International Sheep and Wool Handbook

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Foreword

It is with great pleasure that the International Wool Textile Organisation (IWTO), the international body representing the interests of the world's wool-textile trade and industry, salutes David Cottle on this comprehensive coverage and most informative handbook on the sheep and wool industry. The handbook will serve both as a reference work to students and to those with a general interest in the sheep and wool industry.

IWTO membership covers woolgrowers, traders, primary processors, spinners, weavers, garment makers and retailers of wool and allied fibres in its member-countries, as well as all kinds of organizations related to wool products and the Wool Industry in general. Thus in this context the book covers the interests of all our members in all parts of the World, from the production of wool at its source through to the finished garment sold in the retail store.

We are indebted to Prof. David Cottle for producing such a comprehensive and interesting study of the sheep and wool industry. This is something which we have not had in the past and thank him and his colleagues most sincerely for the time and effort that they have put into researching and documenting every facet of our industry.

As the drive towards naturally sustainable and ecologically friendly fibres becomes more important, books of this nature will become all the more relevant in showing the benefits of wool.

Günther Beier
IWTO President

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PREFACE

This book is an expanded, updated version of the Australian Sheep and Wool Handbook published in 1991. The 1991 text was widely regarded as the definitive sheep and wool textbook and has been used as the reference text for sheep and wool subjects in many Universities since then. In the 1990s there were few sheep and wool textbooks available compared to the situation in 2010.

Many requests were received over the last 19 years to produce a new edition. The amount of time required to produce a new, substantive book caused some trepidation but a rare window of opportunity to carry out the task opened up in 2008-2009. One massive change that has affected both the sheep and wool industry and the publishing industry is the advent of the internet with its search engines, word processing software and the use of email. This has made multi-authored book writing easier and quicker on the one hand but with the increased problem of possible information overload. Much of the value of this book for readers is the distillation of the mountain of information available in the modern digital, electronic era by the chapter authors sifting through the various sources of information and capturing it in one place. Key websites for further information have been listed at the end of many chapters.

The book has been made more international in scope compared to the earlier 1991 text. There is the collection of new chapters on the sheep and wool industries in the major sheep regions of the world which is unique to this book. There is also a wider range of references to global examples in the various chapters. There are new chapters on meat processing and sustainable production and expansion of some chapters, *e.g.* sheep meat and wool processing.

The 1991 book was written at the time of the wool reserve price scheme collapsing in Australia. There has been much change in the meat and wool industries but some would argue not enough change. All authors were asked to crystal ball gaze about likely future developments. Perhaps this was a recipe for being proven incorrect in future but it was an interesting exercise.

The Meat and Wool Boards were merged in New Zealand and in 2009 the NZ growers voted to reduce the wool levy to zero. Australian producers voted to maintain a 2% wool levy in WoolPoll 2009 but there have been calls to merge the wool (AWI) and meat (MLA) organizations. What changes will the next 20 years bring to the world sheep and wool industries?

DJ Cottle

REPRODUCTION

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Introduction

The full reproductive cycle in female sheep spans 7-10 months. It comprises oestrus or receptivity to the ram, mating, fertilisation, implantation of the embryo, pregnancy, parturition or birth of the lamb, the bonding of dam and new born offspring and lactation up until the time of weaning. This chapter discusses the events in the cycle up to and including fertilisation in the ewe as well as reproduction in the ram. The role of assisted reproductive technologies in sheep breeding is also discussed. Chapter 10 deals with embryo and foetal development, lambing and ewe-lamb interactions around parturition, while Chapter 11 deals with lactation and lamb growth.

High reproductive efficiency is a key determinant of the profitability of most sheep enterprises. In the case of prime lamb production, the benefit of increasing reproduction rate is directly visible in the greater number of lambs per ewe available for sale. For self-replacing flocks, where wool production is paramount, greater reproductive efficiency not only increases the number of surplus animals available for sale but also allows for higher culling rates, more rapid genetic improvement, and a decrease in the number of ewes necessary to maintain the wether flock. Whatever the enterprise, the net reproductive efficiency of a flock is determined by its fertility (proportion of ewes present at joining which lamb), fecundity (number of lambs born per ewe lambing) and survival rate of lambs from birth to marking or weaning. Unfortunately, the reproduction rate in many Merino flocks in Australia is disappointingly low (Lindsay, 1988).

Physiology of reproduction in sheep

A prerequisite to discussing the regulation of reproductive performance in sheep is an introduction to the anatomy and physiology of reproduction. Further information on these topics is available in textbooks by Hunter (1980), Evans and Maxwell (1987), Hafez and Hafez (2000), Dyce *et al.* (2002), Senger (2005) and Cunningham and Klein (2007).

The female

Structure of the reproductive organs

Female reproductive organs comprise the paired ovaries or gonads and the reproductive tract, which consists of the oviducts, uterus, cervix, vagina and vulva (Figure 9.1).

Each ovary is a roughly spherical organ with two principal functions: the production of ova - (or eggs or mature germ cells) and the secretion of the female sex hormones required for conception and pregnancy (primarily oestrogen and progesterone). Each ovary contains enormous numbers of immature germ cells, each of which is capable of development into an ovum.

The Fallopian tubes, or oviducts, are paired tubes that receive the ova and provide the site of fertilisation and early embryo development before the embryos pass to the uterus. The anterior end of each oviduct expands into a funnel called the infundibulum, which embraces the adjacent ovary at the time of ovulation to gather the ova. The remainder of the oviduct is divided into the ampulla and the more muscular and narrow isthmus, which joins the uterus at the utero-tubal junction. This muscular junction can act as a temporary barrier to entry of ova into the uterus and spermatozoa into the oviduct. Epithelial cells lining the ampulla have cilia that beat rhythmically to assist the movement of ova towards the uterus.

The uterus consists of a body and two cornua or horns that are continuous with the oviducts. Its wall comprises two main layers - an outer muscular one (the myometrium) and an inner glandular and stromal layer called the endometrium. The endometrium contains numerous specialised non-glandular areas called caruncles. About 3 days after ovulation, embryos or unfertilised ova enter the uterus, where development of the fertilised egg continues through the embryonic and foetal stages for the duration of pregnancy (see chapter 10).

A muscular and fibrous constriction - the cervix - separates the uterus from the vagina. In pregnancy it is sealed and protects the foetus from the external environment, whereas at around the time of mating and ovulation it is relatively open, enabling the passage of motile spermatozoa into the uterus. The lumen of the ovine cervix is tortuous and convoluted; so it is virtually impossible to pass an inseminating pipette through the cervix into the uterus, as can be done in the cow and mare.

The vagina, a thin-walled tube, connects the cervix to the vulva or external orifice of the genital tract. The urethra, which carries urine from the bladder, opens into the floor of the back of the vagina.

Oestrous cycle, oestrus and the breeding season

The reproductive organs of the ewe function in repetitive cycles of 15-18 days duration, called oestrous cycles. For a limited

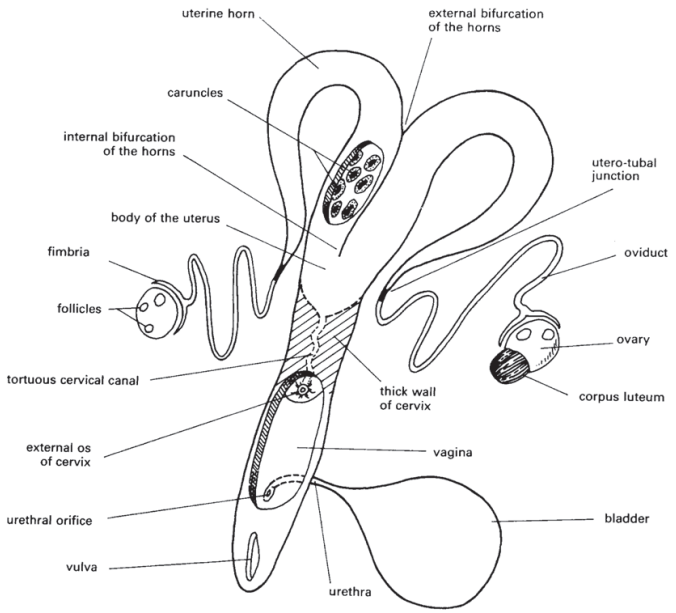

Figure 9.1. The reproductive organs of the ewe. Portions of the walls of the vagina have been cut away to reveal the external os of the cervix and the urethral orifice; a similar 'window' in one uterine horn shows the uterine caruncles. Source: Miller (1991).

period of about 20-35 hours of each oestrous cycle the ewe will accept and may actively seek out the ram. At this time she is said to be in oestrus or 'in heat'. The state of oestrus signifies that the internal reproductive organs are at a stage where fertilisation is possible and pregnancy should ensue if mating occurs. The uterus is pinker and more turgid or less flaccid at oestrus than at other times, due to increased blood flow and capillary permeability. Unlike many other livestock species, the ewe shows no sign of oestrus in the absence of the ram. After oestrus she is outwardly quiescent, but internally the reproductive organs are undergoing important changes that ensure the maintenance of pregnancy or, if she is not pregnant, the return to oestrus after another oestrous cycle.

Because ewes are seasonally polyoestrous, a high proportion of them exhibit regular oestrous cycles only during particular months. In the Southern Hemisphere, including Australia, this breeding season usually lasts from approximately February to June, but the actual duration of breeding activity is highly variable and depends on several factors, to be discussed later. During the remainder of the year or non-breeding season, British-breed ewes are in anoestrus and their ovaries secrete relatively little hormone and do not ovulate. However, Merino and Merino crossbred ewes are often bred in spring and early summer in Australia (particularly in South and Western Australia), and satisfactory reproduction rates may be obtained (Kleemann *et al.*, 1989, 2006).

Under natural conditions, mating in sheep usually precedes the spring by an interval of about 5 months, or the duration of pregnancy. The sheep is called a short-day breeder, since the stimulus that controls the onset of breeding activity is the decrease in the ratio of light to dark hours. It requires about 6-10 weeks of a decreasing light:dark ratio to stimulate breeding, and breeding activity often ceases within 1-3 months after the winter solstice. Most breeding activity occurs during March-April. Joinings at this time for as little as 4 weeks may result in 90% or more of ewes being in lamb.

Ovulation and formation of the corpus luteum

The large numbers of primary oocytes or immature germ cells in each ovary are surrounded by epithelial cells to form primordial follicles. As oestrus and ovulation approach, one or more of the follicles enlarges rapidly, with multiplication and differentiation of the cells surrounding the oocyte to form internal granulosa

and external theca layers. Continuing growth results in a fluid-filled cavity (antrum) within the granulosa, which gives the mature or Graafian follicle a vesicular or blister-like appearance. Concurrent with follicle growth, the oocyte develops into a mature ovum. During maturation the nucleus of the primary oocyte divides twice (meiosis), so the mature ovum is haploid or contains only half the number of chromosomes present in the immature germ cell. The first or reduction division occurs shortly before ovulation.

The rupture of each large Graafian follicle to release one ovum (ovulation) - occurs about 30 hours after the onset of oestrus. The Merino usually sheds one ovum (actual average is 1.4/ewe), but other breeds and crossbred sheep commonly shed one, two or three ova. Ovulation occurs regardless of whether mating has occurred, so the ewe is called a spontaneous ovulator. Some animals, such as the rabbit and cat, are called induced ovulators and will only shed ova after mating. Following ovulation the granulosa and thecal cells within the ruptured follicle undergo reorganisation and rapid proliferation within the remaining cavity to form the *corpus luteum.*

Ovulation rate

The mean number of ova released at each oestrus, or ovulation rate (discussed in detail later), is a major determinant of lambing rate and is controlled by several genetic, nutritional and environmental factors. For any particular breed, ovulation rate appears to be maximal at around the time of the autumn equinox, but may remain near maximal for some 2 months thereafter.

Puberty

At puberty the young sheep first attains the ability to produce mature germ cells and mate. Although the ewe lamb may become pregnant after reaching puberty, she will not reach full reproductive capacity or sexual maturity until some time later. Most of the development of the reproductive organs in both sexes is delayed until around the time of puberty, when hormones from the anterior pituitary gland stimulate ovarian or testicular growth and sex hormone secretion. Regardless of season of birth, puberty is always reached in late summer-autumn (Dyrmundsson, 1983).

Sperm transport and fertilisation

At mating or service, the ram deposits a volume (0.5-2 mL) of semen in the ewe's anterior vagina, adjacent to the external opening of the cervix. Large numbers of spermatozoa 'swim' promptly into the cervix, which forms a reservoir of live spermatozoa from which relatively small numbers constantly migrate forwards towards the site of fertilisation. Movement of spermatozoa through the uterus and into the oviducts is effected mainly by muscular contractions of these organs. While some spermatozoa may be recovered from the oviducts as few as 5 minutes after service (Mattner and Braden, 1963), it takes a minimum of 8 hours for sufficient numbers of spermatozoa to enter this region to ensure fertilisation (Hunter *et al*., 1980). Here spermatozoa bind to the caudal region of the isthmus for 17-18 h (Hunter *et al*., 1980) until ovulation occurs, whereupon small numbers of spermatozoa are released from the oviduct epithelium and transported to the ampulla-isthmic junction for fertilisation (Hunter *et al*., 1982). During this period of exposure to the fluid secretions of the female reproductive tract and interaction with the oviduct epithelium, spermatozoa attain the ability to fertilise the egg (Hunter and Rodriguez-Martinez, 2004). This process is known as capacitation.

Only a minute fraction of the spermatozoa in an ejaculate ever reaches the site of fertilisation. A single spermatozoon penetrates the ovum while the second maturation division (meoisis) occurs within the ovum. The spermatozoon must first penetrate an outer coat called the *zona pellucida* and then an inner vitelline membrane. This occurs only if the spermatozoon has undergone its final stage of maturation, known as the acrosome reaction. Penetration of the *zona* triggers a reaction or change within the egg called the 'polyspermy block', which makes it impenetrable to other spermatozoa. Fertilisation is completed with fusion of the male and female pro-nuclei and pairing of the chromosomes. The period of time during which the germ cells remain fertile within the ewes' reproductive tract is quite limited. Whereas spermatozoa may remain fertile within the cervix for up to 50 hours after ejaculation, ova generally remain fertilisable for less than 24 hours after ovulation (Hunter and Nichol, 1993).

The fertilised egg or zygote is an unusually large cell of about 120 - 180 µm in diameter. It promptly cleaves or divides to form a 2-cell embryo, and its further development is outlined in Chapter 10.

Endocrinology - the control of reproduction by hormones

A hormone may be defined as a substance produced in one organ or endocrine gland and secreted into the blood-stream to elicit a specific effect in a different target organ or tissue. Hormones may influence the metabolism of tissues in a mature target organ (metabolic hormones) and/or stimulate cell growth and multiplication as well as function in particular tissues or organs (trophic hormones).

Three principal endocrine glands control reproduction: the pituitary gland, the gonads and, in pregnant ewes, the placenta. The pituitary gland is located at the base of the brain and actually comprises two distinct endocrine organs, the anterior and posterior pituitaries. Three further organs secrete hormones or hormone-like substances that regulate pituitary and gonadal function. These are the hypothalamus, situated within the brain and adjacent to the pituitary, the pineal gland, situated behind the third ventricle of the brain and, in the ewe, the uterus.

The anterior pituitary secretes several protein hormones, some of which regulate the function of other specific endocrine organs. The two gonad-stimulating hormones or 'gonadotrophins' are follicle-stimulating hormone (FSH) and luteinising hormone (LH). As the name suggests, FSH stimulates the growth and development of follicles and regulates the number of non-atretic antral follicles within the ovaries. Sustained elevations of the plasma FSH concentration towards the end of the oestrous cycle increase ovulation rate (McNatty *et al.*, 1985). Together with FSH,

LH stimulates the maturation of ovarian follicles and follicular secretion of oestrogen. The pre-ovulatory surge of LH causes the rupture of Graafian follicles and their transformation into *corpora lutea*. A third anterior pituitary hormone, prolactin, is concerned primarily with the maintenance of lactation and is discussed in Chapter 11. Prolactin and LH act together in the ewe as a 'luteotrophic complex', maintaining the structure and progesterone-secreting function of the *corpus luteum.*

The posterior pituitary secretes only one hormone that influences reproductive function. This is the small peptide oxytocin, which also stimulates milk 'let-down' in the lactating animal. Oxytocin stimulates contractions of the smooth muscle of the uterus, especially at mating (when the contractions appear to aid the transport of spermatozoa through the uterus) and at the later stages of parturition (to aid in expulsion of the foetus).

Two principal hormones are secreted by the ovaries – the steroids oestrogen and progesterone. The ovaries also secrete some other hormones, including oxytocin, inhibin and relaxin. Oestrogen is produced by the maturing ovarian follicle and has two main functions: it acts on the central nervous system to stimulate oestrus; and it stimulates the organs of the reproductive tract, causing growth of the uterus, swelling of the vulva and increased mucus secretion by glands within the cervix. Progesterone is secreted by the *corpus luteum*, commencing shortly after the time of ovulation. Adequate production of it is essential for the establishment and maintenance of pregnancy. Progesterone conditions the uterine endometrium to accept and nourish the developing embryo, and suppresses contractile activity in the myometrium. In non-pregnant ewes the *corpus luteum* produces progesterone for about 12-14 days after ovulation, and the ewe cannot return to oestrus and ovulate again until the *corpus luteum* regresses or 'luteolysis' occurs. The induction of oestrus by oestrogen requires a prior period of progesterone priming of the central nervous system. Figure 9.2 shows the changes in the levels of several reproductive hormones during the oestrous cycle. Inhibin suppresses the release of FSH by the pituitary. The role of relaxin is discussed in Chapter 10.

Specific groups of cells within the hypothalamus produce several different releasing and inhibiting hormones, which pass to the anterior pituitary in venous blood via specialised portal vessels to regulate the pulsatile secretion of anterior pituitary hormones. A single hypothalamic peptide hormone, gonadotrophin-releasing hormone (GnRH) regulates the secretion of both FSH and LH, but especially of LH. The large pre-ovulatory surge of LH secretion (Figure 9.2) that causes ovulation in the ewe is triggered by a surge of GnRH secretion only minutes beforehand.

The onset of seasonal breeding activity triggered by a decreasing light:dark ratio is mediated by the retina, the pineal gland and altered secretion of the pineal hormone, melatonin (see Figure 9.3).

Termination of *corpus luteum* function in the non-pregnant ewe requires a positive 'luteolytic' signal from the uterus. Prostaglandin produced by the endometrium passes via a local uterine vein-ovarian artery pathway to the ovaries to cause luteolysis.

Hormonal interactions between the hypothalamus, pituitary, ovaries and uterus

The reproductive cycle in the ewe is complex, and several key

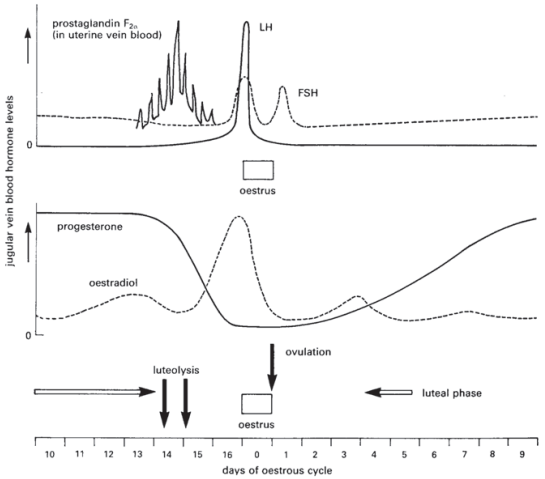

Figure 9.2. Changes in the levels of reproductive hormones in blood during the oestrous cycle of the ewe. Source: Miller (1991).

events require precise timing. Hence the production and secretion of the hormones that control oestrus, ovulation, fertilisation, pregnancy, parturition and lactation must also be tightly controlled. This is achieved by feedback interactions between the hormones from different endocrine organs (Figure 9.3). Such feedback may be either negative (that is, an increase in level of one hormone results in a decrease in the level of another) or positive. Gonadal hormones may influence gonadotrophin secretion by acting either indirectly on the hypothalamus to regulate GnRH release or directly at the pituitary level, and since the site of action is sometimes unclear it is safer to describe the steroids as acting on the 'hypothalamic-pituitary axis'. Neuro-endocrine pathways involving other portions of the brain, such as the pineal gland, also regulate GnRH release by the hypothalamus (Karsch, 1984).

In the non-pregnant ewe with a functioning *corpus luteum,* negative feedback by high levels of progesterone reduces the pulsatile secretion of GnRH and the gonadotrophins FSH and LH, so follicles in the ovary cannot develop fully and secretion of oestrogen is suppressed (Figures 9.2 and 9.3). At the time of luteolysis, decreasing progesterone results in increased gonadotrophin secretion. In particular, the basal level of LH secretion and LH pulse frequency both increase. This altered pituitary function results in further follicular development and oestradiol secretion. The rising blood level of oestrogen just before oestrus stimulates large pre-ovulatory surges of LH and FSH secretion at about the onset of oestrus (positive feedback). The LH surge results in decreased oestrogen secretion, ovulation and progesterone secretion by the new *corpus luteum* (Cumming, 1979; Haresign *et al.,* 1983). Luteolysis is triggered by prostaglandin from the uterus, the release and supply of which is regulated by the ovarian steroid hormones and oxytocin (Krzymowski and Stefanczyk-Krzymowska, 2008). The mechanism of luteolysis is outlined in more detail in Figure 9.4. If the ewe is pregnant the presence of the embryo in the uterus blocks this uterine luteolytic mechanism. This 'maternal recognition' of pregnancy occurs at around day 12 and is mediated by interferon tau – a protein produced by the embryo.

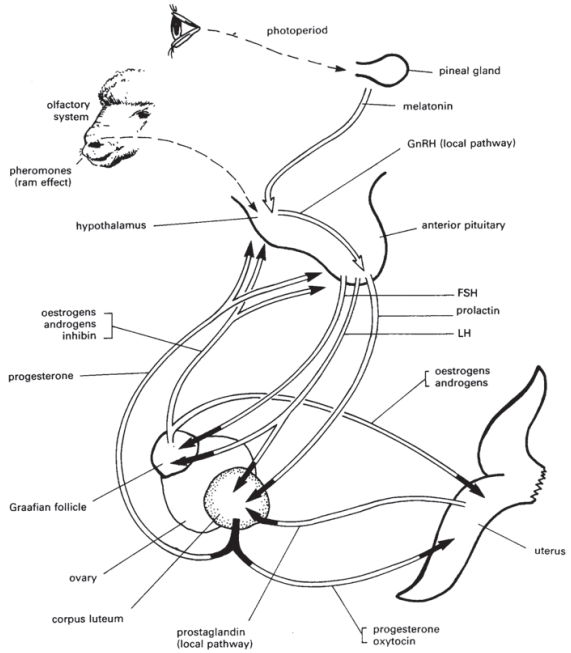

Figure 9.3. Some pathways of hormonal interactions between different organs in the non-pregnant ewe. The diagram also indicates the influence of the external stimuli, photoperiod and pheromones. Source: Miller (1991).

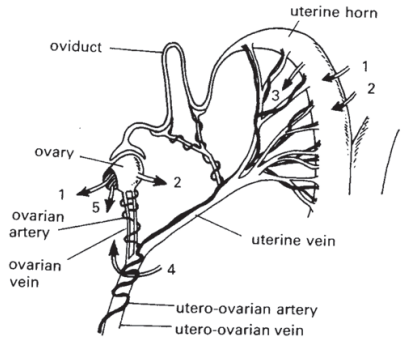

Figure 9.4. The mechanisms of luteolysis in the ewe involves the following steps: 1. Progesterone secreted by the corpus luteum during the luteal phase of the cycle constricts uterine arteries preventing retrograde transfer of prostaglandin $F_{2\alpha}$ from the uterus to the ovary. 2. Oestrogen is released from the ovary during the late luteal phase to assist prostaglandin E_2 in relaxing arterial vessels. 3. This facilitates release of prostaglandin $F_{2\alpha}$ into the uterine vein (and lymph circulation) in increasing amounts. 4. Prostaglandin $F_{2\alpha}$ reaches the corpus luteum to initiate luteolysis via a local uterine vein-ovarian artery pathway and lymph. 5. Oxytocin secreted by the ovary and hypothalamus increase the frequency and strength of uterine contractions, exerting pressure on tissue, blood and lymph containing prostaglandin $F_{2\alpha}$. Thus the levels of prostaglandin $F_{2\alpha}$ reaching the corpus luteum increase to result in complete luteolysis during days 14-15 of the oestrous cycle. Source: Miller (1991).

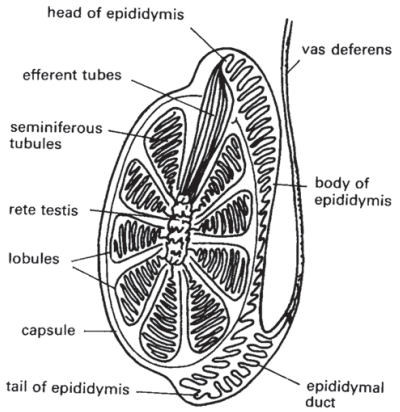

Figure 9.5. Structure of the testis and epididymis. Source: Miller (1991).

The male

Structure of the reproductive organs

The male reproductive organs comprise the paired testes or gonads, the duct system and the accessory sex glands.

During foetal development, ovoid testes form inside the abdomen before descent into the scrotum. As is the case with the female gonad, the two principal functions of the testes are to produce spermatozoa (or mature germ cells) and to secrete male sex hormones. Each testis is structurally divided into many cone-shaped lobules, each containing a number of sperm-producing tubes known as seminiferous tubules (Figure 9.5). These are extremely thin, convoluted and long (the total length of seminiferous tubules in the ram has been estimated at approximately 4-5 km). The epithelial cells lining the tubules are of two types - the spermatogenic cells, which differentiate to become spermatozoa, and the Sertoli cells that support this process. Between the seminiferous tubules in each lobule is the stroma. This makes up the bulk of the testis and contains blood vessels, nerves, lymph and interstitial or Leydig cells, which produce testosterone (Setchell, 1978).

The testes are supported and contained within a skin-covered pouch called the scrotum. Its main function is to maintain the temperature of the testes at several degrees below that of the body, since the temperature within the abdomen is too high to allow the formation of normal spermatozoa.

A system of ducts transports the spermatozoa from the seminiferous tubules to the external opening of the urethra (Figure 9.6). The seminiferous tubules in each testis empty into a network of fine ducts called the rete testis. From here several efferent tubules emerge and then unite to form the epididymal duct. The epididymis consists of three parts - the head, body and tail. The head lies against the dorsal pole of the testis and from there the body runs along the surface of the testis to the ventral pole, where it becomes the tail. The duct is extremely coiled in the head and body but less so in the tail (Figure 9.5). It leaves the tail to become the *vas deferens,* which runs alongside the testis and then passes into the abdomen via the inguinal canal. Each duct continues into the pelvic cavity and joins with the urethra near the neck of the urinary bladder. The final part of each deferent duct is thickened to form the ampulla (an accessory sex gland). The urethra becomes the common excretory duct for the urinary and male genital systems. It proceeds backwards through the pelvis before becoming incorporated into the structure of the penis (Figure 9.6).

The penis (Figure 9.7) consists essentially of cavernous tissue connected with special blood vessels, muscle, and the urethra. Upon sexual excitement, the penis becomes rigid or erect by engorgement of the cavernous tissue with blood and straightening of the sigmoid or 's-shaped' flexure. A fold of skin holds the penis against the abdominal wall. The prepuce is an extension of this fold with a modified internal layer that, except during mating, protects the sensitive head or *glans* of the penis.

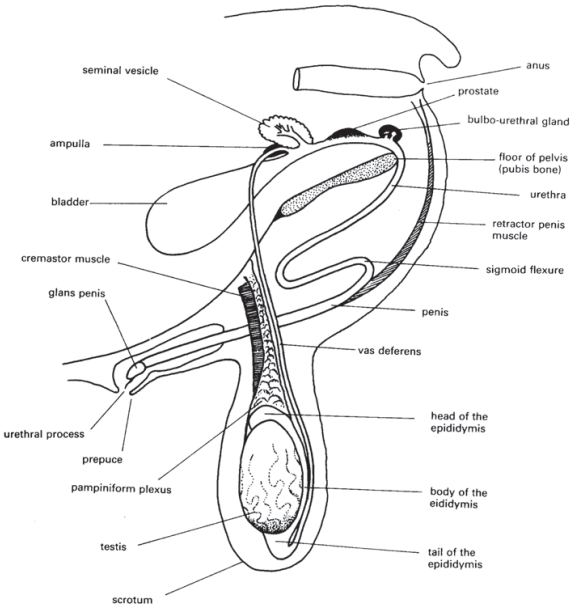

Figure 9.6. The reproductive organs of the ram. Source: Miller (1991).

Figure 9.7. The penis of a ram during veterinary examination. The filiform appendage is held between the forefingers. Source: WMC Maxwell.

In the ram, a urethral process or filiform appendage, which is a continuation of the urethra beyond the *glans,* rotates vigorously and sprays semen about the anterior vagina during ejaculation.

The four accessory sex glands - located behind the neck of the urinary bladder and near to the urethra as it passes through the pelvis - comprise the prostate, the vesicular glands (or seminal vesicles), the bulbo-urethral or Cowper's glands and the glands of the ampulla of the *vas deferens.* At ejaculation secretions of these glands are discharged into the urethra and mix with fluid secretions of the testes and epididymides to form the 'seminal plasma', most of which is derived from the vesicular glands (Setchell, 1978).

Semen and spermatozoa

The male reproductive system produces semen, which is comprised of both cellular (the spermatozoa or sperm) and fluid (the seminal plasma) components. Spermatozoa are produced continuously after puberty, and carry the haploid paternal contribution to the chromosomes or genes of the next generation. A typical ram ejaculate comprises about 0.5-2 mL of semen containing 1.5-5.0 × 10^9 spermatozoa per mL. Continuous matings deplete sperm numbers per ejaculate, but sperm reserves are rarely exhausted in the field.

Spermatogenesis, sperm transport and sperm structure

Spermatozoa are produced from precursor cells called spermatogonia, which are surrounded by the supportive Sertoli cells. The cells within the seminiferous tubules are protected by a 'blood-testis barrier', which precludes the movement of cells and proteins from the blood to the tubules, in effect protecting developing spermatozoa from the animal's own immune system. The full transformation from spermatogonia to spermatozoa through several intermediate cell types and both mitotic and meiotic cell divisions is called the spermatogenic cycle (Figure 9.8), and takes about 47-48 days in the ram (Ortavant, 1956; Cardoso and Queiroz, 1988). As cells pass through this cycle they move away from the wall or periphery and towards the centre of the seminiferous tubule. Upon release by the sertoli cells, spermatozoa travel out of the seminiferous tubules and into the epididymis via the *rete testis* and *vasa efferentia*.

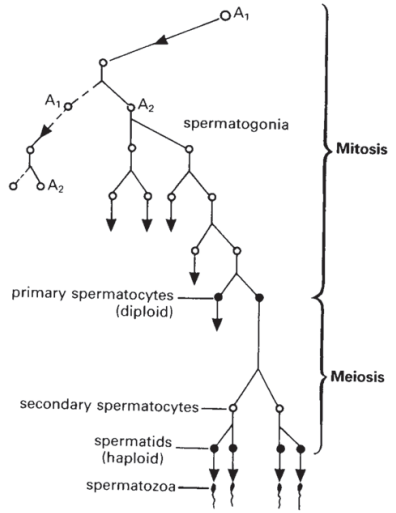

Figure 9.8. A schematic outline of spermatogenesis. Source: Miller (1991).

The epididymis (especially its tail, which has a relatively wide lumen) acts as a reservoir for spermatozoa, storing an estimated hundred thousand million cells ready for ejaculation. A steady supply of spermatozoa flows from the testis, but overflow through the *vas deferens* varies depending on the level of sexual activity. When rams are not sexually active, aging spermatozoa may be resorbed by the ducts or lost into the urine (Setchell, 1984). Generally speaking, spermatozoa spend about 10-14 days passing through the epididymis. During this period they undergo maturation; gaining the potential for sustained motility, increased resistance to thermal and osmotic shock and ultimately the ability to fertilise ova after capacitation.

The mature spermatozoon comprises a head, neck, mid piece, main piece and end piece. The ovoid head is about 5 to 10 µm long and contains the chromosomes. It is capped by an envelope or acrosome. The mid, main and end pieces are together about 40-65 µm long and provide the locomotor system or flagellar apparatus. The structures of a normal spermatozoon and some abnormal ones are depicted in Figure 9.9.

Seminal plasma and semen ejaculation

The mixed fluid secretions of the accessory sex glands, testes and epididymides have several functions. They act as a vehicle to convey the spermatozoa during ejaculation, provide the spermatozoa with a buffering medium (to protect spermatozoa from acidic conditions inside the vagina) and a source of nutrients (for metabolism). Seminal plasma contains many unusual substances. These include inorganic ions, citric acid and organic salts to maintain the osmotic pressure and pH of semen, sugars such as fructose to provide an energy source to sustain sperm motility and a number of proteins, some of which are important in the capacitation process (Maxwell *et al.*, 2007).

Ejaculation requires a sequence of coordinated contractions starting in the epididymides and passing along the *vas deferens.* Simultaneous contractions of the muscles surrounding the accessory glands produce mixing of spermatozoa and seminal fluid within the urethra (known as emission). Contractions of the urethral muscle propel the semen through the urethra and penis. Ejaculation in the ram occurs very rapidly, with virtually complete mixing of the components of the seminal plasma.

Libido and mating behaviour

Ram sexual drive or libido is not easily defined or measured. It is roughly the level of interest in and ability to mount the ewe. Libido is not necessarily related to amount or quality of semen produced, although clearly both strong libido and good semen quality are essential if natural mating is to be effective. Many factors, some of which are quite subtle, may influence ram libido and mating behaviour in flocks in the field (Lindsay, 1979; Fowler, 1984). In artificial insemination programs, care must be taken to ensure that the semen collection procedure is not painful or uncomfortable and that each ejaculation is a satisfying experience to the ram.

The breeding season

Rams have a much less marked breeding season than ewes. Merino rams usually show little evidence of seasonal breeding. Any minor changes in testis weight associated with a change in photoperiod are overridden by seasonal changes in nutrition (Martin *et al.,* 1990). This is not the case in British breeds where marked declines in testis size and semen volume (Setchell, 1984) and libido (Haynes and Schanbacher, 1983; Fowler, 1984) are observed out of season.

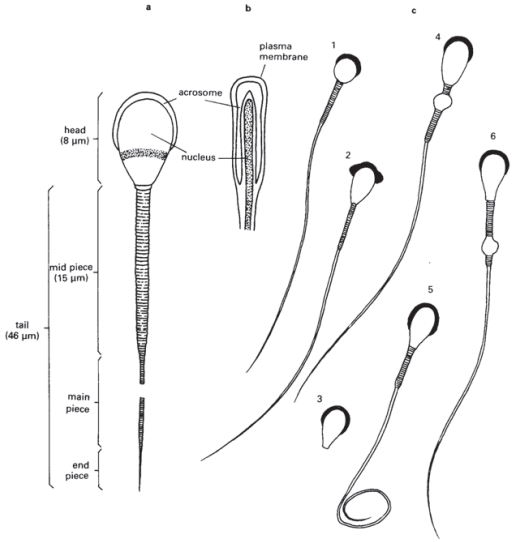

Figure 9.9. Structure of ovine spermatozoa: a) normal spermatozoon; b) the sperm head in cross section; c) some examples of abnormal spermatozoa – 1. round head, 2. degenerating acrosome, 3. separated head, 4. proximal cytoplasmic droplet, 5. coiled tail, 6. abaxial midpiece, distal cytoplasmic droplet. Source: Miller (1991).

Endocrinology

In the ram, FSH stimulates growth of the seminiferous tubules and production of spermatozoa. It also acts on the Sertoli cells, to stimulate their function and the production of inhibin. LH stimulates the secretion of testosterone by the Leydig cells of the testes.

The steroid testosterone is the principal testicular hormone (Setchell, 1984). By comparison with the ewe, rams have rather constant gonadal hormone secretion after puberty, since they do not exhibit similar cyclical breeding activity. Testosterone is required for the maturation of spermatozoa and the development and function of the organs and accessory glands of the male reproductive tract. It also regulates libido and controls secondary sexual characteristics.

Interactions between various reproductive hormones (Figure 9.10) in mature rams seem simple relative to those in the ewe. Both gonadotrophins constantly stimulate different aspects of testicular function, as mentioned previously: GnRH, FSH and LH secretion are in turn regulated by the negative feedback effects, principally of testosterone but also, in the case of FSH, of inhibin from the seminiferous tubules.

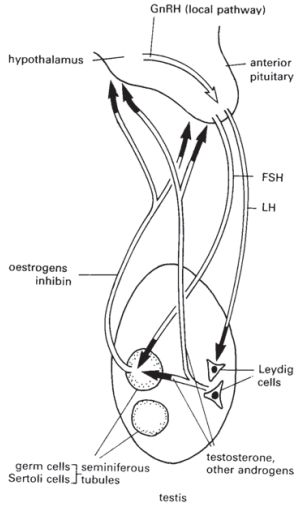

Figure 9.10. Some pathways of hormonal interactions between different organs in the ram. Source: Miller (1991).

Teasers

These are sheep that are sterile but behave like entire rams. They may be used to induce breeding activity out of season (of particular importance to South and Western Australian flocks) or to identify ewes in oestrus in an A.I. program. Rams may be turned into 'permanent' teasers by vasectomy, while wethers may be turned into 'temporary' teasers by injections of testosterone or oestrogen. Rams may also be turned into 'temporary' teasers by the use of 'aprons'.

Regulation of natural reproductive performance in ewes

Definitions

There are several ways of defining the level of reproductive performance in ewe flocks. These include: lamb weaning and lamb marking percentages [which are respectively the numbers of lambs weaned (LW) and marked (LM) per 100 ewes joined (EJ)]; reproduction rate (the number born alive at lambing per ewe joined); fertility (the proportion of ewes in a flock that actually lamb; or ewes lambing per ewe joined *i.e.* EL/EJ); and fecundity (total lambs born per total ewes lambing) or prolificacy (the average number of lambs born per ewe lambing). Clearly, some of these indices of reproductive performance are not easy to determine accurately, and they embrace varying portions of the total reproductive cycle and may or may not be influenced by losses at around the time of lambing (perinatal mortality) or during the interval between marking and weaning. Methods for measuring more discrete events early in the reproductive cycle, such as level of mating activity, fertilisation rate, ovulation rate and rate of embryo mortality are described later. In any event, lamb marking and weaning percentages (LM/EJ and LW/EJ, respectively) are the indices of reproductive performance of greatest significance to the producer and are defined here as net reproductive efficiency.

Levels of reproductive performance in Australia

Lamb marking percentages vary greatly across Australia (Table 9.1). New South Wales alone shows tremendous variation (Table 9.2) with about 8 - 10% of properties marking more than 100% lambs while 4 - 10% mark less than 40% lambs (Restall, 1976; Plant, 1981a). In Western Australia, many flocks are mated during the non-breeding season and lamb markings of 50-60% are typical (ABS, 1995). Overall, Australian producers currently mark 82 lambs per 100 ewes joined (82%; MLA, 2007), a figure which has gradually improved from that observed during the early 1900s (approximately 50-60%; Plant 1981a) By comparison, New Zealand graziers mark 118% lambs (NZ Meat and Wool Board, 2002).

Table 9.1. Average lamb marking percentages for Australian states, summer 2007 to summer 2008.

State	*Lambs marked (%)*
NSW	83.9
Victoria	87.5
South Australia	91.6
Western Australia	72.7
Tasmania	79.7
Queensland	88.7
Australia	82.3

Source: MLA (2007).

Table 9.2. Reproductive performance of 11,642 ewes in 27 flocks lambing in the spring, 1974.

Parameter (%)	*Wagga (13 flocks)*		*Dubbo (14 flocks)*	
	Mean	*Range*	*Mean*	*Range*
Ewes raddled	97	95-99	90	83-100
Returns to service	29	17-56	23	13-59
Ewes dry	12	4-24	11	2-36
Lambs marked to				
Ewes joined	73	49-92	80	31-127
Ewes rearing	111	100-130	114	100-143

Source: Plant (1981b).

It is difficult to categorically define a satisfactory level of flock reproductive performance (Plant, 1981a), particularly in light of the widely differing nature of sheep grazing across Australia. However, in New South Wales an average commercial flock of adult Merinos lambing in spring should usually be able to achieve the following results, expressed as percentages: ewes mating, 95%; ewes in lamb, 95%; pregnant ewes losing all lambs, 10-15%; twins reared by ewes rearing lambs, 15-20%; ewes lost from joining until lamb marking, 5%; lambs marked to ewes joined, 95- 100%. Maiden ewes in the same flock might be expected to mark 75-80% lambs.

Often the causes of low reproductive performance in flocks are misconceived and are in fact multiple. Very careful field investigation techniques are necessary to isolate and determine the principal causes of low reproductive performance, and are discussed in Chapter 10. In the absence of such information producers often partially compensate by extending the length of the joining period, or by joining ewes on more than one occasion through the year. Either practice may increase the number of lambs born per annum, but both have similar disruptive effects on flock management - complicating lambing, shearing, dipping, weaning and classing procedures - and the nutrition of the breeding flock often suffers.

The wide variation observed in flock reproductive performance is most likely due to differing genotype, nutrition, environmental conditions and management practices, rather than to specific diseases (Restall, 1976; Plant, 1981b; Kleemann and Walker, 2005a,b; Kleemann *et al.*, 2006) as is often assumed.

The following section discusses the influence of such factors on reproductive performance in ewes and their potential for manipulation to increase reproduction rate.

Genotype

Breed and strain

Breed and strain of sheep clearly influence reproductive performance. Merinos mated in autumn show no major strain differences in lambs weaned per ewe between the small Saxon-type fine-wool, Peppin and South Australian strains. However, when mated out of season the large South Australian strong-wool strain is the more fertile. This strain is probably the best for joining out-of-season - in fact, it has the least-defined breeding season among Merinos and does not exhibit the marked autumn peak in fertility and fecundity seen in Peppin and fine-wooled strains (Fletcher, 1971; Pattie, 1973). Obviously, with the declining importance of the South Australian strain for the Australian sheep industry (Chapter 2) this fact is increasingly irrelevant.

In general the British breeds achieve considerably higher lamb marking percentages than Merinos, although their performance in Australia is often less impressive than that recorded in Britain. The Border Leicester is generally considered a highly prolific breed, with many lambing ewes having twins or triplets. Their mean ovulation rate in autumn is at least 2, but up to 45% of ewes of this breed may not lamb and low lamb-survival rates are also common (Fogarty *et al.*, 1976). Certain exotic sheep breeds such as the Romanov and Finnish Landrace, as well as the Booroola Merino, have litter sizes well over 2. Merino litters generally average less than 1.3, while the Border Leicester and Dorset have intermediate litter sizes (Hall, 1984).

For prime lamb production, crossbreeds are considered the most popular dams. This is in part due to the hybrid vigour they display in respect of certain reproductive traits (Chapters 1 and 8). Border Leicester × Merino (F1) ewes have generally achieved the highest lamb outputs, with maximum production of about 1.6 lambs born and 1.3 lambs weaned per ewe joined (Hall, 1984).

The Booroola Merino is a strain particularly well known for its prolificacy. This characteristic is due to a segregating autosomal major gene (*FecB*^{B}; Piper and Bindon, 1982) with an additive effect on ovulation rate and a partially dominant effect on litter size (Davis, 2005). This results from a single point mutation in the gene for transforming growth factor (TGF) receptor - bone morphogenetic protein receptor type IB (BMPRIB), also known as activin-like kinase 6 (ALK6) (McNatty *et al*., 2005). Put simply, ewes heterozygous (B+) for the ALK-6 (*FecB*) mutation display an increase in ovulation rate and litter size of 1.5 and 1.0, respectively. While homozygous carriers (BB) of ALK-6 display an increase in ovulation rate and litter size of 3.0 and 1.5, respectively (Davis, 2005). Unfortunately, due to the high reproductive wastage in *FecB* flocks on extensively managed Australian properties (both pre- and peri-/postnatal), net reproductive efficiency remains similar to that of flocks without the *FecB* allele (Kleemann *et al.*, 2005a). Hence the merit of the Booroola Merino as a dam for prime lamb production remains unclear. However, the transfer of this gene into breeds in other countries which practice intensive management has been viewed as successful, despite claims of slower post-weaning growth rate and lighter mature bodyweight of resultant progeny (Gootwine *et al.*, 2006).

There are various other sheep breeds that have been introduced to Australia for the purpose of intensive lamb production (Chapter 2), some of which do exhibit impressive reproduction rates. However, none of these purebreeds have as yet had a major impact on systems of prime lamb production in Australia so will not be discussed.

Breeding and selection

The preferential selection for reproductive traits such as twinning can improve the generally low level of reproductive performance in Merinos (Hanrahan, 1980). However, the heritability of reproductive traits is low: estimates are variable and somewhat unreliable, but typical values for fertility and prolificacy are approximately 0.05 and 0.1, respectively (Fogarty, 1995; Safari *et al.,* 2005, 2007; Huisman *et al.,* 2008). Hence selection is likely to be relatively inefficient.

There has also been concern that selection for body characteristics can influence aspects of reproductive performance. For example, selection for increased skin folds in Merinos in past decades had a substantial and adverse effect on reproductive performance, due to higher percentages of dry ewes (probably ram-related) and higher lamb losses between birth and weaning; the effect being greater in poor years (Dun, 1964; Fowler, 1976; Atkins, 1980). This is of little consequence in modern flocks as graziers have increasingly moved toward a plainer skin. Other aspects of body confirmation have been implicated in decreasing fertility (*e.g.* muffled face), but this has not been clearly established (Restall, 1976). Of greater concern is the effect of selection for decreased fibre diameter. It has been shown that flocks with a genetically lower fibre diameter also have a lower reproduction rate (Adams and Cronje, 2003) and sheep born as multiples tend to have a higher fibre diameter than those born as singles (Kelly *et al.*, 2007). Hence, selection for fibre diameter alone may lower reproductive performance of the flock over time. While the two traits are correlated, multi-trait selection can be used to control changes in fibre diameter without adversely affecting reproductive performance (Chapter 8; Safari *et al.*, 2007; Huisman and Brown, 2008; Huisman *et al.*, 2008).

Nutrition

The ewe's nutritional status is a major determinant of her fertility and fecundity and of lamb survival, yet the way in which nutrition regulates reproduction remains poorly understood. Ideally it should be matched to critical periods during the reproductive cycle, so that the ewe has optimal body weight and condition during the joining period and again during late pregnancy and lactation. In practice, providing ewes with abundant, good pasture both around joining and during late pregnancy and lactation is usually impossible, and uncontrollable variation in

pasture availability from year to year means that any matching of requirements with pasture growth is highly imprecise. Fortunately, the adverse effects of fluctuations in pasture growth can usually be reduced by manipulating one or more of three factors; the energy reserves of the body, the carryover of pasture residues and the use of supplements (Chapter 14).

The importance of nutritional status at around the time of lambing and early lactation is discussed in Chapters 10 and 14. The influences of nutrition on fertility and fecundity are not easy to disentangle, since in Merinos fertility usually increases with increasing ovulation rate.

Nutritional status and ovulation rate

It has long been recognised that ovulation rate and the incidence of twinning can often be increased by giving ewes additional feed before and during the joining period (Lindsay, 1976, 1983). This practice, called 'flushing', is usually most effective when ewes are in medium to poor condition beforehand, but the physiological effect is not well understood (Nottle *et al.*, 1997).

The two most common explanations are known as the 'static' and 'dynamic' theories. The static theory states that ovulation rate is a function of body weight, regardless of how or when this body weight was achieved. The dynamic theory, currently viewed as less important, postulates that the rate of increase in body weight or condition score, rather than absolute body weight or condition score at joining, determines ovulation rate. Others believe that both a 'rising plane' and absolute body weight are involved in twinning performance (Killeen, 1967; Restall, 1976). A slight modification of the static theory (taking into account both liveweight and body condition score) is increasingly recognised as the best way to manage a flock for joining. Within a particular strain or breed of sheep, liveweight at ovulation correlates well with ovulation rate (Fletcher, 1971; Restall, 1976; Kleemann and Walker, 2005b; Kleemann *et al.*, 2006). Some studies have indicated that, for Merino and crossbred flocks within a range of body weight of 35-50 kg, increases of about 2-4% in ovulation rate and twin lambs born per 100 ewes joined occur for each additional kilogram of liveweight at joining. More recent studies place this gain in ovulation rate at 1.6% (Hatcher *et al.*, 2007a) and 1.8% (Kleemann and Walker, 2005b) per kg increase in liveweight in flocks from NSW and South Australia, respectively, though this response to liveweight varies widely between seasons (Fletcher *et al.*, 1970; Cumming, 1977; Kleemann and Walker, 2005b;) and flocks (Hatcher, 2007). In NSW fine and superfine flocks, each increase in condition score (1-5) boosts ovulation rate by 12-13% (Hatcher *et al.*, 2007a). Combined, such studies show that both liveweight and condition score account for a significant proportion of the variability of ovulation rate, at least in mature ewes. In maidens, it would appear that liveweight is considerably more important in determining ovulation rate than condition score (Kleemann and Walker, 2005b; Hatcher *et al.*, 2007b). Though ovulation rate continues to increase with liveweight and condition score in both mature and maiden ewes, economic considerations combined with increases in lamb mortality above condition score 3.5 places an upper limit on these characteristics. Therefore, it is now recommended that most Australian producers aim to join mature ewes at condition score 3 and maiden ewes at 45 kg liveweight.

There are situations in which ovulation rate is independent of both liveweight and condition score. The most relevant example being the use of lupin grains (which have high protein and energy levels) fed to ewes for 2 weeks immediately before and during joining. This feeding regime rapidly increases ovulation rate without significantly altering body weight (Lindsay, 1976; Downing *et al.*, 1995; Van Barneveld, 1999). A significant increase in ovulation rate (20-30%) occurs within 6 days of the commencement of feeding 0.5 kg of lupins per head per day and the response disappears soon after the feeding ceases (Croker *et al.*, 1979; Lindsay, 1983). Of course, if the lupin grain supplement is fed for several weeks, the ewes do gain weight. The means by which this increase in ovulation is achieved is still poorly understood. However, it would appear that lupin supplements increase peripheral insulin levels (Downing *et al.*, 1995), which in turn up-regulate insulin mediated glucose uptake by the ovary. This occurs via the insulin-dependent glucose transporter (GLUT4) in the theca and granulosa cells (Munoz-Gutierrez, 2002). While this mechanism does not fully explain the 'lupin effect', increased glucose levels do directly stimulate folliculogenesis, increasing follicle recruitment and decreasing atresia (Munoz-Gutierrez, 2002; Somchit *et al.*, 2007), resulting in an increased number of follicles available for ovulation. Further exploration of the hormones, biosynthetic pathways and mechanisms involved in the effect of lupins on the ewe's reproduction continues.

Although similar results may be obtained with other high-protein and energy supplements, cereal grain which is high in energy only has little influence on ovulation rate. This is thought to be due to the low starch content of lupins (Van Barneveld, 1999), which increase energy substrates such as acetate available for gluconeogenesis *i.e.* the production of glucose and its uptake.

When flushing is effective it not only increases fecundity but, as previously discussed, usually also increases fertility within the flock (Lindsay, 1976; Croker *et al.*, 1979). This means that ewes may have more than one ovulation and so have a better chance of becoming and staying pregnant, even if they give birth to only one lamb. It is unclear whether this results from increased progesterone secretion and/or an increased mass of foetal tissue in multiple-ovulating ewes. Patterns of change in mean ovulation rate during the year may vary widely between different areas of Australia and different strains of Merino. Thus at Trangie, N.S.W., ovulation rate is usually highest during March-April (Restall, 1976), whereas in South Australia it is usually highest in summer and falls until April with declining body weight and nutritional status (Fletcher, 1971) or does not vary (Kleemann and Walker, 2005a).

Nutrition and the breeding season

Ewes need to be in very poor body condition, bordering on starvation, before they will fail to express oestrus and ovulate during the breeding season. Hence, nutritional status in mature ewes at that time probably has little influence on the onset or

duration of breeding activity (Lindsay, 1976); despite evidence suggesting under nutrition may increase the incidence of anoestrus ewes (Tassell, 1967; MacKenzie and Edey, 1975). It is also possible that under nutrition during the preceding winter and spring may have a delayed effect on oestrous activity at the beginning of the spontaneous breeding season (Smith, 1965; Fletcher, 1974; Oldham, 1990). Although not easily explained, this phenomenon is clearly important in Australia and is observed even in ewes of normal liveweight at the usual time of joining.

In areas such as South and South-western Australia where pasture availability and hence nutrition does not align with the spontaneous breeding season of January-February to April-May, nutrition plays a greater role than photoperiod in establishing the breeding season. Graziers regularly join animals during the 'commercial breeding season' of October to March, with over half of all joinings in Western Australia taking place before January. While the high incidence of oestrous activity during the October to March period can be accounted for by the use of 'teasing' or the 'ram effect', condition score is still positively related to the spontaneous incidence of oestrus at this time (Kleemann and Walker, 2005b; Kleemann *et al.*, 2006). Comparable fecundity between ewes joined in the commercial and spontaneous breeding seasons is most likely due to low bodyweights during the latter.

Nutrition and puberty

Onset of puberty depends on several factors, but principally genotype, season of birth, liveweight and growth rate (Dyrmundsson, 1983). Not surprisingly, there is a wide variation in Australia in the age at which Merinos first show oestrus (150-700 days). Under good nutritional conditions spring born ewe weaners may exhibit oestrus during the following autumn at 6-8 months of age. Certain breeds, such as the Dorset or crossbreds, regularly reach puberty at younger ages. Those lambs that do reach puberty at 6-8 months are not yet sexually mature as evidenced by their poor fertility (Quirke *et al.*, 1983). Less well-grown ewe lambs will not exhibit oestrus until the next summer-autumn, by which time they are 'maidens' and usually show good mating activity and much improved fertility, which however, is still significantly below adult levels (Watson and Gamble, 1961; Restall, 1976; Kleemann and Walker, 2005b).

The effects of season of birth on the live weight and age at puberty are most marked in ewe lambs that have grown rapidly, where spring born lambs will reach puberty by autumn but autumn born lambs will be delayed until the following autumn. This is less obvious in States like Queensland where poorer nutritional conditions result in slower growth rates and delayed puberty

Cycling weaner ewes can be joined at 7-9 months old, but their fertility is low (maximum 60-80% in crossbred ewes of 35 kg liveweight). However, ewes mated as lambs have higher fertility in subsequent joinings, fewer lambing troubles, longer reproductive lifetimes and can be culled earlier facilitating faster genetic progress. This must be weighed against the disadvantages of more maiden ewes in the flock, greater supervision during joining, pregnancy and lambing and the lower marking percentages. In Australia, ewes are seldom first joined as weaners but almost always as 2-tooths at 18 months of age. This generally allows ewes to reach target joining weights of at least 40 kg for medium-wool Merinos and 45 kg for crossbreds (or 75-80% of their mature weight) to maximise fertility and fecundity for maidens. If the disadvantages outweigh the positives of weaner joining, it is advisable to ensure nutrition allows joining at 18 months to avoid a first mating at 30 months which increases costs per animal, increases cull age and either slows down genetic improvement or decreases meat production in prime lamb operations.

Specific components of nutrition

A number of specific nutritional deficiencies reportedly influence reproduction in the ewe. These include deficiencies of selenium, iodine, zinc and manganese, and are discussed in Chapter 13. Ewes grazing pastures dominated by certain cultivars of subterranean or red clover that contain plant oestrogens or coumestans may exhibit infertility. The chronic form of this disease is uncommon (and largely confined to Western Australia and Kangaroo Island in South Australia) and involves severe infertility associated with failure of sperm transport through the cervix and reduced fertilisation rates. The oestrogenic effect is cumulative, causing permanent changes to the structure of the cervix. A second form of the disease, called 'temporary infertility', occurs commonly in several States when ewes feed on oestrogenic clovers for only a limited period shortly before or during joining. It is characterised by a reduction in the incidence of oestrus, a reduction in ovulation rate and lowered fertilisation rates. The disease is controlled, where possible, by removing the affected ewes to paddocks free of the offending clover cultivars for at least 35 days before joining (Wroth and Lightfoot, 1976; Adams, 1979; Plant, 1981b). Pastures high in endophyte perennial ryegrass should also be avoided as conception and lambing rates may decrease by as much as 20%, even without visible ryegrass staggers.

Conclusions concerning nutrition

Nutrient intake and body weight have a critical influence on several facets of reproductive performance in young and adult ewes. However, it is usually difficult to accurately predict the animal response to specific changes in nutritional management, and the producer must balance the importance of these nutritional effects on ewes against the cost of manipulating pasture availability, providing supplements and any potential effects to fibre diameter (Chapter 13).

Physical environment and climate

The first ovulation at the commencement of the breeding season is usually 'silent' - that is, it is not accompanied by oestrus. The few ewes that do come into oestrus at this first ovulation will mate, but display poor fertility at this time (Oldham, 1980).

Breeds differ little in the length of the season during which 100% of ewes can be expected to be ovulating (approximately February-June – see Figure 9.11). However, whereas most of the British breeds, including the Border Leicester, enter the non-

breeding season abruptly in June-July, Merino flocks tend to drift gradually towards a state of anoestrus in October-December and some Merinos continue to cycle all the year round. During June-February, anoestrous Merinos can be stimulated to ovulate by the ram, known as the 'ram effect', whereas rams can stimulate ovulation in most of the British breeds for only about 6 weeks before the normal time of onset of the breeding season in early to mid January (Lindsay, 1983). A few British breeds, notably the Dorset, have somewhat less rigidly defined breeding seasons. Border Leicester × Merino crossbred ewes show a pattern of breeding activity intermediate between those of the parent breeds, and lambing rates after spring joinings are often poor. In contrast to breeds like the Border Leicester and Scottish Blackface, breeds of sheep indigenous to countries close to the Equator are often non-seasonal breeders. Although Merinos can be bred at any time of the year, their reproductive performance is usually superior after an autumn rather than a spring joining; although this depends on location and subsequent nutrition. Extensive studies at Trangie, NSW (Dun *et al.*, 1960) showed that on average spring lambings produced 15% more wet ewes, 25% more twins mothered and 33% more lambs weaned (Table 9.3) whereas those in Western and Southern Australia show fairly comparable lambing rates between spring and autumn (Oldham *et al.*, 1990; Kleemann and Walker, 2005a).

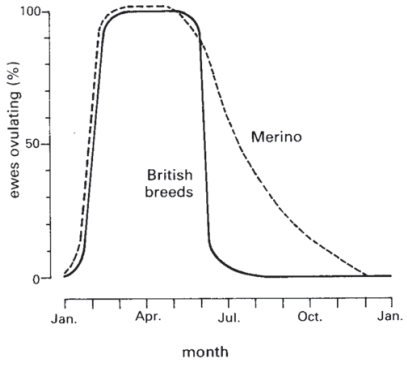

Figure 9.11. Incidence of ovulation in Merino and British-breed sheep in Australia throughout the year.
Source: Lindsay (1983).

Heat stress can reduce breeding activity in Merino ewes. This is observed most often in Queensland. High ambient temperatures in the mid to late luteal phase of the oestrous cycle are reputed to lengthen the cycle by 1-2 days, dampen oestrous behaviour towards rams and reduce the length of oestrus. As a consequence, spells of high temperature during the joining period may lessen the chances of ewes being inseminated and fertility can be reduced (Lindsay, 1983; Kleemann and Walker, 2005b). Even when fertilisation does occur, high temperatures may affect embryo survival (Lindsay *et al.*, 1975) adding to the number of ewes returning to service (Kleemann and Walker, 2005b). If such environmental conditions are experienced during the initial weeks of mating, commercial producers should consider extending the mating period for an additional oestrous cycle (~ 3 weeks).

It is unlikely that extreme temperatures, both hot and cold, have any significant direct influence on the ewe's reproductive system in southern Australia, but fertility may be influenced indirectly by temperature effects on flock behaviour and the extent of ram-ewe contact. The expression of oestrus may also be suppressed by periods of heavy rain or strong winds, but these effects are mostly too fleeting to influence flock fertility.

Management

Several management practices, in addition to the factors already discussed, will influence the fertility and fecundity of ewe flocks (Chapter 17).

Age structure of the ewe flock

Oestrus is often of shorter length and lower intensity in 'maiden' ewes than in adult ewes in the same flock. Further, the length of the breeding season and ovulation, fertilisation and embryo survival rates are commonly lower in 'maiden' than in mature ewes. If joined alongside adult ewes, 2-tooths may have too little contact with the rams. Information about the level of ram-ewe contact and mating can be obtained by harnessing crayons to the rams (see later in this chapter) and if, as is likely, a problem exists with the maiden ewes, they should be joined separately, preferably to older, experienced rams. A longer joining period and/or a higher joining ratio should also be considered. If a longer period is employed, joining should start earlier, so that the young ewes will have time to recover for the next mating (Fowler, 1972).

Table 9.3. The effect of season of mating on reproductive performance of Merino ewes at Trangie, NSW.

Time of mating	*Unmated ewes (%)*	*Wet ewes (%)*	*Multiple births (twins) (%)*	*Wet ewes losing lambs (%)*	*Dead ewes (%)*	*Lambs mothered (%)*	*Lambs weaned (% of ewes joined)*
Spring*	18.0	72.1	12.0	5.6	2.1	75.8	66.7
Autumn**	5.0	87.8	37.5	7.7	2.1	112.8	99.5
Advantage to autumn matings	+ 13.0	+ 15.7	+ 25.5	+ 2.1	0	+ 36.7	+ 32.8

*average of 6 matings. **average of 4 matings
Source: Dun *et al.* (1960).

Ovulation rate and litter size in Merinos increase with age, being lowest in maidens and highest at about 6-7 years of age (Turner *et al.*, 1968). Flock reproductive performance can therefore be adjusted to some extent by manipulating its age structure. Many ewes are culled before their reproductive performance peaks.

Time and duration of joining

Ovulation rate and fertility are usually maximal at the peak of the breeding season. If ewes are to be joined at other times the penalties in terms of reproductive performance should be calculated.

Effects of other management practices

Ewes should ideally be left well alone, but observed, during the joining period. It may be advantageous to crutch, wig and drench them shortly beforehand, and overgrown or diseased hooves should receive attention then. Shearing during the joining period can affect the lambing pattern. Shearing prior to joining may be a useful method of hastening the onset of oestrus in flocks known to be in anoestrus at the time of joining. Interactions between shearing time and ewe fertility are unclear (Kennedy *et al.*, 1982), but it is safest to avoid shearing for at least 2 weeks prior to joining. Ewes to be joined out of season must be well separated from all rams and teasers for at least 1 month before joining.

Regulation of reproductive performance in rams

Rams may vary greatly in their reproductive capabilities, and genetic considerations contribute strongly to this variation. It is easy to identify the relatively small proportion of infertile males, but much harder to rank the remaining fertile rams in order of their reproductive capacity. No tests on the ram will give accurate predictions of fertility in the field. To date, physical examination of the genitalia and measurements of testicular size and of serving capacity in pen mating tests have proved the most useful indices.

Genotype

Testicular size and serving capacity of rams can be improved by selection, with the former having quite high heritability (Huisman *et al.*, 2008). While there is no correlation in rams over 18 months of age, testicular size at 12 months correlates well with serving capacity at 18 months so testis size can be used in selecting hogget rams. Testicular size is moderately correlated with other selection criteria, such as liveweight and fleece weight (Safari *et al.*, 2005; Huisman and Brown, 2008), and is strongly correlated with age at puberty (negative), ovulation rate (positive) and fertility (positive) of female offspring. It is not clear whether the serving capacity of rams is also correlated with such traits (Kilgour and Blockey, 1980; Purvis *et al.*, 1987).

Effects of age and of sexual experience

The ram's testes develop rapidly from 3-4 months onwards. Puberty sometimes occurs at 4-5 months of age, but more commonly fully formed spermatozoa are first released from the testes at 6-7 months (Fowler, 1976). Hence if ram lambs are not weaned until 5 months or more after birth, the ewes may become pregnant to their sons. By 12 months the ram should be producing ejaculates containing a high concentration of highly motile spermatozoa (Courot, 1979). However, the process of maturation is slow and 2-3 year-old rams usually produce considerably more spermatozoa than 1-2 year-olds. Ram lambs should be kept on a high plane of nutrition in order to hasten the development of full sexual maturity.

As mature rams age they run an increasing risk of having lesions within the testes or reproductive tract that may cause loss of fertility (Holmes, 1981). For example, age clearly correlates with the incidence of brucellosis, varicocoele and scrotal dermatitis in rams that have not been subjected to annual physical examinations (see Table 9.4). The incidence of other diseases that affect libido, such as arthritis, also increases. For these reasons it is recommended that about one-third of Merino rams are replaced annually. This ensures that the ram flock remains young and vigorous, and should increase the rate of genetic gain, provided the replacements are genetically superior (Fowler, 1976; Holmes, 1981). In practice, Merino rams are commonly used for 4-5 years, provided they are sound. British-breed terminal sires for prime lamb production may be used for as long as they remain sound.

Some rams, regardless of age, tend to exhibit little mating activity when first run with sexually mature ewes. The incidence of such inactive rams varies between strains, but most inexperienced rams will fairly soon become sexually active after joining commences. A minority continue to exhibit little libido, and as such are of little use to the producer.

Nutrition

In mature rams testis size is very labile and easily influenced by plane of nutrition. The paired testes may range in weight from 100 to 800 g. Assuming adequate libido, the capacity of a ram to impregnate large numbers of ewes each day during joining is presumably determined by his daily sperm production. Unfortunately, this figure is not easily determined and remains unknown for most sheep species. However, in Merinos, it is known that production per unit weight of testicular tissue remains fairly constant over a wide range of nutritional conditions, with each gram of testis producing approximately 20 million spermatozoa per day (Lindsay, 1976). Sperm production correlates well with scrotal circumference, which is relatively easy to measure by calibrated 'scrotal tape'.

Occasionally calipers are used or the testes are compared to a calibrated 'orchidometer' or set of beads ranging in volume from 50 to 400 mL.

In the paddock, testicular volume decreases during the joining period, probably due to the ram spending insufficient time grazing. The protein and energy requirements for rapidly increasing testicular size and the capacity of rams to produce spermatozoa prior to joining are not fully understood. Feeding lupin grain supplements to rams causes a greater increase in testes weight than in body weight (relatively speaking), due primarily to increased seminiferous tubule volume. It could be that feeding lupins or similar supplements to rams before joining offers a cheaper way of providing adequate sperm production for the ewe flock than does buying in additional rams (Gherardi *et al.*, 1980).

In marked contrast to testis size, libido in rams is not obviously related to level of nutrition, except that it is depressed in the extreme case where rams are kept on sub maintenance diets (Lindsay, 1976). Libido in adult rams cannot be increased by treatments with testosterone or LH (Lindsay, 1979).

Physical environment

Season

Testis size and semen production are controlled by season (photoperiod) as well as by nutrition and mating activity (Courot, 1979). Under constant nutritional conditions rams in Europe have heavier testes in autumn than in spring. British-breed rams on pasture in Australia usually show the same seasonal effect, but this is less obvious in Merinos. For example, in Western Australia testis volume in Merinos usually falls during summer and autumn, when forage quality and availability decline and when rams are mating (raising the requirement for semen to its highest). However, British-breeds show increased testis volume during this period, even with decreasing bodyweight. So for Merinos, nutritional effects (*e.g.* lupin supplementation) are able to completely override the influence of photoperiod (Masters and Fels, 1984; Hotzel *et al.*, 2003). This is not the case in British-breed rams such as the Suffolk, where nutrition can only influence testis weight during the normal breeding season. While the quantity of semen produced may vary with season, its quality does not.

Temperature

Rams vary greatly in their response to exposure to high temperatures, in terms of regulating testicular temperature. Raised testicular temperatures have serious, deleterious effects on spermatogenesis (Fowler, 1976). They also cause testicular degeneration, which is probably the most common type of reproductive abnormality or pathology in rams. A period of thermal stress in summer that raises testicular temperature will result in a reduction in semen quality for an interval lasting up to 7 weeks (*i.e.* a full spermatogenic cycle).

Efforts should be made to protect rams from high environmental temperatures by providing them with adequate shade and leaving them undisturbed. If so managed, their testicular temperatures will rarely rise, even if ambient temperatures exceed testicular temperature (34°C - common in summer) or deep body temperature (39°C - during heat waves). In these circumstances the lower testicular temperature is maintained by several mechanisms - which control the positioning of the testes and scrotum relative to the abdomen, the amount of sweating and evaporative cooling from the scrotal skin and the exchange of heat between the blood in the arteries and veins of the spermatic cord or pampiniform plexus (counter-current cooling mechanism) - as well as by panting, which causes whole-body heat loss. If rams are deprived of shade and/or disturbed, the heat load from radiant energy and exercise may cause a breakdown of these regulatory mechanisms. Rams should be carefully trucked rather than driven to mating paddocks if long distances are involved.

The best thickness of fleece when rams are joined at high ambient temperatures is 3-4 months' wool. The scrotum should not be shorn. Shearing immediately prior to joining is not desirable, since some fleece protects the ram from radiant heat. On the other hand a heavy fleece is likely to reduce mating activity and may impair sweating and cooling of the scrotum.

Health and management

Rams should be in good condition but not obese at joining time. Because rams are in a small mob, they are sometimes kept in small, irregular paddocks that are short of feed and infested with weed species. As a result, graziers will often give their rams a 'steaming-up' pre-joining ration in an attempt to compensate for having neglected them for the balance of the year. This may be useful, but is not a substitute for sound, year-round nutritional management. Rams of all breeds should be inspected frequently, noting the condition of the penis (tossle), testes, teeth and toes (known as the four Ts; see next page for details). Frequent inspection should also minimise the likelihood of them gaining unplanned access to ewe flocks (Fowler, 1976). Rams should be shorn twice a year and hooves and horns trimmed at the shearing furthest away from joining. Although ill-advised, the practice of shearing within 6 weeks of joining remains common.

If rams have been run on dry, carotene-deficient pasture for 2-6 months before mating they may lack vitamin A. This condition causes a drop in semen quality and can be avoided by either drenching the rams with vitamin A about 8 weeks before joining is due to commence and again at joining, or providing carotene-rich supplements, such as conserved green feed or maize (Fowler, 1976). The occurrence of footrot, foot abscess, sheath-rot, fly strike or significant internal parasite burdens will cause pain and malaise sufficient to affect mating performance. Several specific diseases of the reproductive tract affecting the testes, epididymides, scrotum or prepuce may interfere with ram fertility. Affected rams should receive prompt treatment or be culled. New rams should be purchased well in advance of joining time, so that they can acclimatise. This may need to be up to 12 months in advance of first joining if young rams

are purchased from temperate regions and transported to the arid pastoral zone of Australia. Rams should preferably have been serologically tested for brucellosis at the time of sale, and may require repeated brucellosis testing where an infertility problem exists.

Mating systems

The management of joining, methods used to assess breeding soundness and serving capacity in rams, selection of joining times and joining rates and some techniques for evaluating levels of mating activity and fertility are examined in more detail in this section.

Controlled natural mating

All rams should have the capacity to successfully cover a large number of ewes during the breeding season. Clearly this depends on the rams being in good health and having high libido and daily sperm production (Lindsay, 1976, 1988; Courot, 1979).

Selection of rams for joining and criteria for culling

Prior to joining, rams should be given a thorough physical examination. This examination and culling are best carried out at the pre-joining shearing. Increasingly, testis size and serving capacity are also being estimated to maximise the efficiency of joining (Plant, 1981a; Blockey, 1983; Galloway, 1983). In the physical examination, the ram is sat on its rump and held to enable inspection of the testes, reproductive tract, teeth, leg joints and feet. Rams should have firm, springy testes of even size and shape. Such testes nearly always produce good-quality semen, while soft or flabby ones often produce poor-quality semen. The epididymides should be of uniform size and shape.

The penis is extended and it and the prepuce are examined (Figure 9.7) for evidence of adhesions or ulcerations that problems such as sheath-rot may cause. The urethral process should be intact. The hind legs are examined for any evidence of swelling of joints or arthritis. Unfortunately, arthritis is not always easy to detect during the physical examination. About 10% of rams may have poor serving capacity due to lameness and arthritis. The feet are examined carefully for horn overgrowth or footrot or foot abscess. If scales are available it is useful to record body weights. Testis size is estimated using scrotal tape.

Such physical examination commonly reveals that 10-20% of rams are unsound for breeding and should be culled (Blockey, 1983; Galloway, 1983; Seaman, 2004). Examinations should be done annually as the rate of unsoundness in rams less than 3 years old averages about 8%, whereas in animals over 6 years old it is about 35%. In one survey of 900 Merino rams in 17 commercial flocks in Queensland, 17% had clinical abnormalities of the testes and/or epididymides and the incidence of abnormalities increased markedly after 2½ years of age (Table 9.4). The abnormalities had a variety of causes, including brucellosis and actinobacillosis (Rival, 1982).

The physical examination can be extended by collecting a semen sample and appraising it, usually by electro-ejaculation. The volume, colour and consistency of the ejaculate are noted. An estimate of sperm motility is usually obtained by placing a droplet of fresh semen on a warmed slide under a low-power microscope and scoring for wave motion. Thin films of fresh semen may be similarly examined to determine the approximate proportion of motile spermatozoa. Occasionally, further tests to determine the percentage of morphologically abnormal sperm (Figure 9.9) and the actual concentration of spermatozoa may be done. Normal ram semen is characterised by a high concentration ($2.5 – 5.0 \times 10^9$ spermatozoa/mL) and intense motility of spermatozoa. More than 85% of spermatozoa should be motile and less that 15% morphologically abnormal (Martin, 1981).

Semen evaluation involves a significant expense to the producer and its practical value has been widely questioned. As a result, few producers bother with the technique, the practice being mostly confined to rams to be used for single-sire matings, hand service or artificial insemination (Martin, 1981; Galloway, 1983). The basic problem is that, except in extreme cases of infertility, its results seem to have little relationship to ram fertility in the paddock; moreover, the sample is usually collected from a sexually rested ram and may differ considerably from an ejaculate collected during the joining period (Fowler, 1976; Lindsay, 1976; Colas, 1983).

Another measure that can be used for ram selection is the standardised serving-capacity test, where the probable libido and mating dexterity of rams in the paddock is estimated by assessment of such characteristics in pens or small yards (Kilgour, 1980; Blockey, 1983). One should draw a distinction between libido (sexual urge or desire) and mating dexterity (ability to mount and inseminate) of rams. Serving capacity is a measure of both these traits, and it is the combination that is important to the producer. In reality, few producers have the time or desire to test for serving capacity, particularly when the use of rams of high or low serving capacity score often result in similar flock fertility and fecundity. However, it may be a useful method of identifying rams which are completely unable to serve.

When to join?

Selection of the best joining and hence lambing time is a critical management decision that influences the timing of most other management activities. Ewes are lambing somewhere in Australia throughout the year. The choice is seldom clear and is influenced by many questions. These may include the following. When do ewes exhibit maximal breeding activity? When will lamb survival not be a serious problem? Will there be sufficient pasture of adequate quality available around both joining and the time of lambing? When is the best time to market lambs or older progeny? Will adequate pasture be available for weaners? Will blowflies be a problem after lamb marking? Will grass seeds be a problem in woolly lambs? Will shearers be available, and when should lambs be shorn? Does the sheep management timetable integrate with other farming operations? Will foot abscess be a problem in lambing ewes?

In practice, a decision is made on the basis of one or a few of these considerations and then the management and nutrition of the flock is adjusted as much as is possible to reduce any negative impact of other factors. In particular, programs for control of parasites must be adjusted to meet the needs of lambs born at different times of the year. Split joinings may sometimes be advantageous, where joining portions of the flock at different times of the year enables the more efficient utilisation of rams and labour and spreads any risk in marketing the progeny.

The joining ratio and duration of joining

These management decisions are interrelated. It is not easy to determine in advance the minimal number of rams that will result in good flock fertility. Similarly, although tight lambings are usually desirable, few producers are willing or able to limit the joining period to 4-5 weeks, preferring instead to join for 6-8 weeks or sometimes longer. In practice, most producers are conservative and are probably using considerably more rams and/or joining for longer periods than is necessary to ensure good flock fertility (Fowler, 1972, 1984). Depending primarily on the purchase price of young rams, significant financial savings can result from the more efficient use of rams.

As a general rule, rams seem able to cover ewes adequately at percentages around 1% (Mattner and Braden, 1967; Gherardi *et al.*, 1980; Kleemann *et al.*, 2006). Individual rams can, on average, impregnate 160-300 ewes to which they are mated in less than 3 weeks. At less than 1%, the probability of the rams mating with all ewes in oestrus declines markedly, but only during the first 18 days of joining (Burton *et al.*, 1982). After this period, by which time much of the flock should be pregnant, very low joining percentages should be adequate. With autumn joinings it may be useful to reduce the number of rams after the first 18 days; surplus rams are then available for joining to other ewe flocks, if required. The percentage of rams should not be decreased during a spring joining. It follows that as the joining percentage declines below 1% the lambing profile tends to become extended. An alternative way to think about the fertilising capacity of rams and hence joining ratios is to join at least 400 g of testes per 100 ewes (Gherardi *et al.*, 1980; Lindsay, 1988).

The problem is that there are often circumstances where a 1% joining ratio may be too low. A number of factors are known to decrease the probability of rams inseminating ewes, making higher joining ratios desirable. Such factors include: poor nutrition in ewes and rams or over-fatness in rams; breeding out of season, when the ewes and perhaps also the rams are not showing full breeding activity; joining maiden ewes, which exhibit relatively weak oestrous behaviour; joining very small flocks, where the use of only one or two rams may be risky; using ram syndicates that contain a lot of 1 year-olds or rams more than 5 years of age (syndicates should ideally contain a good mix of ages with only a minority of previously unmated rams); adverse physical environments, such as hilly tablelands country, or large paddocks in arid zone country where stocking rates are very low and if feed is scarce the ewes break up into small, widely dispersed groups, some of which are well removed from any ram (ram-ewe contact is not always as poor as it appears in such arid zone flocks, since often these small groups of ewes congregate in larger groups at water sources in the early morning and/or the evening); and high temperatures, which reduce activity and perhaps semen quality in rams.

In general, a basal joining ratio of 1% should have one or more rams added to the syndicate per 100 ewes to compensate for each identifiable adverse factor (Fowler, 1976). Often some trial-and-error is involved. If information is available for testis size and serving capacity, the rams can be placed into different categories based on their mating potential and be more precisely matched with appropriate groups of ewes (Blockey, 1983). In spite of these various considerations and recommendations concerning the joining ratio, most producers simply run 2% of rams with their ewe flocks.

Significance of ram-ewe contact

The more services from ram-ewe contact that occur in the paddock the higher will be the pregnancy rate in the first 17 days of joining. Both the ram and the oestrous ewe exhibit active partner-seeking activity (Lindsay, 1979). Such activity in ewes is only seen when the rams are within visual range, while the ram probably requires visual and olfactory contact with the ewe. Several of the nutritional, environmental and management factors discussed earlier in relation to flock fertility may regulate the level of ram-ewe contact, which appears to be the single most important determinant of flock fertility and may also influence ewe fecundity (Fowler, 1976).

The extent of ram-ewe contact in a flock, as manifested by the level of mounting activity, may be determined by the use of crayon raddles harnessed to the briskets of rams (Plant, 1981a).

Table 9.4. Clinical abnormalities in Merino rams of different ages around Goondiwindi, QLD.

Age (years)	*Representation in ram flock (%)*	*Normal rams (%)*	*Clinical abnormalities*		
			Epididymides, head (%)	*Epididymides, tail (%)*	*Testes (%)*
1.5	28.8	88.4	0.8	5.0	8.8
2.5	9.0	100.0	0.0	0.0	0.0
3.5	14.8	88.7	1.5	10.5	4.5
4-6	34.9	78.8	2.9	16.9	10.2
6	12.5	63.4	5.4	23.2	23.2

Source: Rival (1982)

Crayons are available in various colours and different degrees of hardness (for hot, mild or cold conditions), and their use during all or part of the joining period can enable more efficient flock breeding management. If the flock is mustered, raddle colours changed and marks on ewes recorded every 14 days after joining commences, fairly accurate measures of mating activity and of returns to service are obtained, and the levels of breeding activity and flock fertility thus assessed. If more than 30-35% of marked ewes return to oestrus, ram or ewe infertility should be suspected. Since the progress of mating is being monitored, the duration of the joining period can be shortened or lengthened, as necessary. At the end of joining the ewes can be separated into flocks that should lamb either early or late during the lambing period.

It may not be practicable or desirable to muster the flock every 2 weeks during joining. In this case joining may be commenced with unraddled rams and the rams observed in the paddock during the first week for level of mating activity (Plant, 1981a). About 3 weeks after joining commences the flock is mustered and the rams harnessed with crayons. If the rams worked well early and 20% or less of ewes are raddled in the second 3-week period, there will be little point in extending the joining beyond a total of 6 weeks. If more than 25% of ewes are raddled after 6 weeks, the crayon colour should be changed and the joining period extended for a further 3 weeks. When the ewes are examined again after a total of 9 weeks, the pattern of marks on the ewes should indicate whether a low level of breeding activity or infertility was the problem. A disadvantage of using crayons only after most ewes have conceived is inability to separate ewes that never mated from those that simply lambed early.

Ram harnesses and crayons should always be used carefully and with a precise aim in mind, since they involve significant expense and time. Subdivision of the flock into different lambing mobs and dry ewes can also be achieved by pregnancy diagnosis, which is discussed in Chapter 10.

Uncontrolled natural mating

Sometimes producers allow rams to run with the ewes for many months or even year-round. This may reflect no more than disorganised management. If the rams run with the ewe mob year-round, lambings will occur throughout the year, although a seasonal peak is usually observed. This greatly complicates management, making the planning of marking, mulesing, shearing etc. more difficult, and the matching of the reproductive cycle to pasture availability impossible. Nevertheless, reproduction rates in Merinos can sometimes be considerably increased by changing from controlled to uncontrolled mating.

Over their reproductive lives, such ewes give birth to more lambs without producing more twins, a result that would appeal to many Merino breeders. In one trial in Western Australia the average age at first lambing was 1.8 years and subsequent lambings occurred at an average interval of 284 days, yielding about seven lambs in a lifetime (Arnold and Charlick, 1980). Lambings tended to peak in May and June. Weaning was uncontrolled, basic management being limited to annual shearing, tail docking and fly-strike control. These results were obtained at higher-than-normal stocking rates. Lamb mortalities in the first 20 days were only 8%, better than for control-mated ewes; however, level of mortality during the first 2 years after birth was high. This could be substantially corrected by feeding supplements during autumn and winter. In a subsequent study (where weaning was controlled; Arnold and Charlick, 1984), joining for 12 months increased the production of lambs compared with a controlled 6 week breeding, but only when the flock undergoing uncontrolled joining were provided with supplementary feeding. More work is required to establish the possible economic advantages of uncontrolled breeding in Merinos.

Artificial regulation of reproductive performance

A number of techniques are available for use in sheep production to increase reproduction rates and/or the spread of new breeds or strains or superior genotypes of sheep. Further information about these technologies may be obtained from Hunter (1980), Evans and Maxwell (1987), Evans (1991), Hafez and Hafez (2000), Maxwell *et al.* (2004) and Senger (2005).

Artificial insemination (AI)

AI enables a superior ram to inseminate many more ewes than would be possible by natural service (Figure 9.12). The number of ewes inseminated is limited by the amount of semen produced, its characteristics and the number of spermatozoa required for normal fertility after AI. The number of ewes artificially inseminated each year in Australia reached approximately one million in 2005. The value of an AI program depends largely on the expected monetary return from using known superior sires. There is little point in using AI in a commercial flock if the rams have not been performance tested and proved genetically superior. Many AI programs yield poor results, due to poor organisation or failure to recognise common causes of low fertility. Reviews of various aspects of AI in sheep are given by Fairnie (1979), Evans and Maxwell (1987), Evans (1991), Windsor *et al.* (1994), Sanchez-Partida *et al.* (1999) and Salamon and Maxwell (1995, 2000).

Semen collection

For AI, semen is usually collected from rams by using an artificial vagina - an apparatus designed to impart to the ram a stimulus similar to the one received during natural service (Figure 9.13). It consists of an outer cylindrical casing of bakelite or other hard, heat-resistant plastic and a soft inner rubber liner. The space between the case and liner is half filled with water at 42-45°C, and then inflated with air. The inside

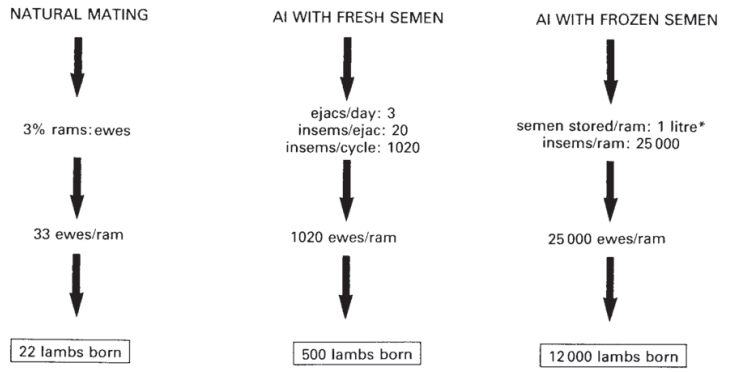

Figure 9.12. The potential number of lambs sired by a ram each year using natural mating, cervical AI with fresh semen and uterine AI by laparoscopy with frozen semen. The calculation is based on 8 months collections from one ram at the rate of nine ejaculates per week. Lamb marking percentages of approximately 65% to natural services and 50% to AI with fresh semen and uterine AI with frozen semen are assumed. Source: Maxwell (1984).

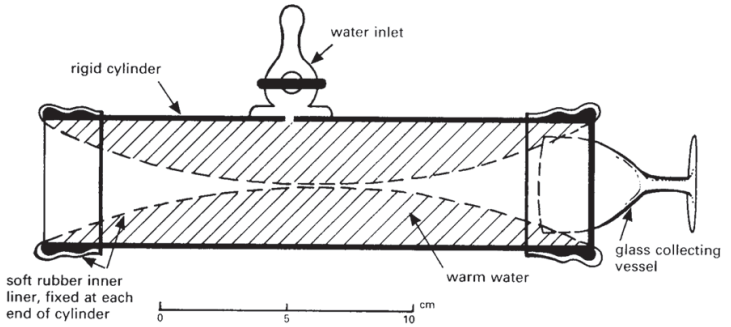

Figure 9.13. Artificial vagina suitable for collecting ram semen. Source: Miller (1991).

Figure 9.14. Semen collection from a ram using an artificial vagina. Source: WMC Maxwell.

of the rubber liner must be clean and dry and is lubricated with a little paraffin or vaseline. A collecting vessel to contain the ejaculated semen is attached to one end. The ram is allowed to mount a restrained ewe, and just before intromission his penis is gently deflected into the artificial vagina (Figure 9.14).

The technique yields superior, more uniform ejaculates than when electro-ejaculation is used. However, rams have to be trained to work to an artificial vagina. Usually they are shedded and fed for 8- 10 weeks before the AI program commences and the training may take place during this interval. Initially the restrained ewe must be in oestrus, but as training advances this becomes unnecessary, and sometimes even a 'dummy' restrained ewe will suffice. Once trained, each ram can supply semen several times a day for an extended period.

Semen evaluation, dilution, storage and freezing

The semen is examined promptly after collection to determine its fertilising ability. Volume and colour and sperm density, motility and morphology are assessed (Evans and Maxwell, 1987). Ejaculates are usually scored from 5, for a thick creamy sample containing more than 5.0×10^9 spermatozoa per mL, down to 0, for a clear watery sample containing very few spermatozoa. Motility and morphology are assessed as previously described. The semen must be handled carefully in clean glassware to protect the fertilising ability of spermatozoa, avoiding cold shock or sudden drops in temperature, contact with water or exposure to sunlight.

For cervical insemination the volume of inseminate should be 0.1-0.15 mL. Good semen samples must be diluted somewhat in order to inseminate the ewe with an optimal number of spermatozoa in this volume. For fresh semen about 100×10^6 spermatozoa per ewe should result in good fertility. Although there is much variation, a good ejaculate should typically contain about 30-40 such doses. Semen is usually diluted l:l or 1:2 with a buffered diluent containing heat-treated cow's milk or egg-yolk glucose citrate.

If diluted semen is not to be used promptly after collection, it may be stored at 2-5°C, at which temperature the spermatozoa retain good fertilising ability for up to 24 hours (Evans and Maxwell, 1987; Salamon and Maxwell, 2000). If longer storage is necessary, the diluted semen may be mixed with a cryoprotective agent, usually glycerol, chilled to 5°C then frozen in pellets or straws. In the popular pellet method, 0.2-0.25 mL of diluted semen is pipetted into a hole engraved on the surface of a block of dry ice (solid CO_2), and the frozen pellet is then transferred to liquid nitrogen for storage at -196°C. Alternatively, semen may be frozen in straws. Frozen-thawed ram spermatozoa have decreased fertilising capacity, and it is necessary to insert at least 250×10^6 spermatozoa into the cervix to obtain satisfactory fertility. The decreased fertilising ability is related to impaired sperm transport through the ewe's cervix. The procedures for diluting, freezing and thawing semen must be rigorously standardised, since processing does affect fertilising ability. There may be large differences between animals, ejaculates and seasons in the ability of ram spermatozoa to withstand freezing and thawing (Salamon and Maxwell, 2000).

Methods of insemination

The three main methods used to artificially inseminate sheep correspond with their site of deposition: vaginal, cervical and uterine. The method employed depends largely on the type and amount of semen available as well as the type of oestrus. Table 9.5 outlines the minimum number of spermatozoa required to obtain satisfactory conception rates and the appropriate circumstances for their use.

In the cervical method a small volume of diluted semen is inserted just inside the external os of the cervix (Evans and Maxwell, 1987). The ewe's hindquarters are elevated, usually by placing them over a fence rail, and the inseminator uses a duck-billed speculum inserted into the vagina and a head lamp to enable visualisation of the cervix for insertion of the inseminating pipette (Figure 9.15). The semen (0.05-0.2 mL) is deposited no more than 1-2 cm inside the cervical canal. The ability of frozen-thawed spermatozoa to traverse the cervix is compromised and as such is not recommended to be delivered by this method. With two catchers, a skilled operator can inseminate 100 ewes per hour by this method.

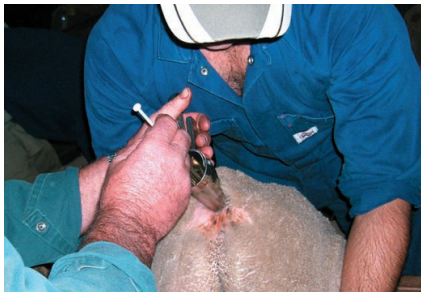

Figure 9.15. Cervical insemination of a ewe.
Source: SP de Graaf.

In the simple vaginal insemination method, semen (0.3-0.5 mL) is deposited 'blind' into the anterior vaginas of sheep standing in a race. This is also known as the 'shot-in-the-dark' (SID) method. In this case a larger volume of diluted semen containing more spermatozoa is required to obtain fertility comparable with that obtained with the cervical method. Frozen-thawed spermatozoa should not be used for this technique for the same reasons outlined above.

More recently, it has become relatively easy and inexpensive to deposit semen directly into the uterus, via the technique of laparoscopy. In this case 0.02-0.10 mL of diluted semen is deposited into the lumen of both uterine horns from a needle and syringe that has been inserted through the ventral wall of the abdomen (Evans and Maxwell, 1987; Figure 9.16). This technique is attractive where valuable, frozen-thawed or sex-sorted semen is to be used; since it permits good fertility with much smaller sperm doses than do the cervical and vaginal methods. It also facilitates insemination of superovulated ewes, which otherwise

experience low fertility due to poor sperm transport through the cervix. An experienced operator can inseminate 40-50 ewes per hour by the laparoscopic method. While other methods of circumventing the cervical barrier have been developed (Buckrell *et al.*, 1992), these so-called transcervical techniques are quite stressful for the animal and provide varied success (reviewed by Salamon and Maxwell, 1995). In Australia, comparisons between insemination by laparoscopy and the transcervical route proved semen deposition by laparoscopy to be the preferred method (Windsor *et al.*, 1994; Sanchez-Partida *et al.*, 1999).

Figure 9.16. Intrauterine insemination of a ewe by laparoscopy.
Source: SP de Graaf.

Time of insemination

Correct timing of insemination is essential for the success of AI (Evans and Maxwell, 1987; Salamon and Maxwell, 2000). In essence, deposition of semen into the female reproductive tract must occur early enough for the spermatozoa to reach the site of fertilisation (the ampulla), but late enough so that the spermatozoa do not exhaust their finite energy reserves before the ovum arrives. As the type of oestrus and method of synchronisation affects time of ovulation, and method of insemination and sperm type influence efficacy of sperm transport and survival, it follows that each factor will impact on insemination time (Evans and Maxwell, 1987).

Although not often undertaken in modern breeding programmes, females in spontaneous natural oestrus can be successfully artificially inseminated. Under such circumstances, oestrus is usually detected by teaser males (wethers treated with 150 mg testosterone cypionate or enanthate at fortnightly intervals, commencing 4 weeks before use and continuing until the end of the AI programme). It is essential that adequate (about 2%) raddled teasers in good health are used if timing of insemination is to be precise. Marked ewes (drafted twice daily) should be inseminated (vaginal or cervical AI) about 12-18 hours after the onset of oestrus. Fresh semen is usually used.

The vast majority of sheep AI programmes inseminate ewes at a controlled oestrus, with insemination occurring at a fixed time post- sponge or CIDR removal. The interval between progestagen device removal and time of insemination is influenced by the aforementioned factors. Using the most common method for synchronisation of oestrus in Australian sheep flocks (progestagen sponge + non-superovulating dose of PMSG; see following section), ewes can be expected to enter oestrus within 36-48 h and ovulate about 60 h post-sponge removal. This interval decreases when ewes have been superovulated for a MOET programme, with oestrus occurring within 24-36 h and ovulation at 48 h following sponge withdrawal. The recommended time of insemination for each of these situations using either fresh or frozen semen for cervical or intrauterine AI is listed in Table 9.6. Occasionally, commercial operators may recommend double inseminations

Table 9.5. Minimum safe numbers of motile spermatozoa per inseminate and appropriate method of AI according to semen and oestrous type.

Method of insemination	*Type of oestrus*	*Type of semen*		
		Fresh ($\times 10^6$)	*Liquid-stored* ($\times 10^6$)	*Frozen-thawed* ($\times 10^6$)
Vaginal	Spontaneous	150	NR	NR
	Controlled	300	NR	NR
Cervical	Spontaneous	100	150	NR
	Controlled	200	300	NR
Uterine (total in two uterine horns)	Controlled	15	15	20
	Superovulated	20	20	30

NR = not recommended; conception rates generally <50%, may be very low.
If ample semen is available, the number of spermatozoa can sensibly be increased somewhat above the relevant number shown above.
Source: adapted from Evans and Maxwell (1987).

for cervical AI, but the minor increase in fertility (5-10%) usually does not outweigh the cost of performing the procedure twice.

Table 9.6. Time of insemination in relation to method of insemination, hormone treatment and semen type.

Method of insemination	*Hormone treatment*	*Semen type*	*Insemination time*[a]
Cervical	Progestagen + PMSG	Fresh	36-48 h
	Progestagen – PMSG	Fresh	48-60 h
Intrauterine	Progestagen + PMSG (non-superovulated)	Fresh	36-48 h
		Frozen	60-66 h
		Sexed-Frozen	57-59 h
	Progestagen + PMSG (superovulated)	Fresh	24-48 h
		Frozen	44-54 h
		Sexed-Frozen	42-43 h[b]

[a]post-sponge removal
[b]optimum insemination time yet to be fully established.

Fertility obtained

When non-synchronised, raddled oestrous ewes are inseminated in autumn with freshly ejaculated semen by the cervical method it is quite possible to achieve 65-70% conception rates (Evans and Maxwell, 1987; Salamon and Maxwell, 1995, 2000). This is essentially the equal of fertility in natural service at a single oestrus. To obtain such results, the program must be carefully planned and executed and the standard of general husbandry of the flock must be excellent. Fertility by cervical insemination is lower using frozen semen, conception rates being around 20-30% and often lower. Conception rates using the SID method may be nearly as good as for the cervical method (Maxwell, 1984). Fertility after intrauterine insemination with both fresh and frozen semen appears to be as good as after cervical insemination with fresh semen (Maxwell *et al.*, 1984). A comparison between the efficacy of a variety of insemination methods and sperm types is given in Table 9.7. Most commercial operators offering intrauterine AI with frozen semen should be capable of approaching 60-70% fertility under most conditions.

Table 9.7. Expected lambing rates after vaginal, cervical or uterine insemination of mature ewes with fresh, liquid-stored or frozen-thawed semen.

Method of insemination	*Type of semen*		
	Fresh	*Liquid-stored*	*Frozen-thawed*
Vaginal	65-70%	<10-20%	<5%
Cervical	65-70%	60-70%	20-30%
Uterine	60-70%	60-70%	60-70%

Quoted percentages assume insemination is conducted at the appropriate time using sufficient numbers of spermatozoa

Synchronisation of oestrus within the breeding season

In an AI program a major decision is whether or not to synchronise oestrus. Without synchronisation, ewes will need to be inseminated over a period of 18 days, which makes the procedure very inefficient in smaller flocks. With large numbers of ewes synchronisation may not prove labour-saving. There are basically two approaches to controlling the time of oestrus in ewes (Cumming, 1979; Robinson, 1979; Cognie and Mauleon, 1983; Evans and Maxwell, 1987).

In the first approach, progesterone or compounds with progesterone-like activity (progestagens) are administered for 12-14 days. Due to negative feedback on the hypothalamus and pituitary, the ewes cannot come into oestrus during treatment. By the end of the treatment period the ewe's *corpus luteum* will have regressed, regardless of the stage of the cycle at which treatment commenced, and cessation should result in all ewes coming into oestrus in the next 2-3 days. There are two ways of administering progestagens. The more common way is to insert a polyurethane sponge ['*Chronogest*' (Intervet) or 'Ovagest' (Bioniche Animal Health), containing 30 mg or 40 mg flugestone acetate; alternative brands may use 60 mg medroxyprogesterone acetate], pessary or controlled internal drug-releaser [CIDR; '*Eazi-Breed CIDR*' (Pfizer Animal Health) containing approximately 300 mg (9%) progesterone] impregnated with an appropriate dose into the vagina of the ewe. Less commonly, progesterone is formulated in a solid slow release vehicle and implanted under the skin. Ewes commence oestrus 24-36 hours after removal of progestagen sponges or CIDRs, with a peak at 48 hours, and nearly all ewes should enter oestrus by 60 hours. If control over the time of oestrus is sufficiently precise, it is not necessary to use teasers and observe oestrus, the ewes being inseminated at a fixed time after sponge or CIDR removal. Usually a minority of treated ewes fail to exhibit oestrus but may still become pregnant if inseminated. The precise time of fixed-time inseminations varies considerably and is discussed on the previous page.

The second and less commonly used approach to controlling oestrus is to administer a single dose of prostaglandin (Cloprostenol, 100 µg, '*Estrumate*', Jurox Pty Ltd or dinoprost-PGF2α, 4-5 mg, '*Lutalyse*', Upjohn Pty Ltd). This induces luteolysis, and the ewe returns to oestrus. However, prostaglandins are only effective when given more than 4-5 days after oestrus, so in order to get all ewes into oestrus at the same time a second prostaglandin treatment must be given, preferably about 12 days after the first (Fairnie and Wales, 1980; Smith, 1982; Evans and Maxwell, 1987). Prostaglandins clearly can be effective only in ewes that are cycling regularly and may cause abortions if given during the first 60 days of pregnancy. They do not give sufficient control over the time of oestrus to enable fixed-time inseminations.

Regardless of the method of synchronisation employed, fertility at the synchronised oestrus is reduced (Robinson, 1979). This is primarily due to depressed sperm transport through the cervix and is observed after both AI and natural mating. The dose of progestagen incorporated into the sponge and the timing of insemination relative to the LH surge are important factors regulating penetration of the cervix by spermatozoa in such ewes (Pearce and Robinson, 1985). Fertility returns to normal at the

next spontaneous oestrus, but in a treated flock this oestrus is less synchronised than the induced one. Subfertility at the controlled oestrus (fertility may drop below 40%) can be largely avoided by increasing the dose of spermatozoa employed for cervical AI (to 200-400 × 10^6 for freshly ejaculated semen) or, in the case of natural mating, by using a high joining ratio of at least one ram for every 10 treated ewes. Alternatively, laparoscopic AI can be employed to deposit semen directly into the uterus, circumventing the cervix. As with AI, good results require careful planning and a high standard of general husbandry.

Extension of the breeding season

The onset of the breeding season may be advanced by up to several weeks by artificial regulation of the light-dark cycle. This can be achieved by yarding ewes into sheds that are thoroughly insulated from daylight for a portion of each day. However, this procedure is obviously too costly and time consuming to be of commercial use. A much more practicable alternative is to administer exogenous melatonin ('*Regulin*', Regulin Ltd.) to ewes for a period during summer prior to the time of normal onset of breeding activity (Karsch, 1984). Melatonin alters the ewe's perception of season allowing an increase in fertility and fecundity in summer matings to that observed during autumn (Brunet *et al.*, 1995). In practice, the effect of Regulin is usually combined with that of the ram effect, with ewes isolated from rams 6 weeks before joining and the melatonin implant administered 30-40 days before joining. Considerably variation between flocks has been observed and the use of Regulin is not recommended for highly seasonal British breeds or in areas where ewes generally cycle during late spring early summer.

Induction of oestrus and ovulation in anoestrous ewes

Seasonal anoestrus

A single treatment with a gonadotrophin can induce ovulation but not oestrus during the non-breeding season. If a period of progesterone or progestagen treatment is given shortly before the gonadotrophin treatment, oestrus and ovulation will occur. Pregnant mare serum gonadotrophin (PMSG; '*Pregnecol*', Bioniche Animal Health), which exhibits both FSH- and LH-like activity, is usually employed, although multiple injections of FSH of animal pituitary origin are also effective. The effectiveness of progestagen/PMSG treatment depends on several factors, including breed, provision of adequate 'ram-power' (*i.e.* larger percentage of rams provided at joining) and stage of the non-breeding season (Cognie and Mauleon, 1983). In general, as the stage of anoestrus deepens, progestagen/PMSG treatment will be less effective, time to onset of oestrus will lengthen (post device removal) and its precision will decrease.

Realistically, these combined treatments are expensive, and factors such as extra ram costs and the unreliability of premiums paid for early lambs make it essential that the economics of this approach to 'out-of season' breeding be critically examined.

A more economical approach to 'out-of-season' breeding utilises the 'ram effect' (Oldham, 1980; Lindsay, 1983; Pearce and Oldham, 1984). If anoestrous ewes are preconditioned by a period of 3 or more weeks of isolation from rams, the introduction of rams will induce them to ovulate. This effect is mediated by pheromones - volatile chemicals released by the rams, which act via the olfactory system of the ewe to modify the pattern of GnRH release from the hypothalamus (Figure 9.3). The ewes do not need to be in physical or visual contact with the rams.

The same effect may be achieved with testosterone- or oestrogen-treated wethers. The pheromones are present in the skin and wool but not the urine of the ram. The frequency of LH pulses from the pituitary in the ewe increases within 10 minutes of ram introduction, and this triggers the normal sequence of endocrine events that lead to ovulation (Martin *et al.*, 1980; Pearce and Oldham, 1984).

The proportion of ewes in a flock in the non-breeding season that will ovulate after ram introduction varies widely. Romney ewes will respond only during a limited period of a few weeks just before the start of the spontaneous breeding season, whereas Merinos can usually respond at any time during the non-breeding season, provided they are in anoestrus at the time of ram introduction. Recent research even suggests that cyclic ewes are capable of responding to the ram effect, increasing pulsatile LH frequency, although do not appear to ovulate (Hawken *et al.*, 2007).

Ewes usually ovulate about 2-3 days after ram introduction. They do not usually come into oestrus at this induced ovulation (Oldham, 1980), but show a relatively synchronised oestrus approximately one cycle length later. In a flock, this oestrus occurs over a period of about 10 days, but with two peaks at about 19-21 and 24-25 days after ram introduction. In the former case the ewes come into oestrus at a normal cycle length after the ram-induced ovulation. In the latter the ewes have an abnormal short cycle and ovulate again about 7-8 days after the ram-induced ovulation. This is also silent, but followed by a normal oestrus and ovulation a further cycle length later. Importantly, a small number of ewes may exhibit oestrus at these early, induced ovulations; any entire rams present may deplete their sperm reserves mating with these oestrous ewes, so it is best to use teaser rams to induce the ram effect.

The reasons for the variable life span of the ram-induced *corpus luteum* and the biphasic spread of the first oestrus are not well understood. Of ewes that mate at the first oestrus about 25-33% apparently conceive (fail to return to oestrus) but do not lamb. This reproductive wastage is probably due to these ewes reverting to the anoestrous state after the first oestrus, without ever becoming pregnant (Oldham, 1980; Pearce and Oldham, 1984). The rate at which non-pregnant ewes cease cycling and revert to anoestrus varies and depends on nutritional status, strain and stage of the non-breeding season - that is, the novelty of the rams wears off after a variable period of time. If a majority of ewes come into oestrus only once then flock fertility will be poor. In some circumstances, where rams are introduced too early, before all ewes are responsive, the effect may be to bring some ewes into oestrus and drive others into a deeper anoestrus, leading to a bimodal lambing pattern,

separated by 1-2 months. Maiden ewes respond less well than mature ewes to the ram effect.

In practice, if the producer wishes the full flush of lambing to take place early in the breeding season, he can usually manipulate mating to achieve this. Vasectomised rams or testosterone or oestrogen-treated wethers (testosterone cypionate or testosterone enanthate, '*Synarot*', '*Banrot*' or '*Tesgro*', 75 mg/ml; 400 mg given 14 days before introduction, or 150 mg, 150 mg and 300 mg at 14, 7 and 0 days before introduction) are introduced about 15 days before the scheduled mating period. Matings should then commence virtually from the first day of introduction of entire rams. If the ewe flock is not teased in this manner, lambing patterns in relation to the date of ram introduction may be more diffuse, especially in flocks where a significant proportion of Merinos were experiencing normal oestrous cycles at the time of ram introduction.

The usefulness of the ram effect for out-of season breeding can be increased by treating conditioned ewes with progesterone or progestagens. If anoestrous ewes are given a single injection of 20 mg progesterone 1-2 days before or at the time of ram introduction, all of the ram-induced *corpora lutea* have a normal life cycle, so most ewes come into oestrus 19-21 days later. However, depending on age of ewe and other factors, some ewes do not ovulate again after the ram-induced ovulation and these do not come into oestrus. This single progesterone treatment does not induce oestrus at the ram-induced ovulation. On the other hand, treatment with progesterone implants or progestagen-impregnated sponges for 6-12 days immediately before ram introduction does induce oestrus at that time (Reeve, 1984; Reeve and Chamley, 1984; Pearce and Oldham, 1984). Ewes enter oestrus at 36-60 hours after sponge or implant removal and ram introduction, so the interval between joining and conception can be reduced by 17-23 days and lambing is concentrated into batches spaced at intervals of approximately 2 weeks. These longer treatments also ensure that the ram-induced *corpora lutea* have a normal life span. Using this approach, good fertility has been obtained in both Merino and crossbred flocks, with up to 60% of ewes lambing to ram-induced ovulations.

Sometimes a higher ovulation rate is observed at the ram-induced ovulation than at the subsequent spontaneous ovulation and in this case a higher twinning rate is seen in ewes becoming pregnant at the first oestrus (Cognie *et al.*, 1980a). It is not clear why this effect on ovulation rate is variable, and it has been suggested that a small dose of PMSG given at ram introduction will ensure an increase in fecundity at the ram-induced ovulation. Selection of the optimal joining date remains important, because responses to the progesterone/ram regime vary during the non-breeding season in both Merinos and crossbreds, and the regime does not work in ewes experiencing spontaneous oestrous cycles or those that, for some other reason, are not responsive to rams. In any case, when the ram effect works well, graziers should expect more than 80% of Merino ewes to lamb.

Lactational anoestrus

In breeds with a well-defined breeding season, lambing will occur late in the breeding season (or after it) and *post-partum* anoestrus will extend into seasonal anoestrus. However, for Merino ewes in areas where nutritional status overrides the effect of photoperiod this will not be the case. If these ewes are in good body condition and lamb in autumn, the first ovulation (which is unaccompanied by oestrus) will occur about 20-30 days after parturition. This will be followed by a short cycle and a second silent ovulation about 6-8 days later. The next ovulation at around 45-55 days *post-partum* is accompanied by oestrus, but fertility then is usually low, possibly due to incomplete involution of the uterus. Normal fertility should be restored by about 60 days after parturition (van Niekerk, 1979).

The procedures described for inducing oestrus and ovulation in the dry, seasonally anoestrous ewe may also be applied in the lactating ewe (Cognie and Mauleon, 1983). In both Merino and crossbred lactating ewes progestagen/PMSG treatment can induce ovulation and oestrus in up to 80% of ewes as early as 2-3 weeks *post partum,* but fertility is *very* low at this stage (less than 10%) and does not reach near normal levels (75%) until 8 weeks *post-partum* (Dawe and Fletcher, 1976). When Merinos in poor body condition are lactating in autumn the introduction of teaser rams can induce ovulations in 50% of animals by 32 days *post-partum,* but does not influence the first occurrence of oestrus, which is seen about 7 weeks after lambing (Geytenbeek *et al.,* 1984). In general, the response to these procedures to induce breeding activity in lactating ewes depends on season, body weight and condition, numbers of lambs born and suckled, breed and the interval from lambing to treatment (van Niekerk, 1979). Controlled joining during lactation is not often attempted in Australia but is much more common in some countries like France and Ireland (Chapter 7).

Control of ovulation rate

Several methods will increase the incidence of multiple births in sheep, each of which acts to increase ovulation rate. Useful increases in numbers of lambs reared often result from treatments that cause only a modest increase in ovulation rate. For example, in Merinos, lifting the mean ovulation rate from 1.2 to 1.7 may result in 20-30% more lambs reared. Excessive ovulatory responses are to be avoided, since rates of embryo and perinatal mortality do increase with increasing ovulation rate. Mention has already been made of the influences of breed, hybrid vigour, season, body weight and flushing (especially grazing lupins) on ovulation rate. By some trial and error it is possible to determine the dose of PMSG that will give an increase in mean ovulation rate of about 0.5 (often around 400 International Units). However, PMSG is rarely used for this purpose, since the cost and the variability in ovarian responses are unacceptable. The use of other exogenous forms of FSH ('*Folltropin-V*', Bioniche Animal Health) will also boost ovulation rate, but are only used for superovulation of ewes in MOET programs (see opposite page).

An alternative means of temporarily increasing ovulation rate is via active immunisation of ewes against polyandroalbumin (*'Ovastim*', Virbac Australia, previously sold as '*Fecundin*'). The immunogen (polyandroalbumin) is a conjugate of the steroid hormone androstenedione and human serum albumin.

Antibodies secreted in response to vaccination partially inhibit the normal negative feedback effect of ovarian androgens and oestrogens on the anterior pituitary gland. This results in higher-than-normal levels of gonadotrophin secretion and hence an increased ovulation rate and fecundity. However, the number of dry ewes does not decrease (Scaramuzzi and Martin, 1984), embryo mortality may be increased (Scaramuzzi and Martin, 1984) and success varies greatly according to ewe weight and breed.

There is a high correlation between body weight at joining and ovarian response to vaccination. Better responses are obtained from heavier animals, and British breeds and crossbreeds respond better than Merinos (Geldard, 1984). In a large series of trials, vaccination increased lambing rates by 28% in crossbreds (range 11-48%) but by only 16% (0-33%) in Merinos. Therefore, the use of Ovastim is not recommended for Merinos. It is a waste of time and money using Ovastim in animals with low condition scores, and producers must carefully consider its likely cost-effectiveness. To ensure good responses the producer must be able to meet the nutrient requirements of ewes bearing various litter sizes and optimise lamb survival. It is helpful if ewes bearing more than one lamb can be identified in mid pregnancy and husbanded separately from ewes bearing singles. The vaccine should not be used in flocks that normally mark less than 70% or more than 150% lambs or in ewes with liveweights less than 45 kg.

In the first year of use the vaccine is given twice, at 6-9 weeks and 3-4 weeks before joining. In subsequent years ewes require only a single booster dose 3-4 weeks before mating. In years when pasture availability is low and twinning is not desired, the booster vaccination can be omitted and ovulation rates are the same as in unvaccinated ewes of the same strain. Vaccination does not interfere with the practice of joining ewes out of season and does not alter the percentage of such ewes that exhibit premature luteal regression.

Ovulation rate will also be increased by vaccination against subunits of the hormone inhibin. Similar to immunisation against polyandroalbumin, negative feedback on the anterior pituitary will be reduced, levels of FSH will increase and a corresponding rise in follicular development and ovulation rate will be observed (Anderson *et al.*, 1998). However, at present, use of this method is restricted to research situations as no commercial inhibin vaccine or agonist is available.

Intensive breeding programs

In several European countries - notably France, Ireland and Greece - lamb production is sometimes markedly increased by increasing both fecundity or litter size and the number of litters born per annum. In the most intensive programs, several components of the reproductive cycle are controlled (Cognie *et al.*, 1980b): the ewes are housed indoors with a controlled light-dark cycle, treated in batches with progestagens and PMSG to control the time of oestrus and ovulation, and induce a mild degree of superovulation, and then artificially inseminated. As batches of pregnant ewes approach term, parturition is induced with the hormone dexa-methasone and the ewes are subjected to intense surveillance to minimise lamb losses. The lambs are weaned early and reared artificially, enabling the ewes to become pregnant again as early as 40-50 days after parturition (van Niekerk, 1979). Success requires careful control of the nutritional status and indoor environment on a year-round basis, the use of naturally fecund breeds or crossbreeds, careful selection of progestagen and PMSG doses according to the physiological state of the animals and the use of higher (250-500 x 10^6) numbers of spermatozoa at insemination. The results obtained can be impressive. In Britain, producers using Finn × Dorset ewes, have achieved levels of 3.5 lambs per ewe per annum. More typically, less fecund ewes may lamb about 1.3 times per year and average about 1.7 lambs per lambing.

Since Australian lamb prices are generally much lower than in Europe, there is little interest in such intensive production in Australia. Of the breeds available, the Dorset and Merino are most suited to 8-monthly lambings (Hall, 1984). Attempts to increase lambing frequency with Border Leicester × Merino ewes, usually in irrigation areas, have generally been unsuccessful. However, there is the potential to reduce lambing intervals to 200 days or 3 lambings every 2 years. Commonly in the Australian environment, increased lambing frequency seriously lowers fertility due to the influences of *post-partum* anoestrus and reduced liveweights. A review of intensive management systems in Australia is given in Chapter 24.

Multiple ovulation and embryo transfer (MOET)

Multiple ovulation and embryo transfer (MOET) enables greater use of selected superior females in breeding programs, in much the same way as AI enables greater use of superior males (Chapter 8). This is achieved by the dissemination of genes from superior females through the mass production of embryos (Evans and Maxwell, 2000). The donor ewe is treated with progestagens (or prostaglandins) and gonadotrophins so as to not only control the time of oestrus and ovulation but also cause the release of a large number (10-20) of mature ova from the ovaries (Figure 9.17). The optimal doses of PMSG and/or FSH required to produce so many ovulations in a particular strain of sheep are largely determined by trial and error, but usually fall within the range of 500-800 IU PMSG and 120-140 mg of FSH (de Graaf *et al.*, 2007a). Many superovulation protocols also make use of GnRH ('*Fertagyl*', Intervet Australia, 40-50 µg) to increase ovulation synchrony. Excessive ovarian stimulation should be avoided due to the large number of persistent follicles, few ovulations, and/or high embryo mortality rate which may result. Fertility in natural mating after superovulation is reduced, due to faulty sperm transport, resulting presumably from excessive oestrogenic stimulation of the cervix. This is overcome by using artificial insemination and depositing semen directly into the uterus (Evans and Maxwell, 1987). As has already been discussed, insemination of superovulated animals has its own peculiarities in terms of insemination time and numbers of spermatozoa used (see Tables 9.5 and 9.6).

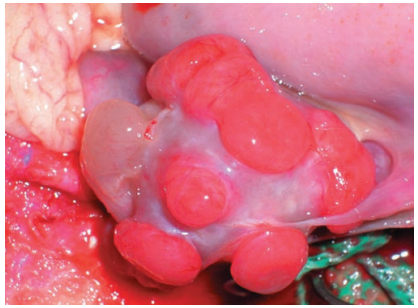

Figure 9.17. The ovary of a superovulated ewe. Note the numerous *corpora lutea* indicating successful hormonal stimulation and multiple ovulations after treatment with PMSG/FSH. Source: SP de Graaf.

At 4-8 days after oestrus (usually day 6), when the embryos have developed to the morula or blastocyst stage but are not implanted within the uterus, the uterine horns are flushed (antegrade or retrograde via mid-ventral laparotomy) with a buffered salt solution to recover the embryos. The embryos are examined under the microscope and, of those judged normal, one or two (usually two) are transferred to each recipient ewe. Transfer is made via laparoscopic insertion into the uterine horn ipsilateral to an ovary containing a corpus luteum. Recipients need not be the same breed as the donors, but their oestrus and ovulation must also be controlled in order to synchronise with the donor. An example of the hormone treatments and timing used for both donors and recipients is given in Table 9.8. Embryos may also be frozen (Fogarty *et al.*, 2000) or vitrified (an alternative method of cryopreservation; Vajta, 2000) for storage and/or transport prior to transfer, although this usually reduces their chances of survival (Maxwell and Wilson, 1994; Fogarty *et al.*, 2000).

The significant biological variation between donors (Cognie *et al.*, 2003) in terms of breed, age and reproductive status (González-Bulnes *et al.*, 2004) as well as the widely varying protocols of hormonal manipulation (Jabbour and Evans, 1991; Blanco *et al.*, 2003; Veiga-Lopez *et al.*, 2005; Simonetti *et al.*, 2008) mean that the number and quality of resultant embryos are very mixed. Despite these issues MOET is a commercial reality in the Australian sheep industry, where the artificial insemination of over 30,000 superovulated donor animals occurs each year, with numbers set to rise as producers transit away from the Merino into newer meat breeds. Table 9.9 gives an approximate indication of the results which can be expected from a well managed MOET programme.

Table 9.8. A sample calendar for treating donors and recipients in a MOET programme.

Day of programme	*Donor ewes*	*Recipient ewes*
0	Insert progestagen sponge or CIDR	Insert progestagen sponge or CIDR
10 (8pm)	Treat with 400 IU PMSG Treat with 22 mg of FSH	—
11 (8am) (8pm)	Treat with 22 mg of FSH Treat with 22 mg of FSH	 Remove sponges; treat with 400 IU PMSG; join harnessed teasers
12 (8am) (8pm)	Treat with 22 mg of FSH Treat with 22 mg of FSH Remove sponges, join harnessed teasers	—
13 (6am) (6pm)	Treat with 22 mg of FSH Treat with 50 µg of GnRH Isolate from feed and water	—
14 (2pm)	Oestrus Laparoscopic intrauterine AI (frozen semen); remove teasers	Oestrus Remove teasers
19 (pm)	Isolate from feed and water	Isolate from feed and water
20	Collect and evaluate Day 6 embryos	Transfer two normal embryos to a uterine horn ipsilateral to an ovary containing a corpus luteum
26-28	Treat with luteolytic dose of prostaglandin analogue; remove skin sutures	—
67-69	—	Score for pregnancy and twins using realtime ultrasound (equals days 53-55, if pregnant)
160	—	Pregnant ewes will all lamb over the next 17 days (if lambs are Merinos); review management of lambing

Table 9.9. Expected results for a well managed MOET programme.

Number of ovulations (corpora lutea)	*10-15*
Recovery rate (ova recovered/no. of corpora lutea)	70-90%
Percentage of recovered ova scored as normal	70-90%
Percentage of transferred embryos surviving to lambs*	60-80%
Average number of progeny per treated donor**	4-6

* Assumes transfer of one or two normal embryos freshly collected from a mature donor; the survival rate will be lower after transfer of frozen-stored embryos, fresh embryos scored as retarded and/or abnormal, embryos collected from lambs, or where the recipient was detected in oestrus more than 12 hours apart from the respective donor.
** This number can be increased to, for example, 20 progeny per donor per annum, if the same donor is reprogrammed 4 times in the same year.

Future developments

MIVET and JIVET

Transferrable embryos can also be produced *in vitro* (IVP) after oocyte pick up (OPU) from a superstimulated donor animal (Morton *et al.*, 2005). This is achieved by aspiration of pre-ovulatory follicles (often by laparoscopy) in donor animals treated with similar hormone regimens to those used for MOET and transfer of the resultant ova to a laboratory for *in vitro* maturation (IVM) and *in vitro* fertilisation (IVF). Embryos are grown to morula or blastocyst stage before transfer to recipients using the same technique described above for MOET. When the oocyte donors are mature ewes, this method of assisted reproductive technology is known as 'mature in vitro embryo transfer' (MIVET). While this technique eliminates the variation in fertilisation rate and superovulatory response observed with MOET (Maxwell *et al.*, 1990), embryos produced *in vitro* remain less viable than those created *in vivo* (Galli and Lazzari, 2008).

Oocyte donors need not be ewes which have reached puberty and sexual maturity. Pre-pubertal lambs as young as 3 weeks old can be stimulated with gonadotrophins and their eggs harvested by OPU (Morton, 2008). Known as 'juvenile in vitro embryo transfer' (JIVET), this procedure offers the opportunity for rapid genetic improvement via a reduction of the generation interval to as low as 6 months (van der Werf, 2005). Unfortunately, oocytes from juvenile animals do not respond as favourably to IVP as those from mature ewes (O'Brien *et al.*, 1996) and embryo/foetal survival post-transfer is also reduced (Kelly *et al.*, 2005; Ptak *et al.*, 2006). Therefore, JIVET remains considerably less efficient than MIVET (Cognie, 1999; Morton, 2008).

In reality, the expense of both JIVET and MIVET excludes most commercial breeders from utilising their benefits and as such few artificial breeding companies offer such services in sheep.

Sex preselection

While economic traits, *e.g.* fibre diameter, are largely unaffected by the sex of the animal, financial gains can be achieved through preselecting the sex of lambs, either by accelerated genetic progress (van der Werf, 2005) or via the commercial exploitation of greater numbers of an individual sex. For example, stud producers who derive the majority of their income from ram sales could increase their profit by producing a higher percentage of rams per lamb batch. Conversely, for graziers transitioning into a new breed the ability to produce predominantly ewe lambs would be of benefit in accelerating flock growth (de Graaf, 2006). In this circumstance, sex-preselection, particularly when coupled with MOET, would decrease the early costs of flock establishment by minimising the number of breeding ewes initially required.

The only effective means of accurately preselecting the sex of offspring is through the use of sex-sorted spermatozoa or "sexed semen". As males are the heterogametic sex with each spermatozoon carrying either an X or a Y chromosome, it is the male, at fertilisation, which decides the sex of the embryo and resultant offspring. Using a procedure known as flow cytometry (Maxwell *et al.*, 2004), it is possible to separate all of the spermatozoa within an ejaculate into X- and Y- chromosome bearing populations (*i.e.* sexed semen) and inseminate one of the populations to produce either a female or a male, respectively. The technical points of the sex-sorting procedure are complex and covered in considerable detail elsewhere (Garner, 2001; Hollinshead, 2004; Garner, 2006), but briefly, it is achieved by exploiting the difference in DNA content (4.2% for sheep) between spermatozoa carrying the X and Y chromosome (Pinkel *et al.*, 1982).

At present, the commercial availability of sexed semen remains limited to cattle (Garner and Seidel, 2008). This is primarily due to the unacceptably low fertilities following AI of sexed semen at low doses (an economic necessity resulting from the slow production of sexed spermatozoa by the flow cytometer; Hollinshead *et al.*, 2003). Fortunately, recent research has increased the fertility of sexed frozen-thawed ram semen to a comparable level with normal frozen-thawed semen in both standard AI programmes (de Graaf *et al.*, 2007b; Beilby *et al.*, 2009) and in superovulated ewes for MOET (de Graaf *et al.*, 2007a). These improvements in fertility (specifically their impact on production costs of sexed semen), should enable the commercial release of "sexed ram semen" onto the worldwide market in the foreseeable future. Further detail on sperm sexing in sheep and future developments in this area are discussed in de Graaf *et al.* (2009).

Cloning

The birth of "Dolly the sheep" (Wilmut *et al.*, 1997) was an amazing step forward in generating genetically identical animals from one superior individual. While technically not the first 'clone', Dolly was the first mammal to be cloned from a differentiated somatic (body) cell. This technique is known as somatic cell nuclear transfer (SCNT) and involves transfer of a

reprogrammed somatic cell nucleus into an enucleated (nucleus removed) oocyte and stimulation with a small electric current to initiate cell division and embryo development. While the birth of Dolly demonstrated the technical feasibility of this procedure, the production of one lamb from 277 initial SCNTs highlights the incredible inefficiency inherent to cloning. Even 10 years after Dolly's birth, only 1-5% of cloned embryos transferred into recipients develop into viable offspring (Oback, 2008). As such, the use of cloned animals in standard farm production systems remains impractical. Perhaps this will change in future years if the inefficiencies and costs associated with cloning are significantly reduced.

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