The Non-Surgical Recovery of Embryos From Red Deer – A Field Clinical Trial

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Abstract

The purpose of the trial was to investigate the feasibility of the collection of red deer embryos by a non-surgical technique similar to that used in other species particularly cattle

A group of 16 donor Red Deer Hinds was programmed for multiple ovulation embryo transfer (MOET) using a standard treatment regime for the species Fertilisation was achieved by trans-cervical insemination Embryo collection was initially carried out after the catheterisation of the cervix with a balloon catheter and flushing the uterus with phosphate buffered saline. Embryos were identified and graded according to standard criteria Following this procedure the hinds were anaesthetised and a surgical flush procedure also carried out. There was difficulty in effectively non-surgically flushing some hinds A significant additional number of embryos were recovered from hinds non-surgically flushed by the surgical procedure

Non-surgical embryo transfer was carried out into a number of recipient hinds but found to be difficult or not possible in some Surgical transfer was found to be a more efficient process in the trial Pregnancy results were similar using either technique

Subsequent donor pregnancy rates were considered normal

Introduction

When Deer Reproduction Services Ltd (DRS) first started applying Multiple Ovulation Embryo Transfer (MOET) technology to deer species in 1985, we were mainly involved with elk and wapiti We initially used the cervical flushing technique, which was already being used successfully in cattle While the animals were large enough, in most cases, to carry out the procedure, we found the recovery rates to be disappointing For this reason we then decided to use the surgical recovery of embryos. Our data from that time showed that the embryo recovery rates improved, so we have continued to use it, now mainly with ET in red deer

For several years there have been claims by ET teams that they have been able to successfully recover and transfer embryos from red deer. Given that it has been some time since DRS had attempted non-surgical flushing in deer it was considered prudent that we should revisit this option in red deer during the ET season in April 2000. There were a number of areas that we were keen to investigate·

1 Could non-surgical flushing be successfully carried out on average red deer hinds.
2 To compare the embryo recovery of non-surgical and surgical flushing of red deer hinds
3 To evaluate non-surgical transfer of embryos into recipient red deer hinds
4 To evaluate the subsequent breeding to natural mating of the red deer donor hinds

Investigation design

16 donor hinds were selected on a Canterbury property that had previously been involved with several years of ET work with red deer These donors were selected primarily on the basis of having reared a fawn successfully in the previous season 30 recipients were selected also on the basis of having reared a fawn in the previous year The usual DRS synchronisation programme was used Donors were treated with 2 CIDR-G's (changed on day 9 with 2 fresh CIDR-G's) and the recipients 1 CIDR-G

The superovulation regime involved the use of three different FSH products and was applied over a standard 4 day 12-hourly injection regime Mating involved the use of trans-cervical AI. This was carried out on one day with 8 donor hinds being inseminated twice at 8-hour intervals and the other 8 donor hinds once. The donor hinds were flushed over 2 days (8 per day) with the non-surgical flushing being carried out first, followed immediately by the surgical flushing of the same donor

All non-surgical work was carried out in a modified Newlands crush which included added rubber padded walls and the hinds were easily held and did not allow too much movement during the procedure

Prior to flushing each donor had a tranquilliser administered along with a muscle relaxant and an epidural anaesthetic to provide better relaxation and easier manipulation of the reproductive tract Following the flush, the trans-cervical catheter was left in place, and the medium used for the surgical flush was collected through it

The recipient hinds (non-surgical) were prepared in the same way as for the donors prior to transfer of embryos. All recipients and donors have since been examined by real time ultrasound rectally for evidence of pregnancy and where possible the pregnancy ages were determined.

Results

Non-surgical recovery from the donors

Of the 16 donors selected, one was removed from the trial on the basis of having not reared a fawn in 1999 (having slipped through the screening system) Of the remaining 15 donors, the flush was abandoned in two hinds without flushing taking place, due to difficulty in passing the catheter through the cervix. Of the thirteen flushed, eight were flushed with little difficulty and were noted as good flushes, the remaining five were recorded as difficult

Embryos were recovered from ten of the donors flushed The range between donors was 1 to 18, with a total of 91 embryos recovered. Of the three donors from which no embryos were recovered non-surgically, embryos were recovered from one following the surgical flush. (Table 1.)

The time taken to flush each donor varied but generally took 30 to 45 minutes to complete the process It was found to be difficult to accurately count the ovarian response rectally.

Surgical recovery from the donors

Of the 15 donors from whom embryos were recovered surgically, all but two had been non-surgically flushed The most recovered additionally was seven from 2 donors, with a total of 35 from the 13 non-surgically flushed donors (Table 2) If the 2 abandoned donors are included, the total recovered surgically becomes 55.

Non-surgical transfer to recipient hinds

Eight transfers were completed from a total of 11 attempts

Three recipients were rejected as being too difficult for one reason or another. From the 8 transfers, there were five resulting pregnancies (62 5%)

Surgical transfers to recipient hinds

A total of 18 embryos were transferred laparoscopically with 15 resulting pregnancies (83%)

Pregnancy results in donors after natural mating following ET

All 15 donors were scanned on the 22/05/00 (day 32 after ET mating) and again on the 27/06/00 Two were pregnant to the ET mating (i e had embryos not recovered at the time of surgery) By the scanning on the 27th June 2000, 12 were pregnant to either the ET mating (2) or natural mating (10) since the surgery date (i e there were three which were not pregnant at the time of examination).

Discussion

Following what was a very successful AI/ET program (106 fertilised embryos from 15

donors), we were able to show that ova could be recovered from red deer donors and transferred to red deer recipients using a non-surgical technique

The non-surgical recovery rates were not as good as we experience with straight surgical approach. The additional ova recovered from the surgical flushing (total 35), when compared to the 91 flushed non-surgically, is significant and when combined with the embryos from the 2 donors which could not be flushed, adds up to a considerable economic loss if non-surgical flushing alone had been done This represents approximately a further 3 ova per donor flushed It should also be noted that doing the surgical collection after the uterine tract had been 'disturbed' following the non-surgical approach, meant that the opportunity to surgically recover further ova was compromised

Table 1 Individual donor result non-surgical trial – April 2000

Tag	No.	Resp.	Rec N/S	Rec surg.	Total rec	Fertilised	Degen	Unfert	Comments
Black	20	19	15	4	19	19	0	0	Good N/S flush
Green	6097	8	4	3	7	6	0	1	Good N/S flush
Green	5112	17	4	7	11	10	0	1	Difficult N/S flush
Black	30	3	0	0	0	0	0	0	Difficult N/S flush
Blue	385	40	18	2	20	2	0	18	Good N/S flush
Orange	5110	16	0	18	18	14	0	4	Abandoned flush
Orange	5618	7	0	2	2	2	0	0	Difficult N/S flush
Orange	5075	20	11	2	13	11	0	2	Good N/S flush
Orange	5619	18	12	4	16	12	0	4	Good N/S flush
Yellow	489	4	0	0	0	0	0	0	Difficult N/S flush
Orange	5120	9	1	2	3	0	0	3	Difficult N/S flush
Green	6125	11	0	2	2	0	0	2	Abandoned flush
Green	6113	24	12	2	14	14	0	0	Good N/S flush
Green	6085	19	3	0	3	3	0	0	Good N/S flush
Green	6104	42	11	7	18	13	0	5	Good N/S flush

Table 2 Totals - non-surgical trial - April 2000

	Response	Rec N/S	Rec surg.	Total rec	Fertilised	Degen	Unfert
Total	267	91	55	146	106	0	40
Average	17 13	6 07	3 67	9 73	7 07	0 00	2 67
Sdev	11 55	6 37	4 61	7 58	6 50	0 00	4 58
High	42	18	18	20	19	0	18
Low	3	0	0	0	0	0	0

The recipient non-surgical transfer, while successful, had a high rejection rate. If this method of transfer was to be selected then a larger than normal rejection rate would need to be included. (i.e. more recipients programmed) The time taken to perform the non-surgical transfer was quite high when compared with the surgical transfer time, and was significant when considering the two options. The difference between the surgical and non-surgical transfer pregnancy rates could not be quantified, as the number to be transferred was too small In this trial the surgical transfers held a small advantage

The technician who carried out the non-surgical flushing and transfer, Lynn Rhodes, is in our experience the best person to undertake this trial work She has many years experience with trans-cervical AI in red deer along with previous experience with non-surgical flushing and transfer Given the relatively 'new' field that this provides for any technician, we were fortunate to have been able to get the services of such an experienced person.

The pregnancy rate following the ET program was within what we consider 'normal' for hinds following surgical alone ET (12/15 pregnant or 80%)

Conclusions

Embryos can be non-surgically flushed and transferred from average sized red deer hinds However, results of this small comparative trial show that there would be a commercial advantage to still complete both the flushing and the transfers surgically

There did not appear to be any lasting reproductive effect from this procedure when looking at the natural pregnancies in the donors or recipients

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