Artificial Insemination in Deer – Practicalities, Problems and Prospects in New Zealand: Jimmy Suttie

Poor conception rates and heroic battles with rutting stags to obtain a decent ejaculate, characterised the early years of AI in Poland and New Zealand. For the last two years the need to use AI to make rapid genetic progress in the New Zealand farmed deer herd, has led to intensive research, both within the public and the private sectors. Gradually the early heroism is giving way to the establishment of routine; conception rates are increasing and the procedure looks set to become normal agricultural practice. In this article I attempt to review the situation in New Zealand and attempt to predict future problems and prospects.

Semen Procurement, Evaluation and Storage

Semen samples are obtained using either electro-ejaculation (in a crush chute or immobilised) or with an artificial vagina.

1. Electro-ejaculation in a crush chute as reported by Jerry Haigh (1985) is not normally carried out with red deer in N.Z. Instead the stags are immobilised and ejaculated while they are recumbent. At Invermay 1 ml of Fentaz (Fentanyl Citrate 10 mg/ml and Azaperone 80 mg/ml) (Janssen Pharmaceutica Beerse Belgium) is added to 5 ml Xylazine (100 mg/ml, Rompun Bayer Ltd) and 1.3 ml of the resultant solution is administered intramuscular to stags weighing about 200 kg. This produces a most relaxed anaesthesia which appears suitable for electro-ejaculation. Straight Xylazine can be used but higher dose rates (to effect) are required. The rectal probe of the ejaculator is 5.5 cm in diameter and 24.5 cm long. It is tapered for 4 cm at either end. There are 3 brass longtitudinal ventral electrodes, 1 cm wide × 15 cm long, 2 cm apart. This was specially designed for red deer by Lane Manufacturing Inc., 5560 E. Pacific Place Denver Colorado 80222 USA, telephone 303 758-5370. Electrical stimulation is provided by a Pulsator III also from the above company. Typically a stag is allowed to lie down quietly after injection with the anaesthetic. After it is recumbent its rectum is emptied of faeces and the penis is exposed. The semen is collected into a rubber cone and graduated tube surrounded by water at 38°C. Care is taken not to contaminate the sample with urine or large amounts of accessory gland fluid. There is considerable individual variation in ejaculate quantity and quality partly due to the area stimulated and partly due to electro-ejaculation procedure. Some stags always urinate while others always produce large amounts of accessory gland fluid. Good ejaculates of about 3 mls contain 4.5×10^9 sperm/ml with 85% live sperm and 75-80% individual motility. The ejaculates are cooled and kept fresh in either Sempak extender (NZ Diary Board, Hamilton, NZ) or egg yolk citrate or frozen after the addition of glycerol in egg yolk citrate. A comparison between time and motility for the two extenders in one experiment revealed that individual motility in fresh samples diminishes rapidly (Table 1) for either extender. Post thaw motility of deer semen is around 75% (85% prefreezing) and semen of less than 50% post thaw is not used for AI. Semen motility post thaw falls from 75% to 15% after four hours, so it is used immediately after thawing. At Invermay 50-60 × 10^6 sperm/0.22 ml straw are frozen.

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At Ruakura Research Centre near Hamilton, New Zealand, Dr Geoff Asher works with fallow deer. His bucks are immobilised with 5 mg of Xylazine and 5 mg Ketamine/kg live weight for electro-ejaculation. Ejaculates are low in volume (0.3 ml) but contain up to 4.5×10^9 sperm/ml. Sperm is frozen in egg yolk citrate, 100 × 10^6 sperm/0.5 ml straw. Post thaw motility of greater than 80% motility is normal. Seminal fluid is contaminated with accessory fluids if electrical stimulation is too powerful. There is some indication that ejaculates are better some 2-3 months after the rut.

Semen has also been collected from Père David stags; they are similar to red deer.

2. An artificial vagina has not as yet been used to collect semen from red deer stags in New Zealand, but an attempt is planned for this mating season to collect semen from certain nominated quiet stags. Oestrogen-primed ovariectomised hinds will be used and the stags will be trained to mount then in pens, prior to collection.

Hind Treatment Prior to Insemination

AI can either be "timed" — when the animal naturally comes into oestrus or "synchronised", if a group of hinds is hormonally manipulated to come into oestrus at about the same time. Due to the difficulties with oestrus detection in red deer, synchronisation using an intravaginal progesterone releasing device is recommended. At Invermay a 9 or 12% controlled intravaginal drug releasing (CIDR) device is inserted for 10-14 days. Retention rates are 95-100% and are much better than intravaginal sponges. In a group of 32 hinds, 27 of them (84%) were mated by a stag 2-4 days after CIDR withdrawal. Synchronisation after the breeding season has started can be achieved with two injections of prostaglandin F2 alpha analogues 10 days apart. PMSG (pregnant mare serum gonadotrophin) can be given at CIDR withdrawal. This tends to advance oestrus to 24-72 hours after CIDR withdrawal (contrast at least 48-96 hours after removal in progesterone only treated hinds).

Fallow does have been treated with a CIDR for 14 days, and then with a single injection of prostaglandin (Estrumate) on day 14.

It is normal practice at Ruakura to run vascectomised stags or bucks with hinds or does during the time the CIDRs are in position and for up to two weeks after AI. These males assist with oestrus detection if they are greased (stags) or wear a raddle harness (bucks) as well as supplying the more physical aspect of the reproduction business which AI essentially fails to give.

Insemination

Two alternative routes of insemination have been used with red deer at Invermay namely intracervical (intravaginal) and intrauterine, using a laparoscope. For intracervical insemination 25 × 10^6 thawed red deer sperm or 40 × 10^6 Pere David sperm were deposited twice; 44 and 68 hours, after CIDR withdrawal. The hinds were restrained in a crush for insemination, a speculum was used to assist with insertion of the pipette carrying the

Table 1. A comparison between sperm motility when kept fresh in two extenders (J. Webster, Unpublished Data)

	Sempak		Egg Yolk Citrate	
Time (Hours)	Gross	Individual Motility	Gross	Individual Motility
0	40	40	40	40
16	40	25-30	30-40	10
24	40	5-10	10	2-3
48	30	5		

semen. The semen was deposited in the uterus (at best), in the cervix, at the os of the cervix or in the anterior vagina (at worst). In another experiment yearling hinds were inseminated at 44 and 68 or 56 and 80 hours after CIDR withdrawal with 15 or 40 × 10^6 sperm per insemination.

Intrauterine insemination was carried out 64 hours after CIDR withdrawal. The hinds were anaesthetised with 0.4 ml Immobilon i.m. and when recumbent a small dose of Thiopentone I.V. was given. The hinds were inseminated with 10-20 × 10^6 live sperm per uterine horn using a laparoscope. About 10 minutes after insemination (to allow time for the Thiopentone to wear off) anaesthesia was reversed using Revivon.

At Ruakura 120 Fallow deer were allocated to either intrauterine or intravaginal insemination with either fresh or frozen semen (80 × 10^6 sperm per insemination). The intravaginal insemination was carried out in the crush 48-50 hours after CIDR removal. A speculum was not used and the dispensing pipette was inserted at best directly into the cervix. For intrauterine insemination the does were immobilised with the same Xylazine/Ketamine mixture as was used for electroejaculation of the bucks, and were inseminated 56-60 hours after CIDR withdrawal. The anaesthesia was reversed with Recervyl (Yohimbine).

Success Rates

Invermay has not been at all successful with AI for reasons discussed below under the Problems section. However, Noel Beatson using intracervical AI with a protocol exactly the same as described for Invermay had 50% success depending on the quality of the semen, based on non-return rate. The conception rate for fallow deer was 70% and 50% for intracervical and intrauterine AI respectively. There was no difference in success rate between fresh and frozen semen.

Problems

As mentioned above success rate at Invermay last year (1986) was poor. This may have been because the hinds had been used for a synchronisation experiment earlier in the breeding season. If so it highlights the necessity to use unmanipulated, stress free hinds for AI. It is also important to use high quality semen. Intra-cervical AI on unanaesthetised hinds demands good facilities and a great deal of operator skill and if a deer can move in the crush then it is difficult to position the dispensing pipette in the correct place.

Use of the CIDR probably drops fertility by about 10% for poorly understood reasons. The hinds/does tend to miscarry 30-40 days after oestrus induced with a CIDR. Until a simple effective technique for oestrus detection in hinds is available, synchronised insemination is a necessary evil, however.

Immobilising the stag for electro-ejaculation is not likely to be the optimal technique. It should be possible to obtain better samples more frequently using an artificial vagina technique. As the behaviour of most stags during the rut precludes close contact with humans, then techniques must be sought for effective collection of semen from stags remotely.

It appears that running a vasectomised stag/buck with the females during the period of synchronisation with CIDRs and after insemination may be worthwhile; Invermay did not do this during the last breeding season, but plan to this season.

Clearly a high standard of hygiene, cleanliness and stock handling are vital to success of AI as they are with any farming enterprise. Deer yards typically are rather dark, dusty places and interested operators would be encouraged to incorporate a readily cleanable "AI room" or "surgery" in their yard design. This room should be well lit. (Indeed it is fair comment that on NZ deerfarms the "darkroom" in the deer yards is becoming a thing of the past as deer have become more domesticated).

Prospects

A simple oetrus detection method would obviate the need for synchronised AI with its attendant problems of hormonal manipulation. At Invermay we are currently working on a system of vaginal mucus evaluation. This system seeks to examine mucus viscosity (spinbarkeit), fern pattern and colour and the way in which stage of oestrus influence these.

continued on page 15

A.I. in Deer

continued from page 9

Preliminary data suggest the day of oestrus can be reliably detected in most hinds. The problem is that day-to-day close handling is called for which commercial deerfarmers may find impractical or undesirable.

If synchronisation is essential then a more reliable system perhaps using CIDRs and/or prostaglandins must be sought. Intracervical AI, being non-surgical is optimal compared to intrauterine and research should be directed on how best to place semen and the optimal timing for insemination. The quantity of sperm per insemination requires further study to determine the minimum number required.

Techniques for semen collection must be rethought — a dummy vagina attached to a tame hind or a vaginal condom have been used in Poland and should be pursued.

Post-thaw motility of semen is often poor. Further research should seek to optimise semen handling in deer — quantity and identity of extender must be studied as well as optimal collection system.

AI in deer offers many advantages in terms of making rapid genetic progress from elite sires. Current problems are not sufficiently great that it may be predicted, with considerable confidence that a viable system of AI will be available within the next few years.

To write this article I have drawn heavily on the work and thoughts of a number of my colleagues whose assistance I gratefully acknowledge; Drs Geoff Asher, Colin Mackintosh, Peter Fennessy, Mark Fisher and Mr Jim Webster. I have deliberately attempted not to paraphrase work written up elsewhere and I refer readers to the proceedings of the Deer Branch of the New Zealand Veterinary Association Deer Course for Veterinarians particularly numbers 2 and 3. These are obtainable from Dr P.R. Wilson, Massey University, Palmerston North, NZ. (Cost $20 NZ).

Reference

Haigh, J.C., Barth, A.D., Cates, W.F. and Glover, G.S. (1985). Electro-ejaculation and semen handling in wapiti. In Fennessy, P.F. and Drew, K.R. eds. Biology of Deer Production. Bulletin No. 22 Royal Society of New Zealand, 197-203.