PARTNERS IN REPRODUCTION

Compendium of animal reproduction

MSD
Animal Health

5 Ovine Reproduction

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, i.e. 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 categorized 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 (e.g. the ram effect; see 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 characterized 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 non-seasonal 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 non-breeding 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 non-breeding 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 (i.e. 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 'post partum anoestrus', also known as 'lactational anoestrus'. The length of this period varies with breed, management practices and the date of parturition, since seasonal and post partum anoestrus may overlap in some instances. Post partum anoestrus is mainly due to the 'anti-gonadotrophic' effect of the sucking lamb, so it normally ends shortly after weaning.
But even when not suckling lambs (e.g. when lambs are reared on milk replacer), the ewes' immediate post partum 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 non-breeding 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, e.g. 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.

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 which include 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 programs, as well as when evaluating their results.

5.1.2 The oestrous cycle

Non-pregnant females separated from the ram, or failing to conceive after mating, have alternate periods of anoestrus and sexual activity. The latter are characterized 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 luteinizing 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 which 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.
Fertilization 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 fertilization, ovine embryos migrate throughout the uterine lumen.

The luteal phase is characterized 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 post fertilization 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 post conception, 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.

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.

Table 1 Definitions of reproductive parameters frequently used in ovine reproduction.

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

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 non-pregnant ewes, and the supplementary feeding of those which 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, minimizing pre-lambing feeding costs, optimizing 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 trans-abdominal 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 abdominal cavity making accurate examination difficult

Doppler and amplitude-depth (A-mode) ultrasound are cheaper alternatives during the second half of gestation.

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 (oPAGs) 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 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 vasectomized ‘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 synchronization of oestrus (see 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 synchronization 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 synchronized) 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 synchronization, 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 trans-cervical 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, which results in the inseminating pipette being diverted away from the lumen.
So 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., 2004). In this case 0.2 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, 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.

Table 2 Typical results from AI in ewes

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	75-100	40-50%	30-60%
	Laparoscopic	26-60	65%	50-90%

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 fertilization 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 synchronization. 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 synchronization and the site chosen for the deposition of semen (see Table 3).

Table 3 Time of insemination in sheep according to the type of oestrus and insemination.

Type of Oestrus	Type of AI	Optimum time for AI
Natural	Cervical or vaginal	12-18 hr after onset of oestrus
Synchronised with Chrono-gest® 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

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/synchronized)
   - 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 (synchronization technique and 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 synchronization in sheep.

The most important factors to be considered before deciding which method to use are:

- The degree of synchronization needed.
- The season.
- Economic and market factors.

The pharmacological methods are effective in the tight synchronization 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 synchronization and is only useful in certain conditions.

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 synchronizing oestrus.

5.3.1 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, which allows 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 last only around 2-3 months.

5.3.2 The Ram Effect

Social influences (e.g., 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 synchronized 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 synchronize oestrus and ovulation tightly enough to allow for fixed time artificial insemination.

5.3.3 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 synchronization 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 synchronization in sheep, the duration of treatment must be at least 12- 14 days, mirroring the length of the luteal phase.

Oestrus synchronization 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 synchronized oestrus. Among the various methods in current use, progestagens offer the most precise synchronization 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 synchronization tight enough for AI.

Progestagens can be administered in a variety of ways (sponges, implants, etc.), via several routes (intravaginal, i.m., s.c.), 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 (Chronogest CR®) or medroxyprogesterone acetate (MAP), and are inserted into the vagina using a dedicated applicator.

As sheep are generally perceived as a more 'green' species than cows and pigs, the new Chronogest CR® sponge with a reduced cronolone load (20mg) offers an interesting option for equally efficient oestrus management and lambing rates, but using less exogenous hormone. Letelier et al. (2009) compared the ovarian follicular dynamics and plasma steroid concentrations in ewes given intravaginal Chronogest® sponges containing either 20 or 40 mg of fluorogestone acetate. None of the features monitored (including by daily ultrasonography) differed significantly between the two groups. Ovulatory follicles grew at a similar rate, with comparable initial and maximum diameters. Moreover, ten days after sponge removal, ovulation rates and plasma progesterone concentrations were similar. These authors concluded that reducing the dose of fluorogestone acetate from 40 to 20 mg in Chronogest® sponges did not significantly affect ovarian follicular dynamics or the other ovarian functions measured.

Use of PMSG/eCG in progestagen-treated ewes

In ewes in anoestrus, progestagens must be supplemented with follicle stimulating treatments (e.g. pregnant mare serum gonadotrophin, PMSG/equine chorionic gonadotrophin, eCG) to induce follicular growth, oestrus and ovulation.

When ewes are being synchronized 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%)

Table 4 Adjustment of PMSG dose in ewes treated with Chronogest CR® method

	Reproductive status	**PMSG (Folligon®) 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

For the superovulation of donor ewes in embryo transfer, eCG/PMSG may be administered at about 28 hours before sponge removal and at a higher than normal dose (Folligon®,1500 IU; Bari et al., 2000; Henderson and Robinson 2000). ECG/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 pre-treatment of ewes with 10mcg buserelin before progestagen-based synchronization (7 day progestagen program in combination with $PGF_{2\alpha}$ administration) resulted in increased multiple birth rates and litter size.

Breeding management in flocks synchronized using progestagens

One of the main advantages of this method is that it can be used to induce and/or synchronize oestrus. The high degree of synchronization 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.

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.

Ram-to-ewe ratio

In synchronized 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 one to five. If the requirement for high numbers of rams poses a problem, then the use of AI should be considered (see 5.2.5).

Lambing period and returns to oestrus

A population of ewes conceiving to a synchronized 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.

5.3.4 Prostaglandins

Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) and its analogues can be used to synchronize 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, and ovulation 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 synchronization. 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 (e.g. Chronogest CR®), followed by an injection of $PGF_{2\alpha}$ at sponge withdrawal, has been shown to be highly effective in synchronizing oestrus during the breeding season.

5.3.5 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 using 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. (2009, in press) reported encouraging results of a synchronization protocol in ewes when progestagen-loaded sponges were used between GnRH and $PGF_{2\alpha}$ injections.

5.3.6 Melatonin

Melatonin, a hormone produced by the pineal gland, mainly during the hours of darkness, is considered to be the chemical trigger which 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 synchronize oestrus and ovulation tightly enough to allow for fixed time artificial insemination. One of the possible options could be a combination of melatonin pre-treatment with precise oestrus synchronization via a progestagen-based program.
DeNicolo et al. (2008) evaluated the effect of treatment with melatonin implants followed by a progesterone-releasing device and eCG/PMSG synchronization. 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 program.

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 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 world-wide, which 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 back-crossing, 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 BCS) rather than when excessively thin or fat (Henderson and Robinson 2000).

On the other hand, 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 and not being stimulated with exogenous gonadotrophins (Downing et al., 1995). O'Callaghan et al. (2000) found that non-stimulated 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 over-stimulation 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 eCG/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 (e.g. pFSH) are short-acting and require frequent injections, so their use is restricted, in practice, to embryo transfer programmes (Haresign 1992).

PMSG/eCG (e.g. Folligon®) is longer lasting and usually used for inducing oestrus and ovulation outside the normal breeding season or for ensuring good conception rates at a synchronized oestrus in a fixed timed 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 Immunization techniques

Immunization reduces the inhibitory effect of the ovarian steroids or ovarian inhibin on the hypothalamus and pituitary, resulting in an increase in the ovulation rate. Immunization 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). There is one vaccine (Androvax®) available commercially. The timing of vaccination is important to the success of this technique. Ewes must be sexually active when the rams are introduced. Therefore, if the technique is to be used out-of-season, the ewe flock needs to be primed with progesterone sponges and eCG/PMSG to stimulate oestrus activity. The dose of eCG/PMSG must then be carefully evaluated, as the effects of eCG/PMSG and the vaccine will be additive (Henderson and Robinson 2000).

5.5 Fertilization rate and embryonic losses in sheep

Data available in the literature suggest that, as observed in cattle, a fertilization rate of 90-95% appears to be normal in ewes, under natural mating conditions. When biotechnical methods involving artificial insemination, and especially with frozen semen, are used, much lower fertilization 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.

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.

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, were 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 (i.e. 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 fertilization 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 non-pregnant ewes had elevated progesterone concentrations necessary for the production of at least one viable embryo/foetus.

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.

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 acclimatization, 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 summarizes the most commonly encountered causative agents and the main signs relevant to each.

5.6.1.1 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 which 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. That is the reason for toxoplasms, circulating in the blood of a pregnant ewe, being able to 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 where they can spread to the rest of the foetus (Buxton et al., 2007).

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, with the lambs being born normal, but infected and immune.

Control measures include good management of food and water sources to limit the contamination with cat faeces, as well as vaccination (Ovilis Toxovax®).
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 be also born in mind that Toxoplasmosis ranks high on the list of diseases which lead to the death of patients with acquired immunodeficiency syndrome (AIDS).

5.6.1.2 Enzootic abortion (EA)

Enzootic abortion 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.

Clinical consequences of infection

Infection during pregnancy may result in abortion, stillbirth, birth of weakly, premature or clinically normal but infected lambs (Tab 5). Abortions occur typically 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.

Table 5 Clinical picture of *C.abortus* infection during pregnancy in sheep (adapted from Kerr et al., 2005)

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.

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 (e.g. Ovilis Enzovax®) 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 which 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 which 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, 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.

5.6.1.3 Q fever

Q fever (short for ‘query’ fever), a zoonosis caused by the obligate intracellular micro-organism *Coxiella burnetii*, is wide spread 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 non-viable 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 which 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 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.

Table 6 The most relevant infectious diseases causing ovine abortion and perinatal losses

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.	Ram: 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.	Non-specific 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

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 the 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 (‘post-parturient gangrene’)

The condition occurs immediately post-partum 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 minimize the incidence.

5.6.4 Puerperal metritis

Factors such as dystocia followed by obstetrical assistance, prolapse of the uterus, retained placenta and post-parturient 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 non-steroidal 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 optimize 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 synchronized 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 producing its own, and luteolysis therefore has no effect. However, both oestrogens and corticosteroids can be used successfully. Some researchers have reported higher rates of dystocia and peri-natal 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 Ram

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.

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.

- 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 ewe in oestrus.

- 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.

- 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, electro-ejaculation can also be used and may well be required in rams not trained in the use of an artificial vagina. Electro-ejaculation 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 electro-ejaculation 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 pre-warmed microscope slide. Morphology of the spermatozoa is studied in microscopic preparations stained with eosin-nigrosin. Table 7 gives the normal parameters of sperm expected in a mature ram during the breeding season.

Table 7 Normal semen parameters in mature ram

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) %

Storage of ram semen

Ram semen may be stored for up to 24 hours by cooling the extended semen down to 2-5°C over 90-120 min. 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 (e.g. France, Australia, Spain).

Management of rams prior to breeding

Properly planned management is necessary to optimize 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.

Table 8 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 be also 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 in the development of the commercial use of emerging techniques such as nuclear transfer and transgenetics.

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6 Caprine Reproduction

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, 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 min), individual sperm motility (2.5–3.5 to over 5) and fertilizing 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 post partum 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 (i.e. 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, generally reported as 36 hours, ranging 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 fertilization 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 which, 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 post partum 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 characterize the industry, but, regardless of the type of goat being produced, their 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, e.g. the male effect, altering the photoperiod, dietary modifications (e.g. flushing), and modifying the breeding pattern (e.g. 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 (i.e. interval between kidding and introduction of the bucks), physiological (seasonal and post partum 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 for 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 which 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, non-pregnant 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 post service. 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 non-pregnancy 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 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 4th week post partum.
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 post breeding 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 post breeding but 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 2-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.

Trans-abdominal 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. By contrast with cows, however, 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 post partum, 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, which 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.

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 non-breeding season, their stored semen should not be used to inseminate does which 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 fertilization 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 trans-cervical route. The kidding rate in the laparoscopically inseminated controls in this trial, was 53%.

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.

Table 1 Timing of insemination in goats.

Type of oestrus	**Insemination time**
Natural*	12-18 hours after onset of oestrus
Induced by Chrono-gest® 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 hours after removal of the sponges

* According to Evans and Maxwell (1987)
** According to Corteel et al. (1988)

6.3 Control of oestrus

The control of oestrus and out-of-season breeding are of increasing interest, as they enable milk producers to maintain regular and consistent levels of production, as well as allowing three kid crops in 2 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 synchronization 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 as well as 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 synchronize 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, which increases semen production per buck by 40% per year and reduces 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 2-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 synchronization during the breeding season
- tight oestrus and ovulation synchronization 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 (i.e. 16-18 days) in order to achieve effective synchronization.
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 (e.g. Chronogest CR®) or medroxyprogesterone and intravaginal devices impregnated with progesterone. There have been some reports of the use of norgestomet implants for oestrus and ovulation synchronization 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; Folligon®) 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 20 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 synchronized 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 (e.g. Folligon®) 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.

Table 2 Treatment schedules for Chrono-gest® sponges in goats.

Treatment	Insertion of sponges	Injection of prostaglandin	Removal of sponges
Short	Day 0	Day 10	Day 12

Table 3 Adjustment of PMSG dose in does treated with Chronogest CR® method

	Milk production	PMSG (Folligon®) 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

6.3.5 Prostaglandins

Prostaglandins or analogues can be used to synchronize 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-synchronized 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 synchronization (94% of animals in oestrus 53 ± 3 hours after the second injection) and a conception rate similar to non-treated controls after natural service (Ott et al., 1980). The most common use of $PGF_{2\alpha}$ in synchronizing 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 synchronization with progestagens generally results in good fertility, irrespective of seasonal effects (breeding or anoestrus season), some breeders are interested in alternative synchronization 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 synchronization 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 pre-determined 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 synchronized 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 have been used, with or without progestagen treatment, the FSHp 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, FSH 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 immunization 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, such as the high rate of premature luteal regression occurring in treated animals and an unusually large number of non-ovulated follicles.

Whereas embryo transfer is an effective method of achieving genetic improvement in cattle, it is not widely used in goats, the main reasons being 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, post-operative adhesions are a frequent complication, limiting the number of possible collections.

A novel, non-surgical 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* fertilization (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 non-productive 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 non-mated 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 4th 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 3rd 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; e.g. Chorulon®, 500 IU) or GnRH (e.g. Receptal®, 2.5 ml) at the time of AI is often used to improve fertility, especially in high-yielding milking goats.

6.6 Induction of parturition

$PGF_{2\alpha}$ and its synthetic analogues 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.

6.7 References

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