CURRENT TECHNOLOGY AND ECONOMICS OF ARTIFICIAL BREEDING OF CERVIDS

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Introduction

While traditional species of domestic ruminants (e.g. sheep, cattle, goats) are the product of several thousand generations of selective breeding, farmed red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) have been husbanded intensively for less than 10 generations and differ little genetically from their wild progenitors. However, considerable research and development has been invested into artificial breeding technologies for farmed deer within the last 10-12 years in order to facilitate increased rates of genetic gain through on-farm selection programmes and to improve performance under New Zealand's unique pastoral conditions. The purpose of this paper is to review the "state of the art" of these technologies, with particular reference to out-of-season breeding, artificial insemination, embryo transfer, *in-vitro* production of embryos and twinning.

Out-of-season Breeding

Rationale: The rigid reproductive and growth seasonality of red and fallow deer imposes certain productive constraints in much of New Zealand's pastoral environment. In particular, there is often a poor synchrony between the high energy demands of lactation in summer and the peak of pasture production and quality occurring in spring. Alignment of the birth season with spring pasture production, by advancing mating by up to two months, could facilitate increased lactational yields and calf/fawn growth rates (30, 33). This has been attempted by two means: the use of intravaginal CIDR devices + PMSG/GnRH in females, and treatment of males and/or females with exogenous melatonin.

Progesterone + PMSG/GnRH. Gonadotrophic stimulation, using pregnant mare serum gonadotrophin (PMSG) or gonadotrophin-releasing hormone (GnRH) in conjunction with intravaginal progesterone treatment (i.e. CIDR devices), has been used to induce ovulation in red deer hinds and fallow deer does prior to the normal breeding season (15, 17, 39, 44, 46, 52, 56, 88).

Dosages of 200-400 i.u. PMSG to red deer hinds , delivered by single intramuscular injection around the time of removal of CIDR devices, is generally successful in inducing oestrus/ovulatory responses 2-3 weeks before the onset of natural mating activity, with efficacies approaching 90-95% for yearling and lactating adult hinds (39, 44). Pregnancy/calving rates of between 69 and 84% to induced ovulation have been recorded following natural mating (39, 52), although the incidence of barren hinds and twinning hinds is occasionally higher than in untreated groups (39). Low libido of stags has been noted as a problem in some studies.

In one study on fallow deer, in which adult does received 500 i.u. PMSG at withdrawal of CIDR devices 3-4 weeks before the natural rut, an extremely low pregnancy rate (i.e. 3/20) was obtained. This was attributed to a high incidence of multiple ovulations and low libido of bucks (17). Subsequent studies using lower dosages of PMSG (100-200 i.u.) have also encountered low overall pregnancy rates (G.W. Asher, unpublished data), and it is now thought that all forms of PMSG delivery to fallow deer (i.e. within season and out-of-season)

are associated with a marked reduction in fertility (70).

Delivery of synthetic GnRH via osmotic minipump (200-500 ng hr^{-1}) during the preovulatory/ovulatory period (i.e. for seven days prior to withdrawal of CIDR devices) has been partially effective in inducing ovulation in yearling and lactating adult red deer hinds (44) and non-lactating adult fallow deer does (15) 3-8 weeks prior to the natural breeding season. However, both studies reported low pregnancy rates to induced ovulation that were almost certainly due to sub-fertility of stags/bucks. Premature regression of induced corpora lutea was also observed for some fallow does.

These techniques of early induction of oestrus/ovulation in female deer have received little practical application within the last 5-6 years This is probably attributed to one or other of the following factors : high costs, variable responses, increased incidence of twinning (PMSG treatment), complex application (subcutaneous minipump insertion) and the recent commercial introduction of melatonin implants that allow coinciding treatment of both males and females

Melatonin: Detailed studies in mammals have shown that the circadian pattern of secretion of the pineal hormone, melatonin, mediates the effect of photoperiod on reproduction (36, 74) Melatonin is secreted only during darkness in all mammals studied, including sheep (7) and fallow deer (21) Numerous studies over the last 10 years have shown that administration of exogenous melatonin during long photoperiods induces early reproductive function in red and fallow deer (30).

The various methods of melatonin delivery applied to deer fall into two broad categories. (a) daily administration at a fixed interval prior to the onset of darkness (i e augmentation of endogenous melatonin secretory patterns) and (b) long-term infusion via slow-release implants While daily administration is analogous to the pattern of endogenous melatonin secretion that would be expected to occur during shorter photoperiods, implants elevate plasma melatonin concentrations for protracted periods (30-120 days), effectively 'swamping' the circadian pattern of endogenous secretion. Both forms of administration have been shown to induce similar overall effects on reproductive seasonality but may differ in the way in which effects are mediated (82).

Daily oral administration of exogenous melatonin, generally by afternoon feeding of melatonin-laced concentrate rations, has been applied to white-tailed deer (*Odocoileus virginianus*; 40), red deer (2, 3, 4, 5, 6, 46, 87, 93, 102) and fallow deer (20). However, unless the appropriate dosage (5-20 mg melatonin per animal) is administered directly to individual animals (e.g. 2, 40) the efficacy of the technique is limited by individual and daily variations in food intake when melatonin is administered orally in feed to groups of deer (20, 46, 102). Daily administration of melatonin via intra-muscular injection has been applied to pubertal red deer hinds (101), and ensures appropriate dosage delivery (e.g 3.75 mg melatonin per hind) but necessitates considerable labour inputs into animal handling

More recent studies on deer have tended to utilise constant-infusion techniques for melatonin delivery, thus reducing labour inputs and frequency of animal handling. Large (42-50 cm^2) silastic subcutaneous implants containing crystalline melatonin (500-1000 mg) have been used in red deer (83) and fallow deer (91) to modify reproductive seasonality. However, the recent development of a novel form of melatonin infusion has provided a more practical alternative to cumbersome silastic implants. Small (< 4 0 mm^3) coated pellet implants (Regulin, Schering, Alexandria, NSW, Australia), each containing only 18 mg melatonin, are capable

of elevating daytime plasma melatonin concentrations to levels comparable to endogenous nighttime levels for periods of more than 40 days when administered singularly to fallow deer (21) or as multiple (two to three) implants to red deer (12, 103) Regulin implants have been used to manipulate reproductive seasonality in a number of studies on red deer (12, 46, 54, 57, 103, 104, 105) and fallow deer (21) Given the ease of application of Regulin implants over all other forms of melatonin delivery, we consider further only those studies involving Regulin usage in farmed deer.

Numerous studies have shown that strategic administration of Regulin implants to pubertal red deer hinds and fallow deer does starting at 10-12 months of age results in advancement of first oestrus, ovulation, conception and parturition (12, 21, 44, 54, 57, 104, 105). However, the degree of advancement of treated females relative to control females has been highly variable between studies, reflecting a wide range of treatment protocols including the time of onset of treatment (i e. 100-200 days after the winter solstice), the duration of treatment (30-180 days) and the potential of social interaction between treated and control animals (i e social facilitation effects). Further to these protocol variables, there are probably confounding effects of environmental variables such as latitude and nutrition. While it is difficult to separate the absolute effects of all these variables, it is possible to reach some conclusions with respect to treatment protocols.

The time of onset of treatment possibly accounts for most of the variation in seasonality responses observed between studies. Advancement of the breeding season of pubertal hinds/does necessitates the initiation of melatonin treatment during the preceding spring/summer months. Recent studies have investigated the effect of timing of initiation of Regulin implantation treatment during this period on advancement of puberty of red deer hinds (12, 59). In one study (12) the potential effects of social facilitation were minimised by maintaining all treatment and control groups in complete isolation from each other Treatments of both hinds and stags were initiated at 100 (Group 1), 140 (Group 2) and 180 (Group 3) days after the winter solstice (shortest day) and continued until all signs of oestrous activity were over. Relative to control hinds, the mean dates of first oestrus, conception and parturition were advanced by 54 days, 50 days and 48 days, respectively, in Group 1, 46 days, 41 days and 38 days, respectively, in Group 2 and 22 days, 22 days and 20 days, respectively, in Group 3, clearly demonstrating a highly significant effect of treatment initiation date However, the intervals between the initiation of treatment and the mean date of first oestrus were 147 days, 109 days and 90 days for Groups 1, 2 and 3 respectively, indicating a curvilinear relationship between treatment initiation and the response A similar relationship was noted by for hinds treated in the absence of stags (59). It seems likely, therefore, that there is an extreme limit, relative to the winter solstice, at which melatonin treatment will not elicit any further response in advancing puberty Administration of exogenous melatonin too close to the winter solstice may in fact prove counter-productive by impinging upon a putative photoperiod entrainment period, whereby exposure to increasing photoperiods is a necessary prerequisite for a reproductive response to decreasing photoperiods (e.g in sheep. 82, 107) Whether this would ablate the entire process of puberty, delay it, or merely render the melatonin treatment ineffective, remains to be tested in deer. Preliminary data indicate that exogenous melatonin may in fact delay puberty in female red deer if administered at eight months of age (about 50 days after the winter solstice), indicating the existence of a period of photoperiodic entrainment in this species (5).

There are only two accounts of exogenous melatonin treatment of pubertal female fallow deer (21, 91). Both involved implantation regimens for periods of more than 120 days, with treated

and control does run together for mating. Treatment of does starting 140 days after the winter solstice (21) resulted in advancement of the mean dates of first oestrus and fawning of 56 days, whereas later treatment initiation at 164 days (91) advanced first oestrus and fawning by 33 days and 27 days, respectively. This indicates an effect of treatment initiation date for this species.

Duration of exogenous melatonin treatment undoubtedly influences the degree of seasonality advancement, but it is difficult to assess the magnitude of the effects from much of the current literature owing to confounding influences of variable treatment initiation dates. However, in one experiment, pubertal red deer hinds were treated with Regulin implants in spring, 160 days after the winter solstice, in the absence of a possible influence from the stag Treatment approximating 60 or 90 days duration resulted in four weeks advancement in the onset of puberty in all hinds, whereas 30 days of treatment resulted in only a portion of hinds responding with early ovarian activity (60).

There are major social facilitation effects on seasonality of red deer It has been demonstrated that red deer stags are capable of influencing the onset of ovulatory activity in herdmate hinds (85, 88). Furthermore, female inductive effects have been documented for red deer (68). It is not surprising, therefore, that melatonin-treated stags and/or hinds appear to influence the reproductive seasonality of untreated herdmates. Fisher *et al* (57) found that control hinds penned together with Regulin treated hinds had a mean calving date 16 days earlier than that recorded in previous years for first calving (2 year old) red deer on the same property Thus, the apparent advancement of treated hinds relative to control hinds was only 11 days, but the real degree of advancement was probably in the order of 27 days (57). In large field trials on the efficacy of Regulin in red deer (104, 105) there was clear evidence that the onset of ovulatory activity in sympatric control hinds was advanced by melatonin-treated animals However, it was not possible, from these studies, to distinguish between the inductive effects of treated stags or hinds Evidence that melatonin treatment of stags can advance ovulatory activity in older, lactating hinds was provided by another study (55) Regulin treatment of both stags and hinds advanced the mean calving date relative to the untreated control group by 21 days. However, untreated hinds run with a treated stag had a mean calving date 13 days earlier than that of the control group. This is clear evidence of a male inductive effect but female inductive effects (i.e. 'sympathetic oestrus') have yet to be investigated

Table 1: Effect of (Regulin treatment of red deer stags and hinds on mean calving date in the Southern Hemisphere (55)

		Mean calving date	Range
Untreated stags			
Untreated hinds	(*n*=9)	4 Dec	(19 Nov-6 Dec)
Treated hinds	(*n*=9)	23 Nov	(15 Nov-6 Dec)
Treated stags			
Untreated hinds	(*n*=9)	21 Nov	(13 Nov-2 Dec)
Treated hinds	(*n*=9)	14 Nov	(8 Nov-22 Nov)

Treatment of adult red hinds and fallow does with exogenous melatonin to advance ovulatory activity is complicated by pregnancy and lactation. For both species, pregnancy lasts for around 234 days (11, 41, 64, 75). The 110-130-day period between parturition and

ovulation/conception represents the period of deep anoestrum coinciding with lactation. The initiation of melatonin treatment, therefore, must occur during either the latter stages of pregnancy or early stages of lactation for a significant advancement effect on the timing of ovulation. Important considerations of treatment include the effects of exogenous melatonin on fetal development, initiation of lactation, maintenance of lactation and the advancement of oestrus/ovulation.

Exogenous melatonin treatments initiated during the later stages of pregnancy (i.e. last 40 days) have been studied in red deer (6) and fallow deer (21). In neither case was any effect of fetal development observed. However, in the study on fallow deer, of six pregnant does treated with Regulin implants, four failed to initiate lactation and subsequently lost their fawns. The remaining two does successfully reared their fawns, as did the six contemporary controls (21). More recently it was demonstrated that Regulin treatment of red deer hinds starting 80 days before parturition was associated with retardation of fetal growth and failure of lactogenesis in all animals (31). However, hinds receiving treatments from 40 days before parturition all reared normal healthy calves, in accord with earlier observations (6) This is suggestive of a crucial phase in late pregnancy when exogenous melatonin treatment interferes with normal fetal and mammary development. In light of these studies, Regulin treatment of pregnant hinds/does is contra-indicated. Regulin implantation has been effective in inducing early reproductive activity in lactating red deer hinds compared with contemporary control animals (44, 57). The degree of advancement achieved for a given treatment initiation date appears to be similar to that observed for pubertal hinds However, because pregnancy frequently extends into mid-December (170 days after the winter solstice), few treatment regimens for adult red deer hinds have been initiated earlier than 180 days from the solstice.

A number of studies on adult red stags and fallow bucks have shown that Regulin treatment initiated in summer advances antler development and the onset of rutting activity by 4-8 weeks (16, 21, 44, 57, 103). Furthermore, treatments conducted in accordance with the herdmate hinds/does appear to result in coincident rutting and oestrous activity, leading to high conception rates of treated females (12, 44, 57) While exogenous melatonin treatments initiated in summer are effective in advancing reproductive function in male deer, there are longer term consequences of treatment that indicate that the mode of action of melatonin-mediated seasonality entrainment is not a simple 'direct-drive' mechanism In a recent study on adult red deer stags, it was demonstrated that Regulin treatment initiated approximately 140 or 170 days after the winter solstice (about 120 days and 90 days treatment duration respectively) advanced changes in scrotal circumference, liveweight, antler state, pelage type and LH response to gonadotrophin releasing hormone (GnRH) injection compared with controls, with the extent of advancement being greater for the earlier initiation of treatment (103). However testes size regressed rapidly and antlers were cast shortly after Regulin implants became exhausted before the onset of the natural rut. This was followed by an additional antler cycle, and reproductive development and decline from mid-winter to early summer. Regulin-treated stags became synchronised with control stags 14-15 months after melatonin treatment began. The extra cycle of seasonal changes was more pronounced for the early initiation of treatment (103).

Advancement of the seasonal pattern of conceptions through the strategic use of exogenous melatonin has been associated with a similar degree of advancement of the ultimate pattern of births for both red deer (12, 44, 57, 104) and fallow deer (21, 91), despite there being a significant increase in mean (±SEM) gestation length recorded for melatonin-treated fallow does compared with control does (238.9±0.6 days vs. 234 5±0.4 days; 21). However,

melatonin treatment may result in a less synchronous birth pattern than that observed for untreated females (21, 104). Therefore, the overall variability in calf/fawn size at weaning is likely to be greater following melatonin treatment. The reasons for the more variable patterns of oestrus, conception and births are unclear, but may relate to the form of melatonin delivery and the time of treatment initiation.

Advancement of the fallow deer birth season into spring in northern New Zealand (39°S) has been associated with a high incidence of neonate mortality due to hypothermia induced by inclement weather (21) and may be exaggerated by decreased birth weights, as observed for treated does in Australia (91). This mitigates against large-scale (more than four weeks) advancement of the fallow deer birth season. Conversely, red deer neonates appear more tolerant of the lower ambient temperatures occurring before the natural calving season Asher (12) recorded a 90% survival rate of calves born on average 45 days earlier than calves from control hinds, with recorded mortalities being unrelated to climate. This is generally supported by data from Regulin field trials (104)

Some evidence indicates that red deer calves and fallow deer fawns born early as a result of treatments may attain puberty in their first autumn Precocious puberty, as evidenced by exceptionally early pedicle and antler development in males, has been recorded for red stags (86, 94) and observed in fallow bucks (30). In so far that pedicle initiation is related to body size it is probable that this precocious puberty is due to large body size at an early age. Pubertal oestrus has also been observed in early-born fallow does, although this did not result in full term pregnancies (30). The performance consequences of precocious puberty have yet to be fully assessed.

Economic Analysis: The simplified analysis compares a number of different scenarios of advanced calving. These involve treatment of stags with melatonin (Regulin) and a proportion of hinds with progesterone-containing CIDR devices and PMSG at withdrawal Table 2 summarizes the data showing the expected advancement of calving in terms of the first calf born and the herd average. Table 3 presents a simplified economic analysis showing costs of treating stags and hinds, together with the estimated value of the liveweight advantage due to the earlier calving (assumes that the calves are sold as weaners) While the potential economic benefits of an earlier breeding and calving season are considerable, there are some risks. The major ones would be the risk of adverse climatic conditions at the time of calving, while precocious puberty may also be an issue in the young males. It is also possible that some of the liveweight advantage at weaning will be dissipated with time so that the advantage in yearling liveweight may be less. However, trials to test this subsequent growth response have given inconsistent results. Overall, the financial benefits of early calving will likely occur to good managers who include the advanced breeding option as part of their total strategy and not as a "one-off" try-out of a good idea.

Table 2: Comparison of different scenarios to advance the calving date in a herd of red deer hinds The days advancement is compared with the normal situation B where the herd average calving date is December 2

Comparison of treatments with Normal B where stags and a proportion of hinds are treated to advance the breeding season						
	Normal		Stags only[1]	Stags + 25% of hinds[2]		Stags and all hinds
	A	B		A	B	
Days Advancement						
First calf	Nil		10	14	28	28
Stags in	*	*	2 Mar	16 Mar	2 Mar	2 Mar
First mating	11 Apr	1 Apr	22 Mar	18 Mar	4 Mar	4 Mar
Hinds per stag	50	50	50	50	50	20
Calving Dates						
First calf expected	1 Dec	21 Nov	11 Nov	7 Nov	24 Oct	24 Oct
First calf actual[3]	25 Nov	15 Nov	5 Nov	1 Nov	18 Oct	18 Oct
Herd average	12 Dec	2 Dec	24 Nov	24 Nov	10 Nov	30 Oct

[1]The early presence of stags alone does not change the basic calving pattern at this stage
[2]Assumes that 70% of hinds will conceive and calve to an induced oestrus and that 90% of non-pregnant hinds will conceive and calve to any natural oestrus
[3]Allows for the variation in gestation length (mean of 234 ± 3 4 (standard deviation) days)

Table 3: Simplified economic analysis of the impact of an advancement in the mean calving date for a herd of 100 red hinds

Simplified economic analysis - per 100 hinds to the stag (90% weaning rate)					
	Normal	Stags only[1]	Stags + 25% of hinds[2]		Stags and all hinds
			A	B	
Days Advanced					
First calf	Nil	10	14	28	28
Herd average	-	8	8	22	33
Costs					
Stags	-	$40	$40	$40	$100
Hinds	-	-	$160	$600	$600
Total	-	**$40**	**$200**	**$200**	**$700**
RETURNS according to value of the increase in live weight advantage					
Value of live weight advantage per calf					
$3 50 per week	-	$360	$360	$990	$1485
$2 50 per week		$255	$255	$705	$1060
$1 50 per week		$155	$155	$425	$635

[1]Stags treated with six Regulin implants (4 + 2)
[2]Hinds treated with a single progesterone-CIDR device + 200 IU PMSG at withdrawal
[3]The economic analysis does not include the costs of any additional labour, feed or animal health or any additional stags required

Artificial Insemination

Rationale: The application of artificial insemination technology within the deer farming industry is still in its infancy. However, its future potential is enormous, particularly in relation to the establishment of genetic improvement schemes and interspecific hybridisation programmes. Artificial insemination (AI) allows for a wider and more rapid dissemination of desirable genetic material than would be remotely possible by natural mating strategies. This is particularly important when considering such rare genotypes as Mesopotamian fallow deer (*Dama dama mesopotamica*), Père David's deer (*Elaphurus davidianus*) or imported blood lines. Moreover, AI provides a safe and cost-effective means of international exchange of genetic material.

We review the present state of artificial insemination technology for red deer and fallow deer by discussing the major components of oestrous synchronisation, semen collection/processing and insemination techniques. While a number of studies have investigated artificial insemination in other species of cervids, e.g. reindeer (*Rangifer tarandus*, 43), wapiti (*Cervus elaphus nelsoni*, 66), white-tailed deer (*Odocoileus virginianus*, 65, 71, 84), they have involved only very small numbers of females with variable, often low, conception rates. Recent trends towards the commercial application of artificial insemination of farmed deer in New Zealand and Australia have prompted rapid progress in the development of more effective techniques that may have application across a number of cervid species.

Oestrous detection and synchronisation. Detection of spontaneous oestrus in farmed deer has generally proven difficult because of intractability of the animals and the limited ability to closely inspect females within a pastoral environment. Direct observation of oestrous behaviour is unreliable because overt oestrus in female deer is usually rather passive compared with other domestic livestock and is often terminated at copulation, within minutes of its onset (10). The use of ram mating harnesses on male deer has been successfully adopted in a number of studies on red deer (22, 64) and fallow deer (9, 18, 19, 25). While the method has proved very effective for fallow deer, with more than 90% of first spontaneous oestrous events being detected under controlled experimental conditions, its effectiveness for detection of natural oestrus in red deer is variable. Red deer stags may exhibit a low mount-to-service ratio during mating (99). This affords little opportunity for stags to mark oestrous hinds with the pressure crayon. Secondly, the propensity of red deer stags to wallow in mud often renders the device ineffective without frequent crayon changes. In summary, high labour inputs required to ensure adequate success of the detection of natural oestrus in both red deer and fallow deer generally mitigate against its usefulness in artificial breeding programmes.

Artificial synchronisation of oestrus (coupled with timed AI) in farmed deer has been adopted as a more cost-effective alternative to detection of natural oestrus. As with other domestic ruminants, synchronisation of oestrus can be achieved either by simulating the activity of the corpus luteum through the administration of progestagens, or by shortening the luteal phase of the oestrous cycle by administration of a luteolysin. 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 (19).

While a wide range of progestagen-releasing devices has yet to be tested for efficacy of oestrous synchronisation in deer, a large number of studies in New Zealand have investigated the use of the intravaginal controlled internal drug release (CIDR) device (CIDR-S or CIDR-G; 9-12% w/w progesterone, InterAg, Hamilton, New Zealand). During prolonged (12-14

days) intravaginal insertion in the fallow deer doe, single CIDR-S or CIDR-G devices (0 365 g progesterone per device) elevate peripheral plasma progesterone concentrations to levels comparable to those observed during the mid-oestrous cycle (18, 19, 25) Exogenous progesterone is cleared from the peripheral system within 2 h of device withdrawal. In this respect, the devices appear well suited for use in fallow deer The efficacy of oestrous synchronisation with the CIDR device in this species, in terms of the proportion of does exhibiting oestrus/ovulation and the degree of synchrony achieved, is clearly dependent on season. Recent studies have shown that device withdrawal just prior to the onset of the natural rut (i.e. period of first spontaneous oestrus) results in a low incidence of oestrus (0-10%). The proportion of does exhibiting oestrus increases, and the mean interval between device withdrawal and onset of oestrus progressively decreases as CIDR devices are removed progressively later relative to the occurrence of the first spontaneous oestrus within the herd (89). Optimal responses appear to occur after the period of first oestrus (rut), at which time the mean interval from device withdrawal to onset of oestrus is between 48 and 58 h (18, 19, 89) The onset of oestrus in fallow does coincides with the onset of the preovulatory LH surge, which attains maximal amplitude (20-30 ng ml^{-1}) 4-6 h later (18, 19, 25) Recent studies have shown that ovulation (follicular rupture) occurs 24 h after the onset of oestrus/preovulatory LH surge following CIDR device synchronisation (25). Therefore, ovulation is synchronised to within the period of 70-80 h after device withdrawal

The use of PMSG at or near CIDR device withdrawal is presently contra-indicated for fallow deer. Studies in which either 500 IU (17), 200 IU (G W Asher, unpublished data, 1989) or 100 IU (70) PMSG were administered by intramuscular injection at CIDR device withdrawal, indicated a high level of ovarian sensitivity to the exogenous gonadotrophin. In these studies, a high proportion of does either exhibited multiple (2-4) ovulations or completely failed to ovulate after device withdrawal. This appeared to result in reduced conception rates to natural mating and increased the incidence of embryonic mortality (particularly with multiple fetuses) This observation is supported by those of commercial inseminators following the administration of between 100 and 200 IU PMSG at CIDR device withdrawal More recent studies on artificially inseminated does have indicated that 50 IU PMSG delivered at device withdrawal reduces the interval to the onset of oestrus and induces greater oestrous synchrony compared with CIDR device withdrawal alone, although fertility was not enhanced (70)

Peripheral plasma progesterone profiles of red deer hinds receiving single CIDR-S or CIDR-G devices differ from those of fallow deer. Concentrations within the first six days of insertion are comparable to those observed during the oestrous cycle (2-3 ng ml^{-1}) Thereafter, levels decline to less than 1.0 ng ml^{-1} by Day 14 (73). This raises questions about the effectiveness of single CIDR devices to inhibit follicular development in red deer (49, 73), although such treatment has generally resulted in acceptable (over 50%) conception rates to artificial insemination (37, 50). The low resultant peripheral progesterone concentrations have occasionally prompted the use of double CIDR-S devices (22) or CIDR-G device replacement on Day 9 (50). However, no improvements in conception rates following artificial insemination have been noted for these treatments (37, 50).

It has become routine practice to administer 200-250 IU PMSG at or near CIDR device withdrawal in red deer (49). There are three main reasons: the first is that in artificial insemination programmes, red deer hinds are often inseminated prior to the onset of the normal breeding season, when progesterone/PMSG is known to improve the incidence of ovulation in hinds compared with progesterone alone (56, 58) Secondly, there is concern that stress of handling may reduce the incidence of ovulation in hinds and extra gonadotrophic

stimulation is required to offset stress effects (49). Third, it is reasoned that PMSG may reduce the spread of ovulation in groups of hinds (49). While the influence of season on the incidence of oestrus/ovulation following CIDR device treatment has been well demonstrated for red deer (56, 58) and fallow deer (89), the other two factors are equivocal. The putative stress effects on oestrus/ovulation have not been conclusively demonstrated for red deer, although it is known that the adrenal glands may secrete physiologically significant quantities of progesterone in response to stress in both red deer and fallow deer (24, 73). However, transient (1-2 h) increases in peripheral plasma progesterone around the time of CIDR device withdrawal (i.e. during handling stress) seem unlikely to have long-term effects on follicular development for deer that are well habituated to the farm environment. Bringans (38) reported that only 50% of red hinds given CIDR devices alone ovulated compared with 90-95% of hinds given CIDR devices+PMSG. While the author linked this result to 'emotional stress', there may well have been confounding effects of nutrition and season. The effects of PMSG on oestrous/ovulation synchrony in red deer require further investigation Recent studies indicated that administration of 200 IU PMSG at CIDR device withdrawal reduced the mean interval to the onset of oestrus (37.4 h; n=7) compared with CIDR device withdrawal alone (44.4 h; n=7) but did not reduce the variance (SD = 5 3 h vs. 2.3 h) For both treatments, ovulation occurred between 20 and 28 h after the onset of oestrus (27). A slightly increased incidence of multiple ovulation has been observed following 200-250 IU PMSG administration to red deer hinds. While this has occasionally resulted in conception and birth of twins in artificial insemination programmes (22), there is little evidence of major production losses through reduced fertility and increased embryonic loss

The duration of CIDR device insertion for red deer normally ranges from 12 to 14 days (22, 37, 51, 56) The optimum duration has yet to be investigated in detail. However, the data of Fennessy *et al* (50) indicate that a 15-day insertion period is associated with lower conception rate to artificial insemination compared with a 12-day insertion period This is indicative of either the low progesterone output of CIDR devices by Day 15 or the inhibitory effects of prolonged progesterone influence on follicular maturation

A limited number of studies have investigated the efficacy of intravaginal sponges impregnated with either fluorogestone acetate or medroxyprogesterone acetate in inducing synchronised oestrus in red deer (1, 22, 67, 76) and fallow deer (92) While synthetic progestagens are well able to control ovulatory activity, some of the studies were plagued by excessive sponge loss rates (76, 92), mitigating against their general effectiveness in deer.

The ability of prostaglandin administration to synchronise oestrus is dependent on the presence of an active corpus luteum at the time of treatment, limiting synchronisation programmes in red and fallow deer to the period after the onset of natural ovulatory activity (i e the rut) Furthermore, recent studies indicate that the cervine corpus luteum may be refractory to prostaglandin treatment before Day 10 of the oestrous cycle (61), necessitating either administration at the correct stage of the cycle or delivery of twin injections at least 10 days apart (52).

A single injection of prostaglandin analogue (500 mg cloprostenol, Estrumate, Imperial Chemical Industries PLC, Cheshire, UK) on Day 13 of the fallow deer oestrous cycle resulted in premature regression of the corpus luteum, clearance of endogenous progesterone from the peripheral system within 14 h, and return to oestrus at an average interval of 43 h (18) More recent studies have shown that ovulation (follicular rupture) occurs 24 h after the onset of prostaglandin-induced oestrus in fallow deer (25). Early observations indicated a reasonable

level of fertility following prostaglandin synchronisation in this species (18) However, recent application of prostaglandin synchronisation to artificial insemination programmes has resulted in lower conception rates than observed following CIDR device synchronisation (28) In this particular study, initial synchronisation of oestrus/ovulation was performed with CIDR devices and may have been conducted too early in the season for an optimum ovulatory response (89) However, in a study conducted 3 weeks later similar trend for reduced conception rates was observed following intracervical insemination (70). The use of prostaglandin synchronisation in red deer has not been investigated extensively. Haigh *et al* (66) recorded briefly the treatment of 39 wapiti-type (elk x red deer) hinds with prostaglandin injections at 13 day intervals. Of these, 16 (41%) became pregnant to intrauterine inseminations of wapiti semen performed 72, 84 and 96 h after the second injection Haigh *et al* (67) recorded the treatment of red deer hinds with double prostaglandin injections: only 8% became pregnant to natural mating, whereas 85% of contemporary hinds became pregnant following treatment for 7 days with intravaginal sponges containing medroxyprogesterone acetate. It is likely that this latter experiment was conducted too early in the breeding season for prostaglandins to be efficacious in achieving oestrous synchrony.

Semen Collection: Semen collection from male deer is one of the more difficult aspects of artificial insemination programmes, and has been one of the major factors limiting its widespread usage within the deer farming industry. Firstly, there are seasonal constraints on semen collection. Male red deer and fallow deer exhibit a pronounced period of testicular quiescence during spring and summer, at which time they are effectively infertile (20, 63, 79, 81). This limits semen collection to the 4-6 month period starting immediately before the autumn rut. This time constraint often conflicts with the need to supply semen for usage within the same breeding season; this is particularly the case for semen export to countries within the same hemisphere, for which there are mandatory pre-collection stag/buck isolation protocols to ensure a suitable health status It also conflicts with the desire of farmers to use the stags/bucks as sires for natural mating during the rut.

Secondly, the temperament of stags/bucks in the presence of their handlers is usually not conducive to the successful implementation of natural semen collection techniques frequently used for more traditional livestock species Depending on the level of habituation to their handlers, farmed stags/bucks are either totally intimidated by their presence or very aggressive towards them. Therefore, semen collection from red and fallow deer has generally been performed by electro-ejaculation while the animals are under general anaesthesia/sedation (20, 22, 23, 48, 50, 63, 72) The use of chemical immobilisation presents obvious risks to valuable sires. Furthermore, electro-ejaculation may generally produce semen of lower quality than that collected by natural methods, although this has yet to be assessed for deer

Cryopreservation of red and fallow deer semen has been described by a number of researchers. Generally, however, semen is extended in sodium citrate-egg yolk glycerol diluent and frozen either as pellets on CO_2 ice (79, 91, 92) or in 0 25 ml straws in liquid nitrogen (22, 23, 50) Fallow deer semen has been shown to be particularly resistant to the rigors of the freezing and thawing procedures, with good post-thaw recovery (motility rates often being in excess of 70% of pre-freezing motility rates (23, 26). This appears to be consistent for different ejaculates from the same buck if collected within the breeding season. However, ejaculates collected at the beginning and end of the breeding season generally exhibit low post-thaw motility rates Post-thaw motility rates of red deer semen appear to be highly variable, both between stags and between consecutive ejaculates from the same stag (50). This warrants further investigation into cryopreservation techniques for this species

Insemination techniques: Laparoscopic intrauterine insemination (77) is presently the preferred method of AI in red deer and fallow deer (29), as it allows precise placement of relatively small quantities of semen close to the site of fertilisation. Early studies on small numbers of red deer hinds clearly indicated the potential of this technique (22, 48) More recent research on larger numbers of hinds have involved comparisons of various insemination times, with frozen-thawed semen, following CIDR device withdrawal/PMSG administration Fennessy *et al* (50) showed no significant difference in conception rate following intrauterine insemination (20 x 10^6 spermatozoa) at 48, 52 and 55 h after device withdrawal. The overall difference in pregnancy rate between treatment with CIDR devices for 12 days or 15 days was not statistically significant (72% vs 44%) but the interaction between the length of progesterone treatment and insemination time (48 h vs 55 h after CIDR device withdrawal) was significant, with the 12 day CIDR/55-h insemination giving a much higher pregnancy rate than the 15 day CIDR/55-h insemination (89% vs 20%) (50) The standard regimen presently applied to commercial laparoscopic inseminations of farmed red deer in New Zealand includes 12 day CIDR device with administration of 200 IU PMSG at CIDR device withdrawal and insemination of 20-40 x 10^6 motile spermatozoa 54-56 h later (37). CIDR devices are frequently replaced after 8-9 days of treatment to ensure that high progesterone levels are maintained until the time of device withdrawal/PMSG treatment.

Early studies on fallow deer does involving intrauterine deposition of 85 x 10^6 motile frozen-thawed spermatozoa 56-68 hours after CIDR device withdrawal resulted in a disappointing 42% conception rate (23). The most recent on-farm studies on laparoscopic intrauterine insemination of fallow deer (28), conducted during the 1990 breeding season in New Zealand (i.e. April/May) investigated variables such as insemination timing, type of CIDR device, CIDR device vs prostaglandin, presence or absence of vasectomised bucks and numbers of spermatozoa per inseminate. The control regimen (i e. treatment common to all farms in the study) was similar to that established by Asher *et al* (26) and, on the basis of ultrasound pregnancy diagnoses, resulted in an overall 68% conception rate. The results indicated a degree of flexibility in the timing of insemination relative to CIDR device withdrawal (60-70 h); CIDR device synchronisation was more effective than prostaglandin synchronisation early in the breeding season; buck presence was not essential during synchronisation treatment, there was little difference in efficacy of the two types of CIDR device (CIDR-G and CIDR-S); and numbers of motile frozen-thawed spermatozoa (10 x 10^6) required for respectable conception rates (60-70%) were lower than presently used commercially (28)

Despite the supposed impenetrability of the cervix of fallow deer transcervical intrauterine insemination (i.e. per vaginam) was performed successfully on anaesthetised does by exteriorising the os cervix (26). Of four does receiving transcervical inseminations of 50 x 10^6 frozen-thawed spermatozoa 68-69 after CIDR device withdrawal, three became pregnant This result demonstrates an alternative to the more invasive technique of laparoscopic insemination and warrants further study

Deposition of semen via the vagina has been attempted in a number of studies on red deer. Insemination sites include intravaginal, intracervical and intrauterine, with a generally low degree of success in achieving transcervical access to the uterus Krzywinski and Jaczewski (79) are credited with some of the first attempts at per vaginam insemination of red deer However, they achieved a conception rate of only 25% to a combination of vaginal and intracervical inseminations with frozen-thawed semen at natural detected oestrus Later studies in New Zealand have proved more successful for fixed-time inseminations following oestrous synchronisation. Single inseminations of about 20 x 10^6motile frozen-thawed spermatozoa

have generally resulted in lower conception rates than two inseminations at 24 h intervals. Fennessy *et al* (50) obtained a pregnancy rate of 39% to a single per vaginam insemination (including some uterine placement) at 48 h after CIDR device withdrawal This was similar to the overall rate of 34% when single inseminations were performed at various intervals (36-68 h) after device withdrawal, with a very low rate (6%) achieved for the latest timing (50) However, double per vaginam inseminations, performed at 44 and 68 h after CIDR device withdrawal, resulted in conception rates of 49%, 45% and 58% in three separate trials (47, 50). This improvement over single inseminations suggests relatively poor synchrony of oestrus in red deer hinds.

Initial studies on intravaginal insemination of fallow does with 85 x 10^6 motile spermatozoa 48 h after CIDR device withdrawal resulted in 50% and 48% fawning rates for fresh and frozen-thawed semen respectively (23) More recently, however, attempts at intracervical insemination with single inseminates containing 20-40 x 10^6 motile spermatozoa have yielded highly variable results amongst commercial inseminators, ranging from 38 to 80% conception rate (29) Recent studies indicate that the success rate of intracervical insemination may be dependent on the method of oestrous synchronisation, the timing of insemination and the number of live spermatozoa per inseminate (70). Intracervical deposition of about 140 x 10^6 motile frozen-thawed spermatozoa 12 h after the median onset of oestrus (i.e about 12 h later than performed in previous studies) resulted in conception rates ranging from 85 to 41% depending on the form of synchronisation. However, such large numbers of spermatozoa are unacceptable commercially

Economic Analysis: Table 4 present a simplified economic analysis of the cost per calf weaned as a result of an AI programme, as affected by the cost of semen and insemination, and the percentage of calves weaned to hinds inseminated It is assumed that hinds that fail to become pregnant to AI will conceive and calve to a chaser stag While AI is expensive, there are a number of advantages which may or may not be readily amenable to economic analysis. For example, access to a superior stag prior to it being generally available is one such situation. With AI often being performed prior to the normal breeding season, there is also the advantage of an earlier calving, especially valuable if the farmer is selling progeny from an elite stag as weaners. In most cases, the major advantage of AI in the commercial deer farming business in New Zealand, considering the current price of semen, is the opportunity for a farmer to breed high quality breeding stags rather than purchasing them This assumes that the quality of the hinds is appropriate for the quality of the semen being used.

Table 4: Simplified costing of an artificial insemination (AI) programme in red deer according to cost of semen and weaning percentage to AI

Insemination	Semen	Cost per calf weaned by weaning %		
per hind[1]	cost	30	50	70
$37	$20	$190	$114	$81
$37	$50	$290	$174	$124
$37	$100	$457	$274	$196
$37	$150	$625	$374	$267

[1]Assumptions - direct cost of $30 per hind inseminated, $1 per hind for a vasectomised stag and $6 per hind for synchronisation Additional labour beyond that in the direct AI costs is not included

Embryo Transfer

Rationale: Multiple ovulation-embryo transfer (MOET) technology has gained rapid entry into the international deer farming industry. MOET offers deer farmers an opportunity to increase numbers of elite stock at faster rates than natural breeding will allow. While artificial insemination permits wide dissemination of the desirable genes of high ranking sires, embryo transfer capitalises on high genetic merit females (donors) by transference of their pre-implantation embryos to lower ranking females (recipients/surrogates). This allows for propagation of pure lines of breeding stock and may have considerable application in the preservation of endangered cervid species via interspecific transfer (e.g European fallow deer serving as maternal surrogates for Mesopotamian fallow deer) (80, 106). Cryopreservation of embryos enhances international transfer of genetic material, as embryos are safer and cheaper to transport than live animals.

Development of MOET technology for cervid species is at a relatively early stage compared with traditional domestic ruminants. While considerable progress has been achieved, MOET has generally been performed in situations where immediate and positive results are required either because of the perceived risk involved in working with endangered species or because economic considerations require it. Consequently much of the development of these techniques has been by trial and error as there is usually a lack of basic information on which to base MOET protocols. Therefore procedures have been adapted from proven cattle and sheep protocols (53). Most of the work to date has involved red deer and wapiti, but there is some information from other species such as fallow deer (90) and white-tailed deer (100).

Superovulation and Breeding: Superovulation procedures are based on those used successfully in sheep and cattle in New Zealand. For red deer, the procedure involves synchronisation of the oestrous cycle with exogenous progestagen and stimulation of follicle development with exogenous gonadotrophin (usually FSH) A typical protocol is the 12-day use of an intravaginal controlled internal drug releasing device (CIDR, containing 340 mg of progesterone) with FSH administered by injection (generally 8 doses) or by osmotic minipump for 4 d through Days 8 to 12 of the cycle. Progesterone is withdrawn at around 12 or 24 h before completion of FSH treatment. Various forms of FSH have been used, although the more purified preparations of FSH with lower LH levels are now preferred The protocol for superovulation of fallow deer is similar to that of red deer except for a longer (14 days) progesterone treatment (98) For mating the female deer are generally run at a rate of up to 10 per male. If mating is prior to the onset of the normal breeding season, males may need to be treated with subcutaneous melatonin implants to advance the rut or mating season. A common dose for red deer is the subcutaneous placement of 4 x 18 mg implants in early December (southern hemisphere) followed by 2 x 18 mg implants about six weeks later Although artificial insemination (AI) could offer considerable advantages in MOET programmes by allowing a greater coverage by genetically superior males, there have been a few attempts to do so in red deer. However, extremely low fertilisation rates with cervical AI or natural mating in MOET programmes in fallow deer have prompted the use of laparoscopic intrauterine AI in this species

Red Deer: An early study (49) with 12 NZ red deer hinds investigated the dose response to Ovagen administered via osmotic minipump (0.14, 0.28, 0.42, 0.56 units over 4 days) The mean ovulation rates and transferable embryos recovered per hind were 0.7, 2 0, 4 3 & 1 5 and 0, 0.7, 2 3 & 5 0. All embryos were at the compact morula or blastocyst stage The data indicate a clear dose response and have been used as the basis of further studies using Ovagen.

This experiment involved the use of minipumps to deliver the FSH but there is only one published comparison (49) of minipumps and twice daily injections for 4 d, this involved eight red deer females and injections were clearly superior (on average of 3.0 and 11.0 ovulations per hind). Despite the increased amount of handling involved, there is a strong opinion that twice daily injections are a more reliable approach The frequency of the FSH injections is also an important consideration. In one experiment (n = 56 NZ red deer) once daily and twice daily injections were compared along with a comparison of two sources of FSH There were no significant differences due to either variable, with the main effects comparison of once versus twice daily FSH giving average ovulation rates of 8.9 and 8.8 and transferable embryos of 2.9 and 2.4 per donor However, once daily injections have generally given disappointing results in subsequent commercial MOET programmes. This may be due to strain differences in that commercial MOET programmes involve the recently imported European strains of red deer (as distinct from the usual NZ red deer which is mainly of Scottish origin) but it may also be that the dose rate with once daily injections should be increased compared with the twice daily protocol. There may also be a difference in the efficacy of the different FSH sources, but any comparison would require dose response data. In one unpublished case, the mean ovulation rates for two strains of red deer were 4.2 and 8.8 (n = 46, SED ± 1.9, $P<0.01$). Commercial MOET programmes do take apparent strain differences into account by manipulating the FSH dose, based on experience

There have been few attempts at AI in the MOET situation with red deer, due to concerns about the potential cost of failure, the stresses of additional handling and lack of knowledge about the timing of ovulation under FSH treatment. However, in one case, natural mating was compared with AI and, although confounding factors preclude a completely valid comparison, only 33% of the embryos recovered from the AI group (one cervical and one laparoscopic insemination per hind) were transferable compared with 66% for the naturally mated group (2.2 and 5.5 transferable embryos per donor n = 20, SED ± 1.7, not significant) Considerably more research would be required to ensure the reliability necessary for AI to be used in a MOET programme in red deer.

The number of embryos recovered as a proportion of total ovulations (corpora lutea) is a variable of interest. In three MOET programmes involving a total of 186 red deer donors, 979 embryos were recovered (65%) of which 662 (68%) were of transferable quality Following recovery, embryos are graded and good quality embryos are frequently frozen and subsequently transferred. For example Dixon *et al* (42) reported an average pregnancy rate of 61% from frozen embryos (previously collected in New Zealand) transferred to 247 recipient red hinds over five properties in Australia. There are three data sets with MOET in red deer which allow evaluation of some factors influencing the success rate These include relationships with the ovulatory response, embryo quality and stage of development of the embryo at the time of transfer. Table 5 illustrates the outcome in terms of pregnancies per donor, where donors have been classified according to their ovulatory response The quality of embryos (assessed visually) also influences the subsequent pregnancy rate (Table 6), with embryos graded 1 and 2 on a 1 to 4 scale (very good to poor) giving higher pregnancy rates The stage of embryo development also influences the pregnancy rate (Table 7) although there are no differences in the rates achieved with embryos from the late morula to late blastocyst stage at transfer.

Table 5: Relationships between ovulatory response in European and hybrid red deer donors, the numbers of embryos recovered and transferred and the subsequent pregnancy rate (c. Day 40) after transfer to recipient NZ red deer hinds

Ovulatory response	Donors n	Embryos (mean/donor)		Pregnancy rate per	
		recovered	transferred	transfer	donor
0	1	-	-	-	-
1-3	6	7	7	0.14	0.16
4-6	8	40	29	0.55	2.0
7-9	9	63	61	0.72	4.9
≥10	10	104	74	0.72	5.3
Overall	34	214 (6.3)	171 (5.0)	0.67	3.4

Table 6: Relationship between quality (1 = very good appearance, 4 = poor) of embryos and pregnancy rate (at Day 40) of recipient hinds on two properties (A and B)

	Embryos transferred (n)		Pregnancy rate per transfer	
	A	B	A	B
Embryo grade				
4	25	0	0.12	-
3	25	20	0.56	0.55
2	39	98	0.80	0.70
1	134	53	0.86	0.64
Total	223	171	0.73	0.67

Table 7: Relationship between the stage of development of transferred embryos and pregnancy rate (at day 40) per recipient or donor hind on two properties (A & B)

	Embryos transferred (n)		Pregnancy rate	
	A	B	A	B
Embryo grade				
4-8 cell	12	0	0	-
Morula	24	10	0.33	0.20
Late morula	53	63	0.81	0.67
Early blastocyst	41	24	0.80	0.71
Blastocyst	50	42	0.84	0.74
Late blastocyst	43	32	0.86	0.69
Total	223	171	0.73	0.67
Mean per donor	3.7	5.0	2.7	3.4

Fallow Deer: While successful artificial insemination protocols are well developed for fallow deer, MOET studies have often met with mediocre results related principally to low ova recovery rates and low fertilisation rates of recovered ova (69, 98). Early studies indicate clear dose responses in ovulation rate to ovine FSH that parallel those in red deer (Table 8), albeit with greater variance at any given dose rate. These studies have generally involved the administration of low doses of PMSG (100-200 I.U.) at the beginning or end of FSH treatment to overcome the apparent "all or nothing" ovulatory response described previously for FSH

alone (98). However, the efficacy of the addition of PMSG over FSH alone does need to be further evaluated. In a recent on-farm study, 32 fallow donors were treated using a variety of superovulation techniques In all, 112 embryos were collected with 67 being of transferable quality. Of these, 30 embryos were transferred at the time of collection with 22 pregnancies resulting, a rate of 73%, which is comparable to the red deer data, 7 of 8 frozen embryos transferred subsequently also yielded pregnancies. However, the low number of transferable embryos per donor in the fallow deer (2 1) meant an overall rate of around 1.6 pregnancies per donor, which is much lower than that generally achieved in red deer.

Table 8: Mean (± sd) ovulation rates and total follicles of fallow deer and red deer (n = 100, 10 per treatment) to increasing doses of ovine FSH (Ovagen)

FSH Units	Fallow deer		Red deer	
	corpora lutea	total follicles*	corpora lutea	total follicles*
0	1 1 ± 1 2	2 5 ± 2 1	0 8 ± 0 3	1 5 ± 1 2
0 25	7 2 ± 5 1	10 0 ± 5 7	7 1 ± 4 2	9 6 ± 5 4
0 50	9 6 ± 7 5	14 9 ± 8 1	9 5 ± 6 3	13 5 ± 5 7
0 75	8 6 ± 7 2	17 3 ± 5 7	6 9 ± 1 8	10 3 ± 2 7
1 00	7 4 ± 6 3	12 9 ± 7 5	6 4 ± 3 9	9 7 ± 3 6

* Total corpora lutea and follicles > 5 mm diameter, including cystic and luteinised follicles

Commercial MOET in fallow deer has mainly involved donors of the Mesopotamian subspecies (*D. d mesopotamica*) or their hybrids with European fallow (*D. d dama*) Limited data from recent studies in European (n = 8) and the larger Mesopotamian hybrid (n = 7) does give similar ovulatory responses (8 6 and 7.0 corpora lutea per donor) and marginally different embryo recovery rates (33 and 49%) but the latter difference was not significant. However, recent attempts to superovulate a small number of pure Mesopotamian fallow does generally meet with complete ovulatory failure (W. Otway pers comm). Given the role of this genotype in international fallow deer farming the development of protocols/regimens specifically for Mesopotamian fallow deer is important The early studies on MOET in fallow deer were plagued by extremely low ova fertilisation (0-50%) and recovery rates (30-50%) following natural mating and/or intravaginal insemination of donors (69, 98) This raised questions about cervical passage of spermatozoa in superovulated donors. Two recent studies have attempted to overcome this problem by laparoscopically inseminating does with fresh semen (25-50 x 10^6 spermatozoa) 36 hours after removal of CIDR devices (i e about 12 hours after observed oestrus) The embryo recovery rates (i.e 30-50%) were considerably improved but were still lower than the more successful red deer programmes (69, 90) It was also notable that there was a wide range of embryo development stages observed in both fallow deer studies

Techniques of Embryo Recovery: Embryos are generally recovered using surgical exteriorisation of the uterus (with or without the ovaries) and flushing with PBS (0 4% BSA) media, with collection using a Foley 10 F catheter The media is initially at 35°C but is allowed to cool to 15°C after collection Laparoscopic procedures are being developed Non-surgical recoveries have been performed on the larger North American wapiti (>200 kg live weight) In all species embryos are recovered 8-9 days after progesterone withdrawal For embryo recovery, females may be anaesthetised by a variety of different treatments, although

a fentanyl citrate/xylazine hydrochloride combination with or without intubation and halothane (49) is generally preferred for red deer and a xylazine hydrochloride/ketamine combination for fallow deer (28). Clenbuterol, a smooth muscle relaxant, is recommended to ease manipulation of the reproductive tract during embryo recovery (49). The anaesthetic/sedative is reversed using nalorphine and yohimbine in red deer (49), and yohimbine alone in fallow deer (28).

Recipient Synchronisation and Embryo Transfer: Recipient females are also synchronised using a progesterone-CIDR device. In red deer the intravaginal CIDR treatment continues for approximately 12 days (49), with an injection of 200 I.U. PMSG at CIDR withdrawal. In fallow deer the treatment omits the PMSG because of highly variable results (29). It is recommended that vasectomised males are run with the females (to ensure normal male-female behaviour around oestrus) from the time of progesterone withdrawal until placement with an intact male some days after transfer, the latter to ensure that donors and recipients have a further opportunity to conceive and carry progeny to term. Embryo transfer is generally effected by a technique involving either a partial exteriorisation of the horns of the uterus (using a laparoscope to locate the uterus) or by a simple intrauterine transfer using a Cassou pipette in the same way as for AI. Normally single embryos are transferred and all recipient females are checked by laparoscopy to ascertain that they have ovulated recently. Procedures for the induction and reversal of sedation are as used for donor animals. Pregnancy status is assessed using a real-time ultrasonic scanner per rectum at around Day 40 of pregnancy. For this purpose the females are restrained in an appropriate crush which limits forward and lateral movement.

Economic Analysis: Table 9 presents a simplified costing for a multiple ovulation and embryo transfer programme. The costs are high and very strongly dependent on the superovulatory response and the pregnancy rate. For example, a lower pregnancy rate of 54% in recipients (i.e. 50% of transferred embryos results in weaned calves) increases the cost per calf by 19, 26 and 29% for 20, 40 and 60 transferred embryos per 10 donors, respectively. As with any reproductive technologies, the financial benefits of embryo transfer depend on the market for the offspring of the presumably high-value donors.

Table 9: Simplified costing of embryo transfer (ET), the influence of the superovulatory response in terms of embryos transferred per 10 donor hinds on the average cost of a calf (i.e. cost in excess of that of a calf born to a natural mating)

Transferred embryos per 10 donors	Pregnancies to ET	Weaned calves to ET	Costs[1]	Weaned calves including donors own calf[2]	Cost per donor calf weaned
20	15	14	$11,800	23	513
40	30	28	$12,800	37	346
60	45	42	$13,800	51	271

[1]Costs: Ten donors at $1,000 each with recipients at $50 each, opportunity cost of seven standard (i.e. calves x natural matings) per 10 recipients (i.e. 10 recipients would be expected to wean nine progeny normally, but for every 2.5 recipients not pregnant to ET, 2.0 will wean a calf and therefore the net opportunity cost per 10 recipients would be $800 - i.e. nine "normal" calves at $111 each less two late calves at $100 each = $800 per 10 recipients). The opportunity cost of not producing a "normal" calf is a cost on the ET procedure.
[2]Assumes a 75% pregnancy rate to ET and a weaning percentage of 93% of pregnancies in the recipients and 90% in the donors.

In-vitro Fertilization

Rationale: *In-vitro* produced embryo (IVP) technology for farmed cervids has the same advantages as for traditionally farmed species, namely, the production of large quantities of embryos for transfer and oocyte/embryos for other manipulations such as embryo sexing, gene injection and cloning. The establishment of *in-vitro* technology would enhance the creation of new hybrid species that would not normally hybridise and assist in captive breeding programmes for endangered cervid species *In-vitro* fertilisation has several advantages over the traditional artificial insemination and embryo transfer methods: circumvention of the problem of timing ovulation for AI, production of largers numbers of embryos at the correct stage of development than can be recovered from hormonally-stimulated donors, reduction in the numbers of viable sperm needed as compared with AI, making use of females with certain types of infertility, salvaging genetic material from females after death, and decreasing generation intervals by using pre-pubertal animals as oocyte donors (80)

Oocyte recovery: Oocytes can be obtained from the follicles of living animals or sourced from abattoir-derived ovaries. Live animals donors are usually females of high genetic value that have blocked/scarred oviducts or unexplained infertility. Laparoscopic follliculocentesis has been used successfully for sheep (32, 97) The females are usually superovulated and the oocytes are aspirated from all visible follicles prior to ovulation. The oocytes are *in-vitro* matured, fertilised and cultured. The advantages of laparoscopic follliculocentesis is that the oocytes from one donor may be fertilised with different sires and oocytes can be obtained from pre-pubertal females (8).

Recovery rates are affected by size of the follicles. A greater number of oocytes are recovered from large follicles vs. small follicles (53% vs 43%; (97); 84% vs 63% (32)) To date, laparoscopic follliculocentesis has not been applied to cervids, but oocytes have been recovered from the follicles of abattoir derived ovaries. Immature oocytes are aspirated from 1-6 mm follicles, with an average of six good quality oocytes per ovary being recovered from red deer ovaries The number of oocytes recovered is affected by pregnancy, with the number of oocytes decreasing as the corpus luteum increases in size (D K Berg, unpublished results)

***In-vitro* maturation:** Oocytes aspirated from preovulatory follicles are immature and need to be cultured before they can be fertilised. During culture, the oocyte resumes meiosis and completes the first meiotic division before fertilisation. Red deer ova resume and complete the first meiotic division within 19-24 hours after the preovulatory LH surge (34) This time period is similar to bovine and ovine ovum maturation. Red deer and reindeer (*Rangifer tarandus*) oocytes mature well in the standard bovine and ovine *in-vitro* maturation system *In-vitro* maturation rates range from 70-80% for red deer (35, 62) and 71% for reindeer oocytes (78).

***In-vitro* fertilization:** Successful *in-vitro* fertilisation is dependant upon a three factors, mature oocytes at the correct stage, capacitated sperm, and a suitable medium for both the sperm and oocyte. Attempts to extrapolate bovine IVF systems for cervids have not been particularly successful. The addition of heparin to bovine IVF medium results in fertilisation rates of 95% for cattle (95). However, only 20% of red deer oocytes and 36% of reindeer oocytes have been fertilised following the addition of heparin to bovine IVF media (62, 78) Low fertilisation rates in red deer have been attributed to poor post-thaw motility of red deer sperm, poor *in-vitro* survival of sperm in traditional IVF media, and a general lack of understanding concerning the events associated with cervine sperm capacitation and

fertilisation. *In-vitro* sperm survival has been extended beyond 24 hours by co-incubating red deer sperm on oviductal monolayers. However no increase in IVF rates were observed (D.K. Berg, unpublished data). Table 10 summarises IVF results using different capacitation systems, different types of sperm and different IVF media. IVF rates remain low relative to other ruminant species.

Several changes in sperm preparation, capacitation medium, and an earlier addition of sperm to oocytes led to a successful red deer IVF protocol. The percentage of fertilised ova ranges from 80-100%, however, polyspermic (more than 1 sperm/ova) fertilisation is a problem Reduction of sperm concentration from 2 million to 300,000 sperm per millilitre reduced the proportion of polyspermic ova from 43 to 6% (Table 11). This reduction in sperm numbers makes cervine IVF a perfect candidate for utilising sexed sperm.

***In-vitro* culture:** Attempts to culture *in-vitro* produced embryos (IVP) have been disappointing. Red deer IVP embryos fail to develop beyond the 8-cell stage (62) and IVP reindeer embryos results are equally low with only two embryos developing to morulae from 154 oocytes (78). However, a recent study utilising *in vivo* derived 1 and 2 cell red deer embryos showed that development can proceed past the 8-cell stage to blastocyst under three different culture systems (34; Table 12). Four *in-vitro* cultured embryos were transferred to recipient hinds and two live calves were born.

Overall status of IVF technologies: Results to date are encouraging for the cervine IVP embryo, with IVF results comparable to bovine and ovine IVF Further research needs to focus on embryo culture, with the aim of producing good quality embryos for transfer.

Table 10: Percentage of IVM oocytes fertilised *in vitro* with different sperm types, media and capacitation systems

Species	Sperm Type	Capaciation Agent	Media	Fertilised (%)	Reference
Red Deer	Frozen-thawed	Heparin (100 µg/ml)	TALP	20	62
Red Deer	Fresh	Heparin (10 µg/ml)	TALP	9	35
Red Deer	Fresh	Cervine oviduct monolayers	TALP	25	35
Red Deer	Fresh	Oestrus deer serum (10%)	Defined Medium	10	35
Reindeer	Frozen-thawed epididymal	Heparin (10 µg/ml)	TALP	36	78

Table 11: Percent of IVM oocytes fertilised and percent of polyspermic fertilisation with different sperm concentration

Sperm concentration	Fertilisation (%)	Polyspermy (%)
2×10^6	70 + 0 09	32 + 0 09
1×10^6	70 + 0 11	43 + 0 08
5×10^5	71 + 0 05	39 + 0 06
3×10^5	61 + 0 09	6 + 0 04

Table 12: Proportion of *in vivo* derived embryos developing to morula/blastocyst stages *in vitro*

	Culture Treatment		
Embryo stage	**Ligated sheep oviducts**	**Oviduct monolayer**	**SOF + HS**
Morula	25 ± 11	26 ± 8	22 ± 6
Blastocyst	50 ± 13	56 ± 9	58 ± 7

Induction of Twinning

Rationale: Biological efficiency, in terms of meat production per unit food energy input, is strongly influenced by reproductive rate in deer farming systems (45) However, with near maximal fertility rate achievable in farmed red deer and fallow deer in New Zealand (~95% pregnancy rates in well managed herds: 14) there is little opportunity for increasing reproductive rate other than by an increase in multiple births (51). The low incidence of natural twinning in red and fallow deer means that the only options for increasing reproductive rate in farmed deer are genetic selection or hormonal induction of double ovulations.

Progesterone & PMSG: A number of studies have investigated the use of the intravaginal CIDR device in conjunction with an intramuscular injection of 200-500 I.U. PMSG at device withdrawal. It may be significant also that in most cases treatments were applied prior to the normal breeding season (39, 44, 51, 88).

In field trials on red deer in the Southland Region, Bringans & Lawrence (39) obtained a low percentage of treated hinds bearing twins (11%), although dose rate of PMSG had a major effect (4% for hinds receiving 200 i.u PMSG vs 14% for hinds receiving 400 i u PMSG) Furthermore, of hinds carrying twins 30% required assistance at calving and the mortality rate of twin calves was considered to be high. Data from other studies were more encouraging, however. Fennessy *et al* (51) found 64% of 92 treated hinds conceived to induced oestrus, and of these, 39 produced single calves, 19 produced twins and one produced triplets. The multiple pregnancy rate for treated hinds was 21 7%. Furthermore, 75% of twins survived compared with 85% of singletons, giving an overall productive advantage to hinds bearing multiples. As with other ruminant species, twin red deer were substantially lighter than singles at birth, proportionately about 30% lighter (cf. 15-25% lighter for other species, 51) Accordingly, most deaths among twins were in calves of low birth weight (⅔ deaths were of calves of ≤6 kg birth weight). The study demonstrated that the red deer hind has considerable capacity to increase milk output adequate to rear twins, although for a given birth weight, twin-reared calves were about 5 kg lighter than singles at 20 weeks (51)

The incidence of freemartinism may also be an important consideration; in this respect several females from sets of unlike-sex twins have been found subsequently to be freemartins (96) As these females are non-reproductive, induction of twinning to increase recruitment rates of breeding female red deer will need to take into account the production of freemartins.

Induction of twinning in fallow deer is also possible by increasing the ovulation rate with the CIDR device + PMSG regimen (17) However, of all 11 induced twinnings recorded on the Ruakura Agricultural Centre, none has resulted in a single live fawn at weaning Typically, the fawns were born non-viable due to excessively low birth weights (ie· ≤2 0 kg) It would appear that the total weight of twins at birth (~4 kg) roughly equates to the normal birth weight

of a singleton. Thus, it is probable that embryonic competition, due to the fallow deer placentation system, exacts a high price on fetal growth On the basis of this work, it would appear that induction of twinning in fallow deer is counter-productive over normal reproductive management (13).

Economic Analysis: Successful twinning in practical farm situations requires a very high levels of managerial skill. The economics of the practice will thus depend greatly on the manager. For this reason no economic analysis is presented

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