

COURSE CODE:	ANP 508
COURSE TITLE:	Controlled Breeding of Cattle, Sheep and Goats
NUMBER OF UNITS:	2
COURSE DURATION:	Two hours per week

COURSE DETAILS:

Course Coordinator:	Dr. Tolulope Julius Williams <i>B. Agric., M.Sc., PhD</i>
Email:	tolwilly@gmail.com
Office Location:	ANP Staff Office, COLANIM
Other Lecturers:	Prof. O.A Osinowo, Dr. I. J. James

COURSE CONTENT:

Definition of Controlled Breeding (CB), Advantages of CB and Disadvantages. Principles of CB, Separation of sexes, Reproductive cycle, Oestrus synchronization, Oestrus detection, Controlled mating, Artificial insemination, Pregnancy diagnosis. Methods of increasing litter size, Flushing, Superovulation, Anti-steroid immunization, Reproductive performance, Herd and Flock structures, Herd and Flock projections, Intensive sheep and goat production, Livestock recording .

COURSE REQUIREMENTS:

This is a compulsory course for all students in the Department of Animal Physiology. Students are expected to participate in all the course activities and have minimum of 75% attendance to be able to write the final examination.

READING LIST:

1. Meat Goat Production Handbook
2. Joe Bearden and John W. Fuquay. Applied Animal Reproduction, Fourth Edition, H. Joe Bearden and John W. Fuquay from Mississippi State University
3. Cognie Y. *Current technologies for synchronization and artificial insemination of sheep*; In *Reproductive Physiology of Merino Sheep* Eds CM Oldham GB Martin and IW Purvis, University of Western Australia, p 207. (1990)
4. Osinowo, O.A. Introduction to Animal Reproduction. Sophie Academic. 2006.

LECTURE NOTE:

Introduction

Controlled breeding is the manipulation of reproductive processes of farm animals to suit management objectives. It is primarily aimed at achieving synchronized breeding and parturition, enhancement of litter size and postnatal survival, controlled parturition as well as optimization of reproductive performance in intensive production systems.

Advantages accruing from controlled breeding include the following:

1. It saves time in oestrus detection
2. It allows for more efficient use of labour
3. It often leads to improved productivity
4. It gives greater control over the production cycle
5. It streamlines and facilitates animal management
6. It enhances accurate record keeping on animal performance.

Disadvantages which may arise from controlled breeding include the following:

1. It often involves additional costs in the provision of extra feed, drugs and breeding aids
2. It may require specialized skills and expertise which may need to be out-sourced
3. It can lead to disastrous outcome such as high peri-natal mortality rate without adequate physical facilities.

Principles of controlled breeding

Controlled breeding involves some basic principles which should be taken into consideration for optimum results. To start with, there is the need for adequate information on the following traits of the farm animals:

1. Age at puberty – It is essential that animals be weaned before puberty in order to avoid indiscriminate breeding (son-dam, half-sib, full-sib mating).
2. Oestrous cycle length – This is required for determining non-return to oestrus.
3. Gestation length – This is required for determining expected parturition dates following successful mating.

The basic principles of controlled breeding include the following:

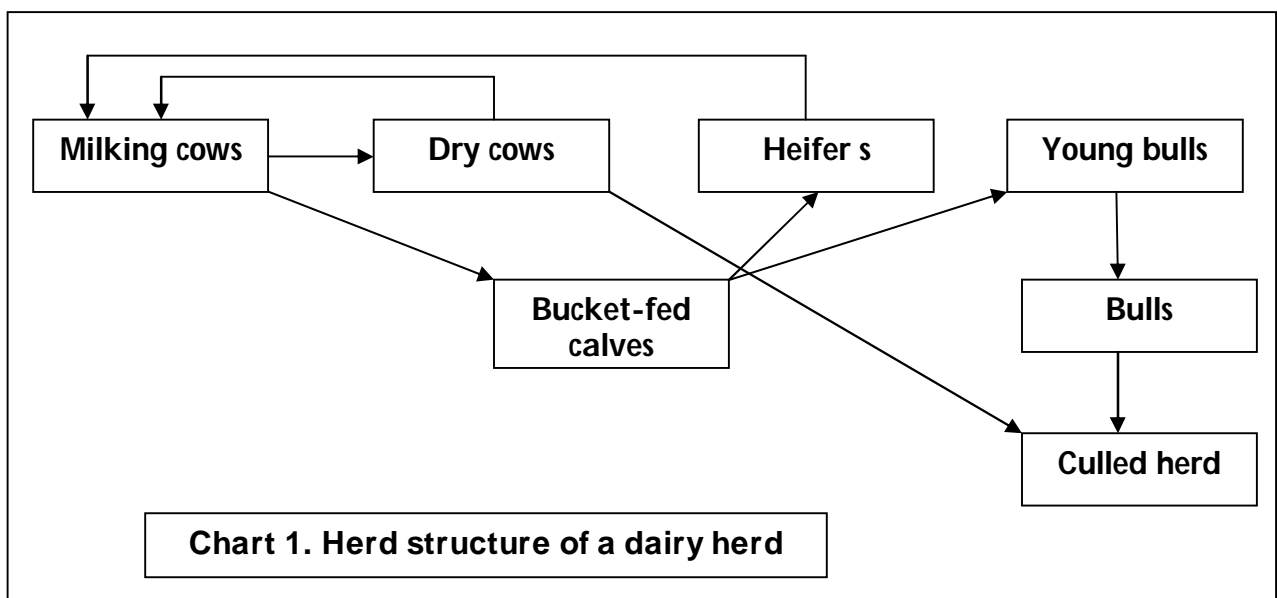
1. Separation of the male and female animals into different herds or flocks prior to the attainment of puberty;
2. Utilization of an efficient oestrus detection method in combination with pen-mating, hand-mating or artificial insemination;

3. Ensuring correct joining ratios of breeding males to females taking into consideration the breeding capacity of the males and the duration of the joining for flock or herd mating;
4. Keeping of accurate breeding records as the basis for planned interventions in the production cycle;
5. Ensuring availability of adequate physical facilities for the care of large numbers of offspring at a time;
6. Ensuring adequate feeding and health management to support high reproductive efficiency in the farm animals;
7. Ensuring controlled-breeding interventions are cost-effective.

Herd/Flock structure

Herds and flocks need to be structured in ways that would facilitate management of the animals. The division into groups could be on basis of age, sex, purpose or physiological state. Such grouping into separate herds or flocks ensures uniformity in the application of necessary specialized management practices to particular groups. For instance, a milking cow herd could be given supplementary feeds to support milk production. Also, a pregnant ewe flock in the last two weeks of gestation can be given supplementary feed to improve neonatal survival of lambs.

Animals in different stages of their productive life have different requirements which are best provided by grouping like animals together. Hence, there could be breeder, grower and finisher flocks of goats or heifer, breeding cow, breeding bull, milking cow and dry cow herds in a dairy operation. Within its productive lifespan, an animal moves through different herds depending on its age or physiological state.



An example of a dairy herd structure is presented in Chart 1. Early weaned calves are moved to the Bucket-fed herd. From there, male calves are moved to the Young bulls herd. Other animal movements depending on age and physiological state are as shown. Sheep and goat flocks have slightly different structure from that of dairy herd.

Flock projection: Sheep

Item	Unit	PY1	PY2	PY3	PY4	PY5
Technical coefficients						
Land area	ha	20	20	20	20	20
Stocking rate	no/ha	40	40	40	40	40
Joining ratio	ewe/ram	20	20	20	20	20
Ewe replacement rate	%	20	20	20	20	20
Ram replacement rate	%	50	50	50	50	50
Lambing rate	%	95	95	95	95	95
Lambing interval	yr	1	1	1	1	1
Avg. litter size	no.	1.2	1.2	1.2	1.2	1.2
Weaning rate	%	80	80	80	80	80
Post-weaning mortality	%	2	2	2	2	2
Flock projection						
Breeding ewes	no.	240	240	240	240	240
Breeding rams	no.	48	48	48	48	48
Lambs born	no.	274	274	274	274	274
Lambs weaned	no.	219	219	219	219	219
Male lambs (3mths-1 yr)	no.	107	107	107	107	107
Female lambs (3mths-1 yr)	no.	107	107	107	107	107
Rams (1-2 yrs)	no.	0	81	81	81	81
Ewes (1-2 yrs)	no.	0	57	57	57	57
Culled rams	no.	24	24	24	24	24
Culled ewes	no.	48	48	48	48	48

- Oestrous cycle
- Oestrus detection
- Oestrus synchronization
- Ram effect
- Synchronized breeding

Flock and herd mating
Pen/hand mating
Artificial insemination
Pregnancy diagnosis

Factors controlling litter size
Flushing
Management of pregnant dams
Steaming up

Peri-natal management
Prewaning management
Weaning/post-weaning management

Rebreeding and parturition intervals
Reproductive performance
Optimization of reproductive performance
Livestock recording
Selection, culling and replacement of breeding animals
Embryo transfer

World trade in frozen semen and embryos

Literature from other sources

MEAT GOAT PRODUCTION HANDBOOK

Reproductive Management of the Meat Goat

Stephan Wildeus

Introduction

Considerable information is available on the reproductive function of goats, but research on reproductive management of goats in the U.S. has focused mostly on milk and fiber production systems and has not been directed at meat as the primary product. In a meat production system, however, reproductive performance is of paramount importance since productivity is largely a function of the number of offspring born and weaned and the frequency with which they are produced. The main reproductive concerns in meat production from goats therefore must be an optimum litter size (2-3 kids) with a high survival to weaning and, secondly, the flexibility to strategically breed does to produce kids that will fit a specific market niche to command a maximum price.

Goat Reproductive Biology

The Doe

Goats are seasonally polyestrous under the temperate climatic conditions of the U.S. During the period of seasonal breeding, reproduction in the doe is controlled by the estrous cycle. Some of the characteristics of the estrous cycle of concern to producers are listed in Table 1. Although the estrous cycle length of goats (21 days) is 3-4 days longer than in sheep, gestation length, duration of estrus and timing of ovulation are similar between the two species. Goats are often considered more prolific than sheep, but there appears to be more variation between breeds within species (i.e. Nubian vs. Angora; Finnsheep vs. Rambouillet) than there is between species.

The reproductive tract of the mature doe consists of the ovaries, which weigh 0.5 to 3 grams dependent on the stage of the reproductive cycle. The ovaries are the primary sex organ, containing the eggs and secreting the female reproductive hormones (i.e. progesterone, estrogen). The oviducts (10-12 cm long) transport the ova to the uterus and act as the site of fertilization. The uterus (15-20 cm long in the non-pregnant state) is the site of fetal implantation and consists of two uterine horns with a common uterine body. The uterus provides the environment that supports

the conceptus throughout gestation. Closure of the uterus is provided by the cervix (4-7 cm long), a muscular canal with several cervical folds or rings that must be at least partially penetrated during artificial insemination. The exterior component of the doe reproductive tract is the vagina which is the site of semen deposition during natural mating; it also supplies a fluid environment to support this process during the appropriate stage of the estrous cycle.

The events of the estrous cycle are largely controlled by the hormonal interactions of the ovaries with the secretory glands (pituitary, hypophysis) located at the base of the brain. In addition to internal stimuli, this system is also responsive to external stimulation such as changes in day length and the presence and absence of males. In short, primary follicles in the ovaries, containing primary oocytes (eggs), develop in successive waves to develop Graafian follicles that will rupture and release a secondary oocyte during ovulation. The released oocyte transverse the oviduct to join with spermatozoa, whereas the Graafian follicle transforms into corpus luteum. The development of the follicle is under the control of gonadotropins (follicle stimulating hormone - FSH and luteinizing hormone - LH) released by the pituitary gland. The gonadotropins, via a hormonal feedback loop, also control the release of estrogens by the ovary, which control the estrous behavior displayed by the doe (flagging, mounting etc.). Following ovulation the luteinized follicle (corpus luteum) secretes progesterone which prepares the uterus for a possible pregnancy and suppresses the secretion of gonadotropins to suspend further follicular development. Failure to establish pregnancy will result in the release of prostaglandin from the non-pregnant uterus, which regresses the corpus luteum and allows a new cycle to proceed. Knowledge of these processes facilitates an understanding of the techniques that can be used to control reproduction (superovulation, estrus synchronization etc.) in the doe.

The Buck

In the buck, the primary sex organs are the testis, which weigh 100-150 grams in the mature animals and fluctuate in size with changes in breeding season. Similar to the ovaries in the female, the testis produces the male gametes (spermatozoa) and sex hormones (i.e. testosterone). The spermatogenic process takes place inside the seminiferous tubules, whereas the Leydig cells in the interstitial tissue are responsible for hormone production. The testes are located in the scrotum, which aids in the thermoregulation of the testes. Spermatozoa produced by the testis enter the epididymis, also located in the scrotum, which serves as the site of sperm maturation (acquisition of motility and fertilizing capacity) and storage. The vas deferens connects the epididymis to the ampulla and accessory sex glands. The latter provide the spermatozoa with the fluids

that make up the ejaculate of the buck and are located in the pelvic region. The penis is the final component of the male reproductive tract and is used to deposit the semen into the female. In the buck, erection is achieved through the extension of the sigmoid flexure that allows an extension of up to 30 cm and the filling of the cavernous tissues with blood. In the non-erect state the glans of the penis is contained in the sheath.

In contrast to the female, where all primary oocytes (eggs) that will be developing are present at birth, primary spermatocytes are produced through mitotic divisions continuously throughout the reproductive life of the male. A further difference between oogenesis in the female and spermatogenesis in the male is that the meiotic divisions of the primary oocytes yield only one functional ova, while primary spermatocytes produce four spermatozoa. The final step of sperm cell production is a process of metamorphosis in which the spermatids, the product of the second meiotic division, develop the characteristics of the functional spermatozoa (head, acrosome, midpiece and tail). Spermatozoa, approximately 60 microns long, are ejaculated in a dense suspension with seminal plasma (Table 1). The seminal plasma activates motility (5-15 mm/min progressive forward motility) and supplies substrates to buffer and nourish the sperm cells. Similarly to the ovaries, the events in the testis are controlled through the gonadotropins LH and FSH.

Onset of Puberty

Sexual development in the goat, as in other mammals, is a process of gradual maturation of the interaction between the hypothalamus, pituitary and gonads, initiated during embryonic development. Postnatal sexual development is dominated by negative feedback of estradiol in association with changes in the secretory pattern of LH. Puberty is generally defined as the point of sexual development at which the animal becomes capable of reproduction (first ovulation in the female and first spermatozoa in the ejaculate of the male), but often animals are not fully sexually competent at this stage. In both the male and female goat, puberty may also often be reached without having achieved adequate physical growth to support reproduction and in the doe first ovulation may not necessarily coincide with first estrus.

Sexual development is influenced by both genetic and environmental factors. In does and bucks the age at puberty ranges from 150 to 230 days, dependent on nutrition, location and season of birth. Nutrition is among the most significant factors influencing reproductive development and the onset of puberty. A low plane of nutrition delays first estrus and reduces uterine and ovarian weights, while having no effect on the partitioning of fat and protein and the weight of other organs. Increasing the overall

plane of nutrition generally advances the onset of puberty, but *overfeeding* will decrease subsequent fertility and impair mammary gland development. Season of birth also has a significant impact on the timing of puberty in both the doeling and buckling, with sensitivity to photoperiodic cues already being in effect in the prenatal stages of development. Puberty in spring-born kids has to be achieved in the same year's fall breeding season or will be delayed until the following year's breeding season. There are some indications that the introduction of bucks may induce estrus and ovulation in the pubertal doe. The physiological basis for this response is attributed to be partly pheromonal and partly neurological.

Seasonality of Breeding

The environmental cue most dominantly affecting seasonal breeding in small ruminants is the annual change in daylength. Photoperiodic control of reproductive patterns is mediated through rhythmic secretions of melatonin by the pineal gland during darkness, which influence the gonadotropin-releasing hormone pulse generation and the hypothalamic-pituitary-gonadal feedback loop. However, following extended exposure to decreasing daylength, animals become photorefractory to the short day stimulus and will cease cyclic activity, unless a period of long day photostimulation is supplied.

Differences exist in the onset and duration of seasonal breeding between various breeds of goats and even between individual animals within a breed. Geographical location, particularly degree of latitude, has a significant impact on timing and length of the breeding season. At locations close to the equator and in tropical breeds of goats animals often are aseasonal and breed throughout the year. In the seasonally breeding does, the breeding season is framed by transitional periods during which cyclic activity can be induced through appropriate management techniques (i.e. introduction of males).

Goat Reproductive Management Techniques

Reproduction should be a vital component of the overall herd management scheme and closely integrated with nutritional and health aspects, as well as form part of a comprehensive recording system. Diets and feed supplies have to be adjusted to account for the physiological stage of production of the goat, particularly in the female (lactation, gestation). Prior to breeding (2-3 weeks) does should be placed on a gaining plane of nutrition to stimulate higher ovulation rates. Once does are bred and pregnancy has been determined, does should be preferentially fed based on pregnancy status (and litter size if fetal numbers were determined; see pregnancy diagnosis below). Does nursing

their kids are nutritionally challenged and may require supplemental feed if pastured to ensure adequate milk supply for multiple litters.

There are currently no major reproductive diseases affecting goats in the U.S., however, goats need to be maintained in good health (dewormed and vaccinated) to ensure proper reproductive function. Meat-type does should be capable of giving birth and raising their offspring unassisted, but help may have to be provided with complications during parturition and the acceptance of the newborn. Records should be collected on kidding and weaning performance (litter size and weight) to be used for selection of breeding stock. Replacement does should be managed closely to achieve a level of sexual maturity that allows an early mating (at 60-70% of adult body weight) within one year of age, thus increasing life time production of the doe. Similarly, young bucks should be mated early in life to decrease the generation interval and achieve maximum genetic progress.

Pregnancy Diagnosis

While not of immediate concern in extensive goat operations that utilize extended natural mating, the early determination of pregnancy can be a useful management tool under more intensive production conditions, or when A.I. and embryo transfer is employed. Pregnancy diagnosis will identify the females requiring repeat breeding or insemination and/or will allow the separation of pregnant and open females for differential management. When fetal numbers can be determined as part of the pregnancy diagnosis, different feeding regimes can be applied to single and multiple litter bearing females.

To be most useful to the producer, pregnant animals need to be identified as early as possible in gestation and provide an estimate of fetal numbers. A variety of approaches have been explored for the early detection of pregnancy and possibly fetal numbers (Table 2). Techniques have either focused on the detection of physical changes resulting from pregnancy (fluid accumulation and presence of a detectable fetus) through palpation and ultrasound, or been concerned with the identification of maternal and fetal physiological signals (progesterone, uterine proteins) associated with pregnancy.

The most promising technique currently available for pregnancy diagnosis in the goat is the use of real-time ultrasound scanning. The arrival of lower cost, portable veterinary scanners, combined with the advantages of their use (fetal number determination, minimal animal restraint, high throughput), has made the application of this technology economically feasible on the farm level. Transcutaneous real-time ultrasonography allows reliable pregnancy diagnosis as early as 35 days

of gestation, whereas transrectal examination will reduce this period further to 25 days. Ultrasound examination can be expanded through the application of fetometry, allowing the aging of the fetus. Guidelines for fetal aging have been developed for the goat, using biparietal diameter as the main measurement. Linear array and sector scanners are available for use in transcutaneous ultrasonography and 5 and 7.5 MHz linear transducers can be used for transrectal examinations. The latter can also be successfully employed for the examinations of ovarian structures.

In view of the versatility and benefits provided by the real-time ultrasonography, many of the techniques listed in Table 2 will find only limited application for routine diagnostic purposes. The use of A- and B-mode and Doppler sound ultrasonic devices has now been succeeded by the real-time linear array and sector scanners. Techniques using hormonal or metabolic (i.e. blood glucose) signals have not found widespread use in small ruminants. With the introduction of animal-side testing for blood and/or milk progesterone by enzymeimmunoassay and the validation of these techniques for goats, the turn-around time for laboratory analysis has been reduced. However, progesterone and estrogen determinations for pregnancy diagnosis should not be expected to find wide-spread application. Success to predict litter size from progesterone and estrogen concentrations has only been moderate (around 60%) and is confounded by breed differences.

Breeding Soundness Examination

A buck should possess characteristics that will advance the production potential of the herd in which he is used, while being able to successfully mate to transmit these characteristics. As was indicated earlier, spermatogenesis is susceptible to outside influences such as elevated temperature, season of year and nutrition and breeding males need to be evaluated for reproductive soundness 3-4 weeks prior to mating season.

Part of such a 'breeding soundness examination' is an evaluation of the overall condition of the buck and includes his health history, physical soundness, particularly of feet and legs, and examination (palpation) of the external genitalia (scrotum and scrotal content, sheath and penis) for signs of infections and other abnormalities. There are currently no age and breed standards for scrotal circumference in meat-type breeds and there is a need for guidelines to be developed. The second part of the examination involves the collection and evaluation of an ejaculate. In trained bucks this is achieved using an artificial vagina, but in most instances an electroejaculator has to be used. The method of collection has some effect on the ejaculate characteristics, the volume generally being larger in an electroejaculate. The ejaculate is immediately scored for motility under low (mass motility) and high magnification (percentage

motile sperm) of a light microscope on a pre-warmed slide. Morphological abnormalities and viability are determined from stained semen smears. In the final part of the examination bucks are allowed access to estrous does to evaluate libido and mating behavior.

Bucks are classified as either sound, questionable or unsatisfactory, based on all components of the examination. No firm guidelines have been developed to assign bucks into these categories and interpretation rests largely with the experience of the examiner. Animals deficient in any part of the examination should be considered questionable and retested after several weeks. A second failed test would indicate reproductive deficiencies and such a buck should not be used in natural mating.

Manipulation of Reproduction in Goats

The utilization of reproductive management techniques has only limited application in an extensively managed herd, but can be an useful tool to improve performance of a more closely managed herd. Additional inputs will be needed in labor and handling facilities and in the area of nutritional management. Unfortunately most of the commercial pharmaceutical products developed for reproductive manipulation in goat and sheep are not available and/or approved for use in the U.S. and have only been applied in the U.S. on an experimental basis. However, a description of these techniques is relevant to familiarize the producer with the options that may become available or can be applied under extra-label use in cooperation with a licensed veterinarian. There are some reproductive manipulations that can be performed without the aid of pharmaceutical compounds, such as the use of the male effect and controlled lighting and they will also be discussed briefly.

Estrus synchronization

Approaches towards synchronizing estrus in livestock have to focus on either the manipulation of the luteal or the follicular phase of the estrous cycle. In the doe the window of opportunity is generally greater during the luteal phase, which is of longer duration and more responsive to manipulation. Different approaches have been concerned with either extending the luteal phase by supplying exogenous progesterone or with shortening this phase through removal of the corpus luteum. Successful techniques must not only establish synchrony, but also provide a reasonable level of fertility in the synchronized cycle (Table 3).

The treatment of choice for estrus synchronization, and also out-of-season breeding, in goats has been the intravaginal sponge, impregnated with 45-60 mg of a synthetic progesterone (Table 4). Sponges are widely used either in conjunction with pregnant mare serum gonadotropin (PMSG),

FSH or prostaglandin to more tightly synchronize and/or induce a superovulatory response. Under research conditions sponges impregnated with natural progesterone in higher doses (400-500 mg) have been used and similar synchrony and fertility to that of commercial sponges were achieved. An alternative means of supplying continuous, exogenous progesterone has been the intravaginal pessary (CIDR-G®) developed for goats in New Zealand. The CIDR device is constructed from a natural progesterone impregnated medical silicone elastomer molded over a nylon core. In large scale trials with cashmere goats in Australia CIDR devices were equally effective to intravaginal sponges in controlling ovulation and fertility following A.I.

A number of synchronization systems for goats have been evaluated under research conditions that use compounds approved for other species and/or applications (Table 4). One of these systems is based on the extra-label use of the norgestomet ear implant supplied with the estrus synchronization system Synchromate-B®, developed for cattle. Does are implanted with the norgestomet implants for a period of approximately 14 days and a gonadotropin, either FSH or PMSG, is administered around the time of implant removal. There will usually not be an adequate response and synchrony of estrus without the gonadotropin treatment. The estradiol valerate injection provided in the product combination for cattle should not be used for goats due to their increased sensitivity to estrogens. Studies have indicated that the implant dose provided for cattle (6 mg norgestomet) can be reduced to 2-3 mg by cutting the implant. Following synchronization does and ewes come into estrus within 72 hours. Melengestrol acetate (MGA) is an orally-active, synthetic progestogen, approved for use in feedlot cattle, that can be used for the induction and synchronization of estrus in does in conjunction with zeranol and PMSG. Prostaglandin F_{2a}, or rather its analogues, are widely used for estrus synchronization in cattle, but results have not been as satisfactory in goats. A functional corpus luteum is required for prostaglandin to regress, thus making this technique only suitable for synchronization during the breeding season. Synchronization with prostaglandin analogue generally produces a more synchronized estrus than that obtained with a progestogen-gonadotropin treatment, but subsequent fertility is somewhat reduced.

The application of estrus synchronization schemes requires an increased level of management either through the utilization of A.I. or the proper management of bucks. With a larger number of females showing estrus at the same time, the female : male ratio should not exceed 7:1, or alternatively, timing of the induced estrus should be staggered (i.e. spreading the removal of intravaginal sponges over several days). Hand mating of males, as a modification of A.I., can also be used. Fertility of the synchronized estrus is generally high, but responses to PMSG and

prostaglandin co-treatment have at times been variable. The repeated use of PMSG in conjunction with progestogen treatment has resulted in reduced fertility in subsequent years and was attributed to an active immunization against PMSG.

Out-of-Season Breeding

Some of the pharmaceutical techniques used for out-of-season breeding in small ruminants are essentially the same progestogen-gonadotropin treatments described for estrus synchronization above. Estrus response and subsequent fertility for the out-of-season application of intravaginal sponges are similar to that reported for does during the breeding season. An alternative pharmacological means of modifying the seasonal breeding patterns is through the manipulation of the melatonin signal. Exogenous melatonin can administered to supplement the endogenous release and thus mimic the 'short days' associated with the onset of breeding season in fall. Melatonin can be supplied either as an orally active compound, by injection or as an implant (subcutaneous or intravaginal), all of which have been similarly effective. For the successful application of this treatment the melatonin stimulus has to be continuous and in case of the orally active form requires daily feeding between 1500 and 1600 hours. A prerequisite for the advancement of the breeding season through melatonin treatment is for animals to have experienced a sufficient period (30-60 days) of long days. The response to melatonin treatment is related to the timing of the treatment in relation to onset of breeding season for a given breed at a specific location. A commercially available, subcutaneous slow release melatonin implant (Regulin®, see Table 4) has been marketed overseas, no commercial products are currently available in the U.S.

Artificial lighting, either by itself or in conjunction with melatonin and/or the male effect, can provide effective manipulation of the breeding season in goats. Since melatonin can be conveniently used to mimic short days, artificial lighting under practical conditions is mostly employed for 'long day' simulation. Long days under artificial lighting are usually administered as 16 hours of daylight to 8 hours of darkness. To simulate long days it is, however, not necessary to provide the entire 16-hour light period, but treatment can be divided into the natural daylight period followed by an appropriately timed 1 hour light stimulus at the time of desired dusk. Goats will distinguish between a gradual decrease in daylength as opposed to a sudden shift from short to long days. Models for light controlled year-round breeding of goats have been proposed and experimentally validated and would subject animals to a 2-month short day-long day cycle. Results indicated that the period of cyclic activity was extended, but that periods of acyclicity remained and a lack of continuity in cycles developed. Most practical systems have focused on

the extension of the natural breeding season, combining a period of long days followed by melatonin treatment for short day simulation.

Exposing anestrus females to intact males or androgen-treated castrates, following isolation, has been demonstrated to induce estrus and ovulation in the doe. The physiological basis for this response is partly pheromonal and partly neurological, with neither aspect completely accounting for the response. However, it is documented that the stimulus will elicit a pulsatile LH release sufficient in length and magnitude to initiate the ovulatory process. The male-induced estrus is usually synchronized, with ovulation occurring within 2-3 days of stimulation. The response to male stimulation can be quite variable and is influenced by breed, completeness of prior isolation, "depth" of anestrus, nutrition and stage postpartum. Unless male-induced cyclic activity is initiated preceding the natural breeding season of a given breed at a given location the response is transient in nature. Hence the practical application of the 'male effect' lies primarily in inducing an early breeding season, or in combination with some pharmacological out-of-season breeding manipulation.

Goats generally respond more favorably to out-of-season breeding using melatonin, artificial lighting and the male effect than sheep. Differences have been attributed to the higher and more variable endogenous night-time melatonin levels in sheep compared to goats, as well as to the need for progesterone priming before estradiol will generate behavioral estrus.

Superovulation

As multiple litter bearing animals, ovulation rate and litter size have a major impact on the reproductive efficiency of goats. Ovulation rate is influenced by the stage of breeding season, nutrition, genotype and parity. However, it can also be manipulated by pharmacological means. Superovulation through gonadotropins (primarily FSH and PMSG), used in higher (pharmacological) doses to elicit a superovulatory response, is commonly used to prepare does for ova collection in embryo transfer. PMSG is more easily administered than FSH, usually as a single injection of up to 1500 to 2000 i.u., but the superovulatory response to PMSG can be quite variable and is usually lower than in a FSH-induced superovulation. Problems associated with PMSG-induced superovulation are a high number of non-ovulated follicles and short, irregular estrous cycles. FSH is usually administered in decreasing doses of 1 to 5 mg, injected in 12 hour intervals over a period of 3 to 5 days around the time of termination of the progestogen treatment. Acceptable ovulation rates in does following FSH range from 10 to 25, but the number of viable embryos may be significantly lower. Improvements in the consistency and predictability of the superovulatory response have been achieved through co-treatment with prostaglandin and LH. The latter acts in synergism with

FSH to achieve follicular stimulation and the ratio of FSH to LH has been considered of some importance in achieving a satisfactory superovulation response.

Increases in ovulation rate have also been achieved through the immunization of does to steroids. Steroid immunization has become commercially available overseas as Fecundin®, which immunizes females to androstenedione (Table 4). Immunization is achieved through two subcutaneous injections (2 ml) administered initially 2-3 weeks apart and in a single annual boosters thereafter. A period of 3 weeks is suggested between the booster immunization and the time of optimum ovulation. Due to the long term effects and the relative ease of application of the product, steroid immunization can be used for the improvement of ovulation rate and subsequently litter size in more extensively managed flocks. The animal response in ovulation rate and litter size varies with breed and location, but improvements of ovulation rate (+1.0) and litter size (+0.5) have been achieved in does.

A number of other pharmacological treatments to manipulate reproductive function in goats are subject to investigation and development under research conditions. However, it is not clear to what extent these approaches may prove to be biologically and/or economically feasible. Among the concepts under investigation are (i) the immunization against inhibin, which selectively suppresses FSH, but not LH, (ii) the use of GnRH in conjunction with progestogen-based superovulation treatments, and (iii) the administration of betamethasone for the induction of kidding.

Assisted Reproduction in Goats

The techniques of artificial insemination (AI) and more recently embryo transfer (ET) in livestock production present producers with unique opportunities to maximize the number of progeny from animals with superior genetic make-up and move their germplasm around with relative ease. Drawback of these technologies are the need for experienced personnel with the appropriate equipment to achieve the desired success. The costs involved are most likely prohibitive for producers of goats that are marketed for meat, but has great potential for producers of breeding stock, propagating animals with outstanding production characteristics. In the fledgling meat goat industry the recent introduction of the Boer goat is an excellent example for the need to apply assisted reproductive technologies for the dissemination of stock. As other superior meat-producing germplasm is identified, the application of AI and ET is likely to rise in the area of meat goat production.

Artificial Insemination

The process of AI can be broken down into semen collection, semen processing and storage and the actual insemination. The first two parts (collection and processing) are usually not of great concern to the producer, unless bucks are collected on the farm. The actual insemination process, however, is often carried out by producers with frozen semen shipped to the farm. Does can be inseminated with fresh and extended, non-frozen semen stored chilled for up to 48 hours, but for most practical purposes semen originates frozen from outside the farm.

For AI, semen is usually collected from bucks trained to serve an artificial vagina, adjusted to the appropriate temperature and pressure. Once a collection schedule is initiated, bucks can be collected 2-3 times daily on alternate days. Semen is immediately evaluated for quality and the concentration is determined. The semen is then diluted in a medium containing egg yolk, sugars and buffer to provide an insemination dose of 20 million (frozen, laparoscopic intra-uterine) to 300 million (fresh, vaginal) spermatozoa, dependent on the intended insemination technique. When semen is intended for frozen use, glycerol is included in the diluent as a cryoprotectant. Semen can be either frozen as a pellet, using an engraved block of dry ice, or aspirated into straws and frozen using liquid nitrogen vapor. Once frozen in liquid nitrogen, semen can be stored for extended periods of time.

The success of the actual insemination depends to a large degree on the appropriate timing in relation to estrus and ovulation. Does must be observed closely for the onset of estrus (flagging, restlessness, frequent urination, vaginal swelling and mucus discharge), or can be synchronized (see above), and should be inseminated 12-18 hours after the onset of estrus. In case of transcervical insemination the thawed semen will be deposited in the restrained doe either in the cervix or in the uterine body, adjacent to the cervical opening, using an insemination pipette and speculum. In case of intra-uterine insemination, the semen is deposited into the uterus through the abdominal cavity via an insemination pipette manipulated through a laparoscope. When using this technique the doe is restrained in a cradle in a ventral position. Though laparoscopic insemination is more involved, fertilization rates are high, even when using small doses of frozen semen.

Embryo Transfer

The ET process can be broken down into the basic steps of 1) estrus synchronization of the donor and recipient, 2) superovulation of the

donor, 3) fertilization of the donor, 4) recovery of the embryos and 5) the actual transfer of embryos to recipients. Success in all of the above steps is vital to achieve implantation and carriage to term of the transferred embryo. The ability to culture embryos following collection has allowed us to transfer the fertilization from inside the donor to the culture dish and also to further manipulate the embryo (embryo splitting and gene transfer).

Techniques used for estrus synchronization of donor and recipient and for the superovulation of the donor with gonadotropins (FSH, PMSG) are similar to those described above. Insemination of donor does should occur either naturally or through vaginal AI, rather than intra-uterine AI, to avoid additional manipulation of the uterus and oviducts. For the actual collection, the uterus of the donor is flushed 3-5 days following mating. Traditionally this is done in goats under anesthesia using a midventral or flank laparotomy, involving the exteriorization of the uterus. Particularly in case of repeated collections this may cause adhesions interfering with subsequent collections. More recently collection techniques using laparoscopy have been developed and reported good success in goats (76% pregnancy). Following collection, the flushing medium is examined to identify fertilized (cleaved) ova, determine the recovery of ova (based on the number of corpora lutea) and evaluate ova quality. Only high grade embryos should be used for frozen storage, whereas embryos of less quality may be used for fresh transfer. Embryos should be transferred into the uterine horn of the same side containing an ovary with a corpus luteum. Multiple transfer into recipients without a corresponding number of corpora lutea is not recommended. Following a sufficient period of rest donor does can be repeatedly collected.

Conclusions

Appropriate reproductive management is vital to a successful meat goat enterprise. Much of the profit to be realized will depend the frequency with which litters are produced, the size of litters and the survival to weaning of multiple litters. A number of the reproductive techniques described here may not have an immediate application for the producer of goat meat, but any success in meat goat production will require sound knowledge of the reproductive biology of these animals. Since goat meat production in the U.S. as a primary enterprise is still in its beginnings, much of the germplasm evaluation and multiplication, completed for many other livestock breeds, will still have to take place. The application of reproductive technology (AI, ET) will form an important part of this process.

Table 1: Reproductive Characteristics of Does and Bucks		
Criteria	Mean	Range
Doe		
Cycle length (d)	20	17-24
follicular phase (d)	4	-
luteal phase (d)	17	-
Duration of estrus (hrs)	30	16-50
Ovulation after estrus (hrs)	33	30-36
Gestation length (d)	150	144-155
Litter size	-	1-4
Buck		
Daily testicular sperm production (billion)	6.0	4.8-7.2
Ejaculate volume (ml)	1.0	0.5-1.5
Ejaculate concentration (billion/ml)	3.0	1.5-5.0

Table 2. Comparisons of techniques available for pregnancy diagnosis in the doe				
Technique	Sensitivity Range (Days)	Fetal Numbers^a	Accuracy (%)	Practical Application
Sterile harnessed male	> 20	no	65 - 90	high
Abdominal palpation	60 - 115	no	60 - 90	moderate
Progesterone assay	18 - 22	no	90 - 95	moderate
Estrone assay	> 60	no	90 - 95	low
Real-time ultrasound	40 - 100	yes	90 - 95	high
A/B-mode ultrasound	60 - 120	no	85 - 95	high
Doppler ultrasound	60 - 90	no	85 - 90	moderate
Radiography	>50	yes	90 - 95	low

a techniques allowing the determination of litter size with high degree of

accuracy (>95%)

Table 3. Advantages Associated With The Synchronization of Estrus in Does

- Facilitates the use of artificial insemination
 - Prepares for the use of embryo transplantation
 - Assists in the induction for out-of-season breeding
 - Concentrates time of breeding and parturition for closer management
 - Allows for optimal nutritional management of dam and offspring
-

[About VEIN](#)

[Research databases](#)

[Links](#)

[Library catalogue](#)

[Request an article or book](#)

[Publications & proceedings](#)

[Research & e-learning](#)

[OLIVER](#)

[LIDA](#)

[How to...](#)

[Your questions answered](#)

[Search the Internet](#)

[VEIN Community](#)

Sheep Health & Production

Chapter 8. Controlled breeding; artificial insemination (ai) and moet

[Introduction](#) | [Control of the time of mating](#) | [Increasing the number of progeny](#) | [Induction of abortion](#) | [Induction of parturition](#) | [Artificial insemination \(AI\)](#) | [Multiple ovulation and embryo transfer \(MOET\)](#) | [Some other technologies in sheep related to AI and MOET](#) | [General references](#)

Return to [Sheep Health & Production Index](#)

Introduction

This second chapter on reproduction is concerned with various direct interventions to manipulate and enhance reproductive function in sheep flocks. These procedures should be used only on healthy and well managed sheep. Flocks which are malnourished, suffering from parasites or other diseases or otherwise mismanaged are not suitable. The procedures usually involve considerable cost to your client, and their use should not be lightly recommended.

There are five broad areas of reproductive intervention, as follows:

- limitation
- control of the time of mating
- increasing the number of progeny
- induction of abortion
- induction of parturition

Limitation embraces practices such as the castration of male lambs and the isolation of rams and ewes except when mating is desired, and requires no further discussion here. *Control of the time of mating* and *increasing the number of progeny* are the main areas of interest in Australia. *Induction of abortion* and *of parturition* are seldom employed in Australia, but veterinarians are occasionally asked to perform one or other procedure.

Control of the time of mating

The basic requirements for an effective method of control of the times of oestrus and mating are:

- it should simulate the normal sequence of endocrine and ovarian events (and permit normal or near-normal fertility)
- that it is effective in nearly all ewes (and at all stages of the oestrous cycle in the case of cyclic ewes)
- that it yields marked precision in the times of oestrus and ovulation
- it should be simple, and
- it should be cheap, readily available and result in no unacceptable side-effects or residues

There are two physiological states to consider:

- cyclic
- anovular (seasonal, post-partum, pre-pubertal)

Cyclic ewes

In these ewes control is achieved by either (a) artificially extending the 'luteal phase' of the oestrous cycle by administering progesterone or other progestagens, or (b) prematurely inducing luteolysis, by giving prostaglandins. Figure 8.1 depicts the levels in peripheral plasma in the ewe of LH, oestradiol and progesterone during the normal oestrous cycle.

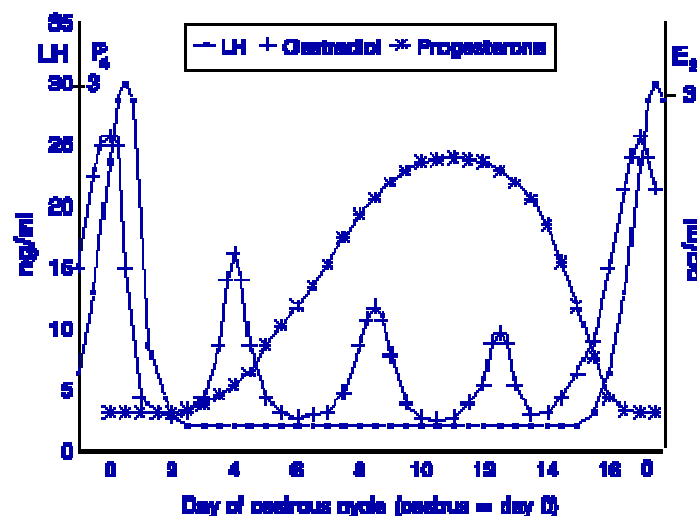


Figure 8.1 Levels of LH, oestradiol and progesterone in peripheral plasma of the ewe during the oestrus cycle.

The oestradiol level peaks at or just before the onset of oestrus and the LH peak occurs 6-18 hours later. Oestrus lasts for about 20-40 hours, being longer and more intense in multiple ovulating breeds. Ovulation occurs at around the end of oestrus. Thus the interval between the onset of oestrus and ovulation in Merinos is usually about one day, with considerable variation; but in more fecund genotypes it may be considerably longer. This has practical implications, in deciding when to hand mate or more especially inseminate different types of sheep in relation to the onset of oestrus (the time of ovulation will usually not be known).

The luteal phase progesterone (Figure 8.1) has three important functions in relation to the next ovulation. This progesterone ensures that :-

1. the ovulation is accompanied by oestrus
2. the corpus luteum resulting from this ovulation has a normal life-span and function, and
3. the uterine endometrium is primed to permit survival and normal development of the embryo (if mating and fertilization occur).

The peaks in oestradiol level that occur during the luteal phase are not required for the maintenance of pregnancy, but they permit non-pregnant ewes to return to oestrus and have another chance to conceive. This luteal phase oestradiol has this effect in non-pregnant ewes by priming the uterus to respond to ovarian oxytocin. This, in turn, results in uterine $\text{PGF}_{2\alpha}$ release and luteolysis.

Systems for oestrus synchronization should enable the insemination (or natural mating) of groups of ewes on a pre-determined date and preferably at a pre-determined time. If the degree of synchrony is sufficiently good, the ewes can be inseminated at a fixed time, without detection of oestrus. As well as requiring satisfactory synchrony and fertility in ewes regardless of the stage of the oestrous cycle at which treatment commences, the treatment should also produce a mild degree of superovulation, resulting in about an extra 0.5 ovulations per ewe. These extra ovulations should boost both conception and twinning rates. In practice, a 'trade-off' is often observed between the precision in timing and fertility. For example, giving progestagens for longer than the recommended period may enhance precision at the expense of reduced fertility.

Progestagens to synchronize oestrus

The use of progestagens is the preferred method of oestrus synchronization in the ewe. When, as is usual, the stage of the oestrous cycle is not known, treatment should be spread over at least 12 days

and preferably be given for 12-13 days. If treatment commences on day 5 or later in the cycle (day 0 = oestrus) the life-span of the existing corpus luteum is unaltered. If treatment commences on day 4 or earlier, the time of luteolysis is advanced (to around day 12). Can you recall the physiological explanation of this phenomenon? In the past progestagens (including progesterone) have been given orally, by daily injection in oil or by a subcutaneous implant. However, these methods of administration generally give unsatisfactory results, with inadequate precision in the interval between cessation of treatment and oestrus (*ie* they do not give a sharp 'end-point' to treatment). The following two procedures have proved equally and highly successful and are commonly employed. Both can be considered to be portable, intra-vaginal 'corpora lutea' which can be inserted and removed at will:

1. administration by polyurethane sponge. The progestagen is dissolved in ethanol and injected into the sponge and dried to give a fine dispersion of crystals within the network of the sponge, to provide a large surface area to aid absorption. There are 3 products available :
 - a) *Repromap* (Upjohn Pty Ltd), containing 60 mg medroxyprogesterone acetate
 - b) *Chronogest* (Intervet), containing 30 mg or 40 mg flugestone acetate
 - c) *Ovakron* (Heriot Agvet), containing 30 mg flugestone acetate
2. administration by silicone elastomer device (mounted on a nylon spine) containing 300 mg (9%) progesterone - *CIDR-G* (constant internal drug release device, Carter Holt Harvey Plastic Products; Australian distributor: Riverina Artificial Breeders).

Table 8.1 shows typical data for the interval between progestagen sponge removal and oestrus in cyclic ewes. Chronogest 30 mg sponges were used. PMSG was given to some ewes at the time of sponge removal to further stimulate folliculogenesis.

Table 8.1 : Interval between sponge removal and oestrus

Dose of PMSG	Percent of ewes in oestrus			
	Hours after removal			
(IU)	24	36	48	>48
NIL	0	5	80	15
300—600	0	40	60	0

The dose of PMSG depends mainly on bodyweight and breed (*eg* Merinos, 300-400 IU; Border Leicester, 600 IU). The optimal dose of PMSG is determined only after some trial and error. These data are based on twice daily inspections for oestrus. The small dose of PMSG both advances and increases the precision in the time of onset of oestrus. It also slightly increases ovulation rate. The degree of precision obtained with PMSG should probably be adequate to allow insemination at a fixed time (around 48 hours) after sponge removal. Fertility at a controlled oestrus is usually reduced (as compared to a spontaneous oestrus), but returns to normal at the second, spontaneous and partially synchronized oestrus after treatment. The extent of the depression of fertility at the controlled oestrus is highly variable and results primarily from faulty sperm transport through the cervix. This defect can usually be overcome by depositing more semen in the ewe (use more rams for natural mating or more spermatozoa for cervical AI) or by depositing semen directly into the uterus (laparoscopic AI).

Prostaglandins for synchronizing oestrus

A single injection of a prostaglandin is likely to be effective in only about 70-75% of ewes (those at Day 5 or later in the cycle). Possible alternative approaches to using prostaglandins are:

- detect oestrus and treat once between 7 and 14 days after oestrus
- treat all ewes twice, 10 days apart or
- treat all ewes and mate or inseminate on detection those ewes which show oestrus. Treat those which do not show oestrus 10 days later, and mate or inseminate on detection.

Cloprostenol, 100 µg, (*Estrumate*, Jurox Pty Ltd) or dinoprost-PGF_{2α}, 4-5 mg, (*Lutalyse*, Upjohn Pty Ltd) are effective. Oestrus occurs 2-3 days after treatment but not with sufficient precision to allow fixed-time inseminations. Further, these treatments will be quite unreliable in any situation where a significant portion of the ewes are not cycling regularly. In consequence of these factors and in contrast to the case in cattle, prostaglandins are little used for controlled breeding in sheep.

Anovular (or anoestrous ewes)

In these sheep it is necessary to induce as well as control the times of oestrus and ovulation. Two distinct types of anoestrus occur (*viz* seasonal and post-partum). In the context of the Australian sheep industry seasonal anoestrus is much the more important of these two states; many AI and MOET programmes, especially on studs, are performed outside of the breeding season. Post-partum anoestrus becomes an issue only when lambing occurs at intervals of less than one year. This is quite common in some European countries, but unusual in Australia. Note that seasonal anoestrus may involve prepubertal ewes which would have reached puberty had seasonal anoestrus not intervened. Since Merinos and Dorsets are less rigorously seasonal than most other sheep breeds in Australia, it is easier to manipulate reproduction in these breeds and their crosses in the non-breeding season.

There are basically four approaches that can be used to induce oestrus and ovulation in ewes in seasonal anoestrus. These are:

1. selection towards breeds/strains which cycle spontaneously for all/most of the year
2. manipulation of the light-dark cycle or the administration of melatonin implants
3. administration of hormones; usually progestagens plus PMSG (requires a higher dose of PMSG than in the cyclic ewe - 500-700 IU, depending on type/breed)
4. use of the 'ram effect'. This may be
 - a) used alone
 - b) used in conjunction with the introduction of some ewes in oestrus - 'social facilitation effect'
 - c) further exploited by pre-treatment of ewes with progestagens.

Various combinations of these four approaches are also possible. While approaches 2, 3 and 4 may lead to good conception rates and flock fertility after an out-of-season joining, responses are quite variable and are influenced particularly by breed and type, body weight/condition score, environment and month of joining. If natural mating is employed, more rams are required, especially in the case of British breed rams like the Border Leicester, Suffolk and Romney.

Melatonin implants to stimulate oestrus

Direct regulation of the light-dark cycle is too costly and time consuming to find commercial use. The use of exogenous melatonin is

much more practicable. The implants (*Regulin*, Regulin Ltd) are inserted subcutaneously near the base of the ear. Regulin is recommended for October, November and December joinings. The resulting increased plasma melatonin levels appear to simulate those which occur spontaneously after the summer solstice. The ewes are isolated from rams for 6 weeks before joining and receive a single implant 30-40 days before joining for 6-8 weeks. Hence these recommendations are utilizing both the light-dark cycle and ram effects (*ie* Regulin seems to prime the ewes or reduce the depth of anoestrus, to respond better to the introduction of rams). Extensive trials have yielded the average results shown in Table 8.2.

Table 8.2 : Responses to Regulin

	per 100 ewes joined		First cross (BL/Mo)	
	Merinos / Comebacks / Polwarths / Corriedales		Untreated	Regulin
empty ewes	22	12	9	7
single bearing ewes	71	68	72	57
twin bearing ewes	7	20	19	36
triplet bearing ewes	0	0	0	0
lambs born	85	108	110	130
Period (days) in which 80% of ewes lambed	28	19	41	28

Note the considerable stimulation of ovulation rate. These data do not reveal substantial variation in the Regulin effect in individual flocks. The decrease in percentage of dry ewes induced by Regulin is more marked in maiden than in mature ewes. There may be little effect of Regulin in occasional situations where some Merino types show spontaneous oestrous cycles in late spring/early summer. Regulin gives poor results and is not recommended for use in the very seasonal British breeds. Regulin should not be used if the spring joining may have to be abandoned; such treatment appears to lower performance if the ewes are re-joined in autumn.

Gonadotrophic hormones to stimulate oestrus

When suitably administered, gonadotrophins with FSH activity and GnRH (LHRH) will induce ovulation in the anoestrous ewe, but the ovulation is not usually accompanied by oestrus. If treatment is preceded by giving a progestagen for a minimum of 6 days, the ovulation is accompanied by oestrus. In practice the usual regime is to insert sponges or CIDRs for 12 days and give PMSG at the time of device removal. A slightly higher dose of PMSG than is used in the cyclic ewe is required. Sometimes the CIDRs are used for only 6 days,

washed and re-used! The use of progestagens plus PMSG is expensive. The response to such hormone treatment is critically influenced by stage of anoestrus (or month of joining). Table 8.3 lists some effects in (Border Leicester x Merino) XB ewes of stage of anoestrus, in terms of (a) % of ewes coming into oestrus (b) % of ewes in oestrus which conceive and (c) the probability of further breeding (or returns to oestrus) in ewes which do not conceive at the induced oestrus. The XB ewes were treated with a sponge or CIDR for 12 days and received 500-700 IU PMSG at device removal.

Table 8.3 : Response of BL/Mo XB ewes to progestagens plus PMSG varies with stage of anoestrus

Stage of anoestrus	Effectiveness of treatment		
	Ewes in oestrus (a)	Ewes pregnant (b)	Further breeding (c)
deep (Aug-Oct)	50% or less	50% or less	nil
late (Nov-Dec)	50-80%	50-60%	possible
very late (Jan-Feb)	80% or more	60% or more	probable

For natural mating, the number of rams required for large flocks may be prohibitive. The number can be much reduced by dividing the ewe flock into subgroups and staggering the hormone treatments by intervals of 2-3 days. For best results, the number of ewes in oestrus per day per ram should not exceed three where British breed rams are used.

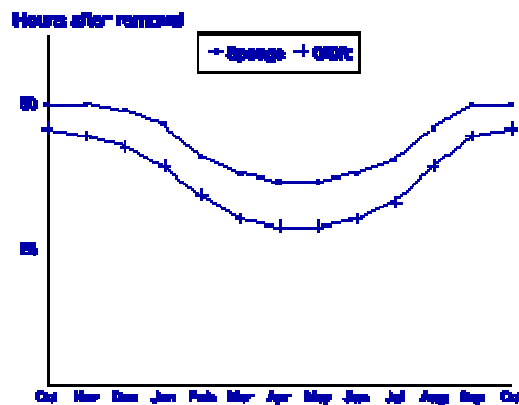


Figure 8.2 Seasonal pattern in the mean time to onset of oestrus following CIDR or sponge removal and treatment with 400 IU PMSG.

Equivalent tables for the effects of stage of anoestrus in Merino and British breed flocks are not shown. How would you expect the data in such tables to differ from those above for XB ewes? For Merinos, you

would expect the same trend, but generally better results. For Nov-Dec joinings, for example, 80-90% should come into oestrus and perhaps 70% of these will conceive to the treatment; and further breeding is probable in animals which do not conceive at the induced oestrus. Conversely, in breeds such as the Border Leicester and Romney, the results will be worse, and there is little point in attempting controlled breeding until January-February (very late anoestrus).

Returning to Merino and XB ewes, Figure 8.2 shows the influence of season on the mean time interval to onset of oestrus following sponge or CIDR removal and a fixed dose (400 IU) of PMSG. The interval is extended out of season, to about 40-56 hours. The standard deviation for interval is also higher out of season. Of course, the interval is also sensitive to PMSG dose and, as this figure shows, type of progestagen device. Oestrus occurs about 10 hours earlier after CIDR than after the sponge. This probably reflects higher rates of metabolism and/or clearance of progesterone versus synthetic progestagens. The reduced precision obtained in times to oestrus and ovulation in ewes in anoestrus is an important reason why large scale AI programmes are best scheduled in or just before the normal breeding season (in very late anoestrus).

The 'ram effect'

The unaccustomed presence of rams with ewes can advance the onset of breeding activity, synchronize oestrus in groups of ewes to some degree, and may increase ovulation rate. Much out-of-season breeding of Merinos relies to a considerable extent on this 'ram effect', although many producers are probably unaware of this mechanism. The ewes must be isolated from the sight and smell of rams for at least one month before joining. In Merinos, ram introduction in spring and summer (Oct-Jan) causes a high proportion of ewes to breed. In the absence of conception, most will show 1-3 consecutive cycles, then revert to spasmodic breeding or full anoestrus. When the ram effect works well, 80% or more of Merino ewes should lamb.

In breeds with a more sharply defined breeding season and deeper anoestrus, the rams must be introduced within 6 weeks of the time at which breeding would normally commence. If introduced earlier, the ewes' responses are highly variable and usually poor. Sometimes the rams induce only an LH response, but not ovulation. There are big differences between rams in their ability to induce ovulation in anoestrous ewes. Merino rams are generally very potent. Experience in New Zealand has shown that Romney rams are fairly useless and Dorsets much better. Hogget rams are not as good as mature rams. The ram effect relies principally on pheromones secreted by the glands of

the wool follicles. Their secretion is androgen dependent. These volatile chemicals (plus, perhaps, the sight and sound of rams?) increase the frequency of LH pulses in the ewe. This usually leads (if anoestrus is not too deep) to ovarian follicular development, oestrogen secretion and an LH surge, culminating in ovulation within 3 days of joining.

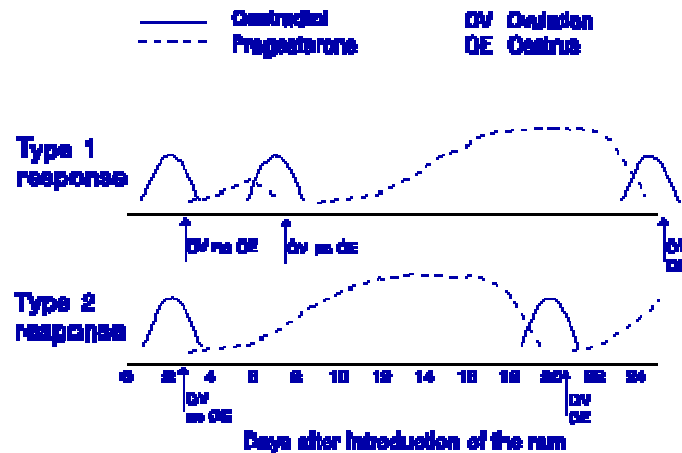


Figure 8.3 Ovarian responses in anoestrus Merino ewes to the introduction of rams. Varying portions of the ewe flock will exhibit Type I or Type II responses.

Merino ewes usually react to the introduction of rams in one of two ways. After the silent, ram-induced ovulation on days 2-3, (rams introduced day 0), most ewes experience a short cycle and have another silent ovulation on days 7-8 (Type 1 response, Figure 8.3). This ovulation is silent because it is not preceded by an adequate period of progesterone priming. These ewes next experience a normal cycle, so that they ovulate a third time around days 24-25, and this ovulation is accompanied by oestrus. The balance of the ewes in the flock do not experience the short cycle, so that after the ram-induced ovulation they ovulate a second time around days 20-21 and this ovulation is accompanied by oestrus (Type 2 response, Figure 8.3). The proportion of the flock exhibiting the short cycle is variable. In practice, there should be a high incidence of oestrus around days 19-25.

For AI, testosterone-treated wethers are best used. Even with natural mating, it is best, if feasible, to induce the ram effect with 'teaser' wethers for the first 14-16 days, so that Merino rams do not take out their frustrations on each other waiting for the ewes to come into oestrus. Testosterone cypionate or testosterone enanthate (*Synarot*, *Banrot* or *Tesgro*, 75 mg/ml, registered for the control in wethers of pizzle rot) is administered subcutaneously next to the base of the ear.

Well grown, large wethers should be used. Note that, in this case, the wethers are only required to exude pheromones and not to mate actively with the ewes (contrast to the preparation of teasers for AI). A single dose of 400 mg given 14 days before use and 1% wethers are probably adequate. However, some veterinarians recommend using 2% wethers which have received 150 mg, 150 mg and 300 mg at 0, 7 and 14 days (inserting the 'teasers' at 14 days and the rams at 28-30 days).

The ram effect with 'social facilitation'

In New Zealand, where common ram breeds often fail to induce a strong 'ram effect', there has been considerable interest in the 'social facilitation effect'. In this case a minor portion (*eg* 1/3rd) of the flock is treated with CIDR/PMSG so that they come into oestrus at the time of ram introduction. This strengthens the ram's effect on the balance of the flock. The effect of the ewes in oestrus almost certainly acts via pheromones to stimulate the rams. The same effect may be seen when the rams are exposed to other ewes in oestrus prior to introducing the rams into the flock. This seems to be another expression of the quantitative nature of the ram effect. This is potentially a way to reduce the costs of hormone treatments for out-of-season breeding in New Zealand. There seems to be little interest in the 'social facilitation effect' in Merinos in Australia.

The ram effect with progesterone pre-treatment

Table 8.4 shows two ways that the ram effect can be further exploited by certain hormonal pre-treatments of the ewes.

The use of a single dose of progesterone should cause essentially all ewes to come into oestrus around days 19-20 (or ovulate on days 20-21). This enables a relatively cheap approach to AI. Potentially, the whole flock may be inseminated over 4-5 days, using teasers for oestrus detection. Fertility should be high, since it is a natural oestrus. However, depending on breed/bloodline and stage of anoestrus, only a variable proportion of the ewes may respond. The first or ram-induced ovulation usually has a higher ovulation rate (an extra 0.2-0.3 ova). Hence pre-treatment with sponges/CIDR not only advances the time of first oestrus by some 3 weeks, but also enables a higher twinning rate.

Table 8.4 : Effect of progesterone pre-treatment on ram effect and oestrus

Pre-treatment	Days after ram introduction			
	2-3	7-8	20-21	24-25
NIL	OV	OV?	OV	orOV
Progesterone; single 20mg dose at or 1-2 days prior to ram introduction	OV	—	OV	—
Progestagen sponge or CIDR for 6-12 days, removed at ram introduction	OV	—	OV	—
	OE	—	OE (returns)	—

OV = ovulation; OE = oestrus

In all cases the times of oestrus and ovulation are to a varying degree synchronized. In order to obtain high conception rates use 5% or more rams or stagger the ewes so that 3 of the ewes are introduced each 3-4 days and use 2% rams. Prepubertal ewes which are of sufficient age and weight to have reached puberty will show the ram effect. What will be the resulting pattern of lambing? How will this be modified if a significant portion of Merino ewes are spontaneously cyclic at the time of ram introduction?

Oestrus stimulation in ewes in post-partum or lactational anoestrus

In ewes which lamb in the latter half of the breeding season, or in spring-early summer, post-partum anoestrus will usually extend into seasonal anoestrus. If ewes lamb just before or earlier in the breeding season, the first, silent post-partum ovulation occurs 20-30 days later. This first ovulation is usually followed by a short cycle and a second silent ovulation 6-8 days later. This, in turn, is followed by a normal cycle and a third ovulation with oestrus at around 45-55 days post-partum. Note the similarity of this sequence of events in the post-partum ewe to that seen in dry, seasonally anoestrous ewes after the introduction of rams. Hence, during most of the year, treatment to induce oestrus and ovulation will be necessary in order to breed recently lambed ewes. The recovery of the uterus post-partum to provide an environment which allows fertilization and embryo development (involving a regrowth of an epithelial cell layer back over the caruncles) takes longer in ewes which are lactating and/or lamb in spring. More generally, the conception rate to a hormone-induced oestrus and ovulation in post-partum ewes is dependent upon:

1. the time after lambing
2. lactational status
3. season of year at which the ewe lambs, and

4. degree of seasonality of the breed/genotype.

In the irrigation districts of south-eastern Australia attempts are occasionally made to produce prime lambs from XB ewes at intervals of less than one year. To be successful, such programmes require high standards of nutrition and management. Table 8.5 gives a guide to the pattern of return to normal fertility in BL/Mo XB ewes. It is important in such programmes that a high percentage of fertile rams are used (or, in the case of AI, ample doses of high quality semen). In practice, the minimal lambing interval achievable is about 200 days (3 lambings in two years or possibly 5 lambings in 3 years).

Table 8.5 : Days post-lambing for return of normal fertility

Season of lambing	Lactational status	
	lactating	non-lactating
Autumn	60-70	50-60
Spring	>70	60-70

Increasing the number of progeny

The procedures available for increasing the number of progeny from rams and ewes are listed below and, in some cases, discussed. Some of the procedures have already been discussed; while AI, MOET and related techniques will be examined in detail later in this chapter.

The ram

1. Advance the age of puberty. This can be achieved by increasing the plane of nutrition and perhaps also treating young rams with melatonin (depending on season)
2. AI
3. Increasing sperm production in mature rams; again by better feeding and perhaps treatment with melatonin in the more seasonal breeds

The ewe

- Join at an earlier age
- Retain to an older age
- Increase the frequency of breeding
- Select for increased litter size
- Cross-breed with more fecund breeds or types
- Induce a temporary increase in ovulation rate, by
 - a) hormonal treatments (usually PMSG)
 - b) immunizations (against androstenedione, inhibin)

- Insert transgenes which regulate fecundity
- MOET, embryo splitting, cloning, *et cetera*

Before discussing some of these procedures in the ewe, it is well to repeat the general caution that the achievement of high fecundity in ewes (especially Merinos) requires higher standards of management and feeding and the acceptance of higher lamb losses.

Join at an earlier age

Joining at an earlier age requires a consideration of the factors which determine the age of puberty in ewes. These can be listed as follows:

1. genotype and the youngest age at which puberty is possible;
2. nutritional status and growth rate;
3. duration of the breeding season; and
4. date of birth.

Suppose, for example, that the breeding season in a highly seasonal breed is March-June and the earliest age at which puberty is possible in well fed lambs is 8 months. In this case, the ewe lambs from ewes which conceive in March-May could reach puberty at 8 months of age, but those from ewes which do not conceive until June cannot reach puberty until they are 16 months old. If we say that 10 months is the earliest age at which ewe lambs can reach puberty (which better approximates most situations in Australia) then the daughters of ewes which conceive in March can reach puberty at 10 months of age, but those of ewes which conceive in April-June cannot reach puberty until 16-18 months of age.

Select for increased litter size

Selection for increased litter size within a breed is possible but difficult and not often pursued in practice. Estimates of the heritability (h^2) of *number of lambs born* vary but are usually about 0.15 and genetic progress in reproductive rate of up to 1.5% annually has been achieved by selecting rams on the dams' reproductive performance. Genetic responses occur principally in ovulation rate. The increased OR seems to result from both (a) increased production of gonadotrophins and (b) increased ovarian sensitivity to gonadotrophins.

Cross-breed with more fecund types or breeds

Cross-breeding with more fecund breeds or types will probably also exploit heterosis. Much of the increase in lambing performance of (Border Leicester x Merino) XB ewes is due to heterosis, since lambing performance drops when the first-cross is consolidated by

interbreeding. The first-cross produces 0.3 - 0.5 more lambs per ewe joined than the mean of the two parent breeds. Crossing of Booroolas[1] with other Merino bloodlines increases ovulation rate and numbers of lambs born (especially if the Booroolas are homozygous for the Fec^B gene). Merino ewes heterozygous for the Fec^B gene typically have ovulation rates of 2.7-3.1. However, depending on nutrition, the severity of the environment and perhaps predators, the number of lambs weaned often does not increase and may even decline. The distribution of OR in a Booroola cross-bred flock will be complex, since the flock will contain FF, F+ and ++ ewes. The Fec^B gene has an autosomal locus. It has an additive effect on OR, but seems not to be expressed in the ram.

Induce a temporary increase in OR

The induction of a temporary increase in OR may be achieved by the use of progestagen sponges and PMSG, as previously discussed, aiming for a mean increase in OR of about 0.5. Alternatively, various immunization procedures can induce a short-term increase in OR. For some years the vaccine *Fecundin* was available to sheep producers. The immunogen in *Fecundin*, polyandroalbumin, contained androstenedione as an antigenic determinant. Vaccination resulted in decreased plasma free androgen (and probably oestrogen) levels and an increase in gonadotrophin secretion. Vaccination was carried out 5 and 2 weeks before joining for 6-8 weeks; in subsequent years a booster dose only was given to the flock 2 weeks before joining, depending on the season and the desirability of obtaining more twins.

The results achieved in terms of lambs weaned were somewhat erratic and the vaccine never became very popular, for several reasons. Commonly the subsequent management of flocks was not good enough to permit high survival rates in multiple-status lambs. Ovarian responses were generally better in high rather than low condition score ewes and in XB rather than Merino ewes. It was generally wise to not treat maiden ewes. Vaccination did not correct a fertility problem and there was no decrease in the number of dry ewes. Research studies suggested that vaccination sometimes increased the rate of embryo mortality, possibly by altering the oviductal/uterine environment. Other research studies have shown that a modest increase in OR can be obtained in ewes by vaccination against inhibin, an ovarian regulator of FSH secretion. Perhaps a commercial vaccine based on inhibin or an inhibin agonist will become available in the future?

Induction of abortion

The maintenance of pregnancy in the ewe ceases to be dependent on the corpus luteum at around day 50. Prior to this time, abortion can be reliably effected with a single luteolytic dose of prostaglandin (for doses, see earlier). In cases of misalliance, the earlier treatment is given the better, but treatment before day 5 is ineffective. After day 50 abortion is not easily induced with even large and/or repeated doses of prostaglandins. These treatments with higher doses are dangerous and the retention of foetal membranes is a serious side-effect.

Glucocorticoids are unreliable before day 140. You should dissuade your client from attempting treatment after day 50. If another pregnancy is required, allow the ewe to go to term and then remove the lamb.

Induction of parturition

Parturition is usually induced with glucocorticoids. This should initiate most of the normal prepartum maturational events, thereby ensuring adequate foetal preparation for postnatal life. 15 mg dexamethasone should result in parturition some 24-48 hours later. The optimal treatment time depends on breed. Treatments on days 144 and 148 are recommended for British breeds and Merinos, respectively. Following a synchronized mating, the treatments are given when 5% of the flock have lambed. Some increase in the rate of perinatal mortality should be expected after these treatments.

Artificial insemination (AI)

Only a very small proportion of ewes in Australia are artificially inseminated. It is difficult to get accurate figures on the use of AI. Nevertheless, it appears that some hundreds of thousands of ewes are artificially inseminated annually and that the majority of these inseminations are carried out or supervised by veterinarians. Increasing significance is being given to progeny testing of rams and the sale of semen by Merino studs. As an historical trend, the role of veterinarians has shifted away from large-scale AI programmes in autumn using natural heat detection and cervical insemination and towards more compact or concentrated programmes using controlled oestrus and uterine insemination. These programmes, which are usually on studs, are often required in December - February. It is also reasonably common in some districts for commercial wool producers to ask veterinarians to inseminate (uterine AI) 100 (say) ewes with purchased semen, to produce their own superior young rams. Allowing for 60% culling of ram lambs, this should yield about 10-12 replacement rams at a cost that is often below that of buying in replacements. This

exercise is probably only worthwhile where the flock size exceeds 2000. Successful involvement of veterinarians in sheep AI programmes requires good planning and organization and an uninterrupted allocation of time, which is often difficult in a busy practice situation.

Selection and preparation of rams

This requires much care. Rams should have a thorough clinical examination to check both general health and the reproductive organs (Chapter 7). A test for brucellosis may be necessary. Collect a semen sample by electroejaculation to confirm the presence of ample normal, motile spermatozoa. Assess any information about the genetic merit and commercial value of the ram. Allow sufficient time between selection and use for training to the artificial vagina, and check ram management, especially feeding and conditions of shedding. Be wary of the stud breeder who wants a salvage operation for a ram which is not performing well in natural mating.

Collection of semen

Two procedures only are potentially available. The relative advantages and disadvantages of using electroejaculation and the artificial vagina are as follows:

Electroejaculation

Advantages:

1. No training is required; potentially suitable for all rams
2. Collections can be made from rams with low libido or which are injured
3. Large volumes of semen can often be collected at the one time
4. Useful for testing large numbers of rams for fertility, including vasectomized rams

Disadvantages:

1. Distressing to the ram and requires considerable restraint
2. Semen is sometimes not collected, which may be misleading for diagnostic purposes
3. Ram may urinate during collection
4. Possible damage to rectum
5. Does not provide any indication of libido
6. Frequent collection of ejaculates of high quality semen suitable for AI is not possible

Artificial vagina

Advantages:

1. High quality semen is usually collected
2. Ram indicates the characteristics of his normal ejaculate and libido
3. Frequent collections are possible, at least 2-3 times per day or 20 times per week
4. No stress to ram

Disadvantages:

1. Rams have to be trained
2. Not suitable for rams with low libido or with injuries
3. Frequent collections are needed when large volumes of semen are required in an AI programme

In summary, electroejaculation is the procedure employed on most sheep properties, where semen collection is required as an adjunct to ram soundness and fertility investigations, whereas the artificial vagina must be used for any significant AI programme. Electroejaculation is achieved by stimulating per rectum the nerves around the accessory glands and the base of the penis. The artificial vagina should simulate the characteristics of the ewe's vagina, in respect of temperature, pressure and lubrication. The apparatus and procedures required for each method of collection, as well as the procedure for training rams to the artificial vagina, will be examined in the practical classes.

Handling of semen

A number of factors may adversely affect the viability of semen during handling. These are:

Change of temperature

A sudden drop in temperature to less than 10EC causes 'cold shock'; an irreversible loss of viability. Exposure to temperatures greater than 42EC causes rapid death of spermatozoa, whereas storage at greater than 30EC reduces the fertilizing life of the sperm, due to exhaustion of energy sources and a drop in pH (resulting from the accumulation of lactic acid). An ideal temperature for short term (1-2 hours) storage is 25-30EC. Slow cooling over 2-3 hours to around 5EC will maintain good viability and fertilizing capacity for at least 12 hours.

Sunlight

Exposure to direct sunlight damages spermatozoa, mainly due to the effects of ultraviolet radiation.

Exposure to heavy metals

Copper, lead, mercury and cadmium are toxic to spermatozoa; glass or plastic containers should be used for semen.

Contact with water

The rapid change in osmotic pressure kills spermatozoa. Artificial vaginas and collection and storage vessels must be dry.

Contaminants

Urine, micro-organisms, dust, *et cetera*.

Disinfectants

Nearly all antiseptics, bactericides and detergents are spermicidal. What are the preferred cleaning and sterilization procedures? Glass or plastic containers should be washed thoroughly, then rinsed repeatedly and thoroughly with distilled water to remove detergents. Sterilization by dry heat, gas (ethylene oxide) or UV irradiation is effective. There is a danger with autoclaving of contamination with heavy metals or other materials previously placed in the autoclave.

Evaluation of semen

Semen can be directly evaluated by measuring pregnancy or lambing rates but in practice the substantial costs and time lags involved are major problems. Thus the indirect evaluation or assessment of semen in the field is a critical factor to the success of any AI programme and expert opinion should be sought by inexperienced veterinarians.

Table 8.6 : Scoring system based on macroscopic examination

Score	Density	Mean (range) in number of spermatozoa/ml (x10 ⁹)
5	thick creamy	5.0 (4.5-6.0)
4	creamy	4.0 (3.5-4.5)
3	thin creamy	3.0 (2.5-3.5)
2	milky	2.0 (1.5-2.5)
1	cloudy	1.0 (0.3-1.5)
0	watery	<0.3

Scores 3, 4 and 5 are suitable for AI

Estimates of probable fertilizing capacity can be made by visual examination and, if necessary, various biochemical tests. Semen samples can be examined **macroscopically** for volume, colour, consistency (or density) and motility. Average ejaculates to the artificial vagina have a volume of around 1.0 ml (range 0.5 - 1.5 ml). The colour should be milky white; other colours indicate contamination. The consistency of the ejaculate can be scored subjectively, according to the criteria in Table 8.6. Motility can be only roughly assessed macroscopically; swirling on the side of the collecting vessel indicates high motility.

Microscopic examination of semen may be used to determine motility, density, % dead sperm, morphology and viability. Motility can be scored subjectively, with the criteria in Table 8.7.

Table 8.7 : Scoring system based on microscopic examination

Score	Motility	Percent of sperm active	Description
5	excellent	>80	rapid, dense waves changing direction rapidly
4	good	65-80	good wave motion but slower changes in direction
3	fair	40-65	slow thin waves
2	poor	20-40	no waves; individual sperm can be seen
1	very poor	<20	few sperm show progressive movement
0	none	0	no movement

Scores 4 and 5 are suitable for AI

Scoring must be carried out on a warm slide (37EC), preferably on a warm stage and at low magnification (40-100x) with a thin sample. Score rapidly, because the sample will soon dry. Assessment of wave motion is difficult or impossible where semen has been extensively

diluted or frozen and thawed; in these cases the percentage of progressively motile sperm is estimated. In practice the subjective scoring of semen samples for density and motility is usually sufficient in a commercial AI programme. Microscopic or other objective determination of density (haemocytometer, colorimeter, packed cell volume) and determination of % dead sperm (stain method), morphology and viability after *in vitro* incubation and biochemical tests (respiration, fructolysis, pH, *et cetera.*) are not usually necessary, and will not be detailed here. Every semen sample contains a proportion of abnormal sperm. In good quality ejaculates the percentage may be 5-10%. Increasingly, the use and sale of ram semen is being underpinned by objective measurement of semen characteristics. Commercial laboratories use computerized image analysis systems to give accurate information on submitted semen samples. This enables measurement of progressive motility and velocity, motility, motile sperm concentration, *et cetera.* Progressive motility is the proportion of spermatozoa moving forwards at more than a defined speed. Some healthy caution is still required in assessing the practical significance of these various objectively measured semen characteristics. What significant correlations have been demonstrated between objectively measured characteristics and fertility? There is some experimental evidence for significant correlations, but a reliable *in vitro* test for prediction of fertility still eludes us.

Dilution of ram semen

The dilution of ram semen is generally essential but may not be necessary if only a modest number of ewes are to be inseminated promptly after semen collection. Semen is diluted for both technical and biological reasons. The technical reason is to enable more ewes to be inseminated with a minimal volume of inseminate (in effect, to get the right number of motile sperm into the right volume for insemination). The biological reason is to enhance the survival of sperm, by:

1. providing energy substrates (eg glucose, fructose)
2. using buffers to stabilize pH
3. using antibiotics to protect against micro-organisms present in semen or the female reproductive tract, and
4. using cryoprotectants to protect against freezing damage (usually glycerol and egg yolk)

Formulae for the preparation of various diluents suitable for both the fresh use and freezing of ram semen can be found in the text by Evans and Maxwell (1987). Antibiotics should be added to all diluents (eg penicillin, 200 IU/ml; streptomycin, 1.0 mg/ml). For the fresh use of ram semen, various synthetic diluents, including Dulbecco's phosphate

buffered saline, or UHT cows' milk can be used. Other types of cows' milk require pre-treatment to inactivate toxic factors. For the busy veterinarian, freshly opened UHT milk is probably the diluent of choice, for both cervical and uterine insemination. Note the following points about the dilution procedure:

- carry out the dilution promptly after collection and examination of the semen
- the diluent and semen must be held at the same temperature (25-30EC), preferably in a water bath
- add the diluent slowly to the semen and mix gently
- the rate of dilution depends upon the concentration and motility of the sperm in the ejaculate, and the route of insemination

Evans and Maxwell (1987) provide recipes for diluents containing glycerol and egg yolk which are suitable for one-step freezing of ram semen in pellet form or in straws. The composition of these diluents may have to be adjusted for different dilution rates. All presently available freezing diluents seem to reduce the quality of frozen-thawed spermatozoa and there is ongoing research to devise superior diluents.

Storage of ram semen

Semen must be stored in a manner which decreases or arrests metabolism and hence prolongs the fertilizing life of the sperm. Semen is stored either (a) chilled, at 2-5EC, or (b) frozen in pellets or straws and held at -196EC. For liquid or *chilled storage* the semen is cooled slowly over 2-3 hours. For use, it may be warmed promptly to 30EC or inseminated without warming. Chilled semen maintains good motility (as seen in re-warmed samples) for several days. However, the fertilizing ability of this semen after cervical insemination declines progressively, so that for acceptable fertility (>45% conception rate?) the semen should be stored for not more than 12-24 hours. If uterine insemination is used, the semen may be used after storage for up to 3 days. For *frozen storage*, the semen is diluted with diluent containing cryoprotectant and chilled to 2-5EC, as before.

Two methods of freezing and thawing are used. In the *pellet method*, the rate of temperature change is fast. Aliquots (0.2-0.5 ml) of chilled semen are dropped into holes made on the surface of a block of dry ice (solid CO₂) and the resulting pellets are transferred with forceps into liquid nitrogen. For use, the pellets are transferred promptly into a dry vessel at 37EC to obtain rapid thawing and maximum recovery. In the *straw method*, which is semi-fast, the straws are loaded with chilled semen and placed in liquid N₂ vapour; then stored in liquid N₂. For use, the straws are thawed at 20-30EC. The proportion of spermatozoa which survive these procedures (as indicated by subsequent motility)

varies widely. 40-70% should survive in good semen samples. Semen from some individual rams will not tolerate freezing and thawing. The inseminating dose must be adjusted according to the survival rate (12 - 2x number of sperm inseminated fresh). The conception rates obtained after cervical AI are only 30-40% at best and usually lower. Sperm which survive the procedure have a decreased fertilizing life. Uterine AI is always recommended, and conception rates by this route can be good. The procedures for freezing, storing and thawing semen are examined in more detail in the practical classes.

Detection of ewes in oestrus

An important decision in any AI programme is whether to inseminate to oestrus detection or at a fixed time after treatment. Even where the latter policy is adopted, it is probably worthwhile to run raddled teasers with the ewes and inseminate first those ewes which came into oestrus first, since the inseminations may take many hours or all day. In any event, you will probably want to run some teasers with the ewes to monitor the success of the hormone treatments in the ewes, and it is possible that the presence of teasers enhances the ewes' responses. Where insemination is based on oestrus detection, the quality of detection will clearly be an important determinant of the conception rate to AI. Detection is necessarily based on the use of teasers with crayon bearing harnesses. The selection, preparation and management of teasers were previously discussed. The following percentages of teasers are recommended:

natural cycles: \$ 2%

synchronized at previous cycle: \$ 4%

synchronized at this cycle: 6-10%

Rams are occasionally used as teasers. They may be entire rams fitted with aprons, but this system is not reliable. Alternatively, sexually active young rams are vasectomized at least 6 weeks before use, and immediately before use semen samples are collected by electroejaculation to confirm the absence of spermatozoa. Techniques employing caudal epididymectomy or penis sheath deviation are not recommended. The teasers used today are usually testosterone-treated wethers. If the wethers are given 150 mg testosterone cypionate (or enanthate) at fortnightly intervals, commencing 4 weeks before use and continuing until the end of the AI programme, they should exhibit good mounting activity.

Insemination of the ewe

During coitus, semen is deposited in the vagina. Ejaculation is rapid, with a small volume of semen containing a high concentration of sperm. AI should at least simulate this and preferably deposit semen in a more favourable position for fertilization (cervix, uterus). However, in the ewe it is very difficult and usually impossible to pass an inseminating pipette through the cervix. Three methods of insemination are employed in Australia; vaginal, cervical and uterine. The apparatus and facilities required and the procedures involved for each of these three methods are examined in the practical classes.

The volume of inseminate can vary slightly within set limits. The upper limit will be determined by the capacity of the organ or site of insemination to retain the semen. Volumes below 50 µl are not practicable. Recommended volumes are:

vaginal insemination: 0.3-0.5 ml

cervical insemination: 0.05-0.20 ml

uterine insemination (per uterine horn): 0.05-0.10 ml

The *minimal safe numbers of motile spermatozoa in the inseminate* (required to obtain satisfactory conception rates) are determined by the route of insemination, type of semen (fresh/liquid-stored/frozen-thawed) and whether oestrus in the ewes is spontaneous or controlled. Some workers have obtained good fertility with considerably lower numbers of sperm, but the numbers of motile sperm shown in Table 8.8 are recommended for general use.

Table 8.8 : Minimal safe numbers of motile spermatozoa per inseminate

Route of insemination	Type of oestrus	Type of semen		
		Fresh (x10 ⁶)	Liquid-stored (x10 ⁶)	Frozen-thawed (x10 ⁶)
vaginal	spontaneous	150	NR	NR
	controlled	300	NR	NR
cervical	spontaneous	100	150	NR
	controlled	200	300	NR
Uterine per uterine horn)	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.

The *time of insemination* is related to the time of ovulation. Ovulation occurs around the end of oestrus. Insemination should be performed at a time sufficiently before ovulation so that by the time of ovulation a large population of sperm is established in the ampulla, the site of fertilization. In general, this means insemination 12-24 hours before ovulation. However, time of insemination is adjusted according to whether the semen is fresh or frozen-thawed, since the former should have a fertilizing life in the female reproductive tract well in excess of 24 hours, while for the latter the fertilizing life may not be more than 12 hours. For *insemination of ewes in spontaneous oestrus* (vaginal or cervical AI), aim for 12-18 hours after the onset of oestrus, with twice daily inspections for detection. With once daily inspection in the morning, ewes in oestrus should be inseminated soon after inspection.

For *insemination of ewes in controlled oestrus* (cervical or uterine AI), insemination is usually performed at a fixed time in relation to the synchronization treatment. (However, some commercial operators still prefer to inseminate in relation to onset of oestrus). The interval between progestagen device removal and time of insemination is influenced by season, type of intra-vaginal device (shorter for CIDR than progestagen sponge), type of semen (fresh, frozen), the use of PMSG and the dose employed. If progestagen sponges are used in conjunction with a non-superovulating dose of PMSG and 5-10% teasers, the majority of ewes will be in oestrus within 36-48 hours and ovulate about 60 hours after sponge withdrawal. Superovulated donor ewes for MOET programmes, which have been treated with larger doses of PMSG and/or other gonadotrophins will be in oestrus within 24-36 hours and ovulate about 48 hours after sponge withdrawal. Insemination is recommended at the following times after progestagen sponge withdrawal, where a single insemination is employed:

cervical insemination+	PMSG, fresh semen	36-48 hours
" "	- PMSG, fresh semen	48-60 hours
uterine insemination +	PMSG	
(not superovulated)	fresh semen	36-48 hours
" "	frozen semen	60-66 hours
uterine insemination		
(superovulated)	fresh semen	24- 48 hours
" "	frozen semen	44- 54 hours

These times can all be shortened a little if CIDRs are used instead of progestagen sponges.

Ewes are usually inseminated only once. Double inseminations are not recommended where uterine insemination is used. In the case of cervical insemination, you must balance the likely small (5-10%) increase in conception rate against the extra costs associated with double insemination. If employed in ewes not receiving PMSG, inseminate at 48-50 and 58-60 hours after sponge removal. Use the recommended number of motile sperm at **each** insemination.

What *conception rates* can reasonably be expected? Done carefully, they should be about the same as those that could be obtained with natural mating with ample ram power. But in practice they are commonly somewhat less. You will hear occasional claims from commercial operators of extraordinary conception rates (up to 90%), but treat these claims cautiously. Rates of 60-70% using uterine AI are very acceptable and seem to be often achieved by better operators.

Overall planning and management of an AI programme

Individual AI programmes may involve as few as 5 or as many as 20,000 ewes. The larger programmes are laborious and expensive, but also very challenging. They represent a major investment on the part of your client. In the case of uterine AI the equipment that you require is expensive and some time is needed to develop expertise. Veterinarians will probably need to do at least 1000 animals per annum to maintain their skill and justify this investment. However, do not be too ambitious initially, as small 'crashes' are better than large 'crashes'! The optical telescope in particular is delicate and liable to be damaged, so a spare should always be carried. In contrast, vaginal insemination programmes, which are still used to a limited extent, require little equipment and can be carried out by one person. There seems to be little role for veterinarians in these programmes.

A full discussion of planning and management is beyond the scope of this chapter. However, some of the issues that need to be addressed are indicated by the following questions:

- Does the programme advance your client's breeding objective and/or commercial prospects?
- Is the programme to be based on natural or controlled oestrus?
- How are the ewes selected; maidens may be a problem; how many ewes can be inseminated in one day; are sufficient teasers available?
- What type (fresh/frozen) of semen and route of insemination will be used?
- How are the sires selected; are sufficient rams or purchased semen available?
- Is the quality of sheep husbandry adequate; consider particularly the condition scores of the sires, ewes and teasers?

- Has sufficient time been allowed for the (a) training of rams, (b) preparation of teasers and (c) purchase or construction of necessary equipment or facilities?
- Are adequate trained personnel available, and what role will you play?
- Are the standards of animal and semen identification and record keeping adequate?
- What will be done with the ewes which do not conceive to AI (these will usually be at least 30% and often more of the flock)?
- How will the conception rate be determined and is it satisfactory?
- How will the inseminated flock be managed during pregnancy, lambing and lactation?

It should be clear that the programme does not end on the day or days of insemination. In view of the high costs involved 'up front', extra attention must be given to subsequent flock management in order to maximize lamb survival. Remember that because of the treatments employed, the twinning rate is likely to be higher than is normal for the type of sheep involved, and most of the lambs will be born over just a few days.

Multiple ovulation and embryo transfer (MOET)

The ovaries of the ewe contain vast numbers of oocytes, only a minute proportion of which ever develop into lambs. Almost all the oocytes become atretic and are lost. This represents a loss of potentially valuable progeny in genetically superior ewes. MOET provides a method whereby some of these otherwise wasted oocytes can be used. The successful planning and execution of MOET programmes in sheep are difficult and challenging work for veterinarians. The level of usage of MOET in sheep in Australia remains modest, for several reasons, including:

1. the high cost of the procedures
2. the high variability in the results obtained
3. the difficulty of recognising genetically superior ewes, and
4. a poor understanding of the procedures by flock owners

Clients are usually poorly informed of the underlying biology, the procedures involved and the potential benefits and costs. These issues should be discussed carefully with any enquiring client. You should consider how many donors the client can afford to submit to a programme, and how many donors you or your team can sensibly handle. Depending on the resources available, the embryos from 5-10 donors can be comfortably collected and transferred in one day. To programme less than 5 donor ewes risks too much variation in embryo yield. The standard of general husbandry should be excellent. Donors and recipients should be shorn and managed to be in condition score 22 - 32 and on above-maintenance feed. For the surgical procedures,

clean up the shearing board area thoroughly and ensure that good lighting is available. The apparatus and facilities required and the procedures involved in MOET are examined in the practical classes. The technique involves the collection of early embryos from superovulated donor ewes and their assessment and transfer to synchronized, recipient ewes.

Superovulation of donor ewes

Various gonadotrophins rich in follicle stimulating activity will induce superovulation when given during the latter stages of the luteal phase of the oestrous cycle. PMSG used to be the gonadotrophin most widely used in practice, because of its availability, relative cheapness and ease of use. However, when used alone at doses of 1200-1500 IU, a significant proportion of ewes show ovarian responses characterized by many persistent large follicles which fail to ovulate and relatively few ovulations. Hence the dose response line for ovulations is not linear. Supplementation of PMSG treatment with GnRH or antiserum or monoclonal antibody to PMSG can reduce the incidence of unovulated follicles, but these treatments are rarely used commercially.

Purified FSH of porcine, bovine or ovine origin is also available commercially and is widely used for superovulation. It gives more ovulations and fewer persistent large follicles than PMSG, but its use is more tedious and some ewes fail to exhibit any superovulatory response (regardless of the dose of FSH employed). FSH has a half-life in the sheep of around 2 hours, which is much less than the equivalent interval of about 20 hours for PMSG. PMSG is given by a single intramuscular or subcutaneous injection, whereas FSH, because of its much shorter half-life, has to be given as a series of injections, usually twice daily over 3 consecutive days.

A 'cocktail' of FSH and PMSG seems now to be the preferred superovulatory treatment. In this case, both hormones can be given as single injections at the same time (*eg* 500-800 IU PMSG **plus** 12-20 mg FSH). There seems to be a higher ovulation rate and more transferable embryos than when either FSH or PMSG is used alone at equivalent doses, and, compared to using FSH alone, there is an increase in the percentage of ewes exhibiting a superovulatory response. Some trial and error may be necessary to determine the optimal doses of PMSG and FSH in the 'cocktail'.

In order to effectively programme the transfer operations, it is essential to control the times of oestrus and ovulation in donor ewes, preferably by using progestagen sponges or CIDR for 12-13 days. If PMSG alone or a 'cocktail' of FSH and PMSG is used, it is given 36-48 hours

before removal of the intravaginal device. The ewes should be in oestrus 24-36 hours after sponge removal, and, at least following PMSG or 'cocktail' treatment, the ewes probably ovulate around 42-54 hours after sponge removal. However, several factors appear to influence the timing and spread of ovulations following a superovulatory treatment, and some experience is required in determining the optimal time for insemination. The uncertainty about the time of ovulation is a real problem where frozen semen is employed. There is a good case here for a supplementary GnRH treatment, to reduce the interval from first to last ovulation and to shorten the time elapsing between sponge removal and the median time of ovulation. For example, 20-40 IU GnRH (*eg Fertagyl*, Intervet) are often given at 24 hours after sponge removal. There is probably no need to use GnRH where fresh semen is employed. Suggested optimal times for insemination were listed previously. In the case of fresh semen, inseminations at 24 hours after sponge removal appear to give higher ova recovery rates but slightly lower fertilization rates than do inseminations at 48 hours. Can you suggest why this may be so?

Variation in the superovulatory response

As already noted, even today the variability in ovarian responses (numbers of ovulations and persistent large follicles which fail to ovulate) remains substantial and it is normal for some treated donors to yield no transferable embryos. It is important to realise that in the practice of MOET it is relatively easy for you to master the surgical and manipulative skills required and considerably more difficult to gain precise control over the superovulatory responses. Factors which influence the ovulatory response in donors include:

- The treatment regime; especially the type, batch and dose of gonadotrophin employed and timing of treatments. Variation in the relative amounts of FSH and LH activity in different gonadotrophin preparations is probably a factor here. Inhibitory factors produced by the ovaries (inhibin and other peptides) are presumably involved
- Breed, genotype and age of donor. Merino ewes selected for multiple births and Booroola ewes are more responsive to PMSG than are unselected Merino ewes
- The stage of the oestrous cycle at which the synchronization treatment commences. The ovulation rate is lower when treatment commences mid-cycle (days 8-10) and higher when treatment commences early (days 1-3) or late (days 13-15) in the cycle
- Body weight and condition score of donor. The literature concerning the influence of these factors is not consistent and more investigation is necessary
- Standards of sheep health and flock husbandry
- Season of year (?)
- Adverse environmental conditions (?)

Mating

Fertilization in donors following natural mating or cervical insemination can fail, due to faulty transport of sperm through the cervix, following exposure of the cervix to abnormally high levels of ovarian hormones, principally of oestrogens. The problem is overcome by uterine insemination. It is a good idea to run raddled teasers with the donor ewes, for reasons that were previously outlined.

Embryos

The surgical collection, recovery from the flushing medium, assessment and storage of embryos are examined in the practical classes. Embryos are usually collected 3-7 days after mating. Collections may be done by either (a) backward flushing of the uterine horns and oviducts or (b) flushing from the tip of the uterine horn only to a suitable catheter inserted at the external bifurcation of the horns. The latter approach is less invasive and can be used repeatedly in the same ewe, but it yields lower recovery rates and can be used only after day 4. Balanced salt solutions (*eg* Dulbecco's phosphate buffered saline, pH 7.3) supplemented with 10% sheep serum and antibiotics are commonly used for flushing and storage. Veterinarians usually use complete, commercially prepared media. Fresh medium is used each day and stored at 30EC for use. Embryos should be located in the flushing medium and transferred to fresh medium early after flushing. They can then be stored in the medium at 20-25EC for 3-4 hours. A stereoscopic microscope (magnification 40-60x) is used to locate and assess the embryos in the flushings and fresh medium.

The assessment of embryo viability is done morphologically and is based on general appearance and stage of development. This method is quick, cheap and non-invasive, but subjective and the correlation with viability is certainly less than perfect. In the evaluation, consider size, fragmentation and granulation of blastomeres, symmetry of the cell mass and the appearance of the zona pellucida. There is no substitute for a lot of practice and determination of subsequent lambing rates. Systems for scoring embryos for quality or viability are often used, but are probably of limited value for the inexperienced.

Variation in the numbers of normal embryos recovered from donors

The recoveries from individual donors can range from none to 20 or more. Factors responsible for the substantial variation observed include:

- the superovulatory response (see above)
- premature regression of the corpora lutea arising from superovulatory treatment
- day of collection and site of embryos within the reproductive tract
- method of surgical or non-surgical collection
- interference with ovulation and/or ovum transport at insemination
- whether the ewes have been used previously as donors

Transfers to recipients

Transfers to recipients should be carried out as soon as reasonably possible after collection. The recipients must be in oestrus within 12 hours of their respective donors. Note that this implies that synchronizing intravaginal devices must be removed from recipients about 24 hours before they are removed from donors. Recipients need not be of the same breed as the donors. Mature (BL x Mo) XB ewes make attractive recipients, especially if two embryos are transferred to each recipient. Transfers are best done using a cradle, local anaesthesia and laparoscopy. A uterine horn ipsilateral to an ovary containing a corpus luteum is identified and then about 5 cm of the ovarian end of this horn is exteriorized through the wound made by the trochar for the transfer. There is always debate about the relative merits of transferring one or two good quality embryos per recipient. It seems reasonable to assume that pregnancy rates are higher after the transfer of two embryos, but in this case there may be a lower rate of conversion of embryos into live lambs. Thorough studies of this question are lacking. In commercial practice, decisions are often made 'on the run' and are based on the relative numbers of embryos and recipients available, and the viability scores allotted to embryos. For maximal speed and efficiency in commercial MOET programmes it is best to deposit two embryos per recipient. Some operators using this policy claim 60% survival of transferred embryos. Programme five recipients per donor (this allows effective use of up to 10 embryos per donor). The general approach with retarded embryos is to transfer these last if recipients are available.

It should be self evident that, even more so than in the case of an AI programme, the subsequent feeding and management of the recipients during pregnancy, lambing and lactation should be excellent, in order to optimize lamb birth weights and maximize lamb survival.

Expected results for a MOET programme

The following results should be attainable in a well managed programme, but in practice the average number of progeny per treated donor seems often to be less than 4-6.

Number of ovulations (corpora lutea): 10-15

Recovery rate (ova recovered/no. of: 70-90%

Percentage of recovered ova scored as: 70-90%

Percentage of transferred embryos: 60-80%

Average number of progeny per treated donor**: -6

* Assumes transfer of one or two normal embryos freshly collected from a mature donor; the survival rate will be lower after transfer:

(a) of frozen-stored embryos

(b) of fresh embryos scored as retarded and/or abnormal

(c) of embryos collected from lambs

(d) where the recipient was detected in oestrus more than 12 hours apart from the respective donor.

** This number can be increased to, say, 20 progeny per donor *per annum*, if the same donor is re-programmed 4 times in the same year.

Schedule for the preparation of donor and recipient ewes

At this stage it should be useful revision to construct a calendar which shows the days and times of all treatments and procedures carried out on the donors and recipients in a MOET programme. The finer details of such a calendar will obviously vary, for reasons which have been discussed. Table 8.9 shows a sample calendar.

Storage of embryos

Sheep embryos can be successfully stored frozen in liquid nitrogen, probably for unlimited periods. At best, with present methods, about 70% of embryos survive frozen storage. Hence, in the case of frozen embryos, you could expect about 40-50% to survive to lambs after transfer to synchronized recipients. The techniques used for freezing and thawing are still evolving. Slow and rapid procedures, as well as vitrification, are employed. There is only very limited commercial use of these procedures in Australia, mostly associated with the international transport of sheep embryos under quarantine conditions. Interest is inhibited by the low survival rates and restrictions on the export of Australian Merino embryos.

Table 8.9 : A sample calendar for a MOET programme

Day of programme	Donor ewes	Recipient ewes
0 (Thurs)	insert progestagen sponge or CIDR	insert progestagen sponge or CIDR
11 (Mon)	treat with 1200 IU PMSG or a 'cocktail' of FSH & PMSG	—
12 (Tues, am)	—	remove sponge and treat with 400 IU PMSG; join harnessed teasers
3 (Wed, am)	remove sponge, join harnessed teasers	—
13 (Wed, pm)	isolate from feed and water	—
14 (Thurs, am)	optional GnRH treatment	—
14 (Thurs, am/pm)	oestrus	oestrus
14 (Thurs, pm)	intra-uterine insemination with fresh semen	—
15 (Fri)	intra-uterine insemination with frozen-stored semen, remove teasers	remove teasers
19 (Tues, pm)	isolate from feed and water	isolate from feed and water
20 (Wed)	collect and evaluate Day 6 embryos	transfer one (or two?) normal embryo 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 real-time ultrasound (equals days 53-55, if pregnant)
160	—	pregnant ewes will all lamb over the next 1-7 days (if lambs are Merinos); review management of lambing

Some other technologies in sheep related to AI and MOET

These technologies generally remain somewhat experimental and so far have found little or no commercial application. Nevertheless, some promise exciting developments in sheep breeding. The technologies include the following.

***In vitro* culture of embryos**

Success depends on the medium used, stage of embryo at commencement of culture and duration of culture. For example, the viability of day 5 embryos cultured *in vitro* from the zygote stage is now quite good for some flocks, but is always poorer than for control embryos cultured *in vivo*. How well do available media and incubation

conditions simulate *in vivo* conditions? Co-culture with oviductal or uterine epithelial cells can improve the results obtained. Embryos can also be cultured *in vivo*, in ligated rabbit or sheep oviducts.

***In vitro* maturation and fertilization of oocytes (IVM and IVF)**

This requires optimal *in vitro* conditions for the maturation of follicular oocytes, capacitation of spermatozoa and fertilization. There is an enormous potential for IVF of oocytes collected from abattoir material. There has been much recent progress in developing these methodologies.

Insertion of desirable transgenes

Genes regulating fecundity, wool growth and other production traits are of interest. The insertions are usually made into zygotes at the pronuclear stage.

Embryo splitting

Embryo splitting is best carried out on late morulae and blastocysts (days 6-10). The split late morulae or blastocysts should develop directly in final recipients without a zona pellucida. This procedure potentially enables an increase in the numbers of offspring in an MOET programme. The embryos are split with a fine glass needle or blade. There is a strict limit to how many times splitting can be repeated. The embryonic anti-luteolytic signal in recipients will be diluted, so it is wise to transfer in pairs to increase the strength of the signal. If embryos are split into portions of less than 3 embryo, trophoblastic vesicles (lacking an inner cell mass) rather than normal embryos are likely to develop.

Nuclear transfer and clone formation

Morulae (16-32 cells) are used as nuclei donors for cloning. Alternatively, embryonic stem cells in suitable culture potentially represent an inexhaustible source of genetic material for nuclear transfer cloning. Clone formation requires good techniques for the maturation and enucleation of oocytes, the fusion of nuclei with anucleated oocytes and *in vitro* culture of the resulting embryos to the late morula/blastocyst stage. 'Electrofusion' is used to activate the oocyte during nuclear transplantation. Culture of the resulting embryos in ligated sheep oviducts may be preferable to *in vitro* culture.

Semen sexing and ICSI

The possibility of separating X- and Y-chromosome bearing spermatozoa has long tantalized animal science researchers. The production of sexed semen on a scale sufficient for normal AI still is remote, but the successful separation of relatively small numbers of spermatozoa that can be used for IVF has been achieved in a number of species.

At present, there is a very low yield of correctly sexed cells from the flow cytometric sorting process used to separate the X and Y sperm. Fertilisation of *in vitro* matured oocytes by injection of a single spermatozoon (intracytoplasmic sperm injection or ICSI) is a method for producing viable embryos with a very small number of sperm. This technique has been widely used in human assisted reproduction but only a small number of offspring has been produced by ICSI in sheep.

Embryo sexing

The available methods include cytogenetic analysis, male-specific (H-Y) antigens and Y-specific molecular probes. In practice, hybridization using Y-specific DNA sequences and 8-10 biopsied trophoblast cells seems to be the method of choice. There are significant problems of cost, loss of viability of embryos after biopsy, and inaccuracy. The scope for commercial application of embryo sexing in sheep is questionable. It will halve the efficiency of existing MOET procedures, assuming embryos of only one sex are required.

Juvenile *In Vitro* Embryo Technology (JIVET)

The production of offspring from very young pre-pubertal ewes has the potential to increase genetic gain by reducing the generation interval. Although still under experimental development, large numbers of viable embryos have been produced from superovulated lambs as young as 5 weeks of age. If commercially applied, JIVET has the potential to not only reduce generation intervals but also dramatically increase the efficiency of MOET programs in sheep and cattle.

General references

Cognie Y (1990) *Current technologies for synchronization and artificial insemination of sheep*; In *Reproductive Physiology of Merino Sheep* Eds CM Oldham GB Martin and IW Purvis, University of Western Australia, p 207

Evans G and Maxwell WMC (1987) *Salamon's Artificial Insemination of Sheep and Goats* Butterworths, Sydney

Geldard H (1984) *Field evaluation of Fecundin: an immunogen against androstenedione* Proc Aust Soc Anim Prod **15** 185

Gordon I (1983) *Controlled Breeding in Farm Animals* Pergamon Press, Oxford

Haresign W (ed) (1983) *Sheep Production* Butterworths, London

Holt NA (1988) *Semen collection and storage AI programs and embryo transfer* In *Sheep Health and Production* Proc 110 Post Graduate Committee in Veterinary Science, p 517

Hunter RHF (1980) *Physiology and technology of Reproduction in Female Domestic Animals* Academic Press, London

Killeen ID (1983) *Artificial insemination and synchronization of oestrus* In *Sheep Production and Preventive Medicine* Proc 67 Post Graduate Committee in Veterinary Science, p 302

Lindsay DR and Pearce DT (eds) (1984) *Reproduction in Sheep* Australian Academy of Science, Canberra

Maxwell WMC, Szell A, Hunton JR and Ryan JP (1990) *Artificial breeding: embryo transfer and cloning* In *Reproductive Physiology of Merino Sheep* Eds CM Oldham GB Martin and IW Purvis University of Western Australia, p 217

Miller SJ (1981) *Artificial breeding techniques in sheep* In *Sheep* Proc 58 Post Graduate Committee in Veterinary Science, p 71

Signoret JP (1990) *The influence of the ram effect on the breeding activity of ewes and its underlying physiology* In *Reproductive Physiology of Merino Sheep* Eds CM Oldham, GB Martin and IW Purvis University of Western Australia, p 59

Tomes GL Robertson DE and Lightfoot RJ (eds) (1979) *Sheep Breeding* Butterworths, London

[1] Booroolas are a strain of medium-wool Merino with very high lambing rates due to the effect of a major gene - the Fec^B gene

VEIN

Partners:



University of Sydney
Faculty of Veterinary
Science



Post Graduate Foundation in
Veterinary Science of the
University of Sydney



University of Sydney
Library



Veterinary Science
Foundation of the
University of Sydney

Top

[Contact](#) | [Feedback](#) | [Site Map](#)

[Disclaimer](#) | [Copyright](#) © University of Sydney 2006

Last Modified: Wednesday 28 July, 2004

Contact: vein@library.usyd.edu.au



About CU Academic Admin Admissions Student Affairs People

EMBRYO TRANSFER HOME PAGE

- [Records of Current Embryo Transfer Project](#)
- [Records of Embryo Transfer from 1996/97](#)

Dr. Bennett's
Presentation

Project
Summary

Press Release
12/1/97

Links Page

Embryo Transfer is the process of removing embryos from a superior cow and placing them in a surrogate cow where they develop into a calf. The process is comparable to artificial insemination, which uses superior sperm to reproduce quality bulls. By combining the two processes, a quality herd can be developed in a short period of time. At Cameron University, we are using the process to help teach an animal reproduction course. Information and materials about the process are being developed to expand the knowledge of students and professionals alike.

The first step in the process is the [superovulation](#) of the superior cow, also referred to as the *donor cow*. A follicle stimulating hormone (FSH) is injected into the cow 40 hours before the cow reaches estrus. The hormone induces production of a large quantity (#) of embryos, which will be [flushed](#) out of the cow.

At the same time, the *recipient cows* are [synchronized](#). They are injected with leutelase, a hormone that regulates the estrus cycle. This will insure the cows are synchronized with each other, and with the donor cows. It is very important that the estrus cycles be at the same point for a successful transfer to take place. [Estrus detection](#) is crucial to the process.

When the *recipient cows* are synchronized with the *donor cows*, and the embryos are in the 250 cell stage, the embryos are *flushed* out of the donor cows. A catheter is inserted through the cervix into the uterus. A saline solution is then used to remove the embryos from the lining of the uterus. As the solution drains out of the cow, it is collected and the embryos are removed.

The embryos are then [processed](#). They are taken from the saline solution and examined under a microscope. They can then be frozen for later use, or immediately transferred to a waiting *recipient cow*. The embryos can also be *split*, which consists of dividing the viable embryo into two halves. Each half can then be frozen or placed into a cow.

The embryos are *implanted* into the cows using a technique similar to artificial insemination. A tube containing the embryo is inserted into the uterus, then the embryo is pushed out. If all goes well, the embryo will attach to the lining of the uterus, and a normal pregnancy will continue from that point.

The cows are watched until their next estrus cycle. If the cows do not come into estrus, it is fairly certain the process was successful and they are pregnant. If they do go into estrus, the first attempt was not successful. They can then be re-implanted with superior embryos, or bred using their own.

More information about [history of embryo transfer](#) is available [here](#).

[About CU](#) [Academic](#) [Admin](#) [Admissions](#) [Student Affairs](#) [People](#)

Page maintained by [Julie A. Duncan](#), [Information Technology Services](#)

Copyright 2003 [Cameron University](#)

Last modified: 01/29/01

Department of Agriculture

Search

Contact CU

News @ CU

Cameron Wel

About CU Academic Admin Admissions Student Affairs People

Estrus Detection

There are several methods of estrus (heat) detection. The first and most obvious is to be observant. Observation is a necessity in effective cattle production. Even more effective methods are available including heat mount detectors (the one we are using for our project), chin ball markers, and tail chalking techniques.

[Heat mount detectors](#) are devices attached to the posterior side of the cow just above the tailhead. They are sensitive to the pressure of another animal mounting and pressing against the detector. The detector then sends a signal to a computer where the mount is recorded.



This method can be a valuable tool for detection, but may have limitations for some breeding operations. Therefore, a thorough investigation of this program should be accomplished; it is expensive and may not be suited for some operations.

Chin ball markers are another effective method of estrus detection. Chin ball markers are attached to the chin of the animal that will be doing the mounting. The markers are large ball-point markers mounted in the halter-like device. The marker rubs on the back of the animal leaving a distinct mark. The animals doing the mounting are referred to as "mounting animals." Mounting animals are commonly bulls that have been surgically altered to prevent penis insertion or steers that have been androgenized to increase sexual desire.

Tail chalking is another way to detect the estrus cycle. This method is limited by the fact that it takes a trained eye to interpret the marks. The marks need to be applied every day to maintain a readable line. The line is rubbed on by a person working with the animals and is applied from the cow's hooks to pins. In the summer a thin band is applied and in the winter a thicker band is applied. Animals riding the cow will rub the chalk lines off or smear them. It takes a trained eye to tell if riding has smeared the chalk lines or if they have been licked off, or lost from other means.

There are many other ways to detect the estrus (heat) cycle. In any case the combined use of observation, good herd records, and an electronic detection system will bring about more effective breeding practices.



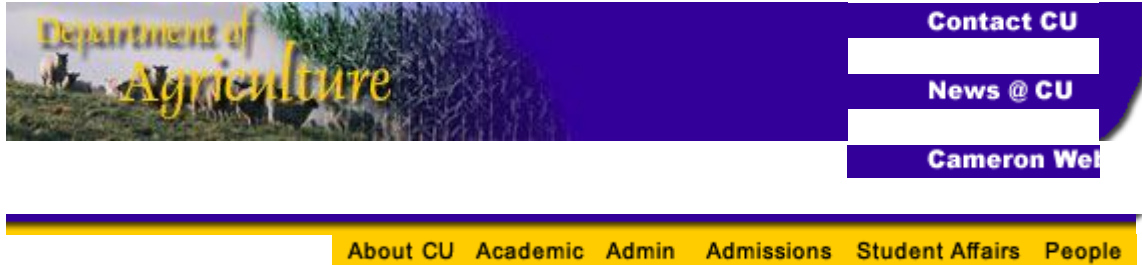
RETURN TO [EMBRYO TRANSFER PAGE](#)

[About CU](#) [Academic](#) [Admin](#) [Admissions](#) [Student Affairs](#) [People](#)

Page maintained by [Julie A. Duncan](#), [Information Technology Services](#)

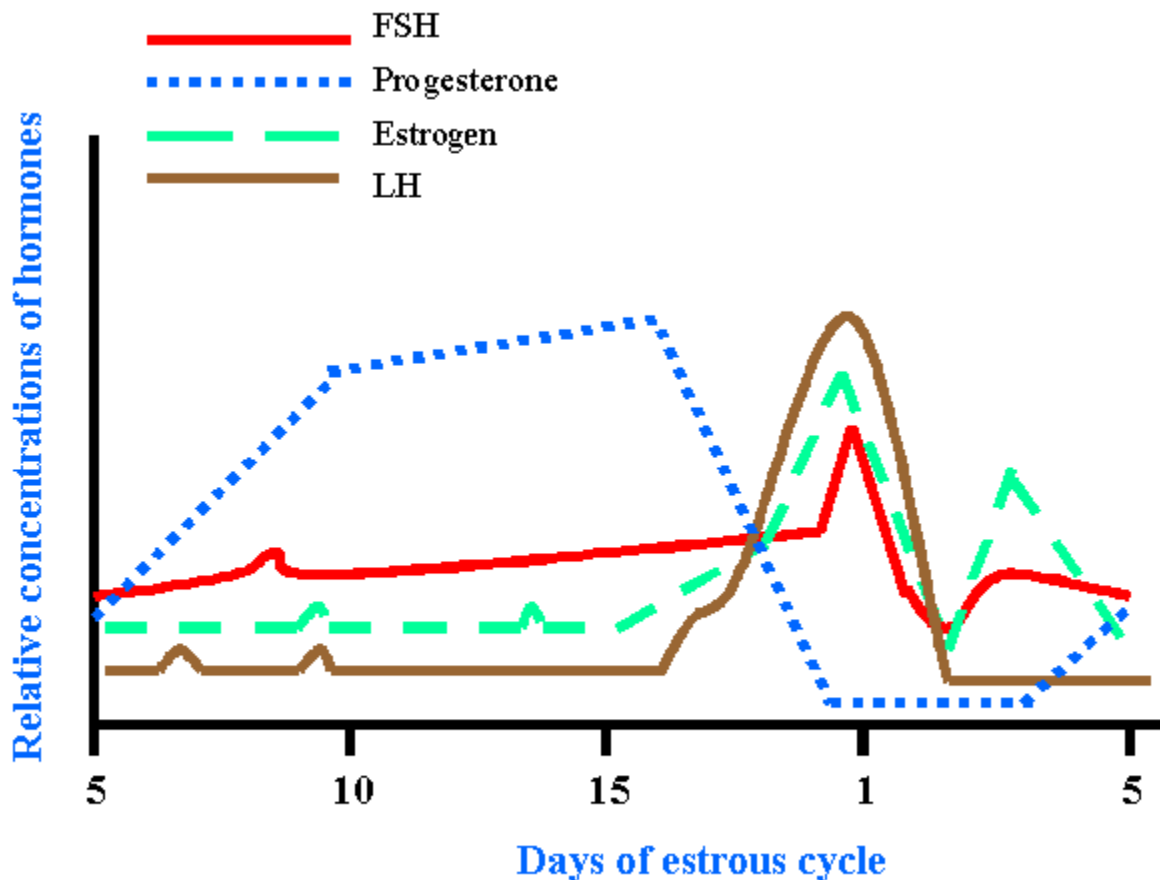
Copyright 2003 [Cameron University](#)

Last modified: 01/29/01



Synchronization

Synchronization is the procedure of getting the recipient cows into the same stage of estrus as the donor cows. Synchronization of recipient cows should be done while superovulating the donor cows. The way to accomplish this is while administering the first injection of FSH on the third day to the donor cows, inject the recipient cows with 25 mg of prostaglandin. The recipient cows should come into estrus about 36 to 72 hours later, approximately the same time as the donor cows come into estrus. Prostaglandin should only be administered to females that are between day 5 and day 17 of their estrous cycle.



The above chart shows the relative concentration of hormones during the estrous cycle. The injection of synchronizing hormones must be carefully timed so that each cow is prepared for the flushing and transfer of embryos at the same date.

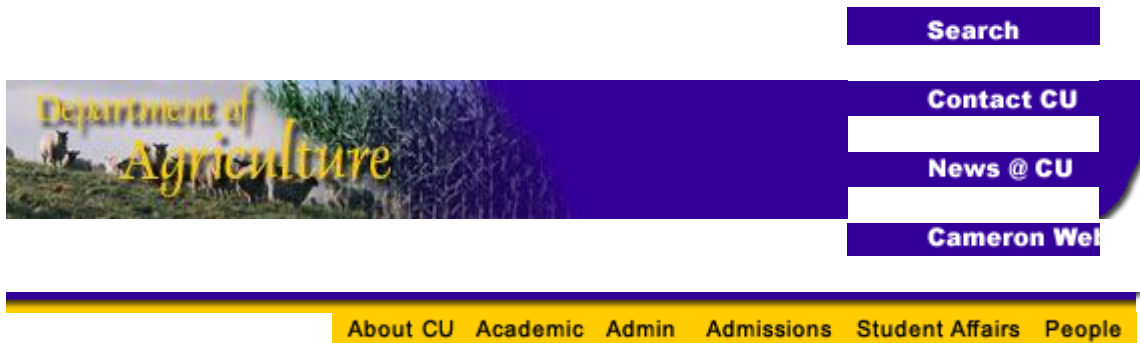
Return to [EMBRYO TRANSFER PAGE](#)

[About CU](#) [Academic](#) [Admin](#) [Admissions](#) [Student Affairs](#) [People](#)

Page maintained by [Julie A. Duncan](#), [Information Technology Services](#)

Copyright 2003 [Cameron University](#)

Last modified: 01/29/01



Superovulation

Before beginning the actual process of superovulation some background information about the hormones of estrus must be presented. There are five major hormones that will be discussed: FSH, LH, estrogen, progesterone, and prostaglandin.

The first hormone we will discuss is the follicle stimulating hormone or FSH. FSH is released by the pituitary gland. FSH promotes the growth of the Graafian follicle on the mature ovary of a female. FSH also plays a role in the production of estrogen.

The second hormone is known as the luteinizing hormone or LH. LH is also released by the pituitary gland. LH causes the release of the egg contained in the Graafian follicle (ovulation) and initiates the formation of the corpus luteum. The corpus luteum is a yellowish colored body located on the wall of the ovary.

When the Graafian follicle breaks open and releases the ovum, it also releases the hormone estrogen. Estrogen promotes the onset of what is known as heat, the physical signs of estrus. Estrogen also stimulates the vascular tissue lining the walls of the uterus causing the "fold curtain" affect. The fold curtain affect increases the tissue size and creates wrinkles in the tissue to catch the fertilized zygote after conception. The vascular tissue is also the immediate nutritional source for the fertilized zygote.

The corpus luteum releases the fourth hormone progesterone, which is responsible for the maintenance of pregnancy. The corpus luteum releases this hormone 10-15 days after its formation. If a zygote is not present by the 10-15 day after the heat period the uterus will secrete the estrus ending hormone - prostaglandin. The final hormone prostaglandin will cause the corpus luteum to regress and begin the estrus cycle all over again.

The actual superovulation should be done on any day of the estrous cycle between day 6 and day 15. There are a couple different ways to superovulate females.

1. Two times daily inject the female with 5 mg of FSH for 4 to 5 days. Estrus is then induced on the third day by injecting 25 mg of prostaglandin at the same time as the sixth shot of FSH. Estrus should occur on the fifth day, if not, continue FSH injections through the day 5. The ovaries should be palpated rectally everyday to

- make sure that overstimulation does not occur. Overstimulation can lead to irreversible damage to the ovaries.
2. Once again the regimen calls for two shots daily of FSH, but instead of 5mg per injection the dosage decreases by 1 mg daily (day 1-5 mg, day 2-4mg, etc.). This is the method we used. Once again on the third day a 25 mg shot of prostaglandin is administered with the sixth shot of FSH. Estrus should occur on the fifth day, if not, continue FSH injections through day 5.

Artificial insemination should take place 4 - 22 hours after the onset of estrus.

Return to [EMBRYO TRANSFER HOME PAGE](#)

[About CU](#) [Academic](#) [Admin](#) [Admissions](#) [Student Affairs](#) [People](#)

Page maintained by [Julie A. Duncan](#), [Information Technology Services](#)

Copyright 2003 [Cameron University](#)

Last modified: 01/29/01

Department of Agriculture

Search

Contact CU

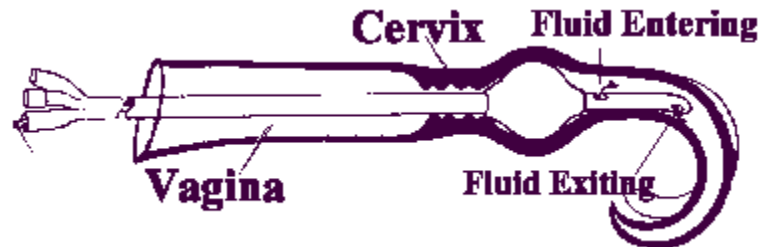
News @ CU

Cameron Wel

About CU Academic Admin Admissions Student Affairs People

Flushing

Removal of embryos from the donor cows is one of the most critical aspects of a successful embryo transfer program. The original means of embryo removal involved surgery, which sometimes meant the sacrifice of the donor cow. Today a safer more effective way to remove embryos is flushing. Flushing is the method of removing the embryos with as little intrusion as possible. The popular method of flushing is the use of what is known as a French Foley catheter (two-way flow catheter).



The French Foley catheter is a device that simultaneously allows flushing fluid to be passed into the uterus and out into a collecting receptacle. This is possible because there are two different tubes inside the catheter. The first tube that pumps the flushing fluid into the uterus is located close to what is known as the balloon of the catheter. The other tube that pulls the flushing fluid out the uterus terminates at the end of the catheter.

The actual process is done by inserting the catheter into the uterus through the cervix, inflating the balloon to prevent expulsion, and the introduction of fluid. The vacuum tube is held clamped shut until the uterus becomes turgid (hard due to the flushing fluid). The vacuum tube is then opened to allow the flushing fluid and embryos to pass out of the uterus. The microscopic embryos are then filtered out of the flushing fluid by the collecting receptacle.



Once the flushing is complete the donor cows are given injections of antibiotics and prostaglandin. The antibiotics are used to prevent infection, and the prostaglandin prevents pregnancy if all embryos were not removed. The embryos are then removed from the collecting receptacle for processing.

Return to [EMBRYO TRANSFER HOME PAGE](#)

[About CU](#) [Academic](#) [Admin](#) [Admissions](#) [Student Affairs](#) [People](#)

Page maintained by [Julie A. Duncan, Information Technology Services](#)

Copyright 2003 [Cameron University](#)

Last modified: 01/29/01

Department of Agriculture

Search

Contact CU

News @ CU

Cameron Wel

About CU Academic Admin Admissions Student Affairs People

Processing Of Embryos

Once the embryos have been removed from the donor cow the evaluation process begins. The solution containing the embryos is placed into a divided petri dish to locate the embryos using a light microscope. The microscopic inspection serves a two-fold purpose: 1) determining the stage of development of the embryo and 2) evaluating the embryo by using some basic criteria.



The embryos should be recovered from the donor cows in the morula or early blastocyst stage of development. These stages of development are usually between 6 to 8 days following fertilization. If the embryos removed are not quite to the morula or blastocyst stage of development they can be grown in a culture *in vitro* till they reach the blastocyst stage. The recovered embryos should also be evaluated for possible abnormalities including:

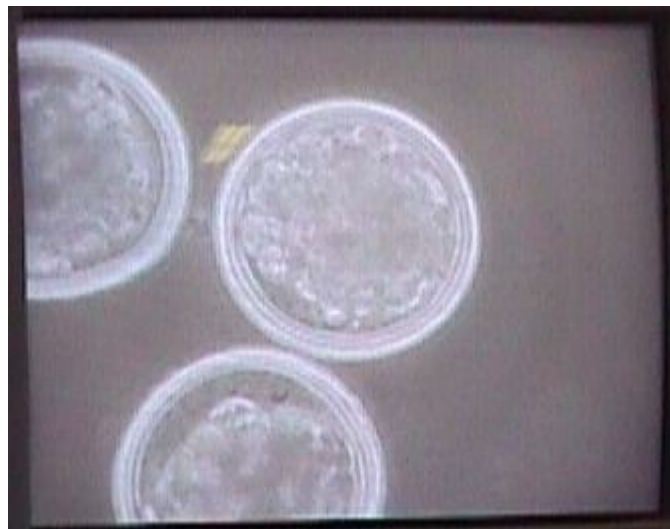


- compactness of cells – a normal embryo is compact rather than a loose mass of cells
- regularity of shape – spherical as opposed to oval
- variation in cell size – blastomeres of similar size
- color and texture of cytoplasm – should be neither very light or very dark
- presence of vesicles – cytoplasm should not be granular or unevenly distributed and should contain some moderate sized vesicles.

- normal embryo size
- presence of extruded cells – there should be no disassociated cells
- regularity of the zona pellucida
- presence of cellular debris – no cell fragments should be visible around the blastomeres.

These few guidelines for embryo evaluation should greatly improve the success rate of embryo transfer. Once the embryos have been evaluated they can either be frozen or put directly into a recipient cow.

If the embryos are to be transferred immediately to a recipient cow the embryos should survive in the flushing media for 2 hours. The embryos must be transferred to recipients that have had their estrus cycles synchronized with the donor. The embryos are placed into a "straw" which has been described for packaging semen. The embryo is then inserted in much the same way as semen is during artificial insemination.



If the embryos are to be frozen they should be placed in 1.5 M (molar) ethylene glycol in the center column of a 0.25 ml straw with a column of phosphobuffered saline on the other end of the straw. The columns are separated by an air bubble. The recommended ratio of ethylene glycol to phosphobuffered saline in the straw is 1:3. The freezing process is as follows:

1. Prepared straw is placed directly into an alcohol bath freezer (-7°C).
2. The straw is "seeded" by touching a metal rod, cooled by immersion in liquid nitrogen, to the straw at a location away from the embryo.
3. After a 5-minute hold, the prepared straw is cooled at 0.5°C per minute to -35°C .
4. The straw is held for 15 minutes and then plunged into liquid nitrogen.

The embryos can be stored in liquid nitrogen almost indefinitely.

Once a donor cow is chosen the embryo can be thawed by placing the straw in a 30°C water bath and then transferred to the recipient cow.



RETURN TO [EMBRYO TRANSFER PAGE](#)

--Some information on this page was found in *Applied Animal Reproduction*, Fourth Edition, H. Joe Bearden and John W. Fuquay from Mississippi State University

[About CU](#) [Academic](#) [Admin](#) [Admissions](#) [Student Affairs](#) [People](#)

Page maintained by [Julie A. Duncan, Information Technology Services](#)

Copyright 2003 [Cameron University](#)

Last modified: 01/29/01

Flock projection: Sheep

Item	Unit	PY1	PY2	PY3	PY4	PY5	
<u>Technical coefficients</u>							
Land area	ha	20	20	20	20	20	
Stocking rate	no/ha	40	40	40	40	40	
Joining ratio	ewe/ram	20	20	20	20	20	
Ewe replacement rate	%	20	20	20	20	20	
Ram replacement rate	%	50	50	50	50	50	
Lambing rate	%	95	95	95	95	95	
Lambing interval	yr	0.5	0.5	0.5	0.5	0.5	
Avg. litter size	no.	1.2	1.2	1.2	1.2	1.2	
Weaning rate	%	80	80	80	80	80	
Postweaning mortality	%	2	2	2	2	2	
<u>Flock projection</u>							
Breeding ewes	no.	240	240	240	240	240	
Breeding rams	no.	48	48	48	48	48	
Lambs born	no.	547	547	547	547	547	
Lambs weaned	no.	438	438	438	438	438	
Male lambs (3mths-1 yr)	no.	215	215	215	215	215	
Female lambs (3mths-1 yr)	no.	215	215	215	215	215	
Rams (1-2 yrs)	no.	0	186	186	186	186	
Ewes (1-2 yrs)	no.	0	162	162	162	162	
Culled rams	no.	24	24	24	24	24	
Culled ewes	no.	48	48	48	48	48	
			420				

Herd projection: Pig

Item	Unit	2007	2008	2009	2010
<u>Coefficients</u>					
Farrowings per yr	No.	2	2	2	2
Avg. litter size	No.	8	8	8	8
Pre-weaning mortality	%	20	20	20	20
Avg. Weaning wt.	kg	6	6	6	6
Post-weaning mortality	%	5	5	5	5
Yearling Wt	kg	120	120	120	120
Boar joining rate	%	40	40	40	40
Breeding sow increase rate	%	40	40	40	40
Replacement rate for b/sows	%	25	25	25	25
Replacement rate for b/boars	%	50	50	50	50
<u>Herd structure</u>					
No. of breeding sows		10	12	13	15
No. of breeding boars		4	5	5	6
No. of piglets born		160	184	212	243
No. of piglets weaned		128	147	169	195
Yearling males		61	70	80	92
Yearling females		61	70	80	92
Culled males		2	2	3	3
Culled females		3	3	3	4
No. of males for sale		55	63	73	84
No. of females for sale		62	71	82	94

Flock projection: Sheep II

Technical coefficients	Unit		year 1	year 2	year 3	year4
land area	ha		100	100	100	100
stocking density	sheep/ha		20	20	20	20
parturition interval	per year		1	1	1	1
lambing rate	%		85	85	85	85
Av litter size	no		1.2	1.2	1.2	1.2
weaning rate	%		75	75	75	75
joining rate	%		5	5	5	5
post weaning mortality	%		2	2	2	2
culling rate of rams	%		50	50	50	50
culling rate of ewes	%		20	20	20	20
Flock Projection						
Breeding ewes	no		600	600	600	600
Breeding rams	no		30	30	30	30
No of lambs born	no		612	612	612	612
No of lambs weaned	no		459	459	459	459
Yearling rams	no		210	210	210	210
Yearling ewes	no		105	105	105	105
culled rams	no		15	15	15	15
culled ewes	no		120	120	120	120
		unit				
		price				
sales						
culled rams	Naira	7000	105,000.00	105,000.00	105,000.00	105,000.00
culled ewes	Naira	3000	360,000.00	360,000.00	360,000.00	360,000.00
yearling rams	Naira	5000	1,049,550.00	1,049,550.00	1,049,550.00	1,049,550.00
yearling ewes	Naira	6000	629,460.00	629,460.00	629,460.00	629,460.00
gross income	naira		2,144,010.00	2,144,010.00	2,144,010.00	2,144,010.00