

# General Technical

# Current Reproduction Technology As Applied To The New Zealand Deer Industry J.W. Hunter

#### Introduction

Natural mating provides the basis of on-farm reproduction in all production species. However, current understanding of the science relating to the process of reproduction has allowed the development and utilisation of techniques which manipulate and modify this These techniques have the aim of improving and advancing the natural process. productive parameters we demand of a particular species. The rapid development and commercial success of deer farming has seen the successful implementation of a number of these current techniques within the industry. There has been historical and continued research by the staff of scientific research institutions, universities, practice veterinarians and deer farmers both nationally and internationally which has made a huge contribution to the reproductive knowledge of the species in a very short time. This knowledge has been gathered from a wide number of breeds of cervidae in many countries. This paper will consider technology as applied to the breeds commonly farmed within New Zealand, all of which have a characteristic and rigid reproductive seasonality. In consideration of these processes emphasis will be placed upon the practical aspects of the implementation of these techniques currently in use along with the results and expectations.

Other than natural mating the practices to be considered are :

**Embryo Transfer (E.T. / M.O.E.T)** being defined as the result of super-ovulation and oestrus induction with fertilisation of donor females resulting in the subsequent collection of viable embryos for transfer, either fresh or after freezing, into recipient females.

**Artificial Insemination (A.I.)** as applied to deer is the timed application of semen, either fresh or thawed frozen, to the reproductive tract of an oestrus synchronised female. Semen is applied either directly into the uterus with the procedure facilitated using a laparoscope or via catheterisation of the cervix .

**In-vitro fertilisation (I.V.F) or In-vitro production (I.V.P)** of embryos is an area of reproductive technology receiving an increasing amount of research for application to cervid breeding. This technique complements ET whereby viable embryos are produced by the harvest of ova from the ovary of females with the subsequent fertilisation 'in vitro' and maturation in culture to a point where transfer is made into synchronised recipient females.

**The Hormonal Manipulation** of cervine reproductive seasonality is a practice which has the potential to be a routine farming procedure providing the farmer with the opportunity to cost effectively enhance the breeding potential of his available herd genetics.

# Artificial Insemination (A.I.)

This procedure as applied to cervids has been reported and reviewed (1, 2, 3, 4) on a number of occasions in recent years. While the use of A.I. has been reported in a number of breeds the significant results relate to the commercial breeds viz. Red deer, Fallow deer and the Elk / Wapiti. The basic methods and technology currently utilised have not changed greatly than those described. In a paper presented at this conference in 1994 (3) Asher stated that ' the application of artificial insemination within the deer industry is still in its infancy'. At this time in 1997 it may still be regarded as such with no substantial growth in the total numbers inseminated. This may be is a reflection of the status of the industry dynamics through this period rather than upon the procedure itself. Some of the results obtained from A.I. could have influenced individual decisions to utilise the technique with some poor conception rates reported. The use of A.I. however does have the potential to influence and increase the rate of genetic improvement within a herd than that given by natural mating. As selection of sires and dams for productive performance becomes more intense the need for this procedure will be more easily justified.

A.I can be used to influence the reproductive process in a number of ways:

- to greatly increase the number of offspring born each year to an individual sire
- to spread the genetic potential of a sire amongst different properties. The relatively short breeding season does not allow for great flexibility in the use of stags and does limit the potential number of offspring to be born. The dairy industry provides the best example of the use of A.I. with high production index males having a broad influence in the population base. This capability allows for the referencing of sires to compare the individual sire performance between and within different properties and their individual genetic base.
- to allow the use of sires where there is a physical inability to mate. This may be as the result of injury.
- to facilitate the international transfer of genetics. Given the risk of disease transmission by live animal import / export it is considered much more acceptable to move genetics by the use of frozen semen (or embryos).
- to allow the hybridisation between breeds which may not occur naturally
- to allow storage of semen from a genetically superior sire to enable its use in future years and as an insurance against the loss of the animal.

Factors influencing the success of A.I are:

- the quality of the semen to be used and its collection and processing.
- the technique of semen placement

- the ability of the operator to do this
- the induction and synchronisation of oestrus in the female
- the post insemination management of the female

#### Semen Collection

Semen is commonly collected from Red , Fallow, Wapiti and Elk breeds (2, 3, 4). This procedure has always been and continues to be a difficult and unpredictable aspect to overcome in the species. Limitations are; the seasonality of sperm production, the need for sires to be used during the breeding period and the wide variation in the volume density and quality of semen obtained as well as the vagaries of the freezing process it may be subjected to.

Electro ejaculation is the principal method used for the collection of cervine semen although historically other techniques such as internal artificial vaginas and dummy females have been used. (1)

Anaesthesia has generally been used to facilitate this procedure with the drug combination 'Fentazin 5' most commonly used. Recently the collection of semen from restrained standing males particularly the Elk / Wapiti breed has been achieved with success. Bringans (pers comm.) reports collecting semen from Elk bulls standing in restraint with little (25 mg Xylazine) or no tranquillisation. Last season (1996) from 100 collects, semen was frozen from 99 of these animals with straw totals ranging from 20 to 250, averaging about 100. This was loading at 40 x  $10^6$  sperm per straw. In comparison the success rate from collections using anaesthesia was much lower.

As in all species the care taken in the handling of raw semen is a major factor in the successful outcome of processing. The assessment of semen quality uses the same criteria as with other species. Deer semen freezing uses techniques adopted for other production species with the basis being a citrate egg yolk extender with glycerol added as a protectant. The semen is loaded into 0.25 ml straws and cooled progressively to a point where it is stored in liquid nitrogen. At this time a target dose of  $15 - 20 \times 10^6$  live sperm is the aim. Good quality thawed semen will be 40% plus live so a 0.25 ml straw will require to be loaded with  $50 \times 10^6$  to ensure this criteria is met. Good quality raw semen contains up to or more than  $3000 \times 10^6$  sperm per ml. Therefore a good quality raw semen collection of 1 ml would allow the processing of 60 straws for freezing. Used fresh and extended, such semen would allow for 150 doses @ 0.25 ml to be used. This gives an indication of the potential for the genetic spread of one collection from a sire.

#### Hind preparation and management

The basis of hind selection and preparation is unchanged from that reported earlier (1,2) in both Red and Fallow deer. The use of progesterone impregnated controlled internal drug release (Cidr -G, Livestock Improvement Corporation Ltd) devices for 12 days with the injection of 200 units of PMSG upon withdrawal is the treatment basis for synchronisation and heat induction in Red hinds. The use of PMSG is not required in

Fallow does. The insertion of Cidr-G devices into red hinds needs to be correct, with placement into the anterior aspect of the vagina important to their retention. In the larger elk breed the use of bovine cidrs is preferred with no great problems reported to their use. The impact of hind selection, their age and temperament along with the standard of onfarm management aspects such as nutrition and handling cannot be understated. The use of vasectomised stags prior to and during preparation has not had any recorded effect on results to my knowledge. However, given the known effect by them when treated with melatonin, on the oestrus advancement of red females it is considered worth doing where they are available. A.I. programmes are usually carried out early in the breeding season and their influence is likely to be beneficial.

#### Insemination technique

The placement of semen into the female reproductive tract is achieved by catheterisation of the cervix with or without entry into the uterus or by direct insemination into the uterine horn. The latter method is facilitated by the use of a laparoscope and is a surgical procedure requiring anaesthesia. The results achieved using these techniques varies significantly with breed. Depositing semen either through or into the cervix of Fallow and Elk / Wapiti females can result in conception rates of 60-80%. (Table 3.) However the same method used in Red deer has given poor results and the laparoscopic technique therefore is the method of choice. The convoluted anatomical features and size of the cervine cervix have some influence upon the ability of operators to pass a catheter into the uterus of Red and Fallow females. The ability and skill of the operator to carry out these procedures obviously will have some influence upon the result. With laparoscopy adverse sequelae to the abdominal trocharisation are not common but likely instances to be experienced are intestinal tract penetration, bladder penetration, haemorrhage from blood vessel damage and adverse anaesthetic response. The rectal manipulation of the cervix may result in tissue damage to the rectum, therefore operators with 'small hands' are preferred. Epidural anaesthesia may be required to facilitate the technique.

Insemination is carried out at a timed interval after removal of the Cidr device. Some variation is made depending on the breed with 52 - 54 hours considered routine for red deer, 65 hours for fallow and 60 hours for elk / wapiti.

#### **Results and Expectations**

Recent results of Red deer laparoscopic insemination follow.

# Table 1: Pregnancy rates using thawed frozen semen

Pregnancy rates of laparoscopic artificial insemination using thawed frozen semen - red deer 1995 / 1997				
Source	No inseminated	No. pregnant	%	
Hunter	·····	، ا		
Property 1	20	16	80	
Property 2	220	133	60	
Property 3	319	154	48*	
Property 4	291	185	64	
Beatson				
Property 1	56	28	50	
Property 2		71	58	
Property 3				
Property 4	218	131	60	
Walker	· ····································			
1997 / 1	173	128	74	
1997 / 2	32	17	53	
1996 / 1	20	12	60	
1996 / 2	10	9	90	
TOTALS	1498	900	60	

\* An age related comparison of conception rate was made to this programme.

Table 2

Property 3 - Age comparison of conception rate laparoscopic insemination - red deer - March 1997				
Age of hind	No. inseminated	No pregnant	%	
Rising 3	97	39	40	
Rising 4	84	36	42	
Rising 5	33	16	48	
Mixed age	105	63	60	
Totals	319	154	48	

Pregnancy rates of laparoscopic artificial insemination using fresh semen - red deer - 1996/1997				
Source	No. inseminated	No pregnant	%	
Hunter				
1996	45			
Beatson	···· · · · · · · · · · · · · · · · · ·			
Property 1	96	68	70	
Property 2	85	53	62	
Property 3	67	53	79	
	72	27	37	
Walker				
1997	24	19	79	
TOTALS	389	255	65	

#### Table 3

\* same stag - different sample

The per-cervical insemination of elk / wapiti has been reported by both Beatson and Bringans (pers comm.)

#### Table 4

Conception rates of cervical insemination in elk				
Source	No inseminated	No pregnant	%	
Beatson				
Herd A	98	58	59	
Herd B	190	126	66	
Herd C	28	18	64	
	26	20	76	
Bringans		· · · · · · · · · · · · · · · · · · ·		
1996	400		68	

tresh semen

Bringans reports following A.I. he has noted the Elk gestation length being in the range of 240 - 255 days with an average of 247 days. This being somewhat less than that reported in earlier literature as being 249 - 262 days duration.

# **Embryo Transfer**

Multiple Ovulation Embryo Transfer (MOET / ET) as a procedure is a commonly adopted method of manipulated breeding in many species and has been used in most food production animals for many years. It has become widely used within the deer industry in New Zealand after original work in the mid 1980's established the parameters for the successful programming of red deer females. A number of published papers have reported on the technique and some results since that time. In comparison with A.I. it has

the additional impact of combining the genetic potential of both the male and female individuals.

E.T. is applied in the following circumstances:

- 1. To speed up the reproductive process and get animals 'on the ground'. A good example of this was the Angora goat industry in New Zealand in the mid-1980's.
- 2. As on-farm production recording identifies genetically superior individuals ET can be used to increase the rate of dissemination of these genetics into the population. An increased selection pressure can be made upon the population. A greater number of offspring are obtained from a smaller number of selected animals which will permit a greater rate of genetic gain to be made. An opportunity also arises for a commercial return by the sale of these animals into the industry.
- 3. To facilitate the international movement of genetic material. The risks and costs associated with live animal importation can be overcome by this process. While the possibility of some disease transfer has not been proven to be eliminated by embryo transfer, it is likely to become an increasingly more acceptable means of international genetic movement.

As far as the deer industry in New Zealand is concerned ET was used initially to increase the rate of reproduction of some breeds, particularly the imported animals i.e. East European, German, and English. The reproductive capacity of this small population was increased to provide a base for selection parameters i.e. velvet or growth rate to be applied as well as for individual sales to provide some commercial return to the importers.

ET has created the opportunity for both the export of genetic material from outside New Zealand and the importation of new genetics for information into the farmed deer population. Deer embryos have been exported from N.Z to Australia ,Canada, U.S.A and Ireland. Importations to date have been sourced from England using donors of English, East European and German origin.

A limited amount of work has been carried out on Fallow deer with some success. (1.) Elk have been a difficult breed to apply MOET to. Both Bringans and Dixon report extremely variable superovulatory responses by Elk cows. However, where embryos are recovered the conception rates using Red or hybrid recipient females are similar to those found with red deer.

# Embryo Transfer - The Technology

In comparison to other production animal species deer have created a number of new challenges to veterinarians involved in ET. The breeds we have in New Zealand have evolved with a short annual breeding season and a producing a single offspring, although twinning is occasionally seen. The short breeding season places considerable pressure

upon the time available to carry out ET and yet allow the donor animal to recover and become pregnant naturally within an acceptable time frame.

Deer are temperamentally a less domesticated species than other production animals commonly used for ET. As such the complexities and stresses of the ET procedure is likely to compromise the results.

In other species of production animals ET has been successfully combined with artificial insemination to effect fertilisation. This has proved to be more difficult in deer although some success has been achieved. However, the negative aspects have so far outweighed the positive.

While some research into ET in the species has taken place (2,), a large proportion of the development has been carried out by veterinarians in a commercial environment which has precluded, to a large degree, the evaluation of "risk concepts" and other possible options.

Despite the above reservations the technique has been successfully and widely applied to a number of breeds of red deer and to a lesser extent in elk and fallow deer.

# The 'Process' of ET

This can essentially be divided into the following areas :

- 1. The reproductive manipulation with the superovulation of the donor hind
- 2. The collection and handling of fresh embryos.
- 3. The synchronisation and preparation of recipient hinds
- 4. The transfer of fresh embryos.
- 5. The freezing for embryos.
- 6. The thawing and transfer of frozen embryos.
- 7. Embryo splitting.

#### 1. The Superovulation of Donor Hinds

Following the priming of the donors with progesterone impregnated intra- vaginal controlled internal drug release device (CIDR- G', National Dairy Board Hamilton), a regime of treatment with follicle stimulating hormone (FSH) takes place. The delivery of this hormone can be achieved by an injection regime or by the use of a small osmotic pump device (Alza Corp , California, USA) placed under the skin for a given length of time. Both methods have their advantages and disadvantages.

Injections ensure the dose of hormone is received but some problems occur with the stress of repeated handling of the animals, with twice-daily treatments for four days being required. The pump devices have the potential to vary in delivery rates and in the worst situation can fail completely. However, the handling requirement is less.

The withdrawal of the progesterone treatment allows the development of 'heat', and then mating will take place. The use of A.I. with a timed insemination has not given the results that are possible with natural mating and at this stage it is not considered a commercial option.

FSH dose rates are varied depending upon the deer, breed and size. The products of choice are either "Follitropin" (Vetrepharm, Canada) or "Ovagen" (Immunochemical Products Ltd, Auckland).

#### 2. Embryo Collection

Except for elk, collection is carried out surgically, requiring general anaesthesia and aseptic surgical procedures. This takes place between six and seven days after mating when the embryos are flushed from the exteriorised uterine horns using phosphate buffered saline ('Dulbeccos Solution') via a Foley Catheter. Occasionally fallopian tube flushing will be carried out to remove embryos likely to be present in this area of the tract. The ovulation rate is assessed by visual examination of the ovaries giving some indication of numbers of embryos likely to be found. Embryos are flushed into petri dishes using warmed Dulbeccos solution (about 35<sup>°</sup> C) containing bovine serum albumin or neo- natal calf serum. They are then examined and graded visually using the same criteria as used for bovine embryos.

A species comparison of embryo recovery following treatment with FSH is shown in Table 5.

#### Table 5

Numbers of viable embryos recovered - species comparison				
Species	Cattle	Goats	Deer	
No donors programmed	214	1593	284	
No viable embryos recovered	934	17836	1426	
Average embryos per donor	44	11 2	5	

#### 3. Recipient Hind Preparation

Recipient hinds are selected on the basis of being proven mothers having produced weaners of good body weight. Preferably four years or older.

The aim is to synchronise these animals to be at the same time and stage of their reproductive cycle as the super-ovulated donors.

Hinds are synchronised with the donors using the same CIDR-G progesterone impregnated intra-vaginal devices (NZ Dairy Board). A low dose (250 units) of

pregnant mare serum gonadogrophin "Folligon" (Intervet) is given at the withdrawal of this device to enhance oestrus,

Vasectomised stags have been used to run with the recipients prior to and during the programming. It is unclear whether they have any significant effect on the conception rate obtained following embryo transfer. However, care must be taken to ensure no entire stags have access to these hinds during the programme.

#### 4. The Transfer of Fresh Embryos

Following anaesthesia of the recipient hind her reproductive tract is examined visually by laparoscope. Ovulation is confirmed by the presence of a corpus luteum on an ovary. This is followed by the exteriorsation of the ipselateral uterine horn via a small abdominal incision.

Single embryo transfer is the usual technique used bearing in mind the fact that a single offspring is normal and the growth rate of twin offspring is reduced. The embryo is transferred from the culture media using a fine catheter and placed in the uterine horn through a puncture made with a needle. The abdominal wound is closed using absorbable sutures.

It is important that post transfer stress be kept to a minimum and nutrition maintained both for recipients and donors.

#### 5. Embryo Freezing

The basic method of freezing embryos in other species has been applied successfully to deer thus allowing the transfer of genetic material between properties within New Zealand and internationally.

For freezing, embryos should be of good quality and at the post - compaction late morula or early blastocyst stage, which in red deer is about 6.5 days after mating. A 10% glycerol media is used as the cryoprotectant . Embryos are washed in trypsin media as and when demanded by international protocols.

The 0.25ml loaded straws are cooled to  $-6^{\circ}$ C at 2°C per minute, 'seeded' and taken to  $-30^{\circ}$ C at 0.5°C per minute and then plunged into liquid nitrogen where they can be stored for an indefinite period.

#### 6. Embryo Thawing

The straws are taken from the liquid nitrogen and briefly thawed in water at 30°C. The glycerol is removed in two steps, using 0.5M sucrose and the embryos are then washed in culture medium prior to transfer.

Transfer technique is the same then as for fresh embryos.

#### 7. Embryo Splitting

This technique aims at increasing the number of conceptions for a given number of embryos. A limited number of deer embryos have been successfully split and transferred. Splitting is carried out using a relatively simple piece of equipment which allows the operator to halve the cell mass of the embryo at the blastocyst stage. At this time the cell mass has the ability to reorganise itself and continue development. The split embryos are then transferred by the usual method.

# **Results and Expectations**

The present treatment regimes have allowed the following conclusions to be made. Between four and five transferable embryos per donor is the average recovery. The greatest number transferred from one donor in my experience is 27 embryos. It is usually possible to freeze three to four of the average five embryos collected.

Conception rates from single embryo fresh transfer in the deer species are very high. It is not uncommon to achieve average conception rates of 75 to 80%.

Pregnancy rates obtained from the transfer of 'fresh' red deer embryos 1996/1997 data				
	No implanted	No. pregnant	%	
Property 1	84	63	75	
Property 2	35	27	77	
Property 3	40	33	82	
Property 4	51	42	82	
Property 5	7	4	57	
Property 6	52	47	82	
Property 7	53	48	91	
Property 8	93	71	76	
Property 9	104	58	56	
Property 10	35	30	86	
Totals	554	423	76	

Table 6

The conception rates obtained from thawed frozen red deer embryos are generally lower but it must be remembered that only good quality embryos are frozen. This may have an impact statistically on the overall conception rate of fresh programmes where a number of grade 1 embryos are removed for freezing and the grade 2 or less embryos are transferred fresh. Embryos frozen for export purposes are also subjected to a trypsin wash procedure which imposes extra handling of them.

### Table 7

	No. Implanted	No. pregnant	%
Property 1	99	63	63*
Property 2	56	40	71**
Property 3	16	13	81*
Property 4	8	8	100*
Property 5	. 91	79	85**
Property 6	34	23	67*
	25		32*
Property 7	55	35	63*
Property 8	38	32	84*
Property 9	50	38	76**
Property 10	180	135	75*
Totals	652	474	72
export processe	ed embryos	69	
• •	cessed embryos	79	

\*\* = non-export processed embryos

This result (Table 7) suggests a lower conception rate in export processed embryos. The above data can be compared with that produced earlier representing results obtained over the 1989/90 period.

#### Table 8

Pregnancy rates obtained from exported thawed frozen red deer embryos - 1989/90 data					
No. Implanted No. pregnant %					
Property 1	95	62	64		
Property 2	17	11	61		
Property 3	25	18	72		
Property 4	40	20	50		
Property 5	70	42	59		
Totals	247	153	61		

A species comparison of pregnancy rates following the transfer of both fresh and thawedfrozen embryos is presented in Table 9.

#### Table 9

Pregnancy rates obtained from fresh and thawed frozen embryos - A species comparison				
	<i>Cattle</i>	Goats	Deer	
No. fresh embryos transferred	1008	1449	1472	
No pregnancies confirmed	673	1011	1156	
Pregnancy rate	66.8	57.4	78.5	
No. frozen embryos transferred	1413	755	403	
No pregnancies confirmed	892	433	282	
Pregnancy rate	66.1	57.4	69.9	

The transfer of split embryos does not appear to appreciably increase conception rates. This is due to the high conception rate achievable with fresh embryos. As such the use of this process is questionable at this stage.

The recent development of blood typing and DNA identification techniques allows for the accurate confirmation of offspring parentage at any future time. Records of the profiles of both donors and sires used in ET programmes is considered a routine requirement to ensure this can be carried out.

#### The Economics of ET in Deer

While the cost of the programme is influenced by size and complexity, some general guidelines can be given. Given the average results outlined above, total programme costs can vary from \$1000 to \$1600 per donor or approximately \$400 per embryo. Thus it is possible to get pregnancies from ET for about \$500 per conception. It must be stressed that ET is a complex procedure, any factor of which can be affected by a number of influences. The management and on-farm logistical requirements are a significant factor in deciding whether ET has a role in the breeding programme on a property. Careful consideration of all factors is a must before proceeding into ET.

The methods and techniques so far applied in deer have resulted in ET playing a significant role in the genetic progress of the species and contributing to the production aims of the New Zealand deer industry.

# In-vitro technology (I.V.F./I.V.P)

The development of this technology has been reported earlier (3). Potentially this process can deliver the same advantages to cervids as that given to more traditional farmed species. The salvage of genetics from elite production index females and the oocyte harvest from prepubertal donors along with this technology providing the basis for the preservation of many endangered species will be important aspects of its future use. There has been continuing fundamental research into this technology in the cervid species (3,

Berg. pers.comm., Bringans. pers comm.) with considerable progress made. Theoretically large quantities of embryos can be produced for ET with the possibility for manipulations such as embryo sexing, gene injection and cloning to be applied. It has the potential to overcome some of the problems associated with ET and A.I. e.g. timing of insemination , poor embryo recovery and semen quality. However, the technology infrastructure and logistics that are required at this time to allow the introduction of this process into the commercial reality of the industry is debatable. Bringans sees IVF as a key to resolving the problems with ET in Elk as well as providing a means to effectively source genetics from other feral populations.

The efficient use of sperm is a fundamental advantage of the technique. Work at Invermay has seen the production of 7 red deer calves per semen straw from IVF technology compared with 0.43 calves per straw using fixed time A.I. of red deer females (Berg pers comm). The results were similar using Canadian Elk semen. One straw of semen produced 6 hybrid calves from IVF compared with 0.63 calves from A.I. Bringans reports the birth of two IVF offspring at the same time as the donor females reached one year of age. This resulted from the prepubertal collection of ovum from calves.

Oocyte recovery in cattle is achieved by the transvaginal aspiration by needle of the ovary using ultrasound visualisation to facilitate the process. Restraint facilities and epidural anaesthesia allow this technique to be carried out twice weekly and an average transfer of 1 embryo per session can be currently achieved. However, in sheep and goats general anaesthesia with a laparoscopic recovery technique is required. This necessity has seen ovarian superstimulation used to increase the oocyte yield. Berg has also reported upon a similar technique being used in a trial at Ruakura using red deer females. Overall she has reported the average aspiration of 15.1 follicles per hind with a high quality oocyte recovery of between 1.5 and 10 oocytes per donor depending upon the stimulation response.

Individual male differences have been found in fertilisation and embryo development rates. This requires each IVF sire to be characterised using abattoir sourced oocytes before fertilising valuable donor oocytes. Current fertilisation rates range from 50 -90% and blastocyst development from 0 - 15% depending upon the sire. Berg's current work is aimed at increasing the embryo quality and yield to the blastocyst stage by developing an in vitro culture medium based upon the analysis of red deer oviduct fluid.

Bringans reports being able to consistently grow embryos to the blastocyst stage but losing a lot of pregnancies after 35 days gestation. He is achieving conception rates of 15 - 30% overall.

The problems associated with the fertility of thawed frozen IVF sourced embryos have also been identified as a further challenge to resolve.

Limitations such as the need for an on-going herd of synchronised recipients and the costs associated with the technology will determine the future role and use that the IVP of embryos has in the deer industry.

# The hormonal manipulation of the seasonal reproductive behaviour of red deer

The techniques that are used to achieve this are by the administration of exogenous biochemical and / or hormonal agents to modify the seasonal reproductive behaviour in both the male and female.

The drugs that are used are the hormone progesterone in the female and melatonin in the male. The effects of both these products upon the seasonal reproductive behaviour has been studied and reported earlier in a number of cervids (1,2,3,8).

Progesterone is commonly administered using a vaginally inserted 'controlled internal drug release' device (CIDR-G).

Melatonin is infused internally by the insertion of the coated pellet implants ('Regulin' - Schering) subcutaneously into the ear region.

From a practical aspect the use of melatonin is contra-indicated in pregnant females with the effective time frame requiring administration prior to parturition and the possibility of interference with normal foetal and mammmary development (3). As such the reproductive benefits obtained from the use of melatonin have relied upon the advancement of sexual activity in the male.

The use of both these products has been common within the deer industry for some time. The use of melatonin treated vasectomised stags within hind groups prior to mating and the synchronised mating of hinds by the use of the progesterone Cidr-G devices are two procedures that have recently been used by some veterinarians and breeders. Currently a large and on going study initiated by South Canterbury deer farmers and assisted by the GIB ('South Canterbury Deer Master') is assessing the productive effects of these procedures 'on farm' and quantifying the commercial impact any advantages gained may have. N. Beatson reports (pers comm.) on this project and has given some indication of results and progress to date.

These products are applied with the aim of :

- 1. Advancing fawning date particularly in yearling hinds. It has been known for some time that weight at weaning is related to birth date as well as mothering ability of the hind. This relies upon the the introduction of melatonin treated vasectomised stags to the hind group prior to the mating period.
- 2. Synchronising the mating of a group of adult hinds to complement the natural mating group of a particular sire. There is evidence to suggest that the currently accepted number of hinds mated to sires is conservative and can be significantly increased without compromising conception rates.

- 1. The protocol for advancement of fawning date in yearling hinds has been based upon the study of three separate groups on each property.
  - Group 1: Isolated from stag contact and conventionally mated from the 1st or 2nd week in March using yearling stags.
  - Group 2: Using melatonin treated vasectomised stags (Nov. and Dec. treated) which are run with the group in mid January and then removed. The sire stags are then introduced in the 1st or 2nd week of March.
  - Group 3: Using melatonin treated vasectomised stags (Nov. and Dec. treated) which are run with the group in mid January. Also added to the group are Cidr-G treated adult 'dry' hinds (at a 1 to 10 ratio) with the Cidr's removed early in March just prior to the removal of the teasers and the introduction of the sire stags.

All sires are removed in early May with the first pregnancy scanning being carried out in late May followed by a second scan in mid June.

At this stage birth date results from last year suggest an advantage to the melatonin treated sires and cidr'd hinds over the melatonin treated sire group only and these had an advantage over the control group. Numbers were statistically small and suggest a trend until this years results are obtained The conventionally mated group, the yearling hinds run with yearling stags had only had a small number of early fawns with some born in late October, some in early November but still a large number fawning in late December. At scanning it is not uncommon to find up to 25 - 30 % of groups of yearling hinds not in calf. It is observed that where higher conception rates are obtained the mating period is longer and there will be a significant number of calves that will be born in January.

Following the pregnancy scanning all the negatives were processed through a slaughter plant. In excess of five hundred animals had their reproductive tracts examined of which 2 -300 of these were yearling hinds. The significant feature of these yearlings was the high percentage that had poorly developed and immature reproductive tracts. There did not appear to be any weight correlation to this feature with a wide weight range of such animals.

Other details of interest from the DSP slaughter study were 2 significant findings. Rectal abscesses were noted in some lines. Some properties had none. Identified as a property problem it suggests an operator induced condition. The time of search, the type of restraint and whether rescanning is done is probably a factor in this condition. Some of these carcases were condemned.

The second finding was the numbers of pregnancies within 'dry' hinds This varied between 0 to 20% with one operator having a significant problem.

Pathological study of the reproductive tracts led to no significant findings with respect to infective or clinical abnormality. Although there were a number of hinds noted that had not bred for a number of years with some incidental pathology there was no real pattern to infertility. Blood analysis, histology and bacteriology has not suggested any common condition having a role in infertility.

2. This project aims to study and expand the mating potential of a single sire. To significantly increase the size of a mating group has an obvious contribution to genetic progress where an 'elite' sire is available. The study is evaluating mating up to 130 hinds to each stag using a group of Cidr-g treated hinds, a number of which are added to the natural mating group on a daily basis after the removal of the treatment. The protocol has allowed for up to 5 synchronised hinds to be added each day over a 2 week period mid to late March. This effectively adds 60+ hinds to the natural mating group of 60 hinds over this period.

The information and results from last year over 8 properties was such that only one 'failure ' was experienced with a stag. In all other properties it was such a success that some of these properties have this year used 3 - 4 such mating groups with up to 130 hinds being mated. There is a suggestion five matings per day is conservative and in some cases this has been increased to six per day. At this time scanning results suggest the upper limit has yet to be reached with respect to mating capacity. Natural mating groups of 100+ hinds that have been examined in comparison appear to have a greater spread of pregnancy ages than the groups running synchronised hinds. Beatson suggests that 80 hinds may be the limit to recommend and minimise the spread of calving. In cidr'd groups some have used 2 yr old stags mated to 90 hinds with 30 Cidr treated and 60 natural mated. Only very few dry hinds have been noted in this situation.

This South Canterbury Deer Master study is on-going and the information when quantified is likely to have considerable impact on future management practice within the deer industry.

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