

EMBRYO TRANSFER IN DEER M Bringans



INTRODUCTION

The implications of embryo transfer in deer are very exciting. There is potential in both the importation and export of superior genetic material, without the disease complications and costs that we have experienced so far in the New Zealand deer farming industry.

The latest treatment of embryos intended for export may include 10 washes with 100 times dilution of the medium at each wash. Provided the zona pellucida is intact then this will rid the embryo of most viruses. A Trypsin wash (to digest any debris on the zona) will be necessary for some viruses (G Struthers, MAF., Pers. comm.)

Once superior female and male lines of various deer breeds have been identified, embryo transfer will be a valuable tool in improving the performance of the national herd.

The aim of this paper is to give an update on the results and treatments involved in embryo transfer in deer since E Dixon's 1986 paper entitled "**Embryo Transfer in Deer - The State of the Art**" in the NZVA Deer Branch proceedings No 3.

1. BIRTHS OF E.T. PROGENY SINCE NOVEMBER 1986.

- (i) Winton, Southland, New Zealand: 4 Pure elk calves
were born by
caesarian out of red
hinds.

Interesting points to come out of this were:-

- a Donor Elk 1 - non-surgically flushed 2 blastocysts - 2 calves
- Donor Elk 2 - non-surgically flushed 3 'good' morula - 2 calves
 1 'poor' morula - 0 calves

Obviously the hold rate of both morula and blastocysts are acceptable.

- b The spread of calving from almost the same conception date was 8 days. Also the red dam carried their pure fawns for the elk gestation length.

Births

Subsequent	Sex	Gestation	Birth wt	length
birth				
<u>Donor Elk 1</u>	Bull calf	260 days	39 lbs	20 days after
	Female	262 days	37 lbs	ET Progeny
<u>Donor Elk 2</u>	Bull calf	255 days	30 lbs	18 days after
	Female	255 days	32 lbs	ET Progeny

Note: A recipient which fails to 'hold' an elk embryo, but conceives at its next natural heat to a red stag will produce a fawn at approximately the same time as the recipients that have 'held' the elk embryos. Don't be tempted into performing a caesarian (as this author did in one case) without first checking the size of the calf's feet!

c Subsequent calvings

Donor Elk 1 - 4 January 1987 (20 days after birth of ET progeny)

Donor Elk 2 - 23 December 1986 (18 days after birth of ET progeny).

A good conception rate may be possible at the first oestrus after flushing.

d Calves born were of good weight (the natural calf of Donor Elk 1 was 40 lb) and if left till the recipient begins calving then the viability can be 100%. All four calves are thriving with no obvious size difference to the naturally born purebreds.

ii) **Australia** - 8 elk-cross animals were born naturally, resulting from transfers performed by Dixon/Hunter 1986.

2. **Results of Flushing 1986-87** (see next page).

3. **Recipient programme**

There seems to be no problem with this particular aspect of embryo transfer in deer.

E Dixon (1986) reports good success with 2 injections of lutalyse [e]¹ 11 days apart. In Taupo, New Zealand, E Dixon and J Hunter report 5 pregnancies out of 6 recipients used in 1985.

¹[e] lutalyse - Upjohn, Kalamazoo, USA

FSH - 7 F out of 2 donors
 PMSG - 3 F/2 NF out of 3 donors.

(iii) Mating - Most donors were observed mating or signs of mating were seen (unlike E Dixon's observation 1986).

In Winton, New Zealand, these were observed from 30 hours post CIDR withdrawal to 50 hours post withdrawal in non-lactating donors. An animal treated with FSH was the first noted. The one lactating donor was the last observed to mate.

In Taihape, New Zealand, the first animal was noted mating at 24 hours post CIDR removal. This was also an animal treated with FSH (G Joyce pers. comm.)

This spread of mating is also indicated by the development stage of embryos which were all collected 8 days post CIDR withdrawal. For example in Winton the embryos ranged from:

16 cell	----	morula	----	hatched blastocysts
(in a lactating donor)		(in 2 donors)		(in 1 donor)

In Taihape 2 donors had both a morula and a blastocyst. In Taupo, fertilized embryos collected ranged from early morula to expanded blastocysts.

Some interesting data on use of FSH-P comes out of this work done in Taupo 1987 (J Hunter pers. comm.). On one property 7 red deer hinds were flushed:-

Failure to superovulate	- 1
Unfertilized ova only	- 3
Fertilized ova	- 3 (7 embryos in total)

On the other property 6 red deer hinds were flushed and all 6 produced fertilized embryos (12 embryos in total). A good pregnancy rate, after ultrasonic scanning of recipients, is reported with 13 scanning positive out of 19 done.

(iv) Condition stress

We found that the quieter natured animals were more likely to be in better condition, and on rectal palpation prior to programming were less likely to have anoestrous ovaries. This obviously affects their chance of flushing successfully.

This was highlighted in Canada in October 1986. Cows were only weaned the day CIDRS were put in and then these donors were put in a small enclosure with the bulls, in a very stressful situation. Most of these cows, however, were actually seen mated.

Note: The hinds on farm 1 were mated by a stag (not treated with melatonin) with 8 pregnancies resulting. There were two sets of twins. The births were spread over 7 days in the last week of October 1986.

(ii) F.S.H. This season we conducted a trial with PMSG and FSH-P

The FSH treated donors showed more consistent flushings of fertilized eggs than did the PMSG treated donors. One batch of PMSG produced no fertilization.

			Flush Result	Bull Used	
Winton	FSH	Elk 1 (lactating)	5 F	Bull 1	
		2	not flushed	Bull 2	
			(palpable stimulation)		
		3	3 F	Bull 3	
		PMSG (Batch a)	Elk 1	1 NF	Bull 2
			2	2 NF	Bull 3
	3		0	Bull 3	
	(Batch b)	Elk 1	3 F	Bull 2	
		2	3 F	Bull 2	
	FSH - 8 F out of 2 donors				
	PMSG - 3 NF/6 F out of 5 donors				

Taihape	FSH	Elk 1	3 PF/2 NF	Bull 1	
		2	2 F	Bull 2	
		3	1 F	Bull 3	
	PMSG	Elk 1	6 F	Bull 1	
		2	2 F	Bull 1	
		3	2 NF	Bull 2	
		4	not flushed (poor stim.)	Bull 2	
		5	0	Bull 3	
		6	1 NF	Bull 3	
	FSH - 3 TF out of 3 donors				
	PMSG - 2 F out of 6 donors				

F - fertilized PF - poor fertilization (ceased development)

NF - non-fertilized

TF - transferable fertilized embryo

Winton	FSH	Red Hind 1	5 F) Same stag
		2	2 F	
	PMSG (batch b)	Red Hind 1	1 NF	
		2	3 F	
		3	1 NF	

FARM 1 : 10 hinds, CIDR in 11 days, PMSG on CIDR withdrawal

	Deer	Result
4 @ 200 IU Pregnecol	- 1	1 C.L., 1 follicle
	- 2	1 C.L., 2 follicles
	- 3	1 C.L., 1 follicle
	- 4	1 C.L.
3 @ 500 IU Pregnecol	- 1	4 C.L., 3 follicles
	- 2	1 C.L., 3 follicles
	- 3	1 C.L., 1 follicle
3 @ 800 IU Pregnecol	- 1	1 C.L.
	- 2	1 C.L., 8 follicles
	- 3	6 C.L., 4 follicles

**FARM 2 : 7 hinds 4 with CIDR for 11 days) followed by [f]²
3 with CIDR for 4 days) 1000 IU folligon**

	Deer	Result
11 days	- 1	3 C.L., multiple large follicles
	- 2	4 C.L.
	- 3	2 C.L., 3 massive follicles
	- 4	--
4 days	- 1	3 C.L., 2 follicles
	- 2	2 C.L., 2 massive follicles
	- 3	--

**FARM 3 : 8 hinds 4 with CIDr for 11 days) followed by
4 with CIDR for 4 days) 800 IU folligon**

	Deer	Result
11 days	- 1	4 C.L., 2 follicles (large)
	- 2	2 C.L.
	- 3	4 C.L., 1 follicle (large)
	- 4	--
4 days	- 1	4 C.L., 2 follicles
	- 2	1 C.L.
	- 3	1 C.L., 1 follicle (large)
	- 4	1 C.L., 2 follicles (large)

Obviously the numbers done were too small to be emphatic, but it appeared that early in the season, length of CIDR insertion was not too important, and we chose Pregnecol as it appeared not to cause the same follicle "stimulation."

²[f] Folligon - Intervet, Australia

I found in April 1986 in Winton, New Zealand, that use of the CIDR alone (inserted for 12 days in red deer hinds) did not produce oestrus and formation of a subsequent c.l. in all cases. In fact 11 recipients had to be operated on to find 6 suitable. However the 'hold' rate in those used was acceptable with 4 pregnancies out of 6 eggs implanted. It should be noted that the donors used in this transfer had been given PMSG at the time of CIDR withdrawal; so the recipients (which had their CIDR's removed at the same time) would probably have been out of synchrony with the donors by 12-24 hours.

This season, April 1987, in Winton, New Zealand, 14 recipients were used. (CIDR in for 7 days and 200 IU PMSG on CIDR withdrawal). These were all at a suitable stage for implantation when the ovaries were visualized. One recipient actually had 2 c.l.'s.

In Taihape 1987, 7 recipients (CIDR in 7 days and 200 IU PMSG on CIDR withdrawal) were surgically checked with 6 being suitable.

In Taupo 1987 (CIDR/PMS) only 2 out of 21 recipients checked were unsuitable for transfer. Heat detection is obviously not of primary concern with recipients!

Fennesy et al (1986) report that PMSG treatment following progesterone priming may tighten synchrony of oestrus and help offset any stress which may inhibit ovulation.

Note 1: The embryo is put into the lumen of the ipsilateral horn to the C.L., either by laparotomy or by exteriorization of the horn of the uterus with use of a laparoscope and small stab incision.

Note 2: Out of 200 'goat' CIDRs placed into deer this season in our practice, there was 100% retention. Out of 100' sheep CIDRs placed into deer, there was a 95% retention.

4. Donor Programme

This is still the area of most frustration in embryo transfer in deer.

(i) PMSG - a drug with both LH & FSH qualities. There may be variations in effectiveness of the drug between brands and even batches (D Marshall pers. comm.).

We conducted a trial in March 1986 in Dipton, New Zealand, with red deer, prior to programming the donors, following a protocol suggested by Dr P Fennesy.

Canada	Ovary Palpation	Condition	Flush result
Elk 1	both inactive	thin	not flushed
2	" "	"	0
3	1 C.L., one ovary inactive	"	1 NF
4	2 C.L., " " "	average	2 NF
5	1 C.L., both ovaries active	"	2 NF
6	1 C.L.	very thin	0
7	1 C.L., both ovaries active	good	1 F

(hadn't reared calf)

compared with Winton 1986

New Zealand

Elk 1	both active	2 C.L.	very good	2 R
2	" "	1 C.L.	" "	4 F

Both groups of elk were on the same programme and had the same batch of PMSG. As noted by E Dixon (1986) because of the flacid nature of deer C.L's rectal palpation is often an underestimate.

I recently palpated the elk programmed in Winton 1987 at the time of CIDR insertion and found that only one had 'anoestrous' ovaries (about the size of a large rice grain).

Note: The one lactating donor we flushed came into oestrus 72 hours post CIDR withdrawal, so was 24 hours behind the majority of matings. Fennessy *et al* (1986) reports that for lactating animals to respond to programming, lactational and seasonal anoestrus must be overcome. It would appear that provided the donor is in good condition, it may be possible to flush successfully.

(v) Flushing non-surgically as described by E Dixon (1986) is possible in elk and wapiti. With Maidens, however, it can be difficult to pass a Rusch (German) catheter through the cervix.

For example - Winton 1987 - 10 elk programmed

6 flushed non surgically, 1 flushed surgically and 3 not flushed. (1 maiden, 3 first calvers).

- Taihape 1987 - 9 elk programmed
 3 flushed non surgically (1 maiden, 2 adults)
 5 flushed surgically (maidens)
 1 not attempted.

5. Freezing

We transplanted 6 frozen red deer embryos this season into red deer recipients in a trial. By ultrasonic scanning at the 40 day stage it appears that there are 4 pregnancies. Al Saga (pers. comm.) reports that an elk morula was frozen in 1985 and subsequently thawed without any disruption to the embryo.

Conclusion

Embryo transfer in deer still may not be viable commercially. The pregnancy rate per donor overall is not high. Due to the small numbers being done worldwide, not a lot of new data is available each year, but each year I feel that some progress is being made; and that there is light at the end of the tunnel.

References

"Manipulation of Reproduction in Red Deer". P F Fennessy, M W Fisher, J.R. Webster, C.G. Mackintosh, J M Suttie, A J Pearse and I D Corson. Proceedings, NZVA Deer Branch, Course No 2.