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Artificial insemination of ewes with frozen semen

By David Windsor, Sheep Industries Research Officer, Great Southern Agricultural Research Institute, Katanning

The judicious use of artificial insemination (AI) of ewes with frozen semen by ram breeders offers substantial gains for wool producers, but it promises even greater benefits if it can be used more widely within commercial breeding flocks.

In the Western Australian dairy industry, for example, genetic gains between 1971 and 1986 are estimated to have been three times as great in herds bred by AI as in herds that were mated naturally.

AI in the Australian sheep industries

The widespread commercial application of laparoscopic insemination and the sustained high wool prices of the late 1980s resulted in a steady increase in the use of frozen semen within the wool industry; 84 per cent of all ram semen frozen in Australia is collected from Merino rams.

The number of ewes inseminated with frozen semen rose from 22,000 in 1983-84 to 250,000 in 1988-89, although this has since declined in line with wool and ram prices.

Western Australian breeders adopted this technology enthusiastically, and more ewes (42,200) were inseminated with frozen semen in 1986-87 than all other states combined (40,900). This activity was largely confined to the stud sector, however, and at its peak represented less than 1 per cent of matings in the national ewe flock.

Advantages of AI

The most widely discussed advantage of AI is its ability to increase the number of offspring born to superior sires (see The role of artificial insemination in genetic improvement on page 24). Other major benefits include the ability to collect and store semen to insure against the death of a genetically valuable ram, or to maintain a flock's genetic diversity without retaining live sheep.

Frozen storage and transport of semen make it possible for progeny tests to be performed in different environments and at different times.

The use of progeny testing has become easier with the development of sire referencing schemes. These schemes are one of the most significant breeding advantages that AI offers to the wool industry. They allow ram selection to be based upon direct comparison of progeny performance in the commercial breeder's environment.

It is, after all, progeny performance rather than the physical characteristics of the sire that determine a ram's genetic value to the end user.

Progeny testing of rams over time is a useful check of genetic progress toward breeding objectives; if today's progeny perform no better than those of a ram that's been dead for 20 years, then it may be time to rethink your approach!
Cervical or vaginal AI would be ideally suited to group breeding schemes or large scale progeny tests where there is no need to buy semen. Subject to the development of a market for cheap 'flock' semen, it would also be well suited for use by commercial breeders.

In the above example, changing the cost of semen to $3 per pellet gives all up costs for cervical and laparoscopic AI of $9 and $21 respectively.

However, in 1986-87, semen from 'home' sires accounted for 86 per cent of all AI (both fresh and frozen semen) in the wool industry. Under such conditions, where semen cost is negligible, a viable method of cervical AI becomes more financially attractive.

Laparoscopic AI, therefore, is likely to remain the preferred choice when performing AI with expensive frozen semen, even if conception rates following cervical AI improve dramatically. For example, a $30 semen pellet may be used to inseminate one ewe cervically for a total cost of $36 ($30 for semen and $6 for AI) or three ewes laparoscopically (costing $10 for semen plus $20 for AI, totalling $30 per ewe).

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Artificial insemination tends to be regarded as 'new' technology, but this is not the case. Italian physiologist, Lazarro Spallanzani, artificially inseminated a bitch successfully in 1784, and AI with freshly collected semen became widespread in the Russian sheep industry during the early 20th century.

Freezing semen from domestic animals became practicable with the discoveries that egg yolk and glycerol protect sperm from the effects of cooling and freezing.

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Effective procedures for freezing ram semen were developed in the 1960s and 1970s, but conception rates after vaginal or cervical AI with frozen semen remain poor. (See The role of artificial insemination in genetic improvement on page 24.) It was not until the early 1980s and the use of laparoscopy to deposit semen directly into the ewe's uterus, that conception rates using frozen semen became commercially acceptable.
There has been considerable industry interest in developing procedures to enhance conception rates following cervical or vaginal AI with frozen semen. The Department of Agriculture, therefore, maintains a continuing program of semen research within the Sheep Industries Branch at Katanning.

**Barriers to conception**

Post-thaw motility in frozen ram semen is superior to that in many species, but the fertility of sperm when used for cervical or vaginal AI remains poor. This is because of the barrier presented by the cervix, which connects the vagina to the uterus.

In some species, notably the cow, the interior of the cervix is sufficiently wide to allow passage of an insemination pipette into the uterus. By comparison, the ewe has an extremely narrow cervix so that semen must be deposited at the point where the cervix joins the vagina. As a result of damage during freezing and thawing, only a small proportion of frozen sperm are able to establish themselves within the cervix, and fewer still traverse its full 5 to 6 cm length to reach the uterus.

However, frozen sperm are capable of fertilising ova if deposited into the uterus by surgery or laparoscopy, suggesting that failure to pass through the cervix is the major cause of fertilisation failure from frozen semen.

One recent development that offers a possible solution to the problem of cervical passage is a trans-cervical AI technique devised at the University of Guelph, in Ontario, Canada. This method permits passage of a pipette through the cervix to deposit semen in the uterus (similar to AI in cattle).

The Department of Agriculture is evaluating the Canadian technique under Western Australian commercial conditions, in conjunction with the University of Guelph and Mr Stan Dorman, a Beverley farmer. This work is funded by the Wool Research and Development Corporation and the Meat Research Corporation. This, and subsequent experiments, will determine the relevance of the trans-cervical technique to the Western Australian sheep industry.

Regardless of the success or otherwise of the Guelph AI method, sperm must still be frozen efficiently enough for them to cross the cervix unassisted.

**Effects of freezing on sperm**

Freezing affects the sperm cell in many ways. The most dramatic of these is widespread damage to the plasma membrane covering the cell. This has much the same effect as a large hole in the hull of a submarine; important components escape or are disabled by the influx of external substances, and the machine ceases to function. Freezing also damages the cell components responsible for fusing with the egg at fertilisation, and the mechanisms of energy production.

The quality of frozen semen is usually assessed by making a subjective estimate of the sperm’s motility. The motility apparatus in the tail suffers little damage during freezing; the loss of motility being caused by damage to the plasma membrane. Therefore, an estimation of motility only provides an indirect measure of the real damage to the cell. This may be one reason why studies on improving the fertility of frozen ram semen, many of which were based on subjective estimation of motility, have made so little progress in the past 20 years.

Given the poor fertility of semen containing literally hundreds of millions of motile sperm, it seems likely that while current techniques for freezing semen prevent gross membrane damage caused by freezing, the fact that we are measuring damage to sperm indirectly means that more subtle injuries remain undetected.

Therefore, part of the Department’s research program on frozen semen is to identify techniques to directly measure damage to different parts of the sperm cell. The Department also wants to evaluate the potential for assessing frozen ram semen for research purposes or for routine evaluation.

**Variation in donor semen**

Frozen semen from different rams differs in fertility following AI, but there is less information on the extent of variation in fertility between ejaculates from the same ram. Our preliminary results indicate there is considerable variation in fertility between ejaculates, and we are now looking for definable differences between the more and less fertile samples. Such differences will help our understanding of which facets of freezing injury are most critical to fertility, and perhaps form the basis of improved systems for semen assessment.
The role of artificial insemination in gen

Artificial insemination allows sheep breeders to use semen from genetically superior males over a larger number of ewes. The following hypothetical scenarios demonstrate how the semen from a single ram may be used to mate more ewes with the aid of AI.

These examples are based on conservative assumptions. Some workers estimate that a single ram may be joined with up to 100,000 ewes per year with the aid of laparoscopic insemination and frozen semen.

**Natural mating**
- Ewes joined with 1 per cent rams
- Seven-week (three-cycle) joining period
- 90 per cent conception rate

This will result in 90 pregnant ewes.

**Cervical AI with fresh semen**
- Semen collected daily throughout a 17-day (one oestrous cycle) period
- Each ejaculate has a volume of 1 mL and a concentration of three billion sperm per millilitre
- Semen is diluted threefold to give a total semen volume of 51 mL, or 510 insemination doses of 0.1 mL
- 50 per cent conception rate

This ram may be joined to 510 ewes, resulting in 255 pregnancies per insemination cycle.

**Cervical AI with frozen semen**
- Semen collected 250 times a year
- Ejaculate characteristics as above
- Semen diluted threefold before freezing
- 0.2 mL insemination dose
- 15 per cent conception rate

The ram may be used to mate 3750 ewes, resulting in 562 pregnancies.

*Injection of semen into the uterine horn during laparoscopic insemination.*

*The ewe's reproductive organs viewed through the laparoscope: (a) uterine horn (b) oviduct (c) ovary (d) corpus luteum (not present at insemination).*

These photographs were taken with an endoscopic camera loaned by King Edward Memorial Hospital, Perth. The hospital uses endoscopic photography as an aid in some gynaecological diagnoses.
Laparoscopic AI with frozen semen

- Semen collection and characteristics as above
- Post-thaw sperm motility of 50 per cent
- 20 million motile sperm inseminated per ewe
- 50 per cent conception rate

This permits insemination of 18,750 ewes for 9375 pregnancies.

The following example illustrates the genetic value of AI to the wool industry under ideal conditions. A breeding pool of 15,000 ewes will produce (at 80 per cent weaning) 6000 ram lambs per year. Under the natural mating system outlined above, 150 (2.5 per cent) of these rams will be required to service the ewe flock. Assuming that optimal selection procedures ensure that only the best rams are used, this could achieve an increase in fleece weight of about 230 g per generation.

Using AI as described, the whole flock could be inseminated with fresh semen from 30 (0.5 per cent) rams, or the frozen semen of a single ram (0.02 per cent). Assuming a 50 per cent AI conception rate, the flock's rate of genetic gain would be increased by 13 per cent with the use of fresh semen or 35 per cent with frozen semen. In the case of frozen semen, this represents an extra 2400 kg of wool cut per year across the flock by the end of two generations (about seven years) as a result of the judicious use of AI.

While this simple example demonstrates the value of using AI to increase selection intensity, it rests on the assumption of optimal ram selection by the breeder. AI can, of course, be used to propagate unfit genotypes just as readily as superior ones.

Progeny testing will greatly help effective ram selection. In the case of fleece weight, measuring 100 of a ram's progeny will increase the accuracy of selection by 52 per cent compared with measuring the ram alone, thereby increasing the selection response. Such progeny tests are facilitated by the use of AI to produce larger numbers of offspring than natural mating, and to permit progeny to be tested in remote environments.

Some of the individualejaculates used for cervical AI in our experiments this year produced conception rates comparable with those achievable using laparoscopic AI. If the exceptional properties of these samples can be defined, we may be able to use that information in the search for methods to reduce damage to critical aspects of sperm function during freezing, or in preliminary screening of semen to ensure that only the most suitable ejaculates are used for cervical AI.

The ability to merely exclude those ejaculates that failed to produce pregnancies would bring about a substantial increase in overall conception rates.

Most importantly, this relative success of a small number of ejaculates demonstrates that cervical AI does have the potential to be effective when used with frozen semen under Western Australian conditions. With the aid of continued research, frozen semen technology may eventually become accessible to a much larger proportion of Western Australian sheep breeders.

Further reading


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