REPRODUCTIVE PHYSIOLOGY AND CONTROLLED BREEDING OF FARMED FALLOW DEER

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Introduction

It has been five years since the last address on fallow deer reproduction to the Deer Branch NZVA conference (1). Since that time there have been considerable advances in our understanding of reproductive physiology and controlled breeding of fallow deer. This paper attempts to condense this information into a package that may serve as a useful reference for veterinarians.

Reproductive physiology

(i) <u>Seasonal nature of reproduction</u> : Both subspecies of fallow deer, European fallow (*Dama dama dama*) and Mesopotamian (Persian) fallow (*Dama dama mesopotamica*), exhibit highly seasonal patterns of reproduction, with mating activity occurring in autumn and parturition occuring in summer. The importance of the seasonal variation of the daily light:dark ratio (photoperiod) in mediating the timing of annual reproductive cycles of many non-equatorial mammalian species has long been recognized, either from the relationship observed between photoperiod and sexual activity or by the results of experimental photoperiodic manipulation (2). The generally accepted hypothesis that the strict seasonality of reproduction in fallow deer is mediated by the prevalent photoperiodic regimen (3,4,5) has been inferred largely from experiments on red deer (6) and sika deer (7) in which artificial photoperiodic regimens altered the timing of the annual antler and testicular growth cycles of males. Recent studies on fallow deer, involving artificial manipulation of light:dark ratios (8) or by strategic administration of exogenous melatonin (9,10) have confirmed photoperiodism in this species.

(ii) <u>Oestrous cyclicity</u> : The onset of the breeding season in female fallow deer is generally regarded as the period during which first overt oestrus occurs within the doe herd. This is confined to within a 12- to 14-day period, starting between 15-20 April in New Zealand (11,12). However, first oestrus is invariably preceded by one or more silent ovulations associated with short-lived (8- to 10-day) corpora lutea (Figure 1).

The onset of the first oestrus period is remarkably consistent between regions and years, although mating groups composed entirely of pubertal does may exhibit oestrous activity 7-10 days later than groups containing mature does (13). Contrary to recent findings for red deer (14), limited data on fallow deer (15) suggest that the presence, and strategic introduction, of males does not strongly influence the onset

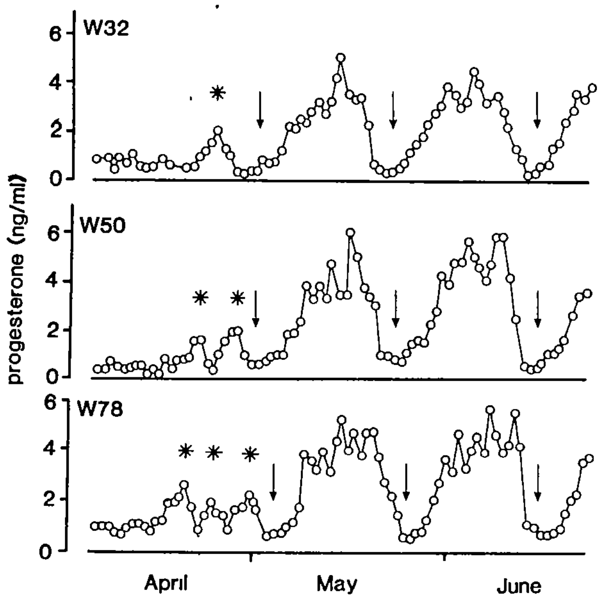

Figure 1 : Plasma progesterone profiles of fallow does through 2 oestrous cycles (asterisks indicate silent cycles; arrows indicate oestrus).

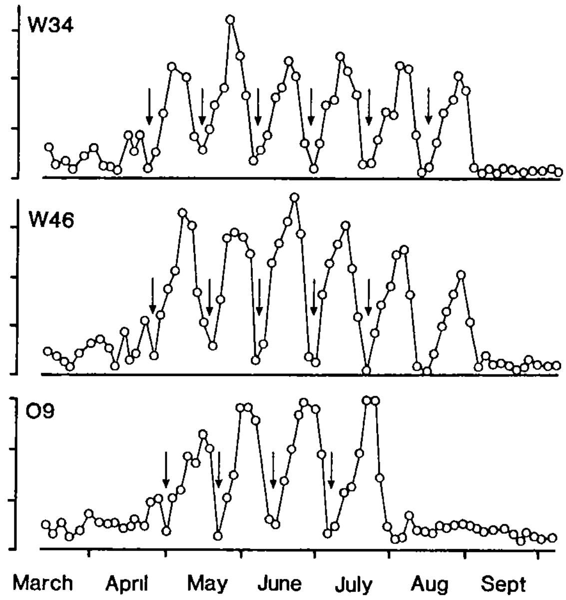

Figure 2 : Plasma progesterone profiles of fallow does run continuously with vasectomised bucks in 1984 (arrows indicate oestrus).

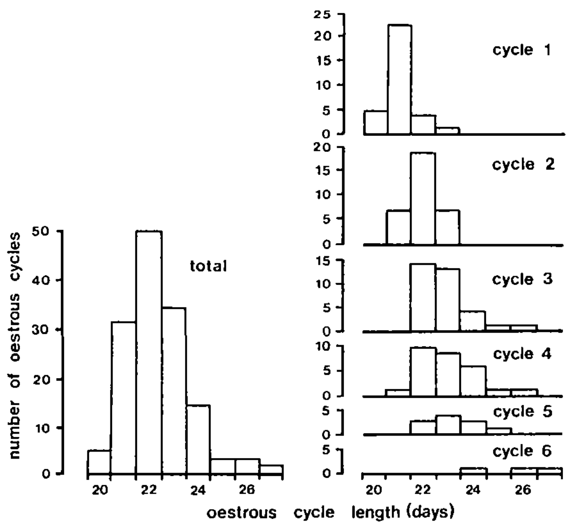

Figure 3 : Frequency histogram of fallow deer oestrous cycle lengths by cycle number.

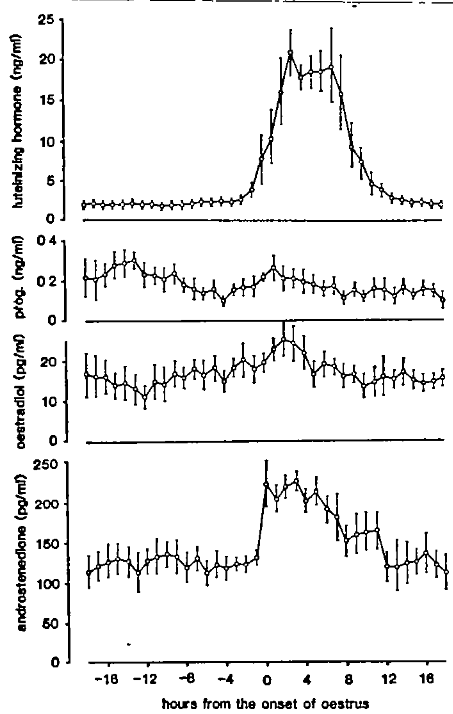

Figure 4 : Mean (±s.e.m.) serum values of LH, progesterone, oestradiol and androstenedione, recorded hourly about oestrus for 4 fallow does

and duration of the first oestrus period. However, oestrous does may have a strong influence on synchronising herdmates (16).

The first oestrus period coincides with the rut; the period of intense sexual activity and vocalisation of bucks. Under natural on-farm mating conditions, the majority (85%) of does conceive to first oestrus matings (17). However, in the absence of conception/pregnancy, fallow does will exhibit regular 20 to 22-day oestrous cycles (11; Figure 2). The first oestrous cycle of the breeding season is of remarkable uniformity, with a mean ($\pm$s.d.) length of 21 ± 0.64 days. However, later cycles tend to become longer and more variable (11; Figure 3).

The duration of the female breeding season, determined by the period of potential cyclic activity, extends from April to early September; during which the does may exhibit up to six oestrous cycles (Figure 2). Younger does ($<$2 years) tend to exhibit fewer oestrous cycles and a shorter breeding season (11).

(iii) Hormonal control of oestrus/ovulation : Fallow does are almost invariably monovulators (4) with the onset of oestrus preceding ovulation (follicular rupture) by 24 hours (18). The natural endocrine regulation of oestrus/ovulation in fallow does shares many similarities with other domestic ruminants (9; Figure 4). The pre-ovulatory luteinizing hormone (LH) surge is initiated at the onset of oestrus, and has a duration of 13-14 hours. Its initiation is probably triggered by decreasing secretion of progesterone following luteolysis and increased secretion of follicular oestradiol and androstenedione. The relative roles of pre-ovulatory increases in oestradiol and androstenedione secretion in inducing oestrous behaviour have yet to be evaluated. Exogenous oestradiol (benzoate) is efficacious in inducing oestrous behaviour and a "pre-ovulatory" LH surge when delivered in pharmacological doses to ovariectomised does (19) but the role of androstenedione in inducing such events is equivocal and often discounted in other livestock species (9).

It is interesting to note that oestrous behaviour in fallow does is normally terminated at copulation, with an average duration of fifteen minutes. However, in the absence of copulation oestrus will persist for at least 12 hours (15).

Following ovulation, luteal development progresses in a similar manner observed in other domestic ruminants. Maximal progesterone secretory capacity of the resultant corpus luteum is attained 12-13 days after oestrus; as is clearly observable during the oestrous cycle (Figure 5).

(iv) Hormonal control of luteolysis : In the absence of a pre-implantation embryo, natural regression of the fallow deer corpus luteum is initiated on about Day 19 of the oestrous cycle (Figure 5). The luteolysin, prostaglandin (PG) F-2α, is clearly of uterine origin, as indicated by failure of luteal regression following midcycle bilateral hysterectomy (20). Luteal regression is preceded by secretory episodes of luteal oxytocin, eventually leading to episodic secretion of PGF-2α (evinced by circulatory concentrations of its pulmonary metabolite, PGFM) on Days 19 and 20 (Figure 6). Demise of the corpus luteum, as indicated by depletion of circulating progesterone concentrations, occurs over a 48-hour period (20).

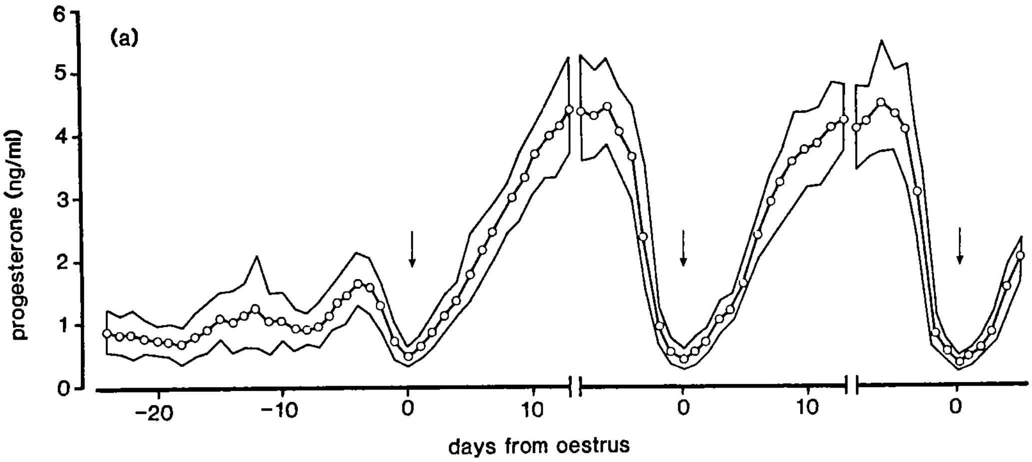

Figure 5 : Composite progesterone profile (mean ±95% CL) of fallow does (n=13) with normal oestrous cycles (arrows indicate oestrus)

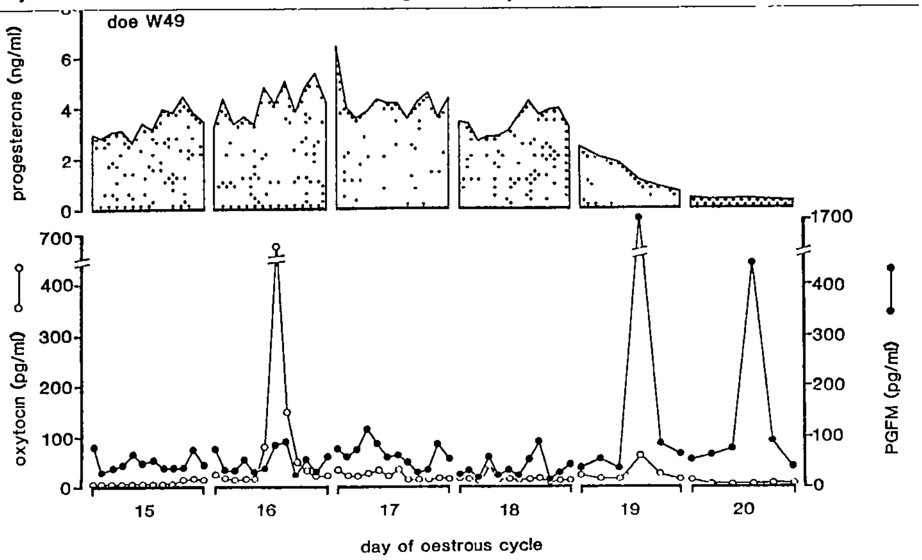

Figure 6 : Profiles of plasma progesterone, oxytocin and PGFM concentrations on Days 15-20 of the oestrous cycle of a fallow doe

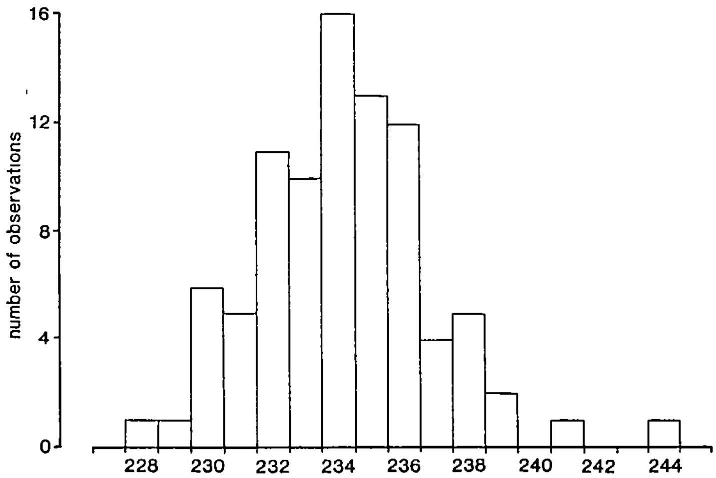

Figure 7 : Frequency histogram of fallow deer gestation lengths recorded at Ruakura in 1983 and 1984

(v) Pregnancy and parturition : The mean ($\pm$ s.e.m.) gestation length of fallow deer is 234.2$\pm$0.3 days (17; Figure 7). Pregnancy is associated with continuously elevated plasma progesterone concentrations (4-10 ng/ml), although the relative secretory contributions of the corpus luteum and the placenta have yet to be determined. Parturition is associated with a rapid decline in progesterone secretion (Figure 8), either through destruction of the corpus luteum and/or removal of the placenta.

The fawning season of fallow deer generally starts later than that of red deer, but tends to be more condensed (21). In the northern regions of New Zealand, the first fallow births usually occur in early December, whereas red deer births may start in early-mid November (Figure 9). However, fallow births are heavily concentrated within the 20-day period between 6 December and 25 December. Fawns born well before this period have been observed but these may be premature births in some cases. Fawns born after Christmas day will generally include those conceived to return oestrus matings (i.e. late May matings).

The facts that fallow does exhibit high conception rates (85%) to their first oestrus and that first oestrus is synchronised to within a 12- to 14-day period, explain the occurrence of a concise fawning pattern despite there being a certain amount of variation in gestation length.

Very late-born fawns do occur on some farms. This is particularly so if sire bucks remain with does throughout the autumn and winter months. This allows persistently cyclic does to conceive at their third, fourth or even fifth oestrus. As it is generally believed that fawns born in February and March have low survival rates and complicate management, it is becoming common practice for farmers to remove sire bucks from the breeding herds in early June, effectively allowing does only two chances to conceive.

(vi) Extra-ovarian progesterone secretion : While the corpus luteum appears to be the principal source of progesterone in female fallow deer, particularly during the oestrous cycle (11) and early pregnancy (15,17), certain anomalies in plasma progesterone concentrations observed occasionally cannot be explained in terms of luteal secretion of the hormone. Unusual incidences of progesterone secretion have been observed in some individuals during the prepubertal (15) and peri-oestrous (9) periods, when active luteal tissue is not normally present. Recent studies in which ovariectomised prepubertal fallow does were challenged with ACTH provided strong evidence that the adrenal glands are secondary and major sources of progesterone in this species (22). ACTH elicited a marked secretory output of progesterone (Figure 10), a situation that can be mimicked by extreme levels of stress. It is concluded that progesterone of adrenal origin may occasionally confound interpretation of plasma progesterone profiles as indicators of ovarian status if animals are not habituated to the handling/sampling procedures.

(vii) Male reproductive cycle : Apart from siring offspring, fallow bucks contribute nothing to the growth and development of fawns. Yet their entire annual cycle is dedicated to reproduction. Bucks invest large amounts of energy into mating activity during the rut, a period of only 12-14 days each year. The remainder of the year seems to

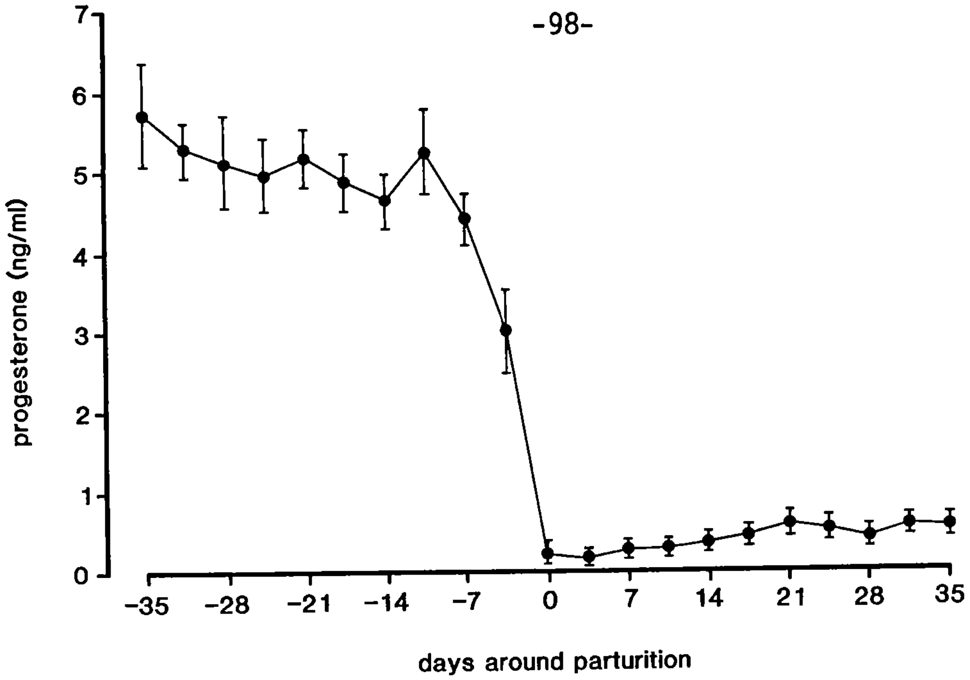

Figure 8 : Mean (± s.e.m.) plasma progesterone concentrations during the parturient period of fallow does.

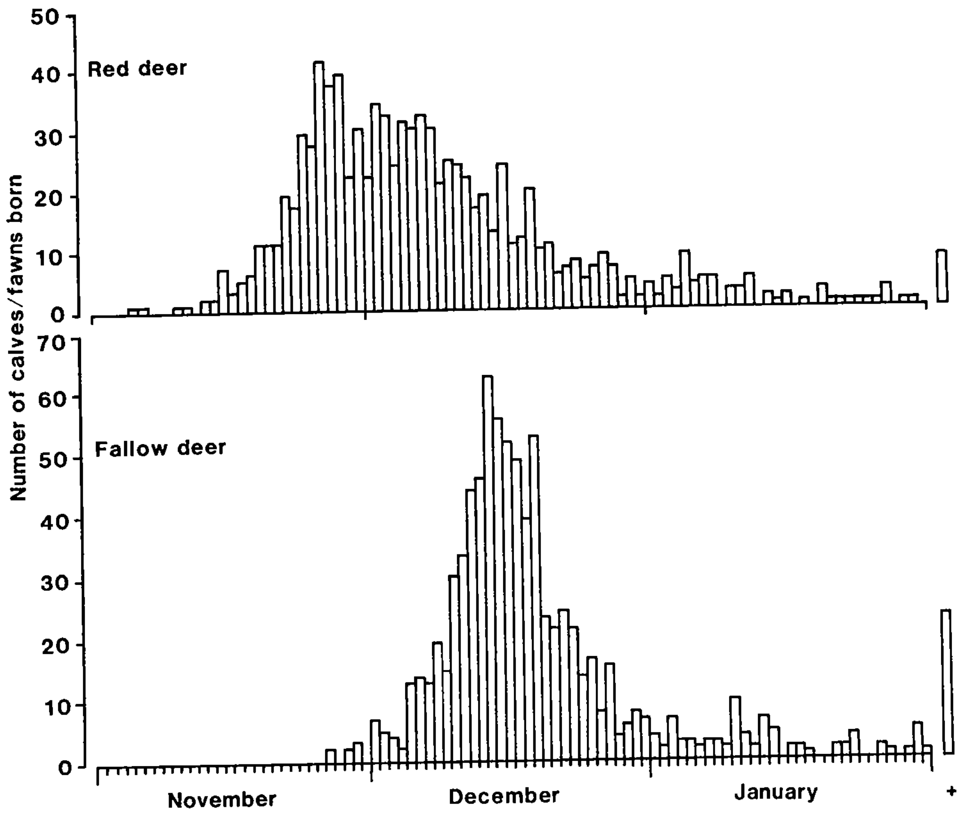

Figure 9 : Frequency distributions of birth dates for red and fallow deer on commercial farms in northern regions of NZ between 1980 and 1984.

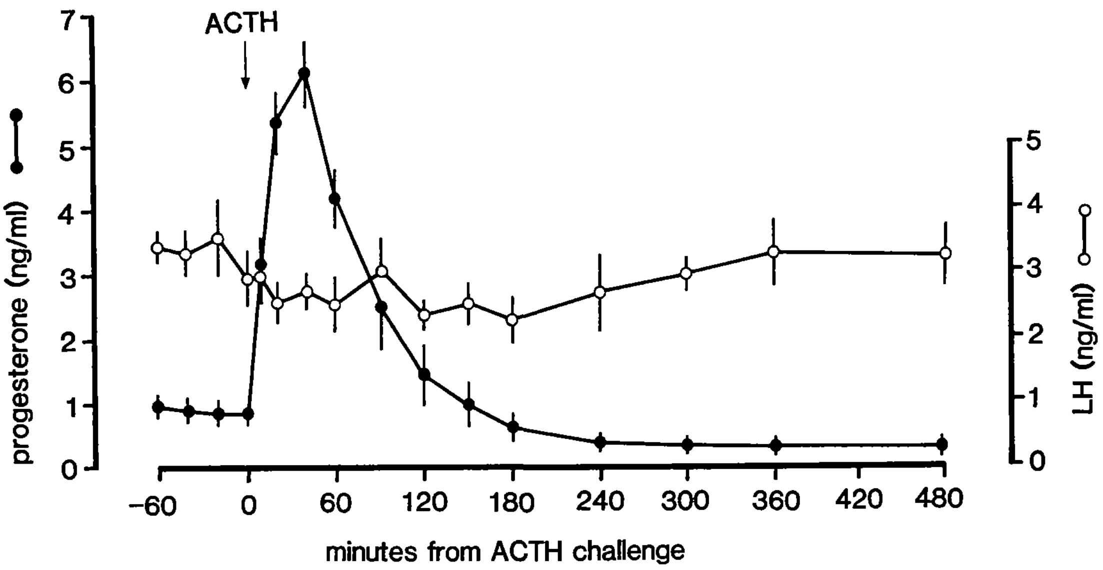

Figure 10 : Mean (± s.e.m.) plasma progesterone and LH concentrations following i.v. administration of ACTH to ovariectomised fallow does.

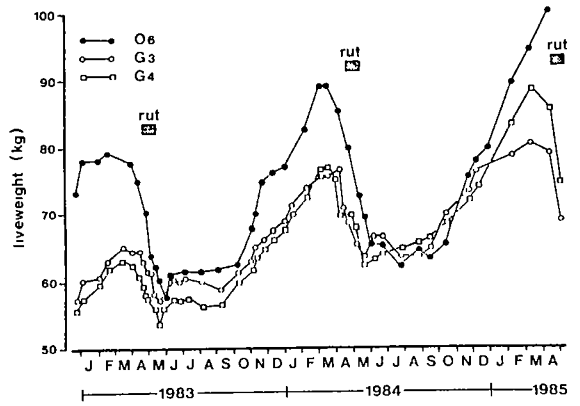

Figure 11 : Seasonal liveweight profiles of 3 mature fallow bucks from December 1982 to April 1985

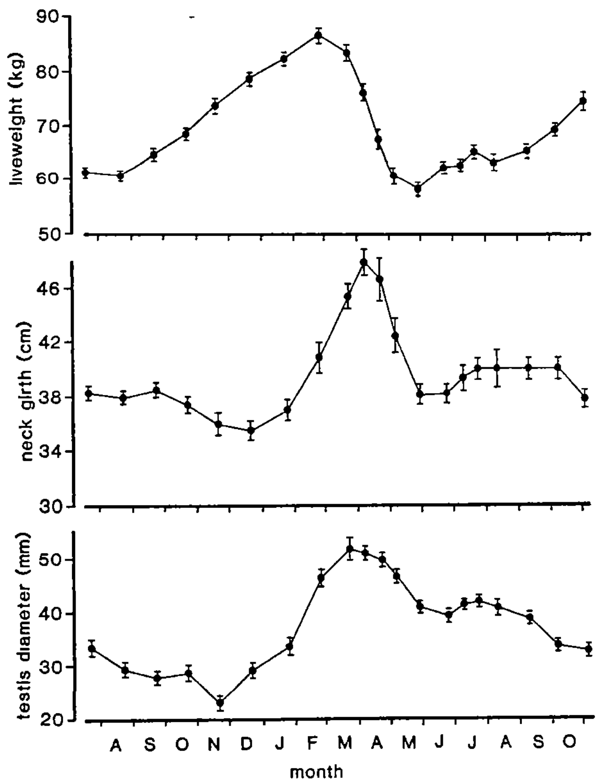

Figure 12 : Seasonal changes in mean (± s.e.m.) liveweight, neck girth and testicular diameter of 4 mature fallow bucks

involve recovery from the effects of the rut or preparation for the next rut.

The annual liveweight profile of adult fallow bucks is typical for various temperate species of deer for which there is a definite rutting season (Figure 11). Rapid liveweight gains occur during spring and summer months (September-February) and bucks reach their peak annual liveweight immediately prior to the rut in autumn. The increases in liveweight mostly represent increased deposition of subcutaneous and depot fat, as well as increased neck muscle mass. However, during the rut (late April-early May) bucks reduce feed intakes and markedly increase mobile activity. The resultant negative energy balance leads to very rapid mobilisation of fat reserves and some catabolisation of muscle, such that bucks may lose up to 30% of total body weight in 3-4 weeks. For a 3 to 4-month period following the rut (i.e. during winter) bucks regain very little of this lost weight even though they increase grazing activity. Bucks, therefore, overwinter in very poor body condition. It is not until the onset of spring (September-October) that the growth and fat deposition cycle starts over again.

Testicular development in adult fallow bucks also undergoes annual cyclic changes (Figure 12). This is primarily controlled by marked changes in secretion of LH from the pituitary gland in response to changes in photoperiod (22; Figure 13). LH is secreted in pulses. These pulses alter in amplitude and frequency during the year, being of low amplitude and frequency during the non-breeding season (November-December) and of high amplitude and frequency leading up to the onset of the breeding season in autumn. These changes in LH secretion in late summer and autumn directly influence testicular activity by promoting testis growth and increasing testosterone secretion. As testicular size increases in response to increased LH stimulation towards the rut, there is a concomitant increase in spermatogenic activity such that, by the onset of the rut, large numbers of viable spermatozoa are present in ejaculates. The testes remain active throughout winter, secreting modest levels of testosterone and producing large numbers of spermatozoa (9). However, towards the start of spring, LH secretion diminishes and the testes regress in size; secreting only very low levels of testosterone. Spermatogenesis is completely arrested by early summer (November); the bucks becoming effectively infertile (Figure 14). It is during this phase of testicular quiesence that liveweights begin to increase rapidly. The animals remain infertile for about two months, gradually regaining fertility towards the end of summer.

The antler cycle of bucks is closely linked to the testicular-testosterone cycle. Old antlers are cast during spring when the testes regress. Casting is in response to a marked decline in testosterone secretion. The new antler grows during the following period of minimal testosterone secretion through spring and early summer. As blood testosterone levels increase in late summer/early autumn, the velvet antler mineralises rapidly and eventually the soft outer layer is stripped off. The hard antlers are retained throughout autumn and winter.

Annual changes in testosterone secretion also have marked effects on some muscles. In particular, rising testosterone levels in late summer/early autumn cause hypertrophy of the neck muscles. This results in a massive increase in neck muscle mass by the start of the rut (Figure 12). Loss of liveweight over the rut results in a

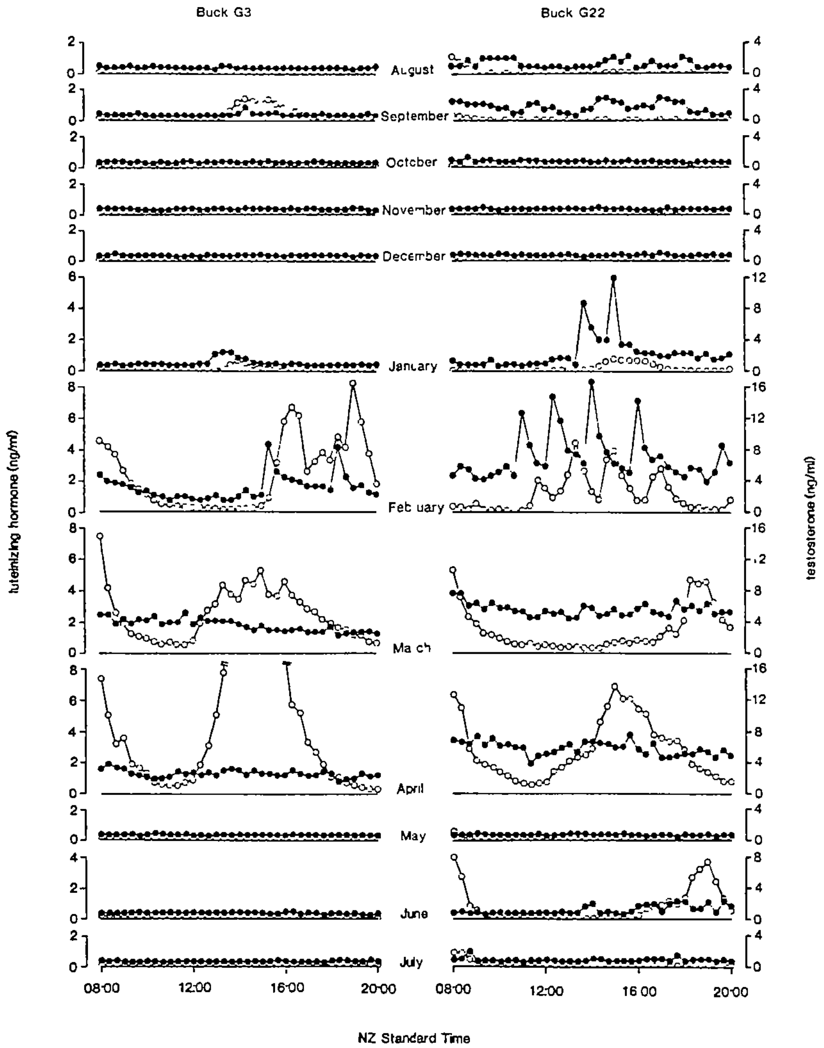

Figure 13 : Twelve-hour profiles of plasma LH (•) and testosterone (o) concentrations for 2 mature fallow bucks recorded monthly

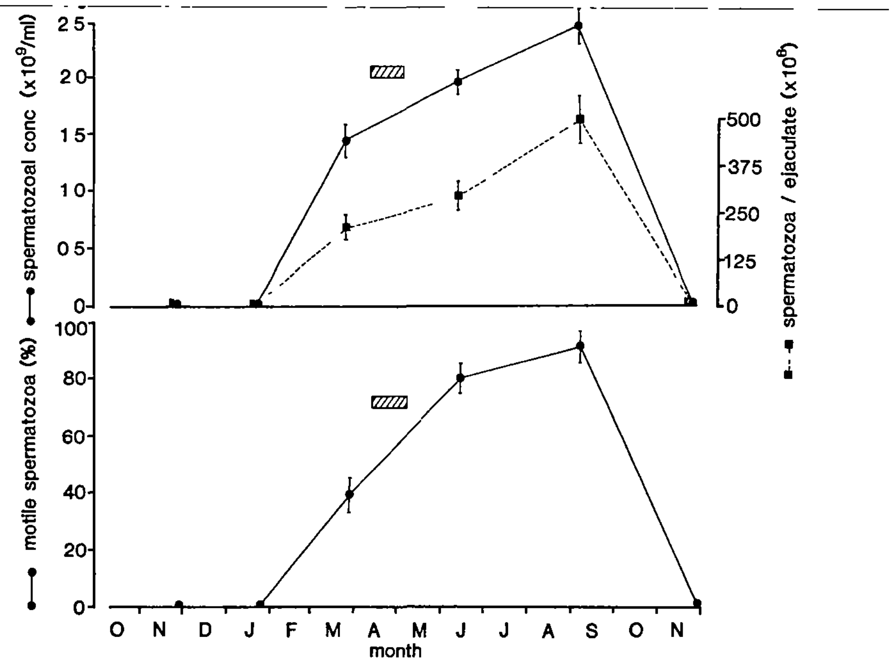

Figure 14 : Seasonal profiles of mean (± s.e.m.) parameters for ejaculates collected by electro-ejaculation of 5 mature fallow bucks

decrease in neck girth. However, when the testes fully regress and liveweights begin to increase in spring, neck girth decreases further in the relative absence of testosterone (Figure 12). This phenomenon of cyclic changes in neck muscle mass is unique to male deer (24).

(viii) Buck rutting behaviour : The fallow deer rut lasts for only a few weeks in late April/early May and is associated with profound and spectacular changes in buck behaviour. The most characteristic behaviour of rutting bucks is their vocalisations; a series of low gutteral grunts often referred to as "groaning". Groaning appears to be confined largely to the period of first oestrus of the does, and there is an apparent close relationship between the intensity of vocal activity and the daily occurrence of oestrous does (Figure 15).

Control of seasonal breeding patterns

(i) Justification : Fallow deer have evolved in the temperate regions of the northern hemisphere where it was clearly advantageous to fawn in summer for optimum survival of offspring. However, summer fawning is not necessarily the ideal situation on NZ pastoral farms, where peak pasture production and quality occur earlier in spring. There is, therefore, a poor alignment between optimum pasture production and the high energy demands of lactation. Closer alignment could lead to more efficient utilisation of pasture resources and better fawn growth rates. Advancement of the fawning season into spring necessarily requires a shift in the previous mating season from autumn (April-May) to late summer (February-March).

(ii) Oestrus/ovulation control with CIDRs + PMSG or GnRH : Early attempts to advance oestrus/ovulation in fallow does using intravaginal CIDR devices (type S or G; 9 or 12% progesterone) in conjunction with an exogenous gonadotrophin (PMSG) or gonadotrophin-releasing hormone (GnRH) were successful in inducing oestrus/ovulation up to six weeks earlier than normal (16,25). However, conception rates were generally low; possibly due to suboptimal buck fertility/libido. Furthermore, there was the additional problem of some does conceiving twins following PMSG treatment.

There has been very little commercial application of intravaginal CIDR devices for induction of early oestrus/ovulation in fallow deer. Their main use appears to be in oestrous synchronisation within the breeding season (i.e. AI).

(iii) Exogenous melatonin usage : More recent studies on out-of-season breeding in fallow deer have centred on the administration of the pineal hormone, melatonin, as its mode of action is common to both sexes.

Melatonin is the main messenger of the photoperiod signal. Blood melatonin levels are elevated naturally only during darkness. During summer, the total duration of secretion within each circadian period is short. However, this secretory period increases as days become shorter (Figure 16). The increasing levels of melatonin secretion as autumn approaches stimulates breeding activity in both males and females. Artificial control of the onset of the breeding season involves supplementation of natural melatonin secretion during summer

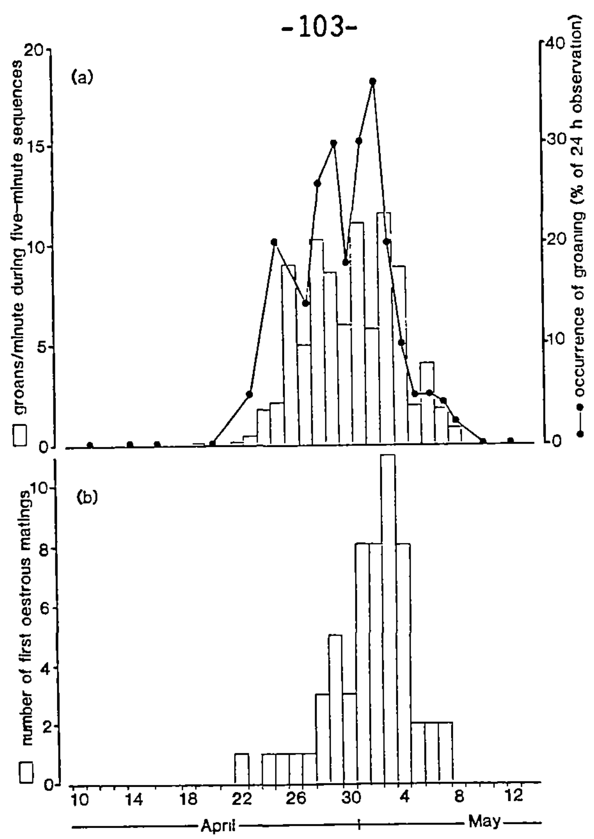

Figure 15 : Frequency of vocalisations of fallow bucks and occurrence of oestrous does during the rut.

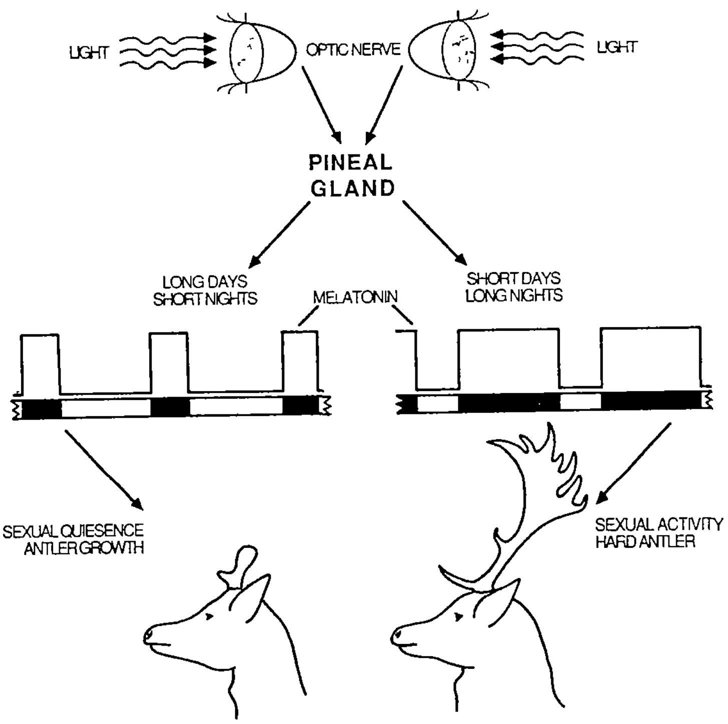

Figure 16 : Schematic representation of changes in melatonin secretion during summer and winter.

with exogenous melatonin; thus inducing a physiological "short-day" state.

The first attempts at such supplementation involved feeding bucks with melatonin-laced pellets at 3.00 p.m. every day during part of summer (9). This was designed to elevate blood melatonin levels several hours before they would be naturally at night, thus augmenting night-time profiles. This form of melatonin administration has several drawbacks, not the least of which are the vagaries of voluntary intake. However, some treatment responses were observed relative to control bucks. Treated bucks exhibited increased neck muscle hypertrophy during and immediately after the treatment period. More encouraging, however, was the observation that treated bucks exhibited an earlier attainment of fertility, as indicated by the presence of viable spermatozoa in ejaculates as early as January (9).

More recently, subcutaneous melatonin implants (Regulin; Young's Animal Health NZ Ltd) have become in vogue. These implants provide continuous release of melatonin, resulting in a perpetual elevation of blood melatonin concentrations for 30-40 days. The effect is actually additive at night, with exogenous levels superimposed on natural endogenous levels (10; Figure 17).

Recent studies involving administration of Regulin implants to fallow does and bucks in summer produced spectacular results (10). Treatment was applied to pubertal, non-pregnant adult and pregnant adult does and adult bucks. Each animal received single implants on four occasions at 30 day intervals from 10 November 1986. The rut of the treated deer occurred in mid February - early March; about 7-8 weeks earlier than for contemporary controls. It is significant that, not only did the treated does exhibit oestrus early (Table 1), the treated sire bucks also exhibited a marked advancement in reproductive development (Figure 18) and expressed full rutting behaviour in response to the early oestrous activity. Furthermore, 94% of treated does conceived to their first oestrus; the remaining 6% conceiving to a return oestrus 21 days later. The melatonin-treated does fawned in October 1987, 7-8 weeks before the control does. Mortality of early born fawns was appreciably greater than fawns born to controls in December; this was primarily attributable to inclement weather in October.

While the three types of does (i.e. pubertal, non-pregnant adult and pregnant adult does) appear to have responded similarly to melatonin treatment, most of the does treated while still pregnant failed to lactate following the 1986 fawning and subsequently lost their 1986-born fawns. It is probable that the initation of lactation was suppressed by melatonin treatment; suggesting a contra-indication to the use of melatonin in pregnant does.

Strategic administration of melatonin implants to farmed fallow deer is a useful tool for advancing the fawning season. However, high mortality rates of early-born fawns due to cold weather may seriously limit the desirable degree of advancement.

Artificial insemination

(i) <u>Justification</u> : Application of artificial insemination (AI) technology within the fallow deer farming industry is in its infancy. However, the future potential of AI is enormous, particularly in

Table 1 Mean date of first oestrus, conception and parturition for (a) melatonin-treated, and (b) control fallow does

	n	Mean (± s.e.m.) date of first oestrus 1987	Mean (± s.e.m.) date of conception 1987	No. does conceiving	No. does fawning	Mean (± s.e.m.) date of fawning 1987
(a) melatonin-treated does						
Pubertal does	6	2.0 Mar (±7.4)	5.5 Mar (±8.1)*	6	3	19.7 Oct (±1.7)
Non-pregnant adult does	6	28.3 Feb (±5.2)	28.3 Feb (±5.2)	6	4	23.5 Oct (±0.6)
Pregnant does	6	24.3 Feb (±2.6)	24.3 Feb (±2.6)	6	6	23.0 Oct (±0.8)
	18	27.6 Feb (±3.0)	28.7 Feb (±3.2)	18(100%)	13(72%)	22.4 Oct (±0.6)
(b) control does						
Pubertal does	6	26.5 Apr (±0.4)	30.0 Apr (±3.8)*	6	4	16.0 Dec (±1.0)
Non-pregnant adult does	6	21.8 Apr (±1.3)	21.8 Apr (±1.3)	6	5	13.6 Dec (±1.3)
Pregnant does	6	20.5 Apr (±0.9)	20.5 Apr (±0.9)	6	6	10.8 Dec (±0.7)
	18	22.9 Apr (±0.8)	24.1 Apr (±1.7)	18(100%)	15(83%)	13.1 Dec (±0.4)

* One pubertal doe in each of the treated and control groups was observed to exhibit a subsequent oestrus

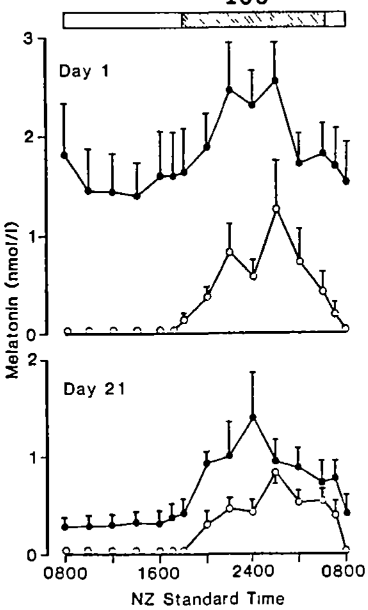

Figure 17 : Circadian profiles of mean (± s.e.m.) plasma melatonin values of 4 fallow does receiving single Regulin implants (•) and 4 control does (o).

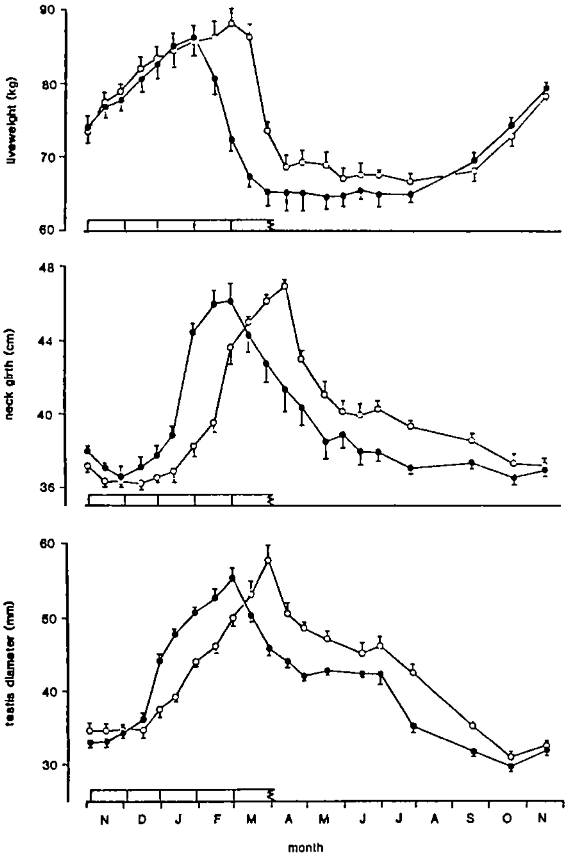

Figure 18: Seasonal profiles of mean (± s.e.m.) liveweight, neck girth and testic diameter of 4 melatonin-treated (•) and 4 control (o) fallow bucks. Horizontal bars indicate the period of melatonin implantation.

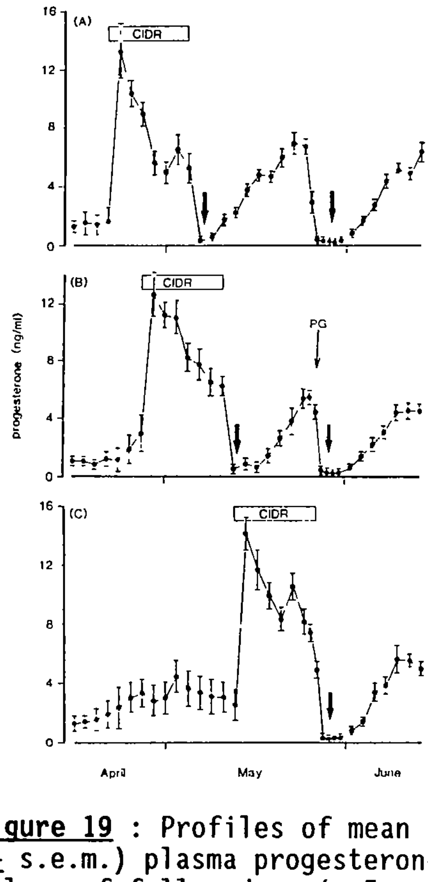

Figure 19 : Profiles of mean (± s.e.m.) plasma progesterone values of fallow does (n=5 per profile) during different treatment regimens designed to synchronise oestrus on 28 May. (a) initial 14-day CIDR followed by a 21-day oestrous cycle; (b) initial 14-day CIDR followed by an i.m. injection of prostaglandin analogue on Day 13 of the subsequent cycle; (c) 14-day CIDR treatment alone. Arrows indicate the mean times of oestrus.

relation to the establishment of genetic improvement schemes. AI allows a far wider use of the genetic material from superior bucks than would be remotely possible by natural mating. This is particularly important when considering such rare genotypes as Mesopotamian fallow deer. AI will also provide a cheaper and safer means of importing or exporting genetic material.

(ii) Oestrous synchronisation : Detection of natural oestrus in fallow does can be performed successfully by fitting bucks with ram mating harnesses (9,10,11,15,16,25). However, the procedures are time consuming and, for the purposes of AI, fixed-time insemination following artificial oestrous synchronisation is more practical than following natural detected oestrus (26). Oestrous synchronisation in fallow does is not difficult and employs similar methods used for other livestock species. The proportion of does exhibiting induced oestrus and the degree of synchrony of oestrus are dependent on the time of year the treatments are administered. Generally, results are most consistent after the onset of the natural breeding season in late April, although this could be modified by prior use of melatonin implants.

The three main methods of oestrous synchronisation in fallow deer are (a) 14-day CIDR (type S or G, 9% progesterone) insertion, (b) prostaglandin injection between Days 12 and 15 of the oestrous cycle, and (c) natural return oestrus 21 days following prior artificial oestrous synchronisation (27; Figure 19).

While a wide range of progestagen-releasing devices has yet to be tested for efficacy of oestrous synchronisation in fallow deer, a large number of studies have been conducted at Ruakura on the use of the intravaginal CIDR (11,12,13,15,16,18,25,26,27). The retention rate of CIDR devices is very high (98%-100%) and during the period of insertion they release sufficient progesterone to elevate blood concentrations to a level comparable to natural endogenous concentrations observed during the mid-oestrous cycle (Figure 19). Clearance of exogenous progesterone from the blood stream following CIDR removal is very rapid and occurs within two hours (Figure 20). This stimulates an increase in LH secretion from the pituitary gland, culminating in the onset of oestrus and the pre-ovulatory LH surge between 40-55 hours (11,12,13,15,27; Figure 20). Ovulation occurs about 24 hours after the onset of oestrus (18).

Induction of premature regression of the corpus luteum by injecting the powerful luteolytic hormone, prostaglandin F2α (or one of its analogues) results in a very tight synchrony of oestrus in fallow deer (27). However, the corpus luteum appears to be insensitive (refractory) to prostaglandin before Day 10 and it is necessary to administer the luteolysin either as a single injection between Days 12 and 15 of the oestrous cyle (necessitating prior oestrous synchronisation with CIDR devices) or as two injections 10-12 days apart. Recent studies on fallow does have shown that a single injection of cloprostenol (Estrumate; Imperial Chemical Industries PLC, England) on Day 13 or 14 of the oestrous cycle will result in rapid regression of the corpus luteum and clearance of endogenous blood progesterone over a 12 to 14 hour period (Figure 21). The onset of oestrus and the pre-ovulatory LH surge occur between 48 and 56 hours from prostaglandin injection (27). As with CIDR withdrawal, ovulation occurs about 24 hours after the onset of oestrus (18). Further

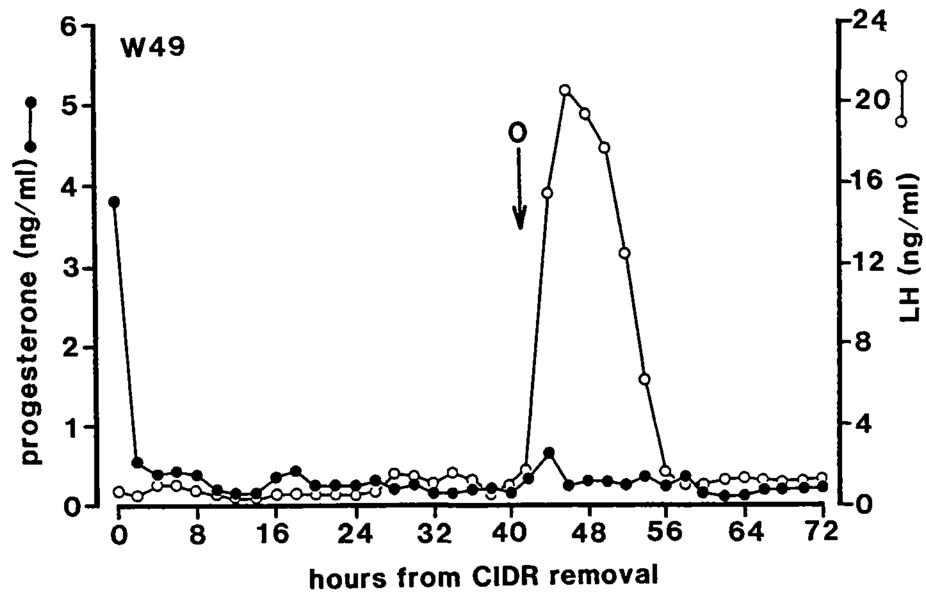

Figure 20 : Profiles of plasma progesterone and LH concentrations following CIDR removal from a fallow doe in early May. The arrow indicates the onset of oestrus.

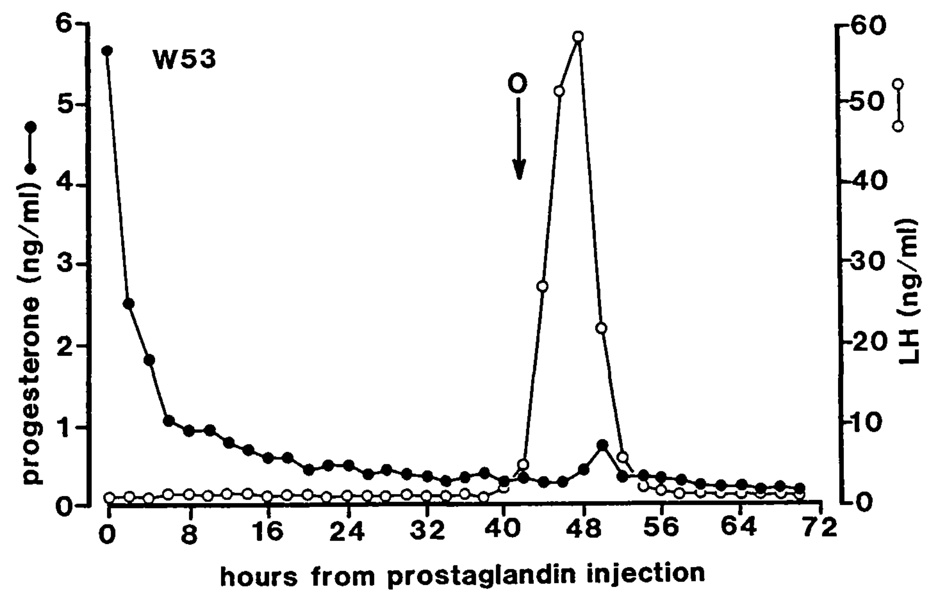

Figure 21 : Profiles of plasma progesterone and LH concentrations following administration of prostaglandin analogue on Day 13 of the oestrous cycle of a fallow doe in early May. The arrow indicates the onset of oestrus.

studies are required to establish a suitable twin-injection protocol for oestrous synchronisation in fallow does.

The first oestrous cycle of the breeding season in fallow deer is remarkably uniform in length (11). Therefore, it is possible to obtain a high degree of synchrony of a return oestrus following synchronisation of the first oestrus (27). This may provide a practical alternative to insemination at the first synchronised oestrus, as there is a suggestion that embryonic mortality rates are slightly higher following CIDR-induced oestrus than to natural oestrus (26). However, within the framework of the potential breeding season of fallow deer, there is little scope for utilizing the return oestrus following a synchronised oestrus without accepting the consequences of fawns born late in summer. The answer may be with manipulation of the onset of the breeding season by strategic use of melatonin implants.

There are two further important considerations with respect to oestrous synchronisation in fallow does. Firstly, non-parous (pubertal) does are generally unsuitable for AI programmes as they often fail to exhibit a suitable degree of oestrous synchrony following CIDR withdrawal/prostaglandin injection. Secondly, the use of PMSG at or near CIDR withdrawal/prostaglandin injection is contra-indicated due to an unacceptably high incidence of multiple ovulation (even at dose rates as low as 100 i.u. per doe). Multiple ovulation is associated with lower conception rates and higher embryonic mortality in fallow deer (16). Furthermore, PMSG undoubtedly alters the temporal relationship between synchronisation treatment, the onset of oestrus and ovulation, requiring different timing schedules for AI.

(iii) <u>Semen collection and processing</u> : As fallow bucks are not fertile throughout the year (Figure 14), collection of semen is highly seasonal. This, coupled with the fact that fallow deer tractability leaves much to be desired when faced with the problem of collecting ejaculates from bucks, explains why semen collection is the major factor limiting the widespread application of AI in the species.

To date, semen from fallow bucks has been collected primarily by electro-ejaculation. Bucks are generally heavily sedated (e.g. 5 mg ketamine hydrochloride and 2.5 mg xylazine hydrochloride per kg liveweight) and electrically stimulated per rectum. It has been our experience that semen collection by electro-ejaculation produces more consistent results in fallow deer than red deer, particularly between early May and late August. A good quality ejaculate may be 1.0-1.5 ml in volume and contain four billion live spermatozoa.

Collection of semen by natural ejaculation is presently being investigated. To date we have developed a prototype artificial vagina (AV) for insertion in teaser does. Preliminary results are extremely pleasing and we expect to be conducting commercial semen collection with the AV device in the 1990 breeding season. It is expected that the number and quality of ejaculates collected from each buck will increase dramatically. Furthermore, there will be less risk to the buck.

Cryopreservation of fallow semen is very simple and effective. In fact, fallow spermatozoa are remarkably robust and post-thaw recovery rates are often in excess of 85%. A brief description of the method of fallow semen processing used at Ruakura is as follows: ejaculate volume, spermatozoa concentration and spermatozoa motility are measured immediately following collection of each ejaculate. From this data, the dilution rate is calculated. Semen is diluted to a concentration of 200 million live cells/ml in 2.9% sodium citrate/20% egg yolk extender (13,28). The extended semen is then loaded into 0.25 ml straws (50 million cells per straw) and frozen in nitrogen vapour to -125°c in a programmable freezer (6°C per minute reduction) before transferral to liquid nitrogen.

(iv) Insemination techniques : There are two main forms of insemination for fallow deer; (a) intravaginal/intracervical (*per vaginum*), and (b) laparoscopic intrauterine.

Intravaginal insemination is the most easily performed but appears to require large quantities of viable spermatozoa (i.e. >100 million) for reasonable success rates. As this form of insemination is analogous to natural insemination, semen placement is timed to coincide with the onset of oestrus (26). For fixed-time insemination at induced oestrus, the preferable time of blanket insemination coincides with the mean interval from treatment to oestrus, being about 48 hours from CIDR withdrawal/prostaglandin injection (26,27).

Placement of semen within the cervix can be achieved with the aid of an appropriate speculum. Intracervical insemination is likely to be less wasteful of spermatozoa than intravaginal insemination, although present studies indicate that at least 40-50 million live sperm are required. Success rates of such inseminations performed 48 hours from CIDR withdrawal have ranged from 40% to >65% (1989 ultrasound data of commercially inseminated does).

Laparoscopic intrauterine insemination is presently the preferred method of AI for fallow deer. It allows for precise placement of relatively small quantities of semen close to the site of eventual fertilization. Early studies involving intrauterine placement of 85 million live spermatozoa 56-58 hours from CIDR withdrawl only produced 42% fawning rate (26). However, more recent inseminations performed with 30-40 million live spermatozoa at 65-70 hours from CIDR withdrawal have resulted in conception rates between 60 and 70% (1989 ultrasound data of commercially inseminated does). This suggests that the initial inseminations were performed too early relative to CIDR withdrawal and ovulation.

Laparoscopy is performed under general anaesthesia induced with an i.m. injection of 5.0 mg ketamine hydrochloride and 2.5 mg xylazine hydrochloride per kg liveweight. Anaesthetic reversal can be achieved with i.v. injection of 0.4 mg yohimbine hydrochloride per kg liveweight. While it is tempting to inspect the ovaries for evidence of impending ovulation during laparoscopy, manipulation of the reproductive tract close to the time of ovulation can disrupt the ovarian fimbria, leading to ova wastage (18).

(v) Ultrasound determination of pregnancy rate : Ultrasonographic pregnancy diagnosis is a useful tool for management of red deer (29,30) and fallow deer (31). Recent studies at Ruakura (32) have involved the sequential measurement of development of known-age fallow foetuses from first detection at 30 days through to 90 days, using ultrasonography (rectal probe). While these data are still being analysed, it is quite clear that foetal age can be estimated to within a 5 to 10-day period. As the length of the oestrous cycle is 21-22 days (11), differentiation between conception to AI and conception following return oestrus is a simple process. Our preference is to scan does 45 days from AI.

Embryo transfer

(i) Justification : It will be a number of years before multiple ovulation-embryo transfer (MOET) technology will be applied routinely on a commercial basis in the fallow deer industry. The main justification for the present research effort into MOET in fallow is based on the possibility of using a proportion of the base herd of European fallow deer (*Dama dama dama*) as recipients for embryos derived from the very rare Mesopotamian fallow deer (*D. d. mesopotamica*). Needless-to-say, future application may have a much broader base than this, especially once key genetic lines of fallow deer have been identified within genetic improvement programmes.

(ii) Superovulation/embryo recovery : Recent studies at Ruakura (33,34) have concentrated upon induction of superovulation, fertilization of multiple ova and embryo recovery.

Superovulation of 36 mature fallow does was attempted in May 1987 by gonadotrophin administration following intravaginal CIDR (type S; 12% progesterone) insertion for 14 days. Three treatments were applied;
(a) 1000 i.u. PMSG (Pregnecol; Heriot Agencies) administered as a single i.m. dose 48 hours before CIDR withdrawal; (b) 20 mg FSH (Folltropin, Vetripharm) administered i.m. in a decreasing dose regimen twice daily for 4 days, the last dose coinciding with CIDR withdrawal; (c) 750 i.u. PMSG + 14 mg FSH, with PMSG administered as for (a) and FSH as for (b). Immediately after CIDR withdrawal does were joined with crayon harnessed fertile bucks. Onset of oestrus was recorded by frequent observation over 4 days following CIDR withdrawal. Ova recovery (OR) was performed 6-8 days after CIDR withdrawal by uterine flush under surgical conditions. Numbers of corpora lutea (CL) and total stimulation points (TS; including cystic and luteinised follicles) were also recorded (Table 2).

Table 2 : Mean (± s.e.m.) ovarian response, ova recovery and fertilization rate following induced superovulation

Group	n	CL	TS	OR	% fertilized
a	12	9.2±2.5	16.8±2.0	3.7±1.1	70.0
b	12	6.3±2.9	7.0±3.1	1.1±0.5	84.6
c	12	11.2±3.3	20.4±3.0	1.9±0.5	52.2

For does that had superovulated, onset of oestrus generally occurred between 15-24 hours after CIDR withdrawl; a significant advancement of oestrus compared with the CIDR-synchronised cycle (11,12,26,27). Eight does receiving FSH alone failed to respond (TS $\leqslant$2), however, the remaining few does ranged in response from 4 to 30 CL, indicating an "all or none" response to this FSH preparation. For both groups receiving PMSG, only one doe failed to respond. Large numbers of cystic and luteinised follicles were observed for these groups, indicating overstimulation and a high sensitivity to PMSG. For superovulated animals, embryo recovery was poor, indicating that overstimulation had led to poor ovum quality, fertilization failure and/or disrupted ovum transport. A wide range of embryonic developmental stages, as well as unfertilised ova, were collected. This is also indicative of overstimulation and also suggests that natural mating may not be effective in ensuring high fertilisation rates.

The effect of various gonadotrophin regimens on ovarian ovulatory responses, endocrine changes and recovery/fertilisation rates was further examined for 50 fallow does in May 1989. Each doe received an intravaginal CIDR (type S; 9% progesterone) for 14 days and one of 5 doses of ovine FSH (0, 0.25, 0.5, 0.75 and 1.0 units Ovagen; Immuno-Chemical Products NZ Ltd). All animals received an i.m. injection of 200 i.u. PMSG (Folligon; Intervet) 12 days after CIDR insertion and eight i.m. doses of FSH administered at 12-hour intervals starting at PMSG administration. After CIDR removal, all does were joined with crayon-harnessed fertile bucks (10:1 ratio). They also received intravaginal inseminations (30 million motile spermatozoa/inseminate) on four occasions at 12-hour intervals starting 24 hours after CIDR withdrawl. Ova were recovered by uterine flush 7 days after CIDR withdrawal. The numbers of CL and unruptured follicles (>5 mm) were also recorded (Table 3).

Table 3 Mean(± s.e.m.) ovulatory response to 200 i.u. PMSG + variable doses of ovine FSH

FSH units	CL	TS
0.00	1.1±0.4	2.5±0.7
0.25	7.2±1.7	10.0±1.9
0.50	9.5±2.5	14.9±2.7
0.75	8.6±2.4	17.3±1.9
1.00	7.4±2.1	12.9±2.5

There was a curvilinear pattern of ovarian response to increasing dose of ovine FSH. The highest numbers of CL were observed following treatment with 0.5 units FSH. Ova recovery rates were low (30.6±5.1%) with no differences between treatment groups. In contrast to the 1987 data, none of the ova recovered had cleaved (i.e. no fertilization). This latter result was particularly disappointing as considerable effort was made to ensure copious quantities of spermatozoa were present in the vagina. Present indications are that sperm transport, and hence fertilization, may be adversely affected by high follicular secretion of oestradiol. It is apparent that studies are needed to define the optimal site and time of semen deposition within the reproductive tract to produce satisfactory fertilisation rates. Future studies will also investigate ovulation synchrony (i.e. strategic GnRH administration) and PMSG neutralisation treatment (i.e. passive immunisation).

(iii) Embryo cryopreservation/transfer : We have yet to investigate embryo freezing and transfer in fallow deer. It is unlikely, however, that these steps will be limiting in the MOET programmes for this species.

References

1. Asher, G.W. (1985) Reproduction of farmed fallow deer (*Dama dama*). *Proceedings of a deer course for veterinarians : Course No. 2.* Deer Branch NZVA; Ashburton : 107-125.
2. Thibault, C., Courot, M., Martinet, L., Mauleon, P., du Mesnil du Buisson, F., Ortavant, R., Pelletier, J. and Signoret, J.P. (1966) Regulation of breeding season and estrous cycle by light and external stimuli in some mammals. *J. Anim. Sci. 25, Suppl.*: 119-142.
3. Chaplin, R.E. and White, R.W. (1972) The influence of age and season on the activity of the testes and epididymides of the fallow deer, *Dama dama*. *J. Reprod. Fert.* 30: 361-369.
4. Chapman, D.I. and Chapman, N. (1975) *Fallow Deer : their History, Distribution and Biology*. Terence Dalton Ltd, Lavenham, U.K.
5. Eaton, D. (1980) Factors affecting the behaviour and reproductive cycle of fallow deer. *Ratel* 7: 5-8.
6. 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.
7. Goss, R.J. (1983) *Deer Antlers : Regeneration, Function and Evolution*. Academic Press, New York.
8. 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.
9. 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. Fert.* 79: 353-362.
10. Asher, G.W., Barrell, G.K., Adam, J.L. and Staples, L.D. (1988) Effects of subcutaneous melatonin implants on reproductive seasonality of farmed fallow deer *(Dama dama)*. *J. Reprod. Fert.* 84: 679-691.
11. Asher, G.W. (1985) Oestrous cycle and breeding season of farmed fallow deer, *Dama dama*. *J. Reprod. Fert.* 75: 521-529.
12. Asher, G.W., Barrell, G.K. and Peterson, A.J. (1986) Hormonal changes around oestrus of farmed fallow deer, *Dama dama*. *J. Reprod. Fert.* 78: 487-496.
13. Asher, G.W. (1988) Reproduction. In *Progressive Fallow Farming* : Proceedings of a course on fallow deer farming; Ruakura Agricultural Centre; February 1988. Eds P.L. Allen and G.W. Asher : 1-38.
14. McComb, K. (1987) Roaring by red deer stags advances the date of oestrus in hinds. *Nature* 330: 648-649.
15. Asher, G.W. (1986) Studies on the reproduction of farmed fallow deer *(Dama dama)*. PhD. thesis, Lincoln College, University of Canterbury, Christchurch, N.Z.

16. 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. Fert.* 81: 113-118.
17. 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, N.Z. : 247.
18. 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 preovulatory LH surge and ovulation in farmed fallow deer, *Dama dama* (unpublished manuscript).
19. Jabbour, H.N., Asher, G.W., Smith, J.F., Morrow, C.J. and Langridge, M. (1990) Induction of oestrus in ovariectomised fallow does *(Dama dama)* with progesterone and oestradiol benzoate (unpublished manuscript).
20. Asher, G.W., Peterson, A.J. and Watkins, W.B. (1988) Hormonal changes during luteal regression in farmed fallow deer, *Dama dama*. *J. Reprod. Fert.* 84: 379-386.
21. Asher, G.W. and Adam, J.L. (1985) Reproduction of farmed red and fallow deer in northern New Zealand. In *Biology of Deer Production*. Eds P.F. Fennessy and K.R. Drew. The Royal Society of New Zealand, Bulletin No. 22 : 217-224.
22. 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. Fert.* 85: 667-675.
23. 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. Fert.* 85: 657-665.
24. Field, R.A., Young, O.A., Asher, G.W. and Foote, D.M. (1985) Characteristics of male fallow deer muscle at a time of sex-related muscle growth. *Growth* 49: 190-201.
25. 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. Fert.* 78: 693-697.
26. 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(3): 487-492.
27. Asher, G.W. and Thompson, J.G.E. (1989) Plasma progesterone and LH concentrations during oestrous synchronisation in female fallow *deer (Dama dama)*. *Anim. Reprod. Sci.* 19: 143-153.
28. Krzywinski, A. and Jaczewski, Z. (1978) Observations on the artificial breeding of red deer. *Symposia of the Zoological Society of London* 43: 271-287.
29. 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.
30. Revol, B. and Wilson, P. (1989) Ultrasonographic pregnancy diagnosis in red deer. *Proceedings of a deer course for veterinarians : Course No. 6* Deer Branch, NZVA; Queenstown : 36-53.
31. 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(8): 257-258.

32. Smith, J.F., Asher, G.W., Jabbour, H.N., Morrow, C.J., Langridge, M. and Parr, J. (1990) Estimation of foetal age in fallow deer *(Dama dama)* by rectal ultrasonography (unpublished manuscript).
33. Thompson, J.G.E. and Asher, G.W. (1988) Superovulation and ova recovery in farmed fallow deer *(Dama dama)*. *Proceedings of the Australian Society of Reproductive Biology* 20: 4.
34. 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 deer *(Dama dama)*. (unpublished manuscript)