ARTIFICIAL INSEMINATION

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INTRODUCTION

As the deer industry develops, the emphasis will move more towards specialist venison or antler production. In a developed industry where hind numbers are stable, less than 25% of hinds will be needed to produce replacement females. For meat production, the most efficient system involves the use of a genetically large male over a genetically small female, such as a wapiti X red hybrid stag over a red hind (see Fennessy 1987). Consequently as the industry specialises and develops, there will be considerable scope for artificial breeding techniques. This paper deals particularly with artificial insemination (AI) where we see considerable potential in:

- the opportunity to greatly increase the rate of genetic progress through a large increase in the number of hinds covered by a single stag.
- the use of semen from genetically superior red deer to produce replacement females and top quality sire stags.
- the use of wapiti or wapiti X red hybrid semen over red or "megared" hinds to produce hybrids for venison production thereby obviating the need for farmers to run such sire stags.
- export/import of semen rather than live animals.
- overcoming animal health concerns.
- hybridisation between species which for "social" reasons do not interbreed freely (eg, Pere David's X red deer).

When cheap systems of semen sexing become available, the potential for artificial insemination is further increased because of the cost benefits of using semen of defined sex (eg, specifically producing hinds for sale, velveting stags, trophy stags, sire stags).

Before artificial insemination can become a routine part of deer farm management there is a considerable amount of research necessary. Particular areas include semen collection and dilution, synchronisation or detection of oestrus, timing of insemination and semen dose. Many of these topics were covered in previous conferences (Fennessy et al 1986; Haigh 1985) so this paper will simply update the situation and present some recent results.

SEMEN COLLECTION

There are basically two alternative methods for collection of semen, namely electroejaculation or natural service into an artificial vagina. The latter technique has been used successfully by Polish workers on a small scale using either a dummy or very tame hind and quiet stags (Krzywinski

and Jaczewski 1978; Jaczewski et al 1984). We are currently investigating some of the possibilities with the specific objective that any techniques be applicable on a moderate scale to enable more frequent collection from selected stags. The necessity for frequent collection of semen precludes the use of any anaesthetics currently available - hence the need for an alternative technique not requiring anaesthetics.

As a routine current technique, we do use electroejaculation on anaesthetised stags with fentanyl/azaperone/xylazine [1 ml Fentaz, i.e. 10 mg fentanyl per bottle of Rompun dry substance (500 mg xylazine) made up to 5 ml at a dose of 1.3-1.5 ml per stag] and the anaesthetic is reversed with yohimbine/lethidrone. The electroejaculator, manufactured by Lane Manufacturing Inc, 5560 E. Pacific Place, Denver, Colorado 80222 is a Pulsator III with a 240 mm long x 50 mm diameter probe. However, we certainly do not regard electroejaculation as a satisfactory means of collection on which to base large scale use of artificial insemination.

The response of stags to electroejaculation is variable with some individuals seldom providing a good sample in terms of volume and/or density while others do so consistently. Similarly, some stags almost always urinate or produce quantities of fluid from the seminal vesicles ("thick egg yolk-like" fluid). It is our opinion that with experience the success of semen collection (in terms of quality and obtaining a sample) has improved. With respect to the time of collection, very good results are generally obtained post-rut (May to June) while it can be difficult to get good collections pre-rut in March. By late June/July the semen quality tapers off.

Immediately after collection, a drop of semen is examined for gross motility (50-100x power) and a drop of semen is diluted with 1-2 drops of saline and examined under high power for individual motility. An aliquot is also taken and diluted for later determination of sperm density. If semen quality is satisfactory the rest is extended using an equal volume of Dairy Board Part A extender (egg yolk-citrate buffer). The tube of semen is then immersed in a 37°C water jacket and placed in a refrigerator to bring the temperature down to 4°C in about 90 minutes. An equal volume of 4°C Part B extender containing 16% glycerol is added prior to freezing (see Appendix A for extender recipes). Semen is routinely frozen in straws. Generally we freeze approximately 50 million sperm per sample with the expectation of a minimum of 50% post-thaw motility giving 25 million live sperm at insemination. Semen may also be frozen in pellets but in our experience, the above Dairy Board extender is not suitable, and poor post-thaw motility results. However, other workers have had some success freezing semen pellets using other extenders.

THE HIND

Successful artificial insemination depends on a highly accurate system for the detection of oestrus or a highly repeatable synchronisation procedure. The latter would allow successful insemination at prescribed times following synchronisation. There is currently no satisfactory technique for the routine detection of oestrus in red deer and we have decided not to pursue this approach at this stage. The reasons include the observation that the period of standing oestrus in hinds appears to be quite short (12-18 hours; J M Suttie and I D Corson, pers comm), and frequent yarding, with its associated stresses, would be necessary to inseminate hinds relative to standing oestrus. Consequently our approach has been to inseminate at fixed times following treatment to synchronise oestrus.

The basic insemination technique involves 12-15 days treatment with a 9% progesterone CIDR (controlled intravaginal drug release) with an injection of 200-250 iu PMSG at CIDR withdrawal. In cases where it has been recorded, most hinds exhibit oestrus 24-72 hours post PMSG treatment. Preliminary results would suggest that about 40-50% of hinds are being settled using a double intravaginal/intracervical insemination technique on the second and third days following CIDR withdrawal. In one recent such trial 75/152 hinds have been diagnosed as pregnant (day 35 pregnancy specific protein, PSP-B; G Sasser and C Ruder).

Considering the techniques used and the stage of development of AI in deer, we consider such a result as very good, although it is lower than what would be expected in a cattle AI programme. By comparison with the usual procedure for dairy cows, we use frozen rather than fresh semen (though at a higher dose) and we synchronise oestrus and inseminate at a fixed time rather than inseminating at standing oestrus. In order to overcome possible problems with the appropriateness of the timing, we have used a double rather than single insemination procedure. Further studies are planned using a single insemination and with varying semen doses. We are also planning further work in an effort to obtain a tighter synchronisation of oestrus and ovulation.

Intrauterine insemination via laparoscopy (under Rompun/Fentaz anaesthesia) has also been successful in red hinds with 15/29 hinds becoming pregnant in 1987 (pregnancy detection by rectal ultrasound at about 40 days) to a single insemination with frozen red deer semen at about 54 hours post CIDR withdrawal. Using the same technique, however, no red hinds have been confirmed pregnant to Pere David's semen. The reasons are unknown but could include semen quality or simply the difficulty of crossing the species barrier.

The choice of anaesthetic in intrauterine insemination can be important. Opiates and allied drugs are probably contraindicated because of their effects on gonadotrophin secretion (see Brooks et al 1986). This could explain the failure of the Invermay group to achieve pregnancies in hinds inseminated via the intrauterine route in 1986; these hinds were anaesthetised using Immobilon/thiopentone. Whether the thiopentone had any effect is unknown but it seems unlikely to have been an important contributor to the lack of success (Clarke & Doughton 1983). However other groups did achieve some success with Pere David X red deer hybridisation by intrauterine AI (G. van Reenan and P. Bowmar; G. Asher and D. Barnes; W. Otway and M. Anderson, pers. comm). These groups used Rompun/Ketamine or Rompun/Fentaz anaesthesia.

There are a number of other issues relating to artificial insemination which may be relevant. There has been considerable discussion about the use of two CIDR's rather than one but in our only trial to evaluate this, the pregnancy rates were the same (16/51 with one CIDR; 14/48 with two CIDR's). It is probably sound thinking to run a vasectomised stag with hinds to be inseminated on the grounds that this could conceivably improve success rates, due to the possibility of a stag effect on ovulation.

PREGNANCY TESTING

From the farmer's point of view, confirmation of pregnancy in an AI programme may well be very important. Rectal ultrasound scanning at 40-45

days post insemination is proving very satisfactory. At this stage the foetus and pregnant uterus are readily visible and there is no chance of any confusion between pregnancy to AI and pregnancy one cycle later. However to be positive about pregnancy status to AI, it is essential that "chaser" stags are withheld for at least 10 days post insemination.

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Appendix A

Semen Extender (Dairy Board)

Part A:	84 ml	2.9% sodium citrate solution
	3 gm	glucose
	1 ml	antibiotics
	15 ml	egg yolk (Fresh)
	100 ml	
Part B:	69 ml	2.9% sodium citrate solution
	16 ml	glycerol (Analar grade)
	15 ml	egg yolk (fresh)
	100 ml	