

**RECENT DEVELOPMENTS IN
ARTIFICIAL BREEDING OF ANIMALS**

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Since the domestication of animals, attempts have been made to improve their productivity by selection. Using conventional selection methods annual genetic gains of 1.5 to 3 percent are possible in various farm species (Smith 1984). Such rates of genetic change can be sustained in practice over many generations and are both cumulative and permanent.

More recently the efficiency of selection has been increased with the aid of techniques for manipulation of reproduction. Artificial insemination (AI) is probably the best example of a technique for increasing efficiency on the male side, and embryo transfer can improve efficiency on the female side.

Other new techniques for the control of the reproductive process include synchronisation of oestrus, semen storage (both chilled and frozen), embryo storage, embryo division (splitting), embryo aggregation (synthesis of chimeras), and removal or injection of parts of embryos (cloning and gene transfer). Methods for synchronisation of oestrus, semen storage, artificial insemination and embryo transfer are well established for the domesticated species, but the other techniques have been utilised only to a limited extent. This paper will attempt to give a brief description of some of these techniques, with particular reference to their application in sheep breeding.

SYNCHRONISATION OF OESTRUS

Extensive research has been done on the control of oestrus and ovulation in domesticated animals. The methods used may be divided into two categories: 1. those involving management practices, and 2. those involving the use of exogenous hormone treatment.

Management practices generally involve the use of nutritional or environmental manipulations (e.g. the nutritional "flushing" of ewes and the batch weaning of piglets from sows) or the use of "teasing" by males. An example of the latter method is the recent widespread use of the "ram effect" for synchronisation of oestrus in sheep for AI in Western Australia (Pearce and Oldham 1984).

The use of exogenous hormones for synchronisation of oestrus is generally based either on the shortening of the luteal phase of the oestrous cycle during the breeding season (e.g. the use of prostaglandins in cattle and sheep), or the suppression of cyclic activity in all animals by treatment over a number of days with an agent which is either orally active (e.g. altrenogest in pigs, Webel and Day 1982) or continuously absorbed from an implanted source (e.g. the progestagen-impregnated intravaginal sponge in sheep, Robinson 1965). Such hormone treatments have led to the development of

highly accurate systems for synchronisation of oestrus in cattle and sheep. In sheep, for example, the treatment of ewes with progestagen sponges for 12-14 days combined with an injection of serum gonadotrophin (PMSG) at sponge removal, provides sufficient synchrony of oestrus and ovulation to allow a fixed-time insemination to be performed without the need for oestrus detection (Maxwell *et al.* 1984).

ARTIFICIAL INSEMINATION AND SEMEN STORAGE

Practical systems for the AI of pigs, cattle and sheep have been available for many years. Many millions of cattle and pigs are inseminated throughout the world every year, the majority of cattle with frozen semen whereas most pigs are inseminated with fresh or chilled semen. Commercial AI in pigs and cattle is generally carried out as part of a full insemination service (with private or government organisations providing both semen and a trained inseminator), a semen delivery service with AI carried out by the producer (as is used for pigs in Britain and North America) and to a lesser extent nowadays an on-farm programme, where semen collection, processing and insemination is performed by the producer with, or without, technical assistance.

The latter method has also traditionally been used with sheep in Australia. Recently, however, there has been a movement away from on-farm AI using 'home-grown' rams, to the use of 'top' sires in studs or commercial artificial breeding (AB) centres, or in nucleus type group breeding schemes. Another recent application of AB in sheep has been the establishment of sire referencing schemes (SRS). SRS aim to provide a means for accurate identification of sires of high breeding value across studs. All of these developments have been associated with an improvement in the technology of sheep AI, particularly in regard to the use of frozen semen.

During the 1960's and early 1970's there were many reports on successful AI of sheep with frozen ram semen (reviewed by Maxwell 1984), and ram semen has remained viable following storage for long periods in liquid nitrogen (Salamon 1980). However, the procedure of freezing and thawing ram semen inevitably reduces the motility and viability of spermatozoa, and hence fertility following cervical insemination with frozen semen tends to be considerably lower than after using fresh or chilled semen (Maxwell *et al.* 1980). The major obstacles to higher fertility have been the establishment of a large enough population of viable spermatozoa in the cervix and impaired sperm transport from the cervix through the genital tract of the ewe (Lightfoot and Salamon 1970). A number of attempts were made to overcome the problem of transport of frozen-thawed ram spermatozoa by bypassing the cervix, and placing semen directly in the uterus. The most successful method (Killeen and Caffery 1982) utilises a rapid laparoscopic location of the uterus, and direct injection of semen into the uterine horns from a fine pipette passed through a canula in the abdominal wall. Recent work has confirmed that the use of the technique with frozen semen is as effective as cervical insemination of fresh semen or natural mating over one synchronised oestrous cycle (Maxwell 1984).

The use of the laparoscopic insemination technique has contributed to an expansion of sheep AI in the stud Merino industry over the past three years. A survey of licenced AB centres indicates that some 40-50,000 ewes were inseminated commercially with frozen semen by laparoscopy during the 1984/85 breeding season. The benefit to the industry of this new technology

now depends on accurate identification of genetically superior sires, and their use in programmes designed to achieve long term genetic gain.

EMBRYO TRANSFER AND STORAGE

Multiple ovulation and embryo transfer (MOET) can allow a doubling of the rate of change in growth rates in beef cattle (Land and Hill 1975) and faster rates of change in dairy cattle (Nicholas and Smith 1983). Applications of embryo transfer in pigs and sheep have so far been on a relatively small scale. The high fecundity and reproductive rate of pigs compared with cattle and sheep does not provide the same economic incentive to apply the techniques for the purpose of genetic improvement. There was a flurry of MOET activity directed towards the multiplication of numbers of Angora goats during the early 1980's which may be repeated for the improvement of Cashmere goat strains. However very little use has been made of MOET in sheep breeding.

Surgical methods for embryo collection and transfer in pigs, sheep and goats, together with non-surgical techniques for cattle, are well advanced (Seidel and Seidel 1981). Treatment regimes for superovulation vary according to species, but most embryo transfer donors are treated with pregnant mare's serum gonadotrophin (PMSG) or follicle stimulating hormone (FSH) to induce the maturation and ovulation of a larger than normal number of ova. Variability of response to superovulation remains one of the major problems of embryo transfer in all species. It is still not possible to accurately predict the degree of success for an individual animal.

Following mating or artificial insemination, embryos are generally collected from donors some time between fertilisation and implantation, usually after migration to the uterus, some 3-7 days after oestrus. For specialised applications, such as in in vitro fertilisation or embryo freezing, the time of collection must be very precise. Embryos representing 50-80% of the ovulations can be recovered nonsurgically in cattle (Betteridge 1977), and the technique can be repeated an unlimited number of times without reducing subsequent fertility (Elsden *et al.* 1976). Surgical recovery is the method of choice in sheep, goats and pigs as it is difficult to pass a collection device through the cervix into the uterus in these species. Embryos in early stages of development (1-3 days after oestrus in cattle, goats and sheep, and 1-2 days in pigs) must be deposited surgically in the oviducts, and this may result in lower fertility than the transfer of older embryos to the uterus (Betteridge 1977, Moore 1977). Uterine transfers may be performed surgically or nonsurgically in cattle, and until recently only surgically in sheep, goats and pigs.

Nonsurgical embryo transfer in cattle is an adaptation of the AI technique. The embryo is introduced into the uterus through the cervix in a plastic straw using an inseminating gun (Brand and Drost 1977). The development of methods for intrauterine insemination for use with frozen semen in sheep has also stimulated work on the use of laparoscopy for nonsurgical transfer of sheep embryos (Mutiga and Baker 1984) and even the collection of embryos from ewes by laparoscopy (McKelvey and Robinson 1984a). Recent results from the transfer of sheep embryos by laparoscopy are presented in Table 1.

From our findings it appears that the transfer of sheep embryos by laparoscopy is as efficient as the surgical technique. The method requires

less time to perform (approximately 3 minutes per ewe) and is less traumatic.

Table 1. Recent studies in which two ovine embryos were transferred non-surgically to one uterine horn in each recipient.

Authors	Age of embryos (days)	Method of transfer	No. embryos transferred	proportion developing(%)
Mutiga and Baker (1984)	not stated	laparoscopy	6	66.7
McKelvey and Robinson (1984b)	4	laparoscopy	12	25.0
Schiewe <u>et al.</u> (1984)	5-6	laparotomy laparoscopy	6 6	16.6 50.0
Walker <u>et al.</u> (1984)	4	laparoscopy	108	32.4
J.P. Ryan, J.R. Hunton and W.M.C. Maxwell (1984, unpublished)	6	laparotomy laparoscopy	86 59	59.3 45.8

The benefits which have accrued from frozen storage of spermatozoa of several species of farm animals have been well documented. Equal, and perhaps greater benefits result from the frozen storage of embryos. The ability to freeze and store embryos in liquid nitrogen for long periods has greatly improved the use and application of embryo transfer in cattle. So far, pig embryos have not been frozen and thawed successfully. This appears to be a consequence of the higher lipid content of the cells of pig embryos than embryos from other livestock species. Methods for the frozen storage of mouse embryos were first developed in the United Kingdom with continued survival of frozen-thawed embryos following transfer to recipients (Whittingham 1974). Since then there have been several reports of frozen storage of sheep (Willadsen et al. 1980), goat (Bilton and Moore 1976a) and cattle embryos (Bilton and Moore 1976b). Embryo survival, expressed relative to the number of embryos frozen, is generally around 30 to 40% (Tervit 1983).

Current research on the storage of sheep embryos is directed towards the use of plastic mini-straws, the same as used for storing cattle semen, simplification of the method for addition and removal of cryoprotective agents, and simplifying methods for cooling and freezing. It is now possible to rapidly cool cattle embryos in straws to subzero holding temperatures before direct plunging into liquid nitrogen for storage (Bui-xuan-nhuyen et al. 1984). For transfer, the straw can be rapidly thawed at 37°C, and the embryos placed directly into the uterus in a similar manner to AI (Massip and van der Zwalmen 1984). The development of similar freezing and thawing methods for sheep embryos, combined with the laparoscopic transfer method, will enable the practical utilisation of frozen-thawed sheep embryos on-farm.

EMBRYO SPLITTING AND CLONING

The technique of continued replication, or cloning, of an individual has been developed and used in amphibia (Gurdon 1976). Recently, a similar method was used to produce identical mice (Illmensee and Hoppe 1981, McGrath

and Solter 1983). These techniques involve the development of an embryo after introduction of a foreign diploid nucleus. The egg's own pronuclei (within the nucleus) are replaced microsurgically with a new diploid nucleus from, for example, a body cell. This new nucleus takes over the "running" of the embryo to which it transcribes its genotype. Theoretically, mass production of identical multiples may be generated from one animal by this means. However, there are no reports of the successful application of this technique in domesticated animals.

Identical or monozygotic twins are in fact clones, being derived from an embryo split early in development to yield two "carbon copies". Such twins appear naturally in many species. The artificial production of monozygotic sheep and cattle twins has been attempted with considerable success. These twins have been developed from microsurgically separated blastomeres from two-eight cell embryos (Willadsen 1979) or split halves from day 4-6 embryos or "morulae" (Williams *et al.* 1982). Such "half" embryos appear to possess normal viability, however, only limited success has been obtained after splitting four-cell or larger embryos into four or more genetically identical individuals. Williams *et al.* (1984) recently reported a 60% pregnancy rate following transfer of single split day 7.5 cattle embryos (early blastocysts). When used with normal embryo transfer procedures, this technique provides a method for routine production of identical twin animals.

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