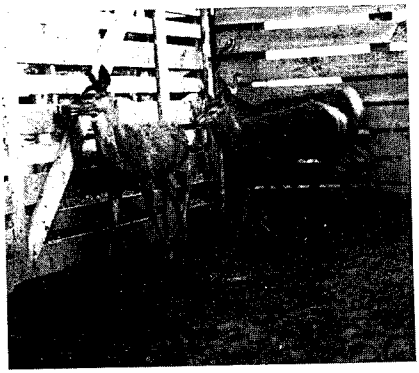

Techniques of Oestrous Synchronisation and Artificial Insemination of Farmed Fallow Deer and Red Deer

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Summary

- Controlled breeding and artificial insemination offer the deer breeder the opportunity to rapidly introduce desirable genetic material into the herd and to increase profitability on the farm.
- CIDR devices are more suitable than prostaglandin for the synchronisation of oestrus and ovulation of Fallow and Red deer. The administration of PMSG at or near CIDR device removal is routinely practiced with Red deer. However, for Fallow deer the exogenous gonadotrophin is believed to reduce conception rates and increase embryonic mortality.
- Semen collection from Fallow deer bucks and Red deer stags is performed by electro-ejaculation. Recently, an internal vagina has been developed for semen collection from Fallow deer bucks. The new device will reduce the health risks on the animals, increase the frequency of semen collection per buck and improve the quality of semen collected.
- Laparoscopic intrauterine insemination is currently the preferred method of artificial insemination for both Fallow deer and Red deer. Conception rates in the range of 70-75% are commonly achieved following the placement of relatively low doses (20-25 x 10^6) of frozen-thawed spermatozoa.
- Rectal ultrasonography is a reliable technique that can be used to estimate conception rates following artificial insemination.

Introduction

Fallow deer and Red deer are the major deer species farmed in New Zealand, Australia, North America and Europe. In the short history of deer farming, considerable improvement in the reproductive efficiency of both species has been achieved by suitable adaptation to the methods of breeding, feeding and management that are generally applied to other domestic farm animals. However, in recent years there has been international recognition of the genetic and reproductive gains that can arise from appropriate application of controlled breeding and artificial insemination programmes. Such programmes allow for a more rapid spread of desirable genetic material than would be remotely possible by natural mating. This is particularly important when considering rare genotypes such as the Pere David's deer, Mesopotamian Fallow deer or imported blood lines. Moreover, artificial insemination provides a safer and cost-effective means of international exchange of semen. There is also the important possibility of employing artificial insemination to identify genetically superior sires (e.g. sire referencing schemes).

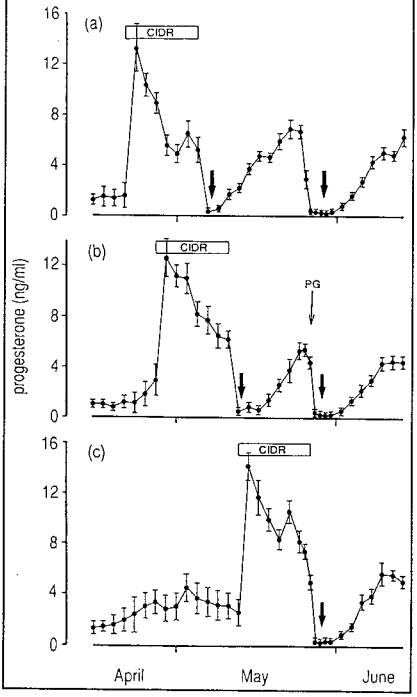
This paper reviews recent research and commercial advancements of oestrous synchronisation, semen collection/processing and artificial insemination techniques for Red deer and Fallow deer.

Oestrous Synchronisation

Natural oestrous detection in Fallow deer and Red deer can be performed by using stags or bucks fitted with ram mating harnesses and crayons. However, fixed-time artificial insemination following

oestrous synchronisation is more practical and cost-effective than following detected natural oestrus. Synchronisation of oestrus and ovulation can be achieved either by simulating the activity of the corpus luteum through the administration of progesterone or by shortening the luteal phase of the oestrous cycle by administering a luteolysin (Figure 1). For Fallow deer, it is also possible to obtain a high degree of synchrony of a return oestrus following artificial synchronisation of the first oestrus (Figure 1). However, within the framework of the potential breeding season of Fallow deer, there is little scope for utilising the return oestrus without accepting the consequences of fawns born late in summer.

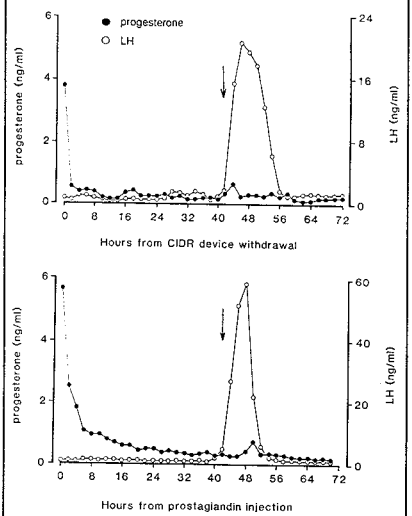
Fig 1: Three methods of artificial oestrous synchronisation in farmed Fallow deer (Asher and Thompson, 1989). Profiles of mean (± s.e.m.) plasma progesterone values of Fallow does (n = 5 per profile) during (a) initial 14 day CIDR device followed by a 21 day oestrous cycle; (b) initial 14 day CIDR device followed by i.m. injection of prostaglandin analogue on Day 13 of the subsequent cycle; (c) 14 day CIDR device treatment alone. Arrows indicate the mean time to onset of oestrus.



CIDR Devices in Fallow Deer

The intravaginal CIDR [controlled internal drug releasing] device has been comprehensively tested for its efficacy in synchronising oestrus in Fallow deer does. The device is commonly inserted for 14 days with a very high retention rate (98-100%). During insertion it elevates blood progesterone concentrations to levels comparable to those observed during the mid-oestrous cycle (Figure 1). Clearance of exogenous progesterone from the blood stream following CIDR device removal is very rapid and occurs within two hours (Figure 2). This stimulates an increase in luteinising hormone secretion from the pituitary gland, culminating in the onset of oestrus and the pre-ovulatory LH surge 40-55 hours later. Ovulation occurs about 24 hours after the onset of oestrus.

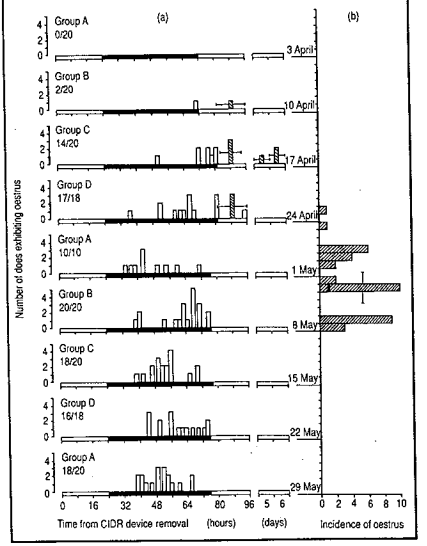
Figure 2: Profiles of plasma progesterone and LH concentrations of individual Fallow does following CIDR device withdrawal or prostaglandin injection. The arrows indicate the time of the onset of oestrus (Asher and Thompson, 1989).



However, recent studies have shown that the efficacy of the CIDR device in synchronising oestrus, in terms of the proportion of does exhibiting oestrus/ovulation and the degree of synchrony achieved, is clearly dependent on season. C J Morrow (unpublished data) has shown that the

incidence of oestrus is low (0-10%) following CIDR device withdrawal just prior to the onset of the natural rut (i.e. period of first spontaneous oestrus). However, the proportion of does exhibiting oestrus increases, and the mean time to onset of oestrus progressively decreases as CIDR devices are removed progressively later relative to the occurrence of first spontaneous oestrus within the herd (Figure 3). Best responses appear to occur after the period of first spontaneous oestrus, at which time the mean interval from device withdrawal to onset of oestrus is between 48 and 58 hours (Asher and Thompson, 1989).

Fig 3: (a) Frequency histogram of the time to onset of oestrus from CIDR device withdrawal at seven day intervals, plotted relative to (b) the incidence of first spontaneous oestrus observed for contemporary Fallow deer does. The solid portion of the abscissa axes represents the period of continuous observations following CIDR device withdrawal, while the shaded bars indicate observations of crayon mating mark (+ time ranges for onset of oestrus). For each treatment group in (a), the proportion of does observed to exhibit oestrus is presented (C J Morrow, unpublished data).



The use of PMSG at or near CIDR device withdrawal is presently not recommended for Fallow deer. The administration of 500 i.u. (Asher and Smith, 1987), 200 i.u. (G W Asher, unpublished data) or 100 i.u. (H N Jabbour, unpublished data) PMSG at CIDR device withdrawal results in a high proportion of does either exhibiting multiple ovulations or completely failing to ovulate. This reduced the conception rate to natural mating and increased the incidence of embryonic mortality (particularly with multiple foetuses). More recent studies on artificially inseminated does have indicated that the administration of 50 i.u. PMSG at CIDR device withdrawal reduces the interval to the onset of oestrus and induces greater oestrus synchrony compared with CIDR devices alone, although fertility was not enhanced (H N Jabbour, unpublished data).

CIDR Devices in Red Deer

The duration of CIDR device insertion for Red deer normally ranges from 12 to 14 days (Fisher et al., 1986; Asher et al., 1988b). Peripheral plasma progesterone profiles of Red deer hinds following the insertion of single type-S or type-G CIDR devices differ from those of Fallow deer. Within the first six days of insertion, concentrations are comparable to those observed during the luteal phase of the oestrous cycle (2-3 ng/ml). However, progesterone concentrations decline to <1.0 ng/ml by Day 14 (Jopson et al., 1990). Although this has resulted in acceptable conception rates (>50%) to artificial insemination (Fennessy et al., 1990), the use of single CIDR devices has raised questions about the effectiveness of such treatment to inhibit follicular development during CIDR device insertion in Red deer. This has prompted the use of double CIDR devices (Asher et al., 1988b) or CIDR device replacement on Day nine (Fennessy et al., 1990). However, this has not improved conception rates following artificial insemination (Bowen, 1989; Fennessy et al., 1990).

The administration of 200-250 i.u. PMSG at or near CIDR device withdrawal has become routine practice in Red deer. This is believed to reduce the spread in the time to onset of ovulation in groups of hinds (Fennessy et al., 1989) and improve the incidence of ovulation in hinds, particularly when treatment is applied prior to the onset of the breeding season (Fisher et al., 1986; 1989). Moreover, it is believed that extra gonadotrophic stimulation is essential to offset possible reduced incidence of ovulation due to stress effects of handling (Fennessy et al., 1989). However, this has not been demonstrated conclusively for Red deer, although it has been shown that significant quantities of progesterone are secreted by the adrenal glands of Red deer (Jopson et al., 1990) and Fallow deer (Asher et al., 1989). However, long-term effects of the transient increase (one to two hours) in peripheral plasma progesterone

around the time of CIDR device removal on follicular development seems unlikely.

The effects of PMSG on oestrus/ovulation synchrony and fertility of Red deer requires further investigation. Recent studies indicated that the administration of 200 i.u. PMSG at CIDR device withdrawal reduced the mean interval to the onset of oestrus (37.4 hours; n = 7) compared to CIDR device withdrawal alone (44.4 hours; n = 7) but did not necessarily reduce the variance (s.d. = 5.3 vs 2.3 hours). Moreover, PMSG treatment did not alter the time to onset of ovulation from oestrus. For both treatments, ovulation occurred between 20-28 hours after the onset of oestrus (G W Asher, unpublished data). However, the administration of 200-250 i.u. PMSG to Red deer hinds is believed to increase the incidence of multiple ovulations (G W Asher, unpublished data). While this has occasionally resulted in conception and births of twins in artificial insemination programmes (Asher et al., 1988b) this is not correlated with major production losses through reduced fertility and increased embryonic loss.

Prostaglandin

The effectiveness of prostaglandin F_{2a} (or one of its analogues) to synchronise oestrus is dependent on the presence of an active corpus luteum at the time of treatment. This limits the use of the luteolytic hormone for oestrus synchronisation programmes of Red deer and Fallow deer to the period after the onset of natural ovulatory activity (i.e. the rut). Moreover, studies on Wapiti deer indicate that the cervine corpus luteum may not be responsive to prostaglandin treatment before Day 11 of the oestrous cycle (Fennessy et al., 1989), necessitating the administration of the hormone either at the correct stage of the oestrous cycle or in a twin injection regime at least 10 days apart.

The administration of a single injection of prostaglandin analogue (500 mg cloprostenol) on Day 13 of the oestrous cycle of Fallow deer results in premature regression of the corpus luteum, clearance of endogenous progesterone from the peripheral system within 14 hours, and return to oestrus at an average interval of 43 hours (Figure 2).

More recent studies have shown that ovulation occurs 24 hours after the onset of prostaglandin induced oestrus in Fallow deer (Asher et al., 1990a). The administration of 50 i.u. PMSG at the time of prostaglandin administration has been shown to reduce the mean (± s.e.m.) time to onset of oestrus (33.5±1.7 vs. 47.1±2.9 hours), but the gonadotrophin does not affect the interval between oestrus and ovulation in Fallow deer (H N Jabbour, unpublished data).

Although earlier observations indicated a reasonable level of fertility following prostaglandin synchronisation in this species, recent application of the luteolytic agent to artificial insemination programmes has resulted in lower conception rates than observed following treatment with CIDR devices. In one study, the conception rate following laparoscopic intrauterine insemination was 52.9% (27/51) for prostaglandin-treated does (G W Asher, unpublished data). In this study, initial synchronisation of oestrus/ovulation was performed with CIDR devices which may have been inserted too early in the season for an optimum ovulatory response (C J Morrow, unpublished data). However, a similar trend was observed following cervical insemination in a study conducted three weeks later (H N Jabbour, unpublished data). In this case the conception rate following prostaglandin administration was 40.7% (11/27), compared with 84.5% (22/26) following CIDR device withdrawal, even though inseminations for both treatments were performed at similar intervals from the mean onset of ovulation.

The application of prostaglandin for oestrous synchronisation of Red deer has not been fully investigated. Haigh (1984) reported a 41% conception rate (16/41) for Wapiti type (Elk x Red deer) hinds following treatment with prostaglandin at 13 day intervals and intrauterine insemination with Wapiti semen at 72, 84 and 96 hours after the second prostaglandin injection. In another study only 7.7% (1/13) Red deer hinds became pregnant following double prostaglandin treatment and natural mating. Whereas 84.6% (11/13) of contemporary hinds became pregnant following treatment with medroxyprogesterone acetate intravaginal sponges for seven days, it is probable that the latter experiment was conducted relatively early in the breeding season for prostaglandin to effectively synchronise oestrus and ovulation.

Semen Collection

Semen collection from male deer is one of the major factors limiting the widespread application of artificial insemination. First, semen collection from Red deer stags and Fallow deer bucks is highly seasonal due to circannual pattern of spermatogensis (Lincoln, 1971). This limits the semen collection season to a four to six month period starting around the onset of the natural rut.

Secondly, the intractable temperament of male deer has prevented the application of more natural

methods of semen collection commonly used for other livestock species. To date, semen collection from Red deer stags and Fallow deer bucks has been performed generally by electro-ejaculation while the animals are under general anaesthesia (Asher et al., 1987; Fennessy et al., 1987). In addition to the obvious health risks to valuable sires from chemical immobilisation, electro-ejaculation limits the frequency of semen collection and generally produces semen of lower quality than that collected by natural methods.

Polish researchers have designed external artificial vaginas that were successful in obtaining ejaculates from Red deer stags (Krzywinski, 1976; Krzywinski and Jaczewski, 1978). However, the techniques generally require a high level of stag training and habituation. This has limited the widespread application of the technique because of limited opportunities to train stags of high genetic merit.

Jabbour and Asher (1990) describe the development of an internal artificial vagina for Fallow deer, but also having potential application in other cervid species. For semen collection with the artificial vagina, ovariectomised Fallow deer does are treated with CIDR devices for six days and 0.05 mg oestradiol benzoate (OBD) 24 hours after CIDR device removal. The does are fitted with the internal artificial vagina at the mean time to onset of oestrus, generally 18-24 hours after ODB injection (Jabbour et al., 1990) and then exposed to the bucks within their pastoral environment. Following mating, the artificial vagina is removed and the semen is aspirated and assessed for quality. While this technique has yet to be fully evaluated, the potential advantages over electro-ejaculation include reduced risk to the bucks, more frequent semen collections per buck and the potential for obtaining ejaculates of higher quality.

For cryopreservation, Red deer and Fallow deer semen is extended in sodium citrate-egg yolk-glycerol diluent and frozen either as pellets on CO_2 ice (Mulley et al., 1988; Mulley, 1989) or in 0.25 ml straws in liquid nitrogen (Asher et al., 1988a). The current techniques seem very suitable for Fallow deer semen with post-thaw recovery rates in excess of 70% of pre-freezing motility rates commonly achieved (Asher et al., 1990b). However, post-thaw motility rates of Red deer semen appear to be highly variable, both between stags and between consecutive ejaculates from the same stag (Fennessy et al., 1990). This warrants further investigation into cryopreservation techniques for this species.

Artificial Insemination

Artificial Insemination of Fallow deer

(a) Intravaginal/Intracervical Insemination: Earlier attempts at intravaginal insemination of Fallow deer does with 85 x 10^6 motile spermatozoa in the os cervix have resulted in conception rates ranging from 38% to 80%, (G W Asher, unpublished data). However, more recent work indicated that the success rate to intracervical insemination may be dependent on the method of oestrous synchronisation, the timing of insemination and the number of spermatozoa deposited (H N Jabbour, unpublished data). Intracervical insemination of approximately 140 x 10^6 motile frozen-thawed spermatozoa 12 hours before the median time of ovulation resulted in conception rates ranging from 84.5% to 40.7%, depending on the oestrous synchronisation regimen used (Table 1).

Table 1: Conception rates of Fallow deer does following intracervical insemination with 140 x 10^6 motile frozen-thawed spermatozoa 12 hours before the median time to onset of ovulation (H N Jabbour, unpublished data).

Synchronisation treatment	No. of does inseminated	No. of does pregnant	Conception rate (%)
CIDR device	26	22	84.5
CIDR device + 50 i.u. PMSG	26	16	61.5
Prostaglandin	27	11	40.7
Prostaglandin + 50 i.u. PMSG	26	17	65.4
Total	105	66	62.9

(b) Intrauterine Insemination: Laparoscopic intrauterine insemination is presently the preferred method of artificial insemination for Fallow deer (Asher et al., 1990b). This method results in good conception rates following placement of relatively small concentrations of spermatozoa. Initial attempts at intrauterine deposition of 85 x 10^6 motile frozen-thawed spermatozoa 56-58 hours after CIDR device withdrawal resulted in 42% fawning rate (Asher et al., 1988a). However, later work has revealed that inseminations, with 20-40 x 10^6 motile frozen-thawed spermatozoa, closer to the time of ovulation (65-70 hours after CIDR device

withdrawal) resulted in an overall 68% conception rate (Asher et al., 1990b).

More recent on-farm studies conducted during the 1990 breeding season in New Zealand have indicated more flexibility in the timing of insemination relative to CIDR device withdrawal (60-70 hours) and a more effective synchronisation of oestrus following treatment with CIDR devices than prostaglandin. Moreover, the study revealed that the presence of vasectomised bucks is not essential during the oestrous synchronisation treatment, that there is little difference in the efficacy of the two types of CIDR devices (type-G and type-S) to synchronise oestrus and that the numbers of motile frozen-thawed spermatozoa required for respectable conception rates (60-70%) are lower than presently used commercially (Table 2).

Table 2: Conception rates of Fallow deer does following laparoscopic intrauterine insemination with frozen-thawed semen (G W Asher, unpublished data).

(a) Effect of time of insemination (Farm 1)

Time from CIDR with-drawal	No. of does inseminated	No. of does pregnant (Day 45)	Conception rate (%)
60 h	36	24	66.7
65 h*	62	41	66.1
70 h	40	29	72.5
Total	138	94	68.1

(b) CIDR device vs prostaglandin synchronisation (Farm 5)

Synchron-isation treatment	No. of does inseminated	No. of does pregnant (Day 45)	Conception rate (%)
CIDR device*	54	38	70.4
Prostaglandin	51	27	52.9
Total	105	65	61.9

(c) Effect of buck presence/absence (Farm 2)

Buck	No. of does inseminated	No. of does pregnant (Day 45)	Conception rate (%)
Present*	53	36	67.9
Absent	50	31	62.0
Total	103	67	65.1

(d) Effect of CIDR device type (Farm 4)

CIDR type	No. of does inseminated	No. of does pregnant (Day 45)	Conception rate (%)
type-G*	44	31	70.5
type-S	47	31	66.0
Total	91	62	68.1

(e) Effect of sperm/inseminate (Farm 3)

Sperm No. (x 10^6)	No. of does inseminated	No. of does pregnant (Day 45)	Conception rate (%)
50*	36	22	61.1
25	38	29	76.3
10	36	25	69.4
Total	110	76	69.1

* Control treatment (i.e. 14-day type-G CIDR device; insemination 65 h post-device withdrawal with 50 x 10^6 spermatozoa; vasectomised buck present during CIDR device insertion). Semen from 5 F1 hybrid (European x Mesopotamian) Fallow deer bucks, balanced by buck across farm and treatment.

Artificial Insemination of Red Deer

(a) Intravaginal insemination: Deposition of semen in the vagina and cervix of Red deer have been attempted, with only 25% conception rate observed following placement of frozen-thawed spermatozoa at natural detected oestrus (Krzywinski and Jaczewski, 1978). Later studies in New Zealand proved more successful following oestrous synchronisation and fixed-time artificial insemination. Fennessy et al. (1990) achieved a pregnancy rate of 39% to a single intravaginal insemination at 48 hours after CIDR device withdrawal. This was similar to the overall rate of 34% following single inseminations at various intervals (36-68 hours) after device withdrawal, with a very low conception rate (6%) achieved for the latest timing (Fennessy et al., 1990). However, this is lower than the conception rates observed in three separate studies (49%, 45% and 58%) following double vaginal inseminations performed at 44 and 68 hours after CIDR device withdrawal. This improvement over single inseminations suggests relatively poor synchrony of oestrus in Red deer hinds.

(b) Intrauterine Insemination: As with Fallow deer, laparoscopic intrauterine insemination is currently the preferred method of artificial insemination of Red deer (Bowen, 1989). Recent research has shown no significant differences in conception rates following intrauterine placement of 20 X 10^6 spermatozoa at 48, 52 or 55 hours after CIDR device withdrawal, with an overall conception rate of 53% (Fennessy et al., 1990). In another trial, the difference in pregnancy rate between treatment with CIDR devices for 12 or 15 days was not statistically different (72% vs 44%) but the interaction between the length of progesterone treatment and insemination time (48 vs 55 hours after CIDR device withdrawal) was significant. Conception rates were higher when semen was deposited at 55 hours in hinds treated with CIDR devices for 12 (89%) than 15 (20%) days (Fennessy et al., 1990). The standard regimen presently applied to commercial laparoscopic artificial insemination of farmed Red deer in New Zealand includes 12 day CIDR device insertion with administration of 200 i.u. PMSG at CIDR device withdrawal and insemination of 20-40 x 10^6 frozen-thawed spermatozoa 54-56 hours later (Bowen, 1989).

Pregnancy Diagnosis

In early work, conception rate was estimated by observing non-return rates and measuring plasma progesterone concentrations on Day 21 after insemination. However, there was a discrepancy between the estimated conception rates and actual observed fawning rate, suggesting either a high level (>10%) of embryonic mortality or an overestimation of the conception rate (Asher et al., 1988a). More recent studies on artificial insemination of both Fallow deer and Red deer have relied on ultrasonography performed between Days 40-60 post insemination to estimate conception rate (Asher et al., 1990b; Fennessy et al., 1990). This technique has resulted in a high correlation between estimated conception rates and observed fawning rates, suggesting low levels of embryonic mortality following artificial insemination.

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