ARTIFICIAL INSEMINATION, EMBRYO TRANSFER AND GAMETE MANIPULATION OF FARMED RED DEER AND FALLOW DEER

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(1) INTRODUCTION

(1.1) Application of controlled breeding technology

Red deer (*Cervus elaphus*) and fallow deer (*Dama dama*) form the basis of a rapidly growing deer farming industry within the world's temperate regions, with large numbers of breeding stock being found in New Zealand, Australia, North America and Europe. The application of controlled breeding 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 hybridization programmes. Artificial insemination (AI), embryo transfer (ET) and various forms of gamete manipulation allow for wider and more rapid dissemination of desirable genetic material than would be remotely possible by natural means. This is particularly important when considering such rare genotypes as Mesopotamian fallow deer (*Dama dama mesopotamica*), Pere David's deer (*Elaphurus davidianus*) or imported blood lines. Moreover, AI and ET provide a safe and cost-effective means of international exchange of genetic material.

This paper reviews the present state of controlled breeding technology for red deer and fallow deer by discussing the major components of AI (oestrous synchronization, semen collection/processing and insemination techniques), ET (superovulation, embryo recovery, cryopreservation, and embryo transfer) and gamete manipulation (oocyte recovery, IVM, IVF, embryo splitting, cloning and sperm/embryo sexing).

(1.2) Reproductive seasonality

Red deer and fallow deer exhibit highly seasonal patterns of reproduction that are entrained by prevailing photoperiod regimens (76, 107, 112, 122). These patterns have evolved to maximise survival of offspring within highly seasonal environments (96) but impose certain constraints on the application of manipulative reproductive techniques within populations of farmed deer. The onset of the breeding season for females of both species, as indicated by the initiation of ovulatory cycles, occurs during the decreasing daily photoperiods of autumn and is preceded by a period of deep anoestrum/anovulation in summer (4, 79). The first oestrus of the breeding season is usually preceded by one or more silent ovulations associated with the formation of short-lived (10-12 days) corpora lutea (Figure 1) that may serve to synchronize first overt oestrus within a herd (4). Subsequent oestrous cycles undergo luteal and luteolytic phases fairly typical of domestic ruminants, as indicated by ovarian progesterone secretion (Figure 1) and uterine prostaglandin secretion (14). However, the average length of the oestrous cycle differs for the two species, being ~18 days for red deer (63, 86) and 21-22 days for fallow deer (4, 102). In the absence of conception, oestrous cyclicity in both species can persist for 4 to 6 months (Figure 2), with a gradual increase in oestrous cycle length being evident with later cycles (4, 63). Of particular interest, however, is the remarkable uniformity and predictability of the length of the first oestrous cycle of fallow deer (21.0 + 0.64 days; mean + s.d.) (4).

In New Zealand, first oestrus of the breeding season generally occurs 2-3 weeks earlier for red deer (late March) than for fallow deer (mid April). This is clearly reflected in the relative onset of the birth seasons for the two species (7) (Figure 3)

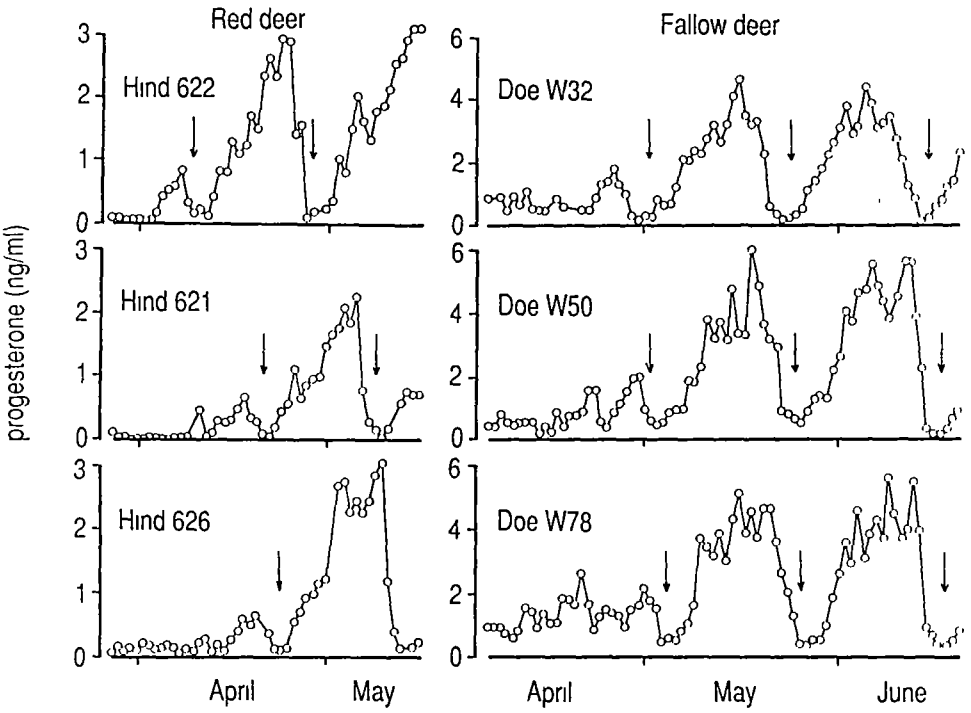

Fig. 1. Plasma progesterone profiles from daily sampling of female red deer and fallow deer at the start of their respective breeding seasons in New Zealand. Arrows indicate overt oestrus. The low-amplitude progesterone cycles prior to first oestrus are indicative of silent ovulations and transient corpora lutea (4, 8).

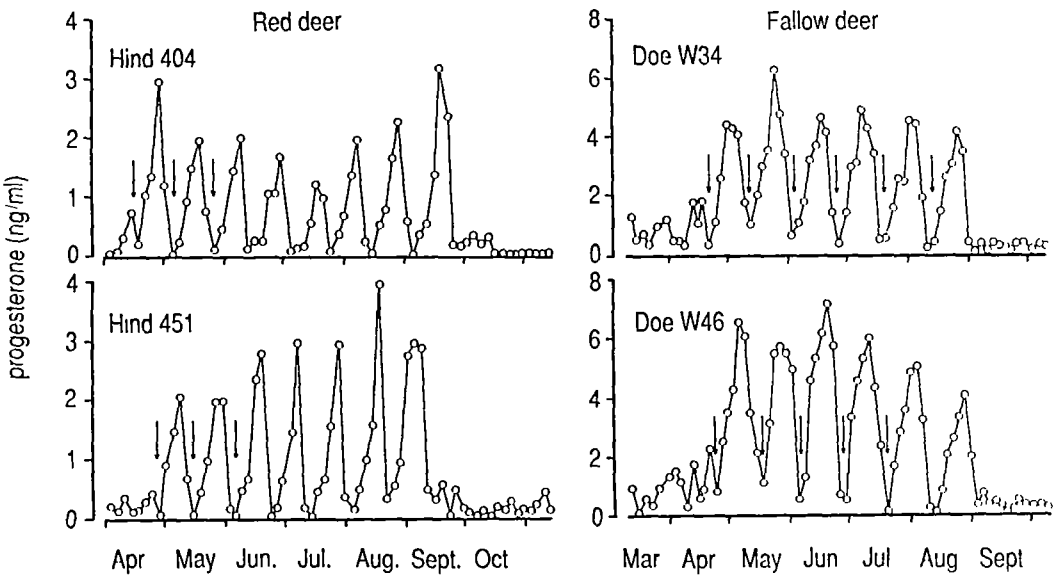

Fig. 2. Plasma progesterone profiles of female red deer and fallow deer in New Zealand that were run continuously with vasectomized males. Arrows indicate overt oestrus (4; M.W. Fisher, unpublished data).

given that both exhibit similar average gestation lengths (234 days) (6, 39, 63, 82). It is important to note that both species appear to exhibit an unusually high level of fertility to first oestrus of the breeding season. Conception rates following natural mating to spontaneous oestrus are in the order of >80% (5, 6, 51). As with other domestic ruminants, pregnancy is associated with cessation of further oestrous cyclicity (5, 6) and the maintenance of the progesterone secretory output of the corpus luteum and/or placenta (2, 6, 83).

Adult male red deer and fallow deer exhibit marked annual fluctuations in liveweight that reflect hormonally-controlled seasonal changes in voluntary feed intake (47, 81, 122) and rutting activity (13, 17, 93, 95). Such liveweight changes are related to pronounced annual reproductive cycles, which have been described for red deer stags (93, 94, 121) and fallow deer bucks (13, 17), and are essentially similar for the two species. In the adult fallow buck, for example, testicular development undergoes annual cyclic changes (Figure 4). This is primarily controlled by marked changes in pituitary LH secretion (17). LH secretory pulses alter in amplitude and frequency during the year, being of low amplitude and frequency during the non-breeding season (early summer) and of high amplitude and frequency leading up to the onset of the breeding season in autumn. The subsequent effects on promoting testis growth and testosterone secretion result in a concomitant increase in spermatogenic activity such that by the onset of the rut large numbers of viable spermatozoa are present in ejaculates (13, 62, 102). The testes remain active throughout winter, secreting modest levels of testosterone (17) and producing large numbers of spermatozoa (13). However, with the onset of spring, LH secretion diminishes and the testes regress in size and secrete only very low levels of testosterone. Spermatogenesis is completely arrested by early summer; the bucks becoming effectively infertile. They remain infertile for about 2 months, gradually regaining fertility towards the end of summer (13, 62).

Annual changes in testosterone secretion also have marked effects on the antler cycle (93) and on some muscles (52). In particular, rising testosterone concentrations in later summer/early autumn cause hypertrophy of the neck muscles, resulting in a massive increase in neck muscle mass by the start of the rut (Figure 4). The annual reproductive cycles of fallow deer in the southern hemisphere are schematically summarized in Figure 5. The patterns are essentially similar for red deer.

(2) ARTIFICIAL INSEMINATION

(2.1) Introduction

While a number of studies have investigated AI in various species of cervids (e.g. reindeer *Rangifer tarandus*, 43; wapiti *Cervus elaphus nelsoni*, 65; white-tailed deer *Odocoileus virginianus*, 64, 98), they have involved only very small numbers of females with variable, often low, conception rates. Recent trends towards commercial application of AI of farmed deer in New Zealand, Australia and USA have prompted rapid progress in development of more effective techniques.

(2.2) Oestrous detection

Detection of spontaneous oestrus in farmed deer has generally proven difficult to achieve 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 to other domestic livestock and is often terminated at copulation, within minutes of its onset (5). The use of ram mating harnesses on male deer has been successfully adopted in a number of studies on red deer (15, 63) and fallow deer (4, 11, 12, 19). While the method has proved very effective for fallow deer, with >90% of first spontaneous oestrus being detected under controlled experimental conditions (4, 5), its effectiveness for detection of natural oestrus in red deer is variable. Red deer stags appear to exhibit a low mount-to-service ratio during mating when compared with fallow deer (5, 127). 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.

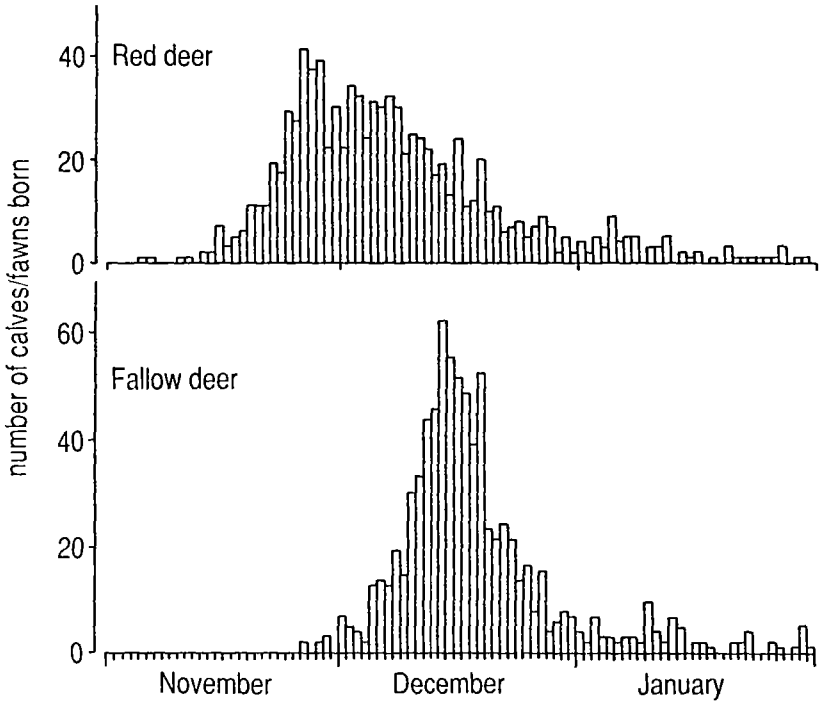

Fig. 3. Frequency distributions of birth dates for red deer and fallow deer on some commercial farms in northern regions (38^{o}-39^{o}S) of New Zealand between 1980 and 1984 (8).

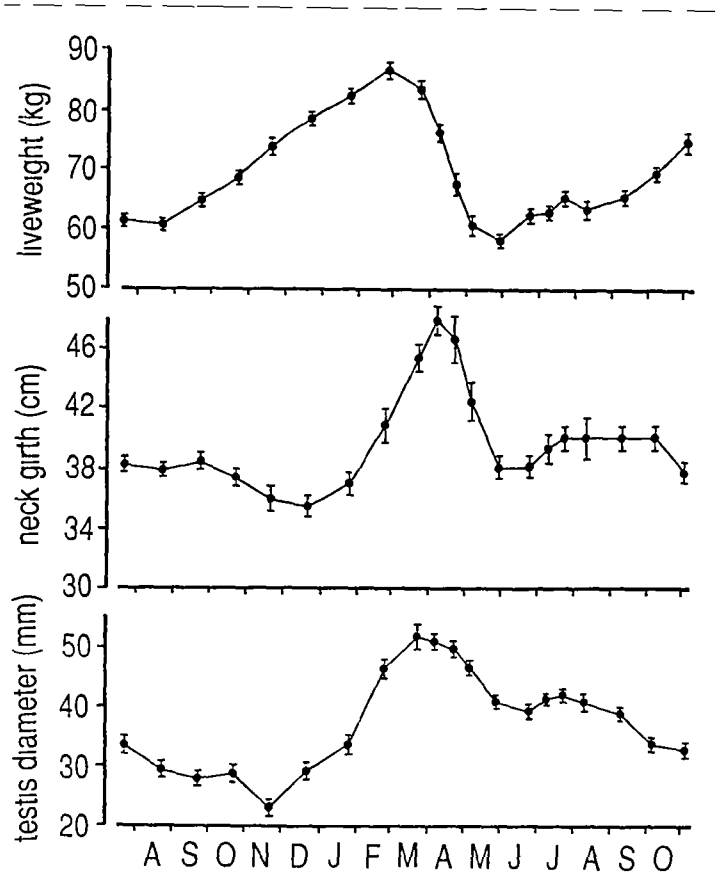

Fig. 4. Seasonal profiles of mean (± s.e.m.) liveweight, neck girth and testis diameter of 4 entire adult (5 years) fallow bucks in New Zealand (17).

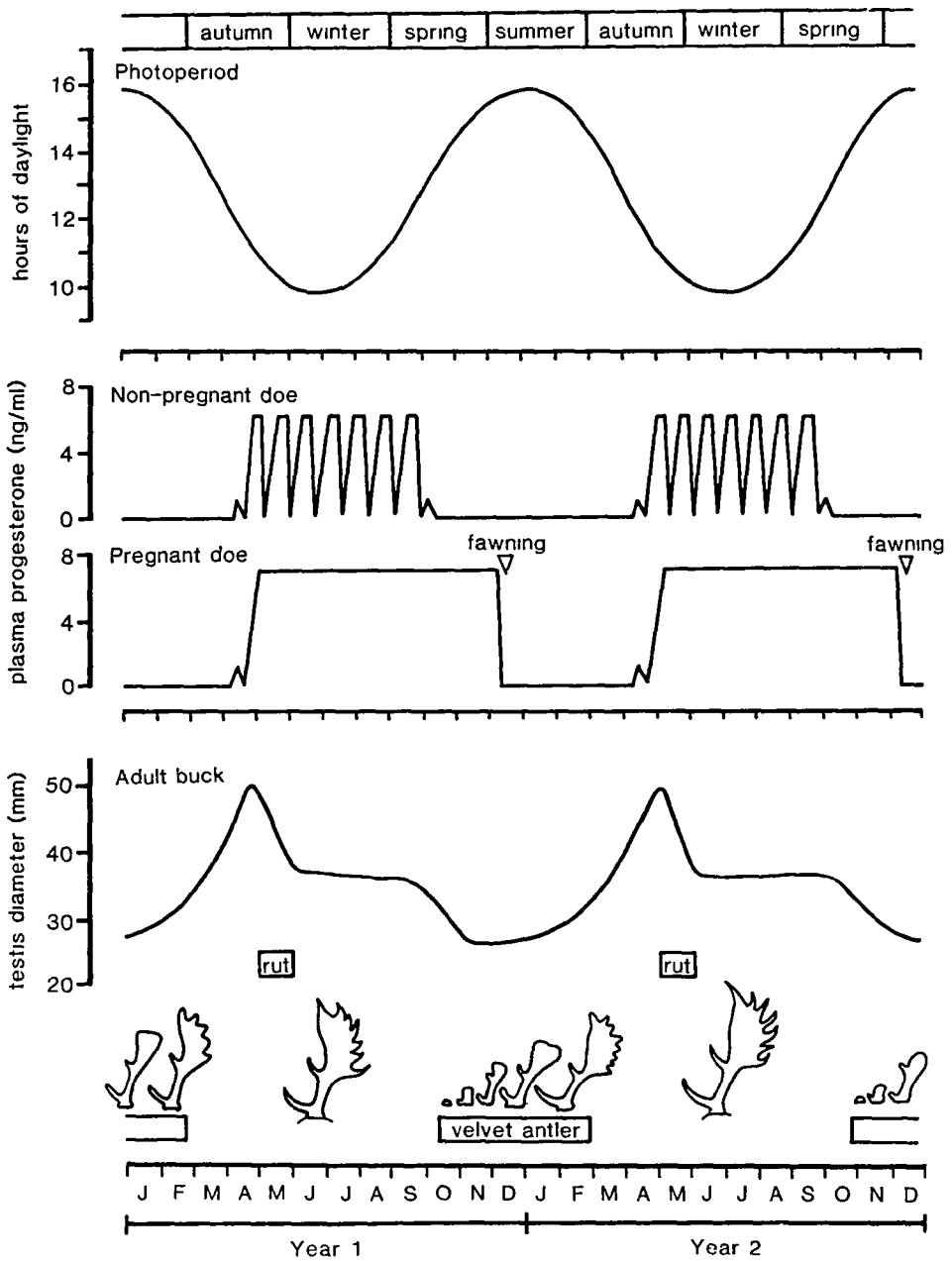

Fig. 5. Schematic summary of the annual cyclic reproductive changes that occur for adult fallow deer in relation to photoperiod and season (southern hemisphere).

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.

(2.3) Oestrous synchronization

Artificial synchronization of oestrus in farmed deer has been adopted as a more cost-effective alternative to detection of natural oestrus. As with other domestic ruminants, synchronization 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 (Figure 6). For fallow deer, it is also possible to obtain a high degree of synchrony of a return oestrus following artificial synchronization of the first oestrus (12) (Figure 6).

(2.3.1) CIDR device in fallow deer: While a wide range of progestagen-releasing devices has yet to be tested for efficacy of oestrous synchronization in deer, a large number of studies in New Zealand have investigated the use of the intravaginal CIDR [Controlled Internal Drug Release] device (CIDR-type S or CIDR-type G; 9-12% w/w progesterone; Agricultural Division, CHH Plastic Products Group Ltd, Hamilton, NZ).

During prolonged (12-14 days) intravaginal insertion in the fallow deer doe, single type-S or type-G CIDR devices (0.365 g progesterone per device) elevate peripheral plasma progesterone concentrations to levels comparable to those observed during the mid-oestrous cycle (11, 12, 19). Exogenous progesterone is cleared from the peripheral system within 2 h of device withdrawal (Figure 7). In this respect, the devices appear well suited for use in fallow deer. Efficacy of oestrous synchronization 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 dependant 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%; C.J. Morrow, unpublished data). 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 first spontaneous oestrus within the herd (Figure 8). 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 (11, 12; C.J. Morrow, unpublished data). The onset of oestrus in fallow does coincides with the onset of the pre-ovulatory LH surge, which attains maximal amplitude (20-30 ng/ml) 4-6 h later (11, 12, 19). Recent studies have shown that ovulation (follicular rupture) occurs 24 h after the onset of oestrus/preovulatory LH surge following CIDR device synchronization (19). Therefore, ovulation is synchronized to within the period of 70 to 80 h after device withdrawal.

Studies have yet to be conducted on the optimum duration of CIDR device insertion for fallow deer. On the basis of a 21 day first oestrous cycle (4), 14 days of device insertion has been most commonly applied to artificial insemination programmes (16, 20). However, insertion for as little as 9 days has been effective in synchronizing oestrus in this species (12) although the level of fertility has not been assessed.

The use of PMSG at or near CIDR device withdrawal is presently contra-indicated for fallow deer. Studies in which either 500 i.u. (10), 200 i.u. (G.W. Asher, unpublished data) or 100 i.u. (H.N. Jabbour, unpublished data) PMSG were administered by i.m. 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 ovulations or completely failed to ovulate after device withdrawal. This appeared to result in reduced conception rates to natural mating and increased incidence of embryonic mortality (particularly with multiple foetuses). This observation is supported by those of commercial inseminators following the administration of between 100 and 200 i.u. PMSG at CIDR device withdrawal (M. Bringans; personal communication). More recent studies on artificially inseminated does have indicated that 50 i.u. PMSG delivered at device withdrawal reduces the interval to the onset of oestrus and induces greater oestrous synchrony compared with CIDR device withdrawl alone, although fertility was not enhanced (H.N. Jabbour; unpublished data).

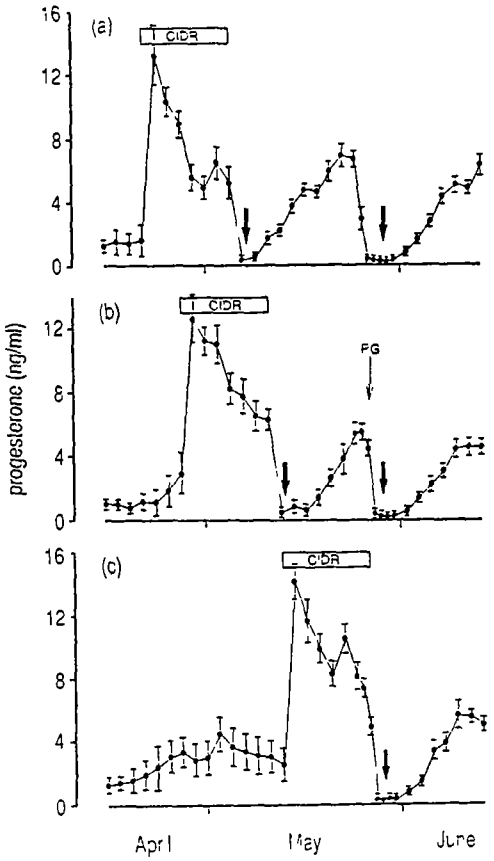

Fig. 6. Three methods of artificial oestrous synchronization in farmed fallow deer (11). Profiles of mean (± s.e.m.) plasma progesterone values of fallow does (n=5 per profile) during different treatment regimens designed to synchronize oestrus on 28 May. (a) initial 14-day CIDR device followed by a 21-day oestrous cycle; (b) initial 14-day CIDR device followed by an i.m. injection of prostaglandin analogue on Day 13 of the subsequent cycle; (c) 14-day CIDR device treatment alone. Arrows indicate the mean times to onset of oestrus.

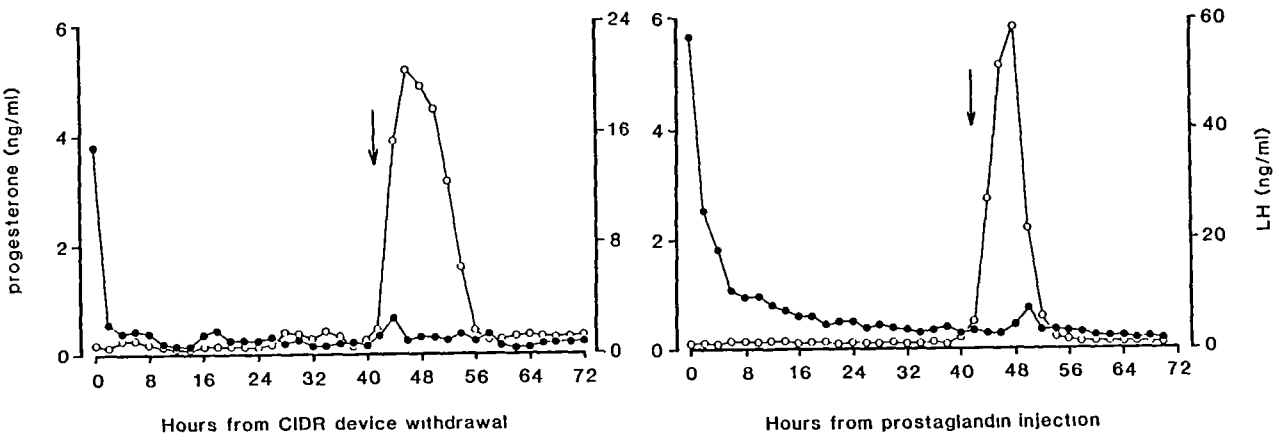

Fig. 7. Profiles of plasma progesterone (•) and LH (o) concentrations of individual fallow does following CIDR type-S device removal or prostaglandin injection. The arrow indicates the onset of oestrus (11).

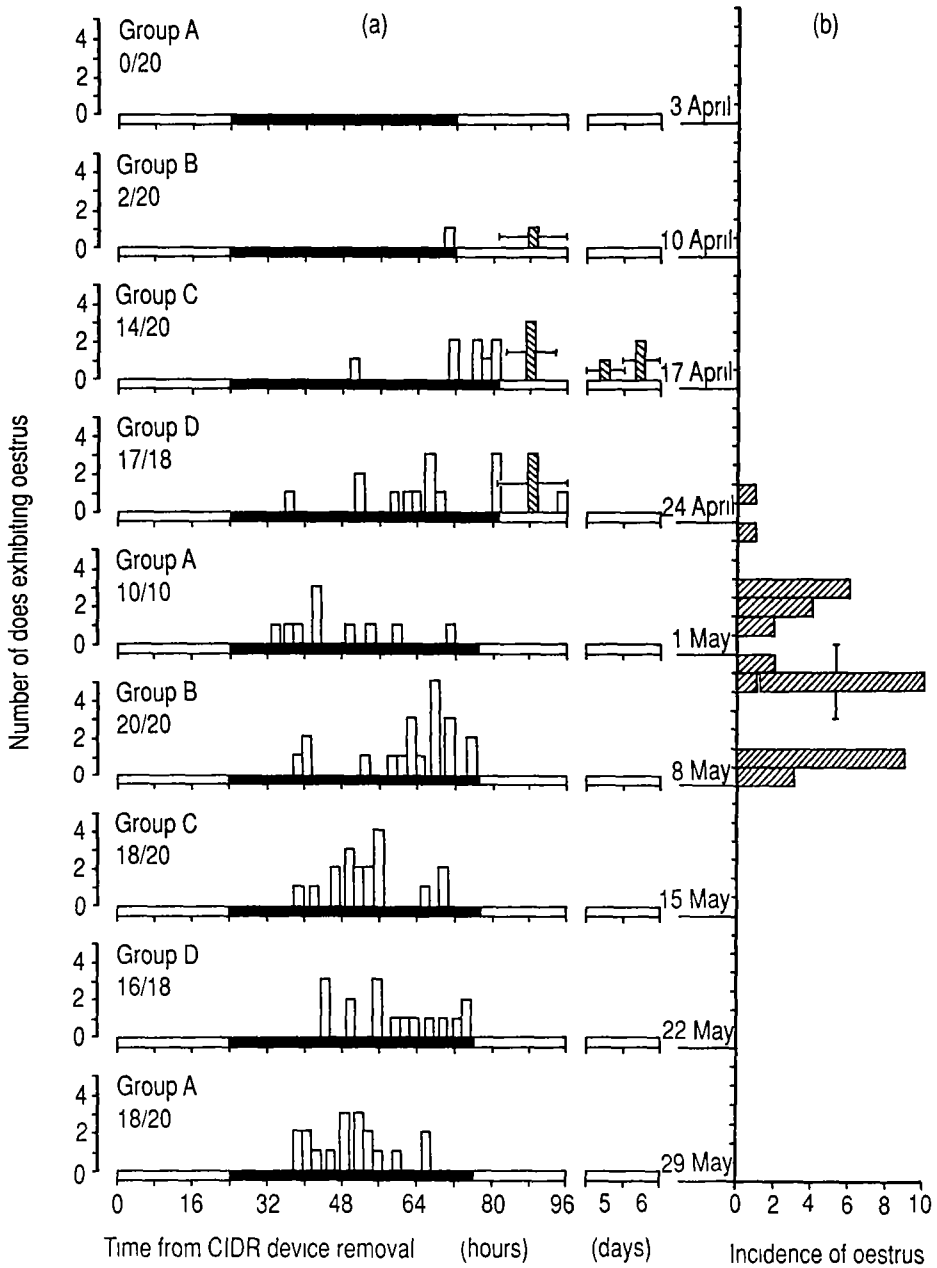

Fig. 8. (a) Frequency histogram of the time to onset of oestrus from CIDR device withdrawal at 7-day intervals, plotted relative to (b) the incidence of first spontaneous oestrous observed for contemporary fallow deer does. The solid portion of the abscissa axes represents the period of continuous observations 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).

(2.3.2) CIDR device in red deer: Peripheral plasma progesterone profiles of red deer hinds receiving single type-S or type-G CIDR devices differ from those of fallow deer. Concentrations within the first 6 days of insertion are comparable to those observed during the oestrous cycle (2-3 ng/ml). Thereafter, levels decline to <1.0 ng/ml by day 14 (79). This raises questions about the effectiveness of single CIDR devices to inhibit follicular development in red deer (50, 79), although such treatment has generally resulted in acceptable (>50%) conception rates to artificial insemination (29, 51). The low resultant peripheral progesterone concentrations have occasionally prompted the use of double CIDR (type-S) devices (15) or CIDR device replacement on day 9 (51). However, no improvements in conception rates following artificial insemination have been noted for these treatments (29, 51).

It has become routine practice to administer 200-250 i.u. PMSG at or near CIDR device withdrawal in red deer (50). 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 (55, 57). Second, 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 (50). Third, it is reasoned that PMSG may reduce the spread of ovulation in groups of hinds (50). While the influence of season on the incidence of oestrus/ovulation following CIDR device treatment has been well demonstrated for red deer (55, 57) and fallow deer (C.J. Morrow, unpublished data), 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 (79) and fallow deer (18). 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 (33) 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 oestrus/ovulation synchrony in red deer requires further investigation. Recent studies indicated that administration of 200 i.u. PMSG at CIDR device withdrawal reduced the mean interval to the onset of oestrus (37.4 h; n=7) compared to CIDR device withdrawal alone (44.4 h; n=7) but did not necessarily reduce the variance (s.d. = 5.3 vs 2.3 h). For both treatments, ovulation occurred between 20 and 28 h after the onset of oestrus (G.W. Asher; unpublished data).

A slightly increased incidence of multiple ovulation has been observed following 200-250 i.u. PMSG administration to red deer hinds (G.W. Asher, unpublished data). While this has occasionally resulted in conception and birth of twins in artificial insemination programmes (15) 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 (15, 29, 51, 55). The optimum duration has yet to be investigated in detail. However, the data of Fennessy et al. (51) 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.

(2.3.3) Progestagen sponges: A limited number of studies have investigated the efficacy of intravaginal sponges, impregnated with either fluorogestone acetate or medroxyprogesterone acetate, in inducing synchronized oestrus in red deer (1, 15, 66, 83) and fallow deer (103). While synthetic progestagens are well able to control ovulatory activity, some of the studies were plagued by excessive sponge loss rates (66, 103), mitigating against their general effectiveness in deer.

(2.3.4) Prostaglandin: The ability of prostaglandin administration to synchronize oestrus is dependant on the presence of an active corpus luteum at the time of

treatment, limiting synchronization programmes in red and fallow deer to the period after the onset of natural ovulatory activity (i.e. the rut). Furthermore, studies on wapiti (*Cervus elaphus nelsoni*) indicate that the cervine corpus luteum may be refractory to prostaglandin treatment before day 11 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 (50).

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 (Figure 7) (11). More recent studies have shown that ovulation (follicular rupture) occurs 24 h after the onset of prostaglandin-induced oestrus in fallow deer (19). Administration of 50 i.u. PMSG at the time of prostaglandin administration in fallow deer does has been observed to reduce the mean (+ s.e.m.) interval to onset of oestrus (33.5 $\pm$ 1.7 vs 47.1 $\pm$ 2.9 h; H.N. Jabbour; unpublished data). Early observations indicated a reasonable level of fertility following prostaglandin synchronization in this species (11). However, recent application of prostaglandin synchronization to artificial insemination programmes has resulted in lower conception rates than observed following CIDR device synchronization. In one study on laparoscopic intrauterine insemination, the conception rate following prostaglandin administration was 52.9% (27/51) compared with 70.4% (38/54) following CIDR device withdrawal (G.W. Asher, unpublished data). In this particular study, initial synchronization of oestrus/ovulation was performed with CIDR devices and may have been conducted too early in the season for an optimum ovulatory response (C.J. Morrow, unpublished data). However, in a study conducted three weeks later (H.N. Jabbour, unpublished data) a similar trend was observed following intracervical insemination. 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 were performed at similar intervals from the mean onset of oestrus (and hence, ovulation). It is interesting to note from this study, that the administration of 50 i.u. PMSG appeared to increase the conception rate for prostaglandin synchronization (65.4%; 17/26) but to lower the rate for CIDR device synchronization (61.5%; 16/26) (H.N. Jabbour, unpublished data). The use of prostaglandin synchronization in red deer has not been investigated extensively. Haigh et al. (65) recorded briefly 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. (66) further recorded treatment of red deer hinds with double prostaglandin injections. However, only 7.7% (1/13) became pregnant to natural mating; whereas 84.6% (11/13) 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 oestrous synchrony.

(2.4) 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 quiesence during spring and summer, at which time they are effectively infertile (13, 62, 86, 93). This limits semen collection to the 4 to 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; being 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 (13, 15, 16, 48, 51, 62, 77). The use of chemical immobilization 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.

Polish researchers have pioneered studies on the use of natural semen collection methods in red deer (85, 86). They designed a variety of artificial vaginas (AVs) that were either hand held or worn externally by oestrous hinds or dummy hinds treated with oestrous hind urine. While they were successful in obtaining ejaculates with the AV's, the techniques generally required a high level of stag training and habituation. As such, these techniques have limited application for farmed red deer because of limited opportunities to train stags of high genetic merit.

Jabbour and Asher (71) describe the development of a prototype internal AV, primarily for semen collection from fallow deer, but also having potential application in other cervid species. For semen collection with the device, ovariectomized fallow does are treated with CIDR devices for 6 days and 0.05 mg oestradiol benzoate (ODB) 24 h after CIDR device withdrawal. The does are fitted with the internal AV at the mean time to onset of oestrus, generally 18-24 h after ODB injection (H.N. Jabbour, unpublished data), and exposed to the bucks within their pastoral environment. Following observed mating, the AV 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 buck, more frequent collection per buck and the potential for obtaining ejaculates of higher quality.

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 (86, 102, 103) or in 0.25 ml straws in liquid nitrogen (15, 16, 51). Fallow deer semen has been shown to be particularly resistant to the rigors of the freezing-thawing procedures, with post-thaw recovery (motility) rates often being in excess of 70% of pre-freezing motility rates (16, 20). 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 (Table 1). Post-thaw motility rates of red deer semen appear to be highly variable, both between stags and between consecuative ejaculates from the same stag (51). This warrants further investigation into cryopreservation techniques for this species.

(2.5) Artificial insemination

(2.5.1) AI of fallow deer: (a) Intravaginal/Intracervical: Initial studies on intravaginal insemination of fallow does with 85 x 10^6 motile spermatozoa 48 h after CIDR device withdrawal resulted in 50% (13/26) and 48% (15/31) fawning rates for fresh and frozen-thawed semen respectively (16). More recently, however, attempts at intracervical insemination with single inseminates containing 20-40x10^6 motile spermatozoa have yielded highly variable results amongst commercial inseminators, ranging from 38% to 80% conception rate (G.W. Asher, unpublished data). Current studies indicate that the success rate to intracervical insemination may be dependant on the method of oestrous synchronization, the timing of insemination and the number of live spermatozoa per inseminate (H.N. Jabbour; unpublished data). Intracervical deposition of ~140 x 10^6 motile frozen-thawed spermatozoa 12 h after the median onset of oestrus (i.e. ~12 h later than performed in previous studies) resulted in conception rates ranging from 84.5% to 40.7% depending on the form of synchronization (Table 2). However, such large numbers of spermatozoa are unacceptable commercially.

(b) Intrauterine: Laparoscopic intrauterine insemination (84) is presently the preferred method of artificial insemination in fallow deer (20) as it allows precise placement of relatively small quantities of semen close to the site of fertilization. Early studies involving intrauterine deposition of 85 x 10^6 motile frozen-thawed spermatozoa 56-58 h after CIDR device withdrawal resulted in a disappointing 42% (22/55) fawning

Table 1: Pre-freeze and post-thaw characteristics of semen collected by electro-ejaculation from a yearling fallow buck on the Ruakura Deer Artificial Breeding Centre (Hamilton, N.Z.) in the 1990 breeding season (Buck R1).

Date of collection	Ejaculate volume(ml)	Ejaculate concentration (x 10^6/ml)	Pre-freeze motility (%)	Post-thaw motility (%)	No. of straws*
8 Feb	1.19	<1380	70	60	9
14 Feb	0.48	<1380	70	70	11
21 Feb	0.33	3130	80	75	20
28 Feb	0.70	3000	95	65	43
7 Mar	0.70	1700	90	65	25
15 Mar	0.76	2200	95	75	33
21 Mar	0.52	3190	90	80	32
27 Mar	0.72	2270	90	70	32
4 Apr	0.82	2550	80	70	41
11 Apr	0.90	<1380	90	75	15
19 Apr	1.40	2410	85	85	69
27 Apr	1.32	<1380	85	75	11
9 May	1.67	1720	85	75	61
15 May	0.78	1035	90	90	16
22 May	1.42	1035	85	75	30
29 May	0.78	1040	80	75	15
12 June	0.32	3480	75	75	22
19 June	1.00	890	70	55	18

* processed at 50 x 10^6 spermatozoa per 0.25 ml straw

Table 2: Conception rates of fallow deer does following intracervical insemination with 140 x 10^6 motile frozen-thawed spermatozoa* 12 h before the median time to onset of ovulation (H.N. Jabbour; unpublished data).

Synchronization treatment	No. of does inseminated	No. of does pregnant (Day 42)	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

* Semen from 3 F1 hybrid (European x Mesopotamian) fallow deer bucks randomly allocated within trial

rate (16). It was postulated that the inseminations were conducted too early relative to ovulation (19) and more recent intrauterine inseminations performed with 20-40x10^6 motile frozen-thawed spermatozoa 65-70 h after CIDR device withdrawal resulted in an overall 68% (105/155) conception rate (20); this being a considerable improvement over the earlier studies.

The most recent on-farm studies on laparoscopic intrauterine insemination of fallow deer, 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 (Table 3). The control regimen (i.e. treatment common to all farms in the study) was similar to that established by Asher et al. (20) and, on the basis of ultrasound pregnancy diagnoses, resulted in an overall 67.5% conception rate. The results indicate (a) a degree of flexability in timing of insemination relative to CIDR device withdrawal (60-70 h), (b) CIDR device synchronization is more effective than prostaglandin synchronization early in the breeding season, (c) buck presence is not essential during synchronization treatment, (d) there is little difference in efficacy of the two types of CIDR device (type-G and type-S), and (e) numbers of motile frozen-thawed spermatozoa required for respectable conception rates (60-70%) are lower than presently used commercially (G.W. Asher, unpublished data).

Despite the supposed impenetrability of the cervix of fallow deer (16), transcervical intrauterine insemination (i.e. *per vaginam*) was successfully attempted on anaesthetized does by exteriorizing the *os cervix* (20). Of four does receiving transcervical inseminations of 50 x 10^6 frozen-thawed spermatozoa 68-69 h after CIDR device withdrawal, three became pregnant. This result demonstrates an alternative to the more invasive technique of laparoscopic insemination and warrants further study.

(2.5.2) AI of red deer: (a) *Per vaginam* inseminations: 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 (86) are credited with some of the first attempts at *per vaginam* insemination of red deer. However, they achieved a conception rate of only 25% (3/12) 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 synchronization. Single inseminations of ~20 x 10^6 motile frozen-thawed spermatozoa have generally resulted in lower conception rates than two inseminations at 12-h intervals. Fennessy et al. (51) obtained a pregnancy rate of 39% (13/33) 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% (27/80) 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 (51). However, double per vaginam inseminations, performed at 44 and 68 h after CIDR device withdrawal, resulted in conception rates of 49% (75/152), 45% (9/20) and 58% (23/40) in three separate trials (48, 51). This improvement over single inseminations suggests relatively poor synchrony of oestrus in red deer hinds.

(b) Laparoscopic intrauterine inseminations: As with fallow deer, laparoscopic intrauterine insemination is the preferred method of artificial insemination of red deer (29). Early studies on small numbers of hinds clearly indicated the potential of the technique (15, 48). More recent research on larger numbers of hinds have involved comparison of various insemination times, with frozen-thawed semen, following CIDR device withdrawal/PMSG administration. Fennessy et al. (51) 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 pregnancy rate for 63 hinds was 56%. In another trial, the 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 vs 55 h after CIDR device withdrawal) was significant; with the 12-day CIDR/55-h insemination giving a higher pregnancy rate than the 15-day CIDR/55-h insemination (89% vs 20%) (51). The standard regimen presently applied to commercial

Table 3: 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 withdrawal	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 synchronization (Farm 5)

Synchronization 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. ($x10^6$)	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

laparoscopic inseminations of farmed red deer in New Zealand includes 12-day CIDR device with administration of 200 i.u. PMSG at CIDR device withdrawal and insemination of 20-40 x 10^6 motile spermatozoa 54-56 h later (29).

(2.6) Pregnancy diagnosis

A number of methods of early pregnancy diagnosis have been used to obtain an early indication of the outcome of artificial insemination programmes in deer. Early studies on fallow deer relied on non-return rates and day-21 (post-insemination) plasma progesterone concentrations to indicate conception rate. However, there was a disparity between estimated conception rate and actual fawning rate, suggesting either a high level (>10%) of embryonic mortality or an overestimation of actual conception rate (16). More recently, studies on artificial insemination of both red deer and fallow deer have utilized ultrasonography to visualize fetal development between days 40 and 60 post-insemination (20, 48, 51). Fetal age estimation, given that there is usually a minimum disparity of 10 days between conceptions to artificial insemination and those to return oestrus at fertile buck/stag introduction, are based on previous ultrasonographic studies in the species (27, 104, 131, 133). Recent studies show a high correlation between ultrasound results and birth data (51; G.W. Asher, unpublished data) indicating low levels of embryonic mortality following artificial insemination except in hybridization studies between Pere David's deer and red deer (P.F. Fennessy, unpublished data).

(2.7) Conclusions and outlook

As intensive pastoral farming of red deer and fallow deer is a relatively new agricultural enterprize throughout the world, it is not suprizing that application of AI technology is still in its infancy. However, research conducted within the last 10 years has clearly demonstrated that techniques of oestrous synchronization, semen collection/cryopreservation and artificial insemination can be, and in many cases have been, suitably refined for these highly seasonally breeding species. Further research, aimed at increasing the economic efficiency of artificial insemination by increasing conception rates, reducing numbers of spermatozoa per inseminate and reducing operation costs, needs to be directed towards more efficient control of oestrous/ovulatory synchrony, more reliable and safer methods of semen collection, alternative diluents/extenders for increased spermatozoa viability, evaluation of fresh vs frozen-thawed semen and further investigation of transcervical (non-surgical) access to the uterine lumen.

(3) EMBRYO TRANSFER

(3.1) Introduction

Justification for adoption of multiple ovulation-embryo transfer (MOET) technology within the international deer farming industry has been highlighted by Bringans (33, 34). MOET offers the industry an opportunity to increase numbers of elite stock at a faster rate than natural breeding will allow. While AI permits wide dissemination of the desirable genes of high ranking sires, ET capitalizes on high genetic merit females (donors) by transference of their pre-implantation embryos to lower ranking females (recipients). This allows for propagation of pure lines of breeding stock and may have considerable application in the preservation of endangered cervid species by interspecies transferrance (e.g. European fallow deer acting as surrogates for Mesopotamian fallow deer). Cryopreservation of embryos enhances international transfer of genetic material, as embryos are safer and cheaper to transport than live animals (51).

Development of MOET technology for cervid species is at a relatively early stage compared with traditional domestic ruminants. Apart from limited studies on white-tailed deer (129), recent developments in establishing MOET protocols for deer have largely centred on farmed red deer, wapiti and, to a lesser extent, fallow deer (32, 33, 34, 42, 50, 54, 71, 83, 124).

(3.2) Superovulation and embryo recovery

Both the *Cervus* and *Dama* genera consist of species that are almost invariably monovulatory. Reproductive potential of donor females is increased dramatically by the administration of gonadotrophins to stimulate the concurrent shedding of multiple

ova. The induction of multiple ovulation (superovulation) in red deer hinds and fallow deer does has been achieved with various proprietary preparations of either pregnant mare serum gonadotrophin (PMSG; or equine chorionic gonadotrophin, eCG), follicle stimulating hormone (FSH), or combinations of both. The administration of exogenous gonadotrophin preparations before and during the pre-ovulatory phase of the luteal cycle (e.g. near CIDR device withdrawal) promotes recruitment of more primary follicles and reduces the atresia rate of recruited secondary follicles. Provided ovarian stimulation is pitched at the right level, the multiple follicles should undergo ovulatory rupture within a short period after insemination.

The limited amount of literature on superovulation of deer indicates a wide range of ovarian responses to PMSG and FSH, with interpretation being confounded by a number of factors; including

(a) farm and year differences,
(b) donor genotype (e.g. elk, European red deer, N.Z. deer),
(c) the use of gonadotrophin preparations from different sources and with differing biological potencies (e.g. ovine FSH vs porcine FSH),
(d) variable dosages of exogenous gonadotrophins,
(e) method of gonadotrophin administration (e.g. multiple injection vs Alzet osmotic minipump delivery of FSH),
(f) timing of gonadotrophin administration relative to CIDR device insertion/withdrawal.

(3.2.1) Red deer and wapiti: Early attempts at superovulation of red deer and wapiti relied on the use of PMSG due to its availability and cost. However, this gonadotrophin preparation has generally resulted in inconsistent ovulation and recovery/fertilization rates (54). Similar observations have been reported previously for other domestic ruminants, such as sheep (26, 46, 70) and goats (3). This has been attributed to the ovarian secretion of high levels of oestrogen, which may result in an unfavourable oviductal/uterine environment or alter the rate of spermatozoa transport through the reproductive tract (45). These high levels of oestrogen appear to arise from increased numbers of follicles which fail to ovulate (46), a second wave of follicular growth stimulated after ovulation (35), or an increased level of steroid production by the follicles (70, 101).

Neutralization of residual PMSG by the timed administration of PMSG antiserum has been shown to reduce oestrogen secretion, increase the ovulatory response and improve fertility in sheep (72) and cattle (40, 41). Likewise, the induction of earlier follicle ovulation following timed administration of GnRH (gonadotrophin releasing hormone) has produced similar effects in sheep (72, 105). However, the benefits of administering PMSG antiserum or GnRH on the fertility of PMSG-treated red deer hinds are equivocal. In a recent study, mature hinds were treated with CIDR devices (type S, 9% progesterone) for 14 days and 1200 i.u. PMSG (Folligon, Intervet) was administered 72 h before CIDR device withdrawal. A proportion of the hinds were further treated with PMSG antiserum or GnRH (Fertagyl; Intervet), administered at 12 or 18 h after CIDR device withdrawal. The time to onset of oestrus, commencement of ovulation and the total ovulatory response were recorded. Although the administration of PMSG antiserum or GnRH reduced the interval between first and last ovulation, the additional treatments had no beneficial effect on the ovulatory response (Table 4; H.N. Jabbour; unpublished data). This possibility attests to the high sensitivity of the cervid species to PMSG.

In recent years, commercial pituitary FSH preparations have gained considerable popularity for superovulation treatments in MOET programmes. The substitution of FSH for PMSG has permitted progress in obtaining regimens that provide more consistant results in relation to embryo quality (125), mainly due to less hyperstimulation and reduced oestrogen secretion during the pre- and post-ovulatory periods (3, 70).

There have been no comprehensive comparative studies conducted to determine differences in the effect of PMSG or FSH on fertility of red deer and wapiti. However, initial results suggest that there is an improvement in the superovulatory response and quality of embryos recovered following treatment with FSH (33, 34). FSH is

Table 4: The effect of PMSG (1200 i.u.) and PMSG antiserum or GnRH on the ovarian response of red deer hinds (H.N. Jabbour, unpublished data). Data are expressed as the mean (± s.e.m.) number of corpora lutea (CL), large follicles (LF) and the total follicular stimulation (TFS).

Treatment	n hinds	CL	LF	TFS
PMSG	10	8.4 ± 2.7	2.3 ± 0.5	10.7 ± 4.1
PMSG + antiserum at 12 h PCW	10	4.6 ± 0.5	4.9 ± 1.8	9.3 ± 1.7
PMSG + antiserum at 18 h PCW	10	6.9 ± 2.0	3.9 ± 1.3	10.6 ± 2.2
PMSG + GnRH at 12 h PCW	10	7.5 ± 2.1	1.6 ± 0.3	9.1 ± 2.2
PMSG + GnRH at 18 h PCW	10	4.1 ± 1.5	2.4 ± 0.7	6.5 ± 1.3

PCW = post-CIDR device withdrawal

Table 5: The effect of 200 i.u. PMSG and various dosages of ovine FSH (Ovagen) on the ovarian response of red deer hinds (74). Data are expressed as the mean (± s.e.m.) number of corpora lutea (CL), large follicles (LF) and the total follicular response (TFS).

Units of FSH	n hinds	CL	LF	TFS
0	10	0.8 ± 0.1	0.7 ± 0.4	1.5 ± 0.4
0.25	10	7.1 ± 1.4	2.5 ± 1.0	9.6 ± 1.8
0.50	10	9.5 ± 2.1	4.0 ± 0.6	13.5 ± 1.9
0.75	10	6.9 ± 0.6	3.4 ± 0.7	10.3 ± 0.9
1.0	10	6.4 ± 1.3	3.3 ± 1.1	9.7 ± 1.2

administered either as 8 intramuscular injections over a period of 4 days or by continuous infusion via osmotic minipumps (Alzet; Alza Corp., Palo Alto, Ca, USA) implanted subcutaneously. Fennessy et al. (50) provided evidence of a dose response to ovine FSH (Ovagen; Immunochemical Products N.Z. Ltd, Auckland, N.Z.), with mean (± s.e.m.) ovulation rates of 0.67 ± 0.3, 2.0 ± 1.0, 4.3 ± 2.4 and 16.0 ± 4.0 for overall FSH doses of 0.25, 0.5, 0.75 and 1.0 unit respectively. The administration of FSH by injection resulted in a significantly higher mean ovulation rate than the osmotic minipump regimen. However, the recovery rate of transferable embryos did not differ between the two delivery methods (50).

The use of FSH preparations alone to induce a superovulatory response sometimes results in complete ovulatory failure in some females, as observed in sheep (44, 70), red deer (50) and fallow deer (124). In sheep, this problem can be overcome by combining FSH and PMSG in the superovulation regimen (73, 110). The "cocktail" regimen increased mean ovulation rate and numbers of viable embryos by increasing the proportion of ewes exhibiting a superovulatory response and by increasing the mean number of corpora lutea (110). A similar "cocktail" regimen has been applied experimentally to red deer hinds (74). A total of 50 mature hinds were treated with intravaginal CIDR devices (type S, 9% progesterone) for 14 days and 200 i.u. PMSG (Folligon; Intervet) administered 48 h before CIDR device removal. Each hind received one of five dosages of ovine FSH (0, 0.25, 0.5, 0.75, or 1.0 units Ovagen) administered in 8 i.m. doses at 12-h intervals initiated 48 h before CIDR device removal. The hinds were naturally mated but also received repeat intravaginal inseminations of frozen-thawed semen at 12-h intervals between 24 and 48 h after CIDR device removal. The numbers of corpora lutea and large unruptured follicles were recorded during surgical embryo recovery on Day 7. There was a curvilinear pattern of ovarian response to increasing doses of ovine FSH (Table 5). The highest numbers of corpora lutea were observed following treatment with 0.5 units FSH. There were no differences in the ova recovery rates between the treatment groups, although the overall rate of 32.7 ± 5.1% was low. The mean (± s.e.m.) fertilization rate of recovered ova was 50.2 ± 8.2%, with the majority of the embryos at the morula stage (74).

(3.2.2) Fallow deer: Recent studies on MOET in fallow deer have concentrated on the induction of superovulation, fertilization of multiple ova and embryo recovery. Thompson & Asher (124) describe a comparative study investigating the efficacy of three different exogenous gonadotrophin regimens. Thirty six mature fallow does were treated with intravaginal CIDR devices (type S; 9% progesterone) for 14 days and randomly allocated to 3 treatment groups. Group 1 received 1200 i.u. PMSG (Pregnecol; Heriot Agencies, Australia) administered as a single intramuscular injection 48 h before CIDR devices removal; Group 2 received 20 mg FSH (Folltropin; Vetripharm, Canada) administered in a decreasing dose regimen twice daily for 4 days with the last dose coinciding with CIDR device removal; Group 3 received a "cocktail" regimen of 750 i.u. PMSG and 14 mg FSH, with PMSG and FSH administered as for Groups 1 and 2 respectively. Time to onset of oestrus was recorded and ova recovery was performed by surgical uterine flush 6-8 days after CIDR device withdrawal. Treatment with the exogenous gonadotrophins significantly advanced the time to onset of oestrus compared with does treated with CIDR devices only (4, 11), with the onset of oestrus of superovulating does occurring between 15-24 h after CIDR device removal. An "all or none" ovulatory response was observed for does treated with FSH alone (Table 6); only 4 does responded with ovulation rates ranging from 4-30. The response of the does in the other treatment groups was characterized by a large number of cystic and luteinized follicles, indicating an overstimulation effect and a high ovarian sensitivity to PMSG (Table 6). This was additionally characterized by poor embryo recovery and fertilization rates, and embryos at a wide range of developmental stages.

The effect of the cocktail regimen on the ovarian ovulatory responses and ova recovery/fertilization rates were further examined by Jabbour et al. (74). A total of 50 fallow does were treated with intravaginal CIDR devices (type S, 9% progesterone) for 14 days and 200 i.u. PMSG (Folligon) 48 h before CIDR device removal. Each doe received one of 5 dosages of ovine FSH (0, 0.25, 0.5, 0.75 and 1.0 units Ovagen)

Table 6: Mean (± s.e.m.) ovarian response, ova recovery (OR) and fertilization rates (FR) following treatment of fallow deer does with PMSG and/or FSH (124).

Treatment	n does	CL	TFS	OR	FR
1200 i.u. PMSG	12	9.2 ± 2.5	16.8 ± 2.0	3.7 ± 1.1	70.0%
20 mg FSH	12	6.3 ± 2.9	7.0 ± 3.1	1.1 ± 0.5	84.6%
750 i.u. PMSG + 14 mg FSH	12	11.2 ± 3.3	20.4 ± 3.0	1.9 ± 0.5	52.2%

Table 7: The effect of 200 i.u. PMSG and various dosages of ovine FSH (Ovagen) on the ovarian response of fallow deer does (74). Data are expressed as the mean (± s.e.m.) number of corpora lutea (CL), large follicles (LF) and the total follicular response (TFS).

Units of FSH	n hinds	CL	LF	TFS
0	10	1.1 ± 0.4	1.4 ± 0.6	2.5 ± 0.7
0.25	10	7.2 ± 1.7	2.8 ± 0.8	10.0 ± 1.9
0.50	10	9.5 ± 2.5	5.4 ± 0.9	14.9 ± 2.7
0.75	10	8.6 ± 2.4	8.8 ± 1.1	17.3 ± 1.9
1.0	10	7.4 ± 2.1	5.5 ± 0.9	12.9 ± 2.5

Table 8: Maturation and fertilization of red deer oocytes cultured for different durations with different times of gonadotropin addition (8 replicates in each treatment) (58).

Time of FSH/LH addition	Culture duration (h)	% (mean ± s.e.m.) oocytes (No. examined) matured		fertilized	
0	16	2.2 ± 2.1	(52)	8.4 ± 3.0	(86)
	20	64.9 ± 6.3	(56)	13.0 ± 3.6	(94)
	24	76.6 ± 5.6	(54)	20.6 ± 4.0	(104)
	28	74.0 ± 5.8	(56)	5.3 ± 2.3	(91)
	Total	54.4 ± 2.6	(218)	11.8 ± 1.7	(375)
6	16	7.2 ± 3.5	(53)	5.8 ± 2.5	(92)
	20	61.1 ± 6.4	(55)	23.6 ± 4.4	(98)
	24	63.7 ± 6.3	(56)	20.3 ± 3.9	(111)
	28	72.8 ± 6.0	(54)	10.2 ± 3.1	(96)
	Total	51.2 ± 2.8	(218)	15.0 ± 1.8	(397)

administered as 8 i.m. injections at 12-h intervals starting 48 h before CIDR device removal. The does were naturally mated but also received repeat intravaginal inseminations of frozen-thawed semen at 12-h intervals between 24 and 48 h after CIDR device removal. The number of corpora lutea and large unruptured follicles were recorded during surgical embryo recovery on Day 7. As with red deer, there was a curvilinear pattern of ovarian response to increasing dosages of ovine FSH (Table 7). The highest numbers of corpora lutea were observed following treatment with 0.5 units FSH. There were no differences in the ova recovery rates between the treatment groups; with an overall rate of 30.6 $\pm$ 5.1%. In contrast to the previous study, none of the ova recovered had cleaved. This was particularly disappointing as considerable effort was made to ensure that copious quantities of spermatozoa were present in the vagina. Further studies may be needed to define the optimal site and time of insemination to improve the fertility of superovulated fallow deer does.

(3.3) Embryo cryopreservation

While there are no detailed published accounts describing embryo freezing for red deer, wapiti and fallow deer, standard techniques have been applied commercially for red deer and wapiti and have culminated in reasonable success rates (>60%) for the transfer of thawed embryos to recipient hinds (33, 34).

(3.4) Embryo transfer

(3.4.1) Red deer and wapiti: Pregnancy rates following embryo transfer to red deer recipients have ranged from 60%-80% for fresh transplantation and 55%-66% for frozen-thawed transplantation (33, 34, 42). Generally, recipient females have been treated with intravaginal CIDR devices for 12 days and 200 i.u. PMSG at the time of CIDR device removal. PMSG is believed to overcome stress inhibition of ovulation, with only 5-10% of PMSG-treated recipients being rejected for transfer as compared to 50% of females treated with CIDR devices alone (33). The embryo is usually injected into the utero-tubal junction of the ipsilateral horn using a glass pipette or a tom-cat catheter (42).

(3.4.2) Fallow deer: There are no published accounts describing embryo transfer in fallow deer. It is unlikely that this step will be limiting in MOET programmes for this species. It must be stressed, however, that synchronization of recipient does is unlikely to benefit from the use of PMSG, as this has proved to be counter-productive for AI programmes (see 2.3.1.).

(3.5) Conclusions and outlook

MOET technology for cervid species is in the early development stages. It is evident from the available information that the superovulatory response and embryo recovery/fertilization rates are the main limiting factors to the widespread application of the technology, particularly for fallow deer. This necessitates further research into improvement of the response of donor females to exogenous gonadotrophins. The overall response to gonadotrophins may be limited by the number of responsive follicles available at the time of treatment. Future investigations may be profitably aimed at developing ways to increase the numbers of follicles that are recruited into the rapid growth phase in order to respond to exogenous gonadotrophins. This may be achieved by administration of gonadotrophins early in the luteal phase or immunization against inhibin. It may also be profitable to investigate production of homologous and/or monoclonal FSH for superovulation, as this may help reduce variability of ovarian response.

(4) GAMETE MANIPULATION

(4.1) Introduction

For traditional domestic ruminant species, gamete manipulation is, and will be, playing an important role in the propagation of genetically superior females. Gamete manipulation allows the female genetic contribution to be emphasized much as AI enhances the male contribution. Recent and potential developments in gamete manipulation will be discussed. The procedures considered include oocyte recovery, *in-vitro* maturation (IVM), *in-vitro* fertilization (IVF) and culture (IVC) of oocytes, embryo splitting, cloning (nuclear transfer), sperm/embryo sexing and gene transfer.

IVM, IVF and embryo culture are the backbone of reproductive biotechnology. Without a readily available source of oocytes in large numbers and embryos at the correct stage of development, these other procedures (splitting, cloning, sexing and gene transfer) are restricted to expensive superovulation and surgical recovery regimes.

Cloning (nuclear transfer), sperm and embryo sexing, and gene transfer are biotechniques which, at the present, are primitive and inefficient when applied to domestic livestock. However, research is ongoing in these areas, and these technologies are likely to have a major impact on the genetics and economics of the livestock industry within this decade.

The potential of these technologies for the deer farming industry has yet to be assessed but it is likely that some forms of gamete manipulation will have considerable impact on the rate of genetic gain. These procedures have the potential to maximize genetic progress more quickly than conventional breeding methods, especially when applied to the rare cervid genotypes. Through IVF, new hybrid species can be created, the embryos sexed, and the females cloned to introduce a new breeding line in a relatively short time span.

(4.2) IVM/IVF

(4.2.1) Oocyte recovery: Oocyte recovery is conducted either on live animals to collect *in vivo* mature oocytes, or at slaughter. Follicular aspiration of the ovaries of superovulated females has been successfully accomplished in cattle (115). The females are treated with a superovulation regime and the oocytes are aspirated, prior to ovulation, using standard laparoscopic technique. Laparoscopic oocyte recovery in cattle yields approximately 8 oocytes per animal (87), while repeated laparoscopic retrieval yields 6-7 oocytes per collection with no ill effects on fertility or ovarian response (111, 116).

Follicular recovered oocytes are potentially valuable to the deer farming industry. Oocytes aspirated from genetically superior female can be fertilized *in-vitro* with one or many different sires. This leaves the donors available for natural breeding or repeated aspiration within the same breeding season. Follicular aspiration allows oocytes to be collected from a female with blocked or scarred oviducts, or when a female exhibits unexplained infertility.

A major limitation of laparoscopic retrieval is the small number of oocytes obtained per animal. Even when animals are superovulated, the usable number of bovine oocytes per donor ranges from 5 to 10 (87, 90). When large numbers of oocytes are required, immature oocytes need to be obtained at slaughter or ovariectomy by aspiration of 1-5 mm pre-ovulatory follicles. Only those oocytes surrounded by compact cumulus cells and showing evenly grained cytoplasms are selected for culture (90).

Oocyte recovery from pre-ovulatory follicles of slaughtered red deer hinds is comparable to that of other domestic ruminants, with an average of 6 good quality oocytes per animal being recovered during the 1990 breeding season (58). Oocytes collected from both pregnant and non-pregnant fallow deer does in September 1990 yielded only 2 oocytes per animal but superovulated non-pregnant does yielded an average of 8.2 oocytes per animal (D.K. Berg; unpublished data).

(4.2.2) IVM: Immature oocytes harvested from pre-ovulatory follicles need to be cultured *in-vitro* to achieve competency for fertilization. The mechanisms of regulating oocyte maturation for ruminants have been reviewed by Moor and Gandolfi (99). The criteria for judging oocyte maturation involve the progression from the resting dictyate stage of meiosis I through metaphase II and extrusion of the first polar body. In addition to nuclear maturation, cumulus cell expansion and cytoplasmic maturation must also occur. Expanded cumulus cells may enhance sperm capacitation and fertilization rates (22). Failure to complete cytoplasmic maturation has been associated with failure of sperm pronuclear formation (123) and developmental competance of the fertilized oocyte (90, 119).

Several types of culture media are employed for the maturation of sheep and cattle oocytes, including TCM 199 (58, 89, 100, 119), Hams F12 (60) and TALP (22, 89, 92). These media are usually supplemented with gonadotrophins, oestradiol-17β and a whole serum or serum component. Fetal calf serum has been shown to be superior to albumin for cattle (89) and sheep (31) oocyte maturation. Oocytes co-cultured with

supplementary granulosa cells have been shown to increase developmental competence after fertilization in sheep and cattle (97, 119). Nuclear maturation of domestic ruminants can occur over a temperature range of 35-41^{o}C (80, 92), but most oocyte maturation studies involve culture at 39^{o} C in 5% CO_2 in air for 24-28 hours.

In-vitro maturation of cervid oocytes is still in the infancy stages. One study was carried out at Ruakura during the 1990 breeding season using red deer immature oocytes aspirated from slaughterhouse ovaries (58). Maturation was studied from 16-28 h in culture. Media was supplemented with FSH and LH added at the start of the culture or with a 6-h delay for LH addition. Maturation rates are presented in Table 8. The time of gonadotrophin addition did not affect the proportion of oocytes maturing; 54.4% vs 51.2% for 0 and 6 h respectively. Culture for 16 h resulted in a lower nuclear maturation rate than with the longer duration of culture. The proportion of oocytes maturing increased with increased culture duration from 20 to 28 h (58). These results are comparable to IVM percentages achieved in sheep and cattle. Immature oocytes from red deer hinds can be successfully matured *in-vitro* thus providing a greater number of oocytes at a lower cost than might be obtained by laparoscopic retrieval.

(4.2.3) IVF: The mechanisms of IVF in ruminants have been reviewed by First and Parrish (53), while practical aspects of IVF in cattle have been reviewed by Sirard (117). In order to achieve successful fertilization *in-vitro*, sperm must undergo a period of preparation, termed capacitation, which normally occurs *in-vivo* in the female tract (21, 36). In domestic ruminants, the site of *in vivo* capacitation is uncertain. Sperm capacitation can take place in the uterus (21, 23, 24), but is accelerated by exposure to the uterus and then the oviduct (24, 69). Capacitation has been induced *in-vitro* in several different media and include conditions of high ionic strength (30), elevated pH (37), long term storage (130), and by exposure to glycosaminoglycans such as heparin (106). Heparin is the most widely used *in-vitro* capacitation agent for IVF in cattle.

Current IVF technology for any cervid species is limited to one study completed during the 1990 breeding season at Ruakura (58). Fertilization rates are presented in Table 8. The highest fertilization rates (20-23%) were achieved using IVM oocytes cultured for 20-24 h. These fertilization rates are very low and this has been attributed to improper sperm capacitation. Further studies have shown that frozen-thawed red deer sperm has poor viability in standard cattle or sheep *in-vitro* capacitation media, with 80-90% of the acrosomes damaged after one hour in culture (Table 10), and most sperm are dead within two hours of culture. (D. K. Berg, unpublished data).

IVF technology has wide potential application for the deer farming industry. Valuable does and hinds effectively infertile due to oviductal obstructions, infundibular adhesions and low fertilization rates, can be utilized. Male infertility can also be treated. Low sperm count and poor ejaculates can be corrected by semen washing procedures or microinjection of sperm into the egg. IVF also has potential for the creation of hybrids that normally would not occur by conventional breeding methods. It is possible that an *in-vitro* assay can be developed to predict the fertility of red deer stags. IVF also provides a means of producing offspring from dead or dying animals of great genetic value. A strong argument in support of IVF is the ability to produce large numbers of embryos at the correct stage of development for other biotechnologies such as gene transfer and cloning, at less cost than superovulation.

(4.2.4) Embryo culture and transfer: IVM/IVF one-cell embryos must develop to the morula or blastocyst stage for transfer to recipient females. Rabbit and sheep oviducts are used as temporary recipients for cattle and sheep one-cell embryos. Development of one-cell embryos is limited in rabbit oviducts (88), but most develop to the blastocyst stage in sheep oviducts. However, culture in sheep oviducts results in a 40% recovery loss of embryos (97).

In-vitro culture systems avoid embryo losses that occur in recipient systems, but the proportion of embryos developing to the blastocyst stage is generally reduced. Culture systems involve co-culturing embryos with epithelial oviductal cells or culture in simple media supplemented with whole serum or a serum component. IVM/IVF cattle and sheep embryos cultured on an *in-vitro* monolayer of oviductal cells result in 22% development to blastocyst stage (59). IVM/IVF one-cell embryos cultured in synthetic

Table 9: Cleavage of red deer oocytes following *in vitro* maturation and fertilization (58).

Time of FSH/LH addition *1	Culture duration (h)		No. oocytes cleaved *2			Total cleaved (mean % ± s.e.m) *3
			2-cell	4-cell	8-cell	
0	20	79 (5)	2	2	0	4 (6.3 ± 3.2)
	24	75 (5)	1	2	0	3 (5.1 ± 2.9)
	Total	154	3	4	0	7 (5.7 ± 2.2)
6	20	80 (5)	4	6	2	12 (18.2 ± 5.0)
	24	75 (5)	3	1	0	4 (6.8 ± 3.3)
	Total	155	7	7	2	16 (12.5 ± 3.2)

*1 As per Table 8.
*2 Examined at the third day after insemination.
*3 Mean from replicates.

Table 10: Percentage (± s.e.m.) of live and acrosome-reacted red deer sperm cultured in standard ruminant *in vitro* capacitation media. (D.K. Berg, unpublished data).

Culture duration (hours)	Live	Live acrosome reacted	Total acrosome reacted
0	35.4 ± 7.1	41.9 ± 2.2	63.4 ± 7.8
.25	26.9 ± 8.5	60.1 ± 2.1	81.9 ± 8.2
.50	25.7 ± 8.5	66.4 ± 3.2	87.5 ± 6.2
4	27.5 ± 9.3	60.6 ± 2.7	82.8 ± 6.4

Pooled data from 7 different capacitation media.

oviductal fluid (SOF) supplemented with human serum resulted in 31% development to blastocysts for cattle (59) and 36% for sheep (109).

Red deer one-cell embryos cultured in SOF supplemented with 10% fetal calf serum for seven days resulted in no development to morula/blastocyst stage. Cleavage was limited to the 2-8 cell stage. Only 12% of the total number of oocytes cleaved suggesting that either IVM/IVF or the culture system was suboptimal (58). Clearly, considerable research needs to be directed to the area of IVM/IVF technology before it can be successfully applied to the deer farming industry.

(4.3) Embryo splitting

Embryo splitting has been employed successfully to create identical twins and to improve the efficiency of embryo transfer programmes in cattle and sheep. Application in cattle by one commerical company resulted in 422 embryos split to produced 442 pregnancies after transfer, whereas 515 intact embryos produced only 291 pregnancies (91). The calf yield for split embryos was 1.05 times the number of orginal embryos compared to 0.56 in the control group. In sheep, 1410 bisected embryos were transfered and produced 710 pregnancies, while 1252 control embryos produced 771 pregnancies. The lamb yield was 1.06 for bisected embryos compared to 0.61 for controls (128).

Embryo splitting has potential commercial value in MOET programmes to double the number of embryos available for transfer. This increase would be of considerable benefit to the deer farming industry when applied to rare genotypes or animals of high genetic value. However, splitting also has limitations. "Normal" development of ruminant embryos does not occur if the embryo is split into four or more portions. Splitting also reduces the ability of the embryo to survive freezing and thawing, therefore, splitting and transfer must be performed on the same day (134).

(4.4) Cloning

Some of the limitations of embryo splitting have been overcome with the development of transferring blastomere nuclei to enucleated oocytes (132). This technique, known as nuclear transfer (cloning), has produced genetically identical offspring in sheep (118), cattle (108), rabbits (120) and pigs (108). The technique and its potential has been reviewed by Prather and First (108). Briefly, blastomeres are separated from 8-32 cell embryos. These blastomeres are transferred to enucleated metaphase II oocytes. Electrical activation of the oocyte causes the egg cytoplasm to reprogram the nucleus to the metaphase II stage. Fusion of the enucleated oocyte to the transferred karyoplast is accomplished electrically. These embryos are placed in culture, and when they reach the 8-32 cell stage they can be cloned, frozen for future use, or transferred to recipients. The success rate for the above cloning process is 45% in cattle, 68% in sheep and 62% in pigs (108).

Commercial application of nuclear transfer has been established in the dairy industry. A large commercial trial in cattle resulted in 42.4% pregnant at day 35, with 33.1% surviving to term (132). This pregnancy rate is well below that obtained in commercial embryo transfer. However when "quality of the embryos" were taken into account, the results improved. "Good and fair" quality embryos resulted in 55.7% pregnancies at day 35 with 47.4% surviving to term. This pregnancy rate is comparable to pregnancy rates acheived in embryo transfer programmes (132).

Cloning has potential within the deer farming industry where artificial breeding and selection is developing rapidly. Genetically superior animals can be cloned to develop the nucleus of the breeding herd. For example, some of the clones are transferred to recipients while the others are frozen for future use. The clones are placed in progeny testing programmes, and those that are superior are thawed, recloned, and enter the breeding herd. This scheme also applies to rare genotypes where inbreeding must be carefully monitored.

Another advantage of cloning to the deer farming industry is the high numbers of offspring which could be produced from one animal. Considering a 90% efficiency rate for each step, one 16-cell embryo would yield 15 blastomeres to be used as nuclear donors. The 15 newly created clones could be cultured to the 16-cell stage, recloned several times to generate several hundred offspring from one animal. These nuclear transfer embryos may not be identical, because the effects of the donor cytoplasm on development of the embryo or performance of the offspring is not known.

(4.5) Sexing of sperm and embryos

The ability to preselect the sex of domestic livestock would have a significant impact on the genetics and economics of livestock production. Several approaches to embryo sexing are being explored in search of the perfect method, which would accurately identify the sex of the embryo without damaging the embryo, and without impeding implantation and development (134). Currently, there are four methods in use to determine the sex of the embryo; karyotyping, H-Y antigen use, sex-linked difference measurment in enzyme activty, and DNA hybridization specific to the Y chromosome (126).

The most promising of these methods is the Y-specific DNA probe. Only a small portion of cells from the embryo are required. Positive hybridization results indicate the presence of the Y chromosome. This method has resulted in a 40% pregnancy rate with 80% of the pregnancies resulting in live born calves for which the sex was accurately determined (28). In another study, zona-free embryos were frozen after hybridization, and later thawed and transferred to recipients. A 63% pregnancy rate at 90 days was reported (68).

Ideally, sex selection would also involve seperation of X and Y spermatazoa, and introduction of the separated spermatazoa via AI. Currently, there is no evidence that preselection treatment of sperm has resulted in an altered ratio of live young born in domestic livestock. However, one paper has described the preselection of sex using rabbits as a model. Following use of a modified cell sorter/flow cytometer, the sorted Y sperm resulted in 81% males born and the sorted X sperm yielded 94% females born (78).

Sex selection would have an enormous impact on the economics of farmed deer production. A genetically superior female could be manipulated to produce only males for use in a progeny selection programme. For example, commerical velvet producers could manipulate the sex ratio in favour of males and avoid a surplus of females. Conversely, female replacements could be selected without the unwanted males born. Rare genotypes, such as the Mesopotamian fallow deer, could have the correct sex ratios of offspring being born, compatible with needed breeding ratios.

(4.6) Gene Transfer

Gene transfer is a technique which creates the opportunity to develop animals with specific genes that enhance their performance (134). Several reviews have been written discussing methods and potential economic impact on the domestic livestock industry (38, 113). Application of the procedure is dependant on three factors; efficient methods of incorporating genes into host genomes, control of their expression after incorporation, and identification of genes capable of having a desirable effect (134).

The present methods of gene transfer are not efficient. In cattle, only 21% of injected eggs reach the transferrable morula stage. Overall, only 0.48% of cattle eggs injected produce transgenic animals (25). These results are similar to sheep where only 0.84% of injected eggs survived to become transgenic lambs (114).

Gene transfer techniques could have a number of applications to the deer farming industry. For example, a poll gene might be introduced in non-velvet producing males, eliminating the need to continually remove antlers. Disease resistant genes, such as those conferring worm resistance, could eliminate the need for anthelminthic treatment and thereby reducing cost and handling of animals. A growth hormone (GH) gene could enhance growth performance while increasing feed efficiency (GH transgenics have been successfully produced in sheep and pigs; 67). Not only single locus genes can be incorporated into host genomes, but poly-loci genes and genes products have the possibility of incorporation. One such gene product beneficial to the fallow deer farming industry would be a sporodesmin antitoxin to confer resistance to facial eczema.

(4.7) Conclusion and outlook

Currently, the only technique that can be applied commercially to the deer industry is embryo splitting. It requires relatively simple equipment and its success depends upon known synchronization and recipient transfer techniques. The success of *in-vitro* culture, cloning, embryo sexing and gene transfer relies heavily on the low cost

availability of oocytes and embryos. This is achieved most economically with IVM and IVF.

The major impact these technologies might have for the deer farming industry is that preferred genetic traits would be deseminated into the general population at a much faster rate than by conventional breeding. These techniques can also be used to create new hybrids and to create highly specialized animals to meet the requirements for specialized production traits.

Clearly, much work is needed to understand the biology of oocyte maturation, fertilization, embryo implantation and pregnancy maintenance in the cervid species before these gamete manipulations can be applied successfully.

REFERENCES

(1) Adam, C.L., 1985. Recent developments for lowground red deer farming. Rowett Research Institute Annual Report 1985: 34-50.

(2) Adam, C.L., Moir, C.E. and Atkinson, T., 1985. Plasma concentration of progesterone in female red deer (*Cervus elaphus*) during the breeding season, pregnancy and anoestrus. J. Reprod. Fertil., 74: 631-636.

(3) Armstrong, D.T., Pfitzner, A.P., Warnes, G.M. and Seamark, R.F. 1983. Superovulation treatment and embryo transfer in Angora goats. J. Reprod. Fert. 67: 403-410.

(4) Asher, G.W. 1985. Oestrous cycle and breeding season of farmed fallow deer, *Dama dama*. J. Reprod. Fertil., 75: 521-529.

(5) Asher, G.W., 1986. Studies on the reproduction of farmed fallow deer (*Dama dama*). PhD thesis; Lincoln College, University of Canterbury, New Zealand.

(6) Asher, G.W., 1987. Conception rates, gestation length, liveweight changes and serum progesterone concentrations during the breeding season and pregnancy of farmed female fallow deer. Proceedings 4th AAAP Animal Sciences Congress; Hamilton, New Zealand: 247.

(7) Asher, G.W. and Adam, J.L., 1985. Reproduction of farmed red and fallow deer in northern New Zealand. In: P.F. Fennessy and K.R. Drew (Editors), Biology of Deer Production. Bull. No. 22, Royal Society of New Zealand, Wellington, pp 217-224.

(8) Asher, G.W. and Fisher, M.W., 1990. Reproductive physiology of farmed red deer (*Cervus elaphus*) and fallow deer (*Dama dama*). Proceedings 2nd International Symposium on Game Ranching; Edmonton, Canada: (in press).

(9) Asher, G.W. and Macmillan, K.L. 1986. Induction of oestrus and ovulation in anoestrous fallow deer (*Dama dama*) by using progesterone and GnRH treatment. J. Reprod. Fertil., 78: 693-697.

(10) Asher, G.W. and Smith, J.F., 1987. Induction of oestrus and ovulation in farmed fallow deer (*Dama dama*) by using progesterone and PMSG treatment. J. Reprod. Fertil., 81: 113-118.

(11) Asher, G.W. and Thompson, J.G.E., 1989. Plasma progesterone and LH concentrations during oestrous synchronization in female fallow deer (*Dama dama*). Anim. Reprod. Sci., 19: 143-153.

(12) Asher, G.W., Barrell, G.K. and Peterson, A.J., 1986. Hormonal changes around oestrus of farmed fallow deer, *Dama dama*. J. Reprod. Fertil., 78: 487-496.

(13) Asher, G.W., Day, A.M. and Barrell, G.K., 1987. Annual cycle of liveweight and reproductive changes of farmed male fallow deer (*Dama dama*) and the effect of daily oral administration of melatonin in summer on the attainment of seasonal fertility. J. Reprod. Fertil., 79: 353-362.

(14) Asher, G.W., Peterson, A.J. and Watkins, W.B., 1988. Hormonal changes during luteal regression in farmed fallow deer, *Dama dama*. J. Reprod. Fertil., 84: 379-386.

(15) Asher, G.W., Adam, J.L., Otway, W., Bowmar, P., van Reenan, G., Mackintosh, C.G. and Dratch, P., 1988. Hybridization of Pere David's deer (*Elaphurus davidianus*) and red deer (*Cervus elaphus*) by artificial insemination. Jl. Zool., 215: 197-203.

(16) Asher, G.W., Adam, J.L., James, R.W. and Barnes, D., 1988. Artificial insemination of farmed fallow deer (*Dama dama*): fixed-time insemination at a synchronised oestrus. Anim. Prod., 47: 487-492.
(17) Asher, G.W., Peterson, A.J. and Bass, J.J., 1989. Seasonal pattern of LH and testosterone secretion in adult male fallow deer, *Dama dama*. J. Reprod. Fertil., 85: 657-665.
(18) Asher, G.W., Peterson, A.J. and Duganzich, D., 1989. Adrenal and ovarian sources of progesterone secretion in young female fallow deer, *Dama dama*. J. Reprod. Fertil., 85: 667-675.
(19) Asher, G.W., Fisher, M.W., Smith, J.F., Jabbour, H.N. and Morrow, C.J. 1990. Temporal relationship between the onset of oestrus, the pre-ovulatory LH surge and ovulation in farmed fallow deer, *Dama dama*. J. Reprod. Fertil., 89: 761-767.
(20) Asher, G.W., Kraemer, D.C., Magyar, S.J., Brunner, M., Moerbe, R. and Giaquinto, M., 1990. Intrauterine insemination of farmed fallow deer (*Dama dama*) with frozen-thawed semen via laparoscopy. Theriogenology 34: 569-577.
(21) Austin, C.R., 1951. Observations on the penetration of the sperm into the mammalian egg. Aust. J. Sci. Res., 4: 581-596.
(22) Ball, G.D., Leibfried, M.L., Lenz, R.W., Ax, R.L., Bavister, B.D. and First, N.L., 1983. Factors affecting successful *in vitro* fertilization of bovine follicular oocytes. Biol. Reprod., 28: 717-725.
(23) Barros, C., 1974. Capacitation of mammalian spermatozoa. In: Physiology and Genetics of Reproduction, pp. 3-24. Plenum Press, New York.
(24) Bedford, J.M., 1969. Limitations of the uterus in the development of the fertilizing ability (capacitation) of spermatozoa. J. Reprod. Fertil. Suppl. 8: 9-16.
(25) Biery, K.A., Bondioli, K.R. and De Mayo, F.J., 1988. Gene transfer by pronuclear injection in the bovine. Theriogenology 29: 224 (abstr).
(26) Bindon, B.M. and Piper, L.R. 1982. Physiological basis of ovarian response to PMSG in sheep and cattle. In: Embryo transfer in cattle, sheep and goats. Eds: J.N. Shelton, A.O. Trounson and N.W. Moore, Australian Society for Reproductive Biology, Canberra: 1-5.
(27) Bingham, C.M., Wilson, P.K. and Davies, A.S., 1990. Real-time ultrasonography for pregnancy diagnosis and estimation of fetal age in farmed red deer. Vet. Rec., 126: 102-106.
(28) Bondioli, K.R., Ellis, S.B., Pryor, J.H., Williams, M.W. and Harpold, M.M., 1989. The use of male-specific chromosomal DNA fragments to determine the sex of bovine preimplantation embryos. Theriogenology, 31: 95-104.
(29) Bowen, G., 1989. Artificial insemination of deer: cervical and laparoscopic techniques. Proceedings of a Deer Course for Veterinarians; Deer Branch (NZVA) Course No. 6; Queenstown, NZ: 8-10.
(30) Brackett, B.C., Bousquet, D., Boice, M.L., Donawick, W.J., Evans, J.F. and Dressel, M.A., 1982. Normal development following *in vitro* fertilization in the cow. Biol Reprod., 27: 147-158.
(31) Braun, J., 1988. Influence of protein supplement and culture conditions on cumulus-cell expansion and nuclear maturation of sheep follicular oocytes. Theriogenology, 29: 1 (abstr).
(32) Bringans, M. 1987. Embryo transfer in deer. Proceedings of a Deer Course for Veterinarians; Deer Branch (NZVA) Course No. 4; Dunedin, N.Z.: 45-52.
(33) Bringans, M. 1989. Embryo transfer in deer: An up-date. Proceedings of a Deer Course for Veterinarians; Deer Branch (NZVA) Course No. 6, Queenstown, N.Z.: 21-28.
(34) Bringans, M., 1989. Embryo transfer in deer. Proceedings Ruakura Deer Industry Conference; Ruakura Agricultural Centre, Hamilton, NZ: 47-50.
(35) Bouters, R., Moyaert, I., Coryn, M. and Vandeplassche, M. 1983. The use of PMSG antiserum in superovulated cattle: Endocrinological changes and effects on timing of ovulation. Zuchthygiene 18: 172-177.
(36) Chang, M.C., 1951. Fertilizing capacity of spermatozoa deposited into the fallopian tubes. Nature. 168: 697-698.

(37) Cheng, W.T.K., 1985. *In vitro* fertilization of farm animal oocytes. PhD Dissertation, Institute of Animal Physiology, Animal Research Station, Cambridge University, Cambridge, England.
(38) Church, R.B., Schaufele, F.J. and Mecking, K., 1985. Embryo manipulation and gene transfer in livestock. Can. J. Anim. Sci., 65: 527-537.
(39) Clutton-Brock, T.H., Guinness, F.E. and Albon, S.D., 1982. Red deer: behaviour and ecology of two sexes. The University of Chicago Press; Chicago, USA.
(40) Dhondt, D., Bouters, R., Spincemaille, J., Corijn, M. and Vandeplassche, M. 1978. The control of superovulation in the bovine with a PMSG antiserum. Theriogenology 9: 529-534.
(41) Dieleman, S.J. and Bevers, M.M. 1987. Effects of monoclonal antibody against PMSG administered shortly after the pre-ovulatory LH surge on time and number of ovulations in PMSG/PG-treated cows. J. Reprod. Fertil. 81: 533-542.
(42) Dixon, T.E. 1986. Embryo transfer in deer. The state of the art. Proceedings of a Deer Course for Veterinarians; Deer Branch (NZVA) Course No. 3; Rotorua: 96-102.
(43) Dott, H.M. and Utsi, M.N.P. 1973. Artificial insemination of reindeer (*Rangifer tarandus*). Jl. Zool., 170: 505-508.
(44) Eppleston, J., Bilton, R.J. and Moore, N.W. 1984. Effect of FSH dose and treatment regime on ovulatory response in sheep. Proc. Aust. Soc. Reprod. Biol. 16: 68 (abstr.).
(45) Evans, G. and Armstrong, D.T. 1984. Reduction of sperm transport in ewes by superovulation treatments. J. Reprod. Fertil. 70: 47-53.
(46) Evans, G. and Robinson, T.J. 1980. The control of fertility in sheep: Endocrine and ovarian responses to progestagen-PMSG treatment in the breeding season and in anoestrus. J. Agric. Sci. (Camb.) 94: 69-88.
(47) Fennessy, P.F., Moore, G.H. and Corson, I.D., 1981. Energy requirements of red deer. Proc. N.Z. Soc. Anim. Prod., 41: 167-173.
(48) Fennessy, P.F., Beatson, N.S. and Mackintosh, C.G., 1987. Artificial insemination. Proceedings of a Deer Course for Veterinarians; Deer Branch (NZVA) Course No. 4; Rotorua, NZ: 33-37.
(49) Fennessy, P.F., Fisher, M.W. and Asher, G.W., 1989. Synchronisation of the oestrous cycle in deer. Proceedings of a Deer Course for Veterinarians; Deer Branch (NZVA) Course No. 6; Queenstown, NZ: 29-35.
(50) Fennessy, P.F., Fisher, M.W., Shackell, G.H. and Mackintosh, C.G. 1989. Superovulation and embryo recovery in Red deer (*Cervus elaphus*) hinds. Theriogenology, 32: 877-883.
(51) Fennessy, P.F., Mackintosh, C.G. and Shackell, G.H., 1990. Artificial insemination of farmed red deer (*Cervus elaphus*). Anim. Prod., 51: 613-621..
(52) Field, R.A., Young, O.A., Asher, G.W. and Foote, D.N., 1985. Characteristics of male fallow deer muscle at a time of sex-related muscle growth. Growth, 49: 190-210.
(53) First, N.L. and Parrish, J.J., 1987. *In vitro* fertilization of ruminants. J. Reprod. Fert. Suppl., 34: 151-165.
(54) Fisher, M.W. and Fennessy, P.F. 1985. Reproductive physiology of female red deer and wapiti. Proceedings of a Deer Course for veterinarians; Deer Branch (NZVA) Course No. 2; Ashburton, N.Z.: 88-100.
(55) Fisher, M.W., Fennessy, P.F., Suttie, J.M., Corson, I.D., Pearse, A.J.T., Davis, G.H. and Johnstone, P.D., 1986. Early induction of ovulation in yearling red deer hinds. Proc. N.Z. Soc. Anim. Prod., 46: 171-173.
(56) Fisher, M.W., Fennessy, P.F. and Milne, J.D., 1988. Effects of melatonin on seasonal physiology of red deer. Proc. N.Z. Soc. Anim. Prod., 48: 113-116.
(57) Fisher, M.W., Fennessy, P.F. and Davis, G.H., 1989. A note on the induction of ovulation in lactating red deer hinds prior to the breeding season. Anim. Prod., 49: 134-138.
(58) Fukui, Y., McGowan, L.T., James, R.W., Asher, G.W. and Tervit, H.R., 1991. Effects of culture duration and time of gonadotrophin addition on *in vitro*

maturation and fertilization of red deer (*Cervus elaphus*) oocytes. Theriogenology (in press).

(59) Fukui, Y., McGowan, L.T., James, R.W., Pugh, P.A. and Tervit, H.R., 1991. Co-culture with somatic cells is not necessary for *in vitro* development to blastocysts of bovine oocytes matured and fertilized *in vitro*. J. Reprod. Fertil. (in press).

(60) Fukushima, M. and Fukui, Y., 1985. Effects of gonadotropin and steroids on the subsequent fertilizability of extrafollicular bovine oocytes cultured *in vitro*. Anim. Reprod. Sci. 9: 323-332.

(61) Glover, G.J., 1985. Aspects of reproductive physiology of female wapiti. M.Sc. thesis, University of Saskatchewan, Saskatoon, Canada.

(62) Gosch, B. and Fischer, K. 1989. Seasonal changes of testis volume and sperm quality in adult fallow deer (*Dama dama*) and their relationship to the antler cycle. J. Reprod. Fertil., 85: 7-17.

(63) Guinness, F.E., Lincoln, G.A. and Short, R.V., 1971. The reproductive cycle of the female red deer, *Cervus elaphus* L. J. Reprod. Fertil., 27: 427-438.

(64) Haigh, J.C., 1984. Artifical insemination of two white-tailed deer. Jl. Amer. Vet. Med. Assoc. 185: 1446-1447.

(65) Haigh, J.C., Shadbolt, M.P. and Glover, G.J., 1984. Artificial insemination of wapiti (*Cervus elaphus*). Proceedings of the American Association of Zoo Veterinarians, Louisville, Kentucky, USA: 173.

(66) Haigh, J.C., Cranfield, M. and Sasser, R.G., 1988. Estrus synchronization and pregnancy diagnosis in red deer. Journal Zoo Animal Medicine, 19: 202-207.

(67) Hammer, R.E., Pursell, V.G., Rexroad, C.E., Wall, R.S., Bolt, D.J., Ebert. K.M., Palmiter, R.D. and Brinster, R.L., 1985. Production of transgenic rabbits, sheep and pigs by microinjection. Nature, 315: 680-683.

(68) Herr, C.M. and Reed, K.C., 1991. Micromanipulation of bovine embryos for sex determination. Theriogenology, 35: 45-54.

(69) Hunter, R.H.F., 1969. Capacitation in the golden hamster, with special reference to the influence of the uterine environment. J. Reprod. Fertil., 20: 223-237.

(70) Jabbour, H.N. 1988. Studies on fertility of superovulated ewes. Ph.D. Thesis, The University of Sydney, Australia.

(71) Jabbour, H.N. and Asher, G.W., 1990. Artificial breeding of farmed fallow deer (*Dama dama*). Proceedings 2nd International Symposium on Game Ranching; Edmonton, Canada: (in press).

(72) Jabbour, H.N. and Evans, G. 1991. Ovarian and endocrine responses of Merino ewes following treatment with PMSG and GnRH or PMSG antiserum. Anim. Reprod. Sci. (in press).

(73) Jabbour, H.N., Evans, G. and Moore, N.W. 1986. Steroidogenic and ovulatory response of Merino ewes to PMSG and procine FSH. Proc. Aust. Soc. Reprod. Biol., 18: 55 (abstr.).

(74) Jabbour, H.N., Asher, G.W., Thompson, J.G.E., Tervit, H.R. and Morrow, C.J. 1990. Superovulation and embryo recovery in farmed Red (*Cervus elaphus*) and Fallow (*Dama dama*) deer. Proceedings of the Second International Symposium on The Biology of Deer, Mississippi, USA; Abstract No. 45.

(75) Jacobson, H.A., Bearden, H.J. and Whitehouse, D.B., 1989. Artificial insemination trials with white-tailed deer. J. Wildl. Manage. 53: 224-227.

(76) Jaczewski, Z., 1954. The effect of changes in length of daylight on the growth of antlers in deer (*Cervus elaphus L.*). Folia Biol., Praha, 2: 133-143.

(77) Jaczewski, Z. and Jasiorowski, T., 1974. Oberservations on the electroejaculation in red deer. Acta Theriologica, 19: 151-157.

(78) Johnson, L.A., Flook, J.P. and Hawk, H.W., 1989. Sex preselection in rabbits: Live births from X and Y sperm separated by DNA and cell sorting. Biol. Reprod., 41: 199-203.

(79) Jopson, N.B., Fisher, M.W. and Suttie, J.M., 1990. Endogenous and exogenous progesterone in red deer hinds. Anim. Reprod. Sci., 23: 61-73.

(80) Katska, L. and Smorag, Z., 1985. The influence of culture temperature on *in vitro* maturation of bovine oocytes. Anim. Reprod. Sci., 9: 205-212.

(81) Kay, R.N.B., 1979. Seasonal changes of appetite in deer and sheep. A.R.C. Research Review, 5: 13-15.
(82) Kelly, R.W. and Moore, G.H., 1977. Reproductive performance in farmed red deer. N.Z. Agric. Sci., 11: 179-181.
(83) Kelly, R.W., McNatty, K.P., Moore, G.H., Ross, D. and Gibb, M., 1982. Plasma concentrations of LH, prolactin, oestradiol and progesterone in female red deer (*Cervus elaphus*) during pregnancy. J. Reprod. Fertil., 64: 475: 483.
(84) Killeen, I.D. and Caffery, M.G.J., 1982. Uterine insemination of ewes with the aid of a laparoscope. Aust. Vet. J., 59: 95.
(85) Krzywinski, A., 1976. Collection of red deer semen with the artificial vagina. Proceedings VIII International Congress on Animal Reproduction and Artificial Insemination (Krakow), 4: 1002-1005.
(86) Krzywinski, A. and Jaczewski, Z., 1978. Observations on the artificial breeding of red deer. Symposia of the Zoological Society of London, 43: 271-287.
(87) Lambert, R.D., Sirard, M.A., Bernard, C., Beland, R., Rioux, J.E., Leclerc, P., Menard, D.P. and Bedoya, M., 1986. *In vitro* fertilization of bovine oocytes matured *in vivo* and collected at laparoscopy. Theriogenology, 25: 117-133.
(88) Lawson, R.A.S., Rowson, L.E.A. and Adams, C.E., 1972. The development of cow eggs in the rabbit oviduct and their vivability after re-transfer to heifers. J. Reprod. Fertil., 28: 313-315.
(89) Leibfried-Rutledge, M.L., Critser, E.S. and First, N.L., 1986. Fetal calf serum is the preferred supplement for *in vitro* maturation of cumulus-oocyte complexes. Biol. Reprod., 35: 850-857.
(90) Leibfried-Rutledge, M.L., Critser, E.S., Eyestone, W.H., Northey, D.L. and First, N.L., 1987. Developmental potential of bovine oocytes matured *in vitro* or *in vivo*. Biol. Reprod., 36: 376-383.
(91) Leibo, S.P. and Rall, W.F., 1987. Increase in production of pregnancies by bisection of bovine embryos. Theriogenology, 27: 245 (abstr).
(92) Lenz, R.W., Ball, G.D., Leibfried, M.L., Ax, R.L. and First, N.L., 1983. *In vitro* maturation and fertilization of bovine oocytes are temperature-dependent processes. Biol. Reprod., 29: 173-179.
(93) Lincoln, G.A., 1971. The seasonal reproductive changes in the red deer stag (*Cervus elaphus*). J. Zool., 163: 105-123.
(94) Lincoln, G.A., 1985. Seasonal breeding in deer. In: P.F. Fennessy and K.R. Drew (Editors), Biology of Deer Production. Bull. No. 22, Royal Society of New Zealand, Wellington, pp 165-179.
(95) Lincoln, G.A. and Guinness, F.E., 1973. The sexual significance of the rut in red deer. J. Reprod. Fertil. Suppl., 19: 475-489.
(96) Lincoln, G.A. and Short, R.V., 1980. Seasonal breeding: Nature's contraceptive. Recent Progress in Hormone Research, 36: 1-52.
(97) Lu, K.H., Gordon, I., McGovern, H. and Gallagher, M., 1988. Production of cattle embryos by *in vitro* maturation and fertilization of follicular oocytes and their subsequent culture *in vivo* in sheep. Theriogenology, 29: 272 (abstr).
(98) Magyar, S.J., Biediger, T., Hodges, C., Kraemer, D.C. and Seager, S.W.J., 1989. A method of artificial insemination in captive white-tailed deer (*Odocoileus virginianus*). Theriogenology, 31: 1075-1080.
(99) Moor, R.M. and Gandolfi, F., 1987. Molecular and cellular changes associated with maturation and early development of sheep eggs. J. Reprod. Fertil., Suppl. 34: 55-69.
(100) Moor, R.M. and Trounson, A.O., 1977. Hormonal and follicular factors affecting maturation of sheep oocytes *in vitro* and their subsequent developmental capacity. J. Reprod. Fertil., 49: 101-109.
(101) Moor, R.M., Osbourne, J.C. and Crosby, I.M. 1985. Gonadotrophin-induced abnormalities in sheep oocytes after superovulation. J. Reprod. Fertil. 74: 167-172.
(102) Mulley, R.C., 1989. Reproduction and performance of farmed fallow deer (*Dama dama*). PhD thesis; the University of Sydney, Australia.

(103) Mulley, R.C., Moore, N.W. and English, A.W., 1988. Successful uterine insemination of fallow deer with fresh and frozen semen. Theriogenology, 29: 1149-1153.
(104) Mulley, R.C., English, A.W., Rawlinson, R.J. and Chapple, R.S., 1987. Pregnancy diagnosis of fallow deer by ultrasonography. Aust. Vet. J., 64: 257-258.
(105) Nancarrow, C.D., Murray, J.D., Boland, M.P., Sutton, R. and Hazelton, I.G. 1984. Effect of gonadotrophin releasing hormone in the production of single-cell embryos for pronuclei injection of foreign genes. In: Reproduction in the sheep. Eds. Lindsay, D.R. and Pearce, D.T. Australian Academy of Science, Canberra: 286-288.
(106) Parrish, J.J., Susko-Parrish, J.L., Winer, M.A. and First, N.L., 1988. Capacitation of bovine sperm by heparin. Biol. Reprod., 38: 1171-1180.
(107) Pollock, A.M., 1975. Seasonal changes in appetite and sexual condition in red deer stags maintained on a six month photoperiod. J. Physiol. 244: 95P-96P.
(108) Prather, R.S. and First, N.L., 1990. Nuclear transfer in mammalian embryos. International Review of Cytology. 120: 169-190.
(109) Pugh, P.A., Fukui, Y., Tervit, H.R. and Thompson, J.G., 1990. Successful use of frozen ram semen for *in vitro* fertilization of *in vitro* matured sheep oocytes. Proc. Aust. Soc. Reprod. Biol, 23: 88 (abstr).
(110) Ryan, J.P., Bilton, R.J. and Hunton, J.R. 1984. Superovulation of ewes with a combination of PMSG and FSH-P. In: Reproduction in the sheep. Eds. Lindsay, D.R. and Pearce, P.T. Australian Academy of Science, Canberra: 338-341.
(111) Schellander, K., Fayrer-Hosken, R.A., Keefer, C.L., Brown, L.M., Malter, H., McBride, C.E. and Brackett, B.G., 1989. *In vitro* fertilization of bovine follicular oocytes recovered by laparoscopy. Theriogenology, 31: 927-934.
(112) Schnare, H. and Fischer, K., 1987. Secondary sex characteristics and connected physiological values in male fallow deer (*Dama dama*) and their relationship to changes of the annual photoperiod: doubling the frequency. J. Exp. Zool., 244: 463-471.
(113) Simons, J.P. and Land, R.B., 1987. Transgenic livestock. J. Reprod. Fertil. Suppl., 34: 237-250.
(114) Simons, J.P., Wilmut, I., Clark, D.J., Archibald, D.L., Bishop, J.O. and Lathe, R., 1988. Gene transfer into sheep. Biotechnology, 6: 179-183.
(115) Sirard, M.A. and Lambert, R.D., 1985. *In vitro* fertilization of bovine follicular oocytes obtained by laparoscopy. Biol. Reprod., 33: 487-494.
(116) Sirard, M.A., Lambert, R.D., Beland, R. and Bernard, C., 1985. The effects of repeated laparoscopic surgery used for ovarian examination and follicular aspiration in cows. Anim. Reprod. Sci., 9: 25-30.
(117) Sirard, M.A., 1989. Practical aspects of *in vitro* fertilization in cattle. J. Reprod. Fertil. Suppl., 38: 127-134.
(118) Smith, L.C. and Wilmut, I., 1988. Factors influencing nuclear transplantation in sheep embryos. J. Reprod. Fertil., 1: 10 (abstr).
(119) Staigmiller, R.B. and Moor, R.M., 1984. Effect of follicle cells on the maturation and development competence of ovine oocytes matured outside the follicle. Gamete Res., 9: 221-229.
(120) Stice, S.L., and Robl, S.M. 1988. Nuclear reprogramming in nuclear transplant rabbit embryos. Biol. Reprod., 39: 657-664.
(121) Suttie, J.M., Lincoln, G.A. and Kay, R.N.B., 1984. Endocrine control of antler growth in red deer stags. J. Reprod. Fertil., 71: 7-15.
(122) Suttie, J.M. and Simpson, A.M. 1985. Photoperiodic control of appetite, growth, antlers, and endocrine status of red deer. In: P.F. Fennessy and K.R. Drew (Editors), Biology of Deer Production. Bull. No. 22, Royal Society of New Zealand, Wellington: 429-432.
(123) Thibault, C., 1977. Are follicular maturation and oocyte maturation independent processes? J. Reprod. Fert., 51: 1-15.
(124) Thompson, J.G.E. and Asher, G.W. 1988. Superovulation and ova recovery in farmed fallow deer (*Dama dama*). Proc. Aust. Soc. Reprod. Biol. 20: 4 (abstr).

(125) Torrie, S., Cognie, Y. and Colas, G. 1987. Transfer of superovulated sheep embryos obtained with different FSH-P. Theriogenology 27: 407-419.
(126) Van Vliet, R.A., Verrinder-Gibbins, A.M. and Walton, J.S., 1989. Livestock embryo sexing: A review of current methods with emphasis on Y-specific DNA probes. Theriogenology, 32: 421-437.
(127) Veltman, C.J., 1985. The mating behaviour of red deer. Proceedings of a Deer Course for Veterinarians; Deer Branch (NZVA) Course No. 2; Ashburton, NZ: 135-142.
(128) Vivanco, H.W., Rangel, R., Lynch, P. and Rhodes, D., 1991. Large scale commercial application of bisection of sheep embryos. Theriogenology, 35: 292 (abstr).
(129) Waldham, S.J., Jacobson, H.A., Dhungel, S.K. and Bearden, H.J. 1989. Embryo transfer in the white-tailed deer: A reproductive model for endangered deer species of the world. Theriogenology, 31: 437-450.
(130) Wheeler, M.D. and Seidel, G.E., 1986. Time course of *in vitro* capacitation of frozen and unfrozen bovine spermatozoa. Theriogenology, 25: 216 (abstr).
(131) White, I.R., McKelvey, W.A.C., Busby, S., Sneddon, A. and Hamilton, W.J., 1989. Diagnosis of pregnancy and prediction of fetal age in red deer by real-time ultrasonic scanning. Vet. Rec., 124: 395-397.
(132) Willadsen, S.M., Janzen, R.E., McAlister, R.J., Shea, B.F., Hamilton, G. and McDermand, D., 1991. The viability of late morulae and blastocysts produced by nuclear transplantation in cattle. Theriogenology, 35: 161-170.
(133) Wilson, P.R. and Bingham, C.M., 1990. Accuracy of pregnancy diagnosis and prediction of calving date in red deer using real-time ultrasound scanning. Vet. Rec., 126: 133-135.
(134) Woolliams, J.A. and Wilmut, I., 1989. Embryo manipulation in cattle breeding and production. Anim. Prod., 48: 3-30.