Artificial Breeding Technologies for Farmed Deer

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INTRODUCTION

Rapid expansion of the deer farming industry around the world within the last 20 years has been accompanied by equally rapid development and adoption of a number of assisted reproduction technologies. These technologies have not only facilitated increased rates of genetic improvement on individual farms, but have also allowed widespread movement of genetic material around the world, have been implicated in genetic "rescue" of rare genotypes/individuals, and have allowed farmers and researchers to cross species boundaries in the production of potentially useful hybrids. The principle tools that have been used include artificial insemination (AI), multiple ovulation-embryo transfer (MOET) and In vitro embryo production (IVEP). These technologies have essentially been adapted from those developed for more traditional agricultural species such as sheep and cattle. However, subtle differences in reproductive physiology between livestock species often result in major obstacles in successful application of artificial breeding techniques across species, and considerable research has been required to fine tune these technologies for deer. Even within cervids there are considerable species/subspecies differences in the effectiveness of standardised techniques, necessitating refinements tailor-made for individual genotypes.

The purpose of this review is to introduce the "state of the art" of various reproductive technologies for two key species, the red deer/wapiti (*Cervus elaphus*) and fallow deer (*Dama dama*).

1. European red deer (*Cervus elaphus* spp.)

The European red deer, especially the *scoticus* subspecies, forms the basis of the deer farming industries in New Zealand (NZ), United Kingdom (UK) and a number of countries in mainland Europe. In NZ alone, approximately 1.5 million red deer, representing 0.8 million breeding hinds, are presently farmed in a pastoral environment. The development and adoption of AI and MOET technologies in NZ goes back to the early 80's. More recently, however, research investment into red deer IVEP technologies may see this latter technology eventually supercede AI and MOET. While the adoption rate of reproductive technologies has been very rapid within the NZ deer industry (ie. within 1-2 years of development), the level of application is still very low relative to some other livestock industries (eg. dairy industry). For example, conservative estimates of AI practice in NZ indicates insemination of 10,000 red deer hinds annually, representing only 1-2% of breeding hinds (c.f. >50% of dairy cows). This highlights the pyramidical breeding structure of the industry, in which a greater

proportion of perceived genetic improvement is actually driven by <5% of deer farming enterprises (ie. the "stud" breeders). Application is, therefore, limited to a small core of breeding operations within the top few levels of the pyramid. This means that the industry as a whole generally receives the benefits of reproductive technologies from the "trickle-down" effect of sire buy-ins from the stud operations. This situation also highlights the "easy care" attributes of the species, with many farmers preferring to allow nature to its course (within limits) without resorting to highly labour intensive and high cost manipulative techniques (this is not a criticism, rather a statement of fact). Future application of reproductive technology will depend largely on the nature of genetic progress (eg. introduction of sire-referencing schemes), changes in cost structures, increased efficiencies of the techniques themselves, and future linkages with associated technologies (eg. marker-assisted selection, sperm sexing, etc.).

Artificial insemination : The efficiency of AI in red deer, assessed as conception rate (pregnancies established per 100 hinds inseminated), generally ranges from 40-80%, with rates of 60-70% considered highly acceptable targets (Hunter 1997). Actual calving rate (calves born per 100 hinds inseminated) often falls slightly below these figures due to a variable degree of fetal wastage particularly when hybridisation is involved (Fennessy *et al.* 1991). Generally, however, red/red AI is not associated with high levels of fetal wastage (eg. <2%).

Semen collection from red deer stags is generally performed by electro-ejaculation. While some researchers have been able to train individual stags to ejaculate into various types of artificial vaginas (Krzywinski and Jaczewski, 1978), the level of animal training required, and the relative danger to animal handlers, often preclude this form of semen harvest across a wide spectrum of valuable sires. Generally, electro-ejaculation is successful in most stags, but some animals may require high levels of sedation. This, and the highly seasonal testicular cycle of stags, often severely limits the frequency of collections during the breeding season (reviewed by Asher *et al.* 1993). Cryopreservation of red deer semen is a relatively straight-forward procedure, with effective diluents/cryoprotectants being similar to those used for ram and/or bull semen (reviewed by Asher *et al.* 1998). Effective dosages for a single insemination range from 10-25 million spermatozoa per inseminate for cryopreserved semen. The number of straws of cryopreserved semen obtainable from a "usable" ejaculate may vary from 5-10 for low volume/density collections to >400 for very exceptional collections. The general range is 30-90 straws (ie. inseminates) per usable ejaculate.

In NZ, semen collection/cryopreservation from valuable sires has generally been aimed at "banking" 100-200 straws per animal. However, particularly valuable or rare animals may be subjected to numerous repeated collections to maximise the number of available inseminates. Using present methods of electro-ejaculation, it has been quite possible to obtain >1000 inseminates from particular stags in a single season, although this very much depends on the "collectability" of the stag.

Oestrous synchronisation of red deer hinds is an important facet of all AI programmes for this species. Natural (spontaneous) oestrus is difficult to detect given the farming systems presently used. Furthermore, natural synchrony is no better than 10-14 days, necessitating successive AI of hinds over this period, a situation which is clearly impractical. Current techniques of oestrous synchronisation are reviewed in detail by Asher *et al.* (1993) and Hunter (1997), and generally involve the use of the intravaginal progesterone CIDR [Controlled Internal Drug Releasing] device (Eazi-breed CIDR type G; InterAg, Hamilton, NZ) with exogenous gonadotrophin (eg. equine chorionic gonadotrophin or eCG; formerly

referred to as Pregnant Mare Serum Gonadotrophin or PMSG) support at device removal. The standard regimen in red deer hinds involves 12 days of CIDR device placement (often with devices replaced on the eighth day) and an intramuscular injection of 200-250 i.u. eCG at final device removal (Asher *et al.* 1993). If performed within the breeding season, most hinds will exhibit oestrus between 36 and 60 hours from CIDR device removal, and ovulate 24 hours after oestrus onset (Asher *et al.* 1992a). The necessity of the presence of vasectomised stags to stimulate hinds is still open to debate. Many practitioners now opt for running treated hinds close to active fertile stags in order that the hinds receive suitable male stimulation (ensuring, of course, that potential fence hopping is eliminated ..... nothing ruins a good AI programme better than an agile teaser stag). The use of prostaglandin injections to synchronise hinds has been investigated, but has generally proved to be of lower efficacy than intravaginal CIDR devices (Asher *et al.* 1993).

Present studies at Invermay aim to reduce further the spread of oestrus onset following synchronisation treatment. The present spread of ~ 24 h indicates that some hinds may not be at the correct stage relative to ovulation at the time of insemination. Studies underway are focused on the dynamic changes occurring with ovarian follicles (eg. Asher *et al.* 1997), particularly the interaction between large, "dominant" follicles and CIDR device usage.

The principle insemination technique for red deer involves placing the thawed or fresh semen directly into the uterus with the aid of a laparoscope. The laparoscopic technique has been adapted from that developed for sheep (Killeen & Caffrey 1982) and was used for red deer because of the relative difficulty of passing the semen pipette through the cervix. Furthermore, intracervical and intravaginal deposition of semen, while relatively easy to perform, have generally been associated with low conception rates (with the possible exception of the use of large, impractical doses of fresh semen). Laparoscopic intrauterine AI is usually performed at an average time of 54-58 hours after CIDR device removal/eCG injection (Asher *et al.* 1993), and the technique has proved to be the most reliable form of AI in red deer. It does, however, require a high degree of skill, chemical sedation of recipient hinds and precautionary aseptic procedures. Such factors often contribute to high operational costs and may be inhibitory to wider adoption of red deer AI.

Clearly, decisions to invest in AI technology take into account overall costs relative to the benefits of acquiring or propagating specific genetic resources. The relatively high costs, which may also include substantial costs of semen purchase, highlight the need for optimising conception rates. This is achieved not only by adhering to the AI protocols (eg. oestrous synchronisation regimen, correct timing of insemination) but also by ensuring that other factors are not limiting to success. Two such factors that should be considered and managed accordingly are hind nutrition and stress. Increasingly, AI practitioners are becoming aware of the importance of appropriate nutrition and minimised stress on conception rates. Malnourished hinds appear to perform poorly in AI programmes, probably reflecting a strong influence of body condition on ovulatory success and/or timing of the onset of the breeding season. Similarly, many practitioners have observed that highly stressed individuals or groups exhibit considerably lower conception rates than hinds better habituated to the farm and handling procedures. There is a strong case for eliminating hinds with obvious temperament problems from any AI programme.

As a final note on red deer AI programmes, it is important to appreciate the influence of seasonality on success. Farmers sometimes attempt AI too early in the breeding season for optimum results, rationalising that non-conceiving hinds returning to oestrus have the opportunity to become pregnant to back-up sire during the natural rut (ie. so as to not produce

late calvings). Although the use of eCG at CIDR device removal may help final follicular maturation early in the season, and thus increase the proportion of responders, it is not a "magic bullet" for out-of-season ovulation. In cases in which the cost of the semen clearly outweighs the value of an ordinary offspring (ie. conceived to back-up sires), it is better to invest in a programme that maximises the conception rate to AI. This may mean delaying the programme several weeks to ensure that all hinds respond appropriately to synchronisation treatment, and accepting that these hinds failing to conceive to AI will likely produce late calves.

Embryo transfer : The principle application of multiple ovulation-embryo transfer (MOET) technology lies in the rapid multiplication of dam and sire lines, particularly where the dam is known to have genetic superiority to other hinds in the herd. In NZ, it has been employed mainly to propagate specific imported genotypes.

The success of MOET programmes is generally measured as the numbers of calves produced per donor hind in a given year. This figure incorporates the numbers of donor progeny that are gestated and raised by surrogates (ie. recipient hinds) plus the donor's own gestated calf (conceived after the MOET programme). The principle components of such MOET programmes include donor superovulation, embryo recovery/cryopreservation, recipient synchronisation and embryo transfer.

There are a number of superovulation protocols for red deer donors, all based on multiple injections or extended infusion of Follicle Stimulating Hormone (FSH) towards the final stages of intravaginal CIDR device treatment (Fennessy *et al.* 1994; Hunter 1997). A number of protocols also include an additional injection of eCG (150-250 i.u.) at or near CIDR device removal. Programmes aim to achieve an average ovulation rate of 6-10, but in reality such averages often mask high variability between donors of 0 to >30 ovulations. Such variability, particularly the high rate of non-responders, is still regarded as one of the most problematical aspects of MOET in red deer. Future modifications of superovulation protocols may include strategic control of dominant ovarian follicles during CIDR device treatment, as it is believed that such follicles may exert a negative influence on the artificial recruitment of multiple follicles (Asher *et al.* 1997). There may also be strong genotype effects on the efficacy of superovulation treatments, with eastern European red deer hinds (*Cervus elaphus hippelaphus*) being reputedly more consistent responders than those of western European origins (eg. *C. e. scoticus*) (Fennessy *et al.* 1994). Recent studies at Invermay likewise showed that F_1 red x wapiti hybrids responded differently than red deer to a standard superovulation regimen, generally exhibiting higher and more consistent ovulation rates. Interestingly, pure elk cows are reputed to be very poor responders to standard FSH regimens (Fennessy *et al.* 1994).

Embryo recovery from superovulated donors naturally mated to selected sires (usually at ratios no more than 1:4) is generally performed 6-8 days after removal of CIDR devices in order to recover transferable grade embryos (eg. morulae/blastocysts). The small body size of red deer hinds generally necessitates laparotomy to achieve adequate uterine flushing. (ie. surgical recovery).

Recipient synchrony is achieved in the same manner as synchronisation for AI (ie. CIDR device & eCG). To ensure compatibility of embryo and recipient, a close temporal match between donor and recipient is required. Thus, the intravaginal CIDR protocol for both is aligned, although it is quite common for recipient's device to be removed 24 h earlier due to effects of additional FSH treatment on advancing donor oestrous by about one day.

Embryo recovery and embryo transfer are usually performed in close tandem to minimise environmental stress on the embryo. Transfer of single embryos to each recipient is generally performed surgically following full laparotomy, although some operators now use a laparoscopic transfer technique that minimises exposure of the reproductive tract. Cryopreservation of red deer embryos, to accommodate surpluses or for later transfer, follows much the same procedures as for other domestic ruminants. Success of embryo transfer, measured in terms of scanned pregnancies in recipients, is in the order of 60-80% for fresh embryos and 50-70% for frozen/thawed embryos (Fennessy *et al.* 1994).

In Vitro Embryo Production (IVEP) : While AI and MOET are well established in the red deer farming scene, IVEP is only now starting to have an impact. Low efficiencies of IVEP presently limit application. However, this technology has the potential to eventually supersede AI and MOET. In vitro systems will eventually allow for greater recovery of elite female gametes, as well as dramatically widening the influence of elite males through more economical use of spermatozoa. The fundamental tenents of IVEP are based on the effective harvest of unfertilised ova (oocytes) from hinds, subsequent "test-tube" fertilisation of each oocyte with low numbers of spermatozoa, and transfer to surrogates/liquid nitrogen. As each female is born with many thousands of oocytes, synchronised harvest is, in theory, possible. However, for theory and actual to meet, much work is required in the areas of oocyte harvest, oocyte maturation, in vitro fertilisation, embryo culture and cryopreservation. It is highly unlikely that the "quantum leaps" will be made with cervids; rather, cervid systems will be developed close on the heels of other domesticated ruminant species.

The present status of the technology for cervids is mainly limited to the red deer/wapiti species (Berg *et al.* 1995). To date, a number of IVEP progeny have been produced in New Zealand (red deer, red x wapiti and red x Père David's deer hybrids) and Canada (wapiti). However, while the level of efficiency of the technology is approaching that of cattle, it is still of marginal commercial significance in the face of more conventional alternatives (eg, AI and MOET). However, it will probably gain strong commercial acceptance over the next decade, particularly with the advent of other associated technologies such as sperm sexing, embryonic cloning and marker-assisted selection.

2. Fallow deer (*Dama dama* spp.)

The European fallow deer (*D. d. dama*) is farmed widely throughout the world, and has proved adaptable to a wide range of climatic environments. Within the last decade, considerable attention has been given to genetic improvement for venison production within a variety of pastoral environments. This has included hybridisation of the common European subspecies with the rare Mesopotamian fallow deer subspecies (*D. d. mesopotamica*), as well as "within-strain" selection (Morris 1993). AI has played a major role in these programmes, particularly in NZ, Australia, USA and Canada (Asher *et al.* 1993). MOET and IVEP technologies have yet to make a significant contribution in this species.

Artificial insemination : The first major studies of AI in farmed fallow deer occurred simultaneously in NZ and Australia in the 1980s (Asher *et al.* 1988a; Mulley *et al.* 1988). Since then >6000 fallow deer have been inseminated commercially around the world, with at least 80% of semen use originating from NZ sources (this includes sires imported into NZ from other countries).

Semen collection from fallow deer bucks is invariably by electro-ejaculation of sedated animals. As with red deer, there are seasonal constraints on harvest of viable spermatozoa,

although good quality semen can be obtained over a 5-6 month period. Fallow deer semen appears to be quite robust and withstands well the rigors of cryopreservation (see Asher *et al.* 1998). Effective dosages for a single insemination range from 10-20 million spermatozoa per inseminate for cryopreserved semen and 1-5 million spermatozoa for fresh semen. The average number of cryopreserved straws per usable ejaculate is about 25-30, although exceptional ejaculates may yield >300 straws.

Oestrous synchronisation of fallow deer does is achieved by the timed withdrawal of intravaginal CIDR devices after 12-14 days placement. There is no need to replace devices as a single device is adequate to maintain high blood progesterone concentrations over the entire treatment period. The use of eCG is contra-indicated for fallow deer as it appears to have a detrimental effect on ovulation at all dosage levels investigated (50-200 i.u.) (Jabbour *et al.* 1993). This further highlights the need to perform oestrous synchronisation treatments within the breeding season to ensure ovulatory success. Thus, most AI of fallow deer is performed 1-2 weeks after the rut. Provided this condition is met, does will generally show overt oestrous behaviour between 47 and 63 hours after CIDR device removal (Morrow *et al.* 1992). The use of vasectomised "teaser" bucks is preferred but not yet shown to be necessary. While strategically timed injections of prostaglandin can induce a high degree of oestrous synchrony in fallow does, this method of synchronisation appears to be associated with reduced fertility when compared with the standard CIDR device regimen, and is therefore not recommended (Asher *et al.* 1992b).

Laparoscopic intrauterine insemination, at an average time of 65-70 hours after removal of CIDR devices, has become the standard protocol for AI of fallow deer. Attempts at intracervical AI have yielded highly variable results, and has generally only been successful with high doses of fresh semen (Jabbour *et al.* 1993). The laparoscopic method, however, appears to be considerably more reliable, resulting in average conception rates of 60-70% for cryopreserved semen and 70-80% for fresh semen (Asher *et al.* 1993). There does appear to be a genotype effect on conception rates; insemination of European fallow does with semen from Mesopotamian fallow deer has generally resulted in slightly lower conception rates (ie. 50-60%) than insemination within genotype (Mylrea *et al.*,1991).

Reduction of stress during the synchronisation/AI process seems to be a critical factor in success. In particular, overnight fasting just prior to insemination may be counter-productive, as the additional stress during the critical period leading to ovulation may influence and delay slightly the ovulatory process. Recommended procedures include yarding of does only 1-2 hours before insemination to avoid such stress-related problems. It should be noted here that fallow deer under heavy xylazine : ketamine sedation seldom regurgitate or exhibit respiratory problems related to rumen weight, eliminating the need for fasting prior to anaesthetisation.

Embryo transfer : Attempts at embryo transfer on fallow deer have not yielded high success rates, largely reflecting low fertilisation rates of recovered ova (Thompson and Asher 1988; Morrow *et al.* 1994; Jabbour *et al.* 1994). From these studies, the overall success rate has been ~ 1.6 pregnancies per donor (including donor pregnancy), which is much lower than that generally achieved in red deer (Fennessy *et al.* 1994).

Commercial MOET in fallow deer has mainly involved donors of the Mesopotamian subspecies or their hybrids with European fallow. Limited data from recent studies in European (n=8) and the larger Mesopotamian hybrid (n=7) does gave similar ovulatory responses (8.6 and 7.0 corpora lutea per donor) and marginally different embryo recovery rates (33 and 49%) but the latter difference was not significant. However, attempts to

superovulate a small number of pure Mesopotamian fallow does generally met with complete ovulatory failure (W. Otway pers. comm.). Given the role of this genotype in international fallow deer farming, the development of protocols/regimens specifically for Mesopotamian fallow deer is important. The early studies on MOET in fallow deer were plagued by extremely low ova fertilisation (0 - 50%) and recovery rates (30 - 50%) following natural mating and/or intravaginal insemination of donors (Thompson and Asher 1988). This raised questions about cervical passage of spermatozoa in superovulated donors. Two recent studies have attempted to overcome this problem by laparascopically inseminating does with fresh semen (25 - 50 x 10^6 spermatozoa) 36 hours after removal of CIDR devices (ie. about 12 hours after observed oestrus). The embryo recovery rates (ie. 30 - 50%) were considerably improved but were still lower than the more successful red deer programmes (Jabbour *et al.* 1994; Morrow *et al.* 1994). It was also notable that there was a wide range of embryo development stages observed in both fallow deer studies.

Induction of ovulation in recipients does not appear to be a limiting factor in MOET programmes for fallow deer. Treatment with CIDR devices alone (ie. without PMSG) within the natural breeding season (late April - late May in New Zealand) has resulted in a high proportion (>90%) of fallow does exhibiting synchronised luteal development and viable corpora lutea present at the time of transfer. This is similar to red deer. Transfer of single fresh or cryopreserved embryos via laparotomy (ie. fully surgical) or laparoscopy (ie. semi-surgical) has resulted in acceptable pregnancy rates (50 - 80%) in a limited number of studies performed to date.

In Vitro Embryo Production (IVEP) : There are no published accounts of IVEP in fallow deer. Attempts at oocyte recovery from slaughterhouse ovaries and subsequent *in vitro* fertilisation of matured oocytes have indicated the feasibility of this technology to fallow deer (D. Berg; pers. comm.). Recent developments in the *ex situ* conservation of Mesopotamian fallow deer at Invermay will likely be the impetus to further advance these studies.

3. Wapiti (*Cervus elaphus* spp)

While red deer and fallow deer account for much of the research emphasis on cervid technology, some aspects of artificial breeding have been investigated for other species/subspecies. North American wapiti (*Cervus elaphus* spp. *nelsoni*; *manitobensis*, *roosevelti*, etc.) are ranched in Canada, USA and, to a lesser extent, NZ. AI of wapiti has been practised widely over the last decade. In general, techniques have been similar to those developed for red deer, with the exceptions that (1) oestrous synchronisation is usually performed with bovine CIDR devices (Ezi-breed CIRR-B) rather than ovine devices, to accommodate the much larger body mass of wapiti cows; (2) slightly lower doses of PMSG are used; (3) transcervical (pipette passed through the cervix) intrauterine insemination, facilitated by rectal manipulation is more feasible in wapiti than red deer, again as a consequence of larger body mass; and (4) average insemination timing is 62 - 68 hours from CIDR device removal.

4. Hybridisation

AI and MOET technologies have found application in the purposeful hybridisation of a number of cervid taxa. In many cases, certain cervid species are closely affiliated genetically but their genomes are isolated by differences in behaviour, seasonality and phenotype (eg. size). Artificial reproductive technologies, especially AI, provide a means for overcoming such barriers to hybridisation. In cases where hybridisation has been known to occur naturally

(particularly between subspecies), AI has been a tool to simply facilitate the speed of such programmes (eg. wapiti x red deer) or allow the production of greater numbers of hybrid progeny when one of the parental genotypes is scarce (eg. Mesopotamian x European fallow deer). In other cases in which barriers inhibit natural hybridisation, AI has been instrumental and critical to the production of hybrid offspring. Notable examples include .....

1) Père David's deer (*Elaphurus davidianus*) x red deer. Attempts to obtain F^1 (ie. first cross) hybrids in NZ by natural mating failed due largely to social and seasonal isolation. Successful production of F^1 hybrids involved laparoscopic intrauterine AI of red deer hinds with cryopreserved Père David's deer semen (Asher *et al.* 1988b).
2) Wapiti x sika deer (*Cervus nippon*). Although closely affiliated species, sheer body size differences between these two taxa (wapiti are >3 times larger than sika deer) would preclude most attempts at natural mating, irrespective of willingness on the part of the deer. Production of F^1 hybrids ("Silks") has been achieved by AI of sika deer hinds with wapiti semen (Willard *et al.* 1996).

The productive benefits of the various hybridisations have yet to be fully assessed (with the exception of wapiti x red deer hybrids), and need to be balanced against such factors as cost of producing hybrids and the biological/ethical consequences of forcing such hybridisation. It would appear that the success of hybrid offspring production using AI techniques is correlated with the genetic inter-relationship of the parental taxa. Closely related subspecies freely hybridise and high pregnancy rates are generally achievable with AI (eg. Mesopotamian x European fallow deer). However, as a general rule, conception rates have tended to decrease, and embryonic mortality rates to increase, with increasing genetic distance between parental taxa. For example, in the production of Père David's deer (chromosome number 2n=68) x red deer (2n=68), average conception rates following AI have been ~ 13% and full-term pregnancies have averaged ~ 8% (ie. 40% embryo mortality) (Fennessy and Mackintosh 1992). Similarly, in the production of sambar deer (*Cervus unicolor*; 2n=56) x red deer (2n=68) hybrids (2n=62), the AI of 400 red deer hinds with sambar deer semen resulted in 31 pregnancies at Day 40 (9% of inseminated hinds), 24 remaining pregnancies at Day 100 (6%) and only 4 full-term pregnancies (1% of inseminated hinds); of these only one female calf was born alive (Muir *et al.* 1997).

SUMMARY

Adoption of various reproductive technologies for farmed red and fallow deer over the last 20 years has been rapid but generally at low levels of application, largely reflecting the nature of genetic improvement programmes within the industry and the accepted "easy-care" farming styles to which the species are suited. However, these technologies, which include AI, MOET and IVEP, will likely play prominent roles in facilitating increased rates of genetic progress, international exchange of genetic material and "genetic rescue" (i.e. propagation of rare or endangered genotypes). AI and MOET technologies presently fulfil these roles to various extents. However, likely developments in IVEP may see this latter range of technologies eventually supercede AI and MOET.

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