crossbred strains of red deer. Elk and elk crosses tend to have faster growth than strains of red deer but also differ in disease and behaviour attributes. Buyers prefer purchasing such animals with DNA-based objective information.

The advent of DNA technology has already revolutionised such recording and will see even greater adoption when DNA marker analysis becomes cheaper than at present allowing for routine parentage testing from multiple sire groups.

The use of DNA markers for marker-assisted selection offers promise, with some chromosome regions (known as QTL) having been identified from strain crosses involving inbred Pere-David deer (Fennessy and Mackintosh, 1992; Tate *et al*, 1995; Goosen *et al*, 1997). These QTL have yet to be validated for within-strain use but offers promise for future selection.

DEVELOPMENT AND APPLICATION OF ASSISTED REPRODUCTIVE TECHNOLOGIES

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The red deer industry in NZ has been quick to realise the potential impact of assisted reproductive technologies (ARTs) on the rate of genetic progress in breeding programmes. However, while the development and application of such technologies has been rapid, overall adoption has been at relatively low levels due to various industry infrastructure and economic reasons. Here we review established and emerging ARTs for red deer and wapiti that have implications for accelerating rates of genetic improvement.

Artificial insemination (AI)

Development of protocols for oestrous synchronisation, semen collection/cryopreservation and insemination of red deer and wapiti was spearheaded in the early 80's, and has been well described in the scientific literature (reviewed by Asher *et al*, 1993b). Present commercial practice for red deer involves:

i) Semen collection by electro-ejaculation of sedated stags. Various cryopreservation techniques

ii) Oestrous synchronisation with 12-14 days treatment with intravaginal CIDR devices followed by injection of 200-250 IU eCG.

iii) Laparoscopic intrauterine insemination of 10-20 x 10^6 frozen/thawed spermatozoa 56-60h after CIDR device removal.

For wapiti, larger size of the cows allows for successful transcervical intrauterine insemination, eliminating the need for laparoscopic techniques.

By the late 80's, AI was already a successful research tool for propagation of unique genotypes and the production of unusual hybrids (Asher *et al*, 1988; Muir *et al*, 1997). However, commercial adoption was also very rapid, with application peaking in the early 90's.

The pyramidical structure to genetic improvement within the deer farming industry effectively resulted in most perceived genetic improvement within the overall industry being arguably driven by about 3-5% of producers (i.e. "stud breeders"). Thus, relatively few elite sires (often imported stags/bulls) attained very high commercial value. With natural service limited to 40-100 hinds only per sire per season, the rapid adoption of AI technology could be viewed as inevitable. Use of AI was further driven by the ability to obtain imported frozen semen from new genetic lineage's. However, despite the reliance on relatively few elite sires, maximal application of AI probably never exceeded 1-2% of the national hind herd per annum (it is doubtful whether >10,000 hinds were inseminated annually during the early 90's).

It could be argued that the NZ deer farming industry was too small, and the AI technology too successful, for even this low level of application to be sustained. The influence of the original elite sires became wide-spread in just a few years and general commercial breeders were able to access cheaper sires from the resultant progeny. Furthermore, as the industry has yet to implement widely recognised and objective measures of true genetic gain, demonstration of further genetic improvements is not generally forthcoming. It must also be recognised that commercial deer farming in NZ features "low labour-input" systems that capitalise on deers' innate "toughness" to fend for themselves, with minimal intervention by man. Highly manipulative techniques, such as AI, require considerable investment in time and resources that often go well beyond normal farm management practice. For producers to invest in such technologies, the commercial advantages over and above the purchase of "good" sires (i.e. offspring of elite sires) need to be demonstrated.

Presently, application of AI seems to be still driven by "stud" operations, particularly with on-going importation of new genotypes. However, consultation with major suppliers of AI services to the NZ deer industry suggest that fewer than 5000 hinds have been inseminated annually over the last 3-4 years. That represents <0.5% of breeding hinds in the country. Moves towards implementing successful, less invasive, cervical AI services over the last season are seen by some operators as potentially increasing AI application.

Multiple ovulation - embryo transfer (MOET)

The development, adoption and application of MOET technologies for red deer have closely paralleled trends for AI. Protocols for superovulation, embryo recovery and embryo transfer are well described in the scientific literature (Fennessy *et al*, 1994). Superovulation of donors is achieved by strategic administration of exogenous FSH over a 4-5 day period towards the end of oestrous synchronisation treatment. Mean ovulation rates ranging from 4-9 corpora lutea per donor have been described from a number of studies. However, wide individual variation in ovulatory response is well recognised. Embryos are recovered, generally following natural mating, 7-9 days after synchronisation treatment. Transfer of embryos to recipient hinds is usually performed surgically or laparoscopically. The overall success rates are characteristically variable but average 3-4 pregnancies per donor for fresh transfer programmes; slightly less when using frozen-thawed embryos (Fennessy *et al*, 1994).

Attempts to superovulate wapiti cows have generally failed, indicating perhaps an insensitivity to exogenous FSH (particularly ovine FSH). However, F_1 wapiti x red deer hybrid hinds have been recently shown to exhibit extremely high ovulatory responses to ovine FSH (Asher *et al*, 2000); unfortunately hybrids are seldom included in the elite genetic pool. The general inability to harvest multiple embryos from pure wapiti donors has seriously limited development and application of MOET in this genotype.

As with AI technologies, application of MOET for red deer has been largely driven by the rarity and high commercial value of imported animals, and probably reached its peak in the early - mid 90's. The main limitations to increased application within the deer farming industry appear to be:

i) Concerns about the invasiveness of the procedures, and the inherent risks to valuable donor hinds.
ii) High costs relative to AI technology.
iii) Variability of results between donors and awareness of occasional, well-publicised failures.
iv) Reducing average commercial value of elite hinds.
v) Perception that other, better, technologies will supersede MOET (e.g. IVP, cloning).

Present application of MOET in red deer appears to be still largely focussed on new genotypes (e.g. imported Eastern European bloodlines) and importation of frozen embryos.

In vitro embryo production

In vitro embryo production (IVP) is a multi-step process that involves the collection of oocytes, the maturation of these oocytes, fertilisation of the oocytes by sperm, and the culture of the putative zygotes to a stage where they can be transferred into a recipient animal. The IVP process is the backbone of more recent reproductive biotechnology and is the vehicle used for the production of cloned and transgenic domestic animals.

Cervine IVP has several potential advantages over conventional assisted breeding techniques. It allows flexibility of sire/dam matings and allows gametes to be "rescued" after death, injury or infertility. IVP has the potential to cryopreserve oocytes and/or early cleavage

embryos of elite and endangered animals. The use of prepubertal animals as oocyte donors can decrease generation intervals for rapid genetic gain. Furthermore, IVP has the potential of overcoming restrictions within the deer farming industry. Frozen-thawed semen is not only a cost limited resource; seasonality constraints, the use of top sires for natural mating and anaesthetisation of the semen donor severely restrict the number of semen collections for each donor. Some breeders may restrict semen supply from elite males, artificially inflating the price of semen. In vitro fertilisation is an efficient use of semen. One straw can fertilise 100 to 300 oocytes, depending upon the concentration and quality of the semen. A half straw will fertilise oocytes from 15 donors. Seasonality also imposes limitations of acquiring multiple progeny from elite female genetics. The short breeding season (4 to 5 weeks) limits MOET collections to one per season. Weekly, trans-vaginal OPU (oocyte pickup) has the potential to extend the breeding season an additional 60 days by collecting donors during the early stages of pregnancy.

It is surprising that different species of deer require different in vitro fertilisation systems, even between closely related sub-species, such as the wapiti and red deer. Wapiti gametes fertilise and develop to blastocysts using a cattle co-culture system (Pollard *et al*, 1995) or Synthetic Oviduct Fluid (SOFaaBSA; Berg *et al*, unpublished data). However, application of bovine IVP systems to red deer results in low fertilisation rates and no embryo development beyond the 8-cell stage (Fukui *et al*, 1991; Berg 1997; Bainbridge, 1999).

IVP in wapiti has enormous commercial value, particularly in the highly speculative North America market. Very little information is available on IVP technique and results. Pollard *et al*, (1995) reported recovering an average of 41.3 oocytes from each pair of ovaries recovered at slaughter with 68% fertilised and 31% developing to the blastocysts stage using bovine epithelial explants during fertilisation and culture. We have investigated on-farm collection of wapiti oocytes using the cattle ultrasound guided trans-vagninal OPU technique (Pie-Med., Philips, Netherlands) combined with the cattle IVP system used at Ruakura (Thompson *et al*, 1998). Four twice-weekly oocyte collections from 7 non-stimulated cows in March yielded 4 oocytes per donor that were suitable for IVP. Fertilisation rates ranged from 7 to 88% depending upon the IVF bull. Thirty-five embryos were transferred, fifteen pregnancies established and thirteen live calves were born. It appears that wapiti IVP can be readily adapted from cattle techniques.

Red deer IVP has presented a major challenge. Red deer ovaries are smaller and appear to have fewer antral follicles present on the ovary than wapiti, which limits the number of good quality oocytes available from each donor. More importantly, the block at the 8-cell stage has been the limiting factor in the development of red deer IVP. This block has been reported from different laboratories using different sources of oocytes and sperm. (Fukui *et al*, 1991; Berg, 1997; Bainbridge, 1999). These embryos do produce healthy calves (23/67 34%) when transferred into the oviducts of recipients as 4- and 8-cell embryos (Berg, 1997). This block has recently been overcome by using fertilisation and embryo culture media based upon the composition of red deer oviduct fluid. Currently 10% of oocytes placed into IVP develop into blastocysts, but none of these embryos have been transferred.

The application of IVP requires the development oocyte collection from live donors. Two different techniques have been applied to red deer: laparoscopic and ultrasound guided trans-vaginal oocyte pickup. Laparoscopic oocyte pickup is similar to AI and requires the donors to the fully anaesthetised during the procedure. Red deer antral follicles do not protrude above the ovarian surface and are covered by a tough tunica albuginea. This makes the follicles difficult to visualise, dulls the aspiration needle making aspiration slow and laborious and resulting in low recovery rates (45-50%). Ultrasound guided trans-vaginal oocyte pickup requires donor animals to be only lightly sedated, if at all, while restrained in a crush for approximately 10 minutes and can be collected on a weekly basis during the breeding season and anoestrum with the potential of collection during early pregnancy. Recovery rates average 66% and may be attributed to improved follicle visualisation and the use of disposable needles. Currently 5% of all oocytes recovered develop to blastocysts.

To increase the efficiencies of cervine IVP further research needs to focus on:

i) Follicular management and manipulation to increase the quality and quantity of oocytes for IVP.
ii) Collection of oocytes during early pregnancy and transition to the breeding season and IVP of these oocytes.
iii) Cervine oocyte maturation requirements.
iv) Improvements of the blastocyst rate by investigating culture system for later cleavage stage embryos.
v) Investigation of the freezing of early cleavage and blastocyst embryos.

CLONING

Using a laboratory procedure termed nuclear transfer, it is now biologically possible to produce sets of genetically identical animals (or clones) from either early embryos or most significantly, from adult animals of proven performance. This was first achieved with "Dolly" the cloned sheep (Wilmut *et al.*, 1997) and has more recently been extended to mice, cattle and goats. The technology is still under development and much remains to be understood as to how the nucleus of an adult cell can be "reprogrammed" to recommence embryo development. In essence, nuclear transfer involves removing the chromosomes from an unfertilised egg and then fusing a cell from the donor animal to replace the genetic material. The reconstructed one-cell embryo is then artificially activated to start embryonic development (mimicking fertilisation) and is transferred to a synchronised female after one week of in vitro culture. The current efficiency of cloning adult cattle at AgResearch in terms of live calves from embryos transferred is 10% (Wells *et al.*, 1999), compared to 40% survival following in vitro fertilisation. It is expected that future research will improve these holding rates. It is also important to determine whether any long term

consequences of the technique exist, either with the cloned animals or their offspring, before it is adopted commercially.

Nuclear transfer relies on a sufficient understanding of the basic reproductive biology and embryology of the species concerned. We are fast approaching this point in deer species. It is therefore timely to consider what new opportunities exist for the deer industry by integrating an efficient cloning method into production systems of the future. Applications exist in the ability to rapidly multiply genetically superior individuals, however, this relies on accurately selecting elite genotypes. Cloning strategies could both increase the rate of genetic gain and the dissemination of this genetic gain from breeding herds to the commercial industry. This could be managed without overly reducing genetic diversity amongst the elite breeding population. Herds of cloned individuals may have more similar performance characteristics, aiding management or production, however, it must be remembered that animal phenotype is an interaction between genotype and the environment. A significant impact of the technology may be in conjunction with gene technology to produced transgenic animals. As understanding of the genes that regulate production traits are better understood in the future, so too will the ability to genetically enhance these by manipulating specific gene sequences in cells growing in the laboratory before nuclear transfer is used to produce animals from these cells. Ultimately, these genetic modifications will be more precise and direct than what can be achieved with conventional breeding. The scientific breakthrough that led to Dolly has opened up a range of potential opportunities for animal industries to embrace.

NEW DIRECTIONS FOR THE CONTROL OF SEASONAL BREEDING IN RED DEER

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Like their wild counterparts, farmed red deer hinds commence breeding each autumn at a time which means that the earliest dates of calving occur well into summer. In New Zealand pasture production reaches a peak in spring, so the productivity of pasture-based deer farming could be improved if deer were to calve much earlier than they do at present.

Techniques used to advance the onset of seasonal breeding in red deer so far have met with variable and limited success. Depending on the length and timing of treatment, melatonin can advance the mean calving date by up to 5 weeks but since it can cause premature cessation of lactation, the procedure has to be restricted to nulliparous females (Asher *et al.*, 1993a). Likewise, attempts to overcome seasonal anoestrus by administration of gonadotrophins or gonadotrophin-releasing hormone (GnRH) analogues have met with limited success (Duckworth and Barrell, 1988; Fisher and Fennessy, 1985; Moore and Cowie, 1986). We