EMBRYO TRANSFER IN DEER

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ABSTRACT

The rapid growth in deer farming and interests in the conservation of various deer species have stimulated development of embryo transfer procedures. The protocols developed are based on those used in sheep and cattle and generally involve surgical embryo recovery following FSH-induced superovulation. Data are limited, but pregnancy rates of around 3.0 per donor female for red deer, and around 1.5 for fallow deer are being achieved.

Key words: embryo transfer, Cervus elaphus, Dama dama, superovulation, FSH

INTRODUCTION

The Cervidae includes a range of species from the diminutive Chinese water deer to the North American wapiti (6). From both conservation and farming perspectives, there is increasing interest in the application of embryo transfer (4, 11). While considerable progress has been achieved, multiple ovulation and embryo transfer (MOET) has generally been performed in situations where immediate and positive results are required either because of the perceived risk involved in working with endangered species or because economic considerations require it. Consequently much of the development of these techniques has been by trial and error as there is usually a lack of basic information on which to base MOET protocols. Therefore procedures have been adapted from proven cattle and sheep protocols. Most of the work to date has involved red deer and wapiti (Cervus elaphus ssp) but there is some information from other species such as fallow deer (Dama dama ssp) and white-tailed deer (Odocoileus virginianus ssp, 11).

MATERIALS AND METHODS

Superovulation and Breeding

Superovulation procedures are based on those used successfully in sheep and cattle in New Zealand. For red deer, the procedure involves synchronisation of the oestrous cycle with exogenous progestagen and stimulation of follicle development with exogenous gonadotrophin (usually FSH). A typical protocol is the 12-day use of an intravaginal controlled internal drug releasing device (CIDR[a], containing 340 mg of progesterone) with FSH administered by injection (generally 8 doses) or by osmotic minipump[b] for 4 d through Days 8 to 12 of the

[a]CIDR-G device, Carter Holt Harvey, Hamilton, New Zealand
[b]Alza Corp, Palo Alto, CA, USA

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cycle. Progesterone is withdrawn at around 12 or 24 h before completion of FSH treatment. Various forms of FSH have been used, although the more purified preparations of FSH[c] with lower LH levels are now preferred. The protocol for superovulation of fallow deer is similar to that of red deer except for a longer (14 days) progesterone treatment (10). For mating the female deer are generally run at a rate of up to 10 per male. If mating is prior to the onset of the normal breeding season, males may need to be treated with subcutaneous melatonin implants[d] to advance the rut or mating season. A common dose for red deer is the subcutaneous placement of 4 x 18 mg implants in early December (southern hemisphere) followed by 2 x 18 mg implants about six weeks later. Although artificial insemination (AI) could offer considerable advantages in MOET programmes by allowing a greater coverage by genetically superior males, there have been few attempts to do so in red deer. However, extremely low fertilisation rates with cervical AI or natural mating in MOET programmes in fallow deer have prompted the use of laparoscopic intrauterine AI in this species.

Embryo Recovery

Embryos are generally recovered using surgical exteriorisation of the uterus (with or without the ovaries) and flushing with PBS (0.4% BSA) media, with collection using a Foley 10 F catheter. The media is initially at 35°C but is allowed to cool to 15°C after collection. Laparoscopic procedures are being developed. Non-surgical recoveries have been performed on the larger North American wapiti (>200 kg live weight). In all species embryos are recovered 8-9 days after progesterone withdrawal. For embryo recovery, females may be anaesthetised by a variety of different treatments, although a fentanyl citrate/xylazine hydrochloride combination with or without intubation and halothane (4) is generally preferred for red deer and a xylazine hydrochloride/ketamine combination for fallow deer (2). Clenbuterol, a smooth muscle relaxant, is recommended to ease manipulation of the reproductive tract during embryo recovery (4). The anaesthetic/sedative is reversed using nalorphine and yohimbine in red deer (4), and yohimbine alone in fallow deer (2).

Synchronisation of the Recipient, Embryo Transfer and Pregnancy Diagnosis

Recipient females are also synchronised using a progesterone-CIDR device. In red deer the intravaginal CIDR treatment continues for approximately 12 days (5), with an injection of 200 iu PMSG[e] at CIDR withdrawal. In fallow deer the treatment omits the PMSG because of highly variable results (1). It is recommended that vasectomised males are run with the females (to ensure normal male-female behaviour around oestrus) from the time of progesterone withdrawal until replacement with an intact male some days after transfer, the latter to ensure that donors and recipients have a further opportunity to conceive and carry progeny to term. Embryo transfer is generally effected by a technique involving either a partial exteriorisation of the horns of the uterus (using a laparoscope to locate the uterus) or by a simple intrauterine transfer using a Cassou pipette in the same way as for AI (5). Normally single embryos are transferred and all recipient females are checked by laparoscopy to ascertain that they have ovulated recently. Procedures for the induction and reversal of sedation are as used for donor animals. Pregnancy status is assessed using a real-time ultrasonic scanner per rectum at around Day 40 of pregnancy. For this purpose the females are restrained in an appropriate crush which limits forward and lateral movement.

[c]Ovagen, Immuno-Chemical Products Ltd, Auckland, New Zealand
Folltropin, Vetrapharm Inc., Ontario, Canada
[d]Regulin, Schering Agro-chemicals Ltd, Australia
[e]Folligon, Intervet, Lane Cove, NSW, Australia

RESULTS AND DISCUSSION

Red Deer

An early study (4) with 12 NZ red deer hinds investigated the dose response to Ovagen administered per osmotic minipump (0.14, 0.28, 0.42, 0.56 units over 4 days). The mean ovulation rates and transferable embryos recovered per hind were 0.7, 2.0, 4.3 and 15 and 0, 0.7, 2.3 and 5.0. All embryos were at the compact morula or blastocyst stage. The data indicate a clear dose response and have been used as the basis of further studies using Ovagen. This experiment involved the use of minipumps to deliver the FSH but there is only one published comparison (4) of minipumps and twice daily injections for 4 d; this involved eight red deer females and injections were clearly superior (on average of 3.0 and 11.0 ovulations per hind). Despite the increased amount of handling involved, there is a strong opinion that twice daily injections are a more reliable approach. The frequency of the FSH injections is also an important consideration. In one experiment (n = 56 NZ red deer) once daily and twice daily injections were compared along with a comparison of two sources of FSH. There were no significant differences due to either variable, with the main effects comparison of once versus twice daily FSH giving average ovulation rates of 8.9 and 8.8 and transferable embryos of 2.9 and 2.4 per donor. However once daily injections have generally given disappointing results in subsequent commercial MOET programmes. This may be due to strain differences in that commercial MOET programmes involve the recently imported European strains of red deer (as distinct from the usual NZ red deer which is mainly of Scottish origin) but it may also be that the dose rate with once daily injections should be increased compared with the twice daily protocol. There may also be a difference in the efficacy of the different FSH sources, but any comparison would require dose response data. In one unpublished case, the mean ovulation rates for two strains of red deer were 4.2 and 8.8 (n = 46, SED $\pm$ 1.9, $P<0.01$). Commercial MOET programmes do take apparent strain differences into account by manipulating the FSH dose, based on experience.

There have been few attempts at AI in the MOET situation with red deer, due to concerns about the potential cost of failure, the stresses of additional handling and lack of knowledge about the timing of ovulation under FSH treatment. However in one case, natural mating was compared with AI and, although confounding factors preclude a completely valid comparison, only 33% of the embryos recovered from the AI group (one cervical and one laparoscopic insemination per hind) were transferrable compared with 66% for the naturally mated group (2.2 and 5.5 transferrable embryos per donor; n = 20, SED $\pm$ 1.7, not significant). Considerably more research would be required to ensure the reliability necessary for AI to be used in a MOET programme in red deer.

The number of embryos recovered as a proportion of total ovulations (corpora lutea) is a variable of interest. In three MOET programmes involving a total of 186 red deer donors, 979 embryos were recovered (65%) of which 662 (68%) were of transferrable quality. Following recovery, embryos are graded and good quality embryos are frequently frozen and subsequently transferred. For example Dixon et al (3) reported an average pregnancy rate of 61% from frozen embryos (previously collected in New Zealand) transferred to 247 recipient red hinds over five properties in Australia. There are three data sets with MOET in red deer which allow evaluation of some factors influencing the success rate. These include relationships with the ovulatory response, embryo quality and stage of development of the embryo at the time of transfer. Table 1 illustrates the outcome in terms of pregnancies per donor, where donors have been classified according to their ovulatory response. The quality

of embryos (assessed visually) also influences the subsequent pregnancy rate (Table 2), with embryos graded 1 and 2 on a 1 to 4 scale (very good to poor) giving higher pregnancy rates. The stage of embryo development also influences the pregnancy rate (Table 3) although there were no differences in the rates achieved with embryos from the late morula to late blastocyst stage at transfer.

Table 1. Relationships between ovulatory response in European and hybrid red deer donors, the numbers of embryos recovered and transferred and the subsequent pregnancy rate (c. Day 40) after transfer to recipient NZ red deer hinds

Ovulatory response	Donors n	Embryos (mean/donor) recovered	Embryos (mean/donor) transferred	Pregnancy rate per transfer	Pregnancy rate per donor
0	1	-	-	-	-
1-3	6	7	7	0.14	0.16
4-6	8	40	29	0.55	2.0
7-9	9	63	61	0.72	4.9
≥10	10	104	74	0.72	5.3
Overall	34	214(6.3)	171(5.0)	0.67	3.4

Table 2. Relationship between quality (1 = very good appearance; 4 = poor) of embryos and pregnancy rate (at day 40) of recipient hinds on two properties (A and B)

	Embryos transferred (n) A	Embryos transferred (n) B	Pregnancy rate per transfer A	Pregnancy rate per transfer B
Embryo grade				
4	25	0	0.12	-
3	25	20	0.56	0.55
2	39	98	0.80	0.70
1	134	53	0.86	0.64
Total	223	171	0.73	0.67

Table 3. Relationship between the stage of development of transferred embryos and pregnancy rate (at day 40) per recipient or donor hind on two properties (A and B)

	Embryos transferred (n) A	Embryos transferred (n) B	Pregnancy rate A	Pregnancy rate B
Embryo stage				
4-8 cell	12	0	0	-
Morula	24	10	0.33	0.20
Late morula	53	63	0.81	0.67
Early blastocyst	41	24	0.80	0.71
Blastocyst	50	42	0.84	0.74
Late blastocyst	43	32	0.86	0.69
Total	223	171	0.73	0.67
Mean per donor	3.7	5.0	2.7	3.4

Fallow Deer

While successful artificial insemination protocols are well developed for fallow deer (1), MOET studies have often met with mediocre results, related principally to low ova recovery rates and low fertilisation rates of recovered ova (7, 10). Early studies indicate clear dose responses in ovulation rate to ovine FSH that parallel those in red deer (Table 4), albeit with greater variance at any given dose rate. These studies have generally involved the administration of low doses of PMSG (100-200 i.u.) at the beginning or end of FSH treatment to overcome the apparent "all or nothing" ovulatory response described previously for FSH alone (10). However the efficacy of the addition of PMSG over FSH alone does need to be further evaluated. In a recent on-farm study, 32 fallow donors were treated using a variety of superovulation techniques. In all, 112 embryos were collected with 67 being of transferrable quality. Of these, 30 embryos were transferred at the time of collection with 22 pregnancies resulting, a rate of 73%, which is comparable to the red deer data; 7 of 8 frozen embryos transferred subsequently also yielded pregnancies. However the low number of transferrable embryos per donor in the fallow deer (2.1) meant an overall rate of around 1.6 pregnancies per donor, which is much lower than that generally achieved in red deer.

Table 4. Mean (± sd) ovulation rates and total follicles of fallow deer and red deer (n = 100, 10 per treatment) to increasing doses of ovine FSH (Ovagen) - (10).

	Fallow deer		Red deer	
FSH units	corpora lutea	total follicles[a]	corpora lutea	total follicles[a]
0	1.1 ± 1.2	2.5 ± 2.1	0.8 ± 0.3	1.5 ± 1.2
0.25	7.2 ± 5.1	10.0 ± 5.7	7.1 ± 4.2	9.6 ± 5.4
0.50	9.6 ± 7.5	14.9 ± 8.1	9.5 ± 6.3	13.5 ± 5.7
0.75	8.6 ± 7.2	17.3 ± 5.7	6.9 ± 1.8	10.3 ± 2.7
1.00	7.4 ± 6.3	12.9 ± 7.5	6.4 ± 3.9	9.7 ± 3.6

[a] Total corpora lutea and follicles >5 mm diameter, including cystic and luteinised follicles

Commercial MOET in fallow deer has mainly involved donors of the Mesopotamian subspecies (D. d. mesopotamica) or their hybrids with European fallow (D. d. dama). 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, recent 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 (7, 10). This raised questions about cervical passage of spermatozoa in superovulated donors. Two recent studies have attempted to overcome this problem by laparoscopically inseminating does with fresh semen (25 - 50 x 10^6 spermatozoa) 36 hours after removal of CIDR devices (i.e. about 12 hours after observed oestrus). The embryo recovery rates (i.e. 30-50%) were considerably improved but were still lower than the more successful red deer programmes (8, 9). 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 (i.e. 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 CL present at the time of transfer. This is similar to red deer. Transfer of single fresh or cryopreserved embryos ipsilaterally via laparotomy or laparoscopy has resulted in acceptable pregnancy rates (50-80%) in a limited number of studies performed to date (10, 11).

Conclusions

The overall efficiency of MOET in red deer approaches that of other domestic livestock. Future developments will be directed at gonadotrophin delivery techniques to reduce handling requirements. Future research on fallow deer will need to focus on improving recovery of fertilised ova. While development of MOET for cervids is gaining momentum through commercial investment in deer farming, the implications of such development on captive propagation of endangered cervid taxa have yet to be realised. The close genetic affiliations between common and rare taxa within Cervus and Dama indicate the possibility of implementing maternal surrogacy programmes.

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