endometritis, without an active corpus luteum being present, can bring certain advantages through a direct beneficial effect of $PGF_{2\alpha}$ on the function of the uterine immune defenses.

As already mentioned, a combination of prostaglandin and intrauterine antibiotics seems to provide the best possible solution for eliminating infection and preventing a relapse during subsequent luteal phases (Lewis, 2004; Kasimanickam et al., 2005; Sheldon et al., 2006; LeBlanc, 2008).

However, there has been much controversy over the claimed value of the routine use of prostaglandins in the early postpartum period, in the absence of a functional corpus luteum. There are conflicting reports of the efficacy of exogenous prostaglandins in increasing the rate of uterine involution, causing the evacuation of bacterial and debris from the uterus, consequently improving conception rates. Prostaglandins would be more consistently effective in these instances if it was administered when there is a corpus luteum present. In most postpartum cows, this would be at approximately 17-24 days postpartum. Many practitioners believe that sequential luteolysis with exogenous prostaglandin treatment at specific times postpartum results in exposing the uterine environment to normal concentrations of progesterone for shorter intervals, thereby reducing the susceptibility of the uterus to bacterial infection. Many published studies have failed to demonstrate a clearly measurable benefit of such a treatment (Burton and Lean, 1995 (meta-analysis; Hendricks et al., 2005), while others have shown a reduction in uterine problems and improved fertility (Etherington et al., 1994; Nakao et al., 1997; Fernandes et al., 2008a,b).

xii. Pyometra

Pyometra can be regarded as a specific form of chronic endometritis, ie, one featuring a persistent corpus luteum and closed cervix. During the progesterone-dominated phase, the uterus has a reduced resistance to infection:

- The pH is lower, which creates better conditions for the common uterine pathogens
- The leucocyte activity is delayed and reduced
- The uterine secretion has no detoxifying effect

In cases of pyometra, prostaglandin $F_{2\alpha}$ release from the uterus is insufficient to cause luteolysis. Prostaglandin injections can, therefore, be used to treat this condition. The corpus luteum regresses, followed by the maturation of a new follicle. The uterine contractility increases, the cervix relaxes, and the purulent material is expelled. The change in the hormone balance (increased oestrogen/decreased progesterone) stimulates the uterine self-defence mechanisms. Treated cows should be closely monitored, as relapse is common. It is strongly recommended that these animals receive a second prostaglandin injection after 12-14 days. Insemination can begin once the endometrium is restored, which usually takes 4-8 weeks. Intrauterine antibiotic therapy can be used as adjunct therapy. In view of the destructive nature of pyometra, any intrauterine infusion should be nonirritant to prevent even more destruction of the endometrium.

xiii. Vaginitis

In heifers, vaginitis is quite a common sequel to natural mating and usually requires no treatment. In adult cows, vaginitis may be due to environmental infection and can easily lead to endometritis. It is often difficult to differentiate between these two conditions. Nonpregnant animals are best treated as for endometritis. Prevention must be based on improving hygiene. A number of specific infections are accompanied by vaginitis and/or endometritis.

c. Anoestrus

When a dairy cow is not observed in oestrus by 60 days postpartum, whether actually cycling or not, the condition is defined as postpartum anoestrus (PPA).

Some definitions will prevent misunderstanding:

- Anoestrus: The cow is not observed in oestrus either because she has not come into oestrus (not cycling) or because oestrus was not detected (cycling)
- True anoestrus: The cow does not come into oestrus because it has inactive ovaries
- Suboestrus: The cow has normal cyclic activity but is not observed in oestrus due to weak or absent oestrus behaviour or inadequate observation

Keep in mind that anoestrus is a possible symptom of cystic ovarian disease, which is a different syndrome altogether.

i. Suboestrus

Suboestrus, or failure to observe oestrus, accounts for most reported postpartum anoestrus. It includes animals showing normal oestrus behaviour, weak oestrus behaviour, or none at all. Differentiation between these is practically impossible. Action must be based, firstly, on improving oestrus detection: knowing what to look for, observing for long enough, often enough, clear identification of individuals, good fertility records, and possibly the use of milk progesterone test kits.

The control of oestrus and ovulation by the use of prostaglandins, gonadotrophin-releasing hormone, or progestagens may ameliorate some of the problems of oestrus detection by defining the period in which the farmer can expect to observe oestrus.

ii. True anoestrus

Resumption of cyclic activity after calving is influenced by nutrition, body condition, suckling, lactation, dystocia, breed, age, season, uterine pathology, and concurrent disease. In most well-managed dairy herds, fewer than 10% of cows fail to ovulate by day 40 postpartum. In beef cattle, this may be up to 60% due to the suppressive effect of suckling, nutrition, season, etc. The duration of postpartum anoestrus is not determined by the emergence of follicular waves, but rather by follicular deviation and/or the fate of the dominant follicle.

Benefiting from the use of ultrasonography and the growing knowledge of follicular dynamics in cattle, Wiltbank et al (2002) proposed the following classification of anovulatory status:

1. Anovulation with follicle growth up to the emergence stage. In this form of anoestrus, cows exhibit very small follicles that grow only to the emergence phase and proceed no further. The authors speculate that this form of anoestrus is related to a relative deficiency in FSH release
2. Anovulation with follicle growth up to deviation phase. In this form of anoestrus, follicular growth takes place and proceeds through emergence and deviation but does not continue to ovulation. It is a form of anoestrus frequently reported. It appears to occur in all cows during the prepubertal period and commonly occurs in the postpartum period in lactating dairy and suckling beef cows. The characteristic signs are small ovaries with no corpus luteum or follicles of ovulatory-size, even though they show continuing growth in a dynamic wave pattern up to the deviation phase. The underlying physiological problem is the inhibitory effect of oestradiol on GnRH/LH pulses that does not allow the growth to the final phase or oestradiol production by the postdeviation dominant follicle
3. Anovulation with follicular deviation, growth, and the establishment of a dominant follicle, which fails to ovulate but becomes a persistent follicular structure. Persistent follicular structures may become follicular cysts or they may luteinise (luteal cysts).

iii. Treatment of anoestrus in cattle

Improved knowledge of the physiological background of various types of anovulatory conditions in cattle allows the use of more adequate and appropriate treatment approaches that address the underlying endocrine disorder.

Improvement in the energy status in dairy cows, by providing optimal nutrition during the transition period and early lactation, can reduce the anoestrus period associated with the lack of LH pulses. In beef cows, an improvement in energy status and/or reduction of the frequency with which the calves are allowed to suck can increase LH pulses and reduce the time to first ovulation. Hormonal treatment can be used to

stimulate anovulatory cows, especially if combined with increased energy supplementation in dairy cows and energy supplementation and/or reduced suckling frequency in beef cows.

In the anovulatory condition, with follicular growth progressing only to the emergence stage (type 1), the possibilities for pharmacological treatment are rather limited. It is often found in cows at pasture, especially in *bos indicus* breeds reared in tropical zones. Such cows usually have poor-quality feed and are exposed to very demanding climatic conditions. Field experience indicates that treatments with GnRH analogues usually have no effect, but FSH treatment can increase follicle growth. FSH-based products used routinely for the stimulation of multiple ovulations in oocyte/embryo donors in cattle are relatively expensive. Careful dosing with PMSG/eCG could be used to stimulate further follicular growth in cows suffering from this type of anoestrus. It must be stressed that without radical improvement in the nutrition of the treated cows, the prospects for success are rather low.

The type of anovulation with follicular growth up to deviation phase (type 2) is especially common in high-yielding dairy cows and in suckled beef cows of relatively good body condition. Regardless of any pharmacological intervention, improvement in energy status by the provision of optimal nutrition during the transition period, as well as during early lactation, should be considered as the primary means of reducing the period of anovulation caused by the lack of LH pulses. In addition, disease conditions should be minimised. Anovulatory lactating dairy cows can be effectively treated with a regular Ovsynch protocol, as many such animals have follicles of a sufficient size and ovulatory capacity to respond to a pharmacologically stimulated LH surge. Beef cows, on the other hand, are preferably treated with progestagens combined with a temporary weaning of their calves. Vasconcelos et al (2009) described a trial comparing the results of temporary weaning and the use of a progesterone-releasing device in anoestrus crossbred bos indicus/bos taurus cows. Their findings indicated that temporary weaning alone increased the incidence of oestrous behaviour, whereas the use of progesterone-releasing devices alone benefited conception. Combining the two improved pregnancy rate, with direct benefits on behavioural oestrus and conception.

iv. Use of progestagens in anoestrus cows

Treatment with progestagens/progesterone is now considered to be the preferred option for the induction of cyclicity and management of oestrus in anoestrus cattle, especially beef breeds (for reviews see: Yavas et al., 200a,b; Peter et al., 2009).

The use of progesterone or progestagens to treat anoestrus is beneficial because it initiates the oestrous cycle with ovulation and facilitates the subsequent luteal phase of a normal length. The best results have so far been obtained with the use of progesterone or progestagens combined with an injection of oestradiol at the start of treatment.

Injection of PMSG/eCG may be used following a period of progesterone treatment to induce oestrus and ovulation in anovulatory anoestrus cows.

Using daily transrectal ultrasonography, Rhodes et al (2000) demonstrated that anoestrus cows treated with small doses of progesterone did not develop persistent ovarian follicles such as those seen in cows treated after oestrous cycles had begun. Therefore, it should be possible to obtain satisfactory results in this group of cows with progesterone or progestagen treatment alone.

Gonadotrophin-releasing hormone analogues may also be used at the start of progesterone treatment to cause the regression of the dominant follicle present and synchronise the emergence of a new cohort of follicles. This protocol has the additional effect of inducing ovulation and the formation of a corpus luteum in a majority of cows, resulting in elevated concentrations of progesterone in plasma, compared with cows not treated with GnRH (Xu et al., 2000a). To ensure the absence of luteal tissue following the removal of a progesterone-releasing device, prostaglandins are generally included in such protocols. Oestradiol has been used in many countries to stimulate ovulation and the expression of oestrus following progesterone treatment. In suckled cows in deep anoestrus, temporary weaning (separating calf and dam for 48 hours) at the time of progesterone/progestagen removal provides additional ovarian stimulation.

v. GnRH analogues in combination with prostaglandins

The capacity of GnRH analogues to induce ovulation during the postpartum anovulatory anoestrus period allows for the use of programmes such as Ovsynch to treat anoestrus in cattle, provided that follicles responsive to LH stimulation are present on the ovaries. Use of this protocol, in conjunction with separating cow and calf, was compared with the use of norgestomet implants and injection of oestradiol valerate in anoestrus beef cows and in cows that had resumed oestrous cycles. Pregnancy rates were similar in previously anoestrus cows treated with either protocol and were equivalent to those obtained in cows that had resumed oestrous cycles before treatment with the Ovsynch protocol (Geary et al., 1998).

In anoestrus grazing dairy cows, use of an Ovsynch protocol resulted in similar conception rates to first insemination and median interval to conception compared with cows treated with CIDR devices and oestradiol benzoate and inseminated at the observed oestrus (McDougall et al., 2001). However, the results suggest that the Ovsynch protocol may be of benefit in treating anoestrus cows in situations in which oestrus detection is a problem, although pregnancy rates are lower than those obtained in cows that have resumed oestrous cycles (Cartmill et al., 2001).
Hormone treatment can effectively reduce the interval to first ovulation, and synchronise oestrus, across a variety of physiological states. However, response to treatment is not uniform either between herds or within herds and appears to be dependent on those factors influencing the prevalence of anoestrus, such as age, body condition, and interval after calving. In postpartum anoestrus cows with low body condition, both oestrus response rates and pregnancy rates are usually disappointing, regardless of the method used. In view of the fact that uterine infections were shown to be associated with the delayed resumption of ovarian activity (Opsomer et al., 2000; Sheldon et al., 2004a,b), anoestrus cows should always be checked for signs of endometritis.

d. Persistent corpus luteum/pyometra

Persistent corpora lutea are generally accompanied by a uterine disorder preventing the release of sufficient prostaglandin for luteolysis. As already mentioned, abnormal luteal activity, often associated with uterine infections, is commonly found in high-yielding dairy herds (Shrestha et al., 2004), especially during the warmer months of the year (Kornmatitsuk et

al., 2008). Treatment consists primarily of the administration of exogenous prostaglandin to cause regression of the persistent corpus luteum. This can be combined with GnRH administration to cause ovulation of the dominant follicle from a new follicular wave, followed by insemination.

e. Cystic ovarian disease

Traditionally, cysts have been defined as anovulatory follicular structures (diameter >25 mm) that persist for 10 or more days in the absence of a functional corpus luteum and accompanied by abnormal oestrus behaviour (irregular oestrus intervals, nymphomania, or anoestrus). However, recent data using ultrasonography indicate that, typically, follicles ovulate when 17 mm in diameter, so follicles that persist at that diameter or greater may be considered to be 'cystic' (Vanholder et al., 2006a), which is the reason for the term cystic ovarian follicles (COF) to be in common use, rather than ovarian cysts.

Ovarian follicular cysts are the most common reproductive disorder in dairy cows, developed by approximately 6%-19% of this class of animal (Garverick 1997). In the early postpartum period, the incidence is probably much higher, because about 60% of the cows that develop 'ovarian cysts' before the first ovulation re-establish ovarian cycles spontaneously (Ijaz et al., 1987). The economic impact of cystic ovarian disease is a function of their impact on days open and other associated costs. Each occurrence of ovarian follicular cysts has been estimated to add between 22 and 64 extra days open and costs US$137 in reduced milk production and veterinary expenses (Silvia et al., 2002).

Though no single cause can be blamed for cystic ovarian disease, high yield, season, stress, and negative energy balance are all considered to be predisposing factors. Postpartum problems such as placental retention, milk fever, and endometritis have been associated with an increased risk of cystic ovarian disease.

There is evidence to indicate the existence of a genetic background to cystic ovarian disease. Moreover, beside the existence of a genetic predisposition for COF, a genetic correlation between cysts and milk production traits has been established, indicating that continuing to select cows for production parameters will increase the incidence of COF. Nutritional factors include β-carotene deficiency and phyto-oestrogens.

Nutritional deficiencies (negative energy balance, NEB) are thought to be one of the most important factors contributing to the formation of cystic follicles during the early postpartum period. Moreover, there seems to be a link between COF incidence and the magnitude and/or duration of the NEB (Vanholder et al., 2006a). Although elevated serum ketone concentrations increased the risk for the formation of cystic follicles in postpartum dairy cows, they were not found to exert any negative effects on bovine follicle cells in vitro (Vanholder et al., 2006b). Therefore, ketone concentrations in the postpartum dairy cow seem to be an indicator of the severity of the NEB, rather than a mediator of the negative effects of the NEB on reproduction at the ovarian level.

COF formation may result from functional disorders at both ovary/follicle and hypothalamus/pituitary levels. The most widely accepted hypothesis to explain the formation of a cystic follicle is the altered release of LH from the hypothalamus-pituitary: the preovulatory LH surge is either absent, insufficient in magnitude, or occurs at the wrong time during the maturation of the dominant follicle.

It is believed that an altered feedback mechanism of oestrogens on the hypothalamus-pituitary can result in aberrant GnRH/LH release, ovulation failure, and cyst formation. A GnRH/LH surge occurring prematurely during follicle growth, ie, when no follicle capable of ovulation is present on the ovary, can render the hypothalamus unresponsive to the feedback effect of oestradiol, which results in the formation of a cystic follicle. An altered feedback mechanism and GnRH/LH release may be attributed to factors interfering at the hypothalamic-pituitary level.

Based on this finding, Silvia et al (2002) proposed a new model for the ethology of follicular cysts in cattle. Ovarian follicular cysts develop due to a lack of the preovulatory LH surge that should take place in response to the preovulatory rise in oestradiol. The primary cause lies in the hypothalamus, which fails to release a surge of GnRH in response to an oestradiol stimulus. Hypothalamic insensitivity to oestradiol may be induced by intermediate (subluteal) concentrations of circulating progesterone. If progesterone is administered at intermediate levels (0.5-2 ng/mL), it will block the LH surge, prevent ovulation, and result in the formation of a follicle with a greater

diameter and persistency than those of normal dominant follicles (Hatler et al., 2003). This concept has been proved with the discovery that treatment with low doses of progesterone, such as delivered by many progesterone-releasing devices used for oestrus synchronisation, can lead to the formation of a persistent dominant follicle.

Primary dysfunction at the level of the follicle may disrupt the hypothalamic-pituitary-ovarian axis, causing the formation of COF. Alterations in LH-receptor expression and content may cause anovulation of the follicle. Besides these, alterations in steroidogenesis by the dominant follicle may also be involved in cystic degeneration.

Macroscopically, cysts can appear as either follicular or luteal, but these are considered to be different forms of the same disorder. Luteal cysts are believed to develop in the presence of LH concentrations that are insufficient to induce ovulation but capable of causing luteinisation of the follicular walls. Luteal cysts are associated with anoestrus, but it is not possible to differentiate between follicular and luteal cysts on the basis of behaviour alone. Luteal cysts have a thicker wall. A high progesterone level in milk or plasma is indicative of a luteal cyst. Care should be taken not to confuse luteal cysts with hollow corpora lutea, which are not pathological at all.

The clinical signs that accompany ovarian cysts can vary considerably. Anoestrus is most common, especially during the postpartum period. Irregular oestrus intervals, nymphomania, relaxation of the broad pelvic ligaments, and development of masculine physical traits are other signs of the presence of oestrogenically active follicular cysts, especially later in lactation. Luteal cysts are almost invariably associated with anoestrus.

i. Treatment of cystic ovarian follicles in cattle

In spite of a relatively high self-recovery rate, the development of ovarian cysts, untreated even when diagnosed, can extend the calving to conception interval by 64 days, leading to economic losses of $55 to $160 per lactation (Bartolome et al., 2005d).

The administration of GnRH is the treatment of choice. It acts by stimulating the pituitary gland to release LH and FSH. The induced LH surge leads to

luteinisation of the cystic follicle. Depending on the type of cyst, and possibly the dose of GnRH, some cystic follicles may be induced to ovulate. Moreover, the GnRH-induced increase in FSH concentration causes recruitment of a follicular wave that usually restores normal cyclicity. Following treatment, 60%-80% of cows will come into oestrus between 18 and 23 days after injection.

It is essential to recognise that it is the stimulation of the normal follicular turnover and the induction of ovulation from a new follicular wave that produces the desired result, not the physical elimination of the cystic follicle. For this reason, manual rupture of the cyst often fails to restore cyclicity. Besides, the transrectal squeezing of cysts carries a considerable risk of damaging the delicate ovarian tissues and inducing adhesions.

Since both follicular and luteal cysts respond similarly to this kind of treatment, differentiation is unnecessary, and authors generally agree that GnRH administration remains the best initial therapy for the majority of cows with cystic ovarian disease. Human chorionic gonadotrophin (hCG) (3000 IU) is another possibility. hCG is a gonadotrophin with strong LH activity. It has a half-life in cattle of nearly 2 days, and thus exerts a long-acting luteotrophic effect directly on the cyst; as a result, it is frequently reserved for recurrent cases.

Various studies have indicated that prior exposure of the effector cells of the ovarian follicle to sufficient levels of progesterone is essential for their sensitisation to further gonadotrophin stimulation. Therefore, the use of progesterone or progestagens is a logical treatment for follicular cysts and has led to very encouraging results, either alone or in combination with GnRH (Calder et al., 1999; Todoroki et al., 2001; Ambrose et al., 2004).

In order to reduce the number of days open, and to reduce the incidence of cystic ovarian disease, a GnRH/prostaglandin-based system was proposed by White et al (1996) and further tested by Lopez-Gatius et al (2002). This regime can be used between 30 and 90 days postcalving and involves the administration of GnRH when the cyst is detected, followed 9 days later by $PGF_{2\alpha}$.

Once luteinisation of the cyst has been initiated by the GnRH, luteal tissue is developed within 9 days of treatment. The resulting corpus luteum should then respond to the subsequent prostaglandin treatment, and a new oestrous cycle begins.

Alternatively, a classic Ovsynch protocol can be used for the treatment of ovarian cysts in lactating dairy cows, as demonstrated by Bartolome et al (2000), who reported that synchronisation of ovulation and timed insemination with an Ovsynch protocol resulted in pregnancy rates similar to those of oestrus synchronisation and insemination at an induced oestrus within 7 days. Further studies by Bartolome et al (2005), De Vries et al (2006), and De Rensis et al (2008) confirmed the suitability of the Ovsynch-type protocols for the treatment of cystic ovarian follicles in dairy cows.

Cows that have not come into oestrus within 23 days of GnRH or hCG treatment must be checked and treated if necessary. The same applies to animals that show signs of oestrus within 14 days, since this indicates that they failed to respond to the first injection.

Prevention of cystic ovarian disease can be approached by identifying and eliminating the contributory causes of the disease (periparturient stress, nutritional inadequacies, and uterine infections). Furthermore, the administration of GnRH on day 14 postpartum has been shown to reduce the incidence of ovarian cysts (Britt et al., 1977). Earlier administration is ineffective because the pituitary gland is not capable of releasing LH in response to GnRH before 12-14 days postpartum.

Prostaglandin therapy is also used to treat cows with luteal-type cysts. However, the response and cure rate are dependent on the presence of luteal tissue and the accuracy of diagnosis that the cyst is indeed luteal. Because palpation has been reported as being inaccurate as a means of differentiating between luteal and follicular cysts, diagnosis is better based on plasma or milk progesterone concentrations or on the use of ultrasonography.

2.6.3 Embryonic mortality

The period from conception to day 45 of pregnancy is known as the embryonic stage. It is followed by the foetal stage that lasts until parturition. Embryonic mortality is regarded as one of the major causes of reproductive failure in cattle, resulting in reduced pregnancy rates, slower genetic improvement, and substantial financial losses to dairy and beef production.

Recent estimates indicated that the average value of a pregnancy was US $278 in high-yielding herds in the USA, whereas the cost of the loss of a pregnancy was substantially greater (De Vries, 2006).

It is generally accepted that the fertilisation rate is on the order of 90% and that embryonic loss accounts for 29%-39% of losses after fertilisation, most of them between days 8 and 16 after fertilisation (Roche et al., 1981; Dunne et al., 2000; Diskin and Morris 2008).

With the advent of ultrasonography, accurate pregnancy diagnosis has been possible as early as 25 days after AI in cattle, thereby facilitating the study of embryonic mortality after the period of maternal recognition of pregnancy. The frequency of this late embryonic loss is estimated at approximately 7%. While the extent of late embryonic mortality is much lower than early mortality, it is nevertheless an important cause of serious economic loss. These losses can be especially severe in seasonally bred herds when cows that lose embryos towards the end of the breeding season will not be rebred, but culled because of reproductive failure.

Embryonic mortality refers to the losses that occur in the period between fertilisation and the completion of the stage of differentiation at approximately day 42.

Early embryonic mortality, ie, before day 15, does not affect the length of the cycle. When the embryo dies after this time, the cow returns to oestrus when the corpus luteum has regressed, and the cycle is thus lengthened. Embryonic mortality in the late embryonic phase (after day 35-45) may be diagnosable. Although in some cases the embryo and the membranes are aborted, the remnants will frequently be resorbed. The corpus luteum may persist for a long time, thus delaying the return to oestrus. Usually the only obvious sign is a return to oestrus behaviour as late as 35-50 days after insemination.

Some of the factors influencing embryonic mortality are:
- Inherent fertility of both the sire and the cow
- Embryonic chromosomal abnormalities
- Age of the cow
- Uterine abnormalities (eg, endometritis)
- Damage to the embryo by rectal palpation (eg, at pregnancy diagnosis)
- Diseases inducing fever
- Heat stress
- Delayed insemination (reduced fertility of the ovum)
- Insufficient luteal function

a. Mechanisms of pregnancy recognition in cattle

During the normal oestrous cycle, an efficient mechanism involving oxytocin and prostaglandin $F_{2\alpha}$ ensures prompt luteolysis of the corpus luteum and initiation of a new oestrous cycle. Oxytocin produced by the corpus luteum binds to the specific oxytocin receptors in the endometrium, thus stimulating the release of $PGF_{2\alpha}$ from endometrial cells (Silvia et al., 1991; Wathes et al., 1995; Mann et al., 2001). Prostaglandin is released into the bloodstream, reaches the ovary, and causes regression of the corpus luteum. Increasing levels of oestrogens, produced by growing ovarian follicles, stimulate the expression of oxytocin receptors.

In order to sustain the corpus luteum and maintain pregnancy, an effective mechanism for pregnancy recognition has to take place. In other words, the developing embryo has to produce a specific signal to prevent luteolysis, which would otherwise be triggered towards the end of the oestrous cycle. It has been demonstrated that early bovine and ovine embryos produce and release a specific pregnancy protein – interferon-τ (IFN-τ) (Farin et al., 1989; Mann et al., 1999). The mechanism of luteolysis inhibition by IFN-τ is now well established and involves inhibition of oxytocin receptors on the uterine luminal epithelium (Robinson et al., 1999) and induction of a prostaglandin synthesis inhibitor (Thatcher et al., 1995). In cattle, mRNA for IFN-τ is first detected in trophoectoderm, the principal site of its production, at approximately 12 days and reaches its maximum levels between days 15 and 16 (Farin et al., 1990). Interferon-τ can be detected in significant amounts, first in the uterine flushings at 14-16 days, coinciding with the start of the embryo elongation (Mann et al., 1998).

If retardation of embryonic development occurs, or if the growth of the embryo and the progress of the maternal oestrous cycle are not synchronous (eg, due to delayed ovulation or late insemination), insufficient or delayed IFN-τ production results, inhibition of luteolysis fails, and the embryo is lost. The main reason for this impaired secretion of IFN-τ by the embryo is supposed to be an aging process in the oocyte, associated with a prolonged period of follicular dominance. It has been argued that due to this extended period of follicular dominance and delayed ovulation, precocious maturation changes take place in the oocyte, which in turn reduce fertilisation and developmental capacity. Poor embryonic development is associated with low interferon-τ production, failed inhibition of luteolysis, and embryo loss (Mann et al., 1996; Mann et al., 1998). As mentioned in Section 2.3.3, a close correlation exists between the early postinsemination rise in progesterone concentrations, embryonic development, and its production of IFN-τ (Kerbler et al., 1997; Mann et al., 1999; 2001).

Figure 25 is a schematic representation of the interactions between the embryo and the cow during the early embryonic phase and pregnancy recognition.

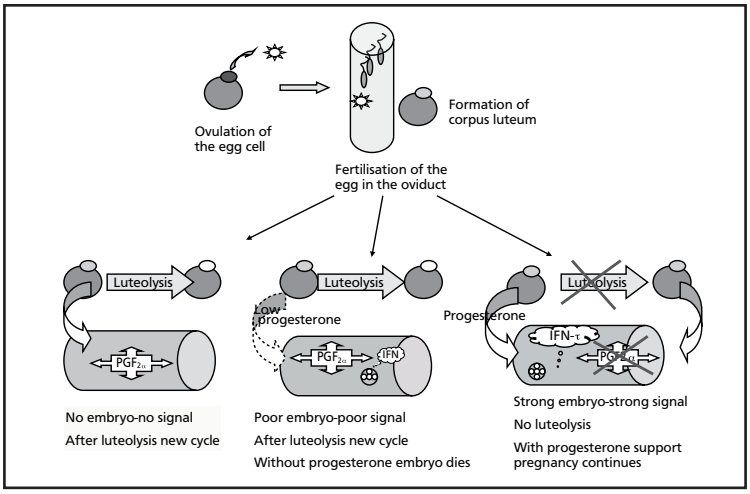

Figure 25. Maternal-embryonic interactions preceding pregnancy recognition in cattle.

b. Pharmacological measures to prevent early embryonic mortality

At present, the most popular strategies and pharmacological treatments aimed at the improvement of pregnancy rates in cattle can be classified into two groups:

1. Prevention of delayed ovulation
2. Support of early luteal function and prevention of precocious luteolysis

2.6.4 Repeat breeder cow

The repeat breeder cow is defined as a normally cyclic cow, with no clinical abnormalities, which has failed to conceive after at least three successive inseminations. In herds where conception rates are commonly 50%-55%, about 9%-12% of the cows would be expected to be repeat breeders. As conception rate decreases, the number of cows requiring additional services increases. Repeat breeding can quickly become a problem when conception rates are low. See Table 15.

Expected repeat breeders at various conception rates		
Conception rate	Percent of cows conceiving in 3 services	Percent repeat breeders
70	97	3
60	94	2
50	88	12
40	78	22
30	66	34
20	49	51

Table 15. Expected repeat breeders at various conception rates.

Repeat breeding is clearly a function of conception. Most repeat breeders are not sterile but suffer from lowered fertility, for some reason. In general, if more than 15% of the cows in a herd require more than three services, further investigation should be initiated. Unfortunately, diagnosing the cause can be very difficult. The cause may be a herd problem or it may be a collection of different individual cow problems. Herd problems are probably more common and may include things such as:

- Improper timing of insemination
- Cows being inseminated that are not in oestrus
- Inaccurate oestrus detection
- Inadequate oestrus detection
- Inadequate semen quality
- Improper insemination techniques
- Improper semen handling or storage
- Infertile bull

Individual cow problems may include things such as:

- Metritis and/or endometritis
- Cervicitis and/or vaginitis
- Delayed ovulation
- Anovulation
- Obstructed oviducts
- Abnormal ova
- Anatomical defects
- Early embryonic death

To effectively diagnose the cause of repeat breeding in a herd of cows requires a complete analysis of the entire reproduction program by the producer, inseminator, and veterinarian working together. Frequently it is not a single factor but several seemingly minor problems that collectively result in a herd problem.

The treatment for a repeat breeding problem depends on the underlying cause(s). A single treatment that successfully resolves a repeat breeding problem is yet to be identified and probably will never be found, due to the multifactorial nature of the problem. Prevention is the best solution. Prevention comes in the form of a very well organised reproduction program with records that are up to date and evaluated on a regular basis by the management team.

2.6.5 Abortion

Abortion in the cow is defined as foetal death and expulsion between day 45 and day 265 of pregnancy. It is a significant cause of reproductive wastage and is of economic importance, especially when there is large-scale abortion in a herd. Moreover, some of the infectious causes of abortion in cattle represent an important zoonotic risk to humans.

An annual abortion rate up to 5% is considered to be normal. An abortion rate in excess of 10% is considered to be an abortion storm. The diagnosis of the cause of abortion is difficult; unfortunately, only 20%-30% of cases are definitively diagnosed. Often, the initiating cause and diagnosis are separated by significant amounts of time, due to delays in expulsion of the foetus after it has died. This leads to poor sample quality. Autolysis develops rapidly before expulsion and can severely affect the quality of samples taken for diagnosis. Serology is not very revealing because of the delay in time of the initiating cause and diagnosis.

A whole range of infectious and noninfectious causes of abortion have been reported.

Noninfectious causes of abortion include physical causes, such as trauma, erroneous insemination of pregnant cows, and hyperthermia (also associated with pyrexia). Nutritional factors rarely cause abortion in cattle. An increase in abortion rate can be observed in herds suffering from severe vitamin A deficiency. Several plant toxins, and mycotoxin, can cause abortion, as well as inorganic poisons such as nitrates/nitrites, lead, or cadmium. Iatrogenic abortions are not common, but abortions induced by the administration of prostaglandins and glucocorticoids have been reported, especially in herds with poor animal identification and inadequate treatment records, and when cows are misdiagnosed as being nonpregnant.

Infectious causes of abortion in cattle include a wide range of bacterial, viral, and protozoan microorganisms (Anderson et al., 2007).

It is important to remember that some genetic and developmental abnormalities can cause foetal death and abortion in cattle. These include so-called complex vertebral malformation (CVM), dyschondroplasia (bulldog calves), and various chromosomal abnormalities.

The summary in Table 16, listing various abortion causes in cattle, is not comprehensive but a list of more common causes.

Noninfectious causes	Infectious causes
Genetic aberrations: Chromosomal abnormalities Phytoteratogens: lupin senecio spp	Viruses: Bovine herpes virus 1 (BHV1) Bovine herpes virus 4 (BHV4) Bovine virus diarrhoea virus (BVDV) Parainfluenza 3 virus (PI-3) Parvo virus
Nutritional: Toxic plants Nitrate poisoning Phyto-oestrogens Iodine deficiency Vit. A deficiency Selenium deficiency Lead poisoning Cadmium poisoning	Bacteria: *Brucella abortus* *Campylobacter foetus* *Chlamydia psittaci* *Leptospira hardjo/pomona* *Listeria monocytogenes* *Staphylococci* *Streptococci* *Salmonella dublin/typhimurium* *Pasteurella spp* *E. coli* etc
Stress: Handling High ambient temperature Trauma Surgery Drying off Anxiety Vaccinations	Protozoa: *Toxoplasma gondii* *Sarcocystis* *Neospora caninum* *Trichomonas foetus*
Miscellaneous: Multiple pregnancy Insemination Corticosteroid therapy Prostaglandin therapy Allergy Dehydration	Fungi: *Aspergillus* spp. *Mycoplasma* spp.

Table 16. Diagnostic investigation of abortion in cattle.

For a detailed investigation of possible causes of abortion with the best chance of identifying the causative agent (especially infectious), it is essential to collect the correct samples and the appropriate additional data. Ideally, the entire foetus and placenta should be submitted to the laboratory for anatomical, histopathological, and microbiological evaluation, accompanied by a serum sample from the dam for serological testing. Farmers and veterinary practitioners should make every effort to include the placenta, as it is often in placental tissues that the evidence of certain infectious pathogens is found.

Formalin-fixed tissues for histopathological examination include foetal brain, lung, heart, liver, kidney, adrenals, spleen, thymus, lymph node, skeletal muscle, abomasum, small intestine, eyelid, and placenta.

Serological evaluation of paired serum samples from an aborting cow may help determine if there had been exposure to an agent, but cannot usually differentiate between vaccination and natural exposure (unless marker vaccines are used) or between recent and previous exposure to infection. Maternal serology is most useful when serum from unvaccinated animals is examined, when several animals from the herd are tested, and when each animal's history is provided.

Additional information should be provided, including:
- The aborting cow: age, stage of production, duration of pregnancy, any general clinical signs preceding the abortion, housing, maintenance details (housed or at pasture), vaccination status, any healthcare measures performed within 1-2 weeks of abortion (especially administration of pharmaceutical preparations)
- The herd: average abortion level in the herd, recent increase in abortions, number of abortions to date, herd vaccination schedule, any recent herd-wide medication, feeding regime and feed changes, recent introductions into the herd

It must be remembered that there are zoonotic considerations associated with bovine abortion pathogens such as those causing brucellosis, leptospirosis, listeriosis, salmonellosis, etc. Appropriate precautions should be employed while sampling and shipping specimens.

Table 17 lists the main symptoms of the most important infectious causes of abortion, with suggestions for the most relevant samples.

Table 17. Symptoms of the main infections causing abortion in cattle.

Infectious Factor Common Names	Abortion Rate	Abortion Timing	Recurrence of Abortion	Foetal Lesions	Samples
Bacterial					
Brucella abortus Brucellosis Bang's disease Zoonosis	Up to 80% of unvaccinated animals infected in 1st or 2nd trimester	6-9 months Abortion or stillbirth 2 wk to 5 mo after infection	Majority abort only once	Placenta: retained, cotyledons necrotic, red-yellow; area between thickened Calf: normal or autolytic with bronchopneumonia	Placenta, foetus, or uterine discharge Diagnosis: maternal serology, IFAT for Abs in placenta, bacteria isolation
Campylobacter foetus venerealis Vibriosis	>10%	5-8 months	Uncommon, convalescent cows resistant to infection	Placenta: mild placentitis, hemorrhagic cotyledons and an edematous intercotyledonary area. Foetus: fresh or autolysed; mild fibrinous pleuritis, peritonitis, bronchopneumonia	Placenta, foetal abomasal contents, vaginal flushing Diagnosis: microscopic detection, isolation
C foetus foetus *C jejuni*	Sporadic	4-9 months	Uncommon, convalescent cows resistant to infection	See above	See above
Leptospira interrogans serovars grippotyphosa pomona hardjo canicola icterohaemorrhagiae Zoonosis	5%-40%	Last trimester Abortion 2-5 weeks after infection	Immunity to the serotype causing abortion but sensitive to other types	Placenta: diffuse placentitis with avascular, light tan cotyledons and edematous, yellowish intercotyledonary areas Foetus: autolysed	Placenta, foetus Diagnosis: IFAT for Abs or PCR testing for leptospira
Arcanobacterium (Actinomyces) pyogenes	Sporadic	Any stage	Not known	Placenta: endometritis and diffuse placentitis, reddish brown to brown colour. Foetus: autolysed, fibrinous pericarditis, pleuritis, or peritonitis	Placenta, foetus Identification in bacterial culture from placenta or abomasal contents
Listeria monocytogenes Zoonosis	Usually sporadic but can reach 50%	Last trimester	May recur	Dam: fever, inappetance Placenta: retained Foetus: autolysed fibrinous polyserositis and white necrotic foci in the liver and/or cotyledons	Placenta, foetus Identification in bacterial culture from placenta or abomasal contents

Infectious Factor Common Names	Abortion Rate	Abortion Timing	Recurrence of Abortion	Foetal Lesions	Samples
Fungal					
Aspergillus sp (60%-80%) *Mucor* sp, *Absidia*, or *Rhizopus* sp	Usually sporadic but can reach 5%-10%	4 months to term most common in winter	May recur	Placenta: severe, necrotising placentitis cotyledons enlarged, necrotic, intercotyledonary area is thickened and leathery Foetus: autolysed~30% have gray ringworm-like skin lesions principally involving the head and shoulders	Foetus, placenta Diagnosis: isolation from the stomach contents, placenta, and skin lesions
Protozoan					
Tritrichomonas (Trichomonas) foetus Trichomoniasis	Sporadic	First half of gestation	Animal gains immunity but probably not life-long	Placenta: retained, mild placentitis with hemorrhagic cotyledons and thickened intercotyledonary areas covered with flocculent exudates Foetus: no specific lesions	Placenta, foetus, vaginal/uterine discharge Diagnosis: detection in abomasal contents, placental fluids, and uterine discharges
Neospora caninum Neosporosis	High in first gestation and when infection enters the naïve herd Up to 30% first outbreak Enzootic: 5%-10%	Any stage, but most often 5-6 months	Decreases with parity but always possible	Placenta, foetus: no specific gross lesions, autolysed Microscopic: focal encephalitis with necrosis and nonsuppurative inflammation, hepatitis	Placenta, foetus (brain, heart, liver, body fluids), serum samples from the dam Diagnosis: detection of antigen in brain histology samples Immunochemistry in tissue samples Abs - PCR, ELISA
Viral					
Bovine viral diarrhoea virus BVD-MD	Usually low	Complex pathology Abortion usually up to 4 months	Uncommon, immunity develops	Placenta: retained, no specific lesions Foetus: no specific lesions, autolysed, mummified	Placenta, foetus (preferred -spleen), dam and herdmates serum Diagnosis: isolation, immunologic staining, PCR, or detection of precolostral antibodies in aborted calves

Bovine herpes virus type I (BHV I) Infectious bovine rhinotracheitis virus (IBRV) IBR IBR-IPV	5%-60% in nonvaccinated herds	Possibly any stage but most common from 4 months to term	Uncommon, immunity develops	In the majority of cases there are no gross lesions in the placenta or foetus Placenta: necrotising vasculitis Foetus: autolysed, foci of necrosis in the liver	Placenta, foetus, serum samples from the dam Diagnosis: Immunochemistry in samples from kidney and adrenal glands, blood serology, PCR
Blue tongue virus Blue tongue	Usually low	Variable	Unlikely	No specific Foetus: autolysed	Placenta, foetus, serum samples from the dam Diagnosis: virus isolation
Epizootic bovine abortion Foothill abortion etiologic agent has not been definitively determined, vector – tick ornithodoros coriaceus	Can reach 75% Limited mainly to California in the US	Usually in the last trimester	Unlikely	Placenta: No specific Foetus: hepatomegaly, splenomegaly, and generalised lymphomegaly. Microscopically - marked lymphoid hyperplasia in the spleen and lymph nodes and granulomatous inflammation in most organs	Anamnesis Diagnosis: elevated foetal Ig-G
Factors not typical for cattle or rarely occurring					
Chlamydophila abortus (Chlamydia psittaci serotype 1) enzootic abortion of ewes Zoonosis	Sporadic	Near the end of the last trimester	Unlikely	Placenta: placentitis, thickening and yellow-brown exudate adhered to the cotyledons and intercotyledonary areas. Foetus: fresh, minimal autolysis, pneumonia, hepatitis	Placenta, foetus Diagnosis: isolation from the placenta, lungs, and/or abomasal contents
Ureaplasma diversum	Usually sporadic, but outbreaks possible	Third trimester	Possible	Placenta: retained, intercotyledonary areas thickened, nonsuppurative placentitis Foetus: no gross lesions, pneumonia	Placenta, foetus Diagnosis: isolation from the placenta, lungs, and/or abomasal contents
Salmonella spp	Usually sporadic but can take form of an abortion storm	Any stage	Possible	Cows: clinically ill Placenta and foetus: autolysed and emphysematous	Placenta, foetus Diagnosis: isolation from the abomasal contents and other tissues
Other infectious factors that potentially can cause abortion in cattle: Parainfluenza 3 virus (PI3V), *Mycoplasma* spp, *Histophilus somni (Haemophilus somnus), Staphylococcus* spp, *Streptococcus* spp, *Pasteurella* spp, *E. coli*, *Toxoplasma gondii*					

a. Neosporosis

Neospora caninum is a protozoan parasite, closely related to *Toxoplasma gondii*, that has emerged as a major cause of reproductive failure in cattle worldwide (Dubey, 2003; Hall et al., 2005; Dubey et al., 2007). The dog and the coyote have been identified as definitive hosts for *Neospora caninum* (Dijkstra et al., 2001; Gondim et al., 2004), while a clinical form of neosporosis has been described in cattle, goats, sheep, deer, and horses (Dubey, 2003).

Cattle appear to be the most important intermediate host for the parasite. The presence of neospora-specific antibodies has been demonstrated in numerous species, but the consequences of seropositivity remain unclear in many of them: sheep (Dubey et al., 1990), goats (Dubey et al., 1992), buffalo (Fuji et al., 2001), foxes (Buxton et al., 1997), coyotes (Lindsay et al., 1996), raccoons (Lindsay et al., 2001), dingoes (Barber et al., 1997), cervids (Tieman et al., 2005), llamas and alpacas (Wolf et al., 2005), and European bison (Cabay et al., 2005). In a recent publication by Sedlak and Bartova (2006), antibodies to *N. caninum* were found in 31 of 556 zoo animals (5.6%), representing 18 of 114 species tested: Eurasian wolf (Canis lupus lupus), maned wolf, fennec, cheetah, jaguar, Eurasian lynx, Indian lion, fisher, blackbuck, European bison, lechwe, African buffalo, eland, sitatunga, Thorold's deer, eastern elk, Vietnam sika deer, and Père David's deer.

The consequence of infection in pregnant cattle will depend on several factors, including the gestational age of the foetus at the time of infection and the immune status of the dam. The stage of pregnancy at which infection/parasitaemia occurs is an important determinant of the severity of disease. Neospora infection in the first trimester of pregnancy may have more severe consequences for the foetus than infection occurring in the final trimester (Innes, 2007). Clinical consequences of infection during pregnancy may include abortion of the foetus, birth of a weak calf, sometimes showing neurological signs, or birth of a clinically healthy but persistently infected calf (Innes et al., 2005).

One of the most interesting, but also still poorly understood, aspects of abortion caused by neospora in cattle is the immunological relationship between the host, its developing foetus, and the parasite. The immune

system of a pregnant dam, regulated through cytokines, plays a major role during gestation, which may be viewed as a complex process in which the mother has to support a semi-allograft. To facilitate the pregnancy, the cytokine environment in the placenta favours the regulatory Th2-type cytokines, whose role is to counteract the pro-inflammatory Th1-type immune responses. Protective immunity to *N. caninum*, similar to many other intracellular parasites, involves Th1-type immune responses, which may become a problem for a cow trying to control the infection during pregnancy (Innes, 2007). An excessive Th1 response can induce loss of the pregnancy and may, therefore, form one of the elements in the abortion process associated with neosporosis.

Abortion occurs in mid-gestation, usually between the fourth and sixth month, with no clinical signs of disease in the dam. The aborted foetuses are usually autolysed with no gross lesions, and placentas are not retained. The brain, heart, liver, placenta, and body fluids or serum are the best specimens for diagnosis, and diagnostic rates are higher if multiple tissues are examined. Although lesions of neosporosis are found in several organs, foetal brain is the most consistently affected organ. The most characteristic lesion of neosporosis is focal encephalitis characterised by necrosis and non-suppurative inflammation (Dubey 2003; Dubey et al., 2007).

Herds infected with neospora can exhibit both endemic and epidemic patterns of abortion. The most important feature is that the parasite persists in the female as a chronic infection, which can then be passed to the foetus during pregnancy. Two methods of within-herd transmission have been postulated. The horizontal route involves a two-host life cycle of the parasite, with the cow becoming infected by the ingestion of protozoan oocysts, which are shed by a definitive host – a dog. Vertical, transplacental, transmission also occurs as foetal infection frequently fails to result in abortion, the foetus surviving as a persistently infected carrier. Heifers resulting from such pregnancies can abort when they become pregnant themselves. By contrast with ovine toxoplasmosis, cows that abort a neospora-infected foetus may carry infected foetuses in subsequent pregnancies.

The major economic losses associated with neosporosis in cattle are caused by reduced reproductive performance (abortions, stillbirth, repeat breeding, increased culling rate for reproductive failure). Indirect costs include

veterinary expenses associated with the diagnostic investigation and the cost of replacement if affected cows are culled.

There is some evidence that seropositive herds achieve lower milk production than seronegative ones (Hernandez et al., 2001; Romero et al., 2005).

Diagnosis is by histopathology and immunohistochemistry of aborted foetuses and serology of the dam or the foetus (indirect fluorescent antibody test (IFAT), enzyme linked immunosorbent assay (ELISA), and direct agglutination test (DAT)).

To date, there is no evidence for the venereal transmission of *N. caninum* in cattle. Studies reported by Serrano-Martinez et al (2007) and Ferre et al (2008) demonstrated the presence of *N. caninum* DNA in the semen and blood of experimentally infected bulls. Their observations indicated an intermittent presence of *N. caninum* in low numbers in semen, associated with a chronic stage of the infection. Nonetheless, the protozoa could not be isolated from mice inoculated with PCR-positive semen samples, and there was no seroconversion in heifers inseminated with pooled semen samples. The possible consequences in terms of disease transmission are still to be fully elucidated.

Although it has been possible to induce vertical transmission of *N. caninum* following experimental infection of rhesus macaque monkeys (Barr et al., 1994a), there is no conclusive evidence to date that *N. caninum* can infect and cause disease in humans.

Control of neospora-associated abortions may involve vaccination (Romero et al., 2004) and/or test-and-cull, aimed at eliminating infected individuals from the herd (Hall et al., 2005). Both of these approaches have limitations to their use. Test-and-cull may only be applicable in herds in which the number of infected animals is relatively small. All neospora control programs should, however, always include measures aimed at reducing the exposure of the breeding animals to the infectious forms of the parasite. This involves prompt detection and removal of the aborted foetus and afterbirth, as well as limiting the access of domestic dogs and wild canines to the cattle feed stores and pasture.

More information can be found at www.neosporosis.com.

b. BVDV infection around insemination time

In cattle, pre- and postnatal infection with BVD virus is associated with a variety of disease syndromes, including immune suppression, congenital defects, abortion, and mucosal disease. In several surveys, BVD was the most commonly diagnosed virus disease in bovine abortion cases. The pathology of BVD in the developing foetus is complex. Infection of the foetus before 125 days of gestation can cause foetal death and abortion, resorption, mummification, developmental abnormalities, or foetal immunotolerance and persistent infection. After 125 days of gestation, BVD may cause abortion, or the foetal immune response may clear the virus. There is increasing evidence that the influence of infection with BVD virus on reproductive performance does not limit itself to the induction of foetal death followed by abortion.

A reduction in conception rates in cattle with acute BVDV infection has been reported and is very often a major complaint in herds in which BVD is identified (Houe et al., 1993; McGovan et al., 1993). Viraemia induced experimentally during the follicular phase has resulted in a 50% reduction in pregnancy rate and a deterioration in the quantity and quality of embryos recovered after superovulation (McGowan et al., 1993; Kafi et al., 1997).

Ssentongo et al (1980), Grooms et al (1998), and McGowan et al (2003) described inflammatory changes (lymphocytic oophoritis) within the reproductive ovarian tissue associated with acute infection with BVDV and viraemia. The inflammatory lesions were demonstrated in both the follicles and the forming corpora lutea of infected cows and clearly contributed to the functional disorders leading to inadequate follicular and luteal function and, in consequence, fertility failure.

i. Impaired follicular growth

Grooms et al (1998) reported that the maximum diameter and growth rate of dominant anovulatory and ovulatory follicles were significantly reduced during two oestrous cycles, subsequent to infection of seronegative cattle with a noncytopathogenic bovine pestivirus isolate. This was further confirmed by the work of Fray et al (1999, 2000, 2002), who showed that in the cows infected with BVD virus, the pattern of follicular growth

was clearly disrupted, with a smaller diameter being attained by the preovulatory follicle and also lesser maximum diameter of the ovulatory follicle, in comparison to noninfected cows. Kafi et al (1997) described a significant decrease in the ovulation rate of superovulated heifers inoculated with noncytopathogenic bovine pestivirus 9 days prior to AI.

ii. Inadequate oestradiol production

The work by Fray et al (1999, 2000, 2002) clearly demonstrated that a cell-free viraemia around the time of breeding has a profoundly negative impact on the reproductive endocrine functions in both cows and heifers. The differences in the follicular growth in the infected cows were associated with disrupted oestradiol secretion pattern with generally lower oestradiol levels and especially delayed preovulatory oestradiol peak (Fray et al., 1999, 2002).

iii. Delay in oestrus expression and delayed LH peak

A changed pattern of oestradiol production might, in turn, explain a delay in the onset of oestrus behaviour and the poorer expression of oestrus signs observed by Kafi et al (1997) and McGowan et al (2003) in heifers infected with BVDV. Furthermore, in the same experimental series, McGowan et al (2003) observed an erratic LH pattern in the infected cows, of which only a few showed a normal preovulatory surge, while in the remaining infected individuals a delayed or low-amplitude preovulatory LH peak was detected. Examination of endocrine profiles of the infected heifers in this study revealed that a majority (83%) had no normal preovulatory peak of oestradiol and LH (McGowan et al., 2003). This could be interpreted as a direct result of inadequate follicular growth and oestradiol secretion incapable of stimulating the proper LH secretion. A delayed and inadequate preovulatory LH surge can lead to delayed ovulation, which may negatively affect the quality of oocytes and also the developmental potential of embryos.

iv. Inadequate progesterone production

In the experiments reported by Fray et al (1999, 2000, 2002) and McGowan et al (2003), cows and heifers experiencing a cell-free viraemia around the time of breeding showed a delay in the postovulatory rise in progesterone as well as generally lower progesterone concentrations between 3 and 11 days after ovulation.

It is possible that suppressed plasma progesterone concentrations observed in the BVDV-infected animals compromise fertility by retarding embryo development. The delayed and inadequate preovulatory peak observed in BVDV viraemic cows and heifers can also cause retardation of embryonic development and affect embryo quality. This, in turn, can reduce the ability of the embryo to produce interferon-τ and prevent luteolysis. This may be supported by the results of a large-scale statistical analysis of the effects of BVDV infection on fertility in dairy herds in Brittany, in which cows in herds exposed to an ongoing BVDV infection had a significantly higher risk of late return to service (later than 21 days) than cows in herds presumed to be not infected for a long time or not recently infected (Robert et al., 2003).

One of the basic approaches to reducing the reproductive losses associated with BVDV infection in cattle is the implementation of tight biosecurity measures, limiting the exposure of the animals to the virus, and vaccination with products that will prevent cell-free viraemia and transplacental infection.

2.6.6 Unwanted pregnancy

While better avoided altogether, the accidental mating of young heifers is a common reason for terminating pregnancy. Feedlot operators also have reason to abort pregnant heifers. If pregnant at slaughter, heifers achieve lower prices and, in any case, feed efficiency is better if they are not carrying calves and calving difficulties are avoided. Up to about day 150 of pregnancy, the corpus luteum is the only source of progesterone in the pregnant animal. Luteolysis with prostaglandins will result in abortion. If mating is observed, prostaglandin can be injected 10-16 days later, or alternatively, it can be administered to mismated animals that do not return to oestrus after 3 weeks.

Between 100 and 150 days of pregnancy, the efficacy of prostaglandin is reduced to less than 90%, because some pregnancies become less dependent on the corpus luteum (CL) for absolute support. Thus, an injection of prostaglandin is never guaranteed to terminate pregnancy. It is always wise to advise pregnancy diagnosis at least 10 days after the use of prostaglandin and repeat the injections until all the animals have been aborted.

After day 150, the placenta produces sufficient progesterone to maintain the pregnancy on its own. The combination of 25 mg of dexamethasone and a dose of prostaglandin $F_{2\alpha}$ usually induces abortion at all stages of pregnancy. However, Thomas (1991) reported an increased mortality in feedlot heifers treated with the dexamethasone/prostaglandin combination.

2.7 Induction of parturition

The main reasons for choosing to induce parturition are:

- To advance calving, to reduce the calving interval, or to tighten the calving pattern
- To reduce the incidence of dystocia by preventing foetal oversize
- To terminate abnormal pregnancies
- To advance the date of calving in late-conceiving cows, where breeding and production is seasonal

In the cow, progesterone is necessary for the maintenance of pregnancy. As already noted, in the first 150 days of gestation and during the last few days before parturition, the corpus luteum is the main source of progesterone. In the period between, the placenta produces sufficient progesterone to maintain pregnancy. Parturition is triggered by an increase in foetal cortisol production. This initiates a rise in placental oestrogen production and of prostaglandins ($PGF_{2\alpha}$). The corpus luteum regresses and the plasma progesterone level drops sharply. Research has focused on the use of prostaglandins, corticosteroids, or a combination of the two to induce parturition.

Corticosteroids

The administration of a short-acting dexamethasone shortly before, or at term, mimics the rise in foetal cortisol and thus initiates the calving process. Most cows will calve within 72 hours.

When induction is attempted more than 7-10 days before the expected time of parturition, the response is more variable and induction fails more frequently. This can be overcome by priming the animal with a medium-acting corticosteroid preparation, and about a week later giving a short-acting product. It is worth noting that 10%-30% of cows will calve within a week in response to the priming injection.

Prostaglandins
Injection of a standard dose of prostaglandin $F_{2\alpha}$ during the week prior to the expected date of calving will also induce parturition, with most cows calving within 48 hours. Combinations of corticosteroids and prostaglandins may be preferable because the former are required for foetal maturation.

Data from both the literature and field experience indicate that an increased occurrence of retained foetal membranes is associated with the induction of calving with prostaglandins, regardless of the type of analogue used. It is important to know the correct service date to avoid inducing a premature birth, which would significantly reduce the viability of the calf. Good breeding records are, therefore, important, as is close attention to the hygiene of the calving environment.

2.8 The bull

On farms utilising natural service, the fertility of the bull is of major importance to herd fertility. Subfertility in bulls leads to delays in conception, prolonged calving intervals, reductions in the calf crop, and increased culling for reproductive failure. Annual evaluation of each bull's suitability for breeding is strongly recommended.

2.8.1 Evaluation of breeding suitability

The standards for evaluating a bull's suitability for breeding are provided by the Society for Theriogenology (www.therio.org). Several criteria are used in the evaluation of bulls (Kastelic and Thundathil, 2008). The final assessment is based on a physical evaluation and basic semen evaluation.

The examination of the fertility potential of a bull consists of four elements:
- General examination
- Examination of the genital tract
- Semen evaluation
- Assessment of libido

a. General examination

Having checked the bull's age and identification, special attention should be paid to the locomotory system, while the animal is standing and when moving over a hard surface. For bulls kept under extensive conditions, eyesight is also of importance.

b. Genital tract exam

A complete examination must include the penis and scrotum as well as rectal palpation.

The penis must be inspected and palpated. However, some defects such as spiral deviation or erection failure are only detectable during mating.

The scrotum is inspected for abnormalities such as inguinal hernia, excess fat, gross disparity between the testes, and their size and consistency, which should be resilient. The epididymis must feel normal, with a soft tail. The scrotum must be well developed. There is a direct relationship between scrotal circumference, which reaches a maximum at 4-6 years of age, and sperm production.

Structures assessable by rectal examination include the urethra, prostate, vesicular glands, ampullae, vas deferens, and internal inguinal rings.
The most common abnormality is seminal vesiculitis, the aetiology and pathogenesis of which are poorly understood. *A. pyogenes, B. abortus, E. coli, Streptococcus* spp., and several others have been isolated. Response to long-term treatment is variable and unreliable.

c. Semen evaluation

Most bulls can be made to ejaculate with an electroejaculator, which is a simple and safe method of enabling semen collection. Some fail to ejaculate or produce only a 'watery' urethral fluid, in which case a supervised mating utilising an artificial vagina may be more useful. The gross motility of semen is evaluated at 37°C, by placing a large drop of semen on a preheated microscope slide for examination under low-power magnification. Gross motility is graded as 1) rapid, vigorous waves, 2) slower waves, 3) no waves but general oscillation, 4) occasional flickering only. Because gross motility also depends on sperm density, a more accurate estimate of sperm motility can be assessed using phase-contrast microscopy.

Sperm evaluation under a light microscope is able to detect gross abnormalities but unlikely to provide any information about more subtle variations in motility that could possibly affect the fertility of the examined bull. A computer-assisted semen analysis (CASA) is a far more objective method that can measure specific motion characteristics associated with the functional status of spermatozoa.

Morphology can be examined at 1000x magnification, using fresh semen stained with eosin-nigrosin.

The threshold for a satisfactory potential breeder is 30% of progressively motile and 70% morphologically normal sperm.

d. Libido evaluation

A simple test for libido is to pen a cow or heifer in oestrus and then turn the bull in for 10-15 minutes. If he manages one or more services in this time, his libido is unlikely to be a problem. If the bull fails, he should be retested. Failure on both occasions gives serious grounds to question his libido.

Several new methods based on molecular biology and germ cell interaction are being studied, with the intention of employing them in the future testing of fertility in bulls (Petrunkina et al., 2007; Kastelic and Thundathil, 2008).

2.8.2 Male infertility

Male infertility may be due to failure to mount, failure of intromission, or failure of fertilisation. A diagnosis can generally be made after careful examination following the above guidelines. Subfertility is much more difficult to diagnose. Testicular infections normally carry a very poor prognosis. Testicular degeneration may be caused by stress, toxins, heat, and nutritional deficiencies. Diagnosis often relies on semen examination, and recovery is variable. The semen of some bulls may return to normal within 8 weeks, while for others it may take up to 6 months. Again, semen testing is essential.

The hormonal treatment of infertile bulls is of limited value. PMSG acts like FSH and will stimulate spermatogenesis. hCG stimulates testosterone production because of its LH activity. GnRH will induce a short-term increase of FSH and LH levels. A good history and clinical examination will assist in arriving at the correct diagnosis. Only then can a specific treatment, or a change in management (including rest), be decided upon.

Several pathogens can cause infertility in bulls or transmit infection via their semen (Givens et al., 2006; 2008). Some of them can directly affect a bull's fertility by causing disease of the reproductive tract or associating with spermatozoa to prevent fertilisation. Viral pathogens that can reduce sperm quality and/or affect the bull's reproductive tract include bovine herpes virus I (BHV-I, the agent of IBR), and bovine viral diarrhoea virus (BVDV). Other viruses that can be transmitted in semen include foot-and-mouth disease virus, vesicular stomatitis virus, rinderpest virus, and lumpy skin disease virus. The risk of transmitting bovine immunodeficiency virus and bovine leukosis virus via semen appears to be very low. Bluetongue virus (BTV) can be detected sporadically in the semen of viraemic bulls and might result in venereal transmission.

Tritrichomonas foetus and *Campylobacter foetus venerealis* are sexually transmitted; they do not cause disease in the bull but can survive in frozen semen; thus, it is extremely important that breeding bulls, especially those used for natural service, should be regularly tested for these two pathogens. Other microorganisms that can be transmitted in semen and might be associated with infertility or the transmission of infection include *Brucella abortus, Leptospira* spp., *Histophilus somnus, Ureaplasma diversum, Mycobacterium avium s*ubsp. *paratuberculosis, Chlamydia, Mycobacterium bovis, Coxiella burnetii,* and *Mycoplasma mycoides* ssp. *mycoides*.

The World Organization for Animal Health (OIE) sets standards for disease control associated with semen production. Bulls placed in AI and semen collection centres should be annually tested for brucellosis, tuberculosis, BVDV, *T. foetus, C. foetus,* and BHV-1. Prequarantine and quarantine testing should confirm the bull to be free of brucellosis, tuberculosis, BVDV, as well as BHV-1 if the herd or AI centre is to be considered BHV-1-free. Additionally, Certified Semen Services recommend that bulls should be tested for leptospirosis.

2.9 Embryo transfer

Artificial insemination helps to achieve the rapid genetic improvement of a herd by making more effective use of top quality sires. The maximum reproductive capacity of the cow is one calf per year. Multiple ovulation and embryo transfer (MOET or ET) techniques increase the reproductive potential of the dam, thus enhancing the effect of the female in cattle breeding.

Some of the reasons for using ET are:
- To obtain more calves from a valuable, high quality cow
- To increase the rate of genetic improvement of a herd
- To facilitate international shipment of animals
- To prevent acclimatisation problems when exporting cattle to tropical areas
- In (international) bull breeding programmes
- For the induction of twinning
- To obtain purebred beef calves from the lower quality end of the dairy herd
- To obtain offspring from cows with fertility problems

Extensive reviews of the steps involved in embryo production and transfer in cattle were provided by Mapletoft and Hasler (2005) and Sirard and Coenen (2006).

There are essentially two sources of embryos used for transfer in cattle:

2.9.1 Embryos produced in vivo flushed from the reproductive tract of a donor cow

These embryos are produced with full knowledge of the genetic potential and health history of the parental animals, ensuring optimal possibility for rapid genetic improvement in the herd. This system is limited by factors such as: the ovulation rate after pharmacologically induced superovulation in the donor, fertilisation rate, and embryo recovery rate. These embryos can be transferred directly to synchronised recipients or frozen and stored in liquid nitrogen for future use.

2.9.2 Embryos derived by in vitro maturation (IVM) and in vitro fertilisation (IVF) of oocytes

These embryos can be derived either from oocytes originating from known, selected donors and harvested by aspiration from the preovulatory follicles (the so-called ovum pickup procedure) or after the in vitro maturation of immature oocytes harvested from ovaries obtained from a slaughterhouse. The latter system, though the most efficient, poses certain challenges such as the unknown genetic status of the donor.

It is well recognised that bovine embryos derived in vivo are of superior quality compared to those derived from in vitro maturation, fertilisation, and culture. Despite the high rate of nuclear maturation obtained, the developmental competence of bovine oocytes matured in vitro is variable. One likely reason for much of this variation could be the intrinsic quality of the oocytes recovered from the ovaries. One negative consequence of both IVP and somatic cell nuclear transfer (SCNT) in cattle, and other species, is that embryos, foetuses, placentas, and offspring can differ significantly in morphology and developmental competence compared with those from embryos produced in vivo (Farin et al., 2004; 2006; Lonergan and Farin, 2008). Collectively, these abnormalities have been referred to as 'large offspring syndrome' or 'large calf syndrome.' These abnormalities have largely been eliminated with the development of better culture media and techniques.

Oocyte maturation in vitro and embryo culture techniques are integral to the process required for cloning and facilitating the breeding of transgenic cattle for the production of valuable pharmaceutical proteins in their milk. The cloning of adult cattle through nuclear transfer and the production of cloned, transgenic cattle has been achieved technically. However, it is an expensive and inefficient technology which, at this stage in its development, could only be used by the pharmaceutical industry and research community (Mapletoft and Hasler, 2007; Galli and Mazzari, 2008).

In vitro fertilisation by intracytoplasmic sperm injection is feasible in cattle, even with freeze-dried semen, but is not yet widely used. A few laboratories have reported successes in producing pregnancies from embryos produced in vitro, based on oocytes harvested from calves, which offers the potential for increasing the rate of genetic improvement by reducing the generation intervals.

The International Embryo Transfer Society issues a series of carefully defined procedures, especially in respect to the sanitary and epidemiological aspects of embryo production and transfer. Infectious factors such as BVD and IBR were identified as potentially transferable with embryos, which led to the adoption of specific procedures to ensure the safety of ET in respect to these pathogens.

A comprehensive review provided by Givens et al (2008b) summarised the current health recommendations for bovine embryo transfer, while emphasising recent research to develop and validate novel approaches to biosecurity. Most of the commercial embryo production systems use culture media supplemented with various animal-derived sources of nutrients. Although many preventive measures are applied (screening of sources, pretreatment with high temperatures, and addition of antibiotics) such systems increase the health risks, especially for in vitro embryos (Givens et al., 2008). A system based on defined components, free from cell constituents or elements derived from blood, would be ideal from the viewpoint of health and quality control. Chemically defined conditions without serum or serum proteins allow for more precise observation of the effects of growth or other embryotrophic factors in any given medium. Reports are available on the use of media for which every component is semi-defined, or fully defined, chemically (Feugang et al., 2009).

2.9.3 Management of the donor cow

Under natural conditions, the cow usually has only one ovulation per cycle. Gonadotrophic stimulation of the ovaries can induce multiple ovulations (superovulation). Although embryo transfer techniques are widely used around the world, variability in response to the superstimulatory treatments

remains an important limitation. Variability in ovarian response has been related to differences in superovulatory treatments, such as gonadotrophin preparation, batch, and total dose, duration and timing of treatment, and the use of additional hormones in the program. Individual variation between animals cannot be ruled out.

The recent development of protocols capable of controlling follicular wave emergence and ovulation has not yet completely eliminated the variability in superovulatory response. Nonetheless, these treatments have had a positive impact on the application of commercial, on-farm embryo transfer, by permitting the initiation of treatments at a predetermined time. Protocols that synchronise ovulation tightly, allow for the insemination of donor cows at a fixed time, thereby eliminating the necessity of oestrus detection during the superstimulatory protocol.

From information generated by ultrasonography, it has been established that approximately 8-12 days after oestrus (equivalent to 7-11 days after ovulation), another follicular wave emerges in cows exhibiting two- or three-wave cycles, and a cohort of growing follicles should be available for the stimulation of multiple ovulation at that time. However, it has been shown that superovulatory response is better when gonadotrophin treatments are initiated precisely at the emergence of the follicular wave rather than later.

Three different types of gonadotrophins have been used to induce superovulation in the cow: gonadotrophins from pituitary extracts of pigs or other domestic animal (extraction FSH); equine chorionic gonadotrophin (eCG)/pregnant mare serum gonadrotrophin (PMSG); and human menopausal gonadotrophin (hMG). At present, the most notable gonadotrophins used in the ET industry in cattle to achieve multiple ovulations are pregnant mare serum gonadotrophin (PMSG) and follicle stimulating hormone (FSH). Both are administered during the mid-luteal phase, usually of a synchronised oestrous cycle, as it has been shown that the superovulatory response is higher when gonadotrophin treatment is instituted precisely when the follicular wave emerges, rather than later. Therefore, it is usual in normally cycling cattle to use treatments that control the timing of the follicular wave.

Natural FSH preparations of porcine and ovine origin are available. Because FSH has a relatively short half-life, it is generally administered twice daily. The usual regimen involves 4 or 5 days of twice-daily treatments with 400 mg of purified extraction FSH. $PGF_{2\alpha}$ is injected to induce luteolysis 48 or 72 hours after the start of treatment. Oestrus occurs between 36 and 48 hours after the prostaglandin injection, and ovulation between 24 and 36 hours later.

Pregnant mare serum gonadotrophin – PMSG, also called equine chorionic gonadotrophin, eCG – has been shown to have a half-life of 40 hours and to persist for up to 10 days in bovine circulation, so a single injection is sufficient. Recommended doses of eCG/PMSG range from 1500 to 3000 IU, with 2500 IU the most common. Forty-eight hours after the PMSG injection, regression of the corpus luteum is induced with a dose of prostaglandin. Donaldson (1983) reported a better luteolytic effect with natural $PGF_{2\alpha}$ when two or three injections were given, but when using analogues, a single dose was sufficient.

The continued stimulation effect of high doses of PMSG may have a negative effect on ovulation and cause the emergence of a second wave of follicles.

Factors affecting the success rate in embryo collection and transfer were evaluated in dairy herds by Chebel et al (2008).

a. Animal related

- Stage of the cycle. Best results are obtained when superovulation is initiated during the mid-luteal phase (day 9-13)
- Follicular status at the time of superovulation. The presence of a large dominant follicle at the time of superovulation reduces the response
- General health and nutritional status
- Lactation phase

b. Management and environment related

- General management of the animals (avoiding stress, adequate housing)
- External temperature and humidity

c. Procedure related

- Synchronisation and induction system (type of system used and compliance)
- Semen quality and insemination at 12-24 hours after the onset of standing oestrus. Repeated inseminations do not appear to give better fertilisation rates. Differences have been reported between bulls
- Embryo harvesting and processing technique

The use of progestagens provides an efficient way of ensuring a tight oestrus synchronisation in embryo and oocyte donors. The exposure to progestagen results in higher quality oocytes/embryos harvested and the possibility of a fixed time insemination.

d. Management of the recipient

Due to the high variability of embryo recovery, it is very common to find that either too many or too few recipients have been prepared. Surplus embryos may be frozen and stored in liquid nitrogen, but only good quality embryos should be selected for freezing. They can be transferred during a normal cycle or, of more practical use, during a controlled cycle. There is no difference in the pregnancy rates of recipients between transfer during a natural or a controlled cycle.

The administration of a GnRH analogue, at the beginning of oestrus, can be used to induce ovulation in recipient cows, at an oestrus synchronised using prostaglandin analogues. Better results are expected with more precise timing of ovulation and improved development of corpora lutea.

Among the factors influencing the success of embryo transfer in recipients (measured as the pregnancy/calving rate) the following are the most relevant (Peterson and Lee, 2003; Looney et al., 2006; Vasconcelos et al., 2006):

- Quality of the embryo and adequate transfer technique
- Adequate timing of the transfer in relation to the recipients' oestrous cycles
- Adequate progesterone concentrations in the recipients' circulation around the time of the transfer (often correlated with milk production)
- Management of heat stress and other stress factors (management, nutrition, housing, etc)

Even if only excellent quality embryos are transferred, the resulting pregnancy rates can vary considerably among recipients. McMillan (1998) developed a model that allowed for the separation of the embryo's and the recipient's contribution to the survival of transferred embryos during the first 60 days of pregnancy. This model demonstrated that it is the variation in the ability of a recipient to carry the pregnancy to term (recipient quality), rather than the ability of the embryo to survive and develop, that leads to this variation in pregnancy rates after ET. Interestingly enough, the quality of the recipient does not contribute, to any great extent, to foetal losses after 60 days of pregnancy. It is important to recognise that the model proposed by McMillan suggests that there may be superior recipients within herds, and in practice, many veterinary surgeons specialising in ET strive to identify such animals, and use them repeatedly to introduce desired genetic material into the herd.

2.10 Use of sex-sorted semen in cattle breeding

The application of sexed semen allows dairy producers to select from their herd's potential dams and produce replacement heifers from only the genetically superior animals.

Current technologies to sort X and Y chromosome-bearing sperm populations require individual identification and selection of spermatozoa in a modified high-speed flow cytometer (reviewed by Seidel, 2007; Garner and Seidel, 2008).

To date, fertility is still variable and is quite dependent on processing after sorting. New processing techniques are under investigation and will likely be able to improve the fertility rates for sex-sorted semen. Selection of the most appropriate bulls and testing the sorted samples on a routine basis are very important to success.

The procedure of sperm sorting has proved to be safe for the sperm's genetic material. Large-scale studies have found no increase in the abortion rate or differences in gestation length, neonatal death, calving difficulty, birth weight, weaning weight, or live births when sexed sperm is compared to unsexed control sperm. Genetic damage to sperm during sex sorting is probably minimal or even nonexistent.

Commercially available sex-sorted semen is often used primarily in heifers because of their inherently higher fertility and the limited amount of sexed sperm available. Conception rates obtained in dairy herds have been reported ranging from 30% to 70%, with the average level of fertility in a given herd, and managerial factors, greatly influencing the outcome of AI (Garner and Seidel, 2008).

2.11 Twinning

In the dairy cow, twins are associated with higher calf mortality, reduced birth weight, higher incidence of still births and dystocia, retained placenta, longer calving to conception intervals, and reduced milk yield. In beef cattle that don't experience all the negative effects on the dam, twinning has been shown to have interesting advantages.

Twinning rates have increased alongside milk production (Lopez-Gatius et al., 2005). In high producing cows, the twinning rate can exceed 9% and the rate of double ovulation may be over 20%.

Twinning has multiple risk factors, both genetic and environmental. These risk factors include genetics, season, parity, stage of lactation, ovulation rate, and milk production (Fricke, 2002; Lopez-Gatius et al., 2005).

Twinning in cattle can be classified into two types: monozygous or identical twins and dizygous or fraternal twins. Identical twins come from a single fertilised oocyte that later divides to form two embryos. Fraternal twins come from the fertilisation of two oocytes at the same time.

Monozygous twinning is rare in cattle. Dizygous twinning accounts for most twin births in dairy cattle (Fricke, 2002). Dizygous twins are no more alike phenotypically or genetically than two siblings with the same parents but from different gestations.

Because dizygous twins arise from double ovulations, understanding the cause requires an understanding of follicle development and dynamics. Normally only one follicle within a wave is selected to become dominant and achieve ovulatory capacity. The point of selection is a process called

"deviation." Deviation provides a mechanism by which a single follicle reaches ovulatory capacity during a given oestrous cycle. Occasionally two or more follicles escape the process and share dominance to reach maturity and ovulatory capacity. The mechanism by which this happens needs further investigation, but the incidence of double ovulations appears to be much higher when deviation occurs in lower progesterone conditions.

Not a lot of information exists on how to manage cows with twins or on how to alter the incidence of twins. Despite this lack of information, there are several strategies that might be considered. Identificaton of cows carrying twins is the place to start. Nothing can be done unless cows carrying twins can be identified accurately.

Once identified, cows carrying twins can be fed a higher plane of nutrition to support the increase in gestational mass, especially during the third trimester. Gestational length in cows carrying twins is reduced by 6 to 10 days. Management changes, dry off dates, and dietary changes all need to reflect a shorter gestation length.

Cows carrying twins have a much higher incidence of dystocia. Providing timely monitoring and assistance at calving can help reduce complications associated with dystocia and reduce associated economic losses.

Twinning in dairy cattle has increased over time, as milk production has increased. More information is needed to develop practical strategies to identify and manage cows with twins.

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3.1 Physiology

3.1.1 The mare: a seasonal breeder

Reproductive activity in the horse is seasonal; the natural breeding season of mares extends from early spring to late summer—April to September in the Northern Hemisphere and October to March in the Southern Hemisphere. The normal cyclic activity of horses is activated primarily by increasing day length (longer photoperiod) in early spring, while in the late summer and early autumn, decreasing day length (shorter photoperiod) triggers the end of the breeding season. Hence, a mare that is not pregnant will display winter anoestrus, followed by a transition to the breeding season in early spring, usually between March and May. In most mares, the breeding season, with its associated regular oestrous cycles, will continue until the beginning of the transition to anoestrus in the autumn. Some mares (around 25%) develop a persistent corpus luteum in late summer. Some mares do not display seasonal anoestrus.

Figure 1 shows the association between changes in photoperiod and seasonal reproductive activity in mares. There is considerable interindividual variation in the number of oestrous cycles at which a mare can be bred. It should also be remembered that this pattern of reproductive activity may only be obvious in maiden and barren mares. This pattern is not seen in mares that conceive in consecutive years because conception at foal heat, within 6-8 days of foaling, is followed by pregnancy (around 11 months).

The age of a horse is measured from January 1, irrespective of its actual birth date; therefore, particularly in the racing industry, it is important that foals are born as early as possible in the year so that they are as mature as possible (in terms of muscle and bone and ability to withstand stress and effort) by the time they start to compete. Racehorses and trotters mainly perform when 2 and 3 years old. Foals that are born early in the year have advantages in terms of maturity at the start of their show or race career and/or their value at yearling sales. This is a challenge due to the seasonal pattern of ovarian activity in mares. However, there are a number of ways to achieve this successfully.

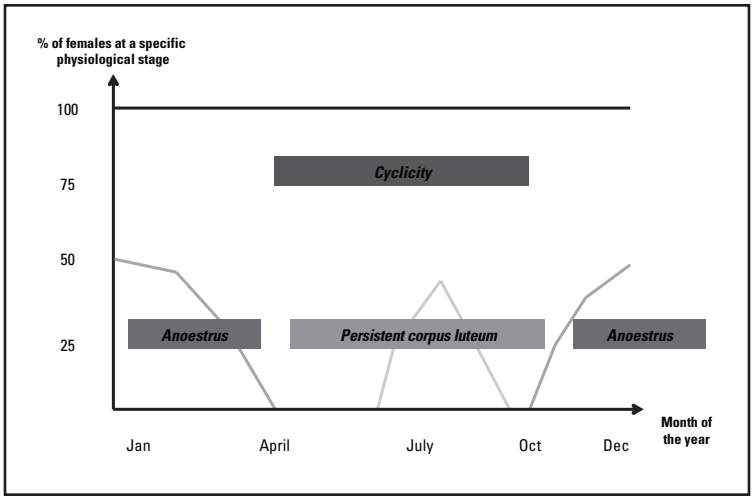

Figure 1 Changes in the proportion of mares displaying anoestrus (in red), a persistent corpus luteum (in yellow), and those cycling throughout the year

3.1.2 Physiology of the oestrous cycle in the cyclic mare

During the breeding season, mares come into oestrus ("heat") on average every 21 days (range 18-24 days). This can be split into luteal and follicular phases. The luteal phase, when progesterone is produced by the corpus luteum, typically lasts 13-15 days, from ovulation until the regression of the corpus luteum. The follicular phase, from luteolysis until ovulation, lasts around 7 days, during which growth and maturation of the ovulatory follicle takes place. However, its duration can be variable, ranging from 4 to 5 days at the end of spring to over 15 days at the end of winter. The timing of ovulation after the beginning of oestrus is not predictable. Thus, the wide variation in the duration of the follicular phase poses a major hurdle in the optimisation of the breeding management of mares. Figure 2 summarises the patterns of corpus luteal and follicular growth and regression during a 21-day cycle.

In most mares, there is only one period of follicular growth ("follicular wave"), starting around days 12-14 and culminating in ovulation on day 21. Two follicular waves have also been detected in some mares, with the first one starting after ovulation, reaching a maximal size during the luteal phase, and regressing around day 12 and the second one, starting around days 12-

14, generating a preovulatory follicle. During a follicular wave, recruitment of a group of medium-sized follicles (10-20 mm in diameter) is usually followed by the selection of a single follicle that becomes dominant and completes terminal follicular growth and maturation before ovulation. Oestrus generally starts around the time when the largest follicle reaches around 25 mm in diameter. The dominant follicle grows at a rate of 3 mm/day, reaching approximately 35 mm at the preovulatory stage. All of the other follicles undergo atresia and regress.

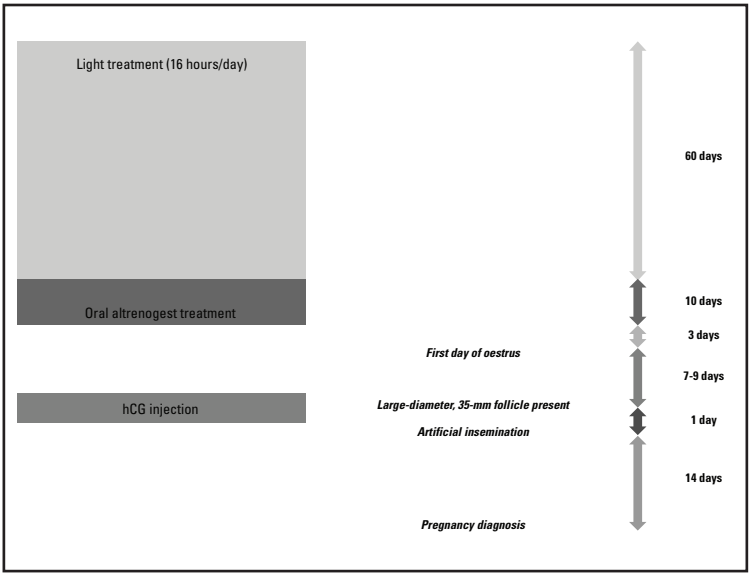

Figure 2 A proposed treatment strategy to efficiently breed maiden and barren mares

Factors affecting the size of the preovulatory follicle include the age of the mare (larger in young mares), season (larger in late winter than in summer), and the number of preovulatory follicles (smaller if a double ovulation) (Davies Morel et al., 2010). Preovulatory follicle diameter appears to be highly repeatable within an individual (Cuervo-Arango and Newcombe, 2008). Sometimes two follicles become dominant, and this is followed by a double ovulation. Factors affecting the incidence of double ovulation include breed (estimated to range from 2% in ponies to 25% in Thoroughbreds) (Ginther et al., 2008), reproductive status, and age. Double ovulation is strongly repeatable in individual mares.

The changes in the concentrations of the two main gonadotropins controlling ovarian function (follicle stimulating hormone [FSH] and luteinising hormone [LH]) during the oestrous cycle in mares are well characterised.

FSH concentrations rise around the mid-late luteal phase (days 10-14 of the oestrous cycle) and act as the trigger for the recruitment of follicles. As the wave of follicles grows, it starts to produce increasing amounts of inhibin and oestradiol, which lead, through negative feedback, to a progressive reduction in circulating concentrations of FSH, with the lowest concentration reached in the presence of the dominant follicle. If there is a double ovulation, FSH concentrations are lowest during the late luteal and follicular phases (Ginther et al., 2008).

The pattern of LH secretion in the mare is rather unique in contrast to what has been demonstrated in most other species. Firstly, LH secretion does not appear to be pulsatile. This may be related to the longer half-life of equine LH. Secondly, the LH surge is spread over at least 5 days; LH concentrations start to rise 3 days before ovulation, peak around the time of ovulation, and do not return to basal levels before 3 days after ovulation. It is not clear why the LH surge is so prolonged in this species.

There appears to be general consensus that the rise in FSH concentrations in the late luteal phase causes the recruitment of small follicles and supports their growth from 10 mm to around 20-25 mm in diameter. By this stage, the two or three largest follicles have developed LH receptors on their granulosa cells (Fay and Douglas, 1987), which allows these follicles to shift from FSH- to LH-dependence and to survive while FSH concentrations are decreasing. The dominant follicle is the follicle with the highest response to LH, likely mediated via an increase in free insulin-like growth factor-1 (IGF-1) concentrations in the follicular fluid, which maximises the response of granulosa cells to LH (Checura et al., 2010). Increased vascularisation of the dominant follicle may also help its development through the preferential delivery of hormones and nutrients. As soon as a follicle becomes dominant, there is an increase in oestradiol concentrations due to peak, LH-stimulated production of androgens by thecal cells and conversion of androgens into oestradiol by the highly active aromatase enzyme in granulosa cells.

Peak plasma concentrations of oestradiol are generally detected 2 days before ovulation (Ginther et al., 2006). However, there is still debate as to whether this oestradiol peak exerts positive feedback on the hypothalamic-pituitary axis, as in other species.

3.1.3 Initiation of pregnancy, pregnancy maintenance, and pregnancy loss

Fertilisation takes place in the oviduct up to 30 hours after ovulation. Transit of the young embryo through the oviduct into the uterus takes about 6 days, by which time it has reached the blastocyst stage. It reaches 2 mm in diameter around day 10 and becomes large enough to be visualised by ultrasonography (as a round, 20-mm diameter vesicle generally in one uterine horn) around days 13-14 after ovulation. Consecutive ultrasound scans in an individual mare show that the embryo moves freely throughout the uterus during this period, a key part of maternal recognition of pregnancy, which occurs around day 17 (Allen, 2001a). Any pathological changes within the endometrium, or large endometrial cysts or septae, can contribute to insufficient maternal recognition of pregnancy. To date, the exact embryonic signal involved in maintenance of equine pregnancy is not fully understood. In pregnant mares, luteolysis does not occur because there is no release of prostaglandin (PG) $F_{2\alpha}$ from the endometrium due to an absence of cyclical upregulation of endometrial oxytocin receptors (Stout et al., 2000). This means that progesterone concentrations remain high from days 16 to 21 and then decrease slightly between days 21 and 40. Progesterone concentrations increase again around days 40-50 following the formation of accessory corpora lutea induced by pregnant mare serum gonadotropin (PMSG, also known as equine chorionic gonadotropin, eCG).

Foetal heartbeats can first be detected by ultrasonography between days 25 and 35 after ovulation. Around days 36-38, cells from the trophoblast migrate deep into the maternal endometrium to form structures, unique to Equidae, called endometrial cups. Large amounts of PMSG are produced and secreted by the endometrial cups between days 40 and 70 (Allen, 2001a). There is close synchrony between the appearance of PMSG in the peripheral circulation and the formation of accessory corpora lutea, although a direct

cause-effect relationship remains uncertain. Starting around day 70, the endometrial cups begin to degenerate and plasma concentrations of PMSG reach a plateau (at 100 international units (IU) per mL). Finally, at around day 100-120, the necrotic endometrial cups detach from the surface of the endometrium and PMSG concentrations decrease, becoming undetectable around day 120. At this stage, the placenta has gained the ability to produce steroid hormones and synthesises large amounts of progesterone or progestins, as well as the oestrogen equilenin. As the corpora lutea regress around day 160, pregnancy is maintained by the high placental output of 5α-pregnanes, a specific class of progestins.

Pregnancy in the mare lasts for around 11 months (average 335 days, range 310-365 days). The variability in the duration is due to a number of factors, including season (pregnancies started in winter and spring are around 10 days longer), body condition (pregnancy is 4 days shorter for mares in good body condition), and the sex of the foal (pregnancy is 2-3 days longer for male foals).

The development of ultrasonography for pregnancy diagnosis (Palmer and Driancourt, 1980) allowed monitoring of embryonic and foetal survival from the first diagnosis of pregnancy (usually before day 20) and foaling. Studies (Ginther, 1985; Woods et al., 1987; Chevalier-Clément, 1989) have established conclusively that about 5%-7% of pregnancies are lost between days 20 and 50 (embryo loss), while about 9% of pregnancies are lost between day 50 and foaling (foetal loss or abortion). Factors increasing pregnancy loss include the presence of twins (twofold increase), old age (twofold increase in mares older than 15 years), and abnormal embryos (sixfold increase). The abortion rate increases considerably in the presence of twins (sixfold increase) and old age (threefold increase in mares older than 20 years) (Chevalier-Clément, 1989). There is no consensus on the possible effect of the physiological status of the mare when bred (lactating or barren) and the rate of embryonic loss or abortion.

The first oestrus after parturition ("foal heat") starts 6-8 days after parturition (range 6-15 days), and most mares ovulate around 10-15 days after parturition. Mares foaling during periods of short day length (winter) tend to display a longer foaling to first ovulation interval (15

days) than those foaling in spring (10 days) (Macpherson and Blanchard, 2005). Given that a short parturition to conception interval is required to maintain the annual production of offspring, breeding mares at foal heat can enhance reproductive efficiency. Fertility at foal heat appears to be higher when ovulation occurs after day 10. Breeding at foal heat should be avoided in mares where uterine involution is incomplete (uterine fluid on ultrasonography) or there are periparturient problems (dystocia or retained placenta) as well as in older mares (slower uterine involution). In addition, the benefit of breeding early needs to be weighed against reduced fertility at the foal heat compared to the following heat.

3.1.4 Seasonal regulation of reproductive activity in the mare

A maiden mare may experience three different transition periods in any given year. In early spring, there is a transition from anoestrus to regular oestrous cycles. In summer, the development of a persistent corpus luteum leads to a cessation of cyclic ovarian activity. In mid-autumn, the mare may revert to anoestrus.

Day length (photoperiod) plays a key role in the regulation of seasonal reproductive activity in mares. Exposure to increasing day length during winter triggers the resumption of cyclic ovarian activity. Other factors affecting the duration of anoestrus are age (young mares more commonly display anoestrus), breed (anoestrus is more common in ponies than in horses), and body condition (anoestrus is longer in lean animals).

As in other species, melatonin, synthesised in the pineal gland (or epiphysis) from the neurotransmitter serotonin (5-hydroxytryptamine) by the enzyme serotonin N-acetyltransferase, forms the link between day length and the hypothalamic-pituitary axis. Rates of synthesis and release of melatonin are low during daylight and peak during darkness. In sheep, melatonin has been shown to act indirectly through a complex neuroendocrine network involving the hypothalamic kisspeptin (Kp) family of peptides and RFamide-related peptide-3 (RFRP-3) (see Chapter 1, Section 1.4.2) (Malpaux et al., 1999). However, although a bolus injection or infusion of equine Kp-10 (eKp10) consistently and transiently increased peripheral concentrations of LH and FSH in pony mares, it did not induce ovulation, irrespective of when

it was administered (Decourt et al., 2014). Thus, the link between melatonin concentrations and hypothalamic-pituitary axis activity in mares is not clear and appears to be different than in sheep.

Prolactin may also be involved in seasonal breeding in mares. Indeed, prolactin concentrations are low in the winter months (Evans et al., 1991), while they are high in summer. Treatment of mares with prolactin or dopamine receptor antagonists (eg, sulpiride) can hasten the first ovulation of spring (Besognet et al., 1997). However, the highly variable response of mares to dopamine receptor antagonists (Daels et al., 2000) appears to suggest that prolactin may act more as a modulator of seasonal breeding in mares.

Resumption of ovarian activity during the spring transition period occurs in a stepwise manner (Donadeu and Watson, 2007). Initially, ovarian follicular growth starts and follicles reaching 25-35 mm in diameter appear. However, this is not associated with oestrus behaviour, and these follicles regress after a few days. These blunted follicular waves are typically associated with high FSH and low LH concentrations. The changes in the pattern of GnRH secretion responsible for this transition, which typically lasts 30-90 days, have not been well characterised, mainly due to the difficulty in collecting hypophyseal portal blood from mares. This is followed by a second period that occurs during the weeks preceding the first ovulation of the breeding season. The pituitary, possibly due to increased activity of GnRH neurons, regains its ability to produce and release LH (Donadeu and Watson, 2007). Increased LH concentrations support terminal follicular growth up to a preovulatory size, promote steroid hormone production by this follicle (by increasing androgen production by thecal cells), and may increase follicle sensitivity to LH (by reducing the concentrations of IGF binding proteins present in the follicular fluid). Eventually, this large-diameter follicle starts producing enough oestradiol to initiate oestrus. The rising oestradiol concentrations increase the sensitivity of the pituitary to GnRH and the follicle to LH, therefore starting the loop that triggers ovulation.

A number of studies have reported that the last LH surge in the breeding season is smaller and that in autumn the first failure to ovulate is associated with the absence of an LH surge (Ginther et al., 2003). The neuronal mechanisms suppressing GnRH secretion and therefore preventing

increases in LH are unknown. Interestingly, the transition to anoestrus is gradual, with an initial stage where follicles continue to grow to large sizes (without ovulating) followed by a stage where follicular growth is blunted and no follicles grow to greater than 20-25 mm in diameter. It is possible that the mechanisms involved are the opposite of those that occur in the spring transition period.

The physiological mechanisms involved in the development of persistent corpora lutea during summer have been partly clarified (Kindahl et al., 2000). $PGF_{2\alpha}$ concentrations fail to increase, possibly due to a decrease in uterine sensitivity to oxytocin and/or ability to secrete PGs.

3.2 Tools available to optimise reproduction management

3.2.1 Oestrus detection

The most common method of detecting oestrus in mares is called "teasing"; on exposure to a stallion, the mare exhibits external signs of oestrus. Mares that are not in oestrus will pull back their ears, keep their tails down, and try to kick when approached from behind by an interested stallion. A mare in oestrus will tolerate and may even encourage the advances of a stallion. The mare squats, raises its tail, urinates, everts its clitoris ("clitoral wink"), and stands still, as the stallion calls, nibbles, licks, and even bites or threatens it. Nibbling of the mare's stifles and hocks by the stallion may lead the mare to tilt its pelvis even further. The rounded-back posture (kyphosis) of equine oestrus is unlike the arched-back posture (lordosis) seen in other species (eg, cats, dogs, cattle, and rodents).

The external signs can be subtle early in oestrus, but gradually become more marked as the time of ovulation approaches. Other external stimuli, such as the presence of a foal or an unfamiliar environment, can reduce the demonstration of oestrus signs. Under these circumstances, the judicious use of a "twitch" can lead to these signs becoming more obvious.

While some mares may display obvious signs of oestrus even in the absence of a stallion, detecting oestrus (particularly in the early stages) may be very challenging in the absence of a stallion in "shy" mares. For such mares, palpation of the tonicity of the reproductive tract, as well as ultrasound

scanning of the ovaries and uterus, may provide very valuable information. The observation of a large-diameter follicle (greater than 35 mm in diameter) on one of the ovaries, together with an "orange slice" aspect to the uterine horns (also known as uterine oedema), is clear evidence of oestrus.

3.2.2 Ultrasonography

Ultrasound has been used to monitor follicular growth and diagnose pregnancy since the early 1980s (Palmer and Driancourt, 1980) and is now used routinely by equine veterinarians and on many stud farms. It relies on the fact that the ultrasound emitted by probes bounces back differently depending on whether tissue (grey images) or fluid (eg, follicles - black images) is encountered. Information on tissue density is reflected by differences in the depth of grey visualised. Depending on the type of probe used, it is possible to visualise small, 5- to 10-mm, follicles (high-frequency probes) or medium-sized, 10- to 15-mm, follicles (3-MHz probes). Similarly, by using a high-frequency probe, pregnancy can be diagnosed 1 or 2 days earlier (days 12-13 after ovulation) than with a 3-MHz probe.

During an ultrasound scan, the diameter of the largest follicle on each ovary and the number of follicles in specific size classes are recorded. Daily scans allow the growth of the dominant follicle to be monitored. However, ultrasonography is not able to indicate how close a preovulatory dominant follicle is to ovulation. Monitoring the softening of this follicle by rectal palpation is still the best way to get an insight into the likelihood of ovulation occurring within the next 24 hours; very soft follicles are close to ovulation. However, this technique requires skill so as not to induce ovulation during handling of the ovary through the rectal wall.

3.2.3 Mating

To maximise fertility, mating needs to occur close to ovulation. Oestrus behaviour is not an accurate predictor of the time of ovulation due to the variability in the interval between the beginning of oestrus and ovulation.

Two different strategies are employed. Where there are a limited number of mares scheduled for the season (no more than 40), each mare in oestrus is usually bred every other day until the end of oestrus. Where stallions are

heavily booked (some Thoroughbred stallions mate more than 150 mares during the 6-month breeding season), there is usually allow only one mating per oestrus per mare. Under such conditions, careful ultrasound monitoring of follicular growth, possibly combined with induced ovulation, is usually used to try to make sure that this single mating closely coincides with ovulation.

3.2.4 Artificial insemination

Artificial insemination (AI) is quite common, depending on the studbook and country, and offers clear advantages in terms of management and health. It allows stallions of high genetic merit to be used to breed a larger number of mares. The risk of injury (associated with transport and natural mating) and infection, and the costs associated with transport, are reduced since mares can be inseminated at home. A mare can be inseminated with frozen, thawed semen from a stallion from a different country, which may also have advantages for the gene pool.

AI can be carried out using fresh, cooled or frozen, thawed semen (Table 1). Fresh, cooled semen is used when there is only a short time (up to around 24 hours) between semen collection and insemination. This technique is now well established, and many stallion owners have made fresh, cooled semen available in response to breeder demand. However, not all stallions produce ejaculates suitable for cooling, and the logistics of semen management must be very well managed, owing to the relatively short viability of fresh, cooled semen (24-48 hours). Typically, insemination within around 1 hour of collection uses semen with 500 million progressively motile spermatozoa (PMS), while 1 billion PMS are used for semen stored for 24 hours at 5°C. It is common for fertility to be high (up to 60% of mares pregnant after AI).

The use of frozen, thawed semen allows for a longer delay between semen collection and AI and usually results in acceptable conception rates (Table 1). The use of frozen, thawed semen is generally thought to allow the widest choice of genetics from the best-performing stallions. However, it is critical to remember that the quality of the semen (number and viability of spermatozoa in the straw post-thawing) and the care used in preparing the mare for insemination may strongly modulate conception rates. One of the main reasons that frozen, thawed semen is not used more

widely is individual variability in the capacity of sperm to tolerate freezing and thawing. The semen from only 25% of stallions generates pregnancy rates comparable to those for fresh, cooled semen or natural mating, even when healthy mares are inseminated at the optimal time (Vidament et al., 1997). Mares to be bred with frozen, thawed semen should be monitored beforehand for regular and normal, cyclical reproductive activity. All mares (except maiden mares younger than 6 years old) should have uterine samples taken for cytology and microbial culture at least once. Maiden mares with any evidence of uterine fluid accumulation must undergo the same procedure. Fertility following the use of frozen, thawed semen in aging (older than 12 years of age) mares is better than in the past due to improvements in diagnostic tools and semen freezing, but can still be disappointing.

1. Fresh, cooled semen (after Sieme et al., 2003)

AI to ovulation interval (hours)	Number of cycles	Pregnancy rate (%)
0 to +12	24	45.8
-12 to 0	28	53.6
-24 to -12	88	59.1
-36 to -24	7	28.6
-48 to -36	22	18.2

2. Frozen, thawed semen (after Sieme et al., 2003)

AI to ovulation interval (hours)	Number of cycles	Pregnancy rate (%)
0 to +12	48	50.0
-12 to 0	75	41.3
-24 to -12	26	30.8

Table 1 Links between the interval between insemination and ovulation and fertility following a single insemination with fresh, cooled semen (1) and frozen, thawed semen (2)

It is important that there is very close synchrony between insemination with frozen, thawed semen and ovulation to obtain acceptable conception rates. This is usually achieved by inducing ovulation with human chorionic gonadotropin (hCG) or the gonadotropin releasing hormone (GnRH) agonist deslorelin once a large dominant follicle has been detected by ultrasonography. Ovulation occurs 36-38 hours after treatment, making it quite easy to set the time of insemination to optimise the insemination to ovulation interval. Typically, frozen, thawed semen containing 400 million-800 million spermatozoa is used. There is still some controversy about whether mares should be inseminated just prior to or just after ovulation.

For many years, the standard procedure was to deposit the frozen, thawed semen in the uterine body. However, several groups have reported differences in pregnancy rates when mares were bred with reduced numbers of sperm placed at the uterotubal junction ipsilateral to the ovary that contained the preovulatory follicle. It appears that inseminating deep in the uterine horn or close to the uterotubal junction maximises sperm usage, increasing the number of sperm in the oviduct, which could result in higher pregnancy rates.

The average pregnancy rate per cycle with frozen, thawed semen is around 30%-40% with 1.8-2.0 oestrous cycles per pregnancy. However, it is not uncommon for pregnancy rates per oestrous cycle to vary between 0 and 100% (Loomis, 2001; Samper, 2001). It is recommended that insemination should take place between 6 hours before and 6 hours after ovulation (Samper and Morris, 1998). However, one retrospective study suggested that this did not lead to significant differences in pregnancy rates (Barbacini et al., 1999). More than one insemination results in a slightly, but consistently, higher pregnancy rate than a single insemination (Vidament et al., 1997).

Recently, low-dose insemination and insemination by hysteroscopy have been used. Insemination using low doses of equine semen can be carried out using manual guidance through the rectal wall or by using an endoscope. Insemination by hysteroscopy, using small numbers of fresh or frozen, thawed spermatozoa, has been used for stallions whose semen is in short supply.

3.2.5 Reproductive tools to breed high-value and problem mares

a. Embryo transfer

Embryo transfer in horses is a technique for maximising foal production from mares with high genetic merit. The main candidates for embryo transfer include older mares that are unable to carry a foal, mares that are competing, and very young mares with the highest genetic merit prior to entering the sport. In all cases, embryos are collected nonsurgically (around 6-8 days after ovulation) and transferred to surrogate mares.

The majority of equine embryos collected originate from spontaneous single ovulations. Following collection, by flushing the donor's uterine lumen 7-8 days after ovulation (Squires et al., 2003), the embryos are placed in appropriate culture media, which includes protein and antimicrobial agents, to ensure a high rate of embryo survival and eliminate any bacterial contamination. Morphological evaluation is used to decide whether an embryo is suitable for transfer.

As in other species, the success of embryo transfer is heavily dependent on the management of the recipient. The highest pregnancy rates are obtained when the recipient ovulates 1 day before to 3 days after the donor mare. Donor-recipient synchrony is relatively easily achieved by a single administration of a $PGF_{2\alpha}$ analog administered to the donor and, 1-2 days later, recipient mares.

Using fresh embryos, pregnancy rates of up to around 50% can be obtained in mares. Embryos can be stored at 5°C before transport to another site for transfer into the recipient mare. Embryos can also be stored frozen for extended periods. This is more difficult than in other species due to the capsule surrounding young equine embryos that limits the penetration of cryoprotectants, thereby reducing survival following thawing. Vitrification, a simple cryopreservation process that is used for equine embryos, uses a kit and minimal equipment (reviewed by Carnevale, 2006). The most critical step is the collection of embryos less than 300 nm in diameter, at the morula or early blastocyst stage.

The collection of embryos from unstimulated mares means that usually only one embryo can be collected per mare (Logan et al., 2007; Squires et al., 2003; Squires and McCue, 2007). Superovulation, using equine pituitary extracts, FSH, or combinations of FSH and LH, allows the collection of more embryos per mare per oestrous cycle. However, in contrast to other species, it has proven difficult to increase the number of ovulations above three to five, irrespective of treatment regimen used. There are good responses from some mares (with up to eight embryos); there are also many poor responses (one or two embryos). In addition, even in the mares with multiple ovulations, there is a large gap between the number of ovulations and the number of embryos collected (two to three). This strongly suggests that this type of treatment reduces oocyte quality. Combination treatment with recombinant FSH and LH appears to minimise this gap (Meyers-Brown et al., 2011).

b. In vitro fertilisation, intracytoplasmic sperm injection, and gamete intrafallopian transfer

In vitro fertilisation (IVF), gamete intrafallopian transfer (GIFT), and intracytoplasmic sperm injection (ICSI) have proven difficult to develop for use in mares.

i. In vitro fertilisation (IVF)

There are two main issues facing embryo production, which have limited the number of foals born following IVF in mares. Firstly, the rate of nuclear maturation of oocytes to the stage where successful fertilisation is possible (metaphase II) is far lower in mares than in other species. Secondly, in vitro capacitation of stallion sperm has been difficult to achieve (Allen, 2005).

ii. Gamete intrafallopian transfer

GIFT is a technique that helps to bypass the difficulties linked to IVF in mares. It relies on the collection of an oocyte from a donor mare, maturation of this oocyte in vitro to metaphase stage II, followed by transfer back to the oviduct of a recipient mare. Immediately before intrafallopian transfer of the donor oocyte, the recipient mare is inseminated and its large-diameter follicle aspirated. GIFT is particularly valuable in mares with high genetic merit that have blocked oviducts or damaged uteri and has led to the production of several live foals. GIFT is not in widespread use and is unlikely to become available commercially.

iii. Intracytoplasmic sperm injection

ICSI, a technique where a single spermatozoon is injected into the cytoplasm of a metaphase II oocyte, has helped to solve the in vitro capacitation issue (reviewed by Squires, 2005). Advantages of ICSI include the ability to use frozen, thawed, and even sex-sorted semen. ICSI is not uncommon and may become available commercially.

3.2.6 Pregnancy diagnosis

The two main factors affecting reproductive efficiency are:
1) fertility per oestrus
2) number of oestrous cycles used for breeding

Early pregnancy diagnosis is an essential tool to maximise the number of opportunities for breeding a mare. It is used to detect those mares in which breeding has not been successful. It is also used to detect twins.

There are a number of methods used for pregnancy diagnosis in mares – lack of return to oestrus, measurement of hormone concentrations, and ultrasonography – that are summarised below.

a. Lack of return to oestrus

This method is simple, if there is access to a teaser stallion, but very unreliable, as the intensity of oestrus signs varies considerably between mares. This means that oestrus may not be detected in "shy" mares, and mares with a persistent corpus luteum may wrongly be assumed to be pregnant.

b. Measurement of hormone concentrations

i. Progesterone

Plasma progesterone can be measured by radio-immunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). ELISA kits are available for use in practice. Between 17 and 22 days after ovulation, progesterone concentrations should be greater than 2 ng/mL in pregnant mares and less than 1 ng/mL in nonpregnant mares. One limitation of this approach is that it is indirect; it confirms the absence of pregnancy when concentrations are low. However, high progesterone concentrations may

be associated with a persistent corpus luteum, pregnancy, or sampling the mare outside the appropriate window (17-22 days after ovulation).

ii. Pregnant mare serum gonadotropin

PMSG appears in the blood of pregnant mares around day 40 after ovulation. Peak concentrations (very high but variable) are reached around 60-80 days after ovulation. PMSG concentrations decrease and are undetectable around day 120. Measuring PMSG would have value as a direct test for pregnancy between 40 and 120 days or to confirm pregnancy in mares already diagnosed as pregnant using ultrasonography. However, there is no standard, commercially available test for PMSG. Locally available tests for PMSG should only be used after confirming that they have been validated.

iii. Placental oestrogens

Circulating concentrations of oestrone sulfate increase from day 65 of pregnancy and peak at around day 200, remaining high beyond day 300. The measurement of high oestrone sulfate concentrations is therefore a useful test for confirming ongoing pregnancy. High oestrone sulfate concentrations are also a good indicator of foetal viability. However, there is no standard, commercially available test for oestrone sulfate.

iv. Ultrasonography

Ultrasonography (using a rectal probe) is the most accurate and useful method for diagnosing pregnancy (see Section 3.2.2). It is possible to diagnose pregnancy in mares as early as 13-16 days after ovulation. When using repeated scans, it is possible to measure changes in the size of the embryo and check its growth rate (Bucca et al., 2005). In addition, this method can detect twin pregnancies soon enough to take action.

The ideal method for pregnancy diagnosis in an individual mare should take at least three parameters into account:

1) The time interval between conception and the test (the precocity of the test)
2) The sensitivity, specificity, and positive and negative predictive values of the method selected
3) Whether the test is direct (detection of the embryo) or indirect (where negative results are informative)

Based on this, the optimal test is ultrasonography because it is a direct test with high sensitivity and specificity that can be performed during the first 3 months of pregnancy and provides information on embryo viability.

3.3 Solutions for efficient horse breeding

There are several reasons why it is useful to try to shift the normal pattern of reproduction and breed successfully as early as possible.

3.3.1 Breeding during the transition period

Breeding as early as possible in the breeding season is a prerequisite to maximise reproductive efficiency of maiden and barren mares. The methods used include:

- Altering day length (photoperiod)
- Progestins
- Gonadotropin-releasing hormone

The response depends on the ovarian activity prior to treatment (Squires et al., 1983; Webel and Squires, 1982). There is a better response in the late transition period (ie, after March 15 in the Northern Hemisphere) than in the early transition period. The lowest response is seen in mares in deep anoestrus (Allen et al., 1980). Thus, regardless of the type of treatment used, it must be remembered that only mares in the mid-to-late transition period (generally defined as those with follicles 25 mm in diameter or larger) may respond favourably to treatment.

a. Altering day length

It has been known for more than 30 years that winter anoestrus can be terminated and the time of first ovulation and conception advanced by altering the photoperiod. This is usually done by exposing a mare to artificially long days (at least 14-16 hours of light) for about 60 days, starting from the shortest day (Palmer and Driancourt, 1983). An alternative, more cost-effective and energy-saving regimen uses incremental increases in day length to mimic what occurs naturally. This starts with the addition of, for example, 3 hours of supplemental light in the evening, starting in early December in the Northern Hemisphere, and adds 30 minutes to the day

length every week until a day length of 14-16 hours is reached. The exact length of the photosensitive phase within the day-night time sequence and the minimum level of light exposure needed to achieve the best results are also well established (Nagy et al., 2000). Although light treatment is easy, there is considerable interindividual variation in the interval from starting treatment to first ovulation and to conception. This may be related to the "depth" of anoestrus when this treatment is initiated.

A combination of light exposure and GnRH has been shown to reduce the variation in the response to treatment compared to light alone (Lowis and Hyland, 1991). However, to date, the use of a GnRH analogue has not proven to be cost effective enough for widespread use.

b. Progestins

Progestins have been used widely to hasten the onset of cyclic ovarian activity during the winter to spring transition period in mares for many years (Allen et al., 1980; Squires et al., 1979; Squires, 1993; Webel and Squires, 1982). Progestins prevent irregular oestrus behaviour (without active follicular growth), which commonly occurs during anoestrus and can be very misleading to breeders. Progestins, through negative feedback on the hypothalamic-pituitary axis, prevent the release of LH from the pituitary. FSH concentrations increase midway through treatment (Squires et al., 1983). After treatment, LH is released, supporting follicular growth, initiation of oestrus, and ovulation.

Progestins can either be administered orally or by intramuscular injection. Altrenogest (allyl trenbolone 0.22%, 0.044 mg/kg once daily) can be administered orally for 10 or 15 days to mares with significant follicular activity (ie, with follicles of at least 20-25 mm present at the beginning of treatment) during the transitional period. Any irregular oestrus behavior is suppressed within 1-3 days of starting treatment (Squires et al., 1983). Treatment in the spring transition period significantly increases the size of follicles and shortens oestrus and the interval from ovulation to conception (Squires et al., 1983; Webel and Squires, 1982). This is often combined with day length manipulation (Figure 2). In addition, regular oestrous cycles occur in more mares (75%) when treatment is in the late transition period rather than the early transition period (55%) or untreated controls (57%) (Webel and Squires, 1982). The mean interval to oestrus after the end of treatment

is 4.4 days (Webel and Squires, 1982). Approximately 90% of mares show signs of oestrus within 5 days, and 60% of mares ovulate 11-14 days after the end of treatment. Fertility at the first posttreatment oestrus reaches around 50% to 60% (Allen et al., 1980).

Progesterone-releasing intravaginal devices (PRIDs) developed for use in cattle have also been used in mares (Ataman et al., 2000; Klug and Jöchle, 2001; Handler et al., 2006). This can be associated with low retention rates and vaginal discharge.

c. Gonadotropin-releasing hormone

As winter anoestrus is mainly associated with low LH concentrations, it is tempting to assume that treatment with GnRH may help to initiate cyclic ovarian activity and ovulation during the spring transition period, at the end of winter. However, a major hurdle in implementing this approach is the very short half-life of GnRH (a few minutes) and GnRH agonists (around 1-3 hours). This means that either an injection needs to be administered several times per day for at least the full duration of the growth of the dominant follicle (at least 10 days) or an intravenous infusion used. Recent studies have looked at the use of osmotic mini-pumps (Thorson et al., 2014a, b) and confirmed that continuous GnRH delivery for 28 days, started in February or March, induced ovulation in 60% and 90% of mares, respectively. The intervals to ovulation (19.3 days) and conception (28.6 days) were shorter than in the control group (51.8 days and 65.3 days, respectively). However, only about 50% of the mares that failed to conceive following insemination continued to display cyclic ovarian activity after pregnancy diagnosis confirmed that they were not pregnant (Thorson et al., 2014a, b). Using GnRH is complex and expensive; therefore, methods using photoperiodic stimulation and/or progestins are likely to remain the most cost effective and applicable for managing the spring transition period.

3.3.2 Breeding season - solutions for optimising fertility and obtaining one foal/year

Obtaining a maximum proportion of mares pregnant at the end of the breeding season may be attained by:

- Achieving high fertility per oestrous cycle
- Maximising the number of oestrous cycles used per annum

a. Achieving high fertility per oestrous cycle
This target can be reached by closely synchronising insemination and ovulation and by using high-quality semen.

The first of these two aims may be easily reached by monitoring the growth of the preovulatory follicle using ultrasound and by inducing ovulation (using hCG or GnRH, see Section 3.3.4) when the follicle has reached 30-35 mm in diameter.

Obtaining details on fertility of individual stallions can be a bit more challenging. A few important factors should be remembered. Simple semen treatment (such as dilution before cooling and transport) may strongly affect the fertilising ability of semen from specific stallions. It is always useful to have sound information on the fertilising ability of fresh, cooled and transported semen before choosing to use it. Frozen, thawed semen should not be used in older mares. Reducing the number of straws of frozen, thawed semen used for insemination is a gamble. Repeated insemination should be avoided in mares that are prone to endometritis after breeding.

b. Maximising the number of oestrous cycles used per annum
The optimal strategy depends on the type of mare involved. There are two strategies.

For maiden and barren mares, the following sequence is likely to maximise the number of breeding opportunities per year (as well as getting conception early in the year). The mares should be exposed to long day length, starting in early December in the Northern Hemisphere, to ensure that cyclic ovarian activity has been initiated by the middle of February. Following the detection of oestrus, together with a soft preovulatory follicle (ultrasonography and palpation), hCG (1500-2500 IU) can be used to induce ovulation, with a single insemination performed 30 hours after treatment (ie, 6-10 hours before ovulation). Ultrasonography should be conducted 14-17 days after ovulation so that any mares that are not pregnant can be bred again as soon as they return to oestrus. For the induction of ovulation at this second oestrus, hCG or a GnRH analogue may be used. Mares that are diagnosed pregnant at the first ultrasound scan should have this confirmed before day 30-35 of gestation. Specific attention should be paid to the possible presence of twins during any ultrasound examination.

For mares that foal during the breeding season, the challenge is to get them pregnant again within a limited time frame (from foaling to the end of the season). There are two strategies that are usually helpful. The first is to breed the mare at the foal heat (to minimise the foaling to conception interval), acknowledging the risk of lower fertility associated with this heat. Early pregnancy diagnosis using ultrasonography (14-17 days after ovulation) is an essential part of this strategy so that there can be an immediate response to any mare that is not pregnant. The second strategy allows the mare to have a foal heat and ovulate without being bred, with the useful associated effect of oestrogens on the quality of the uterine environment, and develop a corpus luteum. PG can then be administered 5-7 days after ovulation, leading to luteolysis and regression of the corpus luteum (Oxender et al., 1975). This is followed within 3 days by oestrus at which the mare can be bred. The remaining steps are similar to those described in the first strategy.

There are two factors that veterinarians can use to help make the best choice. Firstly, the later in the breeding season the mare foals, the stronger the incentive to breed during the foal heat. The older the mare is, the more the second strategy is favored, based on slower uterine involution in these animals. In addition, the second strategy should be used for any mare with uterine fluid accumulation or uterine pathology.

3.3.3 Solutions for synchronising oestrus in groups of mares

Synchronising oestrus in groups of mares is used either as a management aid (to minimise the number of trips to the stud) or for embryo transfer (to synchronise donor and recipient mares). There are two main strategies.

a. Progestins

Synchronisation of oestrus is usually done using a progestin (synthetic or natural) for 10-12 days. This relies on the inhibitory effects of progestins on LH, leading to regression of follicles larger than 25 mm in diameter (Driancourt and Palmer, 1982). This follicular turnover is associated with a rise in FSH concentrations in the last days of treatment. This initiates a new wave of follicular growth. The rise in LH concentration triggered by the end of treatment supports follicular growth and the emergence of a dominant

follicle. The maximum size of a follicle at the end of treatment is inversely related to the interval to ovulation and can be used to predict when ovulation may occur.

Synchronisation of oestrus mares may be achieved by administering progesterone (using a PRID developed for use in cattle, a progestin (eg, daily oral administration of altrenogest at 0.044 mg/kg), or a controlled internal drug release (CIDR) device developed for use in cattle) for 10-12 days. At the end of treatment, PG is administered by injection to induce regression of the corpora lutea resulting from ovulations in the days preceding the start of treatment. Most mares come into oestrus within 3 days. The interval between the end of treatment and ovulation is longer early in the breeding season (10-14 days) than later in the breeding season (5-8 days) (Handler et al., 2005). Ovulation is generally spread over 4 days. If tighter synchronisation is needed, hCG (1500-2500 IU intravenously) can be administered once a follicle greater than 35-40 mm in diameter is detected.

b. Prostaglandins

Two PG injections 14 days apart may also successfully synchronise groups of mares (Palmer, 1978). This is because the corpus luteum in mares becomes sensitive to exogenous $PGF_{2\alpha}$ by the fourth day after ovulation and is fully responsive to its luteolytic effects when 6 days old (Meyers, 1991). Mares that have a PG-sensitive corpus luteum at the time of the first PG injection will also have a PG-sensitive corpus luteum 14 days later, at the time of the second injection. Furthermore, mares in the follicular phase or that have just ovulated at the time of the first PG injection will have a PG-sensitive corpus luteum 14 days later, at the time of the second injection. This method of synchronisation relies on the manipulation of luteal function, meaning that all treated mares must be cycling for it to be effective. At the beginning of the breeding season, an injection of hCG (1500-2500 IU) 6 days after the first PG injection may increase the success of this method of synchronisation.

3.3.4 Solutions for inducing ovulation and timing insemination

In contrast to other species, oestrus in mares is long and very variable in duration, lasting 8-14 days early in the breeding season and 5-7 days later in the breeding season. Moreover, the beginning of oestrus does not allow the

timing of ovulation to be predicted. The fertility of mares is optimal when the interval between AI and ovulation is minimal.

Several strategies for breeding can be applied based on the availability of a stallion/semen and the technical skills of the breeding team:

1) The mare is bred every other day from the start of oestrus.
2) Growth of the ovulatory follicle is monitored every other day, and the mare is bred twice, starting on the day that a large-diameter (greater than 35 mm), soft follicle is detected (by palpation or ultrasound) and again 36-48 hours later.
3) Growth of the ovulatory follicle is monitored every other day. When a large-diameter (greater than 35 mm), soft follicle is detected, ovulation is induced (as described below) and the mare bred once 24-36 hours later. This approach has many advantages, including:
    a) Maximising fertility, owing to the close synchrony between AI and ovulation (Woods et al., 1990, Grimmett and Perkins, 2001)
    b) Allowing easier access to highly popular stallions
    c) Gaining access to most reproductive biotechnologies (eg, frozen, thawed semen, embryo transfer) by increased synchrony between AI and ovulation
    d) Reducing the time difficult mares spend away from home and at the stud

GnRH analogues and hCG have been used for many years to induce ovulation in mares (reviewed by Samper, 2008, and Squires, 2008).

a. Human chorionic gonadotropin (hCG)

The optimal therapeutic dose of hCG is 2500 IU administered intravenously, although lower dosages (1500 IU) have also been shown to work well in some studies (Grimmett and Perkins, 2001). A prerequisite for the full efficacy of hCG is the presence of a large-diameter (usually 35 mm or greater), LH-sensitive follicle on one ovary. Ovulation occurs within 36-48 hours in over 80% of these mares (Barbacini et al., 2000; Grimmett and Perkins, 2001).

While hCG is a potent and reliable inducer of ovulation, it is a human protein and has a long half-life and therefore needs to be used with caution, as it has the potential to trigger neutralising antibody formation following repeated administration (Roser et al., 1979). No such antibodies could be detected in 7 out of 12 mares following repeated administration of therapeutic doses (1500-3000 IU) in six consecutive cycles. However, antibodies to hCG were detected in 5 out of the 12 mares—in 1 mare following five, four, and three injections and in 2 mares following two injections. Interestingly, the presence of antibodies to hCG did not prevent ovulation following the endogenous LH surge (because there was no binding to endogenous LH) or appear to interfere with fertility. Whether antibodies to hCG develop under field conditions (ie, following one or two injections per year) has never been clearly demonstrated. However, the observation that hCG is less efficient in older mares suggests that this may be the case (Barbacini et al., 2000). It is recommended that hCG be used once or twice per breeding season, with focus on the beginning of the season, when its direct LH-like effects on the ovulatory follicle may be better than alternative treatments, such as GnRH agonists, which need a fully functional pituitary to generate a normal LH surge.

b. Gonadotropin releasing hormone (GnRH)

GnRH and its analogues are also used to induce ovulation in cyclic mares. A number of different treatment regimens have been evaluated: intermittent injection (Barrier-Battut et al., 2001; Bott et al., 1996; McKinnon et al., 1997), pulsatile administration (Becker and Johnson, 1992; Johnson, 1986), sustained-release implants (Meyers et al., 1997), and a single injection of a high dose (Levy and Duchamp, 2007). Most mares treated intravenously with buserelin (0.02 or 0.04 mg) twice daily for 4 days ovulated within 48 hours (Barrier-Battut et al., 2001). Ovulation was observed in 42.8% of mares with follicles 32 mm or greater in diameter administered buserelin (0.04 mg) intravenously twice daily until ovulation (Camillo et al., 2004). The number of mares ovulating within 48 hours after treatment increased to 97.6% when hCG (2500 IU intravenously) was administered. A single administration of a very high dose of buserelin (6 mg) induced ovulation in mares during the breeding season, but the cost of this approach is very high and may prevent its use under field conditions (Levy and Duchamp, 2007).

An implant containing the GnRH analog deslorelin has been approved for the induction of ovulation in mares. It is indicated for use in mares displaying behavioural oestrus and with a follicle of 30 mm or greater in diameter (McKinnon et al., 1993, 1997). While this appears to yield a similar response to hCG, in terms of ovulation and fertility, none of the mares treated with the sustained-release implants became pregnant, and return to oestrus was delayed significantly and interovulatory interval prolonged (Vanderwall et al., 2001). In another study, hCG and deslorelin (implant or injection) produced a similar and acceptable response in terms of efficacy and interval to ovulation (within 2 days of treatment) that is suitable for use under field conditions (Berezowski et al., 2004). To avoid a delayed return to oestrus in nonpregnant mares, it is recommended that the deslorelin implant be removed as soon as ovulation has occurred.

Recombinant equine LH has been tested in mares, following improvement in the production yield of the carbohydrate-based recombinant protein technologies (Yoon et al., 2007). Doses of 0.75-0.90 mg intravenously were needed to consistently induce ovulation. Recombinant LH is not available commercially.

There is a better understanding of the mechanisms controlling GnRH secretion in the brain (see Section 3.1.3 of this chapter and Chapter 1, Section 1.4.2), including the role of the hypothalamic kisspeptin (Kp) family of peptides in the stimulation of GnRH neurons. However, two recent studies failed to demonstrate that injection of Kp was able to trigger ovulation in mares when administered during the follicular phase (Decourt et al., 2014; Magee et al., 2012).

3.3.5 Solutions for breeding late-foaling mares efficiently during early summer or late autumn

Breeding mares during early summer or late autumn is uncommon, since all barren and maiden mares are usually bred as early as possible in the breeding season. The only population likely to be bred at this stage is mares that have foaled late in the breeding season. Breeding mares at these times of year is only difficult if a persistent corpus luteum develops following ovulation at the foal heat or if the mare does not conceive following breeding and does not return to oestrus, due to a persistent corpus luteum. It

is recommended that pregnancy diagnosis should be carried out early (by ultrasound on day 14 after ovulation) for mares bred late in the breeding season. All nonpregnant mares should be treated immediately using a luteolytic dose of $PGF_{2\alpha}$ (eg, cloprostenol) and mated when they return to oestrus. If PG treatment is successful, ovulation occurs on average 6.8 days later (Loy et al., 1979). Mares with a follicle of 40 mm or greater in diameter had the greatest variance in time to ovulation due to regression of large follicles and later ovulation of a succeeding follicle.

3.3.6 Solutions for twin pregnancies

Twin pregnancies can occur. Double ovulation is common in some mares during the second part of the breeding season. However, the chance of this resulting in twin pregnancy decreases sharply if the two ovulations are 2 days apart rather than synchronous. If double ovulation is detected at the time of breeding, it is of utmost importance that pregnancy diagnosis is conducted as early as possible (by ultrasound on day 14 after ovulation) to confirm whether there is a twin pregnancy. In twin pregnancy there is either one conceptus in each uterine horn or two conceptuses (generally next to each other) in the same uterine horn. In the first situation, one of the two embryonic vesicles can be compressed manually through the rectal wall. The smaller the conceptuses are, the lower the risk of damage to the second conceptus. This is why early pregnancy diagnosis and treatment is recommended. In the second situation, it is usually recommended that the ultrasonography be repeated after a few hours to days, since embryonic vesicles move freely around the uterus during early pregnancy. It is therefore possible that the first situation (one conceptus in each uterine horn) may be seen at the follow-up examination. If not, it may be possible for an experienced veterinarian to manually compress one of the two embryonic vesicles in a single uterine horn, but it may be tricky to avoid damaging the second embryonic vesicle. An alternative approach, if a twin pregnancy is detected early in the breeding season, is to terminate that pregnancy using a luteolytic dose of $PGF_{2\alpha}$, such as cloprostenol. However, this approach carries the risk of a repeat twin pregnancy.

Twin pregnancy should always be monitored and treated carefully. Twin pregnancy very often ends up in the abortion of both foetuses. When this happens after day 45 of pregnancy (when PMSG production is initiated),

the mare does not return to oestrus following the abortion and remains barren for that year due to presence of endometrial cups. If twin pregnancy goes undetected and is not treated appropriately, natural embryonic death or abortion of one of the twin foals can occur during midpregnancy, but its occurrence is unpredictable. When twin foals are carried to term, they are usually much smaller than a single foal, and this may severely compromise survival and performance.

3.3.7 Solutions for inducing parturition

Owing to the large variability in the duration of pregnancy, it is very difficult to predict when foaling may occur. Approximately 85% of foals are born during the night. It is essential for the breeder to be present, as this allows parturition to be monitored and a veterinarian called in a timely fashion when needed. It also ensures that, once outside the mare, the foal is freed from the placenta.

Inducing parturition is an approach that leads to foaling within a narrow timeslot, thereby avoiding long nights of waiting. Furthermore, parturition may need to be induced in mares with serious problems (eg, colic, endotoxaemia) around the time that foaling is expected. However, this carries the not-insignificant risk of delivering a premature foal (that will struggle to survive or die) if carried out too far in advance of the time that natural parturition would have occurred. Classic signs of immaturity, which can rapidly lead to foal mortality, include:

- Inability or difficulty standing or remaining standing
- Incomplete maturation of the lungs
- Incomplete maturation of the gastrointestinal tract

The prerequisites for the induction of parturition with minimal risk are as follows:

- The mammary glands should be developed and contain colostrum. This is the most important criterion.
- The calcium content of the mammary secretion is a useful predictor of the foal's readiness to be born. In 95% of spontaneously foaling mares tested 12 hours before foaling (using a test strip for assessing the hardness of water), calcium concentrations were in the range of 180-280 parts per million.

- A change in the color of the colostrum from clear to white is also a good indicator that foaling is close enough for parturition to be induced.
- Gestation should have been at least 320-330 days. The previous gestation(s) is a good indicator of a sufficient length of gestation.
- The cervix and the sacro-ischiatic ligaments should have softened.

A number of different agents, sometimes in combination, can be used to induce parturition.

a. Oxytocin

Oxytocin concentrations increase during parturition. Oxytocin increases myometrial contractions. Injection of oxytocin provides a reasonably reliable and rapid (within 90 minutes) means of inducing parturition. Intravenous doses of oxytocin as low as 1 IU appear to be efficient and safe for the induction of parturition in mares at term (Camillo et al., 2000). Subcutaneous doses of 10-20 IU of oxytocin at intervals of 15-20 minutes can also be used. Administration of doses in excess of 60 IU is not recommended, as it carries a number of potential risks, including causing considerable distress to the mare.

b. Prostaglandins

Intramuscular doses of $PGF_{2\alpha}$ (eg, 0.25 mg cloprostenol) mimic what occurs naturally just before foaling and can be used to induce parturition. Safety, assessed by the absence of side effects (such as abdominal discomfort, sweating, and nervousness), is usually better for analogs than for natural $PGF_{2\alpha}$. However, due to the potency of PGs, the timing of administration has to be selected with extreme caution. Induction of foaling prematurely may have very serious consequences for the foal.

c. Other

Combinations of cloprostenol and oxytocin may also be used, although combined treatment does not always have clear benefits.

Glucocorticoids are not as effective as they are in some other species. In addition, complications, such as weak foals, prolonged parturition, dystocia, and poor milk production, have been reported.

3.3.8 Solutions for suppressing oestrus in competing mares

Oestrus behaviour can sometimes be a problem in show mares. The progestin altrenogest exerts negative feedback on the pituitary to reduce LH concentrations, thereby acutely reducing oestradiol production by the dominant follicle and thereafter its regression. Altrenogest may be given for short periods (eg, 10-15 days), corresponding to the timing of training or competitions. Oestrus is usually suppressed within 3 days of starting treatment. However, oestrus will usually resume within a few days (7-10 days) after treatment is discontinued. It is important that any product used in a competition horse is in compliance with local and/or sport governing body regulations.

3.4 Reproductive pathologies: prevention and treatment

3.4.1 Endometritis and endometriosis

The majority of mares that do not become pregnant after breeding have an endometrial disorder. Degenerative changes in the uterus are associated with advancing age, and bacterial and other infections can lead to inflammatory changes. The most critical factor in the uterine defenses against infection is the rapid, physical clearance of inflammatory debris from the uterus. Changes in the conformation of the vulva predispose mares to uterine infection. A number of other defects can interfere with uterine drainage and thus play an important part in the pathogenesis of endometritis (Hurtgen, 2006). Repeated breeding and foaling can lead to anatomical defects, such as poor perineal conformation, an incompetent vagino-vestibular sphincter, vaginal stretching, an incompetent cervix, or a pendulous uterus, as well as degenerative changes, such as an abnormal myometrium, periglandular fibrosis, vascular degeneration, lymphangiectasia, scarring and atrophy of endometrial folds, or damage to the mucociliary apparatus.

Contagious equine metritis (CEM) also plays an important role (see *Taylorella equigenitalis* in Section 3.4.2).

a. Endometritis

Breeding-induced endometritis is a normal physiologic reaction that does not require treatment unless it is persistent. After breeding, there is an inflammatory and immune response, which is necessary to remove any contaminating bacteria and (mainly dead and dying) spermatozoa (reviewed by LeBlanc and Causey, 2009, and Watson, 2000). Inflammatory cytokines are released around 2 hours after breeding in response to the presence of bacteria and spermatozoa. This leads to the recruitment of polymorphonuclear leukocytes and subsequently to the release of $PGF_{2\alpha}$. This, combined with the oxytocin released during breeding, leads to uterine contractions, which aid in the expulsion of excess semen and debris. In the normal, healthy equine uterus, uterine inflammation and contractions return to baseline by around 12 hours, and the uterus is primed and ready to receive the conceptus by around 24 hours after breeding.

b. Persistent breeding-induced endometritis

Persistent breeding-induced endometritis (PBIE) reduces conceptus viability and impairs fertility and is thought to occur in 10%-15% of mares (reviewed by Woodward and Troedsson, 2013). Susceptible mares appear to have an altered innate uterine immune response and impaired clearance of debris from the uterus (reduced opsonisation of bacteria and spermatozoa and reduced coordinated contractile activity (Card, 2005)). Affected mares have been shown to have higher levels of inflammatory modulating cytokines (such as interleukin (IL) 10 and IL6) within 6 hours after breeding compared to other mares. Inflammation persists because clearance mechanisms do not function optimally, and this is accompanied by prolonged influx of lymphocytes and plasma cells, possibly contributing to chronic degenerative changes and further impairment of endometrial function. If left untreated, inflammation persists for more than 5 days and the conceptus arrives into an inflamed and unsuitable uterine environment.

A variety of factors appear to contribute to the susceptibility of a mare to PBIE, including age (older than 12-14 years), conformation (eg, poor perineal conformation, dependent uterus), and endometrial health (eg, scarring from previous PBIE). Mares with PBIE do not generally show clinical signs, although vaginal discharge, a shortened luteal phase, and decreased fertility can be observed.

i. Diagnosis

PBIE is diagnosed based on the demonstration of endometrial inflammation and fluid accumulation in the uterus 3-5 days or more after breeding. The presence of persistent endometritis should be confirmed by endometrial cytology on samples taken by uterine swab (double or single guarded), brush biopsy, or low-volume lavage. There are several schemes for interpreting cytological findings; however, the presence of >5% neutrophils is generally considered to be indicative of endometrial inflammation (Card, 2005). Histologically, endometritis is characterised by infiltration by polymorphonuclear leukocytes (PMN), lymphocytes, and macrophages into the endometrium (Card, 2005; reviewed by LeBlanc and Causey, 2009). The histopathological changes can be classified as inflammatory (acute, subacute, or chronic) or noninflammatory, including endometrial hypoplasia and hyperplasia. Severe chronic degenerative changes, such as elastosis, lymphangiectasia, excessive exudate, loss of epithelium, and epithelial hyperplasia, will interfere with a mare's ability to become pregnant. Retention of fluid within the uterus can be confirmed by ultrasound.

ii. Treatment

Improving pregnancy rates in PBIE-susceptible mares requires the early detection of inflammatory changes and fluid accumulation using rectal palpation, ultrasonography, and/or endometrial cytology followed by timely intervention. Mares presented for breeding that are considered to be susceptible to PBIE should be examined by ultrasound every day or every other day during oestrus and especially for 1-2 days after breeding to identify any patterns of uterine oedema, determine the presence and location of intrauterine fluid accumulations, monitor response to treatment, and record ovulation. The decision to treat a mare for persistent uterine inflammation after breeding should be based on history and clinical signs, such as poor uterine tone, intrauterine accumulation of fluid, exfoliate endometrial cytological evaluation, and bacterial culture and sensitivity. This is generally aimed at assisting the uterus to clear inflammatory debris and other contaminants. Other therapeutic objectives include the correction of defects in the uterine defenses, neutralising virulent bacteria, and controlling postbreeding inflammation (reviewed by LeBlanc and Causey, 2009). The type of treatment depends

on how marked the inflammation is and how much fluid has accumulated in the uterus.

A variety of strategies are used in the management of endometritis. These include uterine lavage to physically clean the uterine lumen, ecbolic agents to remove fluid, antimicrobial agents to stop bacterial growth or kill bacteria, administration of mucolytic drugs to break up mucus associated with bacterial infection, and surgical correction of the reproductive tract to assist in preventing the entrance of bacteria (reviewed by Woodward and Troedsson, 2013). These different modalities are used either prophylactically or as treatment and are often used in combination, with the dose, frequency, duration of treatment, and administration route of agents administered varying considerably. Embryo transfer could offer a means of obtaining foals from mares with chronic endometritis, a history of repeated early embryonic death or abortion, and nonresponsive PBIE.

- Uterine lavage
  Uterine lavage assists in the removal of contaminated uterine contents and stimulates uterine contractions. Uterine lavage can be performed prebreeding or postbreeding. It is most commonly performed in repeat breeder mares and in mares with uterine fluid accumulation that is greater than 20 mm in diameter. Uterine lavage conducted as early as 4 hours postbreeding appears to have no harmful effect on pregnancy rates. To perform the lavage, 1-2 L of warmed isotonic saline or other balanced salt solutions, such as lactated Ringer's solution, are used and infused through a large-bore catheter (eg, 8 mm) (reviewed by Brinsko et al., 2011). Dilute povidone iodine solutions can also be used (eg, 10 mL of 5% or 5 mL of 10% povidone iodine in 1 L sterile saline or lactated Ringer's). The uterus can also be massaged per rectum to facilitate lavage.

- Oxytocin
  If inflammation is relatively mild, then one or two injections of oxytocin can be administered at 4-6 hours and 8-12 hours after breeding (Pycock and Newcombe, 1996a; reviewed by Watson, 2000). Factors that may affect the response to oxytocin treatment include an

inadequate number of endometrial receptors, a pendulous uterus, a closed cervix, and an excessive dose resulting in inappropriate uterine contractions, the abnormal propagation of uterine contractions, or prolonged inflammation.

If inflammation is more severe or is prolonged (>24 hours) and/or there is fluid accumulation, then oxytocin (20 IU intravenously or 20-40 IU intramuscularly) may be used to facilitate uterine contractions and evacuation of fluid from the uterus (reviewed by Brinsko et al., 2011), in combination with uterine lavage and/or PG treatment.

- Prostaglandins
  During oestrus the uterus is better able to fight infection. Administration of a PG only causes luteolysis and regression of the corpus luteum, followed 3 days later by oestrus, if it is administered more than 5 days after ovulation (Oxender et al., 1975). Administration of a PG (eg, cloprostenol) within 12 hours of ovulation does not result in luteolysis but is effective in eliminating accumulated uterine fluid. However, this should be done with care since PG administration can alter the developing corpus luteum and progesterone production as well as pregnancy rates (Brendemuehl et al., 2002; Troedsson et al., 2001). It has been suggested that progesterone supplementation be considered in mares where repeated PG administration is required.

- Antimicrobial agents and disinfectants
  The use of antimicrobial agents is controversial (in both treatment and prophylaxis) since it has not been shown to be superior to uterine lavage alone. Antimicrobial agents should be selected on the basis of culture and susceptibility testing. Prior to instilling an antimicrobial agent into the uterine lumen, uterine lavage should be carried out to remove any exudate present in the uterine lumen, which may dilute or inactivate the antimicrobial agent. Antimicrobial treatment is usually infused daily for 3-5 days during oestrus. Treatment during dioestrus should be avoided, since treatment during the progesterone phase has been associated with the development of resistant bacterial and fungal infections (McDonnell and Watson, 1992). Local instillation of a disinfectant or antimicrobial agent into the uterus can induce

severe local reactions, resulting in persistent fibrosis and intrauterine adhesions. If irritation or hypersensitivity is suspected, the uterus should be lavaged with large volumes of distilled water.

Systemic antimicrobial agents have also been used to treat endometritis. Good results have been demonstrated using the combination of an antimicrobial agent and oxytocin therapy, with pregnancy rates higher than those achieved using intravenous oxytocin or intrauterine antimicrobial agents alone (Pycock and Newcombe, 1996a).

- Other medical treatment
  Corticosteroids have anti-inflammatory and, at higher doses, immunosuppressive actions. A number of studies have been conducted looking at the use of corticosteroids in mares susceptible to PBIE (reviewed by Woodward and Troedsson, 2013). For example, prophylactic administration of 50 mg of dexamethasone intravenously at the time of breeding has been shown to reduce PBIE but did not appear to have effects on white blood cell migration or phagocytosis (Bucca et al., 2008).

  Mycobacterial cell wall extract (MCWE) has been studied experimentally and appears to be potentially beneficial in terms of normalising the uterine inflammatory cytokine response and improving fertility (Rogan et al., 2007).

  There is insufficient data from controlled clinical studies to support the use of corticosteroids or MCWE in the treatment of PBIE (reviewed by Woodward and Troedsson, 2013).

- Vulvoplasty
  Vulvoplasty (also known as Caslick's procedure) is a well-established, simple surgical procedure that has been used, since it was first described in 1937, to aid in the prevention of air, fecal debris, and other external contaminants from entering the reproductive tract.

c. Endometriosis

Endometriosis, or chronic degenerative endometrial disease, is the term used to describe degenerative changes in the endometrium that are seen in older mares. It is considered to be one of the most important causes of infertility, especially in older mares, but little is known about the aetiology and pathogenesis. If severe, it may result in delayed clearance of the uterus after breeding.

Endometriosis can be destructive or nondestructive. The various types of endometriosis appear to represent different stages of a fibrotic process, possibly leading to glandular destruction followed by the development of stromal fibrosis. It has been suggested that fibrotic foci are independent of the hormonal control of the uterus, since cyclic and seasonal endocrine changes seem to have no effect on progression (Hoffmann et al., 2009). The degree of endometriosis increases with age but does not appear to be associated with the number of foalings (Ricketts and Alonso, 1991).

i. Diagnosis

Endometriosis is diagnosed based on endometrial biopsy. Degenerative change of the uterus, such as active or inactive fibrosis around the uterine glands and in the endometrial stroma, cystic dilation of endometrial glands, and glandular necrosis, can be seen on histopathology (Schoon et al., 1992). The first sign of endometriosis is atypical morphological and functional differentiation of periglandular endometrial stromal cells. There are often two to three layers of fibrotic tissue around the glands, but there can be as many as ten layers in severe cases. The initial stage of fibrosis is characterised by the presence of large, polygonal, periglandular stromal cells (type I) that produce collagen. In advanced fibrosis, the histological picture is dominated by metabolically active or inactive stromal cells (type II), without signs of collagen synthesis, as well as myofibroblasts. The contractility of the latter may lead to constriction of the uterine glands, resulting in glandular dilatation. Additionally, myofibroblasts may be able to affect the composition and extent of the extracellular matrix by secreting different mediators.

An internationally accepted scoring system (Kenney and Doig, 1986) can be used to help assess whether a mare is likely to conceive and carry its foal to term (Table 2).

Mare category	Degree of endometrial pathology	Expected foaling rate
I	Absent	80%-90%
IIA	Mild	50%-60%
IIB	Moderate	10%-50%
III	Severe	<10%

Table 2 Expected foaling rates in mares based on the histological classification of the endometrium

ii. Treatment

Endometriosis appears to be irreversible. A number of different treatments have been tried, including physical and chemical curettage (eg, dimethyl sulfoxide). These approaches aim to induce transient superficial tissue damage (ie, inflammation, necrosis, and tissue loss) in the hope that healing will produce a uterus that is more able to support a normal pregnancy. However, there have been few, if any, studies that have critically evaluated treatment under controlled conditions, particularly in terms of improved endometrial structure and/or function after treatment (reviewed by Holyoak and Ley, 2007). Moreover, care should be taken to avoid aggressive physical or irritant chemical curettage, as the potential benefits and long-term complications (eg, adhesions) of these approaches have not been studied.

3.4.2 Early embryonic death and abortion

Early embryonic death is generally defined as loss of pregnancy during the first 40 days of gestation, whereas abortion is used to describe the loss of pregnancy between days 40 and 300.

a. Early embryonic death

Early embryonic death, generally ranging in frequency from 5% to 15%, is assessed by measuring losses occurring between the first diagnosis of pregnancy and follow-up examination at around day 40. This has been confirmed in large-scale studies, such as a study in 3,740 mares where the overall embryonic mortality, based on examinations between days 22 and 44, was 8.9% (Chevalier-Clément, 1989). Another large study (in a group of 1,393 mares monitored closely throughout pregnancy) showed that 63% of all pregnancy losses occurred between days 15 and 45 (Morris and Allen, 2001). A much higher incidence was seen in some specific categories of mares (24.4% in mares with endometrial cysts and 34.8% in mares with an abnormal conceptus) (Chevalier-Clément, 1989).

The timing of breeding in relation to ovulation is important for the prevention of early embryonic death (see Section 3.2.3).

b. Luteal insufficiency

Adequate progesterone concentrations are essential for the maintenance of pregnancy to term. There is no clear evidence that primary luteal insufficiency causes early embryonic death and pregnancy loss prior to day 25 in the mare, although there is evidence in other species. Endometrial oxytocin receptors reappear around day 18 (Stout and Allen, 2001). There is no luteotropic support for the corpus luteum from day 18 until the beginning of PMSG secretion at day 38-40, and the corpus luteum is susceptible to luteolysis during this period, which is also when many equine pregnancies fail.

In experimental paradigms of luteal inadequacy, triggered by PG release (eg, associated with endotoxaemia in colic) during the first 40 days of pregnancy, reduced progesterone concentrations lead to luteolysis and pregnancy loss (Daels et al., 1987).

i. Treatment

Despite the lack of evidence for early luteal insufficiency in mares, attempts to support luteal function through either progestin supplementation or the induction of additional corpora lutea are common In fact, progestins (altrenogest) are used more widely in an attempt to maintain pregnancy in mares than in any other species (Allen, 2001b; Canisso et al., 2013a).

A GnRH analogue can be administered 11-12 days after breeding in an attempt to induce additional corpora lutea. A single intramuscular administration of buserelin (0.02-0.04 mg), a synthetic GnRH analog, during the late luteal phase (8-12 days after breeding) has been shown to improve pregnancy rates by around 10% at day 28-30 in mares (Newcombe et al., 2000). Buserelin, administered 9-10 days after detection of ovulation, has also been shown to increase pregnancy rates at 12-13 days compared to untreated controls without differences in the progesterone concentration (Jackson et al., 1986; Newcombe and Peters, 2014). Improvement in pregnancy rate was the same whether buserelin was administered intramuscularly on day 10 or 11 or subcutaneously on day 8 (Pycock and Newcombe, 1996b).

Treatment with GnRH in late dioestrus, before the luteolytic signal is triggered, may perhaps prevent luteal regression in mares in which the embryo alone is incapable of eliciting the proper signal for the maternal recognition of pregnancy.

c. Mare Reproductive Loss Syndrome

Mare Reproductive Loss Syndrome (MRLS) was first observed and described in the spring of 2001, following the loss of more than 4,500 foals due to early foetal loss, late-term abortions, stillbirths, and neonatal deaths on central Kentucky horse farms (reviewed by Sebastian et al., 2008). Similar syndromes were described in Australia in 2004 and in Florida and New Jersey in 2006. Both the early and late foetal losses were characterised by the absence of specific clinical signs in aborting mares. It is also now generally accepted that ophthalmic and cardiac disease also form part of this syndrome (reviewed by Gwaltney-Brant, 2012).

The pathogenesis of MRLS is still unclear. In the United States, there appears to be a temporal correlation between MRLS and the presence of Eastern tent caterpillars (*Malacosoma americanum*), wild black cherry trees (*Prunus serotina*), and waterfowl and the practice of feeding hay off the ground (reviewed by Gwaltney-Brant, 2012; McDowell et al., 2010). In Australia, this appears to have been linked to processionary caterpillars (*Ochragasterlunifer* species). Affected mares have decreased concentrations of conjugated oestrogens, suggesting that the chorionic portion of the placenta is the target, supported by the fact that pregnancies of less than 35

days (prior to placental development) were largely unaffected (Volkmann et al., 2008).

d. Abortion

Loss of pregnancy can be due to infectious or noninfectious (eg, twinning) causes or of unknown origin. Viral, bacterial, and parasitic infections of the equine reproductive tract are a common cause of infertility, abortion, and premature births. Some of the most common infectious causes of abortion and infertility in mares are listed in Table 3.

Viral	Bacterial	Parasitic
Equine herpesvirus-1 Equine viral arteritis virus	*Streptococcus equi* subsp. *zooepidemicus* *Taylorella equigenitalis*	*Trypanosoma equiperdum*

Table 3 Infectious causes of infertility and abortion in horses (not luteal dependent during middle and late foetal development) (Adapted from Givens and Marley, 2008)

Other bacteria associated with abortion in mares include *Actinobacillus equuli, Escherichia coli,* and *Pseudomonas, Klebsiella, Enterobacter, Leptospira,* and *Chlamydia* spp. Fungal infection can also cause abortion and infertility in mares. Abortion rates between days 44 and 310 of pregnancy are generally around 9%.

i. Viral

- Equine herpesvirus

  Equine rhinopneumonitis is the collective term used to describe highly contagious clinical disease in horses due to infection by equid herpesvirus-1 (EHV-1) or EHV-4, two closely related alphaherpesviruses of the horse. EHV-1 and EHV-4 are enzootic in all countries where there are large populations of horses. Equine rhinopneumonitis is highly contagious among susceptible horses, with transmission occurring by inhalation of an aerosol of virus-laden respiratory secretions. The viruses infect and multiply in epithelial cells of the respiratory mucosa. Clinical signs become apparent 2-8 days after exposure and are characterised by fever, inappetence, depression, and nasal discharge. The severity of respiratory disease varies with the age

and immunological status (eg, vaccination, previous infection) of the horse. Generally, recovery is uncomplicated and occurs within 1-2 weeks. EHV-1 can spread beyond the respiratory tract and is the most important viral cause of abortion in horses, often at more than 7 months of gestation and without any previous clinical signs. The placenta may be oedematous or normal, while the aborted foetus may exhibit subcutaneous oedema, jaundice, increased thoracic fluid volume, and/or an enlarged liver with yellow-white lesions approximately 1 mm in diameter. Histologically, there are areas of necrosis and characteristic intranuclear inclusions. Necrotising bronchiolitis is also a common finding. EHV-1 can also cause perinatal foal death and/or neurological dysfunction (myeloencephalopathy). Recovered animals can remain latently infected. Diagnosis is performed using direct immunofluorescent detection of viral antigen or by virus isolation, eg, on tissues from an aborted foetus. Polymerase chain reaction (PCR) and immunoperoxidase staining methods are also available. Prevention of equine rhinopneumonitis is based on biosecurity and vaccination. For a review of equine rhinopneumonitis, see the World Organisation of Animal Health (OIE) (2008).

- Equine viral arteritis

  Equine viral arteritis (EVA) is an acute, contagious, viral disease caused by equine arteritis virus (EAV), an RNA virus belonging to the genus Arterivirus, family Arteriviridae. EAV is present in the horse population of many countries worldwide (Timoney and McCollum, 1993). Transmission of EAV is by respiratory, venereal, and congenital routes. Infection is often subclinical. When present, clinical signs can include fever, depression, anorexia, dependent oedema, conjunctivitis, an urticarial-type skin reaction, and abortion. Apart from mortality in young foals, particularly those congenitally infected, the case-fatality rate in outbreaks of EVA is very low (Timoney and McCollum, 1993; Vaala et al., 1992). EVA cannot be differentiated clinically from other viruses causing respiratory and systemic disease in horses. Diagnosis of EAV infection is based on virus isolation, detection of nucleic acid (eg, by reverse transcriptase PCR [RT-PCR]) or viral antigen, or demonstration of a specific antibody response. A variety of serological tests have been used for the detection of antibody to EAV. Currently, complement enhanced virus neutralisation (VN) and enzyme-linked

immunosorbent assay (ELISA) are the most widely used, with VN being the most sensitive and specific. EVA control programs employ appropriate biosecurity measures along with, in countries where this is permissible and available, targeted vaccination to prevent the spread of EAV in breeding populations, thus minimising the risk of abortion outbreaks, death of young foals, and the establishment of carrier colts and stallions (Timoney and McCollum, 1993). For a review of equine viral arteritis, see the World Organisation of Animal Health (2013a).

ii. Bacterial

Bacterial placentitis is reported to be the cause of 9.8%-33.5% of abortions, stillbirths, and perinatal losses in horses (reviewed by Lyle, 2014). The majority of placentitis (53%), a condition that occurs in late pregnancy, is caused by ascending bacterial infections. Studies in an experimental model of ascending placentitis in pony mares appear to suggest that it is secondary inflammation of the chorion rather than infection that may lead to premature parturition (reviewed by Macpherson and Bailey, 2008). It appears that there is a complex interplay between the inflammatory response to the pathogen, loss of myometrial quiescence, and activation of the foetal hypothalamic-pituitary-adrenal axis that leads to abortion.

- *Streptococcus equi* subspecies *zooepidemicus*
  Streptococcus equi subsp. *zooepidemicus (Strep. zooepidemicus)* is a Gram-positive bacterium that belongs to the commensal flora of the caudal reproductive tract of mares. It is an opportunistic pathogen and the pathogen most commonly isolated from the uterus of mares. *Strep. zooepidemicus* can cause abortion following ascending infection. It is isolated from around 28% of cases of placentitis. Other bacteria isolated frequently include *Escherichia coli* and *Leptospira*, *Pseudomonas*, *Enterobacter*, and *Klebsiella* spp., other streptococci, staphylococci, and *Actinobacillus* spp. (Giles et al., 1993; reviewed by Lyle, 2014). The placenta can also become infected by viruses and fungi, but this is more commonly associated with abortion in early gestation. Mares with placentitis often present in late pregnancy with signs of premature udder development and premature lactation with or without vulvar discharge (reviewed by Macpherson et al.,2008).

Early detection and treatment of this condition is vital for ensuring the production of a viable foal (reviewed by Cummins et al., 2008). Transabdominal or transrectal ultrasonography is used commonly in the diagnosis of placentitis. Mares with placental infection or inflammation exhibit an increase in the combined thickness of the uterus and placenta (CTUP) (reviewed by Macpherson and Bailey, 2008). Thickening of the amnion and/or separation of the membranes from the endometrium can also be observed. Purulent hyperechoic material may be seen in pockets between the chorioallantois and the endometrium. Definitive diagnosis is based on histopathology of the chorioallantoic membrane. Management strategies are aimed at treating the infection and maintaining the pregnancy to as close to term as possible (reviewed by Cummings et al., 2008). Treatment generally consists of antimicrobial therapy, based on bacterial culture and susceptibility testing, anti-inflammatories, and hormonal support (Cummins et al., 2008; Macpherson, 2005; Macpherson and Bailey, 2008).

- *Taylorella equigenitalis*

  Contagious equine metritis (CEM) is an inflammatory disease of the proximal and distal reproductive tract of the mare caused by *Taylorella equigenitalis*, a Gram-negative, nonmotile, bacillus or coccobacillus. Mares with CEM may present with signs of endometritis, cervicitis, and vaginitis of varying severity and slight to copious mucopurulent vaginal discharge. Prolonged asymptomatic or symptomatic carriage is established in a proportion of infected mares. Stallions can become asymptomatic carriers. The highest risk of transmission is by direct venereal contact from a contaminated stallion or an infected mare. However, venereal transmission can also take place at AI if fresh, cooled or possibly even frozen, thawed semen is used (Schulman et al., 2013). Indirect transmission can also occur (eg, fomites, inadequate biosecurity at time of breeding and/or semen collection, processing, and transport) (Schulman et al., 2013). The level of risk associated with cryopreserved semen and embryos needs further investigation (Schulman et al., 2013). Diagnosis of CEM and detection of carriers is based on bacterial culture of *T. equigenitalis* from swabs taken from the genital tract, particularly the clitoral fossa and recesses of the clitoral sinuses (Platt et al., 1978). This methodology, despite

some limitations, is the gold standard used for all international horse trade and movement protocols (Schulman et al., 2013). Serology is not a reliable means of detecting *T. equigenitalis* infection. However, serum antibody to *T. equigenitalis* can be used to help detect recent (3-7 weeks previously) infection. PCR can also be used to detect *T. equigenitalis* and is rapid, cost effective, and offers high throughput and improved sensitivity and specificity (Schulman et al., 2013). However, a robust, validated PCR assay is needed before regulatory authorities worldwide could consider this as a potential replacement for bacterial culture (Schulman et al., 2013). For a review of CEM, see the World Organisation of Animal Health (2012).

iii. Parasitic

- *Trypanosoma equiperdum*

  Trypanosoma equiperdum is a protozoan parasite that causes Dourine, a potentially fatal, acute or chronic disease that is transmitted during coitus (venereal transmission). It is the only trypanosome that is not transmitted by an invertebrate vector and is found in Africa and South America. *Trypanosoma equiperdum* is unusual in that it is primarily a tissue parasite and is rarely detected in the blood. There is no known natural reservoir of the parasite other than infected equids. It is present in the genital secretions of both infected males and females. The incubation period, severity, and duration of the disease vary considerably. Clinical signs include a fluctuating course of fever, oedema of the genitalia and mammary glands, cutaneous eruptions and plaques (pathognomonic), facial or lip paralysis, incoordination, anaemia, and emaciation, leading to death. Diagnosis is usually based on clinical signs and serology (complement fixation test). The only effective control is through strict quarantine and identification and removal of infected animals. Good hygiene is essential during assisted breeding because infection may be transmitted through contaminated fomites. For a detailed review of Dourine, see the World Organisation of Animal Health (2013b).

3.4.3 Retained foetal membranes

In mares, the placenta is normally expelled within 90 minutes after parturition (Vandeplassche et al., 1971). There appears to be consensus

that retained foetal membranes (RFM) in mares can be defined as a failure to expel some or all of the chorioallantois spontaneously within 3 hours of foaling (reviewed by Canisso et al., 2013b). The incidence of RFM ranges from 2% to 10% of foalings in light breed-type mares and has been reported to be as high as 30%-54% in Friesian mares. The incidence of RFM is increased by dystocia, late-term abortion, prolonged gestation, hydrops, induction of parturition, and Caesarean section. Complications of retained foetal membranes include metritis, septicaemia, toxaemia, laminitis, and even death. In one study, 25% of mares with RFM developed signs of laminitis within 24 hours of foaling. RFM may also result in the delayed involution of the uterus and impaired fertility at the foal heat.

a. Treatment

RFM is generally treated in a stepwise fashion, starting with the administration of 10-20 IU of oxytocin intravenously or intramuscularly every few hours from 3 hours after foaling (reviewed by McCue and Ferris, 2015). Larger doses of oxytocin can lead to intense spasmodic uterine contractions that may cause considerable distress to the mare. Oxytocin (50-80 IU) can also be administered as an intravenous infusion in 1-2 L of sterile saline or glucose solution (5%) (Hospes and Huchzermeyer, 2004). The placenta is normally expelled within 1-2 hours after administration (Blanchard and Varner, 1993). Gentle traction can also be applied to the placenta, but care should be taken to avoid damaging the uterus, tearing the placenta, or causing uterine prolapse. Oxytocin treatment can be combined with uterine lavage, which results in a more complete separation of the chorionic villi and removes small pieces of the placenta and debris. Intrauterine and/or systemic antimicrobial treatment can prevent the development of septicaemia. Nonsteroidal anti-inflammatory drugs (NSAID) are indicated should signs of toxaemia occur (Blanchard and Varner, 1993). The prognosis is favourable if treatment is initiated promptly. The long-term prognosis for survival is poor to moderate for mares that develop severe laminitis, endotoxaemia, and/or metritis (reviewed by Canisso et al., 2013b).

3.5 The stallion

Fertility in stallions is assessed by clinical examination, semen evaluation, and observation of sexual behaviour.

3.5.1 Reproductive performance evaluation

A stallion's reproductive evaluation begins with a clinical examination, focusing on the external genitalia and the hind legs and back (to confirm its ability to mount a mare). The testicles should be palpated for consistency and position within the scrotum and their circumference measured. Libido is then evaluated—in particular, the reaction time from presentation of the mare to the time of covering. Deficiencies in libido, excessive aggressiveness toward the mare or handler, and other abnormalities of behaviour should all be recorded.

a. Semen collection

If the examination takes place before the breeding season, semen should be collected three times 24 hours apart to eliminate existing semen reserves. During the season, the stallion should be rested (from sexual activity) for 3 days prior to semen collection. For testing, semen is collected on two occasions, 1 hour apart, and evaluated for gel-free volume, total number of spermatozoa, percentage of progressive motile sperm (PMS), morphology, and pH. Assessment of the number of PMS of a particular stallion means that it can be managed better:

- For natural mating, a stallion will usually be used twice a day, six times per week
- For AI, sperm quality and quantity will determine how many mares can be inseminated from each ejaculate, and the semen will usually be collected three times a week

b. Semen transportation

The widespread acceptance of the transport of fresh, cooled semen in many parts of the equine industry signifies a major change. Semen transportation offers the advantage that mares do not need to be transported for breeding, reducing the possibility of injury or disease to the mare or foal, and increases access to desirable stallions. The number of mares bred using fresh, cooled semen is increasing annually. Semen is diluted 1:3 with semen extenders to provide an energy source and protect it from cold shock. It is then cooled from 37°C to 5°C, at a rate of less than 0.05°C per minute from 18°C to 8°C, and maintained at a low temperature (3°C-6°C) for up to 36 hours. The semen is placed in an airtight polystyrene container with a separate cooling

system and shipped by express courier. Spermatozoa should not come into contact with the rubber plunger of a syringe or the cooling system.

c. Semen conservation at low temperatures

There are certain limitations to the conservation of equine semen at low temperatures, mainly associated with variability in the ability of sperm from different stallions to tolerate freezing and thawing. It is thought that frozen, thawed semen from only 25% of stallions will produce pregnancy rates comparable to that of fresh, cooled semen or natural mating (Vidament et al., 1997). The majority of equine semen is frozen in 0.5-mL straws at a concentration of 200 million-400 million sperm per mL. Typically, cooling rates in the range of 10°C-50°C/min are employed, with relatively low concentrations of cryoprotectants (reviewed by Squires, 2005).

d. Use of sexed semen

Although flow cytometry has proven to be an accurate method for separating X- and Y-chromosome-bearing spermatozoa, it is still not in widespread use in the equine industry. It has been tested in a large-scale field study with promising results (Panarace et al., 2014). Factors limiting the use of sex-sorted semen include the greater cost of the equipment as well as the license needed to use it. Furthermore, the fertility of sex-sorted spermatozoa is highly stallion dependent, and the logistics of having the mare, stallion, and equipment in the same place can be problematic (Mari et al., 2010).

3.5.2 Cryptorchidism

Cryptorchidism is the condition where one or both testes fail to descend normally into the scrotum. Cryptorchidism is common in male horses (2%-8%), and is generally accepted to be a hereditary condition (reviewed by Edwards, 2008). It is often unilateral, occurring with equal frequency on the left or right, but occurs bilaterally in 10%-15% of cases. In slightly more than one-half of cases the testis (or testes) is (are) abdominal rather than inguinal.

When a cryptorchid stallion is hemicastrated, leaving one testicle in the inguinal canal or abdominal cavity, it will continue to exhibit male characteristics, very often including aggressive and dangerous behaviour. In addition, it is possible that the retained testicle may undergo neoplastic

change. If the testicle can be palpated in the inguinal canal, diagnosis is easy; when the testicle is within the abdominal cavity, it is more difficult.

a. Diagnosis

Cryptorchidism, or failure of testicular descent, can be a challenging clinical diagnosis, particularly in horses with an unknown history (reviewed by Lu, 2005). Diagnosis should include behaviour assessment, physical examination, and hormonal assessment.

The testes of the horse produce testosterone and oestrogens. Hormonal assessment can include baseline hormone concentrations and stimulation tests to examine the function of the hypothalamic-pituitary-testicular axis. Cryptorchids have high basal testosterone compared to geldings, but baseline testosterone concentrations in unilateral cryptorchids may not differ from those found in stallions (reviewed by Lu, 2005). Equivocal results are obtained in around 14% of cases if a single baseline sample is taken for testosterone (Cox et al., 1986). Bilateral cryptorchids have higher oestrogen concentrations than stallions, geldings, or unilateral cryptorchids (Ganjam and Kenney, 1975). The concentration of oestradiol-17β has been shown to be lower in unilateral cryptorchids than in stallions (Coryn et al., 1981).

An hCG (6,000-12,000 IU intravenously) stimulation test has long been used for the diagnosis of cryptorchidism in horses (reviewed by Lu, 2005). An increase in testosterone concentrations following administration reflects the presence of functional Leydig cells. Two blood samples need to be taken, the first prior to hCG administration and the second, depending on the test protocol used, 30 minutes-2 hours later. In one study the accuracy was 94.6% following administration of the higher dose of hCG (12,000 IU), with sampling prior to and 30 minutes after treatment (Cox et al., 1986). This also decreased the number of equivocal results to 6.7%.

Baseline oestrogen (oestrone sulphate) concentrations may be helpful in the diagnosis of cryptorchidism. However, oestrogen concentrations following hCG administration can be difficult to interpret (reviewed by Lu, 2005). Following a single intravenous administration of hCG (10,000 IU) to stallions and cryptorchids, there was a gradual increase in unconjugated

androgen concentrations (testosterone), peaking after 2 days, with higher concentrations reached in stallions than in cryptorchids (Silberzahn et al., 1989).

b. Treatment

Given the likely heritable nature of cryptorchidism, medical treatment of this condition is not advised. In addition, no controlled studies have been conducted to support anecdotal reports on the use of GnRH or hCG in the treatment of cryptorchidism. It is not known how often testicular descent has been achieved and whether the testis is subsequently able to function normally. Cryptorchidism in horses should be treated surgically.

3.5.3 Sexual behavioural disorders

Very few centers specialise in the diagnosis and treatment of sexual disorders of stallions. Sexual behaviour in the stallion is influenced by many factors, including season, hormone concentrations, psychology, and the skills of the handler. Suboptimal libido or poor mating ability appear to be among the more common reproductive complaints in the breeding stallion. Common problems include overuse, illness, or pain (often musculoskeletal) or, in the case of a stallion used for AI, an inadequately prepared artificial vagina (eg, not warm enough, too little pressure) and unsympathetic handling. More research is needed to fully understand the complexities of stallion sexual behaviour.

a. Deficient libido

Pharmacological treatment to stimulate libido or mating ability is a last resort, to be attempted only when clinical examination, careful management and handling, and patient attempts to train and encourage the stallion have failed. Medical intervention is usually only required once in novice stallions that are anxious (diazepam 0.05 mg/kg by slow intravenous injection 5-7 minutes before breeding) or have low libido (GnRH 0.05 mg subcutaneously 2 hours and 1 hour before breeding), because ejaculation is a powerful reinforcing stimulus (McDonnell, 1999). The administration of testosterone to boost libido is not recommended, because too high a dose can suppress spermatogenesis and stimulate aggressive behaviour (Stout et al., 2005).

3.5.4 Testicular degeneration

Numerous factors, including age, trauma, and infection, including parasitism, can lead to testicular degeneration. Inflammation or oedema of the scrotum can interfere with heat dissipation, resulting in an increase in scrotal and testicular temperature, severely affecting fertility. An increase in the temperature of as little as 2°C for 24 hours, if not promptly addressed, sterilises the stallion until new spermatozoa are formed (around 57 days).

Testicular degeneration is difficult to diagnose if there are no results and measurements (testicular size and consistency) from previous examinations for comparison. Histological examination of a testicular biopsy is possible but can lead to rupture of the blood-testicle barrier, leading to the formation of antibodies to spermatozoa, which can hamper reproductive performance, and severe hemorrhage. Ultrasonography is noninvasive and allows investigation of the testicular stroma.

3.5.5 Hemospermia and urospermia

The presence of blood or urine in the ejaculate reduces fertility.

a. Hemospermia

Hemospermia is the contamination of semen with blood. The underlying cause of hemospermia is not always known (Varner et al., 2000). However, blood can enter the semen following infection, trauma, neoplasia, or habronemiasis. It seems that the presence of red blood cells, as much as 20% of whole blood, is the key factor in reducing fertility. Immediate dilution with semen extenders lessens the negative effects of contamination with blood. Treatment involves identification and treatment of the underlying cause and resting from sexual activity for up to 3 months.

b. Urospermia

Urospermia is a sporadic condition that is more difficult to diagnose because the clinical signs can be subtle. The underlying cause is unknown. Although affected stallions may not always urinate during ejaculation (eg, only 30% of the time), it takes very little urine (pH, osmolality) to affect fertility (Griggers et al., 2001). There appear to be no best-practice

approaches to treatment of this sporadic condition, and the results of empirical treatment are often inconclusive.

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4.1 Swine productive and reproductive cycles

Worldwide, reproductive performance of gilts and sows has been consistently improving. In the US, a comparison of PigChamp data between 2006 and 2012 demonstrates an improvement in the average farrowing rate from 77.4% to 83.0% and an improvement in prolificacy (live-born piglets) from 11.99 to 13.4 per litter without significant changes in the proportion of stillborn and mummified foetuses. As a consequence, during this period, the number of live piglets born per sow per year increased from 23.4 to 26.1. Similar trends were observed in Europe, where the average number of piglets born alive per sow per year already exceeds 30 in Denmark, Netherlands, and France. In Brazil, the average value for this parameter is 28.55. Forecasts for the future suggest that genetic progress will continue increasing this parameter by 0.2-0.3 piglets/year, such that litter sizes of around 15 piglets become the norm in the best genetic lines.

Such a rapid improvement is the consequence of three main factors: (1) improved genetics, (2) improved understanding of the parameters (and of the underlying mechanisms) that limit performance (Figure 1), and (3) development of new tools providing solutions to swine producers.

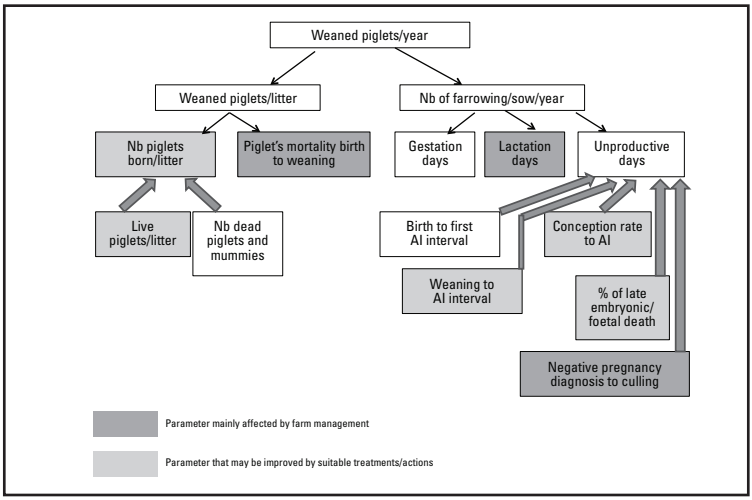

Figure 1 Factors affecting weaned piglets per year

While average reproductive performance obviously improved in recent years, the between-farm variability in reproductive parameters remained large. For example, the PigChamp data show that the number of piglets born alive per sow per year ranged between 20 and 26.9 in 2006 and between 22.4 and 29.3 in 2012 in the best and worst 10% of farms.

The life cycle of a female pig is presented below (Figure 2). It starts with a 6-month-long postnatal/prepubertal phase that culminates in puberty. After two or three cycles, the young gilt is bred and will start her first pregnancy (average gestation length is 113 days). Following a 21- to 28-day (Europe) or 18- to 22-day (US) lactation, the litter is weaned. Within 7 days, most females are back to oestrus and are rebred. Sows usually undergo between 2.2 and 2.3 farrowings/year. Longevity of the breeding sows is usually greater in European pig farms (where parity 5 or 6 sows are not uncommon) than in the US (where retention of sows past parity 6 is uncommon). The main causes for culling breeding sows are either reproductive failure and/or lameness.

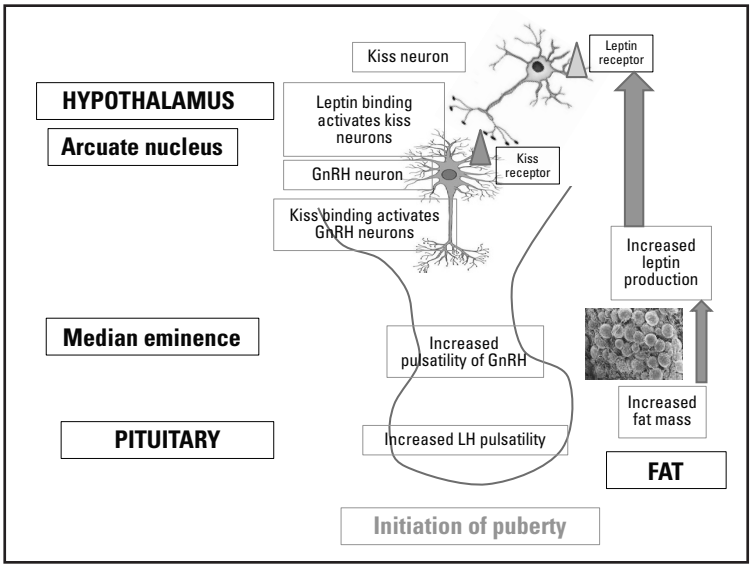

Figure 2 Puberty initiation hormonal factors

The following sections will discuss the mechanisms at work that control the different steps of the breeding cycle and those known to be involved in their variability. Solutions to control this variability will be presented in the last part of this review.

4.2 Puberty

4.2.1 Zootechnical parameters impacting the age of puberty

Onset of puberty in swine results from a combination of events allowing the gradual attainment of the ability to reproduce. A minimum threshold for age, body weight, and fat is necessary for puberty onset. In European breeds, age at puberty normally ranges around 200 days, with a body weight of around 120 kg and a backfat thickness of around 15 mm (Eliasson et al., 1991). However, variability in the age of puberty is very high: in Swedish gilts, it ranges between 151 and 211 days (average of 183 days). Therefore, age or body weight cannot be used to accurately estimate whether a specific animal has reached puberty. In gilts raised in temperate conditions, ovulation occurs toward the end of the first pubertal oestrus. Oestrus without ovulation is very uncommon (less than 5%), except in gilts raised in tropical conditions, where it can reach peak values (78%) during the hot summer months (Tummaruk et al., 2007). Lack of oestrus at the first pubertal ovulation happens in 7% and 21% of gilts maintained in temperate and tropical conditions, respectively (Tummaruk et al., 2007).

The factors responsible for the large variability of age at puberty are partially understood. They include:

- A genetic component: Heritability of age at puberty, at 0.3, has a relatively high impact compared to other reproductive parameters. For example, Chinese Meishan gilts have been shown to reach puberty around 3 months of age, ie, about 3 months earlier than “classic” gilts.

- Stress is known to induce puberty in gilts that are old and heavy enough. Relocation of gilts to another shed or transportation to the breeding unit commonly induces puberty in up to 70% of gilts within a week.

- Interactions with other animals such as boars or foreign sows. Exposure of prepubertal gilts to a mature boar with a high libido stimulates the appearance of puberty, through a combination of olfactory and auditory signals.

- The effect of nutrition on age of puberty is more complex to unravel, as nutrition interacts with growth rate and body composition (Eliasson et al., 1991). Gilts displaying high growth rate display puberty earlier, but reducing growth rate by limiting food supply (to 70% of needs) does not postpone puberty. Gilts with a low backfat thickness display a shallower first oestrus.

Management practices around puberty are very different worldwide. In South America, to minimise the number of unproductive days, gilts are inseminated young (around 6 months of age) at either the pubertal oestrus or the following one. In contrast, in Europe, gilts are inseminated later (at 8-9 months of age in Denmark) in order to have them farrowing around 1 year of age. The rationale for this practice is that the number of ovulations (and hence potential prolificacy) steadily increases from the first to the third oestrus. To maximise lifetime productivity (PigChamp, 2013), it may be recommended to breed around 225-250 days of age, but not before 210 days. Sow body weight should then be within the 145-160 kg range. Reaching such a weight at that age implies a gilt growth rate, between weaning and puberty, in the 700-850 g/day range.

4.2.2 Physiological mechanisms involved in the control of puberty

Between birth and puberty, each gilt transitions through four developmental steps:

- The first one ("immature step") occurs during the first month of life. Ovaries are devoid of large follicles, the uterus is small, and the function of the hypothalamo-pituitary axis is limited.

- The second one ("infancy step") occurs during the second month of life. While there are still no large follicles on the ovaries, the hypothalamo-pituitary axis becomes functional and circulating FSH concentrations increase.

- The following step ("activation step"), which occurs during the third and fourth months of life, is characterised by an increase in circulating LH concentrations. Small follicles start to be visible on the ovaries. Owing to the increased gonadotropin concentrations produced by the pituitary, they start to produce oestradiol and the uterus starts to enlarge.

- The last step ("waiting step") elapses between the end of the fourth month of life and puberty. At this stage, large follicles are present on the ovaries and active follicular growth and regression occurs as consecutive waves that change the ovaries from a "honeycomb type" to a "grape type" take place at regular intervals. FSH and LH concentrations are reduced and fluctuate according to the development and regression of the follicular waves. The endocrine step that prevents initiation of puberty is the lack of high-frequency LH pulses. Hence, the large follicles present on the ovaries fail to produce the high oestradiol concentrations that are needed to initiate the preovulatory LH surge.

Puberty occurs once the body (based on body weight and body fat) becomes able to provide metabolic signals to the brain, indicating that it is ready to initiate cyclic ovarian activity and a possible pregnancy. Leptin, produced by fat, appears to be an essential mediator in the initiation of puberty. At the brain level, leptin concentrations are sensed in the arcuate nucleus of the hypothalamus. In mice, leptin was shown to increase kiss expression in hypothalamic cells. If the same is assumed to occur in swine, puberty might be triggered by a rise in leptin, itself activating the kiss system in the hypothalamus. Kiss activation would result in increased GnRH secretion that would increase production of LH by the pituitary, therefore allowing the large follicles to produce large amounts of estradiol that would finally trigger puberty (Barb et al., 2008) (Figure 3).

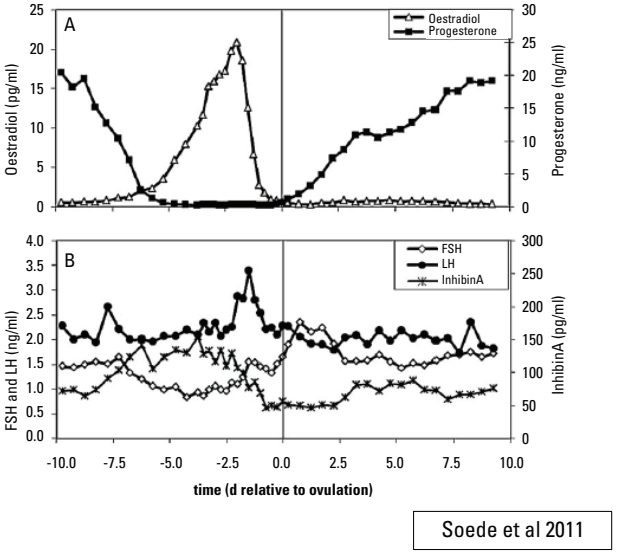

Figure 3 Time related to ovulation and hormonal dynamics

4.3 Oestrous cycle

4.3.1 Ovarian changes throughout the cycle

After puberty, oestrous cycles lasting 18-24 days (typically 21 days) are initiated and will continue until the gilt becomes pregnant. Classically, each oestrous cycle is split between a follicular phase, defined as the interval between luteolysis and ovulation (and lasting four to seven days), and a luteal phase elapsing between ovulation and luteolysis and lasting 14-17 days.

a. During the follicular phase

At the time of luteolysis, the ovaries contain a homogenous population of follicles 2-4 mm in diameter. During the first days of the follicular phase, a large cohort of such follicles (usually including about 50-60 follicles) is recruited and starts undergoing terminal follicular growth. Between days 17 and 19 of the cycle, selection of the ovulatory follicles (about 15-20, depending on the genetic line and parity) usually occurs. Here, two main events can be visualised when monitoring follicular growth. The 15-20

selected follicles go on growing, while the other follicles of the cohort undergo atresia and regress in size. Around day 19 of the cycle, the 15-20 dominant follicles reach 6-7 mm and the first sign of oestrus (vulva becoming pink and swollen) occurs. One day later, preovulatory follicles reach 7-9 mm in diameter and the female fully displays oestrus (standing to the boar, with perked ears). Around 40 hours after initiation of oestrus (Day 21), ovulation of all preovulatory follicles ends the follicular phase. All follicles ovulate over a rather narrow time period (2 to 4 hours). Ovulation rate (ie, the number of ovulations at the end of each cycle) is a major factor controlling prolificacy, as it sets the upper limit of litter size. Factors modulating ovulation rate have been identified and are listed below:

- Genetic line: Hyperprolific lines (with ovulation rates exceeding 30) have been identified (Hyperprolific Large white lines in France) (Bolet et al., 1986) or selected (Nebraska selection line) around the world. Some lines of Chinese Meishan swine also display high ovulation rates.

- Age and parity: Ovulation rate increases steadily throughout the first three cycles of a gilt's life. It continues to slowly increase until the female is fully mature and then remains at a plateau.

- Nutrition: Negative energy balance during lactation may reduce ovulation rate when the wean-to-oestrus interval is short, but not in other cases (Zak et al., 1997).

b. During the luteal phase

Following ovulation, each follicle becomes a corpus luteum. Young corpora lutea are usually presenting a clear ovulation point and range in size between 10 and 15 mm. In each of them, the granulosa and theca cells, which were the somatic cells of the preovulatory follicles, are combined with fibroblasts and blood vessels in a compact structure. Mid-luteal phase corpora lutea are fully grown (generally reaching about 15 mm in diameter and a weight ranging between 6 and 10 g) and are of a light red color. There are no follicles growing over 4 mm in diameter during the luteal phase. Hence, gilts and sows display a single wave of follicular growth per cycle. This is a major difference from ruminants (cattle and sheep), which have two or three waves of follicular growth per cycle and can have one or two follicular waves during the luteal phase.

4.3.2 Endocrine changes throughout the cycle

a. During the follicular phase

Several experimental paradigms interfering with the normal function of the hypothalamo-pituitary axis have established that in swine, follicular growth between 2 and 4 mm depends on a rise of FSH concentrations, while terminal follicular growth is LH dependent (Driancourt et al., 1995). However, it has proven difficult to consistently identify an increase in circulating FSH concentrations at the beginning of the follicular phase (Figure 3). In contrast, a close synchronisation between the increased pulsatility of LH during the mid-late follicular phase and the follicle growth between 4 and 8 mm in diameter has been repeatedly demonstrated. Not only is LH the key gonadotropin supporting follicular growth, but it also plays a key role in maximising oestradiol production by the enlarging follicle. Indeed, several studies have shown that LH has the ability to increase androgen production by thecal cells. These increased amounts of androgens are immediately transferred to granulosa cells, where they are metabolised into oestradiol (by aromatisation). IGF1, which is contained in the follicular fluid in large amounts, acts as a local regulator to increase follicular response to LH. The combined effects of LH and IGF1 on follicular steroidogenesis are responsible for the rise in oestradiol concentration that triggers oestrus and the LH surge (by positive feedback on the hypothalamo-pituitary axis). The LH surge lasts between 12 and 20 hours, and peak LH concentrations usually range between 5 and 15 ng/mL.

b. During the luteal phase

After rupture of the preovulatory follicles at ovulation, tissue reorganisation and angiogenesis (ie, formation of blood vessels) occur to change the hollow follicular structure into a solid mass of luteal tissue. The main steroid produced by corpora lutea is progesterone, and production by each CL is strongly related to its weight. Progesterone reaches peak concentrations (at around 20-35 ng/mL) around day 9 of the cycle and remains at this level until the initiation of luteolysis (around day 15). Such high progesterone concentrations act by negative feedback on the pituitary to reduce pulsatile LH secretion. In addition, production of inhibin (which acts by negative feedback on FSH) is also responsible for low FSH concentrations

during the luteal phase. The combination of low LH and low FSH concentrations explains why terminal follicular growth does not occur during the luteal phase in swine. Toward days 14-16 of the cycle, progesterone concentrations collapse, owing to $PGF_{2\alpha}$ release by the uterus that triggers luteal regression.

4.4 From initiation of pregnancy to farrowing

4.4.1 Zootechnical factors affecting conception, farrowing rate, and prolificacy

a. Effects of the insemination-to-ovulation interval on conception rate
While a close proximity between insemination and ovulation is a prerequisite to maximising fertility in all species, studies assessing the impact of the insemination-to-ovulation interval on farrowing rate could only be conducted from the moment when the time of ovulation could be precisely defined using ultrasonography. Using a model where sows were inseminated once at various times relative to ovulation (from 48 hours before until 16 hours after), Soede et al. (1995) concluded that the optimal interval for insemination is within an 8-hour window before the time of ovulation. Fair conception results were also recorded from 24 to 16 hours and from 16 to 8 hours before ovulation. Inseminations from 32 to 24 hours before ovulation, as well as within 8 hours after ovulation, were associated with reduced fertilisation rate and embryo development. However, under practical conditions, the time of ovulation is not known and the only parameter that can be used to efficiently breed each female is oestrus. Owing to the tremendous variation in the duration of oestrus between sows and to the negative correlation between the wean-to-oestrus interval and the duration of oestrus, it is impossible to breed sows once close enough to ovulation to ensure high fertility and prolificacy. Therefore, sows are generally bred multiple times (two or three) during oestrus) to make sure that at least one of these inseminations is close to ovulation.

b. Effects of the insemination-to-ovulation interval on prolificacy
The relationship between the insemination-to-ovulation interval and prolificacy was explored in two studies that used either a single

insemination (Nissen et al., 1997) or two to three inseminations (Terqui et al., 2000). Both studies demonstrated that the curve relating these two parameters shows its maximum value for inseminations conducted around 10±6 hours before ovulation. The curve was symmetric, with a decrease of 1 piglet for every 10 hours (either preovulation or postovulation) away from this optimum period.

c. Other factors affecting farrowing rate

i. Quality of sperm used for insemination

Storage of semen shortens the optimal period for insemination relative to ovulation. When semen is very fresh (12-38 hours, as in Soede et al., 1995), high rates of fertilisation are obtained with inseminations performed during the 24 hours preceding ovulation. In contrast, Waberski et al. (1994a) showed that semen storage for 48-87 hours strongly reduced the fertilisation rate of sows that were inseminated between 12 and 24 hours before ovulation. Storage for a longer time period (up to 120 hours) also reduced the fertilisation rate of sows inseminated during the 12 hours before ovulation. Hence, for obtaining optimal farrowing rates, the use of fresh semen (ideally less than 48 hours old) is recommended, although the magnitude of the negative effects of storage on fertilisation is obviously modulated by the quality of semen and the type of extender used.

ii. Season

A decrease in farrowing rate is commonly detected following breeding in late summer and early autumn. This is generally associated with an increased proportion of females displaying a delayed return to oestrus (25-35 days after insemination) typical of early embryonic mortality (Bertoldo et al., 2012).

d. Other factors affecting prolificacy

i. Duration of lactation

Litter size of the following litter clearly increases when the duration of lactation increases (Martinat Botte et al., 1985). Another way to maximise the size of the following litter is to postpone the following mating/insemination either by feeding altrenogest (see 4.6.1) or by skipping the first heat following weaning.

ii. Parity

Size of the second litter (generated by breeding of primiparous sows) is at best similar to that of the first litter but usually smaller. Litter size tends to increase with increasing parity, until at least parity 6.

iii. Season

It is generally agreed that prolificacy is not affected by season (Bertoldo et al., 2012).

iv. Genetic line

Two main strategies leading to increased prolificacy have been identified in swine.

Firstly, lines such as Chinese Meishan sows are prolific, owing to a higher embryo survival than in the other European swine lines. This improved embryo survival may be related to a better homogeneity in the morphological features of their embryos around the time of implantation and/or to a faster luteinisation of the corpora lutea, leading to a quicker rise in progesterone concentrations following ovulation.

Secondly, hyperprolific selection lines have been developed (Bolet et al., 1986). Their large litter size is associated with a very large increase in the number of ovulations (to 30-35 ovulations in some sows) associated with a small improvement in uterine capacity.

4.4.2 Embryonic development and interactions with the genital tract involved in initiation and maintenance of pregnancy to term

a. Embryo and foetal development

If there is a close synchrony between insemination and ovulation (less than 12 hours), over 90% of the oocytes are fertilised. Embryos at around the 4-cell stage are moved through the oviduct into the uterus at about 48-72 hours (days 2-3) after ovulation. They remain in the upper uterus until day 6 (embryos are blastocysts at this stage), at which point they are moved slowly down each uterine horn. Around day 7, each embryo gets out of the zona pellucida that enclosed each oocyte. Embryos reach the uterine body

around day 9. Embryos of each horn freely move in the uterine space and mix so that embryos present in one horn may have originated from ovulations from the contralateral ovary. This optimises use of the uterine space and makes sure that at implantation, each embryo has an optimal uterine space available. On day 12, at the beginning of implantation, embryos change shape and develop into filamentous structures that change them into long (1 m) and narrow (1-2 mm in diameter) tubular structures. At this stage, there is a huge heterogeneity between embryos of a specific sow, which may play a key role in embryonic survival (with the largest embryos actively preventing growth of the smaller ones). At 18 days of age, the embryos are fully surrounded by allantoic fluid, and between days 20 and 30, fluid in the allantois regularly accumulates. Most embryonic loss occurs during the first month of pregnancy. Embryonic loss commonly ranges from 20% to 40%, mainly around the time of implantation.

Later on during pregnancy, additional embryonic loss affects only 5% to 10% of the embryos. Specific organs may be visualised around day 30, when embryo weight ranges between 0.8 and 1.6 g. Fetal weight then steadily increases throughout pregnancy, from 20-30 g at 45 days to 500-600 g at 90 days and 1100-1600 g at term (114 days).

b. Mechanisms involved in pregnancy maintenance

Pregnancy maintenance around the expected time of luteolysis relies on steady, high progesterone concentrations that will allow embryo implantation in a receptive and functional endometrium. This implies that secretion of the luteolytic signal, prostaglandin $F_{2\alpha}$, around day 14 of the cycle is prevented. As in other species, the embryos present in the genital tract play a key role in pregnancy maintenance, but in swine the embryonic signal is oestrogen (in contrast to ruminant species, where it is interferon tau). Pig conceptuses secrete oestrogens between days 10 and 15 of pregnancy. The key role of oestrogens in CL maintenance is clearly established by the demonstration that injections of oestrogens result in CL maintenance for a period ranging between 30 and 120 days (Geisert et al., 1990). Oestrogens act at two levels to prevent luteolysis. Firstly, they redirect $PGF_{2\alpha}$ secretions away from the uterine venous drainage (that would convey the luteolytic signal to the ovaries) and release them in the uterine lumen (Bazer and Thatcher, 1977). Secondly, they alter the ratio in the secretions of

the luteolytic $PGF_{2\alpha}$ and luteoprotective PGE2, in favor of the latter hormone (Waclawik and Ziecik, 2007). Finally, oestrogens also act on the endometrium to increase expression of specific growth factors (IGF1, FGF7) that act on the trophectoderm to stimulate conceptus attachment and development (Spencer et al., 2004).

c. Mechanisms involved in embryo survival

By definition, embryonic survival is the proportion of ovulation that results in a piglet born. The opposite concept is embryonic loss. Three main causes of high embryonic loss have been identified and are listed below:

- Excessively high ovulation rates. The curve relating the number of live foetuses to the number of ovulations is curvilinear (Wu et al., 1987) (Figure 5). When the number of ovulations increases from 8 to 18, the number of live foetuses increases in parallel, reaching 12 live foetuses for 18 ovulations. At that point, the number of foetuses stops increasing with increased number of ovulations, as all additional embryos are eliminated via embryonic loss. The plateau in the number of live foetuses when the curve reaches a maximum reflects uterine capacity. This is why increasing ovulation rate over the level using 100% of the uterine capacity has no practical value.

- Genetic line: Chinese Meishan sows display a higher embryo survival than the "classic" swine line. Two possible reasons explaining this have been identified. Firstly, they seem to have a better homogeneity in the morphological features of their embryos. Secondly, luteinisation and the rise in progesterone concentrations following ovulation occur faster in Meishan females.

4.5 From farrowing to a new conception

4.5.1 Zootechnical factors affecting the ability to reconceive

The prerequisites to reconceive after weaning are:

- A consistent return to oestrus after weaning. This may be measured by the wean-to-oestrus interval as well as the proportion of females displaying oestrus during the first seven days after weaning. While average values for the wean-to-oestrus interval are four and five days for

multiparous and primiparous sows, respectively, variability around these average values is large, particularly for primiparous sows. Factors involved in this variability are listed below:

- Nutrition: Feed restriction during lactation delays the occurrence of oestrus in weaned sows (Prunier and Quesnel, 2000). In contrast, feed restriction after weaning does not delay oestrus (Prunier and Quesnel, 2000). The duration of the wean-to-oestrus interval increases in parallel with the loss of fat and muscle protein during lactation. Reduced backfat thickness at weaning is commonly associated with extended wean-to-oestrus intervals.

- Season: The proportion of sows that display longer wean-to-oestrus intervals increases during the summer months (Bertoldo et al., 2012). Increased day length and increased temperatures (that reduce appetite) may be responsible for this change.

- Duration of lactation: Following short lactations, the wean-to-oestrus interval increases and the proportion of sows displaying oestrus in the week after weaning decreases (Xue et al., 1993).

- Parity: The wean-to-oestrus interval is always longer (by one or two days) in primiparous sows. In addition, when sows grow older, this interval tends to become shorter as the number of weaned litters increases. This effect may be related to a better ability of multiparous sows to display a consistent food intake during lactation and to the better ability of older females to mobilise their body reserves.

The variability of the wean-to-oestrus interval implies that repeated oestrus detections are needed to identify when insemination may be made. As variability in the oestrus-to-ovulation interval is also large, it is impossible to predict when ovulation may occur once heat is initiated. This is why ovulation induction, combined with fixed-time insemination, may be an attractive strategy to optimise this step (see Section 4.6.3).

- The release of a normal number of good-quality oocytes at ovulation. Because ovulation rate almost always exceeds uterine capacity, this is usually not a factor limiting reconception.

- A good fertilisation rate and embryonic survival during early pregnancy. While fertilisation rate is generally high if semen quality and time of insemination are adequate, early embryonic mortality may be altered by the metabolic status of the sow, either during the previous lactation (particularly in primiparous sows) or during the first weeks of pregnancy.

4.5.2 Physiological mechanisms involved (Figure 4)

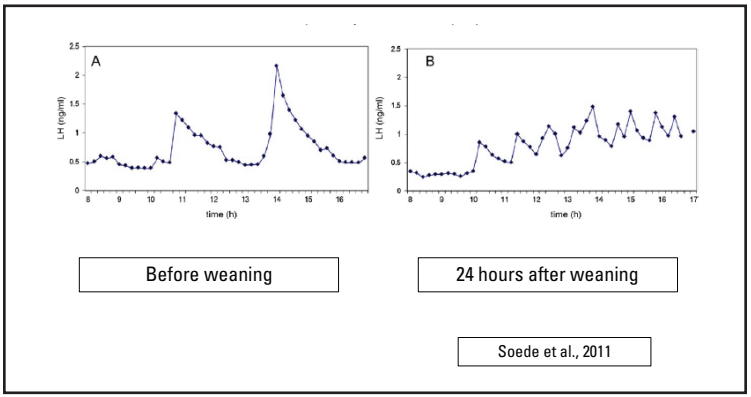

Figure 4 LH dynamic around weaning

a. In parturition

Mechanisms regulating farrowing in swine are more complex than in ruminants, as sows farrow a litter rather than a single young. Therefore, there is a need to maintain the intrauterine integrity of part of the litter while the other part is being born. Changes in hormone concentrations in sows start two days before parturition with a drop in progesterone concentrations, reaching 3-4 ng/mL. This drop is temporally related to a

small rise in prostaglandin $F_{2\alpha}$ concentrations. Increased cortisol production by the adrenals of the foetuses is likely to be responsible for this rise in prostaglandins (First and Bosc, 1979). Luteolysis is then associated with a rise in relaxin production by the corpora lutea. Increased relaxin concentrations (owing to its effects on cervical relaxation) and decreased progesterone concentrations (allowing uterine contractions to be initiated) in the hours preceding farrowing are likely to be the main triggers of farrowing. On the day before parturition, a larger peak in prostaglandin $F_{2\alpha}$ concentrations, together with increased oxytocin concentrations, initiate uterine contractions and delivery of the piglets. Such a claim is supported by data from First and Bosc, 1979, showing that a reduction of prostaglandin secretion around parturition considerably delays it. Such observations provide the rationale for in-field induction of parturition by injecting analogues of $PGF_{2\alpha}$ (see Section 4.6.4).

b. In inhibiting ovarian function during lactation and controlling the wean-to-oestrus interval

During lactation, the inhibitory effects of the repeated suckling bouts prevent GnRH secretion, thereby maintaining low LH concentrations. Consequently, no terminal follicular growth occurs and the size of the largest follicles rarely exceeds 4 mm. Weaning immediately results in an increased GnRH release, generating an increased frequency of LH pulses (Shaw and Foxcroft, 1995; Quesnel and Prunier, 1995). The increased pulsatility of LH secretion supports recruitment of a new ovulatory cohort (days 1-2 postweaning) followed by selection of a new group of ovulatory follicles (days 3-4 postweaning). During the last days of the follicular phase, the largest ovulatory follicles gain the ability to produce oestradiol and to trigger the initiation of oestrus, followed by the endogenous LH surge. This cascade fails to occur in sows weaned after very short lactations (less than 14 days), as LH secretion is minimal and the positive feedback mechanism is not operational during the first two weeks of lactation. In females suffering from strong negative energy balance during lactation, plasma and follicular fluid IGF1 concentrations are decreased (Prunier and Quesnel, 2000). As IGF1 acts synergistically with LH and FSH to support follicular growth and maturation, IGF1 may be playing a key role in explaining the variability in the wean-to-oestrus interval, particularly in primiparous sows.

4.6 Practical solutions to optimize reproductive performance of gilts and sows (Figure 5)

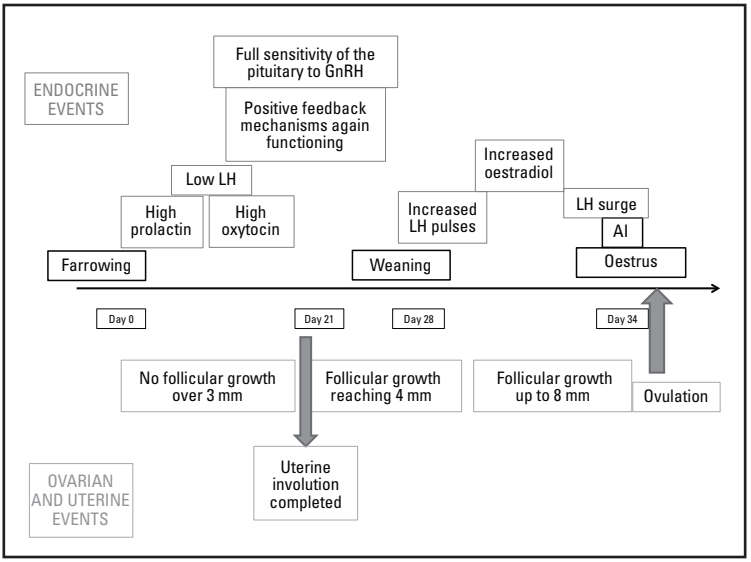

Figure 5 Endocrine, ovarian, and uterine events in farrowing, weaning, and oestrus

4.6.1 To generate batches of breeding females with the use of altrenogest (Regumate and Matrix)

Synchronising ovarian activity of groups of females is a common practice throughout the world to generate batches of animals and incorporate gilts in the breeding stock. While in cattle prostaglandins, GnRH, and progesterone are commonly used for synchronisation, there is a more limited choice in swine. Indeed, prostaglandins cannot be used for synchronisation in swine, as swine corpora lutea are only sensitive to their luteolytic effects when 12 days old (Diehl and Day, 1974). Ovsynch programs (combining GnRH and prostaglandins) do not work in swine as they do in cattle, because swine, do not develop follicular waves during the luteal phase as cattle do (see above). The only way to manipulate ovarian function efficiently in swine is to use progestagens. Two constraints have been solved when developing

synchronisation treatments for swine. Firstly, progestagen treatments need to be administered for a duration similar to that of the luteal phase (14-18 days), as progestagens cannot be combined with prostaglandins in swine (prostaglandins only work in a narrow time window). Secondly, owing to the very large gap in circulating progesterone concentrations in swine (30-50 ng/mL) vs cattle (5-10 ng/mL), very potent molecules (such as altrenogest) rather than progesterone (as in cattle) have to be used. The progesteronic hormonal activity of altrenogest acts by negative feedback on the hypothalamo-pituitary axis, slowing down pulsatile LH secretion and preventing the occurrence of an LH surge. Hence, during altrenogest feeding, the size of the largest follicles does not exceed 4 mm. At the end of treatment, pulsatile LH secretion immediately resumes. This sustains follicular growth and maturation and results in a synchronised initiation of oestrus 5-6 days after the last altrenogest meal. Two clinical recommended doses have been shown to work well: 15 mg/day in the US and 20 mg/day in Europe. Feeding lower amounts of altrenogest carries the risk of triggering the development of cystic follicles. This is why individual feeding of altrenogest is recommended (vs group feeding). If the gilt is in the luteal phase when altrenogest treatment is initiated, the corpora lutea naturally regress during the treatment and, during the last part of treatment, it is altrenogest alone that prevents terminal follicular growth. If the gilt is in the follicular phase at the initiation of treatment, altrenogest acts by negative feedback on the pituitary to prevent terminal follicular growth throughout the treatment period.

The registered treatment for gilts in Europe (20 mg altrenogest/day) for 18 days synchronises initiation of oestrus over a three-day period (93% of gilts over days 5-7 after the end of treatment), with 40%-60% of the sows starting oestrus on day 6 (Martinat Botte et al., 1985). A prerequisite for these precise synchronisation results is that all gilts should have displayed puberty before the altrenogest treatment is initiated. Indeed, half of nonpubertal gilts fed with altrenogest will display a delayed oestrus (around day 10 after the end of treatment), and the other half will not show oestrus or ovulate at all. Oestrus in gilts fed with 15 mg/day will occur, on average, one day earlier than in those fed 20 mg/day.

Following insemination at detected oestrus, conception rate of altrenogest-treated gilts increases 12 fertility points compared to the

controls, reaching 89%. There is one more corpus luteum in the altrenogest-treated gilts, resulting in an extra half piglet (Martinat Botte et al., 1995). Such results are the basis for the widespread use of altrenogest in gilts.

In sows, postponing oestrus after weaning with altrenogest has been tested in several studies using different durations of treatment and different times of initiation of treatment around weaning. While there is no consensus on the optimal treatment strategy, some have reported positive effects on ovulation rate, farrowing rate, or litter size (review: Kemp and Soede, 2012). Such an approach may be helpful for primiparous sows that need more time to recover from their first lactation before being mated again. Recent studies in this model have demonstrated that benefits of treatment on farrowing rate and litter size are most obvious when altrenogest is fed for 14 days (Patterson et al., 2008; Van Leeuwen et al., 2011).

Altrenogest therefore is a key player in generating homogenous batches of breeding females.

4.6.2 To reduce the number of nonproductive days with exogenous gonadotropins (PG 600)

There are situations when the expected changes in ovarian function leading to oestrus and ovulation fail to occur or are delayed. Failures include cases of delayed puberty or of lack of return to oestrus after weaning in primiparous sows during the hot period of summer. Delayed resumption of ovarian activity is commonly observed in primiparous sows not eating enough during lactation and under negative energy balance after weaning. Given the sequential dependence of follicles on FSH (to grow from 2 to 4 mm) and LH (to grow from 4 to 8 mm), injection of a gonadotropin such as PG 600 with this double hormonal activity and a long half-life has the potential to correct the deficiencies listed above. Several studies have clearly established the reproductive performance associated with its use:

- To induce puberty: The timing of puberty is very variable. It is therefore tempting to induce it to control this variability. Injection of PG 600 to prepubertal gilts (older than 150-160 days of age) initiates a synchronised oestrus around 4 days later in a large (around 70%-80%) proportion of the gilts, followed by ovulation. However, for optimal efficacy, all gilts

should be in the "waiting step" of the prepubertal period (see Section 4.2.2) and be consistently exposed to a sexually active boar during the days following treatment (Bartlett et al., 2009). Ovulation rate at the induced oestrus is in the normal range. Therefore, two breeding strategies are proposed. Either all gilts are bred at the induced oestrus, or all gilts are bred when they return to oestrus 21 days after the ovulation induced by treatment. Each strategy has benefits and limits. In the first one, litter size may be somewhat reduced, owing to a suboptimal embryo survival, but this prompt breeding is time efficient. The second one commonly generates better reproductive performance but requires a very high proportion of gilts returning to oestrus, while postponing breeding by 21 days compared to the previous strategy.

- To treat delayed puberty: Due to variability in age at puberty, some gilts may not have displayed puberty when older than 180 days. This subpopulation includes two animal types: on one hand, some gilts may have had a silent heat associated with ovulation, while on the other hand, other gilts may be truly prepubertal. To optimise treatment efficacy, the two subpopulations need to be identified, as PG 600 injection will only work in the truly prepubertal gilts. Such females may be easily identified by performing an ultrasound scan of their genital tract (Martinat Botte et al., 2011). Administration of PG 600 to this subpopulation induces puberty in 96% of them within three or four days, followed by ovulation in all of them. Reproductive performance of the treated gilts mated at their second oestrus appears similar to that of control sows (Martinat Botte et al., 2011).

- To improve reproductive performance of primiparous sows: Due to a more variable metabolic status at the time of weaning, primiparous sows are more likely to display long and variable wean-to-oestrus intervals. Strategic use of PG 600, injected at weaning in this subpopulation, has been shown to allow primiparous sows to achieve the same high reproductive performance as multiparous sows. Indeed, injection of PG 600 at weaning triggers a short and very synchronous wean-to-oestrus interval and increases the proportion of sows bred by seven days after weaning (Kirkwood et al., 1998). Farrowing rate of PG 600-treated females following breeding was similar to that of control sows (87% vs 86%

in control and treated groups, respectively), while litter size was reduced by about half a piglet in the treated sows (Kirkwood et al., 1998). However, productivity (measured by the number of pigs produced per weaned sow) was clearly and significantly improved by PG 600 administration (by 1.5 piglets) (Kirkwood et al., 1998).

- To correct the problems triggered by heat stress in summer: High ambient temperatures during lactation are associated with a reduction in food intake, resulting in an alteration of the metabolic status of the breeding sows. The increasingly negative energy balance, particularly in primiparous sows and/or in sows kept outdoors, translates into a slower resumption of pulsatile LH activity after weaning that may be inadequate to support terminal follicular growth. In such sows, oestrus and ovulation are delayed past seven days after weaning or fail to occur. In all sows that are anoestrous on day 7 after weaning, injection of PG 600 has been shown to trigger oestrus and ovulation within three and five days, respectively. Conception rate following insemination, at around 80%, proves that the induced ovulations are of good quality (Bracken et al., 2006).

The four uses of PG 600 listed above are economically viable approaches maximizing gilt and sow productivity.

4.6.3 To induce ovulation with a GnRH agonist to minimise the number of AIs while maintaining reproductive performance (Porceptal)

As presented above, a prerequisite to obtaining high fertility and prolificacy is to minimise the insemination-to-ovulation interval and have it as close as possible to 12 hours. While altrenogest feeding of gilts and weaning of sows efficiently synchronises the beginning of oestrus, the variability in the onset of oestrus-to-ovulation interval remains large (Kemp and Soede, 1996; Belstra et al., 2004). Multiple inseminations (two to three) must be performed to maximize reproductive performance. As the large variability in the oestrus-to-ovulation interval is mostly generated by the variability in the onset-of-oestrus-to-LH surge interval (Martinat Botte et al., 1997), the potential of a fixed-time insemination following ovulation induction

was tested in several recent studies. Ovulation induction was achieved by injection of compounds that trigger an LH surge (GnRH agonists such as buserelin or triptorelin) (Driancourt et al., 2013; Knox et al., 2011) or of compounds mimicking the endogenous LH surge (hCG or porcine LH) (Brussow et al., 2009; Zak et al., 2009). Two factors have been shown to play key roles in the treatment's efficacy. Firstly, the dose of compound inducting the ovulation should be high enough to ensure that full ovulation occurs and that follicular cysts do not develop. Secondly, the timing of the injection needs to be carefully selected. For example, in gilts, injection of 10 µg of buserelin at 120 hours after the last altrenogest meal, combined with a single insemination 30-33 hours later, maintains farrowing rate and prolificacy at the high levels observed following multiple inseminations at detected oestrus (Driancourt et al., 2013). Furthermore, injection of 10 µg of buserelin to weaned sows at 86-89 hours after weaning, followed by a single fixed-time insemination 30-33 hours later, also generates farrowing rate and litter sizes similar to those of control sows bred twice at detected oestrus (Driancourt et al., 2013). Reproductive performance following ovulation induction with buserelin followed by a single fixed-time insemination is robust (Driancourt et al., 2013) in gilts (no interaction with body condition score or the number of cycles since puberty) and in sows (no effects of lactation length or of the magnitude of fat loss during lactation of multiparous sows) (Driancourt et al., 2013). However, in primiparous sows with a short lactation (less than 21 days), postponing the buserelin injection for 12 hours may be needed to obtain optimal performance.

The benefits of the ovulation induction and fixed-time insemination procedures are well known:

- Reduction in the number of semen doses used (to one)
- Reduction in the time used for oestrus detection (only one detection at the time of insemination is needed)
- Optimisation of the semen logistics and improved use of the workforce for insemination
- And finally, 114-116 days later, a tighter synchrony in the timing of farrowing

4.6.4 To optimise piglet survival at farrowing by farrowing induction (Planate)

Given the key role of the increased uterine $PGF_{2\alpha}$ secretion in the initiation of the farrowing process, it is not surprising that injection of exogenous natural or synthetic $PGF_{2\alpha}$ analogues triggers parturition (Einarsson, 1981) by inducing regression of the corpora lutea that were supporting pregnancy. For example, a single injection of 175 µg cloprostenol (Estrumate or Planate) to sows between days 112 and 114 of pregnancy (which usually lasts 114-117 days if parturition is not induced) results in an injection-to-parturition interval of 21-23 hours, with about two thirds of the sows farrowing in the 25- to 32-hour time window (Willemse et al., 1979; Jainudeen and Brandenburg, 1980) and more than 90% between 16 and 34 hours postinjection. In contrast, farrowing of untreated sows is spread over more than 50 hours. No detrimental effects of parturition induction on piglets are detectable, when piglet body weight, survival, and growth rate are used as end points. Induction of farrowing by injecting cloprostenol should not be attempted before day 111 of pregnancy, as delivery of immature piglets is always associated with increased mortality rates.
To avoid prematurely inducing parturition, it is of utmost importance to keep good breeding records, documenting the day of pregnancy when cloprostenol may be injected. Of course, when ovulation is induced by GnRH agonists and a single fixed-time AI achieved, it becomes very easy to time the day when cloprostenol should be injected for optimal efficiency.

Parturition induction has numerous benefits: it allows for optimal use of the labor force for supervising the smoothness of the birth process, minimising the proportion of farrowing occurring during weekends or at night, and using optimal conditions for adoptions.

4.6.5 Diagnosing pathological causes of reproductive failure

a. Reproductive failure: What is affected by specific pathogens?
The table below summarises our understanding of the effects of the main pathogens involved in reproductive failure. Additional information is presented in the sections below dealing with specific pathogens.

Disease	Sow sick	Abort	<Total born	Mum-mies	Still-born	Weak born	Sick pigs Pre-wean	Sick pigs Post-wean
PRRS	Yes	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
Parvo	No	**No**	**Yes**	**Yes**	**Yes?**	**Yes?**	**No**	**No**
PCV2	Yes/no	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
PRV/ ADV	Yes	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
CSF	Yes	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**	**Yes**
SIV	Yes	**Yes**	**No**	**No**	**No**	**No**	**Yes**	**Yes**
TGE/ PEDV	Yes	**Yes**	**No**	**No**	**No**	**No**	**Yes**	**Yes**

Table 1 Parameters affected by main reproductive diseases

b. Infectious causes of reproductive failure

i. Porcine reproductive and respiratory syndrome virus (PRRSV)
The diagnosis of PRRSV in acute outbreaks in the breeding herd is fairly straightforward, especially if disease is occurring in weaned pigs. In endemic or chronic situations, diagnosis can be very frustrating if samples used to detect the virus are limited to foetal tissues.

Serology is most commonly performed by ELISA. An emerging problem in the USA is the increasing prevalence of singleton reactors (pigs that yield

a positive result with no history of contact with the virus). This has become very problematic for breeding-stock companies that use serology to confirm the negative status of the animals that they offer for sale. Another confusing issue with PRRSV serology is the kinetics of the S/P ratios following infection. The commonly accepted scenario says that PRRSV antibodies are detected by ELISA 7-14 days postinfection, rise to maximal levels at 1-2 months, and then decline to negative levels by 4-6 months. Recent studies and field experience suggest that titer levels do not necessarily decline to low levels so soon. In addition, the S/P ratio positive/negative cutoff level of 0.4 is perhaps too high. Pigs from negative herds rarely have S/P ratios over 0.15-0.20 except for the singleton reactors. On the other hand, many animals from positive herds have titers between 0.15 and 0.40. In positive herds that experience flare-ups of reproductive failure, high S/P (sample to positive) ratios provide a presumptive positive diagnosis. However, one needs to be careful in using only this testing strategy to diagnose PRRSV in this situation. In the strictest sense, serology indicates whether or not an animal has been infected. On the other hand, a negative test result does not necessarily indicate that the animal has never been exposed.

Virus can be detected in serum, tonsil scrapings, lung lavage fluid, and tissues (primarily lung and tonsil) following infection for a variable period of time. Tonsil scrapings appear to provide the best sampling technique with regard to sensitivity and practicality. Detection of the virus in aborted foetuses is confounded by the rapid degradation of the virus that can occur as the foetus autolyses. An alternative sample to aborted foetuses is weak-born pigs, which are sampled prior to suckling to prevent test interference by colostral antibodies and to enable testing for foetal antibodies induced following transplacental infection. In young pigs that are necropsied, lung tissue usually provides the best sample.

Detection of viral antigen in tissue is increasingly done by immunochemistry for a variety of reasons, including convenient sample preservation (fixed in formalin versus refrigeration or freezing) and the ability to visualise the viral antigen within the tissue and appropriate cell types.

If genetic evaluation of the virus is desired, then virus isolation via cell cultures is necessary in order to propagate sufficient virus for genetic analysis. With regard to detecting PRRSV in fluids such as serum or semen, the polymerase chain reaction test (PCR) is commonly used. PCR provides a substantial advantage for detecting PRRSV in semen, because semen can be quite toxic to the cell cultures used for virus isolation. A somewhat controversial use of PCR is testing semen that originates from boar studs that are endemically infected with PRRSV. The test is completed before the semen is shipped out. In the past several months, nearly a dozen large boar studs in the Midwest USA have contracted PRRSV, and transmission to negative sow herds has resulted. Following the outbreaks, the studs are monitoring semen for PRRSV via PCR. Whether or not this strategy is sufficiently sensitive to detect PRRSV-contaminated semen is debatable. On the other hand, it has been shown that the minimum infectious dose for exposure via artificial insemination is higher than by the usual oronasal route.

ii. Porcine parvovirus (Parvo)

Diagnosis of parvo is fairly straightforward if mummified foetal tissue is available for testing. Parvo is very durable, and the antigen appears to concentrate in the tissues as the foetus mummifies. The most definitive test is direct immunofluorescence (FA). Compared to FA tests for other diseases, the parvo FA is one of the better systems. If FA is not available, testing the tissue for hemagglutinating activity can be used to detect parvo. Basically, the foetal tissue is minced and suspended in a buffered saline solution. The suspension is centrifuged to remove the tissue debris, and serial dilutions of the supernatant are mixed with a dilute (0.5%) suspension of guinea pig erythrocytes in buffered saline. The test can be conducted in plastic or glass culture tubes or in microtiter plates. If parvo is present, the virus will agglutinate the erythrocytes, appearing as a jagged-edged button in the bottom of the tube or plate. If the virus is not present, the button will be dense and have a smooth edge.

Unfortunately in many regions, diagnostic labs lack the capability to perform the FA test, and the hemagglutination test is not as definitive. Accordingly, serological diagnosis is the only method available. The hemagglutination-inhibition (HI) test is the primary assay used. The test is

based on the ability of serum antibodies to inhibit the agglutination reaction described above in the presence of a known amount of virus. The HI test can be used to detect antibodies in foetal serum or thoracic fluid, but one needs to be careful with regard to overinterpreting the results because the process of foetal death may result in a false-positive reaction. Testing for immunoglobulins in general by radial or single immunodiffusion will confirm the presence of antibodies and provides support of a positive HI test from foetal serum.

Serological testing of dams is fairly straightforward with regard to performing the HI test, but interpretation can be very frustrating. The antibody response induced by parvo is somewhat different from other diseases in several ways:

1. Following experimental infection, serum antibodies are detectable within 4-5 days and reach their maximum levels within 11-14 days.

2. These titers are quite high and appear to persist at very high levels for the duration of the animal's life. For example, in our laboratory, the highest titer measured is 1:8,192, and many animals have antibody titers that exceed this maximum reported titer.

3. Because of the high titers in the dams, maternal antibodies in the piglets can be quite high and can persist until 5-7 months of age. The half-life decay of maternal antibodies is 17-19 days. Accordingly, the titer will decline one dilution every 17-19 days. As with other diseases, the titer of the sow at farrowing is equivalent to the titers of her offspring when they are approximately 4 weeks old, so the titer of the young pigs will exceed the titer of the sow.

4. Another unique feature of parvo occurs with vaccination. Vaccination of naïve animals induces a relatively low titer, ranging from nondetectable to perhaps 1:32. Following subsequent exposure to field virus, the titer DOES NOT increase in most animals. This is contrary to almost every other disease that we deal with. Eventually, the titers may rise to very high levels, although reproductive failure appears to be NOT associated

with this rise. This is the main reason why a wide variation of titer levels is observed in sow herds.

5. Because the virus is ubiquitous in the environment, many gilts become infected prior to mating, which induces lifelong immunity.

In summary, serotesting for parvo in cases of reproductive failure is only useful for excluding the disease, which rarely occurs because nearly all herds are endemically infected. The typical strategy for paired sampling is useless unless the first sample was collected before breeding. The main justification for parvo serology is monitoring the immune status of gilts prior to breeding as a surrogate measure for assessing acclimatisation programs for infections that are ubiquitous in a swine herd.

iii. Swine influenza virus (SIV)

The role of SIV in reproductive failure has become more of an issue with the emergence of H3N2 in the USA. As nearly all herds were completely naïve and susceptible, outbreaks resulted in severe systemic disease, which led to abortion. Diagnosis of SIV is fairly straightforward, provided that an animal with a productive infection is sampled. A major constraint for diagnosing SIV is the short duration of productive infection. Virus is detectable in nasal swabs for up to five days postinfection, and detection in tissues is often limited to less than seven days postinfection. Human kits for detecting Type A influenza virus can be used for detecting SIV in nasal swabs or lung lavage fluid.

Serological diagnosis in sows is limited by the widespread distribution of SIV in swine populations, with the exception of emerging strains like H3N2. Changes in titers between acute and convalescent samples can improve the accuracy of maternal serology. Presently, hemagglutination inhibition (HI) is the main serological test used in the USA. Because the hemagglutinin reaction is strain specific (H1 vs. H3), a separate test needs to be performed for each type. SIV titer interpretation is a confusing and controversial issue. First of all, the variability in titer levels between laboratories can be significant. Secondly, many diagnosticians report titers of <1:40 as not significant. It is unclear as to where that recommendation originated from and the frame of reference for the significance

designation. In searching for SIV-negative herds for research purposes, pigs that originate from herds that are truly free of SIV are uniformly seronegative. Herds that are infected with SIV exhibit wide variation in titers unless the pigs are vaccinated. Vaccines induce moderately high titers (such as 1:40 to 1:320), whereas pigs that are both vaccinated and infected often exhibit titers that exceed the highest reported titer, 1:640. ELISAs for SIV are becoming available, but experimental data and field experiences regarding their use in general and specifically with cases of reproductive failure are limited at this time.

iv. Aujeszky's disease virus (ADV)

Reproductive failure due to ADV is rare because of the widespread use of vaccines in herds that are located in endemically infected areas. Within the past two months, the number of quarantined herds in the USA has declined to near zero as a result of a national eradication program. The most prominent feature of this eradication program was the use of gene-deleted, modified live virus vaccines that enabled the detection of infected animals regardless of their vaccination status. In addition, these vaccines reduced the level of postexposure shedding such that transmission within a herd was eliminated and endemically infected herds eventually became negative for field virus.

ADV-induced reproductive failure is nearly always accompanied by clinical disease in either dams or pigs on the farm. Occasionally, ADV-infected foetuses and neonates will exhibit multifocal hepatic necrosis. This lesion appears as small, pinhead-size white lesions on the surface of the liver. Oftentimes, the diagnosis is based on detecting virus by immunofluorescence in the tonsils of neonatal pigs exhibiting clinical signs. In situations where only sows are present, foetal tissues, nasal swabs collected from the dams, or tissues (tonsil) collected from the dams following necropsy can be tested by immunofluorescence or virus isolation using a wide variety of cell cultures that are permissive to ADV.

A wide variety of serological tests are available for detecting antibodies in maternal serum. Foetal serum can also be tested, but this is rarely necessary for establishing a diagnosis.

v. Other viruses

Other viruses that have been implicated in reproductive failure include enteroviruses, encephalomyocarditis virus (EMC), Japanese B encephalitis virus, cytomegalovirus, and classic swine fever. The enteroviruses were originally believed to be the causal agent of SMEDI (stillbirths, mummies, embryonic death, and infertility), but after the discovery of parvo, most SMEDI cases were diagnosed as parvo. Multiple enterovirus serotypes exist, and separate serological assays are often required for each serotype. Even though enteroviruses are ubiquitous in most swine populations and are fairly easy to isolate and detect in cell culture, they are rarely incriminated in reproductive failure. Classic swine fever can induce a wide variety of clinical signs, and congenital infection can lead to abortion, foetal mummification, and stillbirth. Occasionally, abortion can occur during TGE virus outbreaks, especially following the feeding of intestinal contents for establishing uniform herd immunity. The other viruses are rarely diagnosed and/or are limited to certain regions of the world. Their impact on reproductive performance is summarised in the table below.

Disease	Sow sick	Abort	<Total born	Mummies	Stillborn	Weak born	Sick pigs Pre-wean	Sick pigs Post-wean
EMC	No	Yes	Yes?	Yes	Yes	Yes	Yes?	Yes?
Teschovirus	No	No	Yes	Yes	Yes	Yes	Yes	Yes
ASF	Yes	Yes	Primarily abort				Yes	Yes
BVD	No	Yes	Yes	Yes	Yes	Yes	Yes	No
PRNS	No	Yes	Infertility				Yes	Yes
JVE	No	Yes	Yes	Yes	Yes	Yes	Yes?	No
PCMV	No	No	Yes	Yes	Yes	Yes	Yes	Yes
Blue Eye	Yes	Yes?	Yes	Yes	Yes	Yes	Yes	Yes
Menangle	No	No	Yes	Yes	Yes	Yes	???	???

Table 2 Rare viruses affecting reproduction

vi. Bacterial and toxin diseases associated with reproductive failure

- *Leptospirosis*

The diagnosis of leptospirosis is primarily based on serological evaluation of the dams in most instances. Detection of the organisms in tissues, urine, or blood by culture procedures is difficult and time consuming and requires specialised laboratories. Immunochemical tests, such as immunofluorescence, are commonly used in diagnostic laboratories to identify leptospires in tissues, urine, and foetal fluids, but they are not as sensitive as culture. Several laboratories have published PCR procedures, but implementation in diagnostic laboratories has been limited to date.

The most widely used serological test is the microscopic agglutination test (MAT). In adult animals, titers >1:1000 are considered positive for pomona. Interpretation of titers with other serovars is not as well defined, and serial sampling to demonstrate rising titers during acute and convalescent stages of infection may be necessary to achieve a definitive diagnosis. Foetal serum or thoracic fluid can be tested for antibodies, and a positive test is considered diagnostic.

- *Other bacteria*

In the USA, an extensive serosurveillance program is in place to identify herds infected with *Brucella suis;* typically, these infected herds are depopulated. Detection of singleton reactors in noninfected herds is a common event. These reactors are often detected in sows at slaughter. In order to rule out the possibility of an infected herd, the single reacting sample may be tested by a different procedure and/or a representative number of animals from the herd of origin are tested. Each state tends to have their own protocol, and the exact follow-up investigation is handled on an individual herd basis.

Bacteria isolated from cases of purulent vulvar discharge are often environmental bacteria such as *E. coli, Streptococcus spp.,* and others. Purulent discharges that originate from the reproductive tract are highly suggestive of metritis or endometritis. Purulent discharges from the vulva are also observed with urinary tract infections.

Quite commonly, a wide variety of bacteria are isolated from aborted foetuses. In many cases, environmental bacteria or the normal flora of the birth canal contaminate the aborted foetus during and after expulsion. In some instances, the bacteria infected the foetal tissues prior to expulsion, suggesting transplacental infection. Further confirmation of transplacental bacteria infection is based on finding inflammatory lesions in either the foetus or the placenta.

The table below summarises the reproductive consequences of these pathologies.

Disease	Sow sick	Abort	<Total born	Mum-mies	Still-born	Weak born	Sick pigs Pre-wean	Sick pigs Post-wean
Leptospirosis	Yes	Yes	Yes	Yes?	Yes	Yes	Yes	Yes
Brucella suis	No	Yes	???	???	Yes	Yes	???	???
Chlamydia sp.	No	Yes	Infertility				Yes	Yes
Carbon monoxide	No?	Yes	No	No	Yes	Yes	Yes	Yes
Zearalenone	Yes	No?	Yes	No	No	No	Yes	Yes?

Table 3 Reproductive consequences of miscellaneous factors

c. Concluding remarks

Diagnosis of reproductive failure is often a difficult and frustrating task. Although our diagnostic capabilities have improved, investigations often fail to identify a specific cause. There are several reasons for this:

1. The clinical manifestations of reproductive failure (reduced litter size, pregnancy loss, or failure to cycle) occur long after the insult has occurred.

2. Reproductive failure investigations tend to focus on infectious causes, while noninfectious causes are minimally considered.

3. Poor application of effective management practices related to reproduction and general animal care at the farm level.

Accordingly, investigation of reproductive failure cases must involve a variety of activities:

1. Record analysis to determine the severity of the problem, identify animal-related risk factors (parity, location), identify seasonal or other time-related relationships, and determine the stages of gestation affected by characterising the clinical manifestations observed. The main benefits of record analysis are narrowing the list of potential causes and developing an effective diagnostic sampling strategy.

2. Herd observation is oftentimes the most critical activity, especially if the underlying cause is related to management factors rather than a specific infectious agent.

3. Examination of individual animals tends to be overlooked. Our emphasis on the "herd" sometimes overshadows the obvious fact that examination of the individual animal may also provide useful clues.

4. Laboratory testing is almost always focused on identifying infectious causes of reproductive failure. Both maternal and foetal sera are tested for antibodies and/or infectious agents, and tissues are tested for the presence of an agent or a lesion. Laboratory testing of feed for mycotoxins is a common practice. The main limitation of laboratory testing is that the causal agent may be no longer present when the samples are collected. Serology provides a means to determine if an infection occurred, but in many cases the timing of infection can't be directly linked to when the reproductive failure occurred.

In overt outbreaks of reproductive failure that are produced by known reproductive pathogens that also cause disease in other age pigs and systemic disease in the sows, such as porcine reproductive and respiratory syndrome virus and Ajueszky's disease virus, diagnosis is fairly straightforward. With outbreaks caused by infectious agents that are not considered to be primary reproductive pathogens but cause significant systemic disease, such as swine influenza virus, one needs to be careful with regard to overlooking other agents that might be present.

4.7 The boar and its use for artificial insemination

4.7.1 Sperm production and quality

In semen production centers, boars are selected for collection at around 8 months of age. An average ejaculate contains 101±36 billion sperm in about 272±86 mL. Ability of spermatozoa to move is commonly assessed by computer sperm analysis (CASA). This technology documents the ability of individual spermatozoa to move (via the "velocity average path" end point) and to have straight trajectories (via the "velocity straight line" end point). A limitation of this approach is that it does not document morphological abnormalities. These tests are sometimes complemented by functional tests using fluorescent probes that provide information on integrity of its membrane (via propidium iodine), on the functionality of the mitochondria (Rhodamine 123), or on the quality of its acrosome (PSA). Another useful approach that provides information on quality of the membranes is resistance to a hypo-osmotic test.

Finding a link between the CASA measurements and fertility in vivo, following the use of insemination doses containing 3 billion spermatozoa, has generally proven difficult (Gadea, 2005; Didion, 2008). In contrast, Druart et al. (2009) demonstrated a link between fertility in vivo and resistance to a hypo-osmotic shock. The optimal way to predict fertility from a specific boar appears to be a combination of sperm parameters (motility and the percentage of sperm with cytoplasmic droplets, together with the ability of sperm heads to form male pronuclei following coincubation with oocytes in an IVF test (Ruiz-Sanchez et al., 2006)).

As seminal plasma has detrimental effects on survival of spermatozoa, following collection, semen is generally quickly diluted using a range of media (BTS, Androhep, and many others), ensuring that spermatozoa will remain viable in vitro for up to 7 days. However, a general prerequisite to obtaining high fertility is to use semen as fresh as possible—not older than 72 hours.

4.7.2 Sperm use for AI

Three insemination strategies may be used in swine:

- The standard one, which is still the most commonly used, is cervical insemination with doses containing 3 billion spermatozoa in around 80 mL. High farrowing rates and good litter sizes are generally obtained with this approach.

- Postcervical insemination may be used in sows, but not in gilts. It requires doses containing only 1 billion spermatozoa, which are deposited about 20 cm past the cervix (Watson and Behan, 2002). Farrowing rate and prolificacy are in the normal range, provided the insemination doses contain more than 1 billion spermatozoa (Rosenboom et al., 2004).

- Deep intrauterine insemination was developed more recently. It uses low volumes (5 mL) containing fewer spermatozoa (0.1 to 0.01 billion) that are deposited as close as possible to the uterotubal junction (Martinez et al., 2002; Roca et al., 2003). It is of value when the amounts of spermatozoa available for insemination are limited (rare genetics, sexed semen). Unless the insemination doses contain too few spermatozoa (around 1 million), this procedure generates acceptable fertility and prolificacy results.

Freezing swine semen is technically possible. However, obtaining high fertility and prolificacy following use of frozen semen is impossible unless there is a very tight synchrony between insemination and ovulation (time lag not exceeding 4 hours). Indeed, survival of frozen spermatozoa in the genital tract is very brief (Waberski et al., 1994b). The development of ovulation

induction strategies using GnRH agonists may be an approach whereby this tight synchrony may be achieved.

Sexed semen has been produced in boars. When sexed semen was used to produce embryos by in vitro fertilization, almost all embryos obtained were of the expected sex. However, the combination of the tedious and time-consuming semen-sexing process and the large number of spermatozoa needed for each insemination dose do not make it a viable strategy nowadays (Bathgate et al., 2008), even if intrauterine insemination is used.

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5.1 Physiology

5.1.1 Seasonality of sexual and ovarian activity

One of the most important features of ovine reproduction is seasonality, though this is not exclusive to sheep, of course. Reproduction follows a seasonal pattern in ewes, ie, alternating periods of anoestrus and sexual activity. In temperate regions, seasonality is regulated by the photoperiod, or daylight length (reducing daylight length stimulates sexual activity, and increasing daylight length induces anoestrus). Sheep are therefore categorised as 'short day' breeders.

Ewes are able to 'monitor' changes in the daily photoperiod by the circadian secretion of melatonin from the pineal gland. Melatonin output is regulated by photoperiod, and elevated concentrations are found in blood only during the hours of darkness (O'Callaghan, 1994; Rosa et al., 2003). The characteristics of the circadian pattern of melatonin secretion vary with changes in the light-dark cycle throughout the year, enabling the animal to 'recognise' the changes in the light/dark ratio. Melatonin has a profound effect on the secretion of gonadotrophin-releasing hormone (GnRH) from the hypothalamus, which modulates the release of pituitary gonadotrophins, and these, in turn, control seasonal reproductive activity.

While the photoperiod is the main determinant of seasonality, other factors can influence reproductive patterns, such as genetics (some breeds being sensitive to daylight variation), management practices (eg, the ram effect; see Section 5.3.2), and social interactions (Henderson and Robinson, 2000).

The length of the breeding season varies between breeds. Dorset Horn ewes are theoretically capable of lambing at any time of the year, although an 8-month breeding season might be expected within a particular flock (Henderson and Robinson, 2000). Mountain breeds, such as the Scottish Blackface, Swaledale, Welsh Mountain, and Cheviot, exhibit much shorter seasons of approximately 4 months. Crossbreeds (Greyface and Mule) are often characterised by only a moderate duration of reproductive activity. Despite these variations, there is a peak of fertility in late autumn (October-November) for most of the breeds in the Northern Hemisphere. Therefore,

the highest lambing rates are recorded in late March and April. Breeds from the intermediate latitudes, such as the Australian Merino and Mediterranean breeds, have a short anoestrus during which a proportion of ewes ovulate spontaneously. In tropical and subtropical environments, ewes are either completely nonseasonal or intermittently polyoestrus, with the availability and quality of food dictating sexual activity. Yearling ewes and ewe lambs have a shorter breeding season than older ewes.

During the nonbreeding season (anoestrus), oestrous cycles as such are not observed. Although the behavioural signs of oestrus and ovulation are absent, dynamic changes in ovarian follicular growth and regression nevertheless occur throughout the nonbreeding season. Anoestrus is due to the failure of antral follicles to proceed to growth and maturation, which normally happens in the pre-ovulatory phase of the oestrous cycle (O'Callaghan, 1994). However, the further development of these follicles can be stimulated artificially, which allows for breeding during anoestrus or the transition periods.

Seasonality not only affects the mature animal, it can also influence the age of onset of puberty. Although genetics plays a major part in determining age at puberty, the season in which birth takes place (ie, the photoperiod at that time) can either advance or delay puberty for several months. Oestrus activity ceases with pregnancy and is not resumed for some time after lambing, due to so-called 'postpartum anoestrus,' also known as 'lactational anoestrus'. The length of this period varies with breed, management practices, and the date of parturition, since seasonal and postpartum anoestrus may overlap in some instances. Postpartum anoestrus is mainly due to the 'anti-gonadotrophic' effect of the suckling lamb, so it normally ends shortly after weaning. Even when not suckling lambs (eg, when lambs are reared on milk replacer), the ewes' immediate postpartum period is mostly spent in anoestrus.

Whilst rams are able to mate at any time of the year, both the lack of libido and the lower quality and quantity of the ejaculate during the nonbreeding season can reduce the efficiency of out-of-season breeding (Henderson and Robinson, 2000).

It is well known that, independent of seasonal influences, nutrition affects many aspects of reproductive performance in sheep, eg, age at puberty

in both sexes, fertility, ovulation rate, embryo survival, parturition to re-breeding interval, testicular growth, and production of spermatozoa (Rosa et al., 2003). Lactation length can also affect the breeding season. Under normal conditions, in highly seasonal breeds, birth occurs during seasonal anoestrus and therefore there is no obvious lactational anoestrus. When the ewes are induced to breed during seasonal anoestrus, however, they lamb during the usual breeding season and the resumption of ovarian activity is known to be delayed in lactating animals.

a. Influence of high ambient temperatures on reproductive function in sheep
Marai et al (2007) recently reviewed the impact of high ambient temperatures on various physiological features in sheep. They concluded that exposure to high temperatures evokes a series of drastic functional changes, including reduction in feed intake and conversion, disturbances of water, protein, energy metabolism, and interference with mineral balances, enzyme-controlled reactions, hormonal secretions, and blood metabolites. Such changes result in an impairment of production and reproductive performance. The effect of heat stress is aggravated when accompanied by high humidity. These data highlight that ambient temperature should be taken into consideration, both when planning breeding programmes, as well as when evaluating their results.

5.1.2 The oestrous cycle

Nonpregnant females separated from the ram, or failing to conceive after mating, have alternate periods of anoestrus and sexual activity. The latter are characterised by a succession of regular oestrous cycles. The length of the oestrous cycle is 16-17 days, with a range of 14-19 days. However, in the transition period between anoestrus and sexual activity (end of summer), short cycles of less than 12 days are quite common. The first ovulations of the season are often not accompanied by oestrus behaviour (known as 'silent oestrus' or 'silent heat').

As in other species, the oestrous cycle can be divided into two phases: the follicular phase of 3-4 days and the luteal phase lasting about 13 days. A wave-like pattern of follicular growth has been recorded in sheep, similar to that observed in cattle, with two to four waves per cycle being the most common (Evans, 2003). In general, follicle waves are preceded by a

transient increase in FSH concentrations, and a hierarchy is established among the follicles of a wave in respect of their diameter and the oestradiol concentration in the follicular fluid. There is no consensus as to whether or not an absolutely dominant follicle develops during each wave. Follicular growth continues even during periods of anoestrus supported by FSH fluctuations, but this does not lead to ovulation.

The duration of oestrus varies with age, breed, and season, ranging between 18 and 72 hours, with an average of 36 hours. In mature ewes of most British breeds, oestrus lasts 30 hours on average, while in lambs it is at least 10 hours shorter. In Merino ewes, heat may even last up to 48 hours. Ovulation is spontaneous and takes place approximately 20-40 hours after the beginning of oestrus (Henderson and Robinson, 2000). As in other species, the overt signs of oestrus result from elevated concentrations of circulating oestrogen, which reach a peak just before the onset of oestrus proper and immediately prior to the luteinising hormone (LH) surge.

Oestrus in the ewe is a less obvious event than in other ruminants. The vulva of ewes in heat is slightly swollen and congested, and a limited discharge of clear mucus can often be noticed. If a ram is present, ewes in oestrus will seek him out and may display tail-wagging and nuzzle his scrotum. Simultaneously, the ram will 'test' the receptivity of ewes in his group by pawing with a forefoot, by rubbing his head along the ewe's flank, and by nibbling her wool. A non-receptive ewe will move away, while one that is fully in heat will stand to be mounted. But in the absence of a ram, or when only an inexperienced ram is present, oestrus can often go undetected.

Ovulation rate (number of eggs released at ovulation) is influenced by a number of factors, including breed, age, reproductive status (dry or lactating), season of the year, nutritional status, and the body condition of the ewe. At the beginning of the breeding season, ovulation rates are usually lower, and oestrus is generally shorter, less intensely demonstrated, and of lower fertility.

Fertilisation takes place in the fallopian ampulla, approximately 25-31 hours after the first signs of oestrus, with zygotes descending into the uterus 60-65 hours later. Until day 15 after fertilisation, ovine embryos migrate throughout the uterine lumen.

The luteal phase is characterised by the maturation of the corpus luteum and elevated levels of circulating progesterone, which reach a peak at about 6 days after ovulation. The luteolytic mechanism is similar to that of the cow, with an increase in the numbers of oxytocin receptors, up-regulated by increasing concentrations of oestradiol produced by the pre-ovulatory follicle of the next wave. Stimulation of the oxytocin receptors triggers the release of $PGF_{2\alpha}$ and both the functional and the structural demise of the corpus luteum (Mann and Lamming, 1995). The luteal phase following the first ovulation of a breeding season is usually shorter in duration.

The gestation period in sheep is about 5 months, 145-152 days on average. Its length varies mainly with breed, parity, and litter size.

Prior to the maternal recognition of pregnancy, the cyclical corpus luteum in the ovary is the only source of progesterone. The corpus luteum of pregnancy continues to be the predominant source between 13 and 55 days postfertilisation in sheep, whereas the placental production of progesterone is sufficient to maintain pregnancy from 55 days of gestation onwards (Sammin et al., 2009).

Similar mechanisms to those in cattle for the recognition and maintenance of pregnancy have been defined in sheep. Briefly, the production of interferon tau by trophoblasts between 8 and 21 days postconception, exerts a local action on the endometrium, which blocks the pulsatile secretion of $PGF_{2\alpha}$, thus prolonging the lifespan of the corpus luteum.

5.2 Flock reproduction management

5.2.1 Introduction

Low productivity is a feature of traditional extensive systems of sheep production. The seasonal nature of production reduces the economic viability of the traditional flock. Therefore, more modern management systems must be associated with various levels of intensification, the success of which are determined to a large extent by the efficiency of reproductive management.

5 Ovine Reproduction

Reproduction may be managed for various reasons:

1. Improvement in productivity of the flock
   - General improvement of fertility
   - Increased prolificacy
   - Increased number of lambings per year
2. Planned reproduction
   - Seasonal demands: meat breeds to cater for periods when price or demand is highest
   - Introduction of ewe lambs to the flock
   - Sustained milk production, ensuring production in periods when the milk price is high
   - Labour efficiency
   - In specific conditions: extensive, small-scale production
   - Sustained supply for the community/family with milk and meat
3. Use of artificial insemination
   - Genetic improvement
   - Scrapie control measures: use of rams with scrapie-resistant genotypes
   - Maximising the use of the best rams
   - Reduction of the number of rams needed within the flock
   - Reduction in the spread of infectious disease

Table 1 presents the basic parameters used to evaluate reproductive efficiency in sheep flocks.

Fertility	= Number of ewes lambing / Number of ewes exposed to the ram or artificially inseminated x100
Prolificacy	= Number of lambs born (dead and alive) / Number of ewes lambing x100
Fecundity	= Number of lambs born (dead and alive) / Number of ewes exposed to the ram or artificially inseminated x100

Table 1. Definitions of reproductive parameters frequently used in ovine reproduction.

Fertility, the proportion of ewes lambing of all those exposed to the ram during a defined period (usually expressed as a percentage), varies with breed, season, age, nutritional status, breeding management, and farm conditions. An average figure of 70% to 80% following natural mating is considered normal to good for autumn breeding and good to very good for

spring breeding. Artificial insemination (AI) produces poorer results than these. Prolificacy (the number of lambs born per lambing ewe), usually expressed as a percentage, varies widely according to the same factors as for fertility. The Merino is recognised as a breed of low prolificacy, commonly 110%-120%, while the Romanoff breed frequently reaches levels of 350%. Fecundity represents the number of lambs born per ewe mated, during a defined period.

5.2.2 Pregnancy diagnosis

Pregnancy diagnosis can help to increase reproductive efficiency. Amongst other benefits are the early re-mating of nonpregnant ewes and the supplementary feeding of those that are pregnant. Moreover, the ability to predict the number of foetuses allows more appropriate nutritional management of the ewes in late gestation aimed at preventing pregnancy toxaemia, minimising prelambing feeding costs, optimising birth weights, viability, and weaning weights of lambs and reducing the incidence of dystocia.

Of the various methods of pregnancy diagnosis in sheep, ultrasound scanning is the most accurate and reliable. A-mode ultrasound (Amplitude-depth or echo-pulse) can be used. It is a quick, convenient, and simple technique, but it cannot predict foetal numbers and the viability of the foetus. Real-time B-mode ultrasonic scanning of the uterus in sheep is very much more common. When performed by a skilled operator, it offers an accurate, rapid, safe, and practical means for diagnosing pregnancy, determination of foetal numbers, and estimation of gestational age. For transabdominal pregnancy diagnosis the probe of the ultrasound scanner is applied flat against the bare area of the right flank, 2 to 3 inches forward of the right teat. Good contact between the ultrasound probe and the skin is essential, so the area should be cleaned adequately before the examination, and the application of ultrasound gel is very helpful.

The optimum time for transabdominal or transrectal ultrasonography in sheep ranges from 25 to 100 days of gestation. Real-time ultrasound can detect pregnancy as early as 23 days of gestation using a rectal probe, and by 40 days using external transabdominal scanning. The number of foetuses can be counted accurately from about 45 to 100 days of pregnancy.

After 100 days, it becomes more difficult to count accurately, so scanning is normally undertaken between the 12th and 13th weeks after the rams are introduced to the ewes.

Possible causes for diagnostic errors include:
- Incorrect probe placement – misdirection of the beam towards the urinary bladder
- Pockets of gas accumulated in the intestines interpreted wrongly as embryonic vesicles
- Ewes in oestrus occasionally accumulate enough uterine fluid to cause the uterus to sink to the bottom of the abdominal cavity, making accurate examination difficult

Doppler and amplitude-depth (A-mode) ultrasound are cheaper alternatives during the second half of gestation.

a. Other methods

The use of the oestrone sulphate assay can detect pregnancy accurately in ewes from day 30 to 35. Detection of specific pregnancy-associated proteins is possible in pregnant sheep. Both pregnancy-specific protein B (PSPB) and ovine pregnancy-associated glycoproteins (PAGs) can be used. These methods, however, are still limited in availability under field conditions and cannot be used to detect the number of foetuses.

5.2.3 Oestrus detection

Oestrus is not generally well expressed in sheep, especially in the absence of rams; the most obvious sign is standing to be mounted by a ram. While oestrus detection is of no importance in natural mating, it is vital for the success of AI or 'hand mating' (see Section 5.2.4), as these can only be successfully performed at a fixed time in relation to ovulation or to the onset of oestrus. For ewes managed in flocks, the most common methods of oestrus detection are the use of entire, 'aproned' rams (the ram's penis is covered to prevent intromission) or vasectomised 'teaser' rams, fitted with harnesses containing marking crayons. For AI, these methods are not very useful because they are time-consuming and labour intensive. For AI using fresh semen, oestrus detection is only useful for large flocks under very special conditions and then only during the breeding season.

An alternative to oestrus detection is the control or synchronisation of oestrus (see Section 5.3), which reduces the period during which the flock is inseminated, requires less labour, and allows the more efficient management of pregnancy and parturition. It can also be used to induce oestrus and ovulation outside the normal season.

5.2.4 Mating

In natural mating conditions, the length of the oestrous cycle and the duration of oestrus mean that about 6%-8% of ewes will be in oestrus each day of the breeding season. Assuming there is a ram for every 50 ewes (50:1 ratio), each will need to mate an average of 3-4 ewes per day. This is compatible with the serving capacity of the ram and allows for good fertility. The high concentration of spermatozoa per ejaculate, together with the repeated mating of the ewe throughout oestrus, ensures a good level of fertility and prolificacy. However, the reproductive performance of rams is affected by seasonal influences (Henderson and Robinson, 2000) and the requirements of out-of-season breeding and the greater number of ewes coming into oestrus as a result of synchronisation impose the need for a more rational use of rams.

Fertility increases as oestrus progresses, reaching a maximum towards the end of the oestrus period. Therefore, the only way to increase fertility, while at the same time optimising the use of the ram, is to practice 'hand mating'. This involves the rams being lined up in a queue in the shedding race and each ram in turn being exposed to a group of (preferably synchronised) ewes. Following an observed mating, the ewe is withdrawn from the group and the ram is taken to the back of the queue. The next ram in line is then exposed to the unmated ewes. The improvement of desirable production traits requires the selection of superior animals for breeding. Since rams are responsible for more offspring than ewes, ram selection is critical. One of the ways of managing selective breeding is batch mating; a group of ewes is mated exclusively by the same ram, using 'hand mating' after oestrus detection or synchronisation, or by artificial insemination.

5.2.5 Artificial insemination

AI is the gateway to the use of top quality sires of both local and international origin. It offers progressive producers the opportunity to make previously unimaginable genetic improvements in a very short period. Considerable progress can thus be gained in respect of commercially important features such as milk production, feed conversion, and growth rates of fattening lambs, as well as wool quality.

Artificial insemination (AI) brings well-known benefits for sheep production, but there are distinct differences between its use in sheep and its more common use in cattle. Because of its different anatomy, the ovine cervix cannot be easily entered with an insemination pipette. This was the subject of extensive investigation by Kershaw et al (2005). Essentially, the lumen of the ovine cervical canal is highly convoluted and tortuous due to the presence of 4-7 cervical rings pointing caudally. These provide a physical barrier to external contamination, but also present the major barrier to transcervical artificial insemination (TCAI) since they not only project into the lumen, but the second and third rings are frequently out of alignment with the first. As a result, the inseminating pipette is diverted away from the lumen, which means semen must be deposited at the entrance to the cervix – intracervical/transcervical AI, or in the fundus of the vagina – intravaginal AI (Haresign, 1992).

In the transcervical method, a small volume of diluted semen is inserted just inside the external os of the cervix. The ewe's hindquarters are elevated, usually by placing them over a fence rail. The inseminator uses a duck-billed speculum inserted into the vagina and a head lamp to enable him to guide the insemination pipette into the cervix. The semen is deposited no more than 10-20 mm inside the cervical canal. With the help of two catchers, a skilled operator can inseminate 100 ewes per hour using this method. With the development of transcervical insemination skills, improved pregnancy rates are now being achieved (Anel et al., 2005; Paulenz et al., 2005).

An alternative is the use of intra-uterine AI, which is performed surgically with the aid of a laparoscope (Wulster-Radcliffe et al., 2005). In this case,

0.02 mL of diluted semen containing about 15-40 x 10^6 sperm is deposited into the lumen of one or both uterine horns from a sharp-tipped glass pipette or needle and syringe, and inserted through the ventral wall of the abdomen. This technique is attractive when valuable, frozen-thawed semen is to be used, since it permits good pregnancy rates with much smaller sperm doses than those used in the intracervical and intravaginal methods.

When properly performed, depositing frozen semen into the uterine horns produces high fertility rates and lambing percentages of 60%-75% (Buckrell et al., 1994; Windsor, 1995; Husein et al., 1998). These are similar to the results obtained using fresh semen, and this method is practised routinely in Australia for AI with frozen semen. Results are good but the procedure is difficult and costs are relatively high. Nonetheless, every year, millions of sheep are inseminated laparoscopically.

Semen type	Insemination method	Dose (in millions of sperm cells)	Typical pregnancy rate reported	Range in pregnancy rate reported
Fresh	Intravaginal	>300	50%	40%-65%
	Intracervical	150	40%	50%-70%
	Laparoscopic	50	70%	60%-90%
Frozen	Intravaginal	>300	10%	0%-30%
	Intracervical	75-150	40%-50%	30%-60%
	Laparoscopic	25-60	65%	50%-90%

Table 2. Typical results from AI in ewes.

For AI to be successful, the timing of the deposition of semen in the ewe must be accurate in relation to the time of ovulation, because the period during which fertilisation can take place is limited. In most ewes, ovulation occurs at about 25 to 30 hours after the onset of oestrus.

As oestrus detection is impractical under most field conditions, AI is only used in flocks using oestrus synchronisation. Artificial insemination is carried out at a fixed time, depending on the breed of ewe, the storage of the semen (chilled or frozen), the method of synchronisation, and the site chosen for the deposition of semen (see Table 3).

Type of Oestrus	Type of AI	Optimum time for AI
Natural	Cervical or vaginal	12-18 hr after onset of oestrus
Synchronised with intravaginal sponges	Cervical or vaginal	48-58 hr after sponge removal Single AI: 55 hr after sponge removal Double AI: 48-50 and 58-60 hr after sponge removal
	Intrauterine	60-66 hr after sponge removal
	Intrauterine in superovulated females	36-48 hr (preferably 44-48 hr) after sponge removal

Table 3. Time of insemination in sheep according to the type of oestrus and insemination.

In general, the following factors determine the success of artificial insemination in sheep:

1. With respect to the ewe herself:
   - Age
   - General heath status and body condition
   - Presence of any bacterial or viral infections affecting reproductive function
   - Seasonality
   - Oestrus type (spontaneous, induced/synchronised)
   - Management and nutritional plane in the post AI period
2. With respect to the insemination procedure:
   - Type of insemination method: intravaginal, transcervical, laparoscopic
   - Type of semen used: fresh, frozen
   - Quality of semen used
   - Service timing and oestrus management in inseminated ewes (synchronisation technique and pregnant mare serum gonadotropin/ equine chorionic gonadotropin [PMSG/eCG] dose)
   - Insemination technique and handling of semen
3. Environmental factors
   - Season
   - Temperature and humidity during peri-insemination period
   - Availability of water and feed during the peri-insemination period

5.3 Management of oestrus

The management of reproduction in ewes can be classified as natural (by altering the photoperiod, flushing, the ram effect) or pharmacological (using progestagens, prostaglandins, and melatonin). Only adjusting the photoperiod, the use of the ram effect and the various pharmacological methods allow for actual oestrus synchronisation in sheep.

The most important factors to be considered before deciding which method to use are:
- The degree of synchronisation needed
- The season
- Economic and market factors

The pharmacological methods are effective in the tight synchronisation of oestrus in the majority of situations, ensuring good production figures following fixed time insemination, but with the disadvantage of the expense of the product and its administration. The natural method is cheaper, but results in less tight synchronisation and is only useful in certain conditions.

5.3.1 Flushing

Flushing involves increasing the ewes' plane of nutrition (intake of protein and energy) approximately 3-4 weeks before the planned beginning of the breeding season. Ewes in improving body condition benefit from increased ovulation and therefore lambing percentages. Flushing is an established method for boosting ovulation rate but the response to the improved quality of forage in the weeks prior to mating varies with the breed and the season. Ewes usually respond best to flushing when they are in medium body condition (2.5-3.5 body condition score [BCS]). Flushing should be used as a method of improving prolificacy and fecundity, not in the hope of inducing or synchronising oestrus.

5.3.2 Altering the photoperiod

This technique involves exposing ewes to an artificially reduced daylight length following a period of extended daylight length. Used alone, it will hasten the onset of the breeding period, but with variable results and an unpredictable spread in the onset of cyclicity.

Nowadays, it is widely used for ewes in intensive production systems in combination with other artificial methods and for rams in AI centres. Sheep and goat AI centres equipped with dark housing use alternate light regimes with a month of long and a month of short days, allowing permanently high levels of semen production in rams and bucks with no seasonal variation in sperm quality. If year-round production of semen is not required, AI centres tend to maintain their rams in open barns and expose them to a period of 2-3 extra months of long days (Dec-Feb) followed either by return to the natural photoperiod or by prolonged treatment with melatonin (subcutaneous implants). Such a treatment stimulates good quantities of high quality semen in spring, mimicking the normal season of sexual activity, which itself lasts only around 2-3 months.

5.3.3 The ram effect

Social influences (eg, chemosensory, tactile, visual) are known to have potent effects on reproductive function in a variety of species. Rams can stimulate gonadotrophin secretion and ovulation in the anoestrus ewe through chemosensory input (Henderson and Robinson, 2000).

The ram effect involves the introduction of rams to ewes that have been separated from males for several weeks beforehand (at least 3-4 weeks). It has only proved to be effective at certain times of the year, usually just before the start of the natural breeding season, when the majority of ewes are not cycling. It is not effective for ewes already cycling or for those in deep anoestrus.

The majority of ewes ovulate within 6 days of the introduction of the ram, but the first oestrus is often silent and is often followed by one or two short cycles (of 6-7 days), or by a cycle of normal length with several peaks of

oestrus activity. It is the reason for this induced oestrus not being synchronised tightly enough to allow for fixed time insemination. It has been shown that the treatment of ewes with progesterone before, or at the time of, the introduction of the rams can improve the efficiency of this stimulatory technique by increasing the percentage of females showing oestrus behaviour at the first ovulation and by reducing the number of unpredictable short cycles.

It should be stressed that the efficacy of the ram effect varies with several factors, including breed, location, the time of year, nutritional status, and the age of the animals. Moreover, use of the ram effect alone does not synchronise oestrus and ovulation tightly enough to allow for fixed time artificial insemination.

5.3.4 Progestagen-based methods

These methods are based on the use of progesterone or its analogues. The latter are usually more potent, allowing for a smaller dose. The degree of synchronisation obtained and the interval between the end of treatment and the onset of oestrus depends on the product used.

In cyclic females, the treatment acts by suppressing the pre-ovulatory pituitary release of gonadotrophins and, therefore, follicular development and ovulation. After the withdrawal of the progestagen, the increasing amounts of gonadotrophin released lead to oestrus and ovulation. Although some progestagens can shorten the life span of the corpus luteum, for effective synchronisation in sheep, the duration of treatment must be at least 12-14 days, mirroring the length of the luteal phase.

a. Oestrus synchronisation with progestagens and artificial insemination
For artificial insemination, accurate oestrus detection and precise service timing are essential. Due to poor oestrus expression in ewes, this can be difficult to achieve, which is the reason for most AI in ewes taking place after pharmacologically synchronised oestrus. Among the various methods in current use, progestagens offer the most precise synchronisation of both oestrus and ovulation and, to all intents and purposes, progestagens are what make AI in sheep feasible. No other method used alone (ram effect, melatonin implants, etc) can ensure synchronisation tight enough for AI.

Progestagens can be administered in a variety of ways (sponges, implants, etc), via several routes (intravaginal, intramuscular, subcutaneous), and at different doses (Haresign, 1992; Godfrey et al., 1999; Bari et al., 2000; Henderson and Robinson, 2000). Intravaginal sponges are by far the most widely used, as they are easy to insert and provide reliable results after natural mating or AI. Sponges are impregnated with fluorogestone acetate or medroxyprogesterone acetate (MAP), and are inserted into the vagina using a dedicated applicator.

b. Use of pregnant mare serum gonadotrophin/equine chorionic gonadotrophin in progestagen-treated ewes

In ewes in anoestrus, progestagens must be supplemented with follicle stimulating treatments (eg, pregnant mare serum gonadotrophin/equine chorionic gonadotrophin [PMSG/eCG]) to induce follicular growth, oestrus, and ovulation. When ewes are being synchronised for fixed time AI using progestagen-based programmes, PMSG/eCG should always be used, to reduce the spread in the timing of ovulation due to individual variation between ewes. A normal fertile oestrus follows the progestagen/PMSG treatment. Ali (2007) demonstrated that PMSG/eCG administration to Ossimi ewes treated with fluorogestone sponges, in the subtropics, stimulated follicular development and increased prolificacy. In this experiment, the administration of PMSG/eCG before sponge removal resulted in a shorter interval to oestrus, and ovulation linked to the earlier development of large follicles. This could be beneficial in the use of fixed-time AI. Furthermore, Luther et al (2007) revealed that pregnancy rates following laparoscopic AI were higher in ewes treated with a combination of progestagens and PMSG/eCG (73.7%) than in those treated with progestagens alone (41.2%).

	Reproductive status	**PMSG dose**
Ewes	In season	300-500 IU
	Out of season	400-600 IU
Ewe lambs	In season	250-400 IU
	Out of season	300-500 IU

Table 4. Adjustment of PMSG dose in ewes treated with intravaginal sponge method.

For the superovulation of donor ewes in embryo transfer, PMSG may be administered at about 28 hours before sponge removal and at a higher than normal dose (1500 IU; Bari et al., 2000; Henderson and Robinson, 2000). PMSG may also be followed by an intramuscular injection of GnRH at the onset of oestrus, for the same purpose (Türk et al., 2008).

Recently, reports have appeared suggesting a positive effect of GnRH administration prior to the standard progestagen+PMSG/eCG regime. Karaca et al (2009) reported that the pretreatment of ewes with 10 mcg buserelin before progestagen-based synchronisation (7-day progestagen programme in combination with $PGF_{2\alpha}$ administration) resulted in increased multiple birth rates and litter size.

c. Breeding management in flocks synchronised using progestagens
One of the main advantages of this method is that it can be used to induce and/or synchronise oestrus. The high degree of synchronisation obtained allows for very good reproductive performance under a variety of conditions. Ewes will begin to come into oestrus from around 24 to 48 hours after sponge removal. The fertility of the oestrus will depend upon a number of factors related to both ewes and rams.

d. Ram introduction
The timing of introduction of the ram following the removal of sponges is crucial. Ewes will begin to show behavioural signs of oestrus from approximately 24 hours after sponge removal. However, most of them will not be in oestrus until 36-48 hours after removal. Consequently, rams introduced immediately after the removal of sponges will repeatedly serve the first ewes to demonstrate oestrus. This may lead to the depletion of their semen reserves, poor conception rates to the induced oestrus, extended lambing, and a poor lamb crop. The ram should not, therefore, be introduced until 36-40 hours after the sponges have been removed from the ewes.

e. Ram-to-ewe ratio
In synchronised flocks, large numbers of ewes are mated over a relatively short period. This means that special attention must be paid to an appropriate ram-to-ewe ratio. During the breeding season, both ram

fertility and libido should be satisfactory and one ram to 10 ewes should be adequate. However, outside the breeding season, both libido and fertility are usually reduced. Therefore, the ram-to-ewe ratio should be increased to roughly 1:5. If the requirement for high numbers of rams poses a problem, then the use of AI should be considered (see Section 5.2.5).

f. Lambing period and returns to oestrus

A population of ewes conceiving to a synchronised oestrus will generally lamb over a 1-week period. None should be expected to lamb during the following week, but any repeat breeders should start lambing in the next 8-10 days. The whole of lambing should be completed in approximately 3-4 weeks if one repeat mating was allowed.

5.3.5 Prostaglandins

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogues can be used to synchronise oestrus in cyclic ewes. Their luteolytic effect leads to regression of the corpus luteum and a lowering of progesterone concentrations in the blood. The resultant increase in the amount of gonadotrophin released by the pituitary gland stimulates follicular development, and oestrus occurs within 2-3 days; ovulation occurs about 24 hours later. Several prostaglandin analogues are available in injectable form but only a few of the commercially available products are, in fact, licensed for use in sheep.

Because the corpus luteum is only responsive to prostaglandins between days 5 and 14 of the oestrous cycle, two injections 10-14 days apart are required for optimum synchronisation. The wide variability of the response, and the need to inject cyclic animals twice, explains the limited used of these products in sheep, under field conditions (Henderson and Robinson, 2000). Furthermore, the fertility of the induced oestrus is generally poor, probably because the reproductive tract has been less than normally exposed to progesterone. However, this can be overcome. An abbreviated period (5 days) of progestagen priming, followed by an injection of $PGF_{2\alpha}$ at sponge withdrawal, has been shown to be highly effective in synchronising oestrus during the breeding season.

5.3.6 Combination of prostaglandins and GnRH

Some authors have recently reported the use of prostaglandins and GnRH in cycling ewes with reasonable results, as long as the ewes are within their normal breeding season and depending on the stage of the oestrous cycle when the treatment is begun (Cardenas et al., 2004; Deligianis et al., 2005). The results of these studies indicate that a modified Ovsynch protocol in cycling ewes can achieve an acceptable conception rate, which could be further improved by modifying the intervals between injections. On the other hand, Husein et al (2005) reported that 4-day progesterone priming ahead of the treatment was essential for the effectiveness of this procedure to maintain follicular response to GnRH. Their results showed an increased response with respect to oestrus and improved pregnancy rates in ewes and goats treated in this way (Husein and Kridli, 2003; Husein et al., 2005). Titi et al (2010) reported encouraging results of a synchronisation protocol in ewes when progestagen-loaded sponges were used between GnRH and $PGF_{2\alpha}$ injections.

5.3.7 Melatonin

Melatonin, a hormone produced by the pineal gland, mainly during the hours of darkness, is considered to be the chemical trigger that allows the photoperiod to control the secretion of hormones by the pituitary gland (Chemineau, 1992). Exogenous melatonin can also be used in controlling the timing of the breeding season.

Many methods involve the continuous administration of melatonin rather than attempting to mimic the natural daily fluctuations. In some countries, melatonin has been marketed as a slow-release implant. Apparently, elevated blood concentrations of melatonin must be maintained for at least five weeks in order to bring the breeding season forward. There is some evidence that this treatment may increase ovulation rate (Symons et al., 1988; Henderson and Robinson, 2000).

Slow-release melatonin implants are often used in conjunction with other environmental techniques, such as the ram effect, especially in extensively managed flocks that are not practising artificial insemination (Zuniga et al., 2002). In the Northern Hemisphere, melatonin implants have been used in

adult ewes, traditionally around the time of the summer solstice, in order to advance the breeding season. In commercial Mediterranean flocks, implants are usually inserted at about the time of the spring equinox, as they have an earlier breeding season than genotypes kept at higher latitudes, even when subjected to the same treatment to adjust the photoperiod (Abecia et al., 2007). These workers concluded that melatonin could be a useful tool for improving lamb production in the three breeds they studied, but within each breed, the degree of success varied according to the farm and the season.

It must be stressed that treatment with melatonin alone does not synchronise oestrus and ovulation tightly enough to allow for fixed time artificial insemination. One of the possible options could be a combination of melatonin pretreatment with precise oestrus synchronisation via a progestagen-based programme. DeNicolo et al (2008) evaluated the effect of treatment with melatonin implants followed by a progesterone-releasing device and eCG/PMSG synchronisation. The results of this experiment suggest that melatonin implants, in conjunction with the administration of progesterone and eCG/PMSG, may be a suitable means of increasing the number of lambs born per ewe in an out-of-season breeding programme.

5.4 Factors affecting oestrus and ovulation

Although most breeds of sheep can carry and rear at least two lambs, lambing percentages are usually lower than 200%. Manipulating the ovulation rate when breeding in or out of season, by pharmacological or natural methods, can improve lambing rates.

5.4.1 Ram effect

This is a method of inducing oestrus and ovulation in anoestrus ewes during the end of the anoestrus period season (see Sections 5.3.1 and 5.3.2).

5.4.2 Genetics

Breeds differ considerably in terms of ovulation rate, and crossbreeding is probably the simplest method of increasing the fecundity of a flock. On the other hand, there are individual animals, or strains of animals, in several breeds worldwide that have a considerably higher ovulation rate than the

mean for their flock or breed. The best-known examples are those Merino sheep carrying the Booroola or 'F' gene. Because this characteristic lies in a single gene, it can be used, by backcrossing, to increase the ovulation rate substantially in any sheep population (Henderson and Robinson, 2000).

5.4.3 Nutrition

Ewes maintained on a low plane of nutrition usually have a low ovulation rate. It has been known for many years that a rising plane of nutrition, commonly known as 'flushing,' may stimulate ovulation and increase litter size. However, the response to better quality feeding in the weeks prior to mating varies with the breed. Ewes generally respond best to flushing when in medium body condition (2.5-3.5 body condition score [BCS]) rather than when excessively thin or fat (Henderson and Robinson, 2000).

It has been demonstrated that low dietary intake can reduce ovulation rate in sheep and that dietary supplements containing high energy and protein can increase ovulation even in ewes in poor body condition without being stimulated with exogenous gonadotrophins (Downing et al., 1995). O'Callaghan et al (2000) found that nonstimulated ewes on a high quality dietary intake had a greater number of follicles compared with the ewes on a lower dietary intake. In general, in order to achieve reliable results, ewes should be allocated to groups, after weaning, depending on their condition score, and each group managed so that the majority are in the appropriate body condition prior to mating.

In Australia, supplementation of the diet with lupin seeds has been found to improve ovulation rate. This effect appears to be independent of body condition and overstimulation seems not to occur. Animals need to be fed lupin seeds at a rate of 500-750 g/head/day for a minimum of 6 days before oestrus, when a modest increase in ovulation rate of 20-30 ovulations per 100 ewes can be expected.

5.4.4 Gonadotrophins

Gonadotrophins, such as PMSG or porcine follicle stimulating hormone (pFSH), can be used to superovulate ewes (Henderson and Robertson, 2000). These treatments need to be administered to cyclic ewes during the follicular

phase of the oestrous cycle or after a period of progesterone priming when used outside the breeding season. The pituitary-derived gonadotrophins (eg, pFSH) are short acting and require frequent injections, so their use is restricted, in practice, to embryo transfer programmes (Haresign, 1992).

PMSG/eCG is longer lasting and usually used for inducing oestrus and ovulation outside the normal breeding season or for ensuring good conception rates at a synchronised oestrus in a fixed time insemination programme during the breeding season (Husein et al., 1998; Henderson and Robertson, 2000). The dose required depends largely on the conditions of use, breed, and season. As a general rule, a dose of 300-500 IU should be used for females in the breeding season and 400-600 IU out of the breeding season. These doses should allow for a moderate increase in the prolificacy of the flock.

5.4.5 Immunisation techniques

Immunisation reduces the inhibitory effect of the ovarian steroids or ovarian inhibin on the hypothalamus and pituitary, resulting in an increase in the ovulation rate. Immunisation against inhibin has been tested experimentally (Anderson et al., 1998; Dhar et al., 2001), but this technique is not yet widely used. Androstenedione, a steroid secreted by the ovarian follicle, has a regulatory effect on ovulation rate through its feedback action on the hypothalamic-pituitary axis (Cognie, 1988; Henderson and Robinson, 2000). The timing of vaccination is important to the success of this technique. Ewes must be sexually active when the rams are introduced. If the technique is to be used out-of-season, the ewe flock needs to be primed with progesterone sponges and PMSG to stimulate oestrus activity. The dose of PMSG must then be carefully evaluated, as the effects of PMSG and the vaccine will be additive (Henderson and Robinson, 2000).

5.5 Fertilisation rate and embryonic losses in sheep

Data available in the literature suggest that, as observed in cattle, a fertilisation rate of 90%-95% appears to be normal in ewes, under natural mating conditions. When biotechnical methods involving artificial insemination, especially with frozen semen, are used, much lower fertilisation rates are to be expected.

Compared to cattle, there are far fewer reports and reviews defining the extent of embryonic losses in sheep. Most studies have focused either on early embryonic survival or perinatal mortality. The fact that a ewe can often ovulate more than one oocyte further complicates the interpretation of the available data.

Most embryonic mortality has been reported to occur before day 18 of pregnancy, while losses between day 18 and lambing are estimated at 9.4% and late embryonic or foetal losses from day 30 to term, as little as 1%-5%. It is now well-established that losses increase with increasing ovulation rate (Knights et al., 2003; Kleemann and Walker, 2005b). It can, therefore, be stated that in sheep, embryo survival rate is a function of ovulation rate.

Dixon et al (2007) investigated patterns of late embryonic and foetal mortality in this species. Cumulatively, a greater percentage of ewes lost 1 or more, but not all, embryos or foetuses of a multiple pregnancy between day 25 and parturition (36.7%) than those that lost a single pregnancy (20.5%) or all of a multiple pregnancy (3.8%). Mean losses of embryos or foetuses averaged 3.7% of embryos from day 25 to 45, 4.3% from day 45 to 65, 3.3% from day 65 to 85, and 11.5% from day 85 to parturition; thus, approximately 3% to 4% for each 20-day period of pregnancy beyond day 25. The authors found that late embryonic and foetal losses occurred at similar rates in the anoestrus and transitional seasons.

5.5.1 Embryonic survival and the age of the dam

There is evidence, at least in some breeds, that embryo survival is lower in ewe lambs than in adult ewes. It is believed that this impaired survival is attributable to the inherent quality of the embryo rather than to any deficiency of the uterine environment. A study by Khan et al (2003) demonstrated that treatment of ewe lambs with 150 IU of hCG at the time of mating improves the growth of the conceptus, placentation, and the number of lambs born.

5.5.2 Luteal function and embryonic losses

Based on the similarities in pregnancy recognition between cattle and sheep, it is quite plausible that progesterone concentration, during the early luteal

phase and placentation, does affect embryonic and foetal survival. In fact, much of the basic research on the relationship between early luteal function and embryonic development, as well as on the mechanisms of pregnancy recognition in ruminants, was carried out in sheep. Moreover, Dixon et al (2007) found that lower concentrations of progesterone on day 25 or 45 of pregnancy were predictive of a greater chance of the complete loss of pregnancy.

Many authors postulate that the timing of breeding (ie, in or out of season) may affect early luteal function and thus contribute to the lower pregnancy rates usually obtained with out-of-season breeding. Mitchell et al (2002) found, however, that season did not affect the numbers of corpora lutea per ewe, nor the numbers of ova recovered, but the proportion of the recovered ova that was unfertilised/degenerate was lower in October than in April. Moreover, their results indicated that during the late, as compared with the peak breeding season, there was an increased incidence of fertilisation failure as a possible consequence of seasonal shifts in LH secretion and/or the associated effects on follicular function. It is, therefore, more probable that the lower pregnancy rates observed in ewes bred outside the normal reproductive season are associated with low ovulation rate and poor oocyte quality, rather than a significant luteal insufficiency following induced ovulation. DeNicolo et al (2009) evaluated plasma progesterone concentrations during early pregnancy in spring- and autumn-bred ewes and found that early luteolysis, low progesterone secretion from corpora lutea, and embryo mortality did occur, but only in a small proportion of ewes. Progesterone concentrations indicated that a majority of mated nonpregnant ewes had elevated progesterone concentrations necessary for the production of at least one viable embryo/foetus.

5.5.3 Nutrition and embryonic losses

Ewes carrying two or more foetuses can suffer from pregnancy toxaemia towards the end of pregnancy as a result of inadequate nutrition. A varying degree of metabolic imbalance, accompanied by hypoglycaemia and ketosis, is caused by a less than adequate feed intake for the number of lambs carried. There may also be other predisposing factors involved. The clinical

signs are anorexia and a range of nervous signs, leading to abortion and/or death of the ewe. As the prognosis is poor unless ewes are treated in the very early stages of the disease, control relies heavily on prevention – identification of ewes carrying more than one foetus, and attention to their nutrition, especially in the last third of pregnancy.

5.5.4 Heat stress and embryonic losses

Heat stress is generally considered to have a direct negative effect on embryo survival rates in sheep. Although normal diurnal variation in temperature and acclimatisation will moderate this effect in the field, it should not be overlooked in areas where high ambient temperatures are expected. Heat stress can also reduce foetal growth by retarding uterine blood flow (Henderson and Robinson, 2000).

5.6 Reproductive disorders

Investigation of reproductive problems in sheep must focus on the flock rather than on the individual. The most relevant losses of reproductive efficiency in sheep can be the consequence of:

- Environmental and social factors causing embryo mortality and infertility
- Infections causing infertility, enzootic abortion, and perinatal losses
- Inadequate nutrition

5.6.1 Infectious diseases

There are several infectious diseases that can interfere with fertility and cause pregnancy losses in sheep (Table 5). Without adequate control measures, many of them carry the risk of severe financial losses due to reduced fertility, limited possibilities for replacements from within the flock, and in some cases, restrictions on the movement of animals. Moreover some infections are potential zoonoses, posing a severe threat to human health. Table 6 summarises the most commonly encountered causative agents and the main signs relevant to each.

Disease	Clinical signs	Lesions	Diagnostic	Control
Brucellosis 1. *Brucella melitensis*	Abortions in the second half of pregnancy. Stillbirth. Perinatal mortality. Systemic effects in the ewe: fever, lameness, etc.	Placentitis with oedema and necrosis of cotyledons.	Culture. Direct microscopy. Complement fixation test. Rose Bengal test. Milk-ring test.	Eradication: test and slaughter. Vaccination. Antibiotics: usually not recommended.
2. *Brucella ovis*	Orchitis. Infertility. Occasionally, abortions.	Rams: Epididymitis Orchitis Ewes: Placentitis	As above. Testicular palpation. Staining of semen/ cotyledonsmears by acid-fast or Kösters.	Eradication: test and slaughter.
Salmonellosis (paratyphoid abortion) *Salmonella abortus ovis*	Abortion that in endemic situations tends to affect only younger ewes. Stillbirth and perinatal mortality. Some ewes and lambs can show diarrhoea.	Nonspecific lesions of the placenta. In cases of perinatal death.	Culture. Serum agglutination test.	Vaccination. Antibiotics.
Enzootic Abortion (Chlamydial abortion) *Chlamydia psittaci*	Late abortions. Premature lambing. Stillbirth. Mummification. Perinatal losses. Usually second gestation abortion. Placental retention.	Placentitis with necrosis of the cotyledons and oedema and thickening of the intercotyledonary spaces. Similar to ovine brucellosis.	Placental smears and smears of vaginal discharges. Fluorescent antibody technique. Chicken embryo culture. Complement fixation test.	Hygienic measures. Vaccination. Antibiotics (oxytetracycline).
Toxoplasmosis (*Toxoplasma gondii*)	Infertility. Mummification. Abortion in late pregnancy that in endemic areas affects only younger ewes. Perinatal losses.	Gross lesions of cotyledons (grey-white foci). Mummified foetuses. Focal leucomalacia in the brain of lambs dying.	Histological examination of cotyledons and foetal brain serological tests.	Vaccination.

Table 6. The most relevant infectious diseases causing ovine abortion and perinatal losses.

a. Toxoplasmosis

Toxoplasmosis in sheep is caused by *Toxoplasma gondii*, an intracellular protozoan parasite. Clinical toxoplasmosis occurs following a primary infection in pregnant sheep, triggered by the ingestion of sporulated *T. gondii* oocysts. In the small intestine, sporozoites are released from the ingested oocysts, and by the fourth day, the next developmental form – tachyzoites – can be found multiplying in the mesenteric lymph nodes (Buxton et al., 2007; Dubey, 2009).

The main source of infection for sheep is feed and pasture contaminated with cat faeces containing infectious oocysts. Although precise data are not available, it is thought that <2% of sheep become congenitally infected with *T. gondii* and less than 4% of persistently infected sheep transmit the organism to their progeny (Buxton et al., 2007; Dubey, 2009).

The pathogenesis of ovine toxoplasmosis resembles, to some extent, the mechanism found in bovine neosporosis. In sheep, due to a specific immune state that ensures the tolerance of a semi-allograft foetus, there is minimal maternal expression of cytokines, such as interleukin 2 (IL-2), tumour necrosis factor alpha (TNFα), and interferon gamma (IFNγ) (Entrican and Wheelhouse, 2006). While allowing a successful pregnancy, these mechanisms also render the placenta and foetus peculiarly susceptible to certain pathogens. This is the reason toxoplasms circulating in the blood of a pregnant ewe can become established in the placenta. They cross the maternal caruncular septa in the placentome before invading the adjacent trophoblast cells of the foetal villi; from there they can spread to the rest of the foetus (Buxton et al., 2007).

i. Clinical consequences of infection during pregnancy

Toxoplasmosis has a profound effect on the reproductive health of the ewe when the animal becomes infected for the first time in mid-pregnancy. Typical signs include abortion or stillborn and/or weakly lambs, often along with a small, mummified foetus. Placental cotyledons of the accompanying placentas will show characteristic 'white spot' lesions, visible to the naked eye.

Following infection, sheep acquire an immunity through a cell-mediated immune response. They remain immune but infected for life (with bradyzoites in tissue cysts in brain and muscle) and will not usually abort due to toxoplasmosis in future pregnancies. This represents a major difference compared with cows infected with *N. caninum* that acquire the infection transplacentally and may abort during each consecutive pregnancy throughout their life (Dubey et al., 2003; 2006). Infection of ewes with *T. gondii* earlier in gestation can result in foetal death and resorption or abortion, while infection in the latter part of gestation, when foetal immunity is relatively well-developed, may have no clinical effect; when this happens, the lambs are born normal but are infected and immune.

Control measures include good management of food and water sources to limit the contamination with cat faeces, as well as vaccination. The diagnosis of toxoplasmosis in sheep is by the histology of samples of cotyledons and/or foetal brain, as well as by various serological tests or PCR.

Toxoplasmosis is an important zoonosis. Humans become infected post-natally by ingesting tissue cysts in undercooked meat or consuming food or drink contaminated with oocysts. Undercooked lamb is considered an important source of infection. Most infections in humans are asymptomatic, but the parasite can produce devastating disease at times. Congenital infection occurs only when a woman becomes infected during pregnancy, and infections acquired during the first trimester are more severe than those in the second and third trimester. A wide spectrum of clinical disease occurs in congenitally infected children. Mild disease may consist of slightly diminished vision, whereas severely diseased children may have the full tetrad of signs: retinochoroiditis, hydrocephalus, convulsions, and intracerebral calcification. It should also be borne in mind that toxoplasmosis ranks high on the list of diseases that lead to the death of patients with acquired immunodeficiency syndrome (AIDS).

b. Enzootic abortion

Enzootic abortion (EA) is caused by a gram-negative, obligate intracellular bacterium *Chlamydophila abortus* (formerly *Chlamydia psittaci*) and represents an important production disease of sheep flocks in many countries (Kerr et al., 2005). It is the most common infectious cause of abortion in

intensively managed lowland flocks near lambing time, and has a major economic impact on sheep farming worldwide.

The infection is commonly transmitted between flocks by means of infected replacement ewes. The main routes of transmission of *C. abortus* are through ingestion of the bacteria shed in vaginal fluids and placental membranes at the time of abortion or lambing or through inhalation of aerosols from the contaminated environment. There is also some evidence of venereal transmission, but so far it has been difficult to estimate the degree to which this route contributes to the epidemiology of enzootic abortion. Another potential route of transmission is through direct infection of the foetus via the placenta, although again it is unclear what contribution this might have to the spread of the infection within the flock.

i. Clinical consequences of infection

Infection during pregnancy may result in abortion, stillbirth, birth of weakly, premature, or clinically normal but infected lambs (Table 4). Typically, abortions occur during the last 2-3 weeks of gestation. An initial outbreak that may be associated with only a few abortions can lead to over 30% of the flock aborting or producing stillborn or weak offspring in the following year (Aitken, 2000). In subsequent lambing seasons, the incidence of abortion is likely to remain at 5%-10% if affected animals are left untreated.

Timing of infection	Clinical consequences
Up to 5-6 weeks prior to parturition	Clinical disease. Abortion in the final 2-3 weeks of gestation or birth of stillborn or weak lambs that frequently die in the first few days of life.
Last 5-6 weeks of pregnancy	Commonly, development of a latent infection, no clinical signs until the next lambing season. Surviving lambs born to infected mothers may be affected in their first pregnancy.

Table 5. Clinical picture of *C. abortus* infection during pregnancy in sheep (adapted from Kerr et al., 2005).

Typically, placentitis represents the major gross pathological feature of chlamydial abortion (Aitken, 2000). The infection is associated with severe and

extensive pathological changes in the foetal membranes. The bacterium targets the placenta, causing tissue damage and inflammation, resulting in abortion (Kerr et al., 2005).

Although there are generally no clinical signs to herald the impending abortion, a vaginal discharge can be observed up to 48 hours prior to the foetus being expelled. The foetal membranes may display varying degrees of necrosis, thickening, oedema, and suppurative exudate (Williams and O'Donovan, 2009). However, the aborted foetuses are usually well-developed and not autolysed, indicating that foetal death has been a fairly recent event. The discharge can persist for 2-3 weeks, adding to the environmental spread of infection. Infected ewes may also give birth to weak lambs that usually fail to survive. Following abortion, ewes develop a protective immunity that prevents abortion from *C. abortus* infection in subsequent pregnancies.

Management of enzootic abortion should always include the rapid removal of aborting ewes, aborted foetuses, and foetal membranes from the lambing pen, followed by cleaning and disinfection. Antimicrobial treatment of ewes with long-acting oxytetracyclines in the face of an outbreak is commonly practised, but the benefit of this treatment is difficult to evaluate. Currently available vaccines are used to prevent enzootic abortion in uninfected ewes and reduce the spread of the disease within the flock.

It is important to remember that enzootic abortion is a zoonosis that can have particularly serious consequences for pregnant women (Longbottom and Coulter, 2003). Infection with *C. abortus* is usually due to exposure to infected foetal fluids and membranes of sheep or goats. In some countries, women who are, or may be, pregnant are advised to avoid involvement with the flock at lambing time.

The definitive method of diagnosis of *C. abortus* or *T. gondii* infection is the isolation of the pathogen from infected tissues. However, this method is labour intensive and time-consuming and relies upon the submission of fresh material to the diagnostic laboratory. Tests such as ELISAs can detect ewes that have seroconverted after exposure to *Chlamydophila*, but it is not possible to distinguish between naturally infected and vaccinated animals. A serum agglutination test and an ELISA can be used to detect antibodies to confirm *T. gondii* infection in ewes, with most infected ewes remaining

seropositive for at least six months following infection. For both enzootic abortion and toxoplasmosis, the PCR technique allows the identification of the antigen in aborted foetuses and foetal membranes.

c. Q fever

Q fever (short for 'query' fever), a zoonosis caused by the obligate intracellular micro-organism *Coxiella burnetii*, is widespread throughout the world and affects a range of animals, including sheep. A comprehensive review of the main features of Q fever in small ruminants was published by Rodolakis (2006). In ewes, *C. burnetii* infections are generally asymptomatic, but can have a negative effect on the reproductive performance of the flock, leading to abortions, stillbirths, and weak or nonviable lambs. In the majority of cases, abortion occurs at the end of gestation without any specific prior clinical signs. Aborted foetuses appear normal, but intercotyledonary fibrous thickening and discoloured exudates can be found in their placenta. Aborting ewes shed large amounts of *Coxiellae* with aborted foetuses and foetal membranes, and in vaginal discharges, urine, faeces, and milk. The abortion rate is usually low.

In humans, the acute disease is associated with flu-like symptoms. However, more severe complications are possible, such as endocarditis in patients suffering from valvulopathy, as well as premature delivery or abortion in pregnant women.

Routine diagnosis of Q fever in sheep is usually established by histological examination of samples from the placenta or by serology. A recently introduced PCR allows for the accurate diagnosis of infection, and even the identification of asymptomatic animals that are shedding the microorganism.

Preventive measures include adequate management of the aborted material and adequate disinfection. Treatment with oxytetracyclines is possible in aborting flocks, although this treatment does not fully suppress the abortions and the shedding of *C. burnetii* at lambing. In ruminants, the only way to prevent the disease is vaccination of the infected flocks, as well as any uninfected neighbouring flocks.

Natural infection with *Neospora caninum* appears to be uncommon in sheep, and only a few cases of abortion or congenital disease have been reported (Dubey, 2003). However, the role of *N. caninum* as a cause of abortion in

small ruminants needs further investigation, since experimental inoculation with *N. caninum* during pregnancy produces similar effects to those observed in cattle.

5.6.2 Pregnancy toxaemia

Ewes carrying two or more foetuses can suffer from pregnancy toxaemia towards the end of pregnancy as a result of inadequate nutrition. A varying degree of metabolic imbalance, accompanied by hypoglycaemia and ketosis, is caused by a less than adequate feed intake for the number of lambs carried. There may also be other predisposing factors involved. Teeth or feet problems, as well as heavy parasitic burdens may also lead to the disease, due to the associated decrease in body condition. Obese or lean ewes are more likely to develop the disorder.

Affected animals are usually in poor condition and exhibit depression, selective anorexia (initially eating only hay and straw, then only straw and finally not feeding at all) and tend to separate themselves from the rest of the flock. Soon afterwards they develop neurological signs such as tremors of the head and neck, wandering, excessive salivation, unusual head carriage, absence of menace reflex, and blindness. Finally, the affected ewe becomes recumbent and comatose.

As the prognosis is poor unless ewes are treated in the very early stages of the disease, control relies heavily on prevention – identification of ewes carrying more than one foetus and attention to their nutrition, especially in the last third of pregnancy.

5.6.3 Clostridial infections ('postparturient gangrene')

The condition occurs immediately postpartum when the external reproductive organs of ewes become infected with *Clostridium chauvoei*. Infection is facilitated if the vulva, vagina, or perineum has been damaged during a difficult lambing or obstetrical intervention (Lewis, 2007).

The infected animal develops high fever. The skin or the mucosa of the infected region may be discoloured, which may be accompanied by subcutaneous oedema, particularly of the perineum. Occasionally, there may

be a sanguineous, malodorous vulval discharge. The infection may extend to the thigh muscles, which become dark and swollen.

Diagnosis is based on clinical and pathological examination. Vaccination of pregnant ewes is essential for the prevention of the disease. Good hygiene during lambing, especially when obstetrical assistance is provided, also helps to minimise the incidence.

5.6.4 Puerperal metritis

Factors such as dystocia followed by obstetrical assistance, prolapse of the uterus, retained placenta, and postparturient ketosis predispose ewes to infections of the uterus and puerperal metritis. The bacteria most commonly isolated are *A. pyogenes* and *E. coli.* The clinical manifestation includes swollen vulva and vagina, vaginal discharge, and retention of foetal membranes, which can be accompanied by more systemic signs such as anorexia, dehydration, fever, and toxaemia.

If the condition remains untreated, it can be life threatening. Treatment should include the systemic administration of antibiotics of an adequate spectrum of activity, oxytocin, and nonsteroidal anti-inflammatory drugs (NSAIDs). Ewes treated at an early stage respond rapidly to treatment and there are usually no consequences for their future fertility.

5.7 Induction of parturition

Parturition may be induced if a very short lambing period is required, whether to optimise supervision for maximum lamb survival, or to simplify the management of the flock thereafter, or both. It is only practical when oestrus has already been synchronised so that mating data are available. Ewes must not be induced before day 144 of pregnancy if the birth of premature lambs is to be avoided.

Prostaglandin $F_{2\alpha}$ cannot be used to induce parturition in sheep because pregnancy does not depend on progesterone from the corpus luteum; the placenta, which produces its own, results in luteolysis that has no effect. However, both oestrogens and corticosteroids can be used successfully. Some researchers have reported higher rates of dystocia and

perinatal mortality following oestrogen treatment. Betamethasone and dexamethasone, at a dose rate of 8 to 16 mg, are the most commonly used corticosteroids. Intramuscular injection at the higher dose rate results in parturition within 26-62 hours of treatment (Henderson and Robinson, 2000).

5.8 Rams

As mentioned in chapter 5.1.1, the sexual activity and breeding efficiency of rams are both subject to seasonal influences. In temperate climates, seasonal variations in the photoperiod and other environmental changes affect rams' reproductive activity, testicular size, gonadal endocrine balance, sperm quantity and quality, and sexual behaviour. In rams, sexual activity is usually stimulated 1-1.5 months earlier than in ewes, so that they are already fully sexually active when the ewes begin to cycle. In subtropical and tropical zones, it is the availability of forage and humidity that seem to have the greatest influence on the seasonality of reproductive efficiency in rams.

5.8.1 Evaluation of ram's suitability for breeding

It is not uncommon for poor fertility in a sheep flock to be caused by the poor quality of the rams. To avoid such situations, rams and bucks should be evaluated by a veterinary practitioner or experienced technician for breeding soundness 30 to 60 days before the breeding season, allowing time to recheck or replace those which are subfertile.

a. Physical examination

This should include careful observation of the general physical condition of the ram, body condition, alertness, and especially the locomotor system. In rams used in so-called harem mating in extensive breeding systems, eyesight is also of prime importance. Signs of any general illness and parasite infestation should be noted. Also, a ram's libido can be assessed in the presence of ewes in oestrus.

b. Fertility examination

Examination of the reproductive tract consists of both an external examination of the reproductive organs and a rectal examination of internal reproductive structures and accessory glands. Scrotal circumference is one of the most

useful measurements of a ram's testicular health and breeding ability. As in bulls, scrotal circumference is closely related to semen quality, quantity, and reproductive success. Careful examination should also include the penis, urethral process, and prepuce. The presence of sores, swellings, or blood clots may indicate penile or preputial injuries. Rams occasionally suffer from adhesions on the surface of the penis, which make it difficult or impossible to extrude the penis for intromission. While this problem may be corrected surgically, it is often an inherited defect, so rams exhibiting it should not be used for breeding.

c. Semen evaluation

Semen evaluation is unfortunately not a common enough practice even on well-managed sheep farms that use natural mating. Nonetheless, it is an extremely important element of the management of rams, since poor quality semen may contribute substantially to a decline in the reproductive performance of the flock, leading to economic losses, if undetected.

Collection of semen can be performed in rams using an artificial vagina and spontaneous mounting. Rams quickly learn to mount a restrained ewe, and intromission and ejaculation are extremely rapid. Alternatively, electroejaculation can also be used and may well be required in rams not trained in the use of an artificial vagina. Electroejaculation is the less reliable method, as samples vary in quality and can be contaminated with urine. The volume of semen collected with the artificial vagina is 0.5-1.8 mL, while the ejaculates obtained by electroejaculation are of greater volume but with a lower concentration of sperm.

Good quality semen will have a milky or creamy appearance and, when examined under a stereomicroscope, will give an impression of boiling or rolling due to the intense motion of the spermatozoa. Much as with bovine semen, evaluation consists of defining the percentage of motile spermatozoa using a simple preparation from a drop of semen on a prewarmed microscope slide. Morphology of the spermatozoa is studied in microscopic preparations stained with eosin-nigrosin. Table 6 gives the normal parameters of sperm expected in a mature ram during the breeding season.

Parameter	Normal value (during breeding season)
Volume	1 (0.8 to 1.2) mL
Sperm concentration	2.5 (1 to 6) billion/mL
Percentage of motile spermatozoa	75 (60 to 80) %
Percentage of morphologically normal spermatozoa	90 (80 to 95) %

Table 7. Normal semen parameters in mature ram.

5.8.2 Storage of ram semen

Ram semen may be stored for up to 24 hours by cooling the extended semen down to 2°C-5°C over 90-120 minutes. This is the approach often used by AI insemination centres during the breeding season when both the supply and the demand for semen are high. Fertility of cooled semen decreases rapidly and is usually too low after 48 hours.

The freezing and storage of ram semen in 0.25-0.3 mL, 3-dose pellets, or in 0.25 mL single-dose synthetic straws in liquid nitrogen at -196°C is successful in maintaining sperm viability, but there may be high variability in post-thaw motility and fertility between rams or batches of semen from the same ram. Semen stored this way is widely used in countries where intensive sheep production and breeding are practised (eg, France, Australia, Spain).

5.8.3 Management of rams prior to breeding

Properly planned management is necessary to optimise reproductive efficiency in rams and thus improve the chances of achieving better lambing percentages. Rams should be in good health and condition, well in advance of the breeding season, in order to correct any possible deficiencies, as well as to allow the evaluation of soundness and semen quality. Infertile rams can also be identified and removed at this stage.

Suggested evaluation of rams before the breeding season:

Time-to-mating	Procedures
12 weeks before mating	• Correction of possible selenium deficiency
6 weeks before mating	• Flushing aimed at achieving 3.5 BCS at the start of mating • Treatment to remove endo-and ectoparasites • Foot care • Separation from ewes at least 3 weeks before mating • Clinical examination
2 weeks before mating	• Detailed clinical examination • Semen evaluation

The general health and performance of breeding rams should also be monitored closely throughout the breeding season. Adjustments can be made to feeding to ensure optimum breeding condition, and replacements can be arranged for any problem animals.

5.9 Embryo technology

Embryo transfer and embryo production in vitro are well established in sheep, although their wide-scale commercial use is very limited. This results directly from the adverse cost:benefit ratio of embryo transfer in sheep, when the value of a single animal, even one of high genetic merit, is usually relatively low. Nonetheless, the production of embryos in vitro provides a rich source of relatively low-cost embryos for basic research, as well as in the development of the commercial use of emerging techniques such as nuclear transfer and transgenetics.

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6.1 Physiology

6.1.1 Seasonality of sexual and ovarian activity

The female goat is seasonally polyoestrous. The length of the breeding season is governed mainly by a combination of genetic and environmental factors. Various climatic elements, such as temperature and photoperiod, regulate the physiological response. In temperate zones, the goat behaves as a seasonal breeder, with a definite anoestrus period dependent on changing daylight length.

The goat is a so-called 'short day breeder' (see Ovine Reproduction chapter). In tropical goats, the photoperiod is less important than temperature, rainfall, vegetation and availability of pasture. The oestrus season of most of the dairy breeds in the Northern Hemisphere is usually restricted to the period between September and December. Meat-producing goats have a short anoestrus period in spring. Anglo-Nubian and Pygmy goats have extremely long breeding seasons. Seasonal influence should always be considered when designing breeding programmes for imported goats, as those recently transferred from another region may take some time to adjust to the difference in the seasons.

The onset of puberty is related to body weight, which, in turn, depends on the level of nutrition, age, type of birth, and the season in which it takes place. Most breeds reach puberty between 5 and 10 months of age, but the more seasonally dependent breeds may approach 15-18 months before being developed enough to exhibit signs of oestrus. The climate, nutrition, and the presence of a buck can modify the age at puberty. It is not advisable to breed young does before they have reached at least 60%-75% of their adult body weight, for the sake of their own development, as well as for the viability of any offspring. Most of the European breeds are usually put in kid for the first time when they reach 7-8 months of age and a body weight of at least 30-35 kg.

Decreasing daylight length also stimulates reproductive activity in the buck. Although most bucks will mate at any time of the year, reductions in libido and semen quality have been observed when they have been worked out of season (Ahmad and Noakes, 1996).

Bucks are at peak reproductive activity in late summer and autumn, in response to declining daylight length. This period, known as the rut, is associated with:

- Peak testosterone production
- High sebaceous gland activity (characteristic odour)
- Agonistic behaviour (fighting)
- Courting behaviour in the presence of females

Testicular weight, in the breeds with a strong seasonality, is usually minimal in spring and maximal in late summer, and is associated with marked changes in sperm production. Alpine bucks display dramatic variations in sexual behaviour between the spring-summer and autumn-winter periods (0-1.5 matings in 10 minutes), individual sperm motility (2.5-3.5 to over 5) and fertilising ability (20%-70% of kiddings after AI) (Delgadillo et al., 1991).

6.1.2 The oestrous cycle

The duration of the oestrous cycle varies widely, from as short as 3 days, to as long as 62 days. The majority of oestrous cycles are 19-21 days in length, but a proportion of them are shorter (<12 days), and others longer (>26 days). The occurrence of short cycles is influenced by the season of the year, the onset of the oestrus season or a transitional period, a 'buck effect' and the early postpartum period. Short cycles are frequently observed, in particular, in does in tropical regions, when they are housed. The longer cycles are commonly encountered at the end of the breeding season before the does enter anoestrus. They can also be associated with embryonic death or persistence of the corpus luteum.

The follicular phase of the oestrous cycle is relatively short, 3-4 days, while the luteal phase occupies the rest of the cycle (ie, about 17 days in a 'normal' cycle). Daily ultrasonographic studies have indicated that between ovulations there is a wave-like pattern of follicular development, as occurs in other ruminant species (Rubianes et al., 2003; Medan et al., 2005). Different authors report the number of follicular waves ranging between two and five waves per cycle, but the pattern in a 'normal' cycle usually consists of four waves (de Castro et al., 1999; Schwarz and Wierzchos, 2000; Menchaca et al., 2002). Evidence of follicular dominance in goats remains equivocal.

Some authors have even postulated that, in goats, more than one follicle may 'cooperate' in exerting a functional dominance over the growth of others (Medan et al., 2005).

Oestrus appears to be variable in length, is generally reported as 36 hours, and ranges from 22 to 60 hours. Ovulation takes place a few hours after the end of visible oestrus. The average number of ovulations varies from 1-4 per cycle, with reduced kidding rates due either to fertilisation failure or early embryonic mortality.

6.1.3 Pregnancy

Pregnancy in the doe is dependent on progesterone from the corpus luteum throughout the whole period, and any interference with the function of the corpus luteum will result in abortion. The caprine placenta produces a considerable quantity of prostaglandin throughout pregnancy that, together with luteinising hormone (LH) and placental lactogen, forms a luteotrophic complex that ensures the continuous production of progesterone by the ovaries and thus the maintenance of pregnancy (Ford et al., 1995).

Gestation length varies from 144 to 151 days, with a typical mean of 149 days.

The length of postpartum anoestrus (between parturition and first oestrus) can vary from 5 weeks (or even less) to 27 weeks, and is influenced by breed, lactation length and nutrition.

6.2 Herd reproduction management

6.2.1 Introduction

Goats are usually classified into four types according to their production: milk, meat and pelt, fibre, and dual-purpose (milk and meat). For small farmers and rural dwellers who are not land-owners, goats are unique among the domestic ruminants because of their ability to survive and reproduce under unfavourable conditions. There is a great diversity in production systems, which makes it difficult to characterise the industry.

However, regardless of the type of goat being produced, its reproductive performance is a major determinant of productivity and, therefore, the economic viability of commercial goat farms.

The control of reproduction may be necessary to avoid undesirable cross-breeding, in-breeding or inappropriate timing, as well as to produce animals better adapted to various environmental conditions. The more sophisticated methods for controlling reproduction are restricted to use in intensive and highly profitable systems. Extensive and low income flocks must rely on more simple measures, such as modifications to the environment, eg, the male effect, altering the photoperiod, dietary modifications (eg, flushing), and modifying the breeding pattern (eg, exogenous hormones, weaning). Both management and pharmaceutical methods can be combined, of course.

The seasonality of reproduction in goats results in lower reproductive efficiency (delayed puberty, prolonged kidding interval, etc) while the seasonality of production leads to variations in market prices. So any improvements in reproductive performance will contribute to improvements in the efficiency of meat or milk production, and, therefore, profitability.

The 'kidding interval,' which can range from 240 to 350 days, is defined as the period between two consecutive parturitions, comprising the very variable period from kidding to conception and the gestation period. The kidding interval is affected by breed, age, and parity of the doe, level of milk production, kidding rate, season of the year, and level of nutrition. These influences can be grouped into husbandry (ie, interval between kidding and introduction of the bucks), physiological (seasonal and postpartum anoestrus, conception rate) and pathological (embryonic death, abortion).

Differences in litter size are mainly associated with breed, season, parity, and body condition. The kidding rate (number of kids born/does giving birth) varies by breed from 1.01 to 2.05. In seasonal breeders, the prolificacy following the autumn mating is generally greater than that of the rest of the year. Kidding rate usually increases from the first to the fifth parity and declines thereafter.

6.2.2 Pregnancy diagnosis

The main indications for pregnancy diagnosis in the goat are better management (feeding strategy, labour, vaccination, etc) and to reduce the number of barren females. Most animals that are not successfully mated will return to oestrus 17-23 days after mating. Towards the end of the breeding season, longer cycles are likely to occur and, in some cases, nonpregnant animals remain in anoestrus. Goats frequently show signs of oestrus during pregnancy. Care must therefore be taken to distinguish between pregnancy, normal cyclical activity, and pseudopregnancy.

Several methods have been devised for pregnancy diagnosis in goats because the signals which are commonly relied upon in other ruminants do not apply in goats. For instance, non-return to oestrus is not reliable. Many does do not exhibit signs of oestrus throughout their breeding season, which may be associated with seasonal anoestrus or pseudopregnancy. Mammary gland development in primiparous goats should not be relied upon either, as 'maiden milkers' are common.

Hormone levels in blood, milk, and urine do provide a means by which to confirm the presence or absence of pregnancy. Oestrone sulphate concentrations in milk and plasma increase steadily during pregnancy and can be used to diagnose pregnancy approximately 50 days postservice. Progesterone secreted by the corpus luteum of a pregnant goat can be detected with RIA or ELISA assays in milk or plasma. Random sampling can produce misleading results, however, because the corpus luteum of cyclic goats, and those with a false pregnancy, also produces progesterone. Nevertheless, a low progesterone level will always indicate nonpregnancy and can be considered to be 100% accurate.

Recently, so-called pregnancy associated glycoproteins (PAGs) have received increasing attention in ruminants, including goats, as potential markers of pregnancy, and are, therefore, useful candidates for the development of tools for early pregnancy diagnosis. Three different PAG molecules have been isolated and partially characterised from goat placenta. During gestation,

PAG concentrations reach maximal levels during week 8, reduce between weeks 12 and 14 and then remain relatively constant until parturition (Sousa et al., 2006). After parturition, concentrations decrease rapidly to very low levels by the fourth week postpartum. Although using RIA or ELISA, these molecules can be detected in goats after day 26 and 32 in plasma and milk, respectively, no test is currently commercially available for routine use in the field.

With the advent of ultrasound, efficient and safe methods of pregnancy detection have become available. A-mode ultrasonography is based on the detection of the fluid-filled uterus and is thus not specific for pregnancy. A-mode units emit ultrasonic waves from a hand held transducer placed externally against the skin of the abdomen and directed towards the uterus. The examination is carried out in a standing doe with the transducer placed against the lower part of the right flank near the udder. Clipping a small area of hair in this region is recommended to allow for optimal contact. Examination between 60 and 120 days postbreeding should allow an accuracy of 80%-85%.

Techniques based on the Doppler effect can detect blood flow in the middle uterine artery, umbilical arteries, and foetal heart as well as foetal movement. Thus, the foetal pulse can be detected after approximately two months of gestation, either via a transrectal or external/transabdominal probe. The accuracy of pregnancy detection approaches 100% during the last half of gestation but the technique is less effective between 50 and 75 days or earlier. The transrectal technique may be attempted as early as 25 to 30 days postbreeding. However, false negative results are common, so it is advisable to wait until day 35 to 40 of gestation.

Real-time (B-mode) ultrasound devices produce a two-dimensional picture on the screen, including a moving image of the uterus, foetus, foetal fluids, foetal heart, and placentomes. With the aid of real-time ultrasound, pregnancy can be detected from 40 days of gestation onwards, but is best done between 50 and 100 days. Ultrasound scanning is estimated to be virtually 100% accurate in determining pregnancy and 96%-97% accurate in diagnosing twins and triplets. The ability to identify multiple foetuses with real-time ultrasonography has a clear advantage over other ultrasound techniques. Feeding management can be adjusted for does carrying multiple

foetuses and appropriate care can be planned in advance of the expected kidding. The optimal time for estimating foetal numbers is probably between 40-70 days, because after 70 days, additional foetuses may lie beyond the depth range of a 5 MHz linear-array transducer. Experienced operators can distinguish pseudopregnancy and resorbed foetuses, as well as identify live kids.

Transabdominal scanning is usually carried out with the goat standing.

6.2.3 Oestrus detection and mating

Oestrus is preceded by pro-oestrus, which usually lasts about a day, during which the doe is followed around closely by the buck but will not stand to be mounted. The only sure sign of oestrus is the female standing and allowing the male to mount (the 'standing reflex'). Does actively seek the presence of the male when in oestrus, and the odour of the buck has a stimulating effect on the expression of oestrus signs. The buck may exhibit the flehmen reaction, flick his tongue and strike the doe with a forelimb (Ott, 1980). Signs of oestrus in does also include tail-wagging, bleating, and urination when near the buck. There may also be swelling of the vulva and a mucous discharge. Some does show no signs other than limited tail-wagging and standing to be mounted by the buck. In contrast to cows, most does will not stand to be mounted by other females, even when in oestrus.

As oestrus progresses, a variable amount of transparent mucus is visible in the cervix and on the floor of the vagina. This mucus later turns cloudy, and finally, cheesy white at the end of oestrus. Conception is most likely to take place if the doe is bred when her cervical mucus is cloudy and the cervix is relaxed.

Silent heat is not as common in goats postpartum as it is in sheep. Under field conditions, oestrus detection is of little importance. Several matings will usually occur within the flock, so timing will not necessarily be of any great interest. However, if artificial insemination (AI) is to be practised, it should be carried out near the end of oestrus. Therefore, with the use of AI in dairy goats, for example, oestrus detection may well be important.

Ovulation is spontaneous and takes place about 30-36 hours after the onset of oestrus. Although it generally occurs late in oestrus, when the cycle is short, it may be after the end of oestrus.

6.2.4 Artificial insemination

In countries such as France, where the genetic improvement of dairy goats is pursued systematically, AI has become part of the management routine. It is important in genetic improvement programmes in allowing the use of semen from males of high genetic merit, even those in distant locations that are likely to have been bred from planned matings between the very best females and males in the population. Moreover, AI is helpful in reducing the spread of infectious diseases by reducing the need to transport animals for natural breeding and the opportunity for venereal transmission.

a. Semen collection and storage

Collection of semen from males requires a teaser and an artificial vagina and is a well-established technique. Undiluted fresh semen can be used where donors and recipients are reared in close proximity. The main advantage is that it requires only simple equipment, but has the disadvantage in that it is difficult to assess semen quality. Diluted, chilled semen allows more time between collection and AI (12 hours) in which to assess sperm motility. Chilled goat semen is usually maintained at 4°C (Leboeuf et al., 2008). However, it requires the use of special diluents and rather more equipment. Because the motility and fertilising capacity of some bucks' sperm is reduced during the nonbreeding season, their stored semen should not be used to inseminate does that have been induced to ovulate out of season.

Goat semen is stored, long term, in 0.2-mL straws containing $1x10^8$ sperm cells and frozen in liquid nitrogen down to -196°C in three progressive steps. The use of frozen-thawed semen is, unfortunately, limited in countries with less advanced levels of technology (Corteel, 1981).

When properly carried out, insemination of does with fresh semen yields fertilisation rates comparable to natural mating. As a rule, the use of frozen semen leads to poorer conception rates. Nonetheless, fertility rates after cervical AI with frozen semen are higher in goats than in sheep. This is mainly due to structural differences in the cervix at oestrus. In a substantial number of does (50%-60%), semen can be deposited deep into the cervical canal or even into the uterus.

With laparoscopic AI, even better, and more consistent, pregnancy rates can usually be achieved. However, the use of this technique is limited by the requirement for elaborate equipment and skilled operators.

Kidding rates of 71% have been reported with another technique, recently described by Sohnrey and Holtz (2005), in which semen is deposited deep in the uterine horns by the transcervical route. The kidding rate in the laparoscopically inseminated controls in this trial was 53%.

Type of oestrus	Insemination time
Natural*	12-18 hours after onset of oestrus
Induced by intravaginal sponges**	Long or short progestagen treatment: two AI about 30 and 50 hours after removal of the sponges Short progestagen treatment: one single AI 43 to 46 hours after removal of the sponges, depending on the breed Kid does about 45 ± 1 hour after removal of the sponges
* According to Evans and Maxwell (1987) ** According to Corteel et al. (1988)	

Table 1. Timing of insemination in goats.

The timing of AI varies according to the method of AI used, the kind of oestrus (spontaneous or induced), the age and breed of the animal, and whether single or double AI is to be performed (see Table 1). Insemination not coordinated with ovulation can be detrimental to fertility. When stored or frozen semen is used, the timing of AI is even more critical. Fixed-time insemination in goats (hormone induced oestrus) has to be gauged specifically for different breeds and physiological conditions.

6.3 Control of oestrus

The control of oestrus and out-of-season breeding are of increasing interest; they enable milk producers to maintain regular and consistent levels of production as well as allow three kid crops in two years from fibre-producing goats. Methods of oestrus control in goats are analogous to those described for sheep, but there are some peculiarities worthy of note. Moreover, it should

be highlighted that the best results are obtained when oestrus induction and synchronisation are undertaken in order to extend the breeding season rather than to breed does out of season, when they are in profound anoestrus.

6.3.1 Buck effect

Introducing bucks to anovulatory females, after a period of complete segregation (which must be at least 4-6 weeks), induces synchronous ovulations in the ensuing days (Pellicer-Rubio et al., 2007). Although an olfactory stimulus plays a predominant part, all the senses are probably involved in the does' response. The contact with males induces the appearance of a pre-ovulatory surge of LH that triggers ovulation. The first induced ovulations are silent in 40% of the does and are followed by a short luteal phase in 75% of them. Oestrous and ovarian cycles return to normal later. The quality of the response depends on the intensity of stimulation and on the depth of anoestrus at the time the males are introduced. Similarly, the fertility of the females is also variable. Generally, the closer to the breeding season, the better the oestrus response and fertility. In more seasonal breeders (Alpine and Saanen), subjecting females to artificial photoperiods may be necessary to improve the response to the male effect. Under these conditions, most does exposed to males were reported to ovulate (99%) and to deliver kids (81%) (Pellicer-Rubio et al., 2007).

The buck effect is more effective in breeds with a low seasonality. However, even in breeds responding well to this stimulus, a progestagen is often needed to obtain good fertility at the first buck-induced ovulation. Artificial insemination can be used, with one or two inseminations over a 24-hour period determined by the occurrence of oestrus or by the introduction of a buck. Relatively high rates of fertility can be achieved in this way, but the required oestrus detection and careful timing of AI are very labour intensive.

6.3.2 Photoperiod regimes

Since the seasonality of reproduction is under the control of day length, reproduction during seasonal anoestrus can be successfully achieved using artificial light, which advances the breeding season but also induces a reproductive state in the middle of the anoestrus period (Chemineau et al.,

1986, 1988, 1999; Delgadillo et al., 2002). While it induces ovulation, it does not synchronise ovulation.

Goat AI centres, equipped with dark housing, use alternating light regimes with a month of long days and a month of short days, which allows for consistently high semen production with no seasonal variation in sperm quality. Currently, in the French national genetic improvement scheme, all bucks (approximately 70 per year) are permanently treated by rapidly alternating long and short days, thus increasing semen production per buck by 40% per year and reducing the duration of the breeding period of males (Cheminault et al., 2008).

On goat farms (always in open barns), males and females are subjected to the other system used in AI centres (long days followed by short days). This more natural treatment needs to be used in conjunction with the buck effect (introducing treated bucks for 45 days after 35-75 of the short day phase) in order to induce oestrous behaviour and ovulation and to achieve high fertility rates. Under such conditions, out-of-season fertility and prolificacy can be maintained at high levels (>75% kidding rate with approximately two kids per kidding). For local breeds in subtropical conditions, where seasonality is less marked than those in temperate latitudes, the treatment of females is not necessary.

6.3.3 Melatonin

It has been shown experimentally that treatment with melatonin can stimulate oestrus and ovulatory activity in anovulatory, out-of-season, dairy goats. For maximum stimulation, the melatonin has to be preceded by a two month period of 'long days' (using artificial light), and followed by the male effect. When used soon after kidding, however, melatonin slightly decreased milk production (Evans et al., 1987).

6.3.4 Progestagen-based methods

The use of progestagens for oestrus management in goats allows for:
- Oestrus synchronisation during the breeding season
- Tight oestrus and ovulation synchronisation for fixed-time AI
- Extension of the breeding season
- Out-of-season breeding

There are some differences in the physiology of goat reproduction that require alterations to the schedule used in sheep. The same progestagens are used as in sheep, but when they are used without complementary luteolytic treatment, the duration of treatment must equal or exceed the lifespan of the corpus luteum (ie, 16-18 days) in order to achieve effective synchronisation. Because progestagens do not hasten luteolysis in the goat as they do in the ewe, a long-lasting treatment is needed.

At present, the progestagens available for oestrus management in goats include intravaginal sponges impregnated with fluorogestone or medroxyprogesterone and intravaginal devices impregnated with progesterone. There have been some reports of the use of norgestomet implants for oestrus and ovulation synchronisation in these species.

The protocol varies according to season, method of breeding, and factors specifically related to the females to be treated (see Tables 2 and 3). When natural mating is to be used, sponges may be withdrawn from 17 to 22 days after insertion. With AI, sponges must not be withdrawn before 21 days (a longer treatment).

In both cases, it is advisable to inject from 400 IU to 700 IU of pregnant mare serum gonadotrophin/equine serum gonadotrophin (PMSG/eCG) at the time of sponge removal (Table 3). During the pre-breeding season or shallow anoestrus periods, and even in deep anoestrus, the same progestagen regimen may be used, but it is necessary to inject even higher doses of PMSG 24-48 hours before the end of progestagen treatment. The fertility obtained after oestrus induced by these treatments ranges from 50% to 70%; the closer to the breeding season, the better the fertility (Corteel et al., 1982).

The interval from parturition to the beginning of treatment greatly influences fertility at the induced oestrus. A minimum of four months is required in the European dairy goat to obtain good results. A shorter treatment regime has been adopted, involving the intravaginal administration of 45 mg FGA sponges for 11-12 days and PMSG/eCG and a $PGF_{2\alpha}$ 48 hours before the end of progestagen treatment (see Table 2). This treatment has advantages over the long treatment: less variable ovulation rate, better synchronised oestrus and higher fertility. It produces good results with a single cervical AI, and can be used in maiden does with satisfactory results, providing the dose of

PMSG/eCG is reduced (250-300 IU).

Goats treated with progestagen-impregnated sponges usually show very strong behavioural signs of oestrus. Oestrus usually occurs approximately 24-72 hours after the removal of sponges, with the optimal time for fixed-time AI at 36-40 hours after sponge removal. Treated goats are usually inseminated once with a thawed dose of frozen semen containing $1x10^8$ spermatozoa.

Treatment	Insertion of sponges	Injection of prostaglandin	Removal of sponges
Short	Day 0	Day 10	Day 12
Long	Day 0	-	Day 17-21

Table 2. Treatment schedules for intravaginal sponges in goats.

	Milk production	PMSG dose
In season	< 3.5L/day	400 IU
	< 3.5L/day	500 IU
Transitional period	< 3.5L/day	500 IU
	< 3.5L/day	600 IU
Out-of-season	< 3.5L/day	600 IU
	< 3.5L/day	700 IU

Table 3. Adjustment of PMSG dose in does treated with intravaginal sponges.

6.3.5 Prostaglandins

Prostaglandins or analogues can be used to synchronise oestrus in cyclic goats. Because luteolysis is provoked only in the presence of a functional corpus luteum (from day 5 to day 19 of the cycle), animals have to be pre-synchronised either by progestagen treatment or by a previous injection of $PGF_{2\alpha}$. Two intramuscular injections of 8 mg $PGF_{2\alpha}$ administered 11 days apart rendered a high degree of synchronisation (94% of animals in oestrus 53 ± 3 hours after the second injection) and a conception rate similar to nontreated controls after natural service (Ott et al., 1980). The most common use of $PGF_{2\alpha}$ in synchronising oestrus is in combination with a short duration progestagen treatment, in which case a single standard dose of

prostaglandin indicated by the producer for goats is used. The wider use of prostaglandins in goat breeding is often complicated by the fact that few $PGF_{2\alpha}$-based products are licensed for use in goats or supplied with detailed information about the dose in this species.

6.3.6 Prostaglandins combined with GnRH

While oestrus synchronisation with progestagens generally results in good fertility, irrespective of seasonal effects (breeding or anoestrus season), some breeders are interested in alternative synchronisation strategies, especially those not involving the use of steroid hormones. Systems based on the so-called Ovsynch protocol developed for cattle (Pursley et al., 1995), involving the sequential administration of GnRH and $PGF_{2\alpha}$ could therefore become an interesting possibility. Holtz et al (2009) compared results of oestrus synchronisation using the Ovsynch protocol and fluorogestone impregnated intravaginal sponges (combined with $PGF_{2\alpha}$ and PMSG/eCG treatment) in Boer does during the breeding season. Does were inseminated at predetermined times (16 hours after the second GnRH injection and 43 hours after sponge removal). Oestrus was identified in 96% of the Ovsynch-treated goats and in 100% of the goats synchronised with progestagen sponges. Kidding rates (58% and 46% in the Ovsynch and sponge groups, respectively) and prolificacy (1.86 and 1.83 in the Ovsynch- and sponge-treated goats, respectively) were similar for both groups, as were the number of ovulations (2.9 and 3.3) and the proportion of does with premature regression of the corpus luteum (29% and 17%). The authors, therefore, postulated that during the breeding season, the Ovsynch protocol may be a useful alternative to progestagen treatment. It is important to bear in mind that, just as in anoestrus in cattle, the treatment of goats outside the breeding season will produce much poorer results in terms of oestrus induction and pregnancy rates. Additionally, although very attractive, such systems have an important limitation in that only a few GnRH and $PGF_{2\alpha}$ products are actually licensed for use in this species.

6.4 Superovulation and embryo transfer

The same methods used to induce ovulation in sheep are also applicable to the goat, but the programme and the doses need to be adapted accordingly.

The main purpose of this treatment is to induce superovulation for embryo transfer programmes. Although both PMSG/eCG and porcine follicle-stimulating hormone (pFSH) have been used, with or without progestagen treatment, the pFSH seems to be superior with respect to ovulation rate and number of offspring born to recipients. Since the half-life of pFSH in goats is only 5 hours, pFSH is administered twice daily for 3-4 days, usually in decreasing doses, beginning between 1 and 3 days before the end of the progestagen treatment (Baril et al., 1990). On average 8-16 ovulations are generated, although this is very variable between individuals. Baril et al (1996) reported very good superovulation results with progestagen pre-treatment followed 12 hours later by administration of a GnRH antagonist.

Two papers published by Medan et al (2003a,b) indicated the suitability of active inhibin immunisation for eliciting multiple ovulations in goats. However, before this method can be used on a larger scale, even in research, there are issues to be resolved. These include the high rate of premature luteal regression occurring in treated animals and an unusually large number of nonovulated follicles.

Whereas embryo transfer is an effective method of achieving genetic improvement in cattle, it is not widely used in goats. The main reasons for this are the lesser value of goats, and the considerably greater technical difficulties involved in collecting and transferring their embryos. Surgical and laparoscopic embryo transfer techniques have been developed, but they still require general anaesthesia as well as the use of sophisticated equipment and considerable technical skill. Moreover, postoperative adhesions are a frequent complication, limiting the number of possible collections.

A novel, nonsurgical method was described by Pereira et al (1998), Holtz et al (2000), Suyadi et al (2000), and Holtz (2005) and has since become standard with various embryo transfer groups.

The various steps involved with the in vitro production of caprine embryos are quite similar to those employed in the bovine. Both the standard in vitro fertilisation (IVF) and 'intracytoplasmic sperm injection' (ICSI) have been reported in goats, resulting in the birth of live offspring (Baldassarre et al., 2003; Wang et al., 2003).

Methods for the cryopreservation of caprine embryos are also similar to those used successfully in bovines. In favourable conditions, pregnancy rates between 45% and >80% may be expected after the transfer of cryopreserved blastocysts, depending, in part, on the number of embryos transferred per animal (Holtz et al., 2000).

Other techniques, such as embryo splitting and nuclear transfer, have been reported in goats, but are a long way from being used on a large scale, even in research. Nonetheless, there is growing interest in these technologies, mainly driven by the desire to breed transgenic animals to provide substances suitable for the pharmaceutical industry.

6.5 Reproductive disorders

6.5.1 Intersexuality (polled gene)

The intersex condition, or hermaphroditism, is a common cause of infertility in does of polled breeds (Smith, 1980). It is an anatomical and functional abnormality which usually involves masculinisation of females and cryptorchid-related abnormalities in the male. The condition is associated genetically with the absence of horns in several breeds of dairy goats (Riera, 1984). The polled trait is dominant while the associated hermaphroditic trait is recessive and sex-linked. If one parent is horned, the offspring will almost never be one of the intersexes. The use of a horned buck is the standard method of avoiding the condition (Smith, 1980).

6.5.2 Pseudopregnancy

This condition, also known as hydrometra, mucometra or 'cloudburst,' consists of an accumulation of varying amounts of sterile fluid within the uterus (Pieterse et al., 1986). It is a significant cause of infertility in the goat (Smith, 1980) that causes permanent anoestrus due to a spontaneous persistence of corpus luteum function (Taverne et al., 1988).

An outward sign of hydrometra is abdominal distension caused by the fluid accumulating in the uterus. This, together with a false-positive pregnancy test, may prolong the nonproductive period in affected goats because they appear to be pregnant. The aetiology of the condition remains obscure. The

term 'cloudburst' refers to those cases in which cloudy (uterine) fluid occurs around the expected time of parturition in nonmated animals (Pieterse et al., 1986). It is relatively easy to diagnose with the aid of real-time ultrasound, and can be treated with prostaglandins, after which pregnancy is once again possible.

6.5.3 Infectious abortion

Abortion is a relatively common cause of loss of reproductive efficiency in goats, as it is in sheep. The most frequent causes of infectious abortion in goats are Brucella spp and Chlamydia (see in Chapter 5). Brucella abortion is caused mainly by *B. melitensis* and occasionally by *B. abortus*. The main feature is abortion, usually in the fourth month of pregnancy, but it can also be associated with other clinical signs such as lameness, mastitis and orchitis. Chlamydia causes enzootic abortion, also known as viral abortion. It usually takes place after the third month of pregnancy, and most frequently during the last two weeks of pregnancy (Smith, 1980). Other infectious diseases associated with reproductive failure and abortion in goats include Q fever (*Coxiella burnetii*), listeriosis (*Listeria monocytogenes*), leptospirosis (*Leptospira spp*), and toxoplasmosis (*Toxoplasma gondii*).

6.5.4 Delayed ovulation/follicular atresia

There is only limited evidence in the literature for these disorders in goats, in comparison with cattle. However, in practice, a treatment to induce ovulation using human chorionic gonadotrophin (hCG, 500 IU) or GnRH at the time of AI is often used to improve fertility, especially in high-yielding milking goats.

6.6 Induction of parturition

Doses of 5.0 and 2.5 mg of $PGF_{2\alpha}$ have been shown to be effective in inducing parturition in does treated on day 144 of gestation (Bretzlaff et al., 1983). However, care should be taken to avoid premature treatment, as high doses of oestrogens or $PGF_{2\alpha}$ analogues will provoke abortion at any stage of pregnancy. Therefore, if the date of mating and the duration of pregnancy are not known for sure, it is more advisable to use corticosteroids which will induce parturition only if the foetuses are ready to signal the initiation of labour (Corteel et al., 1982). In practice, however, they are hardly ever used.

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7.1 Physiology

7.1.1 The oestrous cycle

The female dog (bitch) has only one oestrous cycle, which is much longer than in most other domestic animals, during each breeding season (mono-oestrus) (reviewed by Root Kustritz, 2012). The oestrous cycle can be divided into four phases (Figure 1). A period of sexual inactivity (anoestrus) is followed by pro-oestrus, which is characterised by vulvar swelling and bleeding. Oestrus, the time during which the female dog will accept the male, immediately follows pro-oestrus, and ovulation occurs spontaneously at the beginning of oestrus. If pregnancy does not ensue, oestrus is followed by metoestrus, which blends imperceptibly into anoestrus. The term "heat" is used by dog owners to describe the combination of pro-oestrus and oestrus. There is no specific lay terminology for the rest of the oestrous cycle in the female dog.

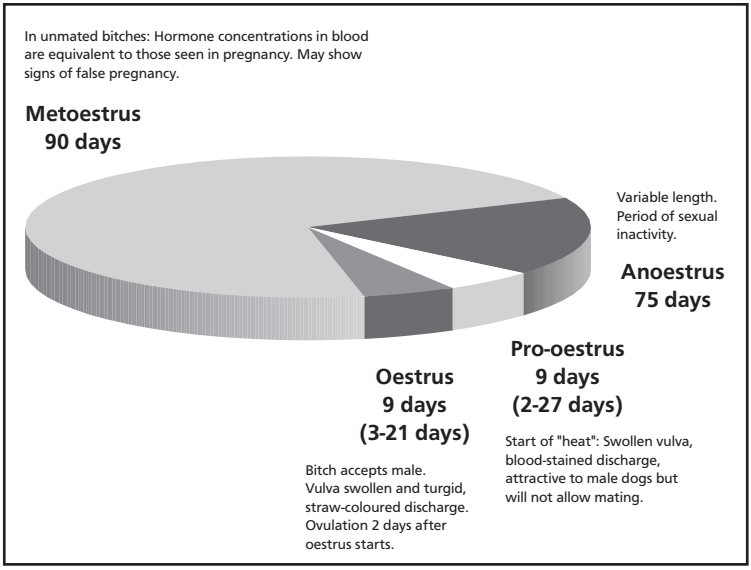

Figure 1. The oestrous cycle of the female dog

a. Duration of the phases of the oestrous cycle

The duration of the different phases of the oestrous cycle can vary considerably between individuals. The situation is also complicated because the duration and intensity of the external changes and behavioural signs — swelling of the vulva, vaginal bleeding, and acceptance of the male — by which pro-oestrus and oestrus are recognised in the female dog are not consistent between animals. Furthermore, the beginning, end, and duration of metoestrus cannot be determined by observation since this phase of the oestrous cycle is not characterised by the presence of specific external signs. All of these factors, together with the fact that the external signs may not mirror the underlying hormonal status, are of great importance when considering breeding or manipulation of the oestrous cycle. Relatively simple techniques, including vaginal exfoliate cytology, the measurement of hormone concentrations (particularly progesterone), and vaginal endoscopy, can reduce these difficulties substantially (Jeffcoate and Lindsay, 1989).

	Early pro-oestrus	Late pro-oestrus	Early oestrus	Late oestrus
Red blood cells	+++	++	+	
Keratinised cells		+	++	+++
Leukocytes	+			+
Debris	+++	++	+	

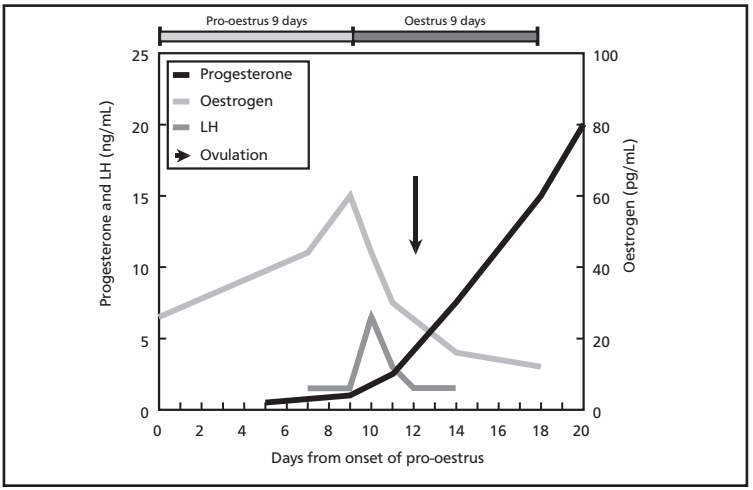

Table 1 and Figure 2. Hormone concentrations and vaginal cytology in pro-oestrus and oestrus

The female dog has a relatively long luteal phase of around 55-75 days, due to genetically programmed luteal cell lifespan (Concannon, 2009). The length of the luteal phase in nonpregnant female dogs significantly exceeds that observed in pregnant animals (Kowalewski, 2012). Metoestrus can be divided into progressive (phase 1) and regressive (phase 2) stages (Table 2 and Figure 3). Originally, this division was based on the histological appearance of the uterus, but the two stages can be related directly to luteal function. Phase 1 refers to the phase of corpus luteum development (approximately 20 days) and phase 2 to the time from the onset of luteal regression until the uterus returns to the anoestrus state, a further 70 days. Thus, metoestrus normally lasts for approximately 90 days (3 months), with luteal function declining after the first 20 days. Endometrial desquamation starts at about day 90 of the oestrous cycle (day 0 = first day of oestrus) and continues for about 21 days, the discarded tissue being resorbed or expelled via the cervix. The endometrium has regenerated completely by day 150, on average.

	"Heat"	Metoestrus
Red blood cells	+++ to +	
Keratinised cells	- to +++	++
Leukocytes	+ to 0 to +	+++
Debris	+++ to +	

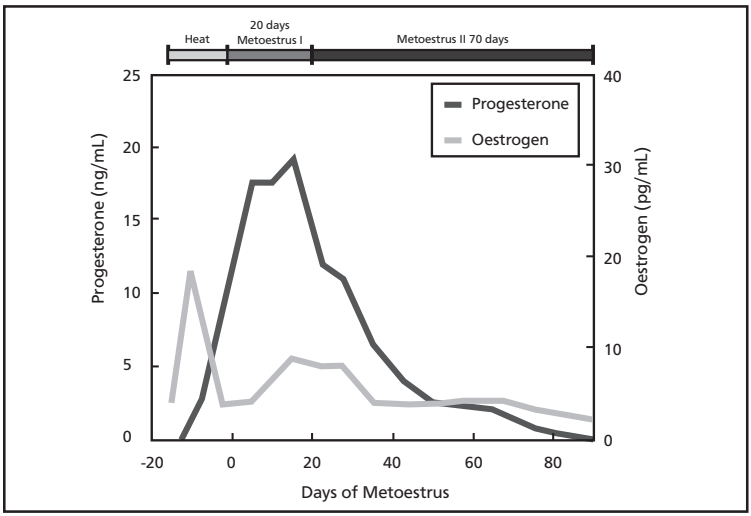

Table 2 and Figure 3. Hormone concentrations and vaginal cytology in pro-oestrus, oestrus, and metoestrus

The frequency of oestrus in the female dog is primarily determined by the length of anoestrus. The length of anoestrus is variable within and between female dogs. The average interoestrus interval in the dog is 7 months (Christie and Bell, 1971) but varies from 4 to 12 months. Breed variability can be striking; eg, German shepherds generally have an interoestrus interval of 4-4.5 months, while African breeds, such as the Basenji, may only come into oestrus once per annum (Fuller, 1956). Pregnancy increases the interval to the next oestrus by 28 days, on average.

Seasonal activity may be slightly increased during the period from February to May (Christie and Bell, 1971), but female dogs commence cycling, breed, and give birth (whelp) at essentially any time of year. There may appear to be some seasonality, since the majority of female dogs housed together often show signs of heat within a limited period. This is also seen where there is a high population density of dogs, eg, shelters, boarding kennels, and some urban areas. This is not true seasonality but "natural" induction (or synchronisation) of oestrus, presumably due to pheromones, and may influence the efficacy of pharmacological intervention.

b. Hormonal changes in oestrus

Hormones from a variety of origins (pituitary gland, placenta, and ovary) are involved in the control of the oestrous cycle in dogs (Concannon et al., 1975; Onclin et al., 2002). Innate cyclical activity and reproductive function are controlled by the hypothalamus, which is sensitive to both external (eg, environmental) and internal stimuli. The oestrous cycle is therefore controlled by the complex interplay between the hypothalamus and the reproductive tract, with the anterior pituitary acting as the "central relay station." A summary of the hormonal changes (reviewed by Concannon, 2009, and Kowalewski, 2012) is presented below.

The length of the cycle is determined by the length of anoestrus, which is likely under neuroendocrine control (eg, dopaminergic), but this may be modified by factors such as the prior luteal phase and exposure to pheromones from other female dogs in oestrus. Anoestrus is not a period of hormonal quiescence but one of preparation for the next oestrus. Follicle-stimulating hormone (FSH) is thought to be associated with the stimulation and maintenance of waves of dominant follicles, follicular aromatase

activity, and basal oestradiol secretion, which suppresses luteinising hormone (LH) secretion by negative feedback. During the last 50 days of anoestrus, there is a further increase in FSH concentrations and an increase in the size and number of LH-sensitive, dominant follicles. Toward the end of anoestrus, high-amplitude LH pulses lead to the selection and terminal development of dominant follicles.

During the 2 to 3 weeks prior to the onset of pro-oestrus, the anterior pituitary secretes FSH in pulses of increasing frequency. FSH controls the development of the ovarian follicles, which principally secrete oestrogen but, as they reach maturity, also progesterone. Low concentrations of oestrogen exert positive feedback on the anterior pituitary, stimulating more FSH to be released, resulting in further follicle growth and increased oestrogen concentrations. This process continues until the follicles are mature and about to rupture. At this stage, the higher concentrations of oestrogen exert negative feedback that inhibits the secretion of FSH and triggers the anterior pituitary to release pulses of LH. Once maximal oestradiol production has been reached, the follicles are no longer capable of producing more oestradiol. Follicles start to produce progesterone, with concentrations in plasma reaching around 5 ng/mL at the time of ovulation. There is a preovulatory surge of LH, which often occurs at the same time as the onset of oestrus, and a large decrease in the ratio of oestrogen to progesterone. This large LH surge is followed by ovulation (Figure 2).

Each ruptured follicle is rapidly converted into a corpus luteum. Depending on the stage of the luteal phase, the development and maintenance of corpora lutea appears to be dependent on LH, prolactin, and prostaglandin (PG) E_2, which appears to have an autocrine or paracrine role (Kowalewski et al., 2013; Okkens et al., 1990). The developing corpora lutea secrete progesterone, and concentrations of this hormone rise rapidly (up to 30-35 ng/mL) during the 15-25 days after ovulation. High concentrations of progesterone exert negative feedback on the production of LH, which maintains these secretory bodies until day 35. Falling concentrations of progesterone exert positive feedback on the release of prolactin, the gonadotropin that maintains luteal function after day 35. Regression of corpora lutea in dogs is caused by periodic cell death, diminution in cell size, low levels of apoptosis, and minimal or modest involvement of endogenous PGF production.

The female dog is unusual in a few respects:

- Low concentrations of progesterone produced by preovulatory follicles are present prior to ovulation, and this, in conjunction with falling levels of oestrogen, is probably responsible for the initiation of oestrus (Table 1 and Figure 2). The signal that marks the end of pro-oestrus and the beginning of oestrus is progesterone concentrations greater than 0.5 ng/mL in conjunction with declining oestrogen concentrations (Table 1 and Figure 2).
- Lack of an acute luteolytic mechanism means that there is a long period of progesterone dominance (Table 2 and Figure 3).

The unique hormonal changes involved in the oestrous cycle in female dogs lead to two distinct phenomena, pseudopregnancy ("false pregnancy") and cystic endometrial hyperplasia (CEH), with or without endometritis, or pyometra. In addition, long exposure to high progesterone concentrations during each oestrous cycle may result in a syndrome of excessive growth hormone (somatotropin) production from the mammary gland, resulting in acromegaly (hypersomatotropism) in some dogs (reviewed by Kooistra and Okkens, 2002).

7.1.2 Mating

Mating in female dogs (reviewed by Christiansen, 1984, and Feldman and Nelson, 2004) is summarised below.

a. Mating behaviour

Female dogs are attractive to male dogs for approximately 9 days while they are in pro-oestrus. Mating occurs when the female dog is in standing oestrus. Before mounting a female dog, the male dog may go through a relatively prolonged courtship but often will simply briefly lick the vulva. As a result of this attention, the female dog will usually stand firmly with its tail held to one side, exposing the vulva. Penetration in the dog is achieved without erection because of the presence of the *os penis*. However, once inside the vagina, the *bulbus glandis* becomes engorged and is accompanied by strong thrusting movements. This results in the ejaculation of prostatic fluid. Once pelvic thrusting ends, the dog will dismount and, by lifting one hind leg over the female dog, end up "tied" to it, tail to tail, locked by the

engorged bulbus, making separation difficult. The tie can last anywhere from 5 to 60 minutes (average 20 minutes), and during this time the female dog and male dog may drag each other around. During the tie, ejaculation of seminal fluid continues. This second part is sperm rich. The tie finally breaks quite spontaneously, and some seminal fluid may be seen draining from the vulva of the female dog. The tie is not essential for conception.

b. Timing of mating

The most common cause of mating failure is inappropriate timing (reviewed by Goodman, 2001). Traditionally, dog owners have their female dogs mated twice, 11 and 13 days after the onset of pro-oestrus, to try to ensure that spermatozoa are present in the female reproductive tract at or around the time of ovulation. This is generally very successful, due to the unusual longevity of dog spermatozoa (6-11 days) in the female genital tract (Concannon et al., 1989).

There is no doubt that many fertility problems result from mating being arranged at a convenient time rather than on the most appropriate day. If the timing of ovulation is determined more precisely, fertility rates are likely to increase and the expected whelping date can be predicted more accurately. In addition, conception failures are less likely and the management of the female dog can be simplified.

c. Detection of ovulation

Three methods of detecting ovulation are available to veterinarians: vaginal cytology, vaginoscopy, and the measurement of hormone concentrations (reviewed by Concannon et al., 1989; Feldman and Nelson, 2004; Jeffcoate and Lindsay, 1989; and Schaeffers-Okkens, 2000).

Vaginal (exfoliate) cytology

Cytological evaluation of vaginal smears can be used to monitor the progress of the so-called vaginal cycle. This is a series of consecutive changes in the number and morphological features of the vaginal epithelial cells that mirror the changes in the endocrine environment and the related changes in ovarian activity during the oestrous cycle.

During pro-oestrus, the number of parabasal and small intermediate cells with easily visible nuclei decreases, while the number of superficial cells increases (Table 2 and Figure 3). As pro-oestrus develops, the number of keratinised superficial cells with pyknotic or indistinguishable nuclei increases, reaching 60%-80% at the transition into oestrus (Table 2 and Figure 3). Red blood cells are usually observed throughout pro-oestrus and slowly disappear as oestrus commences. However, this feature should not be relied upon, since red blood cells can persist. There is no reliable change in the smear indicative of the LH surge or ovulation. In fact, cytology can only be used to detect the time of ovulation retrospectively because the first day of metoestrus is the only stage of the cycle that can be pinpointed precisely using this technique.

The first day of metoestrus is characterised by a dramatic fall in the percentage of superficial cells and the reappearance of white blood cells (Tables 1 and 2 and Figures 2 and 3). In most female dogs this takes place 8-10 days after the LH surge and gives a rough indication that ovulation occurred approximately 6 days earlier. This is of no practical value for breeding management. Thus, vaginal cytology is not a very reliable method for determining the appropriate time for mating female dogs. In addition, it is a rather crude index for predicting the first day of standing oestrus. However, it can be very useful when close monitoring of the consecutive phases of the oestrous cycle is required.

Whenever vaginal cytology is used to time mating, it should never be based on a single sample. Vaginal cytology should be carried out at least 3 times, starting at day 5 after blood-stained discharge was first detected and subsequently every second day. If the percentage of cornified cells has not reached 60% on day 9, another sample should be taken within 2 days. Experienced veterinarians suggest that mating should be first attempted when the amount of cornification exceeds 80% and then repeated every second day for as long as the female dog will accept a male dog.

Vaginoscopy

The changes in the lining of the vagina, as seen through a vaginoscope, parallel those seen in vaginal cytology. However, at the time of ovulation, a skilled observer will note the onset of "wrinkling." The wrinkles become very obvious about 4 days after ovulation, the most critical time for mating.

It is necessary, however, to become familiar with the technique and to examine female dogs at least every other day, from 4-5 days after the onset of pro-oestrus, if the method is to be used effectively.

Measurement of hormone concentrations

The hormonal changes that occur during pro-oestrus and oestrus are shown in relation to the time of ovulation in Table 1 and Figure 2. The peak concentration of LH that occurs before ovulation is generally regarded as the central event of the oestrous cycle. Most of the important events that occur in the oestrous cycle are closely synchronised with the preovulatory LH surge (Table 3 and Figure 4).

Ovulation	48 hours
Oocyte maturation	4-5 days (ie, 2-3 days postovulation)
Peak fertility	0-5 days
Implantation	18 days
Parturition	64-66 days

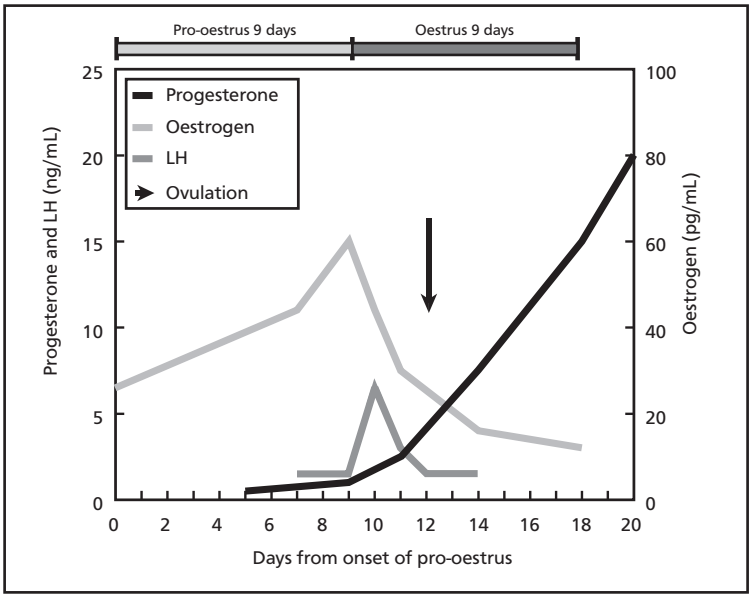

Table 3 and Figure 4. Timing of major events in the oestrous cycle in relation to the peak concentrations of LH

It would be ideal if the LH surge could be detected conveniently and easily. However, this is not practical, since frequent blood sampling would be required to detect increased concentrations of LH, which are elevated only transiently over a period of 1-3 days.

Progesterone concentrations increase during the LH surge and reach concentrations of 2-5 ng/mL around day 2 after the LH concentrations peak. Progesterone concentrations continue to rise throughout oestrus and peak 13-28 days later. It is possible to measure progesterone concentrations in just a drop of blood or plasma. Based on sampling every 2-3 days, the optimal time for mating is around 12±3 days (6-21 days) after the onset of vulvar bleeding (van Haaften et al., 1989).

7.1.3 Pregnancy

a. Duration

The length of gestation in dogs is remarkably constant at 64±1 days, with implantation occurring 18 days after the LH peak (reviewed by Lamm and Makloski, 2012, and Linde-Forsberg and Eneroth, 2000; for a review, see Figure 4). This means that pregnancy generally lasts 63 days, with a range of 56-72 days, after mating. This large variation is due, at least in part, to the longevity of canine spermatozoa (Concannon et al., 1989). There is also some variation between breeds and associated with litter size: female dogs carrying 4 pups or less have a significantly longer gestation than those carrying 5 pups or more (Eilts et al., 2005).

b. Hormonal changes during pregnancy

The endocrine changes that occur during pregnancy in female dogs (reviewed by Christiansen, 1984; Concannon, 1986; Concannon et al., 1989; and Feldman and Nelson, 2004) are summarised below. Circulating concentrations of progesterone, oestrogen, and prolactin in pregnant female dogs, unmated female dogs in metoestrus, and female dogs that have failed to become pregnant are very similar (Figure 3). High concentrations of progesterone are maintained for 50-60 days after the LH peak. However, in the pregnant female dog there are often secondary increases in the

production, but not concentration, of progesterone between days 25 and 40 that may reflect pregnancy-specific mechanisms (ie, relaxin-stimulated increases in prolactin) that result in additional stimulation of progesterone production (Concannon, 2009). Functional corpora lutea are essential for pregnancy: after day 30 of gestation, abortion occurs within 24-72 hours after ovariectomy. During the last third of pregnancy, elevated oestrogen concentrations can be detected. Luteal function in pregnant female dogs is terminated abruptly by luteolysis 62-65 days after the LH surge.

After oestrus, prolactin concentrations rise in both pregnant and nonpregnant female dogs, although the concentrations are somewhat higher in pregnant female dogs and show a transient surge during the rapid decline in progesterone concentrations that occur 1-2 days before parturition (whelping). Prolactin concentrations remain elevated until the litter is weaned. The pregnancy-specific hormone relaxin can be detected in the blood of a pregnant female dog 26-30 days after the LH peak but is not present in nonpregnant dogs (Concannon et al., 1996).

c. Pregnancy diagnosis

Body weight gain in pregnant female dogs is generally 36% (range 20%-55%), with the increase being most marked in the last third of pregnancy. A change in body shape is usually visible by about day 56 of pregnancy, and foetal movements may also be noted around this time. The nipples enlarge and mammary development occurs during the second half of pregnancy, and serous secretion may be present shortly before parturition.

Dog owners frequently want to know whether their female dog is pregnant so that adequate plans can be made in advance of the anticipated whelping date.

Abdominal palpation

Abdominal palpation, usually 3-4 weeks postmating, is used commonly for the diagnosis of pregnancy in the female dog. Although false-positive results are rare in the hands of experienced veterinarians, it is difficult to be certain that a female dog is not pregnant. Moreover, the use of abdominal palpation for pregnancy diagnosis can be problematic in female dogs that are overweight or obese, if the abdomen is guarded, and in certain breeds.

Radiography
Radiography can be used to confirm canine pregnancy, but foetal skeletons do not become radio-opaque until day 45.

Ultrasonography
Ultrasonography can be used to visualise foetal vesicles from day 16 to 20 of pregnancy. Foetal heartbeats can be seen from day 24 to 28.

Hormone measurements
Progesterone concentrations cannot be used to diagnose pregnancy, since high concentrations of progesterone are also present in nonpregnant dogs during the luteal phase. Acute-phase protein concentrations are significantly elevated from day 21 to 50 postmating in pregnant female dogs compared to nonpregnant female dogs (Concannon et al., 1996; Evans and Anderton, 1992). Not all acute-phase proteins are useful for early pregnancy diagnosis, and female dogs must be healthy and mating dates known in order to avoid false-positive or false-negative results (Vannucchi et al., 2002).

7.1.4 Parturition

The events (reviewed by Christiansen, 1984; Concannon, 2009; Concannon et al., 1989; Feldman and Nelson, 2004; Lam and Makloski, 2012; and Linde-Forsberg and Eneroth, 2000) that take place immediately before and during parturition are summarised below.

a. Initiating events

Rising cortisol concentrations, which result from the maturation of the foetal hypothalamic-pituitary-adrenal axis, trigger a whole cascade of events. This leads to a series of hormonal changes, starting with rising oestrogen and falling progesterone concentrations, oxytocin, and the production of luteolytic amounts of $PGF_{2\alpha}$ by the foeto-placental unit for 1-2 days before parturition (Gram et al., 2014). This PG induces the production of relaxin, which results in the relaxation of the pelvis and reproductive tract and causes uterine contractions and abdominal straining, both directly and via the release of oxytocin from the pituitary. However, the precise hormonal mechanisms that precipitate parturition in female dogs have not been fully elucidated.

b. Prepartum signs

During the last 2-3 days before parturition, female dogs usually exhibit characteristic behaviour, such as seeking solitude, restlessness, and nest making. The presence or absence of milk is too variable to be a reliable sign of impending parturition. Just prior to whelping it is not uncommon for the vagina to become edematous, and a slight vaginal discharge may be visible. Female dogs frequently refuse food for 1-2 days prior to parturition. Body temperature decrease, frequently taken by breeders as an indication that whelping will occur within the next 24 hours, is not a reliable indicator of impending parturition in the female dog (Veronesi et al., 2002). There is a significant decrease in progesterone concentrations from 24 hours prior to whelping (Veronesi et al., 2002).

c. Parturition

Classically, parturition is divided into three stages, with the stage altering as each pup is produced.

First stage of parturition: cervical relaxation and dilation
During this stage, which lasts between 4 and 36 hours, the cervix relaxes and dilates. Restlessness, nervousness, shivering, panting, tearing up bedding, and/or vomiting may be seen. Weak uterine contractions may be apparent.

Second stage of parturition: the birth of pups
This stage is characterised by strong uterine contractions and visible straining. Between contractions the female dog will lick its vulva, especially once a foetal sac ruptures and placental fluid is released. Once a foetal head or pelvis is engaged, abdominal straining is stimulated. The duration of the second stage of parturition is extremely variable between individuals and between pups in a litter. As a rule of thumb, however, no more than 6 hours should be allowed to elapse after the delivery of the first pup before an investigation is carried out, since a long delay may result in placental separation and the death of any remaining viable pups. Second and subsequent pups are usually produced after no more than 30 minutes of contractions. Rest periods of more than 3-4 hours should be regarded as abnormal. It is not uncommon for a large litter to take up to 24 hours to be

delivered. Female dogs that are good mothers will clean and suckle the pups between successive births.

Third stage of parturition: the expulsion of the placenta
This is the stage during which the foetal membranes are expelled. Pups may be born with the membranes intact, or they may be born simply attached by the umbilical cord, with the placenta remaining in the genital tract. In the latter case, the placenta will be expelled separately before, with, or after subsequent births. The female dog may eat the placenta; it has been suggested that placental hormones may help to promote uterine involution and milk production. It is probably unwise to let a female dog eat every placenta if the litter is large. The end of whelping is usually signaled by a female dog relaxing and nursing its litter contentedly.

7.2 Management of oestrus

7.2.1 Oestrus prevention

The overproduction of pups necessitates the destruction of large numbers of unwanted dogs. Thus, control of oestrus in female dogs is of socioeconomic importance. Oestrus prevention can be permanent or temporary and based on surgery (ovariectomy or ovariohysterectomy) or medical treatment.

Surgical prevention

Surgical removal of the ovaries and/or uterus (ovariectomy or ovariohysterectomy; spaying) is usually very effective and safe and offers many benefits. It is effective after a single procedure and considerably lowers the risk of malignant mammary neoplasia if performed before approximately 2.5 years after the first oestrus. It also prevents the development of CEH-endometritis or pyometra and progesterone-induced growth hormone excess. There is a trend toward early spaying in a number of countries (reviewed by Root Kustritz and Olson, 2000). However, surgery may not be a suitable option for all female dogs. It is irreversible and obviously not suitable for female dogs that may be intended for future breeding. There is a risk of complications during anesthesia and surgery, and some owners are unhappy about letting their pet undergo major surgery (Burrow et al.,

2005). Side effects, such as urinary incontinence (see Section 7.5.3), infantile vulva, hair loss, and changes in coat color and texture, are possible (De Gier et al., 2008).

Medical prevention

A number of different agents are used for the medical prevention of oestrus in female dogs. These include progestins (synthetic progestagens) or nonsteroidal approaches (eg, gonadotropin-releasing hormone (GnRH) agonists) (reviewed by Gobello, 2006, and Verstegen, 2000). Androgens were used in the past for short-term prevention of oestrus, but products of this type are no longer widely available. GnRH antagonists have been used in research but are not in routine clinical use.

The agent selected and the effect produced (suppression or postponement of oestrus) depend on the stage of the oestrous cycle. The effects are usually most reliable if treatment is commenced during anoestrus. For this reason, some products are only approved for use in anoestrus (eg, some of the progestins), and this should be strictly adhered to. There are three possible approaches:

- Suppression of heat and prevention of conception can be achieved by treatment at the onset of pro-oestrus
- Temporary postponement of heat by treatment in anoestrus
- Permanent postponement of heat by repeated treatment, with the first treatment in anoestrus

a. Progestins

Progestins are commonly used in veterinary practice for oestrus prevention. Their chemical structure is based on modification of progesterone. Progestins can have progestational, oestrogenic, antioestrogenic, and androgenic or antiandrogenic effects; all of them have contraceptive effects. Different progestins may have different affinity and potency for different receptors, and thus the effects of different compounds can vary.

Five progestins, all of them derivatives of 17α-hydroxprogesterone, are used in dogs:

- Delmadinone acetate
- Medroxyprogesterone acetate (MPA)
- Megestrol acetate (MA)
- Osaterone acetate
- Proligestone

Delmadinone acetate (see Section 7.6.1) and osaterone acetate (see Section 7.6.3) are used in male dogs only. MPA, MA, and proligestone are used in female dogs and less commonly in male dogs.

The main pharmacologic effects (including side effects) of the progestins are summarised below.

i. Antigonadotropic

Progestins are more potent than progesterone at reducing GnRH secretion. Progestins affect the hypothalamic-pituitary-ovarian axis, but the exact mechanism of action in dogs remains unclear. Repeated progestin administration is followed by an increase in basal plasma FSH and sometimes also LH concentrations, but there appear to be no changes in basal prolactin concentrations (Beijerink et al., 2007; Beijerink et al., 2008). Pulsatile secretion of FSH and LH continue following progestin administration, although there may be variation from the usual pulsatile secretion, particularly of FSH. This leads to the prevention of oestrus and ovulation, as evidenced by the absence of external signs of oestrus.

ii. Progestational

Progestins mimic the actions of progesterone – the hormone of pregnancy. While abnormal progesterone profiles during pregnancy appear to be uncommon in female dogs, progestins appear to be efficacious for the management of suspected luteal deficiency (Günzel-Apel et al., 2012). Parturition may be delayed, leading to foetal mortality, in female dogs under the influence of progestins during gestation (van Os, 1982).

Progesterone promotes the development of a secretory endometrium that favors implantation. Progestins produce a secretory endometrium. Mucoid vaginal discharge and uterine lesions have been reported in dogs following long-term oral administration of MA (Weikel et al., 1975) and MPA (Von Berky and Townsend, 1993). In clinical field trials with proligestone, the incidence of uterine disorders, including pyometra, was 0.3% but was higher in dogs that had previously been administered MPA by injection (1.4%) (Van Os, 1982). CEH, CEH-endometritis, and pyometra can develop following progestin treatment (see Section 7.5.2). Progestin treatment is contraindicated in female dogs where endometrial pathology has been diagnosed.

iii. Antioestrogenic

Progesterone induces the enzymes 17-hydroxysteroid dehydrogenase, which converts oestradiol into oestrone, and oestrogen sulfotransferase. Progesterone stops oestrogen-elicited mitosis in the endometrium and has an antioestrogenic action in the myometrium, which results in a decrease in uterine contractility and an increase in the viscosity of cervical mucus. These effects interfere with sperm transport, decreasing the likelihood of fertilisation after breeding.

iv. Androgenic and antiandrogenic

Progesterone has little androgenic or antiandrogenic activity. In dogs, some progestins are used for their peripheral antiandrogenic activity (see Sections 7.6.1 and 7.6.3).

v. Somatotropic

Progestins increase growth hormone (somatotropin)-induced production of insulin-like growth factor (IGF) 1 from focal hyperplastic areas of the mammary glands in dogs, particularly following repeated or chronic administration (Bhatti et al., 2007; Mol et al., 1996). Progestins can increase the basal secretion of growth hormone, and this secretion appears to no longer be pulsatile or under the control of usual feedback

mechanisms. Increases in growth hormone production have been exploited for the treatment of pituitary dwarfism in dogs (Knottenbelt and Herrtage, 2002). However, this may also be of importance in the development of mammary hyperplasia and neoplasia in this species (Gräf and El Etreby, 1979). In clinical field trials, the incidence of mammary nodules was lower in proligestone-treated and untreated female dogs than in MPA-treated dogs (Van Os et al., 1981). Female dogs with any neoplastic or hyperplastic changes in the mammary glands should be spayed rather than treated with progestins. Growth hormone also causes insulin resistance (diabetogenic).

vi. Glucocorticoid

It is possible that progestins alter the hypothalamic-pituitary-ovarian axis through their glucocorticoid effects. Progestins act at glucocorticoid receptors and suppress the hypothalamic-pituitary-adrenocortical axis leading to insulin resistance (Selman et al., 1996). Progestins are diabetogenic and can lead to pathologic changes in the canine pancreas (Selman et al., 1997). This can be associated with elevated blood glucose concentrations and glycosuria (glucosuria) and even with clinical signs (eg, polyuria, polydipsia, weight loss despite increased appetite) of diabetes mellitus (see Section 7.5.5). Basal cortisol concentrations and basal and stimulated plasma adrenocorticotropic hormone (ACTH) concentrations appear to remain unchanged during repeated treatment (Beijerink et al., 2007). Behavioural changes, including a transient increase in appetite and associated weight gain, can be seen following progestin administration. Injectable progestins can be associated with local skin reactions, such as hair loss, hair discoloration, and possibly atrophy of the skin and surrounding tissues, often referred to as "pitting," related to the type of formulation (suspension) and glucocorticoid activity. It may be possible to minimise these effects by making sure that the injection is strictly subcutaneous (Evans and Sutton, 1989; Van Os, 1982). Diabetic female dogs should not be treated with progestins.

Progestins should be administered with care. A number of factors can affect the efficacy of progestin treatment. These include individual and/or breed variability, environmental factors, and phase of the oestrous

cycle. Usually, progestins should not be administered at or before the first oestrus.

vii. Individual and/or breed variability

There is considerable variation in the duration of the blocking effect of progestins on reproductive activity in female dogs. This is presumably related, at least in part, to variations in the absorption and metabolism of these agents. After the initial dosing regimen, oestrus can be prevented in the majority of female dogs by a single injection, eg, of MPA or proligestone, every 5-6 months. The progestin should be administered at the manufacturer's recommended dose rate. In some individuals the duration of action of long-acting formulations of progestins is less than 5-6 months. If necessary, the time interval between consecutive treatments can be reduced (eg, to every 4 months). Increasing the interval between treatments is not advised.

viii. Environmental factors

Environmental and/or seasonal influences do not usually affect the efficacy of progestins in female dogs. However, treated female dogs housed together with untreated female dogs may come into oestrus sooner than expected.

ix. Stage of oestrous cycle

Anoestrus is the most appropriate time to start progestin treatment in female dogs. The efficacy and duration of efficacy may be decreased if an injectable progestin is administered during pro-oestrus. Suppression of oestrus during pro-oestrus is better achieved using oral progestins, which have a shorter duration of action.

x. Return to oestrus after treatment

The time between the last treatment and the next oestrus varies markedly between female dogs. The interval to the subsequent oestrus after progestin treatment also depends on which agent, formulation, and treatment regimen have been used. Return to oestrus is usually within 3 (oral progestins) to 6 months (injectable progestins) but can be prolonged (eg, up to 3 years) or not occur at all (3%). In female dogs administered proligestone once during anoestrus, the next oestrus is on average 6

months later, but this interval is less than 3 months in about 3% and more than 12 months in about 4% of treated dogs. In female dogs administered proligestone repeatedly starting in anoestrus (permanent postponement), approximately 4% of cases return to oestrus less than 5 months after the last treatment, but in 7% of cases this interval is more than 12 months.

Owners who may wish to breed their female dog need to be informed that the time between stopping treatment and resuming cyclical oestrous activity varies widely and can be long. Oestrus induction should not be attempted in these animals and may increase the risk of CEH, CEH endometritis, or pyometra. While fertility at the first oestrus following treatment may not be affected, female dogs should ideally not be bred before the second oestrus after stopping treatment.

- Medroxyprogesterone acetate

- MPA is formulated for oral administration or as a suspension for subcutaneous injection. The dosage regimen may vary, depending on the product used.
- Suppression of oestrus: Daily oral administration (10 mg for dogs weighing 15 kg or less, 20 mg for dogs weighing more than 15 kg) for 12 days starting, based on vaginal exfoliate cytology, at the onset of pro-oestrus.
- Temporary postponement of oestrus: A single injection (50 mg) is administered during anoestrus (eg, 3 to 5 months after oestrus).
- Permanent postponement of oestrus: Treatment (50 mg) should be repeated at intervals of 6 months.

- Megestrol acetate

- MA is formulated for oral administration or as a suspension for subcutaneous injection. The dosage regimen may vary, depending on the product used.
- Suppression of oestrus: Daily oral administration at 2 mg/kg for 8 days starting, based on vaginal exfoliate cytology, at the onset of pro-oestrus. Signs of pro-oestrus usually disappear in 2-3 days. The next oestrus usually occurs 4-6 weeks earlier than expected. Treatment will be ineffective if it is not started early enough in pro-oestrus or if too low a dosage is administered. If treatment is started in metoestrus or anoestrus, cyclicity returns shortly after treatment is discontinued.

- Suppression of oestrus – prolonged treatment regimen: Daily oral administration starting at the onset of pro-oestrus has also been described, eg, for animals housed with other cycling female dogs with a history of false pregnancy. There are at least two different regimens described, depending on the product used: 1) at 2 mg/kg for 4 days followed by 0.5 mg/kg for 16 days or 2) at 0.1 mg/kg for 7 days followed by 0.05 mg/kg for 14 days.
- Temporary postponement of oestrus: A single injection (2 mg/kg) can be administered during anoestrus (eg, 3-5 months after oestrus). As an alternative, daily oral administration (0.5 mg/kg) can be started at least 7-14 days before oestrus is expected and continued for up to 40 days. The oral treatment regimen should not be used more than twice in any 12-month period.
- Permanent postponement of oestrus: Treatment (2 mg/kg by subcutaneous injection) should be repeated at intervals of 6 months.

- Proligestone

- Proligestone is formulated as a suspension for subcutaneous injection.
- Suppression of oestrus: A single injection (10-33 mg/kg, depending on the body weight of the dog) should be administered at the onset of pro-oestrus, based on exfoliate vaginal cytology. Female dogs should be kept separate from male dogs until there are no longer any external signs of oestrus (eg, vulvar swelling, bleeding). This may take 5-7 days.
- Temporary postponement of estrus: A single injection should be administered in anoestrus.
- Permanent postponement of oestrus: Repeat injections can be administered starting in anoestrus and then at intervals of 3, 4, and subsequently 5 months.

7.2.2 Oestrus induction

Oestrus induction is used in conjunction with routine breeding management (eg, when breeding opportunities are missed or following conception failure) or as a treatment for primary anoestrus (no signs of oestrus by 24-30 months of age) or secondary anoestrus (no signs of oestrus for more than 13 months after a preceding oestrus). Whatever approach is adopted, proper timing of treatment is crucial for success, particularly if this is regarded not only as oestrus induction but also as leading to ovulation and subsequent pregnancy.

The results of oestrus induction in metoestrus and early anoestrus are usually poor. It is also not unusual for female dogs, in which oestrus induction is commenced in early anoestrus, to have an anovulatory oestrus or corpus luteum insufficiency, resulting in very low pregnancy rates (Chaffaux et al., 1984; Jeukenne and Verstegen, 1997; Verstegen et al., 1999). Generally, the later the induction of oestrus is performed in anoestrus, the better the results, with the optimal time of treatment being 3-4 weeks before the date of the next expected oestrus. More than 40 treatment regimens for inducing oestrus in dogs have been described (reviewed by Kutzler, 2005). Some of these that may be suitable for use in veterinary practice are summarised below.

a. Gonadotropins

The termination of anoestrus in female dogs is associated with increased serum concentration or pulse frequency of LH (Concannon, 1993). Pregnant mare serum gonadotropin (PMSG, also known as equine chorionic gonadotropin, eCG) has potent effects, and signs of pro-oestrus usually occur within 6-8 days of starting daily treatment (Chaffaux et al., 1984). However, the response to treatment varies, and the duration of induced oestrus is often shorter than that of spontaneous oestrus. Since PMSG alone does not appear to be sufficient to restore ovarian activity, this is often followed with human chorionic gonadotropin (hCG). Oestrus can be induced in anoestrus female dogs using PMSG (20 IU/kg) administered daily for 5 days, with a single injection of hCG (500 IU or 25 IU/kg) on day 5 (Arnold et al., 1989; Nak et al., 2012; Weilenmann et al., 1993). A higher dose of PMSG (50 IU/kg), followed 7 days later by hCG (500 IU), also appeared to be effective (Stornelli et al., 2012). Mating at the induced oestrus can lead to pregnancy in up to 80% of female dogs, but rates of 30%-50% or lower have also been reported.

b. Gonadotropin-releasing hormone

Potent, synthetic gonadotropin-releasing hormone agonists can be used to induce oestrus in female dogs when treatment is initiated during anoestrus. However, this is impractical outside a research setting, since replicating pulsatile secretion requires repeated intravenous administration of GnRH for more than 7 days (Cain et al., 1989; Concannon et al., 1997; Concannon et al., 2006; Vanderlip et al., 1987). Sustained-release formulations of

GnRH agonists can be used, but the implant formulations require both administration and removal, which may also prove impractical (Inaba et al., 1998; Trigg et al., 2006, reviewed by Gobello, 2007).

c. Dopamine agonists

Prolactin appears to play a role in determining the interoestrus interval in dogs (reviewed by Kutzler, 2005). Dopamine D2 receptor agonists decrease plasma concentrations of prolactin and shorten the duration of anoestrus but also appear to have other effects, such as increasing the secretion of FSH (Beijerink et al., 2004). The administration of a dopamine agonist, at a dose sufficient to decrease the plasma concentrations of prolactin, from days 90 to 135 of the oestrous cycle can lead to pro-oestrus and fertile oestrus. This translates into a dose rate of 0.005 mg/kg cabergoline orally daily for up to 42 days or until day 2 after the onset of pro-oestrus (Nak et al., 2012). Pro-oestrus takes place from a few days to weeks (eg, 22-29 days) after treatment, depending on how late in anoestrus the treatment is started. While both bromocriptine (Okkens et al., 1985; Zoldag et al., 2001) and cabergoline (Jeukenne and Verstegen, 1997; Nak et al., 2012; Verstegen et al., 1994; Verstegen et al., 1999) have been used successfully, cabergoline produces less marked side effects (see Dopamine agonists in Section 7.3.1 b), making it a more suitable choice for oestrus induction in dogs. Mating at induced oestrus can lead to pregnancy in a large percentage of cases (Nak et al., 2012).

7.2.3 Prolonged or persistent oestrus

If ovulation has not occurred within 25 days of oestrus and external signs of oestrus continue, the female dog is considered to have prolonged or persistent oestrus. The most usual cause of this is ovarian follicles that have failed to ovulate. Young female dogs frequently have prolonged oestrus during their first or second cycles. Individual variability in the duration of oestrus should always be considered. Medical treatment of prolonged or persistent oestrus reportedly has a low success rate in female dogs. Spaying (see Surgical prevention under Section 7.2.1) should be considered.

7.3 Management of mating

7.3.1 Mismating (misalliance) and unwanted pregnancy

It is important that a full history is taken for cases in which mismating (also known as misalliance or mésalliance) is suspected. Where mating has not been observed, the presence of spermatozoa or sperm heads in a vaginal smear is evidence that mating did occur. However, the absence of spermatozoa or sperm heads is inconclusive.

Female dogs not intended for breeding

Spaying is the treatment of choice in bitches that have been mated but are not intended for breeding (see Surgical prevention under Section 7.2.1) and should be advised, especially when the management indicates that there is a real risk that the female dog may escape and be mated again. Surgery can be carried out 3-4 weeks postmating. This time schedule offers the additional possibility for pregnancy diagnosis prior to surgery. Medical termination of unwanted pregnancy provides another option.

Female dogs intended for breeding

A number of different agents can be used to prevent implantation or to terminate gestation in female dogs (reviewed by Verstegen, 2000). Large doses of oestrogen were used to prolong oviductal transport, tighten the uterotubular junction, and prevent implantation (reviewed by Feldman and Nelson, 2004). However, this approach is no longer used widely due to the side effects associated with high doses of oestradiol benzoate (eg, a single dose of 0.3 mg/kg, up to a maximum of 10 mg per female dog) (see Section 7.5.3) and the precise timing required when lower doses (0.02 mg/kg at 3, 5, and sometimes 7 days postmating) were used.

Many of the agents used currently terminate gestation after implantation, leading to abortion. Female dogs treated during the second half of pregnancy should be hospitalised due to the variable time to expulsion of whole foetuses after treatment and potential side effects. Abortion may only be partial in around 5% of treated dogs; therefore, a thorough clinical, and

ideally an ultrasound, examination is recommended at least 30 days after mating and 10 days after treatment to make sure that the uterine contents have been evacuated completely. The dog owner should always be informed about the expected efficacy and possible side effects of treatment.

a. Progesterone receptor antagonists

Progesterone receptor antagonists, or antiprogestins (eg, aglepristone), are synthetic steroids that bind with strong affinity to progesterone receptors, thus preventing progesterone from exerting its biological effects (reviewed by Hoffmann and Schuler, 2000). Aglepristone has about three times higher affinity for progesterone receptors than progesterone. It is also a glucocorticoid receptor antagonist and should not be administered to dogs with or suspected of having hypoadrenocorticism (Addison's disease) (reviewed by Mitchell and Pearce, 2002).

Aglepristone (two doses of 10 mg/kg (0.33 mL/kg) at a 24-hour interval) can be used to terminate pregnancy in dogs (Galac et al., 2000) from the time of mating until day 45 of gestation. Following administration of aglepristone, plasma concentrations of prolactin and the PG metabolite (PGFM) increase, but there are no changes in progesterone, PG, oxytocin, or cortisol within 24 hours (Baan et al., 2005 and 2008). Parturition is associated with incomplete luteolysis (ie, progesterone concentrations are still high), and plasma cortisol concentrations are elevated after parturition. Abortion (or resorption) of foetuses usually occurs within 7 days of the treatment course and can be accompanied by signs of parturition, such as vaginal discharge, expulsion of foetuses, restlessness, and mammary gland congestion, if administered after day 20 of gestation.

Pain on injection and injection site reactions are not uncommon. It has been suggested that reactions can be minimised by injecting into the scruff area of the neck and massaging the site after injection. Severe injection site reactions (eg, edema, thickening of the skin, ulceration, and local lymph node enlargement) may take up to 28 days to resolve. Side effects such as anorexia, excitation, depression, vomiting, and diarrhea have been reported. Administration of aglepristone has been reported to be followed by uterine infection in around 3.4% of cases.

b. Dopamine agonists

Prolactin secretion provides essential luteotropic support and is required to maintain pregnancy in dogs. If prolactin is inhibited, then progesterone concentrations fall. The ergot alkaloids, bromocriptine and cabergoline, are dopamine D2 receptor agonists. A related compound, metergoline, is a serotonin antagonist that has dopaminergic effects at high doses. All three agents are effective abortifacient agents when used after midgestation (reviewed by Feldman and Nelson, 2004, and Gobello, 2006).

- Bromocriptine mesylate can be given by injection at a dose of 0.1 mg/kg once daily for 6 days from day 42 (Concannon et al., 1987). Other dosage regimens have employed lower doses (0.015-0.030 mg/kg orally twice daily for 4 days) in combination with a prostaglandin. Progesterone concentrations fall and abortion occurs 2.5-4 days after commencing the treatment course (Concannon, 1987). Side effects, particularly at higher doses, including vomiting, listlessness, inappetence, and increased drinking, are common and occur 1-3 hours after injection.
- Cabergoline can be given orally at a dose of 0.005 mg/kg once daily for 5 days from day 42 (Post et al., 1988). Plasma concentrations of prolactin fall sharply, but progesterone concentrations fall less sharply and reach basal levels after 3-5 days. Abortion occurs 3-5 days after commencing the course of treatment. Side effects are uncommon, with vomiting reported occasionally during the first 2 days of treatment.
- Metergoline (0.6 mg/kg orally twice daily) causes complete luteolysis and a rapid decline in progesterone concentrations followed by abortion when administered in the last 3 weeks of gestation (Nöthling et al., 2003). Side effects are uncommon.

c. Prostaglandins

PGs work via the induction of luteolysis, stimulation of uterine contractions, and dilation of the cervix. PGs have significant limitations as abortifacients in dogs (reviewed by Feldman and Nelson, 2004, and Verstegen, 2000). In early metoestrus, high doses of PG are required to induce luteolysis and terminate pregnancy. Such high doses produce marked side effects, including vomiting, salivation, diarrhea, and respiratory distress, which usually last for around

20-30 minutes. Low doses of PG analogs (0.03 mg/kg twice daily) have been reported to be efficient in terminating pregnancy from day 35 onwards (Concannon and Hansel, 1977; Wichtel et al., 1990). However, the use of PGs alone is not recommended due to variability in the response to treatment.

d. Dopamine agonists and prostaglandins

A combination of a dopamine agonist and a PG can be used to interrupt pregnancy successfully from day 25 after the LH peak (Gobello et al., 2002; Onclin and Verstegen, 1990 and 1996). Using the agents in combination reduces the risk of side effects associated with the PG. Low doses of either cabergoline or bromocriptine combined with cloprostenol have been shown to be relatively safe and effective (Onclin and Verstegen, 1990 and 1996) and result in foetal resorption if treatment is started on day 25. Bromocriptine mesylate (oral, 0.015-0.030 mg/kg, twice daily), combined with either dinoprost tromethamine (subcutaneous injection, 0.1-0.2 mg/kg, once daily) or cloprostenol (subcutaneous injection, 0.001 mg/kg, every second day) until pregnancy termination, has been reported to be effective and to produce minimal side effects (Gobello et al., 2002).

e. Glucocorticoids

Glucocorticoids are not consistently effective in terminating pregnancy in female dogs (Wanke et al., 1997).

7.3.2 Infertility in female dogs

Infertility, the failure to conceive and produce viable offspring, is most commonly associated with inappropriate breeding management (reviewed by Grundy et al., 2002). Many of the female dogs that are presented for reproductive evaluation are in fact healthy. There are a number of reasons why a female dog may fail to cycle. These include previous spaying (ovariectomy or ovariohysterectomy) and silent or missed oestrus. Before any treatment for infertility is undertaken, a full history and physical examination should be carried out and, where necessary, laboratory evaluations undertaken. The treatment of infertility in female dogs is most commonly centered on appropriate breeding management.

a. Previous spaying (ovariectomy or ovariohysterectomy)

The presence or absence of scar tissue suggestive of abdominal surgery is an unreliable method of ascertaining whether a female dog has been spayed. Vaginal exfoliate cytology is also unhelpful except during pro-oestrus and oestrus. Measurement of oestrogen and progesterone concentrations can also yield equivocal results. Basal plasma concentrations of both FSH and LH are significantly higher after spaying in female dogs but can vary depending on when the sample is taken in relation to spaying (De Gier et al., 2012). A single measurement of the plasma FSH concentration has been shown to be the most reliable (De Gier et al., 2012). A single measurement of serum anti-Müllerian hormone (AMH, also known as Müllerian inhibitory substance) has also been shown to be able to distinguish intact female dogs from those that have been spayed (Place et al., 2011). Plasma oestradiol concentration 2 hours after intravenous administration of the GnRH analog gonadorelin (0.01 mg/kg) can also be used to discriminate between female dogs with and without functional ovarian tissue (De Gier et al., 2012).

b. Delayed puberty

Puberty is usually reached by 6-7 months of age (range 4-22 months); however, there is wide individual and breed variability. Small breeds tend to have a first oestrus between 6 and 10 months of age, but larger breeds may not begin to cycle until 18-20 months old, and, eg, in Greyhounds, this may be as late as 20-24 months of age. Absence of oestrous cycles in female dogs that are 24 months of age may be indicative of hypothalamic-pituitary-ovarian axis malfunction and warrants further detailed evaluation.

c. Prolonged or primary anoestrus

A female dog that has not experienced its first oestrus by 23 months of age is generally considered to have primary anoestrus. Primary anoestrus can be associated with a number of conditions, including hermaphroditism or pseudohermaphroditism, hypothyroidism, or infantilism. Before oestrus induction is attempted, a detailed history should be collected and a thorough physical examination carried out.

Confirming a diagnosis of hypothyroidism can be challenging. Primary hypothyroidism can be confirmed by measuring both total thyroxine (T4) (or free T4) and thyroid-stimulating hormone (TSH) concentrations (reviewed by Scott-Moncrieff, 2012). In a hypothyroid dog, total T4 (or free T4) should be below the reference interval and TSH elevated. Many other factors can decrease total T4, and this alone should not be relied upon to diagnose hypothyroidism in dogs.

If any specific causes of prolonged or primary anoestrus are diagnosed, targeted therapeutic measures can be adopted. Hypothyroidism is treated using oral replacement therapy with levothyroxine. The heritability of any underlying condition should be taken into account prior to inducing oestrus and breeding. If no specific primary cause is found, oestrus induction can be attempted (see Section 7.2.2).

d. Short or prolonged interoestrus intervals and split oestrus

An interoestrus interval of more than 12 months is considered to be a prolonged interoestrus interval in most mature, intact female dogs. Reasons for prolonged anoestrus include hypothyroidism, hyperadrenocorticism (Cushing's syndrome), administration of a progestin or long-term administration of a corticosteroid (glucocorticoid), malnutrition, or starvation. Failure to recognise signs of oestrus and poor oestrus manifestation should also be considered.

When the signs of oestrus are interrupted shortly before ovulation and recommence between 1 and 10 weeks later, this is referred to as "split oestrus" (reviewed by Grundy et al., 2002). The second oestrus is usually associated with ovulation. Split oestrus is common in female dogs experiencing oestrus for the first or second time and is less common in dogs over 2 years of age. Treatment is usually unnecessary, and the timing of insemination can be determined using measurement of progesterone.

7.3.3 Failure to conceive and early resorption

One of the most common causes of failure to conceive is inappropriate breeding management. Differential diagnoses for failure to conceive include inappropriate breeding management (including male-related problems), uterine infection, uterine pathology, and systemic illness.

7.4 Management of parturition in female dogs

7.4.1 Delayed parturition (uterine inertia)

Dystocia means difficult parturition. It can be due to foetal or maternal factors, such as a large or awkwardly positioned foetus or the failure of normal cervical dilation and/or uterine contractions. The latter is probably the most common cause of dystocia in female dogs. It is not entirely clear why this occurs, but a combination of mechanical, physical, genetic, and hormonal factors are likely involved. Two types of uterine inertia are recognised – primary and secondary.

a. Primary inertia

If there is total uterine inertia, the female dog fails to show any signs of impending parturition or fails to progress from the first to the second stage of parturition. Injections of oxytocin have very little or no effect in such cases. Caesarean section is indicated if live pups are to be produced. The production of copious amounts of dark green/black fluid by female dogs not showing any signs of first stage of parturition also indicates the need for Caesarean section.

If there is partial primary uterine inertia, it is important to be sure that there is no obstruction present. If there is no obstruction, medical management is usually successful. Oxytocin, by intramuscular or intravenous injection, is best given in small (1-12 IU intravenously or 2.5-10 IU intramuscularly), repeated doses 30 minutes apart (reviewed by Linde-Forsberg and Eneroth, 2000). If the response is insufficient, each oxytocin injection can be preceded by slow intravenous infusion (1 mL/min) of 2-20 mL calcium gluconate.

b. Secondary inertia

This is most frequently due to exhaustion of the uterine musculature and follows protracted straining in cases of obstructive dystocia or if the litter is large. Unless large numbers of foetuses remain, an injection of oxytocin will often successfully restart uterine contractions. If this is not effective or if large numbers of foetuses remain, Caesarean section is indicated.

7.4.2 Induction of parturition

Inducing parturition in female dogs should only be instituted if the timing of the LH peak and ovulation has been calculated. The progesterone receptor antagonist aglepristone (see Female dogs intended for breeding under Section 7.3.1) can be used (Baan et al., 2005). A number of studies have looked at the combination of this agent with oxytocin, as this appeared to be more effective, in terms of shorter parturition, than use in combination with a PG (Fieni et al., 2001). The combination of aglepristone (15 mg/kg) administered on two occasions at a 24-hour interval from around day 60 of gestation, followed by incremental doses of oxytocin (0.15 IU/kg) every 1-2 hours until the last pup was delivered (Fieni and Gogny, 2009; Fontbonne et al., 2009). There were no significant differences in average expulsion time per live pup and the percentage of live pups at birth, 48 hours, 7 days, or 7 weeks after birth compared to parturition in untreated female dogs. When studied in different breeds and sizes of dog, the first pups were delivered around 25 hours (21 to 30 hours) after treatment was started, and parturition lasted around 10 hours (Fontbonne et al., 2009). However, the interval between pups (2 hours) was significantly longer than in beagles or untreated controls (around 1 hour).

7.4.3 Retained placentas

Expulsion of retained placentas can be stimulated using oxytocin at a dose of 1-5 IU oxytocin per dog, administered subcutaneously or intramuscularly 2-4 times daily for up to 3 days (reviewed by Linde-Forsberg and Eneroth, 2000).

7.5 Other conditions of or related to the urogenital tract in female dogs

7.5.1 Pseudopregnancy

Pseudopregnancy (false pregnancy or pseudocyesis) occurs in intact female dogs usually within 6-8 weeks after oestrus. It is thought to result from the rising prolactin concentrations that are stimulated by falling progesterone concentrations as metoestrus progresses. This is supported by the fact that prolonged lactation occurs if the ovaries are removed from female dogs that have signs of false pregnancy. Moreover, female dogs with false pregnancy have significantly lower progesterone and significantly higher prolactin concentrations than unaffected female dogs at a comparable stage of the oestrous cycle (between 50 and 95 days after the onset of pro-oestrus) (Tsutsui et al., 2007).

The signs of pseudopregnancy vary in intensity from abdominal distension with mammary hyperplasia and milk production to an almost complete replication of parturition (including nervousness, excitability, and panting) and nursing (including the production of variable amounts of milk). The female dog may also show mothering behaviour toward inanimate objects. The incidence of false pregnancy is difficult to assess since the signs may be very mild in some cases. However, it is generally considered that the majority of female dogs (50%-75%) will show some signs of this normal physiological condition. There appears to be no evidence that female dogs that show significant signs of false pregnancy are any more likely to suffer from CEH with or without endometritis or pyometra or from infertility.

The requirement for treatment of false pregnancy depends on the type and severity of the signs shown. The condition is usually mild, and most cases recover spontaneously within a few weeks. In more severe cases, medical treatment with a dopamine agonist or a progestin may be indicated. In female dogs that suffer from severe bouts of false pregnancy after each oestrus, spaying (see Medical prevention under Section 7.2.1 and Female dogs intended for breeding under Section 7.3.1) is the treatment of choice. To avoid persistent lactation, the surgery should not be carried out while signs of false pregnancy are present (Harvey et al., 1999) or being suppressed medically.

a. Dopamine agonists

Dopamine agonists effectively inhibit prolactin via direct action (bromocriptine, cabergoline) on D2-dopamine receptors of the lactotrophic cells of the anterior pituitary gland (see Female dogs intended for breeding under Section 7.3.1). Bromocriptine is more frequently associated with side effects than cabergoline (Harvey et al., 1997 and 1999).

b. Progestins

Progestins inhibit milk production via negative feedback on the anterior pituitary, which inhibits prolactin production. Progestins (eg, proligestone – see Medical prevention under Section 7.2.1) may also help reduce the behavioural signs of false pregnancy due to their effects on the hypothalamus. Following proligestone administration, behavioural signs have usually resolved within 6 days and lactation is reduced or resolved within 9 days (Van Os and Evans, 1980).

7.5.2 Cystic endometrial hyperplasia, endometritis, and pyometra

Cystic endometrial hyperplasia (CEH) is a common uterine disorder in female dogs (reviewed by Verstegen et al., 2008). It can develop spontaneously during the luteal phase of the oestrous cycle and is also seen following progestin treatment.

The pathogenesis of CEH is not completely understood. Sequential periods of oestrogen dominance, which enhance the stimulatory effects of progesterone on the uterus, followed by prolonged progesterone dominance, are thought to lead to the development of CEH. Initially there is endometrial glandular hyperplasia, followed by cystic transformation of the glands in the endometrium – cystic endometrial hyperplasia. If bacterial infection is present, then the condition is more correctly termed CEH-endometritis if the cervix is open (often called open pyometra) or pyometra if the cervix is closed. Endometritis and pyometra are systemic, potentially life-threatening diseases characterised by a fluid-filled uterus and toxemia accompanied by inappetence and vomiting, (initially reversible) glomerulonephritis leading to excessive thirst (polydipsia), peritonitis, shock, and death (reviewed by Feldman, 2000). In CEH-endometritis, the contents of the uterus are lost, at least in part, through the vagina via the open cervix.

In pyometra, there is no vaginal discharge (closed cervix) and the female dog is usually much more acutely ill.

CEH-endometritis and pyometra usually occur 4-6 weeks after oestrus but have been diagnosed in some female dogs as early as the end of oestrus and as late as 12-14 weeks after oestrus. These conditions occur primarily in female dogs aged more than 5 years that have not been used for breeding. However, they can occur in young dogs and have even been recorded after the first oestrus.

Surgical removal of the uterus and ovaries, following adequate rehydration (intravenous fluid therapy), is the treatment of choice (reviewed by Nelson and Feldman, 2004).

Medical treatment of CEH-endometritis or pyometra can be used in female dogs intended for breeding. Medical treatment relies on the combination of an antimicrobial agent(s) with progesterone receptor antagonists (aglepristone), prostaglandins, and/or dopamine agonists. Around three-quarters of female dogs with CEH-endometritis or pyometra may respond within the first 3 weeks of medical treatment (Ros et al., 2014; Trasch et al., 2003). All cases treated medically must be monitored carefully with regard to evacuation of uterine contents and renal function (glomerulonephritis), and surgical intervention may be necessary to avoid mortalities. In addition, up to one-half of female dogs treated medically appear likely to relapse, usually around 10-11 months after the end of treatment (Ros et al., 2014; Trasch et al., 2003).

a. Aglepristone

Aglepristone is a progesterone receptor antagonist (or antiprogestin) that binds with great affinity to uterine progesterone receptors, preventing progesterone from exerting its biological effects (see Female dogs intended for breeding under Section 7.3.1).

b. Prostaglandins

PGs increase myometrial contractions and are luteolytic, decreasing serum progesterone concentrations, but produce variable cervical relaxation in female dogs. The use of PGs in the treatment of pyometra is associated with a very high risk of uterine rupture, a life-threatening complication. PG

administration can also be associated with both circulatory and respiratory depression, serious complications that may easily lead to a fatal outcome and should therefore be administered with great care.

c. Aglepristone and prostaglandins

Repeated administration of aglepristone (10 mg/kg subcutaneously) – usually on 4 occasions – with or without low doses of a PG analog (eg, cloprostenol 0.001 mg/kg subcutaneously), combined with antimicrobial treatment (for around 3 weeks), appears to offer the most effective medical treatment with limited side effects (Fieni et al., 2014; Gobello et al., 2003; Ros et al., 2014).

d. Dopamine agonists and prostaglandins

Dopamine agonists reduce prolactin concentrations, leading to luteolysis and a fall in progesterone concentrations, and have been used in combination with PG and antimicrobial agents to treat pyometra. Cabergoline (0.005 mg/kg orally once daily) has been combined with subcutaneous injection of cloprostenol (0.001 mg/kg once daily or 0.005 mg/kg once every 3 days) (Corrada et al., 2006; England et al., 2007). Gastrointestinal side effects can occur due to the PG treatment. Recurrence during the next luteal phase or relapse has been reported in around one-third of cases.

7.5.3 Urinary incontinence

Urethral sphincter mechanism incompetence (USMI) is the most common cause of acquired urinary incontinence in spayed female dogs (reviewed by Noël et al., 2010). It ranges in incidence from 4% to 20%. More than 90% of cases of USMI are in spayed female dogs, and up to 20% of spayed female dogs develop USMI. There appears to be no difference in the incidence of USMI based on whether spaying was by ovariectomy or ovariohysterectomy (Arnold et al., 1989). The age of the dog at spaying does not seem to play a significant role.

USMI is a multifactorial condition associated with decreased urethral resistance. Urine leakage occurs when the intra-abdominal pressure rises, for example, during barking or when the dog is recumbent. Predisposing factors include urethral tone, bladder neck position, pelvic bladder (ie, more than 5% of the bladder length located inside the pelvis), urethral length, neutering, body size, breed, docked tail, and obesity.

Spaying is associated with structural changes in the bladder and urethra, which may alter the functional integrity of the lower urinary tract (reviewed by Noël et al., 2010). There is a decrease in smooth muscle in both the bladder and urethra, alterations in the total number of striated muscle fibers (type I fibers are involved in resting urethral tone), increase in the volume of type II striated muscle fibers, and an increase in the volume of the urethral vascular plexus, and there may also be changes in collagen content. Alterations in urethral function that are associated with spaying include a significant decrease in mean urethral closure pressure (MUCP), functional profile length (FPL), and integrated pressure (IP) (Nickel, 1998; Salomon et al., 2006).

High-affinity oestrogen receptors can be found in the proximal urethra. There are changes in urethral resistance during the oestrous cycle (Hamaide et al., 2005). High oestrogen concentrations are associated with an increase in FPL and low oestrogen concentrations with a decrease in MUCP and IP (Nickel, 1998). However, USMI is not caused by a simple deficiency in circulating oestrogen concentrations. It is also impossible to differentiate between spayed and anoestrus female dogs based on oestrogen concentrations.

Medical management is the first-line approach to treating USMI. Oestrogenic compounds and sympathomimetic amines are used most commonly to treat USMI in female dogs (reviewed by Noël et al., 2010). Other approaches (including submucosal injection of bulking agents into the proximal urethra, surgical colposuspension, or surgical implantation of an artificial urethral sphincter) are reserved for dogs that do not respond to medical treatment.

a. Oestrogenic compounds

Oestrogenic compounds used in dogs include synthetic compounds, such as the synthetic nonsteroidal stilbene diethylstilbestrol (DES) and esters of oestradiol (eg, benzoate, cypionate), and the natural oestrogen oestriol. Oestrogenic compounds exert their activity via oestrogen receptors. Oestrogens have been shown to have a trophic effect on the urethral epithelium, subepithelial vascular plexus, and connective tissue and to increase the number of α-adrenergic receptors in the proximal urethra and their responsiveness to sympathetic stimulation. They may also act on other receptors (β3-adrenergic, muscarinic).

The potency of an oestrogenic compound is determined by the duration of its interaction with the oestrogen receptor or receptor occupancy. DES is a potent oestrogenic compound due to its inherently long receptor occupancy, which gives it a relatively narrow safety margin. The natural oestrogen oestradiol is also a potent oestrogenic compound, and its duration of action can be prolonged by conversion to an ester. Oestriol is considered to be a weak or impeded oestrogen due to its short receptor occupancy.

b. Synthetic oestrogenic compounds

DES has been in use since the late 1930s but is no longer widely available due to concerns over human safety (eg, genotoxicity, carcinogenicity), based on studies conducted in rodents, dogs, and primates (reviewed by Marselos and Tomatis, 1993). Oral administration of DES (eg, 1 mg daily for 3-7 days followed by 1 mg weekly) has been used widely in dogs to treat USMI. However, there is little or no evidence to support the dosage regimen and few, if any, large-scale, controlled clinical studies on DES in dogs (reviewed by Page, 1991). In a small clinical study, a response to treatment was seen in around 88% of dogs, although it was only partial in 23% of cases (Nendick and Clark, 1987). Esters of oestradiol are reportedly used to treat USMI in female dogs, but there is a lack of large-scale, controlled studies with these agents for this indication. A complete blood count should be assessed prior to commencing treatment with any oestrogenic compound and monitored during treatment.

Myelotoxicity has been associated with administration of diethylstilbestrol, oestradiol benzoate, and oestradiol cypionate to dogs at or in excess of the recommended dose rates (reviewed by Sontas et al., 2009). The pathogenesis of this is not fully understood but appears to involve a myelopoiesis-inhibitory factor that is produced by canine thymic stromal cells. Clinical signs of myelotoxicity can vary in a normal dog through loss of appetite, depression, pale mucous membranes, petechial hemorrhages, unilateral or bilateral epistaxis, vulvar edema, and vaginal bleeding to collapse. Complete blood counts reveal nonregenerative anemia, thrombocytopenia, and/or leukocytosis.

c. Oestriol

Oestriol has been available commercially for use in dogs since 2000. It has been shown to increase the electrical activity of urethral smooth muscle

significantly within 1 week of starting treatment (Hamaide et al., 2006; Noël et al., 2013). It is administered orally once-daily, with the dose titrated to effect. It has been reported that around 80% of female dogs respond to once-daily oestriol treatment (Mandigers and Nell, 2001). Mild, transient short-term oestrogenic effects (eg, vulvar swelling, attractiveness of males) are seen in approximately 5%-9% of the dogs. Chronic administration of oestriol is rarely associated with long-term oestrogenic effects, such as myelotoxicity (Meadows et al., 2013). A complete blood count should be assessed prior to commencing treatment and monitored during treatment.

d. Sympathomimetic amines

Sympathomimetic amines include phenylpropanolamine (DL-norephedrine, PPA), ephedrine, and pseudoephedrine. PPA (1 mg/kg) is administered orally and needs to be administered at least twice daily or even 3 times daily. Its mechanism of action is unclear. It appears not to alter urethral smooth muscle electrical activity via α-adrenergic stimulation but to produce β-adrenergic mediated relaxation of the bladder (Hamaide et al., 2006; Noël et al., 2012; Noël et al., 2013). A decrease in the detrusor threshold pressure and increase in the volume of urine voided have been demonstrated after 2 weeks of treatment (Noël et al., 2013). It has been reported that around 85% of female dogs should respond to 3-times-daily PPA treatment (Scott et al., 2002). Side effects can include hypertension, restlessness, anxiety, and tachycardia. PPA should be administered with caution to dogs with cardiovascular disease.

Ephedrine is administered orally and needs to be administered at least twice daily in the dog. It is a nonspecific sympathomimetic agent (ie, it has effects at both α- and β-adrenergic receptors). It has less predictable efficacy than PPA, but a response to treatment can be seen in around 75% or more of female dogs. It produces similar side effects to PPA but has a narrower margin of safety. Pseudoephedrine is a stereoisomer of ephedrine with a similar efficacy and safety profile (Nendrick and Clark, 1987).

e. Oestriol and PPA

During long-term treatment for USMI, female dogs may become refractory to medical treatment, presumably due to lower availability of receptors in the urethra or bladder. While combined treatment with an oestrogenic compound and a sympathomimetic amine has been proposed, this has not

been investigated in large-scale, controlled studies. Moreover, in one study urethral resistance was lower in dogs administered oestriol and PPA than in dogs administered oestriol alone after 1 week of treatment (Hamaide et al., 2006). This approach requires further investigation but may offer an alternative to an increase in dosage in dogs where clinical signs recur during long-term treatment or in cases that appear to be refractory.

f. GnRH analogs

GnRH analogs have been assessed as a treatment option in small-scale studies but have not been shown to improve urodynamic parameters (Reichler et al., 2006). A relationship between gonadotropins (FSH and LH) and decreased MUCP has not been demonstrated.

7.5.4 Acromegaly (hypersomatotropism)

Acromegaly is characterised by bone and soft tissue overgrowth and insulin resistance due to excessive growth hormone (somatotropin) secretion in mature animals (reviewed by Rijnberk et al., 2003). In dogs this manifests as the development and gradually increasing prominence of skin folds, gradual increases in abdominal distension, and widening of the interdental spaces.

In dogs, growth hormone excess usually originates from the mammary gland (see Progestins in Section 7.2.1 a). This can be due to endogenous progesterone secreted during the luteal phase of the oestrous cycle or exogenous progestins. When associated with progestin administration, the growth hormone secretion is not pulsatile or responsive to normal feedback. Progestin-associated increases in growth hormone are associated with increased IGF-1 concentrations. Growth hormone is an insulin antagonist, and this leads to insulin resistance and can lead to hyperglycemia and eventually diabetes mellitus.

Spaying is the treatment of choice in female dogs with acromegaly. There is a rapid decline in progesterone concentrations after surgery. This is followed by a rapid return of growth hormone concentrations to normal. Treatment with aglepristone (10 mg/kg by subcutaneous injection) on 3 occasions 1 week apart has been shown to significantly decrease growth hormone and IGF-1 in beagle dogs with progestin-induced growth hormone excess (Bhatti

et al., 2006). Clinical signs may take quite some time to resolve in female dogs that develop acromegaly following progestin administration.

7.5.5 Diabetes mellitus

Diabetes mellitus is a common endocrine disorder in dogs caused by immune-mediated damage and/or destruction of pancreatic β-cells, with an incidence of approximately 0.3%-0.5% (reviewed by Davison, 2015, and Rijnberk et al., 2003). In intact female dogs, insulin resistance and even diabetes can occur during the luteal phase of the oestrous cycle, pregnancy, or following progestin administration (Strage et al., 2014).

Diabetes in dogs is usually due to an absolute deficiency of insulin, but, if there is insulin resistance, the insulin deficiency may be relative. Diabetes – persistent hyperglycemia and associated clinical signs – can be preceded by a period of insulin resistance due to counter-regulatory hormone excess (eg, cortisol, growth hormone). However, the situation is complex, and insulin resistance does not lead to hyperglycemia in all dogs. The development of diabetes may depend on pancreatic β-cell reserve, in relation to previous insult or other risk factors, such as pancreatitis. The risk may be increased in intact female dogs that are overweight or obese (based on body condition score) (Wejdmark et al., 2011).

Diabetes mellitus in dogs usually requires insulin treatment (reviewed by Behrend, 2006). Porcine insulin (starting dose 0.5 IU/kg) is a good choice for dogs because it is identical in amino acid composition to canine insulin and is therefore unlikely to lead to anti-insulin antibody formation, which can be a cause of insulin resistance. Most dogs (around 75%) require twice-daily insulin treatment (Monroe et al., 2005).

Intact female dogs with diabetes should ideally be spayed as soon as possible after diagnosis, even before initiating insulin therapy. Remission of diabetes in dogs (4-39 days after spaying) has been reported rarely (eg, around 5% of intact female diabetics) but can occur if the diabetes is related to progesterone (or progestin) dominance (Pöppl et al., 2013). If insulin therapy has been initiated, care should be taken to reduce the insulin dose

to avoid potentially life-threatening hypoglycemia associated with relative insulin overdose as progesterone concentrations fall after surgery. In dogs where spaying is not an option, for whatever reason, the progesterone receptor antagonist aglepristone (4 doses at 10 mg/kg subcutaneously on 2 consecutive days and then twice at an interval of 1 week) has been shown to reduce glycemia and insulin dose after about 2 weeks during the luteal phase of intact female diabetic dogs (Bigliardi et al., 2014).

7.6 Male dogs

In male dogs, secondary sexual characteristics and behaviour occur as a result of the interplay between hormones produced by the anterior pituitary (the gonadotropins), gonads, and hypothalamus. In response to gonadotropin releasing hormone (GnRH), secreted from the hypothalamus, two gonadotropic hormones, FSH and LH, are released from the anterior pituitary. FSH is responsible for spermatogenesis, while LH, also known as interstitial cell-stimulating hormone (ICSH), maintains androgen (testosterone and dihydrotestosterone) production. LH is released continually in an episodic fashion; concentrations vary throughout the day.

The main androgen, testosterone, acts on target organs to maintain the male secondary sexual characteristics and function, including libido, and helps to maintain spermatogenesis. This hormone also exerts negative feedback on the anterior pituitary and/or hypothalamus. Thus it can be seen that androgens not only control reproductive processes but are also associated with behaviour, eg, mounting, aggression, and territory marking.

7.6.1 Hypersexuality

There are two different mechanisms controlling sexual behaviour – parts of the cerebral cortex in the region of the hypothalamus and male sex hormones. These systems are linked because steroid hormones, including sex hormones, control sexual behaviour and hormone release by positive and negative feedback via the hypothalamus. However, it is important to note that there are large differences in the relative dependence of sexual behaviour upon the cerebral cortex and androgens, both between species and between individuals within a species.

Hypersexuality is essentially excessive or aberrant sexual behaviour, although it is sometimes also taken to encompass normal sexual behaviour that is misplaced within modern society. It is more common in male dogs aged less than 3 years old and manifests as:

- Aggression (fear-induced, dominant, or submissive)
- Mounting other dogs, people, inanimate objects
- Territory marking by urination
- Roaming
- Destructive behaviour
- Excitability

Many owners are not concerned about this type of behaviour in their dogs and do not seek treatment. This is probably because this type of behaviour is accepted as part and parcel of owning an intact male dog. In fact, some of these traits are normal in male dogs and it is merely a question of severity, frequency, and place that makes the behaviour unacceptable.

Hypersexuality in dogs can be treated by a combination of behavioural training and surgical or medical castration (Andersson and Linde-Forsberg, 2001).

a. Behavioural training

Behavioural training is often effective, but the efficacy varies depending on the behavioural signs exhibited. The hormonal status of the dog remains unaffected. Behavioural training requires considerable owner time and commitment. The success of treatment depends on the main presenting sign. Intermale aggression often has a poorer response than other manifestations of hypersexuality. Age also appears to significantly affect the response to treatment in aggressive dogs – the older a dog is, the harder it may be to manage.

b. Progestins

Progestins can be effective due to peripheral antiandrogenic activity. If treatment is effective, this may indicate that surgical castration would be effective. Delmadinone acetate (1-2 mg/kg, depending on the body weight of the dog) is a progestin with peripheral antiandrogenic activity that is used in male dogs. In most cases, it takes 2-4 days before signs

of improvement may be seen. Treatment can be repeated after 8 days if there has been no improvement. In male dogs that respond to treatment, follow-up treatment will usually be required after 3-4 weeks. Progestins used in female dogs, such as MA (eg, 2 mg/kg orally once daily for 7 days, then 1 mg/kg daily for 14 days), MPA, and proligestone (10-33 mg/kg, depending on the body weight of the dog), have also been used to control hypersexuality in male dogs. Male dogs intended for breeding should not be treated with progestins. Delmadinone acetate has been shown to reduce libido, ejaculatory volume, and total sperm output in dogs (Taha et al., 1981). Other progestins may not affect libido (England, 1997). Progestins can also produce side effects in male dogs, including decreases in semen quality due to changes in the head of the epididymis and secondary sperm abnormalities (increased detachment of the acrosome from the sperm head) despite little or no decrease in LH concentration (England, 1997; Paramo et al., 1993). Other side effects, such as lethargy, increased appetite, and insulin resistance, are similar to those described for female dogs – including mammary gland changes (see Medical prevention under Section 7.2.1).

c. GnRH agonists

Treatment with a long-acting formulation of a GnRH agonist leads initially to stimulation of then a decrease in testosterone and oestradiol concentrations, which reach basal levels within around 18 days, and cessation of normal spermatogenesis in most of the seminiferous tubules, leading to aspermia in 5-7 weeks (Ludwig et al., 2009; Paramo et al., 1993). Testicular size decreases by about 82% in 17 weeks. Changes in the head of the epididymis are similar to those seen following progestin treatment. Improvement in behaviour may be seen in around 75% of dogs (Goericke-Pesch et al., 2010). However, the initial stimulation associated with GnRH agonism may be problematic in some dogs, leading to a worsening of behaviour. Other reported side effects include weight gain, anxiety, and hair coat changes. An implant containing the GnRH agonist deslorelin is available commercially for use in male dogs.

d. Surgery

Surgical castration may be effective in some dogs. This removes the main source of androgens but has no effect on the cerebral cortex and will have no effect on the production of androgens by other organs, such as the adrenal glands.

7.6.2 Cryptorchidism

The testes of the dog are intra-abdominal at birth and normally descend into the scrotum during the first 7-10 days of life. At 2 weeks of age, the testes can often be palpated in either the scrotum or inguinal canal, although descent may be delayed in some individuals. About 6%-12% of dogs are cryptorchid (one or both testicles have not descended normally by puberty). Dogs more than 12 months of age with retained testicles should be considered as cryptorchid.

The exact cause of cryptorchidism is unknown, but it is likely that there is an underlying, inherited hormonal abnormality since the incidence is noticeably higher in some breeds (eg, Boxers). For this reason cryptorchid dogs should not be used for breeding.

Unilateral cryptorchid dogs are often fertile, since the descended testicle usually functions normally. Dogs that have bilaterally retained testicles are infertile but usually have normal libido and male secondary sexual characteristics. There is a significant risk that the retained testicle(s) will undergo neoplastic change and/or torsion of the spermatic cord.

A GnRH stimulation test can be carried out to confirm the presence of an abdominal testicle (Purswell and Wilcke, 1993). Blood samples are taken for testosterone measurement prior to and 60 minutes after the administration of a GnRH agonist intravenously (0.001 mg/kg) or intramuscularly (0.050 mg/kg).

Treatment is by surgical castration, and both testes should be removed. This should ideally be done before the dog is middle aged (4-6 years old) to avoid neoplasia.

7.6.3 Benign prostatic hyperplasia

Benign prostatic hyperplasia (BPH) is the most common prostatic disorder in dogs. It is seen in middle-aged and older, intact male dogs (over 6 years old) (reviewed by Smith, 2008, and Nizánski et al., 2014). BPH appears to be a progressive disorder where prostatic hyperplasia occurs in young, intact male dogs (under the age of 3 years old) and is followed by cystic hyperplasia

(from 4 to 5 years old). In most dogs (around 75%), BPH is subclinical. Clinical signs, if present, include abnormal gait (hunched back), straining (tenesmus), persistent or intermittent hematuria, or hemorrhagic, preputial discharge. The diagnosis of BPH can be confirmed by transrectal digital palpation of a nonpainful, symmetrically enlarged prostate. Radiography can be used to confirm symmetrical prostatic enlargement, and ultrasonography can confirm the presence of diffuse cystic change. Ultrasound-guided fine needle aspirate biopsy can be used to confirm the diagnosis. There is usually evidence of mild inflammation without sepsis or neoplastic change on cytology.

Surgical castration is the treatment of choice in male dogs not intended for breeding. Prostatic involution starts within 7-14 days of surgery but can take up to 4 months.

In male dogs that are not suitable candidates for surgery or that are intended for breeding, a progestin, GnRH agonist, or finasteride can be used to treat BPH.

a. Progestins

Osaterone acetate, a derivative of chlormadinone acetate, and its 15β-hydroxylated major metabolite have potent antiandrogenic activity, competitively inhibiting androgen binding at prostatic receptors as well as blocking the transport of testosterone into the prostate. Osaterone acetate (0.25 mg/kg orally once daily for 7 days) and delmadinone acetate (3 mg/kg by intramuscular injection) have been reported to have similar efficacy (eg, clinical signs, prostate volume) in the treatment of BPH in dogs (Albouy et al., 2008). However, the reduction in prostate volume in the 14 days following treatment was greater following osaterone acetate treatment. The most commonly reported side effects were a transient increase in appetite in around 20% of dogs and changes in behaviour in around 10% of dogs. Osaterone acetate treatment can produce a reduction in plasma cortisol concentrations that lasts a few weeks. It has been reported to have no adverse effects on semen quality in dogs.

b. GnRH agonists

The implant containing the GnRH agonist deslorelin could also be used in male dogs with prostatic hyperplasia but has not been studied with respect

to prostatic end points (reviewed by Nizánski et al., 2014; see Section 7.6.1). The initial stimulation associated with GnRH agonism may be problematic in some dogs, leading to a worsening of the signs of benign prostatic hyperplasia (Goericke-Pesch et al., 2010).

c. Finasteride

Finasteride (0.1-0.5 mg/kg orally once daily), which blocks the conversion of testosterone to dihydrotestosterone by 5α-reductase, has also been used in dogs (reviewed by Nizánski et al., 2014).

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8.1 Physiology

8.1.1 The oestrous cycle

Female cats, or queens, usually reach puberty by 6-9 months of age or 2.3- 2.5 kg body weight (reviewed by Verstegen, 2000). Sexual activity of free-ranging cats is photoperiod-dependent, thus the onset of puberty can be influenced by the time of year the queen is born (Goodrowe et al., 1989).

The queen is seasonally polyoestrous, with prolonged anoestrus resulting from decreasing or short day length (Johnston et al., 1996). The onset and duration of ovarian activity is also linked closely to day length. In the Northern Hemisphere, queens cycle between January and September, with peaks of sexual activity in February, May, and June, and occasionally in September.

In terms of behaviour, the oestrous cycle of the queen can be divided into heat and nonheat periods (reviewed by Verstegen, 2000). Heat periods are observed every 14-19 days (range 4-30 days) throughout the breeding season (Root et al., 1995). The oestrous cycle can be divided into pro-oestrus and oestrus and has an average duration of around 6 days (range 2-19 days). Pro-oestrus (1-4 days) is followed by oestrus (3-10 days). This is followed by interoestrus, a short period of sexual inactivity, when plasma oestrogen concentrations usually decline to basal values. In the absence of mating or spontaneous ovulation (Gudermuth et al., 1997; Lawler et al., 1993), this cycle of events is repeated until the end of the breeding season. The last interoestrus of the breeding season is followed by anoestrus, a longer nonbreeding period, which lasts until the first pro-oestrus of the next period of sexual activity. Anoestrus usually occurs when natural day length is short (September to late January in the Northern Hemisphere) and can be absent when the day length is constant (ie, indoors).

During oestrus the queen will, as in pro-oestrus, rub its neck against various objects and people's legs. Typically, queens crouch, hold their tails to one side, and show frequent rolling and treading when 'calling.' Vocalisation, often involving the production of a low moaning sound, occurs more frequently than in pro-oestrus. Such signs often go unrecognised in normally

affectionate cats, but may be interpreted by cat owners as a sign of illness or pain (reviewed by Christiansen, 1984; Feldman and Nelson, 2004; Verstegen, 2000). The duration of, and signs evident in, each phase of the oestrous cycle are summarised in Table 1.

Stage of cycle	Duration	Comments
Pro-oestrus	1-4 days	• Not receptive to the advances of tomcats • Can pass unnoticed, with affectionate behaviour being the only sign • Characterised by behavioural changes, such as rubbing the head and neck against objects, vocalisation, posturing, and rolling
Oestrus	3-10 days	• Will accept a male • Lasts up to 10 days if there is no tomcat present, otherwise 4 days (3-6 days) • Ovulation occurs 27 hours (range 24-30 hours) after mating • Similar to pro-oestrus but signs more pronounced • May urinate more frequently, be restless, and show an increased tendency to roam • May be aggressive rather than affectionate
Interoestrus	6-16 days	Period of sexual inactivity
Anoestrus	3-4 months	Prolonged period of sexual inactivity

Table 1 The phases of the oestrous cycle of the cat

8.1.2 Mating

During mating the tomcat bites the neck of the queen firmly and mounts, grasping the queen's chest with its forelegs. Usually at this stage, both cats tread actively and the queen adopts a posture that makes the vulva more accessible. The penis of the tomcat normally points posteriorly but is directed forwards as it becomes erect. Intromission is followed rapidly by ejaculation. This whole sequence of events can occur in as little as 30 seconds and rarely lasts for more than 5 minutes. As the tomcat withdraws, the queen typically gives a loud, piercing 'copulatory call' and the tomcat retires to a safe distance.

Mating is usually repeated 6-7 times at varying, but often quite frequent, intervals until the queen no longer allows the tomcat to mount. Mating may be repeated over 2-4 days (reviewed by Christiansen, 1984; Feldman and Nelson, 2004; Verstegen, 2000).

8.1.3 Pseudopregnancy and pregnancy

Pseudopregnancy, lasting about 36 days (range 25-45 days), can occur following any nonfertile mating or if ovulation is stimulated artificially. Pseudopregnancy in the cat is not usually associated with behavioural changes or lactation (reviewed by Christiansen, 1984). The subsequent oestrus is delayed by 45 days on average (range 35-70 days), ie, about half the normal duration of a feline pregnancy. The delay will be longer if anoestrus follows pseudopregnancy.

8.1.4 Pregnancy

Fertilisation in the queen is presumed to occur in the oviducts, and the blastocysts migrate into the uterus 4-5 days postmating. Implantation is thought to occur at around 15 days postmating. The duration of pregnancy is 63 days (range 61-69 days) under controlled conditions but can range from 56 to 72 days (reviewed by Feldman and Nelson, 2004; Verstegen, 2000). Variation in the coitus-to-parturition interval is probably best explained by the fact that coitus does not always produce an ovulatory surge and ovulation, rather than by breed differences.

8.1.5 Parturition

Parturition, or kittening, in queens can be split into three stages (reviewed by von Heimendahl and Cariou, 2009). The first stage, which usually lasts for about 24 hours, is characterised by restlessness, vocalisation, and nesting behaviour. Some normally affectionate queens may show signs of aggression as parturition approaches. Once the second stage commences, kittens are usually produced quite quickly with relatively little abdominal straining. The birth of the first kitten usually takes 30-60 minutes, and the interval between delivery of subsequent kittens varies from 5 to 60 minutes. The third stage, the expulsion of the placenta, usually occurs after each kitten is delivered.

Most queens will sever the umbilical cords, eat the placentas, and clean the kittens without requiring any assistance.

Parturition in cats (reviewed by Christiansen, 1984; Feldman and Nelson, 2004; Verstegen, 2000) differs from parturition in dogs (see Chapter 7, Section 7.1.4c) in a few key ways (Table 2).

• Delayed parturition may occur in a stressful environment.
• The second stage of parturition may be divided. The queen may produce one batch of kittens, rest for as long as 12-24 hours, and then produce a second batch of kittens.
• Kittening can be as short as 1 hour or as long as 1-2 days.
• Placenta is red-brown in color.

Table 2 Key differences in parturition in cats compared to dogs

The breed and condition of the queen and the number of litters produced affect litter size. Litter size increases up to the fourth parturition and then declines. The number of kittens born alive per litter is 4 (range 1-8) (reviewed by Christiansen, 1984; Root et al., 1995). Mortality by 8 weeks of age is around 30% (range 15%-45%). Pro-oestrus may follow soon after parturition or may be preceded by a period of anoestrus. On average, queens will call 4-8 weeks (range 1-21 weeks) after having a litter. The interval is dependent on the age at which kittens are weaned and, in cats with a nonbreeding season, upon the time of the year when kittens are born.

8.2 Hormonal changes

8.2.1 Gonadotropins

Follicle stimulating hormone (FSH) and luteinising hormone (LH) both play a role in cats.

Feline oocytes exhibit lower sensitivity to FSH in the nonbreeding season (Hobbs et al., 2012). However, the lack of oocyte development in the

nonbreeding season seems to be unrelated to FSH sensitivity since there are no differences in the expression of FSH receptors or FSH-induced genes. During the breeding season, there is higher sensitivity to FSH and oocytes develop.

Stimulation of the vagina at coitus is followed immediately by an increase in neural activity in the hypothalamus, which is followed by the release of LH. Multiple matings may be required to stimulate the release of gonadotropin-releasing hormone (GnRH), which is thought to cause the LH surge that initiates ovulation (Concannon et al., 1980). The LH response varies considerably between individuals and does not correlate with plasma concentrations of oestradiol or progesterone (Johnson and Gay, 1981). There is not a consistent relationship between the number of copulations and the LH response and/or ovulation (Wildt et al., 1981).

8.2.2 Oestrogens

Behavioural oestrus occurs during peak follicular growth. Pro-oestrus is associated with an abrupt rise in circulating oestrogen (oestradiol-17β) concentrations, heralding the onset of the follicular phase. During this phase, oestrogen concentrations rise rapidly from basal values of 15-20 pg/mL to more than 40-80 pg/mL, remain elevated for 3-4 days, and then decline over the next 2-3 days back to basal values.

8.2.3 Progesterone

Ovulation is followed by the formation of one corpus luteum or more. Progesterone concentrations start to rise 2-3 days after mating, peak around 30-60 ng/mL at 20-25 days after mating, decrease, and then remain stable around 15-30 ng/mL until just before parturition (around day 60), when they decrease to below 1-1.5 ng/mL (Figure 1) (Verstegen et al., 1993). The corpora lutea of pregnancy are functional throughout gestation (Goodrowe et al., 1989; Schmidt et al., 1983; Verhage et al., 1976). During anoestrus, plasma oestrogen and progesterone concentrations remain at basal values and gonadotropin concentrations undergo only minor fluctuations.

In the pseudopregnant cat, progesterone concentrations mimic those seen in pregnant animals but, after peaking 20-25 days after mating, return to basal values by around 30-40 days (Figure 1). The decline in progesterone in these animals is slow and progressive, probably due to the lack of a luteolytic factor (reviewed by Verstegen, 2000).

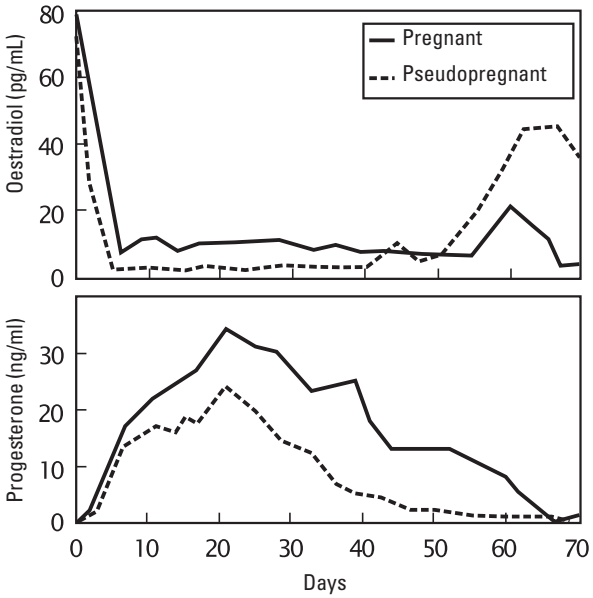

Figure 1 Average concentrations of progesterone and oestradiol in pregnant and pseudopregnant queens (after Verhage et al., 1976)

8.2.4 Relaxin

Relaxin is a pregnancy-specific hormone; it cannot be detected during the oestrous cycle or in pseudopregnancy (Stewart and Stabenfeldt, 1985). It is secreted mainly by the placenta. Relaxin concentrations rise from 25-30 days after mating and reach a plateau at 30-35 days that remains until 10-15 days before parturition, at which time concentrations decrease slowly and are undetectable 24 hours before parturition.

8.2.5 Prolactin

Prolactin concentrations are higher during darkness (31.7 ng/mL) than during daylight (5.5 ng/mL) (Banks and Stabenfeldt, 1983; Leyva et al., 1989a). However, this appears to be a circadian rhythm, and seasonal anoestrus is not associated with significant changes in prolactin secretion. There are no significant changes in prolactin concentrations during oestrus or pseudopregnancy.

Prolactin is a major luteotropin in the second half of pregnancy (from day 30) in cats. It also plays an essential part in preparing the mammary glands for initiating and maintaining lactation. Prolactin concentrations markedly increase in the last third of gestation (from week 6), peaking 3 days before parturition at 43.5 ± 4.5 ng/mL (Banks et al., 1983). During lactation, prolactin concentrations remain high (40.6 ± 7.2 ng/mL) for at least 4 weeks and then decline gradually over 2 weeks before falling steeply at weaning, reaching basal values within 2 weeks thereafter.

8.2.6 Oxytocin

Developing corpora lutea produce oxytocin, which may play a role in regulating prostaglandin secretion by the feline endometrium (Siemieniuch et al., 2011).

8.2.7 Prostaglandins

Prostaglandin (PG) $F_{2\alpha}$ synthase increases in trophoblast cells shortly after implantation (2.5-3 weeks of pregnancy) and in decidual cells near-term (Siemieniuch et al., 2014). PG-endoperoxide synthase 2 (prostaglandin G/H synthase, PTGS2) is upregulated in the placenta, particularly in the last third of pregnancy. This leads to increases in concentrations of $PGF_{2\alpha}$ and its 13,14-dihydro-15-keto-metabolite (PGFM), particularly in the last third of pregnancy (Dehnhard et al., 2012; Siemieniuch et al., 2014).

8.3 Tomcats

In male cats, or tomcats, the testicles have usually descended and are present in the scrotum at birth (reviewed by Feldman and Nelson, 2004; Verstegen, 2000) and can be readily palpated at 6-8 weeks of age. Male cats reach sexual maturity at 9 months of age (range 7-12 months) (reviewed by Christiansen, 1984). Spermatogenesis is seen at around 20 weeks, and the first spermatozoa appear in the spermatic cord at 30-36 weeks of age (reviewed by Verstegen, 2000).

In male cats, the release of LH is controlled by feedback effects of testosterone on the anterior pituitary. LH concentrations vary considerably between individual cats (Goodrowe et al., 1989). Basal values of LH in adult tomcats are similar to those seen in anoestrus queens.

Testosterone concentrations vary considerably between individual cats (Goodrowe et al., 1989). Basal concentrations of testosterone are high (around 4-8 ng/mL) in both intact and castrated male (and female) cats (Verstegen, 2000).

8.4 Pregnancy diagnosis

Pregnancy can be confirmed in cats by abdominal palpation, ultrasound, radiography, or hormone assays.

8.4.1 Abdominal palpation

A series of discrete, firm, spherical uterine enlargements can be readily felt by 17-25 days of gestation (reviewed by Feldman and Nelson, 2004; Verstegen, 2000). Abdominal palpation is easy to perform but can be challenging if there are few foetuses present. It also does not give any indication of the age or viability of foetuses.

8.4.2 Abdominal ultrasound

Abdominal ultrasound is easy to perform and can be used to assess foetal numbers and foetal viability, depending on when it is performed. It can be used to detect pregnancy (discrete spherical swellings) as early as 11-15 days of gestation. The crown-rump length of kittens can be measured during ultrasonography and can be used to help determine the approximate stage of gestation. However, due to the positioning of kittens, the diameter of the head and thorax may be easier to assess. Foetal heartbeats can be detected from 22-25 days. It can be difficult to assess the number of foetuses present, except during early pregnancy.

8.4.3 Abdominal radiography

Abdominal radiography is easy to perform and is accurate after day 45, when it is least likely to yield inconclusive results. Foetal skeletons can be visualised from days 38-45. Radiography is the best indicator of foetal number (litter size).

8.4.4 Hormone assays

Relaxin and PGFM concentrations are pregnancy-specific hormones that can be used to differentiate between pregnancy and pseudopregnancy and for pregnancy diagnosis.

Relaxin is secreted by the placental unit of the pregnant uterus from about 20-25 days of gestation (see Section 8.2.4). Relaxin is excreted in urine but is usually assessed in serum (not whole blood). A rapid immunomigration test is available for use with feline serum.

PGFM increases in the last third of pregnancy in domestic cats and in some captive felid species (eg, sand cat, cheetah) (Dehnhard et al., 2014) (see Section 8.2.7). However, PGFM does not increase in all species (eg, Iberian lynx and Sumatran tiger). PGFM can be measured in feces and is used in some species of captive felid because it is noninvasive (Dehnhard et al., 2012).

8.5 Mismating and prevention of implantation

Veterinarians are not commonly asked to treat mismating or terminate unwanted pregnancy in cats, since this often goes unsuspected. A number of options are available after attempting to determine whether mating actually occurred. These methods, with the exception of a single oral dose (2 mg) of the progestin megestrol acetate during oestrus (reviewed by Feldman and Nelson, 2004), are usually carried out after pregnancy has been confirmed. Surgical removal of the ovaries and uterus (ovariohysterectomy, spaying) after confirmation of pregnancy is possible but is obviously not suitable for queens intended for breeding.

8.5.1 Dopamine agonists with or without prostaglandins

Dopamine D2 receptor agonists, such as bromocriptine, metergoline, and cabergoline, decrease prolactin concentrations. This is followed by a rapid fall in progesterone concentrations and abortion (Jöchle, 1997). Cabergoline has been administered with food, at a dose rate of 0.005-0.015 mg/kg once daily, from day 36 of gestation until pregnancy termination (usually a few days) (Jöchle and Jöchle, 1993). Cabergoline alone may not be effective when treatment is started later in gestation (after day 45) (Erünal-Maral et al., 2004), with 9 days or more treatment being required, and results in premature parturition of live rather than dead kittens and insufficient lactation (Jöchle and Jöchle, 1993). The efficacy can be increased by combining cabergoline (0.005 mg/kg orally once daily) with a $PGF_{2\alpha}$ analogue, such as cloprostenol (0.005 mg/kg every 2 days by subcutaneous injection) (Onclin and Verstegen, 1997).

8.5.2 Progesterone receptor antagonists

The progesterone receptor antagonist aglepristone (at a dose rate of 10 mg/kg by subcutaneous injection on two consecutive days) has been used effectively at day 5 postmating to prevent pregnancy and from day 25 postmating to terminate pregnancy in cats (Georgiev and Wehrend, 2006; Goericke-Pesch et al., 2010). None of the cats treated on days 5 and 6 postmating were confirmed pregnant by ultrasonographic examination on day 25 (Goericke-Pesch et al., 2010). On average, oestrus occurred

18.5 days after treatment, but the range was considerable (5-299 days). Pregnancy rates at the first and second oestrus after treatment were 64% and 82%, respectively. In cats treated from day 25, abortion occurred around 5 days posttreatment (range 4-7 days) in 87% of the cats treated (Georgiev and Wehrend, 2006). Agelpristone is well tolerated by cats, with irritation at the injection site immediately after injection observed infrequently (Georgiev and Wehrend, 2006).

8.6 Contraception

Surgical methods (neutering: ovariohysterectomy (spaying) and castration) are widely used to control reproduction in cats (reviewed by Goericke-Pesch, 2010; Goericke-Pesch et al., 2014b).

8.6.1 Surgical contraception

Surgical contraception is highly effective. Ovariohysterectomy (spaying), complete removal of the ovaries with the uterus, is the method of choice for queens not intended for breeding. Castration, complete removal of both testes, is the method of choice for tomcats not intended for breeding. Surgical neutering is irreversible and is obviously not suitable for cats that are intended for breeding. In addition, some cat owners may be reluctant to consider surgery.

Neutering prior to puberty, also known as prepubertal gonadectomy, has increased in popularity in many countries. This does not appear to stunt growth but may alter the metabolic rate in cats (reviewed by Olson et al., 2001; Root Kustritz and Olson, 2000). The side effects are apparently no greater in cats neutered early (7 weeks) than in those neutered at around puberty (>4 months old).

8.6.2 Nonsurgical contraception

Modern, effective pharmacological alternatives are available for inducing ovulation, suppressing or postponing oestrus, and managing unwanted pregnancy in cats (reviewed by Goericke-Pesch, 2010). The selection of the most appropriate approach for an individual cat requires a detailed knowledge of the physiology of the feline oestrous cycle. Currently there is

no suitable alternative to surgical neutering in male cats. In addition, there is an ongoing search for other alternatives to surgical control of reproduction in cats, some of which are summarised below.

a. Vaginal stimulation

Mechanical vaginal stimulation using a glass rod (eg, 6 mm), introduced at least 4-8 times at 5- to 20-minute intervals for 2.5 seconds per time, has been suggested (reviewed by Feldman and Nelson, 2004). This action will not shorten the oestrus period but, if successful, will delay the onset of the next oestrus.

b. Human chorionic gonadotropin

There is a linear response between the dose of human chorionic gonadotropin (hCG) and the ovulatory response in queens in the dose range of 0-500 IU (Wildt and Seager, 1978). A dose of 50-250 IU of hCG given by intravenous or intramuscular injection will generally induce ovulation and also delay subsequent calling (reviewed by Verstegen, 2000). This is a safe and relatively efficient means of terminating calling in queens with seasonal oestrus. Using this regime, the behavioural signs of heat cease within 1-2 days of the injection and the next period of calling will not take place until the subsequent season starts. In queens with less marked seasonality, the results are not as long-lasting. However, once calling has been interrupted temporarily, the queen can be spayed or progestin therapy initiated.

c. Gonadotropin-releasing hormone agonists

i. Queens

Short-term administration of GnRH (eg, subcutaneous administration of buserelin (0.025 mg); Kanca et al., 2014) increased follicular development leading to oestrus and ovulation. Subcutaneous administration of a deslorelin (4.7 mg) implant initially stimulated oestradiol release, and this was accompanied by treatment-induced ovulation (Munson et al., 2001). Although it happens infrequently, oestrus can be induced around 3 days posttreatment, particularly if the implant is administered during oestrus or pro-oestrus (Goericke-Pesch et al., 2013a). Care should be taken that queens are not mated at this induced oestrus, as they can become pregnant and carry kittens to term but may exhibit decreased maternal behaviour and have inadequate lactation (Goericke-Pesch et al., 2013b).

Sustained exposure to GnRH reduces the GnRH-stimulated secretion of gonadotropins through the down-regulation and internalisation of GnRH receptors and signal uncoupling. This can be used to produce reversible contraception (reviewed by Kutzler and Wood, 2006). The duration of efficacy can vary considerably following deslorelin (4.7 mg) implant administration to queens (ranging from 483 to 1,025 days) (Goericke-Pesch et al., 2013a; Munson et al., 2001). This is an important consideration for cat owners who wish to postpone oestrus for a short period.

ii. Tomcats

Following administration of deslorelin (4.7 mg) implant to tomcats, there is an initial increase in sexual behaviour (eg, libido, mating behaviour, and urine marking) (Goericke-Pesch et al., 2011). The onset of action (nonsurgical castration) is variable, ranging from 4 to 11 weeks before testosterone concentrations are basal (<0.1 ng/mL), and may only be partial (Goericke-Pesch et al., 2011). Treatment leads to a significant reduction in gonadotropin and testosterone concentrations, mating behaviour, and fertility (Goericke-Pesch et al., 2014a). It takes much longer for sexual characteristics, such as testicular volume (60% decrease by 12 weeks) and penile spines (disappearance after about 9-10 weeks), to be affected (Goericke-Pesch et al., 2011). The mean duration of efficacy ranges from 61 to 100 weeks and is followed within 3 weeks by a return to normal testosterone concentrations and within 5-11 weeks by a return of associated sexual characteristics, such as testicular volume and mating behaviour, and fertility (Goericke-Pesch et al., 2014a). The delayed onset of action and initial agonist effect mean that fertile mating can take place in treated tomcats (Goericke-Pesch et al., 2011). Food intake may increase significantly in treated tomcats (Goericke-Pesch et al., 2011).

d. Progestins

Progestins or progestogens are synthetic steroid hormones that mimic the action of progesterone by binding to progesterone receptors.

Progestins have a number of different actions that lead to prevention, reduction, or loss of synchrony in the normal changes in the reproductive tract; prevention of follicle maturation and ovulation; and interference with sperm transport (Table 3). The principal mode of action of progestins during

clinical use is their antigonadotropic action. Many of the other effects are related to the occurrence of progestin-associated side effects. For a detailed review of the effects and side effects of the progestins, see Chapter 7, Section 7.2.1a.

Activity	Actions
Progestational	• Stimulation of endometrial gland development and secretion (especially after oestrogen priming) • Promotion of cervical closure • Direct and indirect (decreased oxytocin receptors) suppression of uterine myometrial sensitivity and contractility • Stimulation of mammary tissue proliferation
Antigonadotropic	• Reduction or modulation of pulsatile increases in secretion of LH and FSH leading to suppression of follicle development and prevention of ovulation
Antiestrogenic	• Pretreatment leads to suppression of oestrogen receptor expression leading to reduction or prevention of vaginal bleeding, oestrus behaviour, and transport in the oviducts
Antiandrogenic	• Reduction, inhibition, or reversal of some effects of androgens, including libido

Table 3 Summary of the activity and actions of progestins

Progestins have been used for many years for nonsurgical contraception in cats. There are 3 agents available: megestrol acetate (MA) and medroxyprogesterone acetate (MPA) are available for oral administration, and MPA and proligestone are available for administration by injection. Progestins should ideally be administered during anoestrus (reviewed by Feldman and Nelson, 2004). In queens, progestins can be used to suppress or postpone oestrus.

i. Suppression of oestrus

To suppress oestrus, a short course (1-3 days) of an orally active progestin is given as soon as signs of calling are seen. Progestins suppress the call and may prevent conception should mating occur.

Following an injection of proligestone (1 mL per cat) or MPA at the onset of calling, the signs of oestrus will usually abate within 1-4 days but may persist for as long as 7 days. Queens may conceive during the first few days after proligestone has been administered for the suppression of calling, even though the signs of oestrus may have already disappeared. Contact with males should therefore be prevented for the first 5 days following injection at this stage of the oestrous cycle.

ii. Oestrus postponement

Progestins can be used to temporarily or permanently postpone oestrus. This is often the method of choice for planned breeding. For temporary suppression, a single dose of progestin is given during anoestrus or interoestrus to postpone the subsequent oestrus. For permanent postponement, progestin treatment is started in anoestrus or interoestrus, and doses are repeated at regular intervals.

Tablets containing MPA (eg, 5 mg per cat per week) or MA (eg, 2 mg per cat per week) can be given orally either daily or once weekly to postpone calling (reviewed by Kutzler and Wood, 2006). Depot injections, usually given at 6-month intervals, have the advantage of convenience.

Proligestone can be used for the permanent postponement of oestrus in cats using a dosage regime similar to that advised for female dogs, namely injections (1 mL per cat) at 3-, 4-, and 5-month intervals (see Proligestone in Chapter 7, Section 7.2.1a - Proligestone). If the timing of the next dose and the breeding season are expected to coincide, it is advisable to shorten the time between injections to 4 months.

iii. Duration of effect and return to oestrus

The duration of effect of any given dose of a progestin depends highly on when in the oestrus cycle it is administered. For this reason, it is not uncommon for the treatment interval to have to be adjusted (eg, to every 4 months) to suit the individual and thus avoid breakthrough oestrus, particularly in queens that exhibit strictly seasonal reproductive activity. Similarly, queens housed together with other intact female cats might require a shorter dosing interval. The dose of progestin should not be increased in an attempt to increase efficacy or provide a longer duration of action due to the risk of side effects.

Following progestin treatment, the onset of subsequent calling will depend on the time of year. If the queen is treated at the end of the breeding season, the next period of calling may not occur until the beginning of the next breeding cycle, so calling could be delayed by as much as 6 months. However, the recurrence of calling after treatment is very variable, and the duration of action can vary widely between individuals. In fact, it is not possible to say precisely when a cat will call again. Generally, cats call sooner after suppression than after postponement of oestrus; 4 weeks after treatment ceases is usual. This means that there is only a slight extension of the normal interval between cycles. Queens may call soon after oral MA or oral MPA treatment ceases, but a delay of 2-3 months is more usual. For the injectable preparations, it is even more difficult to predict when a queen will return to oestrus. Following treatment with proligestone, the majority of queens will call 6-7 months later.

iv. Side effects

Progestins are in general well tolerated by cats, but there are a number of well-known side effects that are related to the pharmacological effects of these agents (reviewed by Feldman and Nelson, 2004; Goericke-Pesch, 2010; Kutzler and Wood, 2006).

Side effects can include:

- Cystic endometrial hyperplasia, endometritis, or pyometra – see Section 8.7.5
- Mammary hyperplasia – see Section 8.7.6
- Mammary tumours (carcinoma, Jacobs et al., 2010)
- Diabetes mellitus
- Other, eg, depression, increased appetite

Mammary neoplasia appears to be linked to the progestational activity of the progestins, but this likely also requires oestrogen and/or prolactin priming. An increased incidence of mammary pathology, including development of mostly benign tumours (adenomas), has been reported following megestrol acetate treatment of queens (Hayden et al., 1989). Growth hormone is produced under the influence of progesterone or a progestin; particularly during long-term treatment. Growth hormone may

also be produced by the mammary gland as it is in dogs (see Somatotropic effects of progestins in Chapter 7, Section 7.2.1). It is possible that this leads to the development of mammary nodules that can then undergo neoplastic change. It is not clear whether the mammary glands of queens produce growth hormone.

Progestins also increase blood glucose concentrations. This appears to be an indirect diabetogenic effect via increased secretion of growth hormone, leading to insulin resistance. In addition, some progestins also bind to (gluco)corticosteroid receptors, and this can be associated not only with increased blood glucose concentrations but also with local alterations in the skin, such as atrophy, discoloration, and alopecia at the site of injection. Moreover, other signs associated with the progestins, such as alterations in mood and increased appetite or thirst, may be associated with glucocorticoid effects (see Glucocorticoid effects of progestins in Chapter 7, Section 7.2.1).

Normal parturition requires progesterone withdrawal. Progestins can prevent or delay the onset of parturition. Care must be taken to ensure queens are not pregnant prior to initiating progestin treatment. If pregnancy is later confirmed, then these animals should be monitored closely and Caesarean section performed if parturition appears to be delayed or not to be occurring.

e. Melatonin

Cyclicity in cats is influenced by day length. Decreasing day length is associated with high concentrations of melatonin, and this is followed by decreased sexual activity (Leyva et al., 1989a and b). Melatonin (4 mg/day) was effective following oral administration to cats, but long-term administration using this route is not practical (Goericke-Pesch et al., 2014b). A subcutaneous implant containing melatonin (18 mg) has been shown to postpone oestrus but not to delay puberty (Faya et al., 2011; Gimenez et al., 2009). The duration of efficacy appeared to be longer if queens were treated during interoestrus (around 113 days) rather than oestrus (around 61 days) (Gimenez et al., 2009). However, another study showed that melatonin given orally or as a subcutaneous implant was only effective for around 63 days (Faya et al., 2011).

f. Chemical vasectomy

Intraepididymal injection of 4.5% chlorhexidine digluconate has been tested in cats (Pineda and Dooley, 1984). Although this successfully neutered the cats, administration was associated with pain and swelling for up to 2 weeks postinjection and with intraepididymal granuloma formation. This approach has not found widespread acceptance.

g. Immunocontraception

A number of components of the reproductive system (including LH and its receptors, oocyte zona pellucida and GnRH) have been identified as suitable targets for an immunocontraceptive vaccine. While immunocontraception appears to offer some promise for the control of reproduction in cats, and further developments are expected in this field, these vaccines have not been developed or commercialised (reviewed by Goericke-Pesch et al., 2014b; Munks, 2012). The efficacy and safety of this type of approach remain to be confirmed.

Oocyte zona pellucida vaccines have been used successfully in many species but to date have proved problematic in queens (Levy et al., 2005). Vaccination of queens with an LH-receptor vaccine has been shown to suppress oestrus for more than 11 months in queens via suppression of corpus luteum function (Saxena et al., 2003).

Development of GnRH vaccines has been problematic, primarily due to the poor immunogenicity of GnRH. A recombinant GnRH antigen has been shown to produce biologically relevant anti-GnRH antibody titres for 20 months in cats following administration on two occasions to 8- and 12-week-old cats (Robbins et al., 2004). Booster vaccination after 20 months resulted in a significant anamnestic response. In female cats, a GnRH vaccine produced good results (93% of vaccinated cats remained infertile for 1 year) following a single administration under controlled laboratory conditions (Levy et al., 2011). The time to conception was significantly higher in treated animals (around 40 months) than in controls (around 4 months) (Levy et al., 2011). However, the duration of efficacy varied from 5 months to more than 5 years. In addition, vaccination induced persistent granulomatous reactions at the site of injection (Levy et al., 2011). In male cats, a single injection of synthetic GnRH coupled to keyhole limpet haemocyanin and combined with

a mycobacterial adjuvant to enhance immunogenicity was effective (basal testosterone concentrations and testicular atrophy) for between 3 and 6 months in two-thirds of the 9 cats tested (Levy et al., 2004).

8.7 Disorders of the reproductive tract

8.7.1 Ovarian remnant syndrome

Ovarian remnant syndrome is a complication of ovariohysterectomy. It is caused by the presence of a remnant of functional ovarian tissue. This remnant can be unilateral or bilateral and is often at or near the ovarian pedicle but can also be at other sites such as in the omentum (Johnston et al., 1996). Affected queens start to show oestrus signs at varying intervals (days to years) after ovariohysterectomy (on average 2 years). Ovarian remnant syndrome manifests as oestrus behaviour of variable intensity with or without cyclical or seasonal pattern.

Ovarian remnant syndrome is diagnosed by confirming the presence of functional ovarian tissue using vaginal exfoliative cytology when the cat is showing signs of oestrus and/or measurement of hormone concentrations. Changes in vaginal exfoliative cytology reflect oestrogen production, and the cells should appear cornified if an ovarian follicle is present. In adult animals, the ovaries are the sole source of anti-Müllerian hormone (AMH, Müllerian inhibitory substance). It has been shown that a single sample for AMH can distinguish between intact animals, animals with ovarian remnants, and animals that have undergone ovariohysterectomy (Place et al., 2011). However, this assay may not be widely available. Administration of the GnRH agonist buserelin (0.0004 mg/kg intramuscularly) stimulates any ovarian follicles present and is followed by an increase in oestradiol to more than 12 pmol/L within 2 hours after treatment (Axnér et al., 2008). However, oestradiol assays are not always widely available.

This condition is treated by surgical removal of the ovarian remnant(s). This is easier to perform when luteal tissue is present, as the remnant is easier to find and there is a lower risk of bleeding (Johnston et al., 1996). Ovulation can be induced using GnRH (0.025 mg/cat) or hCG (250 IU/cat) administered intramuscularly. Progesterone concentrations from the resulting

corpora lutea can be measured 2 weeks later. Surgery can be by exploratory laparotomy or by laparoscopy, the latter using either a 3-port or single-port technique with the animal in dorsal recumbency (Naiman et al., 2014).

8.7.2 Cryptorchidism, testicular remnants, and prior castration

Unilateral or bilateral cryptorchidism can occur but is relatively rare in tomcats. The retained testicle(s) may be situated intra-abdominally or in the inguinal canal. The condition is considered to be hereditary, and for that reason, and because there is some risk that retained testicles will become neoplastic, surgical removal is the treatment of choice.

a. GnRH or hCG stimulation test

In male cats, the administration of a GnRH agonist or hCG causes the release of LH and a consequent rise in circulating testosterone concentrations (reviewed by Verstegen, 2000). Testosterone concentrations peak around 12-16 ng/mL at 20-24 hours after administration. A GnRH or hCG stimulation test can be performed to check for functional testicular tissue. A significant increase in testosterone 60 minutes after intravenous injection of 0.001-0.002 mg/kg of a GnRH agonist or 50-100 IU hCG per cat is diagnostic.

The absence of hormone-dependent keratinised spines on the penis, a test that is quick and easy to perform, is suggestive of prior castration (Verstegen, 2000).

8.7.3 Failure to cycle

Apparently prolonged anoestrus may result from poor oestrus detection and poor management or be secondary to contraceptive administration (reviewed by Feldman and Nelson, 2004; Verstegen, 2000) (see Section 8.6.2). Queens with strictly seasonal reproductive behaviour might give a poorer response when oestrus induction is attempted during anoestrus. The closer the time of treatment is to the beginning of the reproductive season, the better the results. Stress, poor nutrition, systemic disease, temperature extremes, inadequate lighting (lack of exposure to daylight), iatrogenic (progestin or glucocorticoid administration) or cystic follicles all cause oestrus failure in queens. Silent oestrus may result from overcrowding, especially in the case of very subordinate queens.

a. Treatment

The treatment chosen will depend on the underlying cause. It is important to eliminate functional, anatomical, and infectious causes before instigating treatment with exogenous hormones. Adjusting the lighting pattern (exposure to daylight for 14 hours/day or for 12 hours/day after a period of shorter day length) and/or housing with other cycling queens may be successful (reviewed by Christiansen, 1984).

Stimulation of ovarian activity by oestrus induction using 150 IU of pregnant mare serum gonadotropin (PMSG, also known as equine chorionic gonadotropin, eCG) followed 3-4 days later by 100 IU hCG, both by intramuscular injection, may be successful (Donoghue et al., 1993; Swanson et al., 1997). Higher doses of PMSG can cause ovarian hyperstimulation and the development of cystic follicles and abnormal endocrine profiles (Wildt et al., 1978; Cline et al., 1980).

8.7.4 Dystocia

Dystocia is rare in queens. It can result from maternal factors, such as a congenitally narrow pelvis, poorly healed pelvic fractures, uterine torsion or uterine inertia, which may be linked with obesity, or foetal factors, such as relative foetal oversize and foetal malpresentation. Some breeds of cat, notably Siamese and Persians, have been reported to have a higher prevalence of dystocia (von Heimendahl and Cariou, 2009). Remedial action should be considered if unproductive straining occurs for more than 1 hour or if a large quantity of blood-stained vaginal discharge is seen (reviewed by Feldman and Nelson, 2004). If a kitten is present in the vagina, manual removal may be possible, but this must be done carefully.

If uterine inertia is suspected in queens with small litters, oxytocin, at a dose rate of 2-4 IU by intravenous or intramuscular injection, may help. If this has no effect, treatment can be repeated 20 minutes later after administering 1-2 mL 10% calcium gluconate, which can be followed after 20 minutes by 2 mL 50% dextrose intravenously and a further oxytocin treatment (reviewed by Feldman and Nelson, 2004). If there is still no effect, Caesarean section should be performed.

8.7.5 Cystic endometrial hyperplasia, endometritis, and pyometra

Cystic endometrial hyperplasia (CEH), endometritis, and pyometra are the most common uterine diseases in queens but are less common than in dogs (reviewed by Verstegen, 2000). These conditions are more usually seen in queens aged 5 years or older (Potter et al., 1991), presumably due to the elevated progesterone concentrations that occur during pseudopregnancy in cats (reviewed by Christiansen, 1984; Verstegen, 2000). It has been shown that, in cats, short-term exposure to progesterone or long-term exposure to MPA impairs toll-like receptors (TLR) 2 and 4 on the endometrial surface and in the glandular epithelium, presumably enabling pathogens to break through this first natural barrier, thereby increasing the risk of the development of pyometra (Jurza et al., 2014).

Queens with CEH, endometritis, or pyometra do not always show clinical signs; these can be an incidental finding during routine ovariohysterectomy (Potter et al., 1991). If clinical signs are present, they are less prominent than in dogs and usually include vaginal discharge, abdominal distension, dehydration, a palpable uterus, and pyrexia (Kenney et al., 1987).

a. Surgical treatment

Surgery (ovariohysterectomy) is the treatment of choice, particularly in severe cases.

b. Medical treatment

Medical treatment (eg, progesterone receptor antagonist or a prostaglandin combined with antimicrobial therapy) can be attempted (Davidson et al., 1992) but is rarely the treatment of choice.

A preliminary study suggested that aglepristone (10 mg/kg subcutaneously, 2 doses 24 hours apart) was effective and well tolerated (Hecker et al., 2000). Other authors administered up to 4 doses of aglepristone, with treatment on 2 consecutive days, 1 week later and, if required, on day 14 in combination with 1 week of broad-spectrum antimicrobial treatment (Nak et al., 2009). This was effective in 9 of the 10 cats treated and was well tolerated by all of the cats (Nak et al., 2009).

The prostaglandin cloprostenol (0.005 mg/kg, subcutaneous injection, once daily for 3 days) in combination with antimicrobial treatment (for 7 days) has been used effectively to treat open pyometra in cats (García Mitacek et al., 2014). Side effects (eg, diarrhea, vomiting, and vocalisation) were seen within 10 minutes and lasted for up to 30 minutes (García Mitacek et al., 2014).

8.7.6 Mammary hyperplasia

Mammary hyperplasia (also known as fibroadenomatosis or fibroadenomatous hyperplasia) is a non-neoplastic hyperplasia of the mammary glands. Falling progesterone (endogenous or exogenous) concentrations are thought to stimulate prolactin production, which in turn stimulates the growth of mammary tissue (reviewed by Feldman and Nelson, 2004).

Fibroadenomatosis is characterised by a rapid proliferation of mammary stroma and duct epithelium of one or more glands and predominantly affects younger female cats. The condition is progesterone-dependent and develops in postovulatory (including pregnant) and progestin-treated queens and can also occasionally occur in male cats. The clinical picture is generally variable and ranges from mild enlargement to extremely pronounced hyperplasia of all of the mammary glands (reviewed by Feldman and Nelson, 2004). The clinical signs usually include skin ulceration, painful mammary glands, lethargy, anorexia, and tachycardia (Görlinger et al., 2002).

Since this condition is progesterone-dependent, progestins should not be administered to queens with a history of mammary gland enlargement or to queens prior to their first oestrus. Queens with a history of mammary gland enlargement should ideally be spayed, since endogenous progesterone can also produce this condition.

a. Treatment

Treatment options include the withdrawal of progestin treatment or administration of a progesterone receptor antagonist (Görlinger et al., 2002). Progestin treatment can only be withdrawn if a short-acting progestin is being administered orally. Stopping progestin treatment may not always prevent progression of the clinical signs.

Aglepristone administered subcutaneously on one day (20 mg/kg) or two consecutive days (10 mg/kg/day) per week has been reported to be effective in the treatment of fibroadenomatosis (Görlinger et al., 2002). It has been recommended that cats that have previously been administered an injectable progestin (eg, MPA) be treated for 5 weeks (Jurka and Max, 2009). It can take at least 4 weeks for the clinical signs to resolve after starting treatment. Cats developing signs of fibroadenomatosis again months later should be regarded as having a new episode, not a relapse. Cats not intended for breeding should undergo ovariohysterectomy after the clinical signs have resolved. In severe cases, radical surgery or euthanasia may be necessary.

8.7.7 Urine spraying or marking

Cats spray urine as a chemical means of communication and to mark their territory. It has been reported that 10% of all cats spray in adulthood (Dehasse, 1997). Urine spraying or marking is normal behaviour and can occur in both entire and castrated males and also in entire and spayed female cats. Urine spraying and marking must be carefully differentiated from normal micturition, abnormal urination associated with feline lower urinary tract disease (FLUTD), and inappropriate urination (eg, due to separation anxiety syndrome).

a. Behavioural training

It may be possible to reduce urine spraying or marking in cats by implementing environmental and/or behavioural changes. This is primarily aimed at reducing stress and decreasing territorial behaviour. However, the results of one study appear to suggest that behaviour therapy may be redundant in the majority of cases.

b. Additional treatment

Neutering of entire tomcats or queens usually diminishes or stops spraying as well as making the urine smell less pungent. However, this is not universally effective: efficacy rates have been reported to be around 78% in tomcats (Hart and Barrett, 1973). Chemical castration could potentially also be used, but progestins are reportedly not very effective.

Synthetic analogs of feline facial pheromone have been reported to decrease the frequency of urine spraying in 74% of cases when sprayed daily onto items in the home (Frank et al., 1999). The efficacy of feline facial pheromone treatment was similar when a room diffuser was used, and this tended to increase with time (Mills and Mills, 2001). The room diffuser appears to be more convenient for cat owners than daily spraying, and this may improve compliance and thus treatment success.

A number of sedative or psychoactive drugs have also been reported to be of help. The benzodiazepine diazepam is associated with side effects and is not an effective long-term treatment, with more than 90% of treated cats resuming spraying or marking when treatment was gradually discontinued (Cooper and Hart, 1992). The nonbenzodiazepine anti-anxiety drug buspirone has been reported to be more effective than diazepam, with a >75% reduction in 55% of cats treated for urine spraying or marking; 50% of these cats resumed spraying after treatment ceased at the end of 2 months (Hart et al., 1993). Long-term treatment with buspirone has been reported to be safe in cats, and 56% of cats returned to normal litter box usage (Hart et al., 1993). The tricyclic antidepressant clomipramine (0.25-0.5 mg/kg twice daily) has been reported to be effective in more than 75% of cases (Dehasse, 1997). The selective serotonin reuptake inhibitor fluoxetine hydrochloride has also been reported to be an effective treatment but was associated with a reduction in food intake in almost half of the cats treated (Pryor et al., 2001).

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9.1 Introduction

The domestic buffalo, *Bubalus bubalis*, is a distinct species within the Bovidae family. The buffalo population is increasing constantly. It was estimated at over 160 million in 2002 (FAO, 2003), more than 95% of which is located in Asia, where buffalo play a prominent role in rural livestock production, providing draft animals, milk, and meat. In recent decades, buffalo farming has expanded widely in Mediterranean areas and in Latin America.

The swamp buffalo of Southeast Asia (Indonesia, Malaysia, Thailand, and Australia) has 48 pairs of chromosomes. It is mainly used for draft work, being a poor milk producer. The Murrah and Surti river buffalo (India, Pakistan) have 50 pairs of chromosomes and provide a much higher milk yield with a very high fat content (8%). The majority of animals are kept in small village farms under traditional management systems. However, in some countries, such as Italy and Brazil, there are farms engaging in the large-scale production of buffalo milk, benefiting from the overall control of production and reproduction.

9.2 Physiology

The reproductive organs of buffalo are smaller than but quite similar to those of cows. The uterine body is much shorter (1-2 cm) than that of the cow (2-4 cm). The cervix of the water buffalo is smaller (length 3-10 cm, diameter 1.5-6 cm) and its canal is more tortuous, which probably accounts for the lesser degree of dilation of the external orifice during oestrus. Water buffalo have 3 cervical folds on average (Drost, 2007). The smaller buffalo ovary is more elongated than in the cow, and the corpus luteum is not only smaller but also often more deeply embedded in the ovarian stroma.

Puberty in buffalo occurs later than in cattle, with the age of puberty varying widely, ranging from 16-22 to 36-40 months in different countries. Under field conditions, the first oestrus occurs at 15-18 and 21-24 months in river and swamp buffalo, respectively. In well-fed animals, puberty may be reached before 20 months, the timing of which is significantly affected by breed, season, climate, feeding systems, and growth rate. The body weight

of the female is the strongest determining factor, as observed in cattle. The average age at first calving is therefore between 3 and 4 years, but many buffalo cows calve much later.

In river buffalo, the female is active from July until the end of February. The peak of first matings occurs during autumn and winter (Nasir Hussain Shah et al., 1989). The most likely reason for this seasonality is the hot and dry conditions during the summer, and nutrition may also play a part. The swamp buffalo cycles continuously throughout the year, but a crop-associated seasonal pattern is observed. In Thailand, breeding is concentrated between December and February — the postharvest season — when the animals are allowed to graze in the paddy fields.

On average, oestrus lasts 12-28 hours. Buffalo oestrus behaviour is less intense than that of cows and is consequently much more difficult to detect. Mucosal vaginal discharge, swollen vulva, mounting behaviour (far less frequent than in cattle), and the standing reflex are the main signs of oestrus.

The works of Baruselli et al. (1997), Manik et al. (2002), and Ali et al. (2003) confirmed that, as in cattle, follicular development during the oestrous cycle also occurs in waves. Two-wave cycles are most common (63.3%), followed by three-wave cycles (33.3%) and those featuring a single wave (3.3%). Buffalo cows tend to have two or three follicular wave cycles, whereas in buffalo heifers there is a prevalence of two-wave cycles. The number of waves influences the length of the luteal phase and the oestrous cycle. Oestrous cycles with two follicular waves are somewhat shorter than three-wave cycles (21 vs. 24 days). Buffalo that exhibit three waves of follicular growth show a longer luteal phase (12.6±2.9 vs. 10.4±2.1 days), interovulatory interval (24.5±1.8 vs. 22.2±0.8 days), and oestrous cycle (24.0±2.2 vs. 21.8±1.01 days) (Rensis et al., 2007).

The ovulatory follicle reaches a diameter of 10 mm. The diameter of the mature CL ranges from 10 to 15 mm, as compared with 12.5 to 25 mm in the bovine. Ovulation occurs approximately 10 hours after the end of oestrus.

Patterns of hormone activity in buffalo and cows appear to be basically identical, but the progesterone concentrations during the cycle and

pregnancy are much lower in buffalo, especially in the swamp type. Circulating concentrations of LH reach a peak at the onset of oestrus, followed by a sharp decrease, and thereafter remain low during the luteal phase. The duration of the LH surge has been estimated to be 7-12 hours.

During the oestrous cycle, progesterone levels are at basal levels immediately after ovulation, increase during the following 4-7 days, and reach peak levels at about day 15 after the onset of oestrus.

The gestation period of buffalo is longer than that of cows, between 310 and 330 days. Murrah tend to have a shorter gestation period (315 days) than swamp buffalo (330 days). The corpus luteum of pregnancy is invariably located ipsilaterally with the gravid horn.

The calving interval for buffalo varies between 400 and 600 days, although longer intervals are possible. Seasonal, nutritional, and managerial factors play important roles. The first ovulation in river buffalo does not usually occur before 55 days postpartum, but may be delayed up to day 90 postpartum when suckling a calf. The first oestrus is detected after 130 days postpartum in suckled cows but may be delayed much longer, depending on nutritional and climate conditions.

9.3 Reproduction management

Reproductive efficiency is the primary factor affecting productivity and is hampered in the female by the late attainment of puberty, seasonality of calving, extended postpartum anoestrus, and the subsequent calving interval. Pregnancy rates following artificial insemination (AI) are similar (>60%) to those obtained in cattle, indicating that procedures for collection, processing, and cryopreservation of buffalo semen have been well established.

Artificial insemination is still not carried out on a large scale in buffalo because of the discrete expression of oestrus and the variability in its length, which make detection very difficult. Large numbers of buffalo are not bred at all due to the high incidence of silent heat.

Covert or silent oestrus is the single largest factor responsible for poor reproductive efficiency in buffalo. Additionally, the skills required for effective heat detection are often very limited on many buffalo farms. Some of the methods used in cattle can be adapted in buffalo to enhance oestrus detection. Pedometers have proved to be efficient, but the installation of the system itself is often well beyond the financial means of many smaller buffalo farms. Simpler and cheaper methods such as heat-mount detectors are less useful, as mounting behaviour in oestrus is rare in buffalo. That is the reason for pharmacological oestrus management often being the only realistic possibility for increasing the accuracy of AI timing and improving reproductive performance in buffalo herds.

All of the pharmacological systems for oestrus management currently in use in buffalo have been adapted from those used in cattle and are supported by an increasing amount of data reported in the literature. Cattle products are being used in buffalo, although few of them have the indication for buffalo specifically stated in their user leaflets.

a. Prostaglandins

As in cattle, the corpus luteum of the buffalo is sensitive to the luteolytic action of exogenous prostaglandins from the fifth day of the oestrous cycle onwards. In cyclic animals, oestrus can be induced with a single injection of $PGF_{2\alpha}$, provided a functional corpus luteum is present (Figure 1).

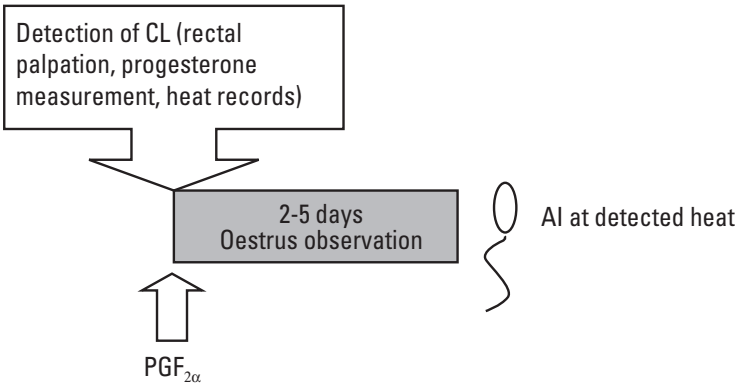

Figure 1 Treatment schedule for oestrus induction with a single prostaglandin injection

Alternatively, a double-injection regime can be adapted using an 11- to 14-day interval (Figure 2) (Singh et al., 2000; Rensis et al., 2007).

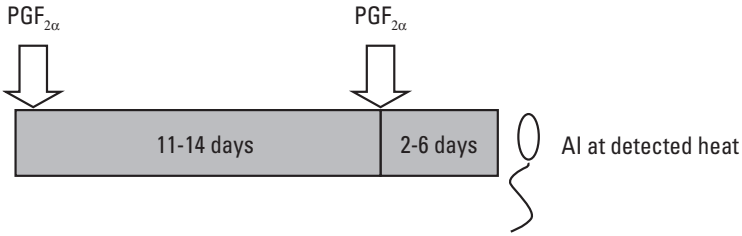

Figure 2 Treatment schedule for oestrus induction with double prostaglandin injection

Warriah and Ahmad (2007) evaluated the follicular dynamics in Nili-Ravi buffalo during spontaneous and $PGF_{2\alpha}$-induced oestrous cycles. Their results indicated that, in most buffalo treated with $PGF_{2\alpha}$ on day 9, the static phase dominant follicle of the first wave was capable of further growth and ovulation. Moreover, the follicular dynamics during the 3 days before oestrus were similar in buffalo undergoing spontaneous and $PGF_{2\alpha}$-induced luteolysis.

The interval from $PGF_{2\alpha}$ treatment to oestrus reported by Brito et al (2002) ranged from 48 to 144 hours, with three quarters of the animals showing heat within 72 to 96 hours postinjection. The time from treatment to ovulation in these studies ranged from 60 to 156 hours, with over 80% of animals ovulating within 84 to 108 hours postinjection. In buffalo, the onset of oestrus and the timing of ovulation after $PGF_{2\alpha}$ treatment are variable. Insemination at the observed heat is recommended.

It is generally thought that both the oestrus response and fertility rates obtained in buffalo are lower than in cattle after prostaglandin treatment. The most probable reasons for these differences are poor body condition and low oestrus detection rates. However, during the reproductive season and with good body condition, single or double $PGF_{2\alpha}$ treatment in buffalo should induce oestrus and ovulation in about 60%-80% of animals. In such favourable conditions, pregnancy rates following prostaglandin treatment can reach 45%-50% on average and appear to be similar to those obtained after natural oestrus.

El-Belely et al (1995) observed a 77% overall oestrus rate after two $PGF_{2\alpha}$ treatments, but there was only 25% response to the first treatment. Phadnis et al (1994) observed a 55.7% oestrus rate after two doses. It should be kept in mind that season has a dramatic influence on the results of insemination in prostaglandin-induced oestrus even if the oestrus response to treatment appears fairly good.

In spite of these limitations, oestrus management with prostaglandins should be recognised as a readily available and valuable tool to facilitate artificial insemination and improve reproductive efficiency in buffalo.

In the search for possible savings in the management of reproduction in buffalo, the intravulval submucosal injection of prostaglandins has been tested by various researchers and practitioners (Chohan, 1998). This route of administration is reported to allow the $PGF_{2\alpha}$ dose to be reduced by 50%. However, care should be taken when using such a reduced dose, because the decline in progesterone concentration and the onset of oestrus were reported to be slower in cows treated with a reduced dose by this route than in those treated with a standard dose intramuscularly (Chauhan et al., 1986; Canizal et al.,1992).

b. Ovsynch-type synchronisation programmes

In cyclic buffalo cows, good results are obtained with the classic Ovsynch protocol (Berber et al., 2002; Baruselli et al., 1999; Neglia et al., 2003; Paul and Prakash, 2005). Some authors, however, point out the beneficial effect of two inseminations at 12-18 hours and 24 hours after the second GnRH treatment (Neglia et al., 2003; Paul and Prakash, 2005). De Arujo Berber et al (2002) reported pregnancy rates of 56.5% under field conditions when the Ovsynch protocol was used in buffalo cows. In the trial reported by Paul and Prakash (2005), the Ovsynch protocol synchronised ovulation effectively in Murrah buffalo and resulted in conception rates (to two fixed-time inseminations) comparable with those achieved with a single AI after an observed oestrus.

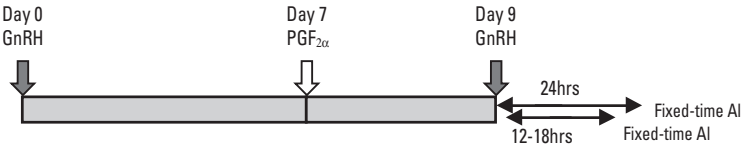

Figure 3 Ovsynch protocol used in buffalo

Ali and Fahmy (2007) evaluated the ovarian dynamics and milk progesterone concentrations in cycling and noncycling buffalo cows during an Ovsynch program (Table 1).

Ovarian response	Cyclic cows	Noncyclic cows
Ovulation to the first GnRH	90%	62.5%
Luteolysis after $PGF_{2\alpha}$	80%	87.5%
Ovulation to the second GnRH	80%	100%

Table 1 Ovarian response to the Ovsynch protocol in cyclic and noncyclic buffalo cows (Ali and Fahmy, 2007).

Conception rates recorded in this study were 60% for cyclic and 37.5% for noncyclic cows. It seems that the lower conception rates observed in the latter group could be due to early/asynchronous ovulation and poor luteal function after induced ovulation. In a study reported by Warriah et al (2008), pregnancy rates in buffalo bred at an observed oestrus (62.5%) or after the Ovsynch protocol (36.3%) during the height of the breeding season did not differ significantly from those of animals inseminated during the low breeding season (55.5% and 30.4%, respectively).

The works of Baruselli et al (1999) suggest that in order to obtain optimum results with the Ovsynch protocol in buffalo, the animals should be treated during their breeding season and must be in good body condition (>3.5).

Ovsynch is of special interest for reproduction management in buffalo because most of them are located in high-temperature zones, where heat stress can affect reproductive performance. As in cattle, treatment with the Ovsynch protocol should bring the benefits of the additional GnRH, and thus LH support for follicular growth and corpus luteum formation.

c. Progestagens

The high incidence of postpartum anoestrus and difficulties with oestrus detection make progestagens a very interesting option for the induction of oestrus and ovulation in buffalo.

Both progesterone-impregnated intravaginal devices and subcutaneous implants releasing norgestomet have been used in buffalo, either alone or combined with other hormone treatment protocols (Singh et al., 1988; Hattab et al., 2000; Bartolomeu et al., 2002; De Rensis et al., 2005; De Rensis, 2007; Carvalho et al., 2013). An example of a treatment protocol is given in Figure 4.

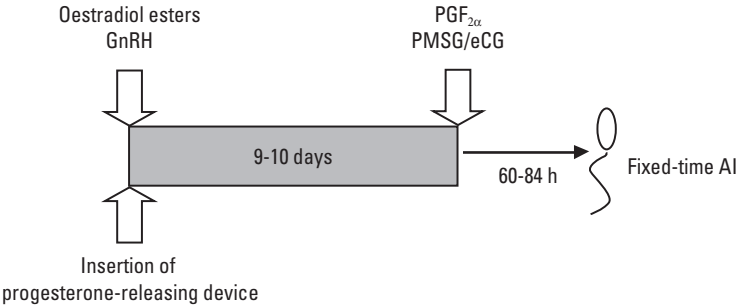

Figure 4 Example of oestrus synchronisation protocol combining progesterone-releasing intravaginal device and PMSG/eCG

Oestradiol, PMSG/eCG, and/or prostaglandin have been used successfully to improve the synchrony of oestrus, as well as conception rates, in progestagen-based protocols. Treatment with oestradiol esters or GnRH at the start of progesterone/progestagen therapy ensured adequate follicular turnover and improved conception rates (Baruselli et al., 2007). Additional treatment with PMSG/eCG at the time the progesterone/progestagen source is removed proved to be especially beneficial in noncycling buffalo cows and

outside the peak of the breeding season. Such a combination can induce the resumption of oestrus in anoestrous buffalo cows, yielding pregnancy rates close to 30% (Zicarelli, 1997; Hattab et al., 2000; Neglia et al., 2003). Barile et al (2001) used PMSG/eCG at the time the progesterone device was removed followed by two timed artificial inseminations 72 and 96 hours later, with a resulting conception rate of 51%.

It should be stressed that the use of progesterone-based protocols during the nonbreeding season allows for insemination and the establishment of pregnancy in animals that would otherwise be nonproductive.

d. Multiple ovulation and embryo transfer (MOET)

In a review by Baruselli et al (2013), the authors concluded that the low adoption of MOET-type technologies in buffalo is due to unsatisfactory numbers of embryos obtained per buffalo donor and reflect the partial understanding of events involved in the manipulation of the oestrous cycle, follicular stimulation, and ovulation in buffalo. Superovulated buffalo cows produce on average 1-3 viable embryos per harvest.

Advances in technique have revealed that buffalo respond satisfactorily to superovulatory treatment, but embryo recovery is not very efficient (Baruselli, 1997; Baruselli et al., 2000). This disparity in ovulation and recovery may be related to failures in collection and/or transportation of oocytes in the oviduct. The reason remains unknown, and further studies are needed to refine MOET as a technique that farmers can widely use to accelerate genetic gain and productivity in buffalo herds.

e. Ovum pickup and in vitro embryo production

Ovum pickup (OPU) with in vitro embryo production (IVP) is an alternative method to in vivo embryo recovery to exploit maternal genetics. In their review Baruselli et al (2013) state that there are two main challenges to utilising this technology in buffalo: low numbers of follicles on the ovary for OPU and poor oocyte quality.

The first problem can be related to the low number of follicles recruited per follicular wave (Baruselli et al., 1997). The second problem with OPU-IVP in buffalo is the more fragile zona pellucida (Mondadori et al., 2010) and a

more fragile bonding between cumulus cells and the oocyte (Ohashi et al., 1998; Gasparrini, 2002).

The results of several studies reviewed in Baruselli et al (2013) indicate that the potential for OPU-IVP in buffalo is a viable technology. Buffalo donors were submitted to OPU sessions every 20-30 days. Viable oocytes were subjected to IVP procedures, resulting in a blastocyst production rate of 44.9%. Pregnancy rates at 30 days were 43.5% and 37.1% for fresh or vitrified embryos, respectively. The rates are not as high as in cows but high enough to make it an alternative in those herds that have access to the technology.

9.4 Reproductive disorders

9.4.1 Uterine disorders

Abattoir surveys suggest that the incidence of endometritis in buffalo is higher than in cows. The data on the frequency of delayed uterine involution in postcalving buffalo are very variable but suggest that a considerable percentage of buffalo cows develop uterine infections and endometritis in the postpartum period (El-Wishy, 2007a). Buffalo are highly prone to dystocia, especially uterine torsion, frequently requiring obstetrical intervention, which subsequently leads to postpartum uterine infection. Poor hygiene, vaginal stimulation for milk letdown, and wallowing may be additional contributory factors. As in cows with puerperal metritis, *E. coli* seems to be the prevalent pathogen (Jadon et al., 2005; Azawi et al., 2008a, b). Buffalo cows suffering from endometritis later in the postpartum period are mostly infected with *Arcanobacterium pyogenes, Fusobacterium necrophorum, Prevotella melaninogenicus,* and other anaerobic bacteria (Jadon et al., 2005; Azawi et al., 2008c).

Diagnosis of uterine infections should be based on clinical examination, preferably including vaginoscopy and/or endometrial cytology, supported by bacterial culture and antibiotic sensitivity tests. However, in the areas where most buffalo are reared, access to the more sophisticated diagnostic methods may be severely limited, especially by the lack of funds. That is the reason for the use of such simple techniques as exploration of the

vagina with a gloved hand and the use of tools such as Metricheck™, which can lead to a dramatic increase in the detection rate of uterine infections. Adequate treatment could then be applied in a higher percentage of animals, with a reduced incidence of repeat breeding and better reproductive performance.

Recent publications have confirmed the existence of negative effects of uterine infections on the ovarian function of postpartum buffalo cows (Hanafi et al., 2008) similar to those found in cattle (Opsomer et al., 2000; Shelton et al., 2002).

Local antibiotic therapy is the treatment of choice. As endometritis is associated with the presence of persistent luteal tissue in a high percentage of buffalo cows, additional treatment with $PGF_{2\alpha}$ is recommended to improve uterine tone and evacuate uterine debris.

9.4.2 Ovarian disorders

The most important ovarian problem in buffalo is true anoestrus, ie, inactive ovaries. This is observed particularly during the hot summer months. Other problems are suboestrus/silent oestrus, delayed ovulation, and persistence of the corpus luteum. Compared with dairy cows, the incidence of cystic ovarian disease is low (1.8%).

a. True anoestrus

Inactive or nonfunctional ovaries are the most important cause of anoestrus and poor reproductive performance in buffalo. In a review by El-Wishy (2007b), it was reported that ovarian inactivity is more frequent (30%) in buffalo on low levels of feeding than in those (3%) on a high plane of nutrition and also more frequent in summer calving buffalo (41%-46%) than in those calving in other seasons (7%-33%). In the literature, a wide range of frequency of true anoestrus is reported, from 8% to 80%.

The administration of a GnRH analogue at 14 days postpartum supports the early resumption of ovarian activity. Induction of ovarian activity can also be achieved by the application of a progestagen implant over 9-10 days in combination with 600-700 IU of PMSG/eCG at implant removal. Fixed-time

insemination at 48 and 72 hours after implant removal is recommended (Virakul et al., 1988; Nasir Hussain Shah et al., 1990).

b. Suboestrus, oestrus synchronisation, and induction

Silent heat is the factor most commonly responsible for poor reproductive efficiency in buffalo. Based on the results of rectal palpation and/or progesterone analysis, a wide variation in the frequency of suboestrus (between 15% and 73%) was reported in buffalo 60-240 days postpartum (summarised in El-Wishy, 2007b). Suboestrus is more frequent during humid seasons, in undernourished buffalo suckling a calf, and in those calving in the hot season (reviewed in El-Wishy, 2007b).

Artificial control of the oestrous cycle has provided an effective means of increasing the reproductive capacity of buffalo by eliminating the need for frequent visual inspection for oestrus detection.

c. Delayed ovulation

If delayed ovulation is suspected, ovulation can be induced with the administration of a GnRH analogue or hCG (1.500 IU). As in cattle, an injection of GnRH or hCG can be administered at the time of artificial insemination. Alternatively, the complete Ovsynch protocol can be used.

d. Persistent corpus luteum

The results of rectal palpation with a 10-day interval, together with progesterone analysis, revealed prolonged luteal activity in 8% of buffalo not exhibiting oestrus before 60-90 days postpartum (Shah et al., 1990). Endometritis was diagnosed in 45% of these cases.

Regression of the persistent corpus luteum can be achieved with an injection of $PGF_{2\alpha}$. As the condition is often associated with uterine disorders such as endometritis or pyometra, it is recommended that the state of the uterus be assessed and treatment administered as necessary.

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There are very few complete accounts of reproduction in the Camelidae. Most of the published articles deal with *Camelus dromedarius* (Arabian camel), but reproduction is comparable across the whole Camelidae family: *Camelus bactrianus* (two-humped camel), *Lama glama* (llama), *Lama pacos* (alpaca), *Lama guanicoa* (guanaco), and *Vicugna vicugna* (vicuña).

10.1 Physiology

a. Camels

Except for the shape of the ovaries, the genital tract of the female camel is roughly comparable with that of the bovine, with two uterine horns in the abdominal cavity, a short cervix (3.5-5 cm), and a long vagina (30-35 cm). The ovaries are relatively small (10 g) and flattened bilaterally, bearing follicles of similar morphology to those of the bovine.

The Arabian camel reaches puberty at the age of 3-4 years, but females are not bred until they are 5-6 years old. Male camels reach puberty at about 3 years, but full reproductive activity is not developed until they are 6-7 years old. The reproductive life of a camel can extend to 20 years.

b. New world camelids

The majority of reproductive research in South American camelids has been undertaken in the two domesticated species, namely the alpaca (*Lama pacos*) and the llama (*Lama glama*). Far less information is available on the two nondomesticated species, the vicuña (*Vicugna vicugna*) and the guanaco (*Lama guanicoe*).

An excellent review of reproductive physiology in new world camelids was presented by Vaughan et al (2006). New world camelids have a bicornuate uterus with the body approximately 3 cm long and 3 cm in diameter. The left uterine horn is usually larger than the right in prepubertal females, and this difference is even more pronounced in multiparous females, because 98% of all pregnancies occur in the left horn. The ovaries of alpaca and llama are round to oval and globular in shape, with an irregular, firm texture and approximately (1.5-2.5) cm×1.2 cm×1.0 cm in size.

Puberty occurs at the age of approximately 6 months, but alpacas and llamas are usually not bred until the age of 12 months. Attainment of puberty is heavily dependent on the body weight of the animal, while conception rate and pregnancy maintenance is affected by the weight of the female at breeding.

10.1.1 Seasonality

The camel is a seasonally polyoestrous animal. The most commonly described season of pronounced sexual activity in the Northern Hemisphere is winter, but it can be altered under zoo conditions. Decreasing day length appears to be the stimulus for the onset of reproductive activity in camels (Musa et al., 1993).

In camels near the equator, factors such as rainfall, nutrition, and management may overcome the effects of photoperiod and allow for breeding throughout the year.

Alpacas and llamas are considered nonseasonal breeders. Breeding and parturition are, however, usually restricted by farmers to the rainy, warmer months of summer in South America (December-April) to ensure the availability of better quality pasture.

Peruvian vicuñas, in their natural habitat, breed in the Southern Hemisphere autumn, which is from March to May. In their nonnatural habitat, new world camelids are bred throughout the year (alpacas in Australia and New Zealand) or in seasons determined by climatic or nutritional factors (North America).

10.1.2 The oestrous cycle

Camelids are induced ovulators. Females require coital stimulation and ejaculation to induce ovulation of the dominant follicle and do not exhibit a luteal phase in the absence of mating.

a. Camels

Follicular growth takes place in regular waves throughout the breeding season. The oestrous cycle, compared to that of cattle, is incomplete and consists of pro-oestrus (follicular growth), oestrus (follicular maturation), and dioestrus (follicular atresia in unmated animals). Heats are observed every 20-25 days.

Oestrus, in which females are agitated and looking for males, lasts about 4-6 days. The external signs of heat are restlessness, bleating, rapid movements of the tail (up and down), mucous vaginal discharge, and swelling of the vulva. Luteinising hormone (LH) pulses, leading to ovulation, occur approximately 2 hours after mating and end 10 hours later (Driancourt, 1991, in: Thibault and Levasseur, 1991).

Ovulation in the camel is induced and occurs within 26-36 hours of mating. Three days after ovulation, serum progesterone rises and reaches peak values of between 3-5 ng/mL on day 7-8 after ovulation and then decreases to basal concentrations (<1 ng/mL) by days 12-14.

b. New world camelids

Ultrasonographic studies have shown that sexually mature llamas, alpacas, and vicuñas that have not been mated exhibit a continuous renewal of follicular waves (Vaughan et al., 2006). Sexual receptivity in South American camelids is associated with a low level of plasma progesterone; females are usually receptive to mating, regardless of the stage of follicular development, and refusal of a male by a female does not necessarily indicate absence of a mature follicle.

The mating behaviour exhibited by a sexually receptive South American camelid female may be divided into courting and copulatory phases. The courting phase occurs when the male actively chases the female. The copulatory phase occurs in sternal recumbency with legs tucked beneath the body. The interval between mating and ovulation is approximately 30 hours (range 24-48 hours) in the alpaca and llama (Tibary and Memon, 1999).

Ovulation occurs with equal frequency from the left and right ovary; however, most pregnancies are located in the left uterine horn.

Three to 5 days after mating (2-4 days after ovulation), a corpus luteum is formed at the site of ovulation concurrent with rising plasma progesterone levels from 4 to 6 days after mating. The lifespan of the corpus luteum is, therefore, 8-9 days. Females should be sexually receptive again approximately 12-14 days after mating if conception does not occur.

10.1.3 Pregnancy and parturition

a. Camels

The gestation length varies from 355 to 389 days as in most of the Camelidae. The cervical plug has unique properties and seals off the external cervical orifice during pregnancy in the camel (Guyton, 1991). The presence of the cervical plug is also an indication of pregnancy in this species.

Parturition occurs either standing or lying down and takes 24 to 40 minutes depending on the size of the foetus. If she had been recumbent, the camel immediately stands up after delivery, and never licks her calf. The placenta is usually expelled at the same time as the calf, though a delay of 15 minutes or longer is considered normal. With good nutrition, heat can occur within a month of parturition, but traditionally it is generally delayed for a year.

b. New world camelids

The literature reports variable gestation length in South American camelids with strong individual and seasonal variability and longest gestations in spring-mated females (351.0± 4.1), followed by summer (346.7±2.1), autumn (336.9±4.3), and winter matings (330.6±2.8) (Vaughan, 2001). The corpus luteum is the main source of progesterone throughout pregnancy and its presence is necessary to maintain pregnancy.

The interval from parturition to resumption of ovarian follicular activity is about 5-7 days. Mating and ovulation are possible by 10 days postpartum. Uterine involution is rapid in camelids and the gross anatomical involution is completed in the majority of the animals by 21 days postpartum.

10.2 Management of reproduction

a. Camels

Reproduction is commonly managed in one of two ways. The traditional one, mainly used in Africa, is based on the pastoral system. It is difficult to compare this with the commercial ranch management found in countries such as Saudi Arabia, Kenya, and Israel.

Under commercial conditions (Kenya), most of the births occur in May/June or November/January. Williamson and Payne (in: Mukasa-Mugerwa, 1985) noted that the young camel is one of the most fragile of mammals, especially during the first three weeks of life. Under traditional management, the mortality rate during the first year is estimated to be around 50%, of which 26% is in the first 6 weeks (Mukasa-Mugerwa, 1985), mainly because the calves are not fed enough colostrum.

b. New world camelids

There are currently two systems of production for South American camelids (SAC) around the world. The first is the traditional pastoral system in the dry Andean highlands, where limited financial and labour resources are available, and climatic conditions such as temperature and altitude can be especially challenging.

In the second system, the animals are maintained in a non-native environment (Australia, New Zealand, North America) where these species are bred not only for fibre but also as companion animals. Interest in applying modern reproductive technologies has increased in the last decade because the products from both domestic and wild species have become appreciated internationally, and several programmes to support such sustainable agriculture have been initiated in the Andean region.

10.2.1 Reproductive parameters

In the main, young females are not mated before they are 4 to 6 years old. It can be earlier in domestic vicuñas, but wild vicuñas are not fertile before 2 or 3 years of age. Most of the literature records a conception rate of 40% to 50%.

In the majority of females, the interval between successive parturitions is generally around 2 years, but can vary, depending on the management system. Under commercial ranch management, intervals between 14 and 18 months were recorded in some milk herds by Wilson (1989). In Watson's report (in: Mukasa-Mugerwa, 1985), 73% of females were not pregnant for 12 months after calving, and 74% of the calves were not weaned under a year old. See Table 1.

Interval between calvings (months)	**Number of camels**
12	1
14	1
15	1
16	1
24	14
30	1
36	7
Parturition-conception interval (months)	
1-3	1
3	2
6	2
7-11	19
Age at weaning (months)	
3	1
6-11	6
12	17
24	3

Table 1 from: Mukasa-Mugerwa, 1985

10.2.2 Mating and artificial insemination

a. Mating

Controlled mating with selected, genetically valuable males has been practised for a long time in the breeding of both camels and alpacas and llamas. The main drawback of controlled breeding is the fact that males are often selected solely on the basis of their external features or sporting performance (racing camels), while their reproductive ability, especially the quality of their semen, is rarely tested.

b. Artificial insemination

Much more research is required before the benefits of AI can be fully exploited, including determination of the optimum time for insemination, semen dose, and the use of hCG or GnRH to induce ovulation.

Collection of semen from camelids presents many difficulties, due to the nature of their copulatory behaviour and the slow (dribbling) process of ejaculation. The main techniques used are the artificial vagina, electro-ejaculation or postcoital aspiration from a female. Of these, the artificial vagina and electroejaculation are most often used and ensure the higher hygienic standards. Successful collection of semen by artificial vagina has

been reported in camels (review: Bravo et al., 2000; Mosaferi et al., 2005), alpacas (Vaughan et al., 2003) and llamas (Lichtenwalner et al.,1996b).

Camel semen has the following characteristics:
- volume: 3.5 mL (1-10 mL)
- colour: white
- appearance: viscous
- concentration: 140-760 million/mL
- pH: 7.8 (7.2-8.8)
- live sperm: 55%
- spermatozoa/dose: 400 million

Artificial insemination with fresh semen is a well-established technique in camels, and an increasing number of reports demonstrate good results after insemination with fresh semen (Al Eknah et al., 2000, Aminu et al., 2003).

One of the problems with artificial insemination in camelids is ensuring that the inseminated females ovulates. Therefore, ovulation is induced with either 3,000 IU hCG or 20 µg of buserelin either 24h before insemination or at the same time as insemination.

In South American camelids, mostly undiluted or diluted fresh semen is used, with pregnancy rates reaching as high as 68%, while insemination with cooled or frozen semen is still not readily available (Miragaya et al., 2006).

10.2.3 Pregnancy diagnosis

Ultrasound is the most reliable method to detect pregnancies in camels from as early as day 18 of gestation. However, in the absence of ultrasound machines, the traditional method for pregnancy diagnosis is the "tail cocking" method. A male camel is led past the females; those that are pregnant curl their tails upwards and those that are not pregnant do not. Some practitioners use rectal palpation (as in the cow) to check for pregnancy and for the presence of a corpus luteum on the ovary. Since the camel is an induced ovulator, a corpus luteum is usually only palpable during pregnancy (Mukasa-Mugerwa, 1985).

Bono et al (1992) proposed a test based on serum levels of oestrone sulphate 15-20 days after mating.

Most pregnancies develop in the left uterine horn (Musa and Sineina, 1976 in Mukasa-Mugerwa, 1985), early embryo migration being very frequent in the camel (and also observed in the llama) (Shalash, 1965 in: Novoa, 1970). Twin births are reported in only a few studies, recording a twinning rate of only 0.4% (Musa et al., 1976). Multiple ovulations occur relatively frequently, but it appears that either only one ovum is fertilised or further embryos perish very early.

10.3 Control of oestrus

Extensive research and development have been undertaken in oestrus synchronisation and controlled breeding in cattle, which is not matched in the Camelidae family.

10.3.1 Induction of oestrus

Both in camels and South American camelids with a functional corpus luteum, oestrus can be induced with an injection of $PGF_{2\alpha}$ analogue.

Various progestagen preparations have also been used in camels and alpacas in order to induce and synchronise oestrus and ovulation. Bono et al (1992) reported the use of progestagens in combination with eCG/PMSG in the dromedary as a safe and efficient technique for inducing fertile heats. Bourke et al (1992) also mentioned the use of implants over a seven-day period in a superovulation programme in the llama.

eCG/PMSG in doses ranging from 1,000 to 8,000 IU has been used to induce oestrus in both the nonbreeding and breeding seasons in camels, but the number of pregnancies achieved has been found to be very low (Al Eknah, 2000).

10.3.2 Induction of ovulation

Ovulation in the camel has been successfully induced by a single treatment with GnRH or hCG. Skidmore et al (1996) administered 20 mcg of GnRH analogue or 3000 IU hCG when the dominant follicle measured 0.9-1.9 in diameter.

A dose of 8 mcg of GnRH at the time of mating has been tested in llamas with good results (McEvoy et al., 1992). Ovulation occurred 29 hours after injection. Cooper et al (1992) observed the same effect in the dromedary using a dose of 20 mcg.

A recent study by Skidmore et al (2009) compared various pharmacological methods of synchronising ovulation in dromedary camels. The reported results indicated that two GnRH (20 mcg buserelin) injections 14 days apart, or two GnRH injections 14 days apart with an intervening dose of $PGF_{2\alpha}$ (500 mcg cloprostenol) 7 days after the first GnRH, were the most effective methods of synchronising ovulation. In a study reported in 2008, Nikjou found the double injection of 20 mcg GnRH analogue, buserelin 14 days apart, to be suitable for synchronising the emergence of a follicle wave in Bactrian camels (Nikjou et al., 2008).

10.3.3 Superovulation

Superstimulatory treatments consist of the administration of gonadotrophic hormones, eCG/PMSG or porcine follicle stimulating hormone (pFSH)) after synchronisation of the follicular wave using a natural luteal phase (induction of ovulation) or an artificial luteal phase (with exogenous progesterone).

eCG/PMSG has been tested using doses from 5,500 to 8,000 IU, 48 to 72 hours before mating. The results showed a 100% calving rate, but the trial only involved 7 animals. In the llama, 1,000 IU is an appropriate dose for superovulation (Bourke et al., 1992). Purified ovine FSH has also been tested in the dromedary (Cooper et al., 1992), but with poor results.

More recent studies have shown that a combination of 2000 IU PMSG/eCG injected on day 1 of treatment and 400 IU porcine FSH given in gradually declining doses over 4 days (days 1-4 inclusive) give better stimulation results with between 4-35 mature follicles developing in the ovaries (Skidmore et al., 2002).

10.4 Reproductive disorders

In common with cattle and horses, the camel can suffer from uterine infections in the postpartum period, with an incidence varying from 53% to 71% (using the bovine classification). The organisms isolated are the same as in the bovine (Wernery et al., 1992).

Both abortion and stillbirth are known to occur in the camel. The incidence of brucellosis (*B. melitensis* and *B. abortus*) varies between countries (1%-26%). Other important infective causes of abortion are trypanosomiasis, pasteurellosis, and salmonellosis.

In new world camelids, the most common reasons for presenting infertile females for examination are repeat breeding (75%) pregnancy loss (18%), visible abnormalities of the genitalia (5%), and continuous rejection of the male (2%) (Tibary, 2004a,b; Tibary et al., 2001).

a. Persistent luteal activity

Persistent luteal activity is encountered relatively frequently in practice. Affected females show a high level of serum progesterone and reject the male. Injection of $PGF_{2\alpha}$ analogues, such as cloprostenol (175 mcg IM for alpacas, 250 mcg for llamas), are recommended, although commercially available products are rarely licensed for use in these species. Dinoprost tromethamine should be used with extreme caution, as signs of toxicity (respiratory distress and death) have been reported in some animals (Fowler, 1998).

b. Ovulation failure

Failure of ovulation occurs commonly both in camels and new world camelids. GnRH analogues (4 mcg) or hCG (1,500 IU) are used in practice, either as a means of prevention or in order to increase pregnancy rates in alpacas and llamas, while the doses of 20 mcg of buserelin or 3,000 IU hCG are used in camels.

c. Uterine infections

Uterine infection is the most commonly acquired reproductive problem, resulting in infertility in camelids. Uterine infection should be suspected in females that have a history of repeat breeding, early embryonic death or dystocia, and/or retained foetal membranes during their most recent parturition.

Postpartum metritis may be accompanied by systemic signs, while chronic endometritis may go undetected. There are no well-established treatment schedules for uterine infections in camelids, as few antibiotic preparations are licensed in these species. Prostaglandins and oxytocin are sometimes used, but with variable success.

d. Embryonic loss

Early embryonic mortality is common in camelids and is estimated to affect 10%-15% of all pregnancies in the first 60 days of pregnancy (Vaughan et al., 2006). The incidence of embryonic loss can be much higher in extreme conditions, since nutritional and climatic factors seem to have a profound effect on the maintenance of pregnancy in camelids. So far, little is known of the efficacy of pharmacological attempts to support luteal function with progesterone, GnRH, or hCG in these species.

10.5 Acknowledgements

The assistance and critical review by **Dr. J.A. (Lulu) Skidmore** (Camel Reproduction Centre, Dubai, UAE) and **Dr. Marc-Antione Driancourt** (MSD Animal Health, Angers, France) are very much appreciated.

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Industrial-scale rabbit farming is found mainly in Belgium, France, Hungary, Italy, and Spain (Szendro et al., 2012). Medium-sized and small rabbit farms are found in other countries, including countries in South America and Asia (Szendro et al., 2012). Development of rabbit farming is based on a better scientific understanding of and focus on the management of reproduction.

11.1 Anatomy and physiology

11.1.1 Female rabbit

The female rabbit or doe has small, oval-shaped ovaries and two independent uteri (about 7 cm long) that open separately through two cervical ducts into the vagina.

The female rabbit does not have an oestrous cycle with regular periods of oestrus or "heat" during which ovulation occurs spontaneously. Female rabbits are in oestrus more or less permanently. From October to December in the Northern Hemisphere, rabbits may molt and many female rabbits do not conceive during this period.

a. Puberty, mating, and reproductive life span

Acceptance of mating appears long before the ability to ovulate and bear a litter. Female rabbits are able to mate at 10-12 weeks old, but do not usually ovulate at this age. The first follicles appear on the thirteenth day after birth and the first antral follicles at about 65-70 days old. The onset of puberty varies greatly with the breed: it occurs earlier in small or medium breeds (4-6 months) than in large breeds (5-8 months). Female rabbits born in summer reach puberty later than those born in other seasons (Kamwanja and Hauser, 1983). Female rabbits fed ad libitum reach puberty 3 weeks earlier than similar female rabbits receiving only 75% of the same daily feed (Lebas et al., 1986). The fertility of female rabbits is influenced by a large number of factors, particularly temperature, light and feeding. Increasing daily light exposure may improve litter size in pubertal female rabbits (Kamwanja and Hauser, 1983).

Female rabbits are usually mated when they reach 80%-85% of the mature weight for their breed. This means that young female rabbits are usually mated for the first time at 16-17 weeks of age but, since puberty is generally reached earlier (Rommers et al., 2001), it is important to take male rabbits away from the litter before 10 weeks of age.

The reproductive life of a female rabbit on a commercial farm is generally around 1 year, with an average of 6 pregnancies (Rosell and de la Fuente, 2009). Selection programs, which focus on the length of the productive life of female rabbits and the number of inseminations after the first fertile breeding, could increase reproductive longevity and decrease the replacement rate (Piles et al., 2006).

b. Hormonal changes associated with ovulation

The rabbit is an induced ovulator (Morrell, 1995). Mating induces a neuro-endocrine reflex, which provokes a luteinising hormone (LH) surge leading to ovulation (Bakker and Baum, 2000). The LH pulse rate increases within 10-15 minutes of sexual stimulation, reaching a plateau for at least 1 hour (Jones et al., 1976). Follicle stimulating hormone (FSH) is released in frequent pulses, while LH returns to basal levels by 5-6 hours after breeding (Dufy-Barbe et al., 1973) (Figure 1). Simultaneously, the hypothalamus secretes oxytocin and the ovaries secrete prostaglandin facilitating ovulation. Ovulation takes place 10-12 hours after breeding.

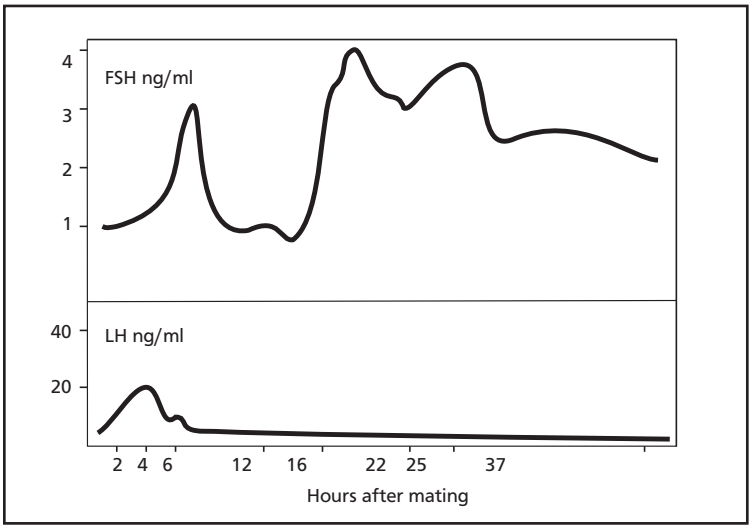

Figure 1. Evolution of FSH and LH secretion after breeding (Dufy-Barbe et al., 1973).

c. Fertilisation

Muscular activity of the oviductal isthmus (11.7-18.7 contractions per minute during oestrus) is increased within 2 hours of ovulation (natural or induced) and this lasts for 2-3 days (Bourdage and Halbert, 1980). This ensures that the ova, which are fertile for 6-8 hours, and sperm cells, which are fertile for 4 hours, meet at the optimal time for fertilisation (Szendro et al., 2012). Preovulatory transport of sperm is rapid, ensuring that it reaches the fertilisation area in the distal ampulla, near the isthmus 30 minutes after coitus. After ovulation the ova is transported slowly though the isthmus, reaching the uterus 72 hours after ovulation, suggesting that gamete transport may be regulated by the oviductal musculature (Bourdage and Halbert, 1980). Implantation takes place 7 days after breeding, at the blastocyst stage. Progesterone concentrations increase from day 3 to 15 postbreeding and remain elevated until just before parturition.

d. Pseudopregnancy

Pseudopregnancy is a normal physiological event that follows an unsuccessful or infertile mating and lasts for 15-19 days before resolving spontaneously. Initially, the corpus luteum and uterus develop as in pregnancy. The corpus luteum does not appear to be susceptible to luteolysis prior to day 9 (Boiti et al., 1998), but normally starts to regress at around day 12. Pseudopregnant female rabbits may have mammary gland enlargement and exhibit nesting behaviour. Rabbits for artificial insemination (AI) should be housed separately for at least 19 days prior to insemination to avoid pseudopregnancy.

e. Pregnancy

Pregnancy in the rabbit lasts 31 days (range of 30-33 days). If pregnancy is shorter than 29 days, the newborn rabbits or kits are not usually viable. A minimum of four corpora lutea may be necessary for the successful maintenance of pregnancy in some breeds, such as the New Zealand White rabbit (Feussner et al., 1992). The minimum number of corpora lutea required may be strain-dependent and may bear a relationship to the normal litter size of the strain (Feussner et al., 1992).

f. Parturition

At the end of gestation, the female rabbit makes a nest with its own fur and any other materials available, such as straw and shavings. This behaviour is linked to an increase in the oestrogen/progesterone ratio and to increasing concentrations of prolactin. Rabbits normally give birth during the day, and day length (photoperiod) plays a role in determining the sensitivity of the rabbit uterus to oxytocin in late gestation (Ninomiya-Alarcón et al., 2004). Parturition or kindling lasts for 15-30 minutes, depending on the size of the litter. On average there are 7-9 kits per litter (range 3-12). Young rabbits are usually weaned at 30-42 days of age.

11.1.2 Male rabbit

a. Anatomy, puberty, and reproductive life span

The male rabbit, or buck, has a scrotum containing oval-shaped testes that remain in communication with the abdominal cavity and can be withdrawn. The testes descend at about 2 months of age. The short, backward-slanting penis points forward when erect.

The first manifestations of sexual behaviour appear at 8-10 weeks of age. Sexual maturity, defined as the moment when daily sperm production ceases to increase, is reached at 32 weeks of age in New Zealand White rabbits in temperate climates. However, a young buck under these same conditions can be used for mating from 20 weeks of age.

On commercial rabbit farms, bucks are usually replaced after 1 year, mostly due to lack of libido. In the wild, male rabbits can remain sexually active for 5-6 years.

b. Mating

Male rabbits are usually kept in their own 'territory,' and female rabbits to be bred naturally are put into the buck's cage. This has the advantage that mating proceeds more quickly since the buck does not need to investigate a new territory. False mounting occurs 1-2 minutes before breeding and this increases the concentration of the ejaculate. Maximum production of spermatozoa is obtained by using the buck regularly once a day.

c. Semen

Rabbit semen has the following characteristics

- Volume 0.5 mL (0.3-0.6 mL)
- Concentration 150-500 million spermatozoa/mL
- pH 6.8 to 7.3

i. Artificial insemination

Rabbit semen is collected for AI using an artificial vagina. Semen quality needs to be evaluated before it is used or preserved and stored. A subjective assessment of the proportion of motile sperm and the motility pattern allows for the identification and exclusion of poor quality ejaculates. There is no real effect of season on semen quality, although ejaculates collected in March are better in terms of volume, raw motility, individual motility, and number of live spermatozoa than those collected in November (Théau-Clément et al., 1991). There is a significant correlation between kindling rate and the percentage of total motile cells (assessed by computer-assisted sperm analysis), linearity index and the percentage of abnormal sperm in the sample (Lavara et al., 2005).

11.2 Management of reproduction in commercial rabbits

Commercial rabbit farming is increasingly profitable due to improvements in the management of reproduction and genetic selection.

11.2.1 Management systems

There are three main systems used in rabbit reproduction.

a. Intensive systems

The intensive system is based on a 35-day cycle. In this system, does are bred 2-4 days after parturition based on gestation lasting 31 days. Young rabbits are weaned at 28 days old or less. This system is associated with lower conception rates and litter size than the semi-intensive system. It is generally considered too intensive for large-scale farms, although it can result in a higher number of weaned rabbits (eg, 50-60 per annum) due to the higher number of litters per female rabbit per year (Szendro et al., 2012).

b. Semi-intensive systems

The semi-intensive system is the most commonly used system in commercial rabbit production and on large farms. This system is based on a 42-day cycle. Does are bred 11 days after parturition based on gestation lasting 31 days. Young rabbits are weaned at 28-35 days old. In this system, if does are always inseminated on a Friday, then parturition will usually always take place on a weekday (Monday, Tuesday, or Wednesday) (Szendro et al., 2012). Semi-intensive systems appear to be better in terms of the reproductive performance of female rabbits and litter viability than intensive management systems (Rebollar et al., 2009). In this system, 45-55 weaned rabbits can be produced per annum.

c. Extensive systems

The extensive system is the system used most commonly by hobby rabbit-keepers and this is based on a 49- or 56-day cycle. In this system, does are bred 18 or 25 days after parturition, based on gestation lasting 31 days. Young rabbits are weaned at 35-42 days old. In this system, 30-35 weaned young can be produced per annum.

11.2.2 Breeding

a. Natural mating

Natural mating, based on mating on one or two fixed days every week, is widely used on small and medium-sized rabbit farms, where there are less than 100 female rabbits. Female rabbits are usually mated twice, using two different male rabbits, one immediately after the other (Szendro et al., 2012). In this semi-intensive system, under favourable conditions, a female rabbit is mated on the same day of the week once every 6 weeks.

b. Artificial insemination

AI has been used in rabbits since the 1950s (see, for example, Murphree et al., 1951). It came into use on commercial rabbit farms in Europe in the 1980s (Théau-Clément 2007) where it is now used widely, particularly on large rabbit farms.

There are five main steps in the AI of rabbits (Szendro et al., 2012)

- Collection of semen from the male rabbits
- Visual and microscopic examination of semen
- Dilution of good quality semen (10 to 20 times)
- Insemination of female rabbits using a pipette
- Administration (intramuscular injection) of a GnRH analog into a hind limb at the time of insemination

AI allows hundreds or even thousands of female rabbits to be inseminated on the same day (Szendro et al., 2012). It also means that well organised cyclical production (eg, insemination, pregnancy diagnosis, parturition, weaning) is possible (Szendro et al., 2012). Additionally, AI brings the same benefits as it does in other species; the control of genetic diversity, rapid progress in genetic improvement, pregnancy in female rabbits that refuse to mate, and reducing the spread of infection.

There has been a lot of research into improving reproductive performance following AI. This has focussed on semen handling (eg, quality, quantity, preservation, and storage) and reproductive performance. Conception rates following AI can be equivalent to, or even better than, those achieved by natural mating. Interestingly, the number of spermatozoa used for AI does

not appear to affect the reproductive performance (Castellini et al., 2006; Viudes de Castro and Vicente, 1997). Reproductive performance is influenced by the physiological status of the female rabbit (ie, parity, stage of lactation, and receptivity) at the time of insemination (Brun et al., 2002). There is an effect of season, with female rabbits mated in July and October having significantly lower fertility than those mated at other times (Théau-Clément and Vrillon, 1991).

i. Fresh semen

High fertility and prolificacy in rabbits is currently only achieved using fresh sperm. Fresh semen usually averages 84% live (unstained) spermatozoa, of which 88% have normal acrosomes (Chen et al., 1989). After quality assessment, the ejaculate is diluted with a suitable semen extender and can be stored for a few hours at 18°C.

ii. Cooled semen

Rabbit semen can be preserved effectively for up to 96 hours at 15°C using Tris-buffer extenders (Roca et al., 2000). Glucose- and fructose-based semen extenders containing gelatin (1.4 g/100 mL) have been tested in a controlled study after storage of semen at 15°C for up to 120 hours (Lopez-Gatius et al., 2005). Semen can also be preserved for 24-36 hours at 5°C using a special diluent, with resultant fertility rates of around 64% (Théau-Clément and Roustan, 1992). Kindling rates for female rabbits inseminated with gelatin-supplemented semen stored for 48 hours (88%) or 72 hours (83%) were similar to those recorded for controls (81%), whereas rates significantly decreased when the semen was solid and stored for longer (Lopez-Gatius et al., 2005).

iii. Frozen semen

The main limitation to AI in rabbits was the ability to preserve semen (Roca et al., 2000). The choice of male (differences in freezing resistance of sperm) may affect the outcome (Moce et al., 2005). Many protocols and extenders (usually with egg yolk and dimethyl sulfoxide (DMSO) or acetamide) have been developed for the cryopreservation of rabbit semen (Mocé and Vicente, 2009). Most of these protocols include slow cooling and cryopreservation in liquid nitrogen (Mocé and Vicente, 2009).

However, semen frozen in liquid nitrogen (44% live sperm, 54% of which have normal acrosomes) generally still produces poorer results than fresh semen (Chen et al., 1989; Roca et al., 2000). This does not mean that it is not possible to achieve good conception rates (Morell, 1995), and there are some studies that report fertility rates (73.9% for a single freeze-thaw cycle) with frozen semen that are similar to those achieved with fresh semen (Si et al., 2006). Despite much research, the reproductive performance following insemination with cryopreserved sperm means that it is still not used in commercial rabbit farming (Mocé and Vicente, 2009).

11.2.3 Pregnancy diagnosis

Pregnancy diagnosis is usually performed by abdominal palpation around 11 days (10-14 days) after breeding. Plasma progesterone concentrations can be measured, using enzyme-linked immunoassay (ELISA) kits developed for use in other species, 17-18 days after breeding to differentiate pregnant from pseudopregnant rabbits (Morrell, 1990 and 1993). Abdominal ultrasound can be used to diagnose pregnancy in rabbits from 7-8 days after breeding, although more accurate diagnosis may be possible on day 9 (Inaba et al., 1986). Pregnant female rabbits can then be housed and fed appropriately during late pregnancy while nonpregnant females can be removed and put with the next batch to be mated.

11.3 Management of reproduction

A number of methods are used to improve receptivity and ovulation and the results of AI in rabbits. Some of these methods rely on treatment with reproductive hormones while others are management tools, such as doe-litter separation and day length manipulation. Frequently a combination of these approaches is used. The effectiveness of any of these methods depends on the status of the does (eg, age) with the best results in female rabbits after the first litter (Szendro et al., 2012).

11.3.1 Receptivity and oestrus synchronisation

Receptivity – the willingness to allow mating – is an important factor that is linked to fertility in female rabbits. Sexual receptivity can be assessed by the colour of the vulva, which is an external sign of oestrogen concentrations

and thus the stage of oestrus (Caillol et al., 1983). The influence of receptivity on fertility, as assessed by conception rates, is shown in Table 1.

Vulvar color	White	Pink	Red	Dark red
Fertility	35%	55%	75%	40%

Table 1. Influence of receptivity (assessed by the colour of the vulva) on fertility at AI (Théau-Clément and Roustan, 1992)

a. Pregnant mare serum gonadotropin

Pregnant mare serum gonadotropin (PMSG, equine chorionic gonadotropin, eCG) is commonly used to increase the receptivity, conception rate, and litter size of lactating does (Szendro et al., 2012). However, it is not effective in female rabbits that are not sexually receptive and it can be associated with lower weaning rates. In addition, repeated use may be associated with antibody production and lower conception rates.

b. Pregnant mare serum gonadotropin plus a gonadotropin-releasing hormone agonist

Administration of PMSG (40 IU) 48 hours before breeding is often combined with the administration of a gonadotropin-releasing hormone (GnRH) agonist (buserelin or gonadorelin, 0.2-0.35 mL) at the time of AI (Molina et al., 1991; Remmen et al., 1979) (Figure 2). It is also possible to use lower doses (20 IU) of PMSG 48 hours before breeding (Castellini et al., 2006; Remmen et al., 1979).

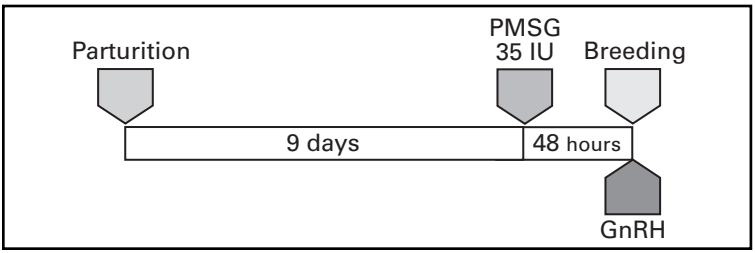

Figure 2. Example of a protocol that could be used to manage receptivity

c. Management methods

Management methods that result in the biological stimulation of does can be used instead of PMSG (Théau-Clément, 2007). There are three different management methods that can be used: photoperiod manipulation, doe-litter separation, and changing nursing methods. All three methods are good alternatives to hormonal (PMSG) treatments and are easy to use on farms (Szendro et al., 2012).

i. Photoperiod manipulation

In the wild, rabbits stop breeding when the day length decreases (from late autumn to early spring). Rabbits start coming into oestrus as day length increases in the spring (Szendro et al., 2012). This effect can be recreated in housed rabbits by artificially increasing day length. This is used commonly to improve receptivity and synchronise oestrus in rabbits (Quintela et al., 2001). Longer day length (12-16 hours light) for 6-8 days before AI improves sexual receptivity and increases the conception rate by about 10% (Quintela et al., 2001; Szendro et al., 2012). The use of photoperiod manipulation before AI may decrease the body weight of kits at weaning, presumably due to effects on milk production and/or feed intake (Maertens and Luzi, 1995).

ii. Doe-litter separation

Most does go into heat shortly after weaning. When milk production ends, the inhibitory effect of prolactin on the oestrous cycle is diminished (Szendro et al., 2012). Female rabbits usually allow the kits to suckle once per day. If more than 24 hours elapses between suckling, prolactin concentrations fall and there is an increase in receptivity and in the response to GnRH administration. Thus, receptivity and fertility can be improved by temporarily separating the female rabbit from its litter (Ubilla et al., 2000), by, for example, closing the nesting box on day 9 after parturition, and only allowing kits to suckle for 10 minutes on days 10 and 11 (Rebollar et al., 2008). Doe-litter separation for 48 hours produces higher conception rates than 24- or 36-hour doe-litter separation (Szendro et al., 2012). Doe-litter separation for 24 to 48 hours before AI produces similar improvements in sexual receptivity as the administration of PMSG (Rebollar et al., 2006 and 2008). Doe-litter separation does not appear to have an effect on litter size but the weight of individual kits at weaning may be lower.

iii. Changing nursing methods

This is a form of doe-litter separation that involves only allowing the doe and litter to have contact for about 20 minutes in the morning for 2-3 days prior to AI (Szendro et al., 2012). The most effective methods of separating a doe from its kits are by using a metal plate or physically moving the nest tray by 5 m (with or without visual contact). This type of management method has been shown to increase receptivity and conception rates by 15%-27% (Szendro et al., 2012).

d. Combined methods

It is not uncommon to combine the management methods described above with reproductive hormone treatment to better synchronise oestrus in rabbits. Photoperiod manipulation is frequently used in conjunction with PMSG not only to improve receptivity and better synchronise oestrus prior to AI but also to increase global productivity, the number of weaned rabbits per 100 female rabbits inseminated (Quintela et al., 2001).

e. Housing

It is also essential that rabbits are housed appropriately. Temperature extremes can reduce productivity. Excessive heat in summer may decrease the number of kits that survive to weaning, due to lower feed consumption, lower milk yield, and higher kit mortality (Szendro et al., 2012). Low temperatures can also be problematic. The effect of season on reproductive performance can be minimised using suitable housing, fixed lighting programs (16 hours light – 8 hours darkness), and appropriate environmental control, including adequate ventilation (Szendro et al., 2012).

11.3.2 Inducing ovulation

Inducing ovulation is an essential element of AI of rabbits. Ovulation can be induced reliably by the presence of a vasectomised buck, or the administration of a GnRH agonist (eg, buserelin, gonadorelin) or human chorionic gonadotropin (hCG). However, hCG is no longer used because, irrespective of the dose, the effectiveness of treatment decreases with repeated use (more than five treatments) (Molina et al., 1991).

a. GnRH agonists

i. Intramuscular administration

GnRH acts on the pituitary to induce the release of both LH and FSH. Plasma concentrations of LH peak 10-30 minutes after the intramuscular injection of a GnRH agonist (eg, buserelin, 0.2 mL / 0.0008 mg; gonadorelin, 0.020 mg). Ovulation occurs approximately 10-12 hours later. Intramuscular administration of GnRH agonists is still common practice on many rabbit farms.

ii. Intravaginal administration

Intravaginal administration of GnRH analogs can also be used. This is most commonly done by adding the GnRH analog to the semen used for AI (eg, 0.0016 mg or 0.01 mg buserelin). This results in similar ovulation rates but a higher number of kits born than intramuscular administration (Quintela et al., 2004; Rebollar et al., 2012). When lower doses of a GnRH agonist are used (eg, 0.0005 mg buserelin), the pregnancy rate, kindling rate, and average kits born live are similar to those obtained following intramuscular administration (0.001 mg buserelin, Viudes de Castro et al., 2007). The pregnancy rate in rabbits administered 0.005 mg buserelin in 0.5 mL semen is affected significantly by the seminal dose. The highest pregnancy rate was seen with the lowest dose of spermatozoa (6 million), although the number of implanted embryos and litter sizes was unaffected.There also appears to be a significant effect of dilution of the seminal dose on the bioavailability of buserelin after intravaginal administration, with higher fertility associated with higher dilution rates (1:20) (Vicente et al., 2011; Viudes de Castro et al., 2014).

11.4 Induction of parturition

a. Oxytocin

Oxytocin is involved in facilitating and maintaining parturition in rabbits. Oxytocin concentrations remain low in rabbits throughout gestation and rise only once uterine contractions start during parturition (Fuchs and Dawood, 1980; O'Byrne et al., 1986). Injections of oxytocin (0.15-0.2 mL) caused a dose-dependent increase in plasma oxytocin concentrations and uterine activity (Fuchs and Dawood, 1980).

b. Prostaglandins

Prostaglandins have been known to play an important role in luteal function in rabbits since the early 1970s. In the rabbit, prostaglandins are used to induce luteolysis and thus to control the timing of parturition. Prostaglandin administration on day 28 or 29 of gestation reduces LH concentrations but does not alter prolactin or FSH concentrations. Cloprostenol 0.004-0.018 mg (Partridge et al., 1985) or etiproston 0.050 mg (Ubilla and Rodriguez, 1989) are followed by kindling within 30-60 hours (Partridge et al., 1985). Live birth weights tend to be lower but kit viability does not appear to be affected. The conception rate after breeding on day 1 postpartum and the milk yield during the first week were also unaffected (Partridge et al., 1985).

11.5 Pet rabbits

11.5.1 Female rabbits

a. Ovariohysterectomy

Female rabbits can be spayed (ovariohysterectomy), from about 4 months of age, to prevent unwanted pregnancy, aggressive behaviour, and urine spraying (territorial marking behaviour) (Brower, 2006).

Ovariohysterectomy is also the treatment of choice for a number of conditions in female rabbits (Redrobe, 2000), including

- Endometrial polyps/cystic hyperplasia and uterine neoplasia, which occur in intact female rabbits over 2-3 years of age
- Pyometra and endometritis, which are common problems in female rabbits (including female rabbits that have never been bred), with *Pasteurella multocida* and *Staphylococcus aureus* isolated commonly

b. Hormonal control of oestrus

There are few reports of the use of progestogens (progestins) to suppress or postpone oestrus and ovulation in female rabbits. One study showed that medroxyprogesterone acetate inhibited mating-induced ovulation for 40-65 days and prevented fertilisation following hCG-induced ovulation from 15 days up to 83 days posttreatment (Chang, 1985). Proligestone has also been used at dose rates of around 30 mg/kg in female rabbits (Keeble, 2001).

c. Mismating

The progesterone-receptor antagonist aglepristone (10 mg/kg by subcutaneous injection on 2 days) has been shown to induce abortion (19-72 hours posttreatment) in female rabbits when administered on days 15 and 16 postmating (Özalp et al., 2008). Aglepristone treatment led to resorption of embryonic fluid and abortion when administered to mid-gestation rabbits (Özalp et al., 2013). Vaginal bleeding and discharge started 1-2 days after commencing treatment, with complete expulsion of foetuses within 2-4 days. Aglepristone appears to be well tolerated by rabbits and can be used as early as 6-7 days after breeding (Özalp et al., 2010). A short nonreceptive period and short period of decreased food consumption may be seen after treatment (Özalp et al., 2008).

d. Dystocia

Dystocia (difficult parturition) is rare in rabbits (Redrobe, 2000). Obesity, nutritional deficiency and foetal deformities, foetal oversize, uterine inertia, narrow pelvic canal (congenital or as a sequel to fractures) may all contribute to dystocia. In nonobstructive dystocia, where uterine inertia is suspected, 5-10 mL of calcium gluconate (10%) followed by oxytocin (1-2 International Units (IU) intramuscularly) 30 minutes later, can stimulate uterine contractions. The female rabbit should be placed in a dark, quiet area and left undisturbed for 40-60 minutes. A Caesarean section or ovariohysterectomy may be performed if no young are produced, depending upon the viability of the foetuses and state of the uterus.

11.5.2 Male rabbits

a. Neutering

Neutering (castration) of male pet rabbits is used to manage reproduction, aggression against other males and humans, and urine spraying (territory marking). Recently an implant containing the GnRH agonist deslorelin (4.7 mg) was tested but did not appear to provide an alternative to surgical castration (Goericke-Pesch et al., 2014). Male rabbits are best neutered shortly after they reach sexual maturity (from 4-6 months of age; up to 9 months of age in giant breeds). Neutered male rabbits should not be put with intact female rabbits for at least 3 weeks after neutering, as live sperm may still be present in the vas deferens, and testosterone levels drop slowly.

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12.1 Introduction

The propagation of fish has been practised from time immemorial in different parts of the world. Aquaculturists, especially those involved in the rearing and propagation of fish, often have the disadvantage of cultivating species whose seed supply depends largely on capture from the wild.

Techniques for the production of adequate quantities of high-quality fish seed from captive broodstock are necessary for the continuation and large-scale expansion of aquaculture. The lack of such techniques has been an important constraint in the controlled and successful culture of several cultivable species. The development of such techniques would provide the opportunity to develop a closed-cycle production system that does not need to rely on catching wild broodstock and/or offspring and would open the door for genetic improvement programs and better disease control.

Top-class fish reproduction management must aim to achieve the physiological potential of each fish species to produce offspring of high quality, quantity, and desired gender, including sterile fish.

This review has a dual purpose: firstly, to discuss briefly the reproductive physiology of fish, with the emphasis on cultivated species, and, secondly, to indicate areas of reproductive physiology in which artificial intervention is required in order to breed cultivated fish successfully in captivity. This evaluation takes into account the teleost fish as a group, without reference to the large variation in reproduction parameters between species.

12.2 Physiology and conditioning

As in mammalian species, the hormonal pathway of reproduction revolves around the hypothalamic-pituitary-gonadal axis (Figure 1). The hypothalamus is stimulated by environmental and chemical factors such as pheromones.

Following this stimulation, different neuropeptides [gonadotrophin-releasing hormones (GnRH)] are synthesized and released. Different fish species possess different forms of GnRH (Somoza et al., 2002; Sherwood and Wu, 2005), ranging between two and three forms per species. Despite the multiplicity of GnRH forms in the fish, only one regulates the production and

release of gonadotrophins (GtH) by the pituitary. This species-specific form of GnRH is produced in the brain's preoptic area and is the only one that projects directly into the pituitary through neurosecretory fibers. The pituitary gland produces two GtH (GtH-I and GtH-II) that act directly on the gonads (Suzuki et al., 1988a). Because of a significant degree of homology with mammalian luteinizing hormone (LH) and follicle-stimulating hormone (FSH) (Suzuki et al., 1988b; Itoh et al., 1990), GtH-I is now clearly identified as fish FSH and GtH-II as fish LH (Yaron et al., 2003).

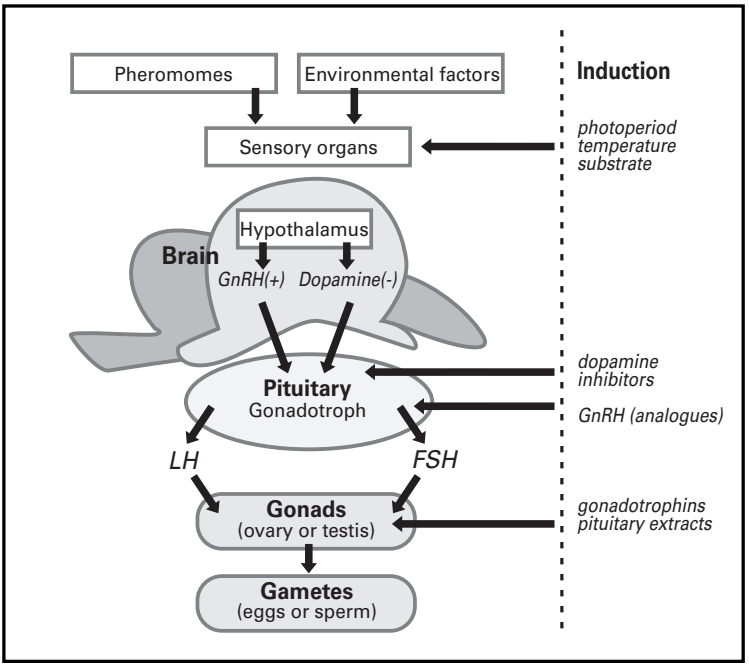

Figure 1. Hormonal pathway in the hypothalamic-pituitary-gonadal axis and levels of external intervention that can be utilised to induce maturation and ovulation/spermiation in teleost fish.

The seasonality of the reproductive cycle is determined by the environmental conditions to which fish are exposed. The environmental cues are transduced into endocrine changes that control gametogenesis.

In poikilothermic species such as fish, there is interaction between water temperature and photoperiod for the control of the reproductive cycle. Depending on the species, one of these factors is the primary transduction mechanism. In cyprinids, temperature plays the major role, whereas, in salmonids and other fish families, photoperiod regulates the endocrine activity (Bayarri et al., 2004). It is assumed that photoperiodicity is perceived in fish through both the eyes and the photoreceptors of the pineal gland, the endocrine organ located on top of the brain. The pineal gland synthesizes and secretes the hormone melatonin, which participates in determining the timing of gonadal development (Bromage et al., 1996). However, data on the relationships between gonadotrophin secretion and melatonin in fish are rather scarce. Generally, melatonin stimulates LH secretion, but its effect depends on the phase of the day-night cycle (Khan and Thomas, 1996).

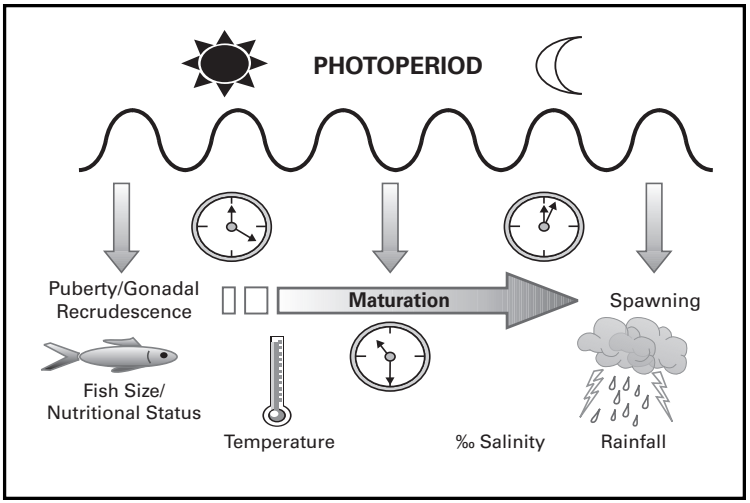

Figure 2. Reproduction and the environment in fish.

a. Reproductive cycles

The majority of teleost fish are seasonal breeders, while a few species breed continuously. Among the seasonal breeders, there is wide variation in the time of year when breeding occurs. Freshwater temperate-zone fish spawn in spring and early summer, while others, such as most salmonids, do so in

autumn (Billard, 1992), the timing of spawning being programmed in order to allow the liberation of offspring into the wild to coincide with optimal food availability.

The seasonality of spawning is a major problem in broodstock management of most fish species. Environmental factors such as photoperiod, temperature, salinity, seasonal rainfall, and several features of the stimuli involved in the interaction between males and females, such as tactile, visual, auditory, and electrical signals, interfere with the reproductive cycle of teleost fish (Chadhuri, 1994; Weerd et al., 1990). In the African catfish, *Clarias gariepinus*, year-round endogenous rhythms of gonadal recrudescence and regression occurring in nature can be avoided in captivity by raising the broodstock from egg to maturity under a constant high temperature (Richter et al., 1995). In salmonids, the programming of long and short photoperiod sequences in the culture of different strains (spring or autumn spawners) can allow reproduction at any time of the year.

b. Hypothalamus

As already noted, it is believed that only one form of GnRH regulates the release of GtH. The relevant GnRH induces the release of both FSH and LH (Zohar, 1996); however, there are conflicting data showing that GnRH cannot stimulate FSH secretion (Breton et al., 1998a). The neuroendocrine regulation of LH secretion in teleost fish is mainly under the control of a dual neurohormonal system. LH release is stimulated by GnRH and inhibited by dopamine, which functions as a gonadotrophin-release inhibitory factor (GRIF).

Dopamine acts directly on the pituitary to modulate the actions of GnRH, as well as the spontaneous release of LH, and also inhibits the release of GnRH (Peter et al., 1993). This tonic inhibition by dopamine on GnRH depends on oestradiol, high levels of which during vitellogenesis (yolk accumulation) prevent LH release. The drop in oestradiol concentration at the end of this process results in the removal of dopamine inhibition (Saligaut et al., 1998).

c. Pituitary gland (hypophysis)

One of the main reasons for the lack of ovulation and spawning in a number of cultured fish is the failure of the pituitary to release LH (Lin and Peter, 1996). Both FSH and LH induce steroidogenesis in specific gonadal cells.

FSH is believed to be mostly involved in regulating earlier stages of gametogenesis, ie, vitellogenesis in females and spermatogenesis in males. Once these processes end, the levels of FSH in the blood decrease, while LH levels rapidly increase. LH is believed to be mainly involved in regulating final oocyte maturation and ovulation in females and spermatogenesis and spermiation in males (Swanson, 1991; Breton et al., 1998a; Chyb et al., 1999).

d. Ovary, oocyte maturation, and ovulation

The ovary in most teleost fishes is a hollow saclike organ into which extend numerous folds lined with germinal epithelium. The germ cells, the endodermally derived oogonia, multiply mitotically and are transformed into non-yolky primary oocytes, with meiosis being arrested at the prophase of the first meiotic division until maturation. Primary oocytes undergo vitellogenesis when yolk is deposited in the ooplasm. During maturation, the first polar body is removed and the second meiotic division is arrested at the metaphase. The eggs are spawned at this stage and the second polar body is only released after fertilisation. In some fish species, ovulation and spawning occur almost at the same time, whereas in rainbow trout and milkfish, ovulated oocytes are retained in the ovarian cavity and spawning takes place later (few days) (Billard, 1992).

e. Hormonal regulation

As mentioned above, the gonadotrophins act on steroidogenesis in the gonads (Nagahama, 1994). In females, the major reproductive steroids are oestrogens (mainly oestradiol-17β), which induces vitellogenin production (yolk) in the liver. Vitellogenin is transported by the blood to the ovaries, where it is incorporated into the yolk granules of vitellogenic oocytes.

Progestagens (mainly 17α, 20β dihydroxy-4-pregnen-3-one and 17α, 20β, 21 trihydroxy-4-pregnen-3-one) induce final oocyte maturation. LH is significantly more active than FSH in stimulating ovarian 17α hydroxy, 20β dihydroxy progesterone (maturation-inducing steroid, MIS) production for the reinitiation of meiosis at the end of the female sexual cycle. A surge of LH is necessary for the in vivo production of MIS (Suzuki et al., 1988c). MIS stimulates the production of maturation-promoting factor (MPF). This nonsteroidal factor involves two components: cdc2 kinase and cyclin B

(Nagahama et al., 1993). MPF triggers the cellular mechanism of germinal vesicle breakdown (GVBD), the reinitiation of meiosis, and the hydration of the oocytes just before ovulation.

f. Fecundity and egg quality

A major difference between fish and many other domestic animals is their high level of fecundity. There are also significant differences between fish species in this regard. For example, flatfish and other marine fish species produce millions of eggs at a single spawning, while other species, such as salmonids, produce only thousands (Bromage, 1988). These species differences are of profound importance to the planning and management of broodstock facilities, with the less fecund species requiring far more fish, and more facilities, to produce the same number of eggs as the marine species.

A number of biotic and environmental factors have been shown to influence fecundity, as well as egg size and quality. Generally, as the size of the species increases, so do both fecundity and the diameter of the eggs produced. The age of the fish seems to be less important (Bromage, 1995). Egg quality is defined as those characteristics of the egg that determine its capacity to survive (Bromage et al., 1992). Many factors have been identified as important in relation to egg quality, eg, diet (the ration and its formulation), spawning methods, husbandry, manipulations, induced spawning, environment, selection, and culture conditions.

g. Testes, spermatogenesis, and spermiation

The testes of teleost fish are in most cases a pair of elongated structures composed of branching seminiferous tubules embedded in the stroma. These thin-walled tubules or lobules contain germ cells, the spermatogonia.

Primary spermatogonia, which are present throughout the year, divide mitotically to give rise to secondary spermatogonia, which are transformed into primary spermatocytes. These divide by meiosis, giving rise to spermatids from which the spermatozoa are formed. The seminiferous tubules are packed with spermatozoa in the prespawning and spawning periods (Winkoop et al., 1995).

h. Hormonal regulation

Testosterone is the main regulator of spermatogenesis, while 11-ketotestosterone and 17α, hydroxy 20β, dihydroxy progesterone are involved in spermiogenesis and spermiation.

12.3 Reproductive management by using hormone preparations

In most cultivated species, gametogenesis generally develops normally in captivity, if fish are kept under the appropriate conditions of temperature and photoperiod, but the final important physiological steps often do not take place spontaneously, leading to poor sperm production in males and the blockade of ovulation in females. This is linked to a lack of environmental stimuli necessary for the release of GnRH and/or a decrease in the inhibitory influence of dopamine to allow for the induction of an ovulatory LH surge.

The most logical point at which to intervene in fish reproduction is at the environmental level, ie, adjusting the environmental conditions to induce spawning. However, while this approach has been successful in some species, in many others it has failed. In the course of improved broodstock management, there are four areas that can be manipulated to provide the industry with the required quality and quantity of offspring at any given period of the year.

a. Maturation and ovulation

Induced ovulation involves the induction of final oocyte maturation (germinal vesicle migration and breakdown) in the female. Various hormones and other pharmaceutical compounds are used to induce maturation and ovulation of post-vitellogenic oocytes. These processes can be induced by fish pituitary extract (FPE), human chorionic gonadotrophin (hCG), 17α hydroxy 20β dihydroxy progesterone, GnRH analogues, and dopamine antagonists (Chaudhuri, 1994; Zohar and Mylonas, 2001). Most species require manual stripping (artificial spawning) after induced ovulation.

b. Spermiation

For most male teleost fish broodstock, spermatogenesis and spermiation are usually adequate and need no hormonal treatment. However, many salmonid hatchery owners encounter the problem of either early or late spermiation

in relation to ovulation in the female, leading to a lack of sperm, low availability of milt, or poor sperm production, which is generally the case in numerous marine species that require the rearing of large numbers of mature males. Goren et al (1995) showed that GnRH analog implants resulted in improved milt volume in Atlantic salmon (70 mL per fish in the treated group compared with 12 mL per fish in the controls).

c. Synchronisation

Synchronisation of a population of broodfish reduces the period in which spawning occurs when compared with untreated groups of female broodfish (for review, see Zohar and Mylonas, 2001). When salmonids are treated with GnRH several weeks prior to normal spawning, up to 90%-100% of them can ovulate within 12-15 days of treatment. In the untreated group, often only about 10% ovulate in the same period, while the remaining controls ovulate in an unsynchronized manner over 30-60 days (Breton et al., 1990; Goren et al., 1995; Haffray et al., 2005).

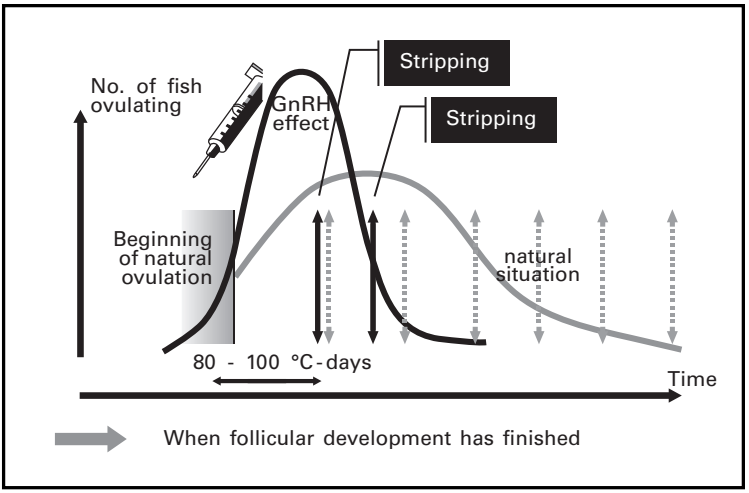

Figure 3. Synchronisation and induction of ovulation in fish.

d. Out-of-season spawning

Of great practical application for altering the rate of maturation and the time of spawning is the use of modified light or photoperiod regimes and temperature manipulation. In Atlantic salmon in particular, even a modest

4- to 6-week advancement in spawning constitutes a considerable commercial advantage, because fry are available and can become S1s (1-year smolts that can be released into the sea) (Bromage, 1995). In general, while egg quality may suffer slightly, GnRH agonists are effective in inducing and advancing spawning, when administered as early as 6 weeks prior to natural spawning. An acceleration of maturation of up to 4 weeks can be achieved (Goren et al., 1995; Haffray et al., 2005).

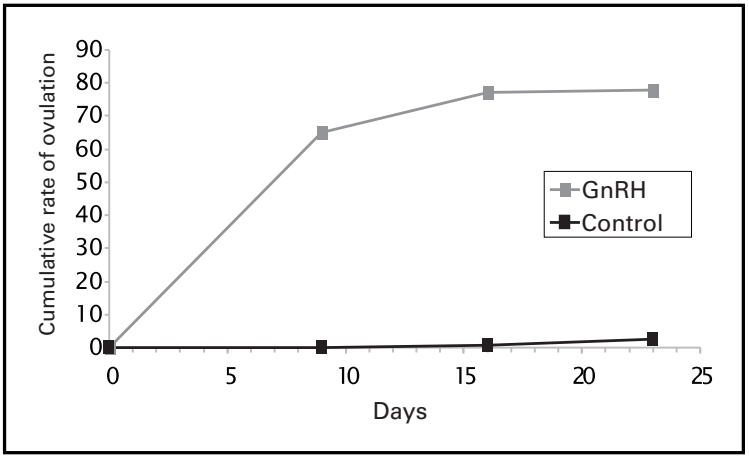

Figure 4. Cumulative ovulation in Atlantic salmon after single injection of the spawning aid, GnRH. Scotland, injected Dec. 7, brackish water 9ºC, 0% natural ovulation at the time of injection.

12.4 Induction of spawning

The experimental work on controlled reproduction in fish, for the purpose of seed production in aquaculture practices, can be divided into those studying environmental parameters and those studying the effect of various hormones (of both piscine and mammalian origin).

a. Environmental manipulation

It is well known that environmental factors influence the reproduction of many animals, including fish. Those factors believed to influence maturation and spawning in fish are temperature, light (photoperiod), salinity, pH, turbidity, and meteorological factors such as rainfall, flood, water current, and lunar periodicity (for review, see Bromage et al., 2001; Glasser et al., 2004).

The seasonality of spawning imposes considerable constraints on trout and salmon farming because the consequent restrictions on the supply of eggs and fry make it difficult for rearing farms to maintain a continuity of production of fish of a suitable size for eating throughout the year (Bromage et al., 1992). Long days early in the reproductive cycle, and short days at any time in the 3-4 months before the summer, advance sexual maturation, whereas short days during the first few months of the cycle, or long days after the summer solstice, delay sexual maturation in rainbow trout (Bromage et al., 1982).

b. Hormonal treatment

The hormonal induction of spawning is usually carried out in fish that do not normally spawn spontaneously in captivity. For the species that spawn naturally in confinement, hormonal manipulation is undertaken in order to synchronise the spawning of a group of females for the mass production of fry (Ayson, 1991; Yaron, 1995; Peter and Yu, 1997).

c. Hypophysation

The term 'hypophysation' in this context means the injection of crude fish pituitary extracts (FPE). This method was developed in Argentina many years ago (Houssay, 1930). FPE includes gonadotrophic hormones that stimulate the maturation of gonads and reproduction in fish. In many countries, pituitary extracts are used extensively, although periodically there are problems with purity, specificity, continuity of supply, potency, and microbiological safety.

d. Gonadotrophin-releasing hormones (GnRH/LHRH)

GnRH (a linear chain of 10 amino acids) is a potent inducer of GtH release. In practice, synthetic analogues of GnRH are used because they are more potent or because they have a longer-lasting action than the naturally occurring hormone (ie, they are better able to withstand enzymatic degradation) (Zohar, 1996). In addition to the induction or release of GtH by GnRH, there is evidence that, in teleost fish, LH secretion is under the control of an inhibitory hormone, dopamine (Peter et al., 1988).

Depending on the species, the inhibitory influence of this hormone can block GnRH action, as in most cyprinids and silurids, whereas, in other species, such as in salmonids and most of the marine species, it is not potent enough to block GnRH action.

When necessary, treatment with antagonists of dopamine, such as pimozide or domperidone, together with GnRH, leads to an enhanced release of GtH as compared to the effect of GnRH alone (Sokolowska et al., 1985; Lin et al., 1986; Mikolajczyk et al., 2004).

e. Human chorionic gonadotrophin (hCG)

From the early 1960s, hCG has been largely used for inducing gonadal maturation and spawning in fish. HCG has one big advantage over several of the other hormones and FPE, namely that its potency can be standardised in International Units (IU), so that the results of different investigations can be properly compared. Chaudhuri (1994) provided quite a long list of positive results obtained by administration of hCG to various fish species.

f. Sex steroids, pheromones, prostaglandins

It is known that gonadotrophins stimulate the production of sex steroids, which in turn induce maturation and ovulation in fish (Resink et al., 1987; Weerd et al., 1990). Experiments on gonadal steroids have not been very encouraging so far. In addition, progestagens, such as 17α, hydroxy 20β, dihydroxy progesterone, are very expensive.

Pheromones are substances secreted by an individual that may provoke a specific reaction in the opposite sex of the same species. They occur in fish, just as in mammals, and may exert strong influences. For example, Weerd et al (1990) showed a significant effect of male pheromones on the gonadosomatic index (GSI) of female African catfish. Prostaglandins have been implicated in the ovulation of oocytes from the follicles in some species (Stacey and Goetz, 1982).

12.5 Mode of administration

There are basically two routes by which to administer different endocrine products to teleost fish. The most common technique is injection of the product (typically the active dissolved in a solvent), whereas newer methods, such as the injection of impregnated implants or oral administration, are either not widely used (ie, not widely registered) or still under evaluation.

a. Injection

The majority of the fish species treated with hormone preparations are injected with a solution either intramuscularly or intraperitoneally. The latter method, unless performed by an experienced operator, risks damaging or infecting the intestine of the fish. Depending on the species, the rapid clearance of injected GnRH analogue from the circulation may require multiple injections in order to achieve an effective response. Excessive handling of fish receiving multiple injections can lead to stress-related injuries, mortalities, and suppression of reproductive processes.

A relatively new method is the implantation of controlled-release delivery systems (Zohar, 1996; Zohar and Mylonas, 2001). The prolonged diffusion of the implant prevents the problems associated with multiple injections (Goren et al., 1995). However, such implants and indeed many other GnRH or GnRH-dopamine antagonist-containing solutions may not be approved for commercial use in many countries.

b. Dietary treatment

Certain fish species are especially susceptible to the stress of handling when they are in a spawning condition. They may fail to ovulate or may even die if they are not anaesthetised prior to netting, handling, and injection, particularly if the environmental conditions are suboptimal (Thomas et al., 1995). Thomas and Boyd (1989) administered 1.0-2.5 mg of a GnRH analogue per kg body weight orally, which resulted in spawning of sea trout after 32-38 hours, with high rates of fertilisation and hatching. Similar results were obtained in African catfish and common carp (Breton et al., 1998b; Mikolajczyk et al., 2002), Thai carp (Sukumasavin et al., 1992), and sable fish (Solar et al., 1990). This method has some disadvantages, as it is usually impossible to achieve the correct dose per individual and some species do not accept feed during the spawning season.

12.6 Propagation

The propagation of fish starts with the collection of eggs and sperm. Generally these gametes are obtained by 'stripping' the ripe spawners or by collecting the fertilised eggs after mating in artificial enclosures (Huisman, 1976).

a. Collection of eggs

To secure the maximum control over the eggs, most species are manually stripped using one of three methods:

i. Eggs are stripped by gently massaging the belly in the direction of the genital pore
ii. The belly is opened surgically and the eggs are removed by hand
iii. A needle is inserted at the posterior end of the belly in order to introduce air to help flush out the eggs from the anterior end

b. Collection of sperm

Collection of sperm from male broodstock can be achieved by stripping the male or by removing the ripe testis by surgery. The quality of sperm is highly variable and depends on various external factors such as the feeding regime, the quality of the feed, and the rearing temperature of the males. The most commonly used parameters for assessing sperm quality are its motility and survival during storage (Billard et al., 1995).

c. Fertilisation

In general, three different methods of artificial fertilisation are in use, all of which involve the manual removal of gametes from the broodfish:

i. Wet method. The gametes are stripped into a pan filled with water
ii. Dry method. Eggs are stripped into a dry pan, dry sperm are mixed with eggs, and water is added afterwards
iii. Super-dry. This method is based on (b), but the eggs are stripped onto a sieve to get rid of the ovarian fluid (Huisman, 1976). If present, ovarian fluid needs to be removed since it inhibits the movement of sperm. This is very important in the salmonids

d. Incubation

After fertilisation, eggs can be incubated. Different incubator systems are in use for the various species, depending on the biological requirements of the incubated eggs and local customs. Optimal hatching temperatures vary, eg, 25°C-28°C for Chinese carp, with hatching after 23-28 hours, but 5°C for halibut eggs, with hatching after 16-19 days (Kjørsvik and Holmefjord, 1995). For salmonids, incubation in cold water lasts from about 2 months for rainbow trout to 6 months for Atlantic salmon or Arctic charr. During incubation, the sensitivity of the eggs varies widely and oxygen supply is of great importance.

e. Hatching

At the end of egg development the eggs hatch. Hatching can be accelerated by increasing the temperature (Sorensen et al., 1966). However, Lilelund (1967) showed that, for pike, when incubation took place at lower temperatures, birth occurred in a more advanced morphological stage, the larvae being bigger than normal.

12.7 Reproduction-related diseases

Broodstock should be managed to avoid subsequent diseases in progeny resulting in inferior-quality offspring.

a. Vertical transmission

A number of diseases are described that can be transmitted vertically from broodstock to offspring. Bacterial kidney disease (BKD) caused by *Renibacterium salmoninarum* is transmitted within the egg. After proper surface disinfection, it was found that 10%-20% of the eggs sterile on their surface were still positive for BKD in one study (Evelyn et al., 1984). While the isolation of broodfish may not be critical, the eggs from individual spawners should be quarantined after fertilisation so that the parents can be screened for these pathogens (Pascho et al., 1991).

b. Contamination

The culture of eggs and larvae of fish in the same environment enhances microbial growth, as a result of the increased volume of available nutrients from the metabolic by-products of the fish and the increased number of surfaces on which organic debris can be trapped and on which microorganisms can colonise.

Another source of nutrients is the various lipid and protein components of fish eggs that are released at hatching. The most frequently isolated bacteria from the surface of live eggs are members of the genera *Cytophaga, Pseudomonas, Alteromonas, Flavobacterium,* and *Aeromonas.* As well as being in the water, these bacteria are often present in the coelomic fluid of the maturing female (Kjørsvik and Holmefjord, 1995).

12.8 Gender control

Gender control is of importance for maximising the economic efficacy of production systems (Donaldson, 1996). A number of techniques are available, all of them aimed at the development of monosex populations, which have major advantages over mixed populations (such as better growth rate, more homogeneity, lower susceptibility to disease, and better meat quality).

a. Sex reversal

The advantage of 100% monosex cultures also includes reduced gonadal development with no production of offspring during the grow-out phase (Komen et al., 1989). Hand-sorting by gender, hybridisation, or hormone treatment are the most common methods of producing monosex stocks in commercial practice (MacIntosh and Little, 1995).

For tilapia, hormonal sex reversal by masculinisation of fry with androgens (typically 17-methyl-testosterone) is widely recognised as having significant advantages over both hand-sorting and hybridisation (McAndrew, 1993; Lin et al., 1995). However, in some countries (eg, EU), monosex female populations are produced by indirect feminisation so as to ensure that fish are not exposed directly to steroids.

b. Gynogenesis

The term gynogenesis implies that the genomic inheritance of the embryo is entirely female. It means that the chromosomes of the fertilising spermatozoon need to be inactivated, without affecting its functional ability to fertilise. Gynogenetic haploid fish do not survive beyond yolk-sac absorption. Diploidy can be restored by interfering with meiosis, by retaining the second polar body with its haploid set of chromosomes, or by interfering with mitosis by preventing the first cell division (Komen et al., 1988).

c. Androgenesis

In androgenesis, eggs are irradiated to destroy the female nuclear material. Fertilising these treated eggs using a homozygous sperm donor results in the production of clones (Bongers et al., 1994).

d. Triploidy

Much of the interest in induced triploidy in fish culture is based on the assumption that triploids would be sterile and may show better growth than diploids at the period of maturation and reproduction of diploids (Purdom, 1976; Johnstone et al., 1991). It can also prevent cultured exotic species from forming self-sustaining feral populations. Cold-shocking of eggs is a successful method for inducing triploidy in the African catfish (Richter et al., 1986). Heat shock is applied for induction of triploidy in salmonid fish (Chevassues et al., 1983; Quillet et al., 1991).

12.9 Transgenesis

Gene transfer has become a very active topic for fish research in recent years (Chen and Powers, 1990). The principal approach used to transfer genes into fish eggs is by microinjection. Cloned DNA sequences are injected into the eggs shortly after fertilisation. Transfer of the genes can then be monitored by the presence of the foreign DNA in the progeny or by expression of the foreign genes. The genetic constructs introduced into fish were aimed at antifreeze protein and growth hormones from different sources (Maclean and Penman, 1990; Delvin et al., 1995).

Public perception and hazards associated with the possible escape of transgenic animals into the wild are the two major elements presently constraining the production and application of transgenic fish in the aquaculture industry (Thorgaard, 1995).

12.10 Acknowledgements

The assistance and critical review by Dr. Bernard Breton (formerly of INRA, Rennes, France) and Dr. Tomek Mikolajczyk and Dr. Mirka Sokolowska (University of Agriculture, Krakow, Poland) are very much appreciated.

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