A NEW APPROACH TO COMMERCIAL EMBRYO TRANSFER PROGRAMMES IN CANADIAN ELK

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SUMMARY

Following superovulation using FSH and controlled internal drug releasing devices (CIDR), 28 mature female elk were allocated to one of five treatment groups. The groups were : 1. a naturally-mated group; 2. females were laparoscopically inseminated once using fresh semen; 3. females were laparoscopically inseminated twice using fresh semen; 4. females were laparoscopically inseminated once using frozen-thawed semen; and 5. females were laparoscopically inseminated twice using frozen-thawed semen. Eight days after removal of the CIDR, all 28 females were surgically flushed and the number of embryos and unfertilized ova recorded.

Females that were inseminated with fresh semen produced 6.3 embryos and 0.3 unfertilized ova cf. 4.3 embryos and 1.0 unfertilized ova from those inseminated with frozen-thawed semen. Females that were inseminated once produced 6.0 embryos and 0.5 unfertilized ova cf. 4.7 embryos and 0.8 unfertilized ova from females that were inseminated twice.

Females that were inseminated once with fresh semen had significantly more embryos and significantly fewer unfertilized ova than females that were naturally mated (7.0 cf. 1.3 embryos and 0.0 cf. 4.0 unfertilized ova for inseminated and naturally mated groups respectively).

INTRODUCTION

Pure Canadian elk are still rare and relatively expensive animals in Australia. Bred for an oustanding ability to provide large quantities of venison and velvet, Canadian elk proved to be a profitable source of income in Canada and U.S.A. With very few pure elk on hand in New Zealand and Australia, there was a great expectation that artificial breeding could provide a quick way of increasing the elk population. Imported semen, sometimes of doubtful quality, proved to be a lengthy and expensive method, so most of the potential breeders turned to embryo transfer (ET) techniques.

Based on their experiences with ET in domestic animals, Canadian specialists designed and tried their first programmes using PMSG and later a mixture of PMSG/FSH as the superovulatory drugs of choice. Despite their lack of success, they were quickly followed by commercial ET units in New Zealand and Australia. The majority of these programmes proved to be very costly and commercially not viable due to very low fertilization rates of flushed ova.

After a thorough analysis of many ET programmes with elk, it became clear that most utilised a natural service, and that in 86% of cases, the mating activities were recorded around 35-60 hours after withdrawal of intravaginal synchronization devices. Because use of superovulatory drugs can produce a negative factor in semen transport within the reproductive tract of treated females and seriously diminish the chance of

fertilization, it was decided to apply laparoscopy techniques to determine the optimal time for semen deposition using intrauterine insemination with fresh or frozen-thawed semen.

MATERIALS AND METHODS

Three independent ET programmes were conducted in April and May of 1990 and 1991. In total, twenty-eight mature female elk (11 pure and 17 7/8 pure) were superovulated by administration of FSH (Folltropin-V, Vetrepharm) following CIDR G (AHI Plastick Moulding Co., New Zealand) insertion for 12 days. Because of the size of the animals, two CIDR G implants were inserted into the vagina of each female and replaced on day 8 of the treatment. FSH was administered from 60 hours before CIDR withdrawal and continued twice daily for eight injections in a decreasing manner (20, 15, 15, 12, 10, 10, 9 and 9% of total dose) to give a total FSH dose of 26 units NIH. Vasectomized red stags maintained continuous male presence.

At the time of CIDR withdrawal, the female elk were randomly allocated to one of five treatment groups. These treatments were :

1. Natural mating. Females were run with a bull elk and time of natural service was recorded.
2. Females were inseminated once using fresh semen.
3. Females were inseminated twice using fresh semen.
4. Females were inseminated once using frozen-thawed semen.
5. Females were inseminated twice using frozen-thawed semen.

In treatments 2 to 5,females were subjected to laparoscopy examinatinon every three hours after CIDR withdrawal. Close monitoring of the ovaries allowed time of ovulation to be recorded and insemination using intrauterine techniques occurred at this time.

Fresh semen was collected from mature elk bulls by electro-ejaculation, using a very tight crush (standing position), under light anaesthesia (50 mg of Xylase- I.M.). Semen for freezing was diluted with citrate/egg yolk/glycerol diluent to the ratio of 200 x 10^6 sperm/ml. Every straw of such prepared semen contained a minimum of 50 x 10^6 spermatozoa (insemination dose).

Eight days after CIDR withdrawal all superovulated females were surgically flushed and the number of total stimulating points (TSP), embryos (EMB) and unfertilized ova were recorded and analysed.

Data were analysed by least-squares methods. The initial analysis examined the effects of semen type (fresh or frozen-thawed) and number of inseminations (one or two) on responses to superovulation. A further analysis compared the effects of treatment group (1 to 5 above) on those responses.

RESULTS AND DISCUSSION

Semen Collection

The time of response to the electro-ejaculation procedure was variable, although most of the semen was collected from 5 to 8 minutes after activation of the inserted anal probe. Collections of 1.5 to 4 ml of milky to thick milky semen followed frequent urination and occasionally some yellow vesicular fluid. The density of collected semen varied from 0.9 x 10^9 to 3.2 x 10^9 sperm/ml. Fresh semen was diluted to approximately 200 x 10^6 sperm/ml and was recorded as containing approximately 90% live sperms with

75-85% progressive motility. After the freeze-thawing procedure, the semen had approximately 60-70% live sperms with 60-75% progressive motility.

Effect of Semen Type and Number of Inseminations

The effect of semen type and number of inseminations on responses to superovulation are shown in Table 1.

Table 1. Least squares mean effects of semen type (fresh *cf.* frozen-thawed) and numbers of inseminations on number of total stimulating points (TSP), embryos and unfertilized ova

	No. animals	TSP	Embryos	Ova
Mean ± s.e.		6.8 ± 0.8	5.3 ± 0.8	0.6 ± 0.2
Semen type				
Fresh	11	7.2	6.3	0.3
Frozen-thawed	11	6.4	4.3	1.0
No. inseminations				
One	10	7.0	6.0	0.5
Two	12	6.6	4.7	0.8

Neither semen type nor number of inseminations had a statistically significant effect on the number of TSP, embryos or unfertilized ova. This may be partly due to the small number of animals used in the study. Use of fresh semen resulted in almost 50% more embryos (6.3 cf. 4.3) and fewer unfertilized ova (0.3 cf. 1.0) than when frozen-thawed semen ws used. Responses to superovulation were also marginally better when only a single insemination was done. This may be because the timing of the single insemination was at the most appropriate time or may result from lower stresses associated with single insemination. From an economic point of view, a single insemination would be preferred because costs of labour and drugs are significantly reduced with a single insemination.

Effect of treatment groups

Responses to superovulation following either natural mating or one of the four insemination treatments are shown in Table 2.

Table 2. Least squares mean number of total stimulating points (TSP), embryos and unfertilized ova within treatment groups

Treatment	No. animals	TSP	Embryos	Ova
Mean ± s.e.		6.7 ± 0.6	4.5 ± 0.6	1.3 ± 0.3
Natural mate	6	6.5	1.3[b]	4.0[a]
Fresh semen/ Single A.I.	5	7.0	7.0[a]	0.0[b]
Fresh semen/ A.I.'d twice	6	7.3	5.7[ab]	0.5[b]
Frozen semen/ Single A.I.	5	7.0	5.0[ab]	1.0[b]
Frozen semen/ A.I.'d twice	6	5.8	3.7[ab]	1.0[b]

Superscripts within a column having a different letter differ significantly ($P < 0.05$)

There were no differences between any of the treatments on the number of TSP. The number of embryos retrieved using a single insemination of fresh semen was significantly ($P < 0.05$) greater than when natural mating was used (7.0 cf. 1.3 embryos). The other treatment groups also produced more embryos than the naturally mated group, though the differences were not statistically significant. The naturally mated group had significantly more ($P < 0.001$) unfertilized ova than any of the other treatment groups.

CONCLUSIONS

This study shows that laparoscopic insemination on detection of ovulation with fresh or frozen-thawed elk semen, following oestrus synchronization, can produce an acceptable number of healthy, transferable embryos.

From both a management and an economic point of view, it is suggested that only a single insemination should be used in future ET programs. Where possible, it is recommended that use of fresh semen should be used to give improved results when compared to use of frozen-thawed semen.

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