ARTIFICIAL INSEMINATION IN RED DEER

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INTRODUCTION

Interest in artificial insemination in deer has increased markedly in this last season with the commercial availability of semen from a number of stags. However, collection of semen, synchronisation of hinds and insemination must still be regarded as experimental techniques. This paper will update our own research at Invermay over the last year. Many aspects of AI in deer have been covered in previous conferences (Fennessy et al., 1986,. 1987; Haigh 1985) and in other articles (Anon 1986; Asher 1987).

LIMITATIONS

The major limitation to the large-scale use of AI in the deer industry in the future is likely to be the cost relative to the perceived benefit to the farmer. Currently both cost and the perceived benefit are largely a function of the name that a particular stag or breeder has, which may or may not be based on some real performance information. The advent of sire referencing will enable some comparisons between stags and result in a more objective ranking of potential sires than is available at present.

The total cost of AI to the farmer includes the obvious actual costs of synchronisation (including labour) plus the costs of semen, artificial insemination (and possibly pregnancy testing). However a potential hidden cost is the effect on the mean calving date due to hinds which fail to hold to AI. One way to overcome this problem is to mate hinds by AI about one cycle prior to the normal mating time (eg, in mid-March).

Our approach to research in AI is to investigate several relevant aspects with the overall objective of reducing the per pregnancy costs of AI. Therefore we have used a very simple cost model (Table 1) to compare the effects of reducing the direct costs and increasing the conception rate using various AI systems in deer.

Figure 1 shows the effect of the price of semen on the cost per pregnancy for 3 basic AI systems:

single intravaginal/intracervical with 40% conception
double intravaginal/intracervical with 50% conception
single intrauterine (by laparoscopy) with 60% conception

The conception rates used for the intravaginal/intracervical inseminations (IC) are based on recent data. However, there are insufficient data available for an accurate estimate of conception rate by the intrauterine (IU) insemination technique but a figure of 60% would appear reasonable. Using these conception rates in Figure 1, the relative costs of the different procedures can be compared. The points of intersection of the lines indicate the semen price at which it would be cheaper to change the insemination method. Where semen costs less than about $30/dose the single IC method will be cheaper than the IU method. It is also apparent that the double IC method will never be cheaper than either of the alternatives. However there are risk factors associated with anaesthesia and surgery with the IU technique which have not been costed. For example a 0.5% death rate in hinds valued at $800 raises the cost of the IU method such that the intersection point of the IU and single IC lines shifts upwards from about $30 to about $40 per dose of semen.

TABLE 1: Costs ($ per hind) used in calculations of the cost of various artificial insemination procedures

	Intrauterine (by laparoscopy)	Intravaginal/intracervical
Synchronisation		
CIDR/PMSG (single 9% CIDR, 250 iu PMSG)	7	7
Veterinary	2	2
TOTAL	9	9
Insemination		
Drugs (anaesthetic, reversal agents, antibiotics)	15	-
Consumables & equipment	7	2
Veterinary/technical & outside labour	12 (16 hinds/hr)	8
TOTAL	34	10
Overall total cost[1]	$43	$19 (single insem.) $29 (double insem.)

[1] The costings assume that 2 farm labour units are available for all procedures; their costs have not been included.

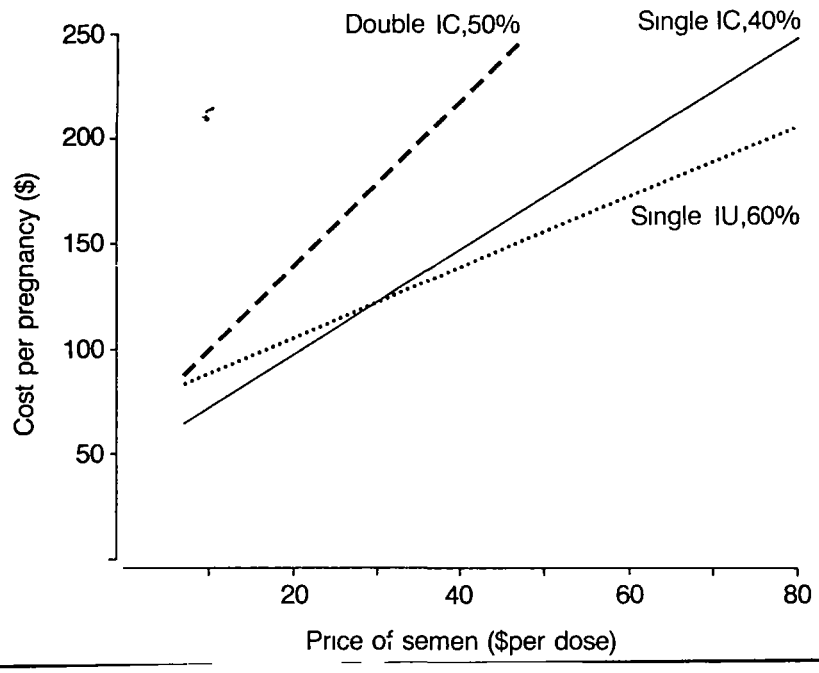

FIG. 1. The calculated cost per pregnancy as a function of the price of semen per dose for 3 basic artificial insemination systems with prescribed conception rates.

Figure 2 shows the effect of improving the conception rate from 40 to 60% with the single IC method. With semen at $25 per dose, the cost per pregnancy drops by 34%. By comparison halving the direct costs of insemination, which could perhaps be possible by greatly increasing throughput and by using a cheaper synchronisation procedure only reduces cost per pregnancy by 22% with a $25 semen dose at a 60% IC conception rate. As the cost per dose of semen rises, the benefit of improving conception rate also increases such that the proportional advantage remains about the same. However the proportional advantage of reducing costs diminishes as the two lines are parallel. Consequently, in our research, we are particularly interested in identifying factors which improve the conception rate, and thus substantially reduce the costs per pregnancy.

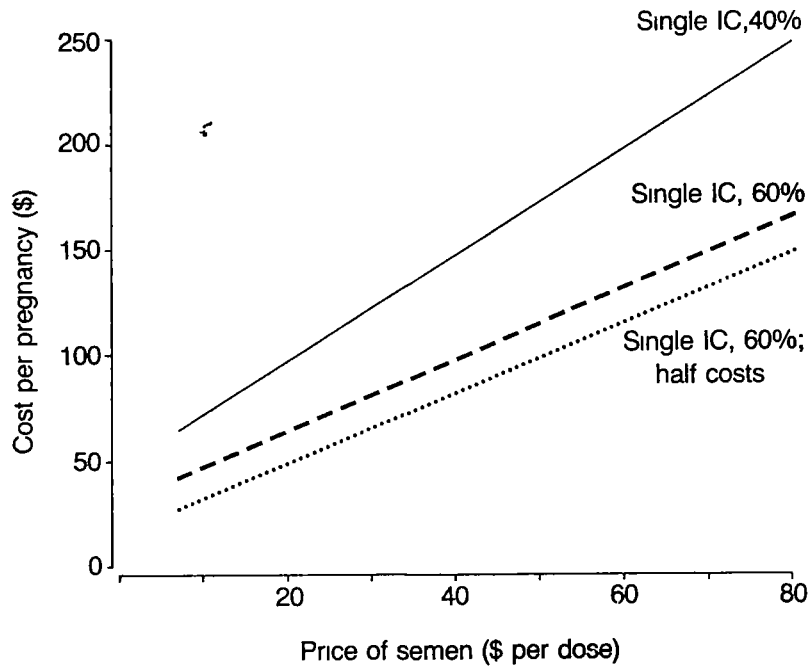

FIG. 2. The calculated cost per pregnancy as a function of the price of semen using a single intravaginal/intracervical insemination with different conception rates and costs.

The interaction of semen price with conception rate is also of importance for the semen sellers. For example, if reducing the semen dose resulted in a drop in conception rate from 60 to 40% using the single IC method, then the value to the farmer would decline. A $50 dose at the 60% conception rate would only be worth about $28 per dose at 40% conception. The converse is also true: if the quality or quantity of semen is improved so that conception rates are raised from 40% to 60% then the seller might be justified in nearly doubling its price. Consequently the effect of semen dose on conception rate and the efficiency of collection of semen from stags are matters of considerable importance to profit for the semen sellers, but of less direct importance to the users of the semen, except in as far as they affect the price of semen per dose. Obviously the price of a straw of semen must reflect not only the perceived value of the stag but it must also reflect the ability of that semen to achieve a reasonable conception rate when used with acceptable AI procedures.

SYNCHRONISATION OF OESTRUS

The overall objective of our research is to increase the conception rate to a single timed insemination (either IC or IU). However a wide spread of oestrus in response to the synchronisation treatment would be expected to limit the conception rate when hinds receive only a single insemination. Therefore our aim is to devise synchronisation methods which will produce a tight pattern of oestrus so that insemination can be better timed to produce higher conception rates. In practical terms, the treatment must synchronise the regression of the corpus luteum (CL) or delay the onset of the major endocrine events leading to ovulation. Therefore the two basic alternative approaches are to use a luteolytic agent to synchronise CL regression or to use progesterone to delay the onset of ovulatory events.

The success of any prostaglandin treatment is dependent on the presence of a CL at the time of treatment. In practice, two injections spaced about half a normal cycle length apart are necessary to ensure that all hinds are treated at an appropriate stage of CL development, since the early CL is apparently refractory to prostaglandin-induced luteolysis (Glover 1985). The use of prostaglandin prior to the start of the normal breeding season cannot be recommended unless hinds have been treated to ensure that they have ovulated and have a functional CL. The alternative method involves delaying the onset of the ovulatory events. Progesterone treatment achieves this although the level of circulating progesterone actually necessary to suppress ovulation in the hind is not known. However an approach which mimics the levels observed during the normal luteal phase should be adequate. In this respect it appears that the plasma progesterone levels in some individual hinds over the last few days of a 12 day 9% CIDR regime may be lower than the normal luteal phase levels (M.W. Fisher et al. unpublished data). Therefore replacing the CIDR at day 9 is a possible approach to ensuring a high level of progesterone over the concluding stages of the treatment in all hinds in the group. The use of a gonadotrophin at or near the end of the progesterone phase also offers theoretical possibilities for improving the timing of ovulation in that it provides an exogenous source of hormone which should stimulate follicular growth and the other events leading to ovulation - hence the use of PMSG which has both LH-like and FSH-like activity, or gonadotrophin-releasing hormone (GnRH).

With that theoretical background, we will now consider the possibilities for improving the synchrony of oestrus in a group of hinds with the objective of improving conception rates to a single insemination at a particular time. The synchronisation method used by most of the groups involved in deer AI in New Zealand is the simple progesterone CIDR/PMSG method (12 day 9% CIDR with 200-250 iu PMSG at withdrawal). There is evidence with sheep that PMSG depresses fertility at the induced oestrus compared with progesterone alone (Harvey 1987; Saywell et al., 1987; Table 2). In deer PMSG has been used for three main reasons. The first is that hinds are often inseminated prior to the start of the normal breeding season, when progesterone/PMSG is known to improve the incidence of ovulation in hinds compared with progesterone alone (Fisher et al., 1986). The second major reason is the feeling that PMSG may overcome "stress" problems in hinds which might otherwise not ovulate due to suppression of LH/FSH release from the pituitary. The third reason is that PMSG, in providing exogenous FSH- and LH-like activity, may reduce the time span of oestrus and ovulation in the group of hinds to be inseminated. However in this respect the data for other species are equivocal (see Roche et al.,

1981). There is evidence in sheep that PMSG reduces the time from progesterone withdrawal to oestrus, with increasing doses from 0 to 750 iu reducing the interval from 49 to 25 hours in one study (Boshoff et al., 1973). Consequently if time of oestrus is influenced by PMSG, then the appropriate timing of insemination will likely be a function of PMSG dose. Although there are no published data for deer, some limited evidence suggests that the time of oestrus is advanced in hinds treated with progesterone/PMSG compared with progesterone alone (M.W. Fisher et al. unpublished data).

TABLE 2: Non-return rates of ewes after intravaginal insemination (n=1150; Harvey 1986)

Synchronisation	Non-return rate %
Natural cycling (AI at oestrus)	72.4
CIDR	70.0
CIDR + 275 iu PMSG	62.3

A further factor to be considered in the synchronisation procedure is the length of progesterone treatment. In studies with cattle, long periods of progesterone have been used in order to give more precise control of ovulation, but at a cost in terms of low fertility (eg, Smith 1978; Roche et al., 1981). Limiting the duration of progesterone treatment to 9-12 days in cattle has overcome the problem of low fertility (Roche 1974; Smith 1978) although as a result there is often a problem of delayed oestrus with such treatments. In particular the stage of the oestrous cycle at the start of treatment can affect the response, and oestrogens have been used to overcome this problem in that they shorten the oestrous cycle (Wiltbank 1966; Roche et al., 1981; Smith 1978). This is much less important if the synchronisation treatment is started prior to the onset of the reproductive cycle; this is a further advantage of inseminating early in the season. One practical approach which is likely to ensure that very few hinds are cycling at the start of treatment is to wean in early March and insert CIDRs at the same time.

Therefore the overall approach to devising a more satisfactory synchronisation procedure involves testing a number of alternatives. Consequently we require a simple method for more accurately pinpointing the time of oestrus so that we can actually compare methods of synchronisation, in terms of their effect on the time of oestrus in groups of hinds. While the most direct method involves using a vasectomised stag, this is not feasible in a large scale research project. Therefore we conducted a small experiment this year where we synchronised hinds with the standard CIDR/PMSG treatment and recorded three factors which might provide indirect assessments of oestrus and/or ovulation. They were electrical resistance of vaginal (cervical) mucus (relative to the value recorded at CIDR withdrawal), cervical penetrability using an inseminating pipette and the stretch of cervical mucus (spinbarkeit). The results are presented in Table 3.

TABLE 3: Mean values for vaginal resistance (relative to the baseline at CIDR withdrawal/PMSG treatment), cervical penetrability (1-3 scale)[1] and the number of hinds with mucus with stretch of >2.5 cm according to time following progesterone/PMSG synchronisation.[2]

Time following PMSG treatment (h)	Relative vaginal resistance (% of baseline)	Cervical penetrability	Cervical mucus
20	86	1.0	0/6
32	75	1.2	0/6
44	63	1.4	3/6
56	69	1.8	3/6
68	49	1.3	3/6
80	85	1.1	3/6
92	80	1.2	0/6

[1] Penetrability: 1, cervical os; 2, mid-cervix; 3, intrauterine.

[2] 8 hinds were used, each hind being tested on either 5 or 6 occasions from 16 to 96 hours after treatment, with 2 hinds being tested every 4 hours; thus 20h is the mean for 2 hinds each at 16, 20 and 24h (ie, 6 in total).

The data reveal a clear pattern with the lowest vaginal resistance, the highest cervical penetrability and the presence of stretchy mucus at around 44-68 hours post CIDR withdrawal/PMSG treatment. When the stretchiness of cervical mucus in individual hinds was compared with vaginal conductivity, it was apparent that the two factors were inversely correlated. However, from an experimental perspective, electrical resistance of mucus has advantages in that a measurement can be taken on all hinds, whereas this is not the case with cervical mucus, where a sample of mucus is not always available. The variability in the baseline vaginal resistance (79 $\pm$ 9, SD; range 68-94) means that a satisfactory baseline value may be required for each hind. The influence of vaginal infection/irritation on electrical resistance must also be considered in future work. The results are encouraging and suggest that vaginal electrical resistance may be developed as a useful method for determining the time of oestrus, although obviously pregnancy rates are the only final arbiter. Such a technique involving recording of changes in vaginal resistance/conductivity of vaginal (cervical) mucus to indicate oestrus has been used in other species including cattle and pigs (Leidl and Stolla 1976; Heckman et al., 1979).

In considering new approaches to synchronisation of oestrus, there are a number of alternative procedures to be evaluated including prostaglandins and also those which might give a higher progesterone level and therefore a greater drop in progesterone at withdrawal (eg, CIDR replacement at day 9 or 10 in a 12 day regime).

INSEMINATION AND PREGNANCY RATES

As a result of a process of guess work, trial and error the simple progesterone CIDR/PMSG treatment with a double IC insemination at 44 and 68 hours after progesterone withdrawal/PMSG has been adopted as a standard procedure (Fennessy et al. 1987). This season we conducted experiments comparing a single CIDR with CIDR replacement (the original CIDR was replaced with a new CIDR at day 9) and single IC inseminations at different times following synchronisation with progesterone/PMSG. In all our studies, a vasectomised stag was run with the hinds from the time of CIDR withdrawal/PMSG treatment to the time when the follow-up stag was introduced (about day 12 after insemination). While there is no specific evidence that the presence of the stag affects conception rate to AI, there is apocryphal evidence for a slight improvement in goats.

In Experiment 1, 33 aged hinds were inseminated by the IC method 48 hours after synchronisation, having been allocated to single CIDR or CIDR replacement groups. There was a suggestion that the conception rate was higher in the CIDR replacement group (8/16 or 50%) than in the single CIDR group (5/17 or 29%). Experiment 2 also featured a comparison of single CIDR and CIDR replacement, but with double IC inseminations (Table 4). The conception rates were virtually the same in each group (55 and 60% respectively with 20 hinds per group). The use of CIDR replacement results in a higher progesterone level at CIDR withdrawal; this could result in a tighter pattern of oestrus than that following the single CIDR treatment. However, unlike the situation in Expt. 1, where a single IC insemination was used, it is theoretically possible that the double insemination could have overcome any disadvantage of low progesterone at CIDR withdrawal. Table 4 also includes the data for conception following a single insemination at 36 to 68 hours after synchronisation. The rates are similar at 36, 44, 52 and 60 hours (37 to 43%) but are much lower at 68 hours (only 6% or 1 of 16 hinds). The 41% achieved with each of the single IC inseminations over the 36-60 hour period, and the observation that the sum of the 44 and 68 hour single inseminations did not reach the 58% level recorded for the double IC inseminations raise questions as to whether the actual amount of semen present may be influencing conception rates in the double IC treatments, as these hinds received twice the quantity of semen given to the single IC hinds.

The quality of semen can also have a considerable effect on pregnancy rates although defining a consistently useful measure of quality other than the actual conception rate is proving more difficult. For example in Experiment 2, 4 batches of red deer semen were used, with 3 of the 4 batches producing a 45-50% pregnancy rate. However the fourth batch (incidentally a different collection from one of the same stags) produced only a 23% (7/30) conception rate. Thus, developing improved methods of quality assessment of semen is an area which could be expected to yield useful data to enable an improved conception rate. Currently, motility at thawing and in some cases, motility following a period of incubation are being used.

TABLE 4: (Experiment 2) Pregnancy rates[1] for various intravaginal/ intracervical insemination protocols following synchronisation with progesterone/PMSG

Treatment	Pregnancy rate	
	Number	Proportion
Double insemination (44 and 68 h after PMSG)[2]		
Single CIDR	11/20	0.55
CIDR replacement	12/20	0.60
Single insemination (time after PMSG)		
36 hours	7/17	0.41
44	7/17	0.41
52	7/16	0.43
60	6/16	0.37
68	1/16	0.06

[1] Pregnancy detection by rectal ultrasound scanning at c. day 40 after insemination.

[2] 9% CIDRs (340 mg progesterone) inserted for 12 days with 250 iu PMSG (Folligon, Intervet) at CIDR withdrawal; in the CIDR replacement group, the CIDR was replaced on day 9.

SEMEN COLLECTION AND PROCESSING

The efficiency of semen collection is an area where considerable improvements can be made. Currently most semen is being collected by electroejaculation of stags under anaesthesia. The use of drugs obviously involves risk, including both the direct risk of anaesthetic complications and the resultant stress on stags due to greatly reduced feed intake over the 3-4 days following the xylazine anaesthesia. Therefore improved methods of collection by natural service would have a major impact.

Semen processing is also an important area. We process straws with 50×10^6 sperm/straw aiming for 25×10^6 post-thaw. However, as yet there are no data on the relationship of conception rates to numbers of live sperm at insemination. Improved methods of freezing and preservation would produce higher post-thaw motilities, while a reduction in the number of sperm per insemination would allow more hinds to be inseminated per collection. However improvements in semen technology will likely have greater impact for semen producers than for those using AI.

CONCLUSIONS

There has been considerable progress in AI developments for the deer industry in the past 2-3 years. Further substantial improvements in conception rate and semen collection and processing can be expected. The impact of improvements in the latter will flow mainly to semen producers although greater use of superior stags will also improve the rate of genetic progress in the industry.

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