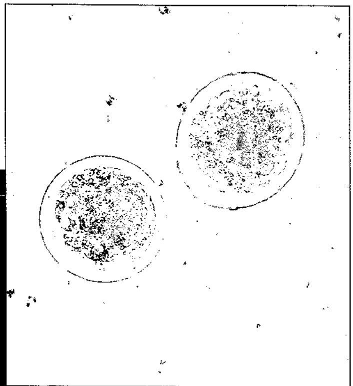
Eggs-eleven

The NZ deer farming industry has been quick to adopt advanced reproductive techniques to increase genetic progress within the national herd. Scientist Geoff Asher has been at the forefront of this work, initially at AgResearch Ruakura and now at Invermay. In this article he talks about superovulation.



Above: Two 7-day old deer embryos
Left: The surgical team in action

Embryo Transfer (ET) is an example of a highly specialised technology that has gained rapid acceptance among Red and Elk/Wapiti breeders as a way of increasing the number of progeny from a single hind-stag mating.

Its relevance to the industry lies in increased numbers of elite animals produced each year, particularly from imported genotypes.

The trend away from live animal imports stems partly from the fact that importation of cryopreserved (deep frozen) embryos is a safer and more cost effective means of acquiring new genotypes.

Not all deer farmers seek to use such forms of artificial breeding — which include artificial insemination, synchronised matings and so on. These techniques place demands on the farmer and the animal that many deer farmers consider unacceptable or unwarranted.

Given the relative ease of normal deer management compared to some traditional livestock systems, the desire to "keep it simple" is understandable.

However, farmers seeking maximum rates of genetic improvement or access to rare genetic material often find the investment of time and money in artificial breeding to be very rewarding.

Like all such technologies, however, the success of ET programmes requires good animal management, an understanding of the principles involved, clear objectives and skilled technical people.

Increased use of ET over the last 10 years attests to some remarkable successes. However, the development of protocols for deer has not been an easy road. The dedication of pioneer farmers, veterinarians and research people in riding through the rough patches cannot be over emphasised.

The separate components of an ET programme have all required much research and development, including donor superovulation, embryo recovery, recipient transfer and embryo cryopreservation.

What is superovulation?

Normally, a Red hind or Wapiti cow produces just one offspring a year. That is her natural limit, despite being endowed with four functional teats. Her pregnancy results from a single ovulation at the time of mating.

This is precisely regulated internally, despite the fact that the ovaries contain upwards of 40,000 oocytes (potential eggs). Administering reproductive hormones at the appropriate stage of the oestrous cycle can induce the shedding (ovulation) of more than one egg just prior to mating. This is called superovulation.

Should these eggs all be fertilised following mating, they are recovered from the donor hind by flushing the uterus with saline fluids. This is usually performed surgically while donors are under full anaesthesia, although in large deer like Wapiti embryos can be recovered non-surgically by carefully weaving a catheter through the cervix to flush the uterus.

Embryos are generally recovered from the donor between seven and nine days after mating, at which stage they resemble round clusters encased by a thick wall. These are unimplanted embryos containing between 64 and 128 cells, and are called morulae or blastocysts, depending on their stage of development.

Embryos at these stages are just visible to the naked eye. Embryos of "transferable" quality (at the correct developmental stage and with no major flaws) are either transferred individually to the uterus of recipient (surrogate) hinds or deep-frozen for long-term storage and transport.

The donor hind is nearly always re-mated in the same season so that she will produce an offspring on her own.

Surrogacy

The recipient hinds are generally animals of lower genetic value than the donor. In effect, they are surrogate mothers who raise progeny that are genetically unrelated to them.

For deer, usually only one embryo is transferred to a single recipient hind. Therefore, in "fresh transfer" programmes — where all embryos are transferred immediately without freezing — there must be enough recipient hinds on hand to receive all embryos recovered.

The $64,000 question is: how many embryos will a donor hind provide? Answer: not sure. Most superovulation regimens aim to produce an average of four to six fertilised eggs of transferable quality per donor hind.

In reality, it is an averages game, as it is not uncommon for some donors to produce up to 20 embryos while others in the same group fail to produce any at all.

There are several factors that can affect the outcome of a superovulation programme. Some we know, but others still baffle the experts. We do know that Red deer of eastern European origin — Hungarian, Yugoslavian — and Red/Elk hybrids tend to be more reliable donors than "Scottish-type" Red deer and pure Elk cows.

We also know that the natural mating of donors results in better fertilisation of multiple-shed ova than does artificial insemination. The dosage and timing of various hormones is critical to success. We also know that highly stressed or malnourished hinds respond poorly to superovulation treatments.

Even given these known factors, there can still be occasional surprises, and farmers should be prepared for some superovulation failures among their donor hinds.

Management of recipient hinds

This is no less important than management of donors. While recipients may not be as genetically valuable, they still need to be capable of having an uncomplicated pregnancy and birth, and be able to rear a very valuable progeny.

The recipient hind needs to be at the correct stage of the ovulatory cycle to establish successful pregnancy following embryo transfer. This necessitates co-synchronisation with donor hinds — achieved with CIDR devices — although donors receive injections of additional hormones.

Again, on the laws of average, four to six recipients are synchronised per donor. However, if the donors are particularly valuable and their previous response history is good, it is not unheard of for upwards of eight to 10 recipients to be co-synchronised per donor.

Generally, 10 to 15 per cent of recipients may be rejected due to ovulatory failure at the time of transfer.

Not all transfers will result in a viable pregnancy. However, for fresh transfer programmes the recipient pregnancy rate is usually around 80 per cent.

This means that on average for every donor hind approximately three to four offspring will be born, including her own pregnancy from subsequent re-mating.

Deep-freeze

Sperm and ova have an amazing ability to survive the rigours of the freezing-thawing process if handled correctly.

The key to success is control over water solidification without ice-crystal formation. Rapid expansion of forming ice does irreparable damage to cell walls. Protectants, such as glycerol, prevent ice crystal formation.

Many embryos recovered at the morulae or blastocyst stage are frozen in liquid nitrogen for later use.

While the procedures for freezing multicelled embryos are a little more complex than for single-celled sperm, the techniques are now fairly routine and generally very successful.

While thawed embryos are not usually as viable as fresh embryos, recipient pregnancy rates following transfer are still usually between 60 and 70 per cent.

Once in a frozen state, embryos can be stored almost indefinitely and transported safely anywhere in the world. In fact, exchange of elite genetic material between countries is more frequently in the form of frozen semen and embryos than live animals, for obvious reasons of safety, disease risk management and cost.

Where to now?

ET in Red deer is a remarkably successful technology, given that its application is only about 10 years old. There is always room for improvement, however, particularly in more predictable and consistent superovulatory responses in donors.

To this end, a number of research programmes are underway that aim to further understand changes that occur naturally in the deer ovary. If we can understand the processes, we stand a better chance of achieving fine-tuned control.

The development of in-vitro produced embryos (IVP) using "test-tube" technology offers some exciting prospects for genetic improvement programmes. This technology is in its infancy, but already there have been some notable successes with Red deer at Ruakura.

In the longer term, such technologies may supersede ET. Meanwhile, ET has enormous significance to deer farming. □