Artificial breeding technology for fallow deer

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G.W. Asher, C.J. Morrow and F.A. Veldhuizen
AgResearch,
Ruakura Agricultural Centre,
Hamilton,
New Zealand

Summary

Rates of genetic improvement of farmed fallow deer can be increased dramatically by the adoption of assisted reproductive technologies; including artificial insemination (AI) and embryo transfer (ET).

AI involves oestrous synchronisation; semen collection, processing and storage; and insemination. Oestrous synchronisation is generally performed by intravaginal insertion of progesterone-releasing CIDR (type G) devices for periods of 13-15 days at the onset of the breeding season. The use of PMSG should be avoided in this species.

Laparoscopic intra-uterine insemination is the preferred method of AI for fallow deer, with semen placement performed 65-72 hours after CIDR device removal. Semen is collected by electro-ejaculation while bucks are under general anaesthesia, and is diluted to 25-50 million spermatozoa per 0.25 ml straw for freezing. Conception rates to laparoscopic AI generally range from 58-75% for frozen-thawed semen and 70-80% for fresh semen. Occasional failures (i.e. < 50%) often relate to failure of oestrous synchronisation due to inappropriate seasonal timing of treatment and high levels of animal stress.

Intracervical (i.e. transvaginal) AI with fresh semen at 60-65 hours post-CIDR removal gives variable results (40-80%). The use of frozen-thawed semen generally gives very poor results (40%) with this technique unless large quantities of spermatozoa (i.e. >100 million) are inseminated.

Embryo transfer (ET) technology is being developed for farmed fallow deer. Results to date have generally been poor due to low recovery rates of fertilized ova from superovulated donors. This seems to relate to impaired passage of spermatozoa through the cervix, and present studies are investigating laparoscopic intra-uterine insemination of donors with fresh semen. The techniques of ET have particular importance to the propagation of Mesopotamian blood lines.

Out-of-season breeding, using melatonin implants ("Regulin") to advance the breeding season, has the potential to increase performance of farmed fallow deer by better aligning lactation with spring pasture production. While significant advancement of the fawning season can be achieved (i.e.>40 days), the costs presently exceed the benefits by a wide margin

Introduction

Artificial breeding techologies have two fundamental purposes; (1) to increase rates of genetic improvement (e.g. artificial insemination, embryo transfer) and (2) to improve environmental performance (e.g. out-of-season breeding). The farming of fallow deer (*Dama dama*) is a relatively new livestock industry, with modern concepts of management arising only in the last 10-15 years. It is not

surprising, therefore, that adoption of assisted reproduction techniques is in its infancy.

Indeed, artificial insemination (AI) has been applied commercially only within the last 5-6 years, with application being largely based out of New Zealand (e.g. of an estimated 4000 fallow deer inseminated within the last 6 years, at least 90% received semen originating from NZ sources). Embryo transfer (ET) techniques have been applied recently to fallow deer but have been of limited success due to the paucity of research in this area. However, these techniques have considerable potential to alter rapidly the genetic constitution of farmed fallow deer populations around the world, as cryopreserved gametes can be transported internationally with comparative ease and safety. In this paper we summarise our current understanding of artificial reproduction techniques that have been developed for farmed fallow deer within the last decade

Artificial insemination

Application of artificial insemination technology within the fallow deer farming industry has grown rapidly over the last few years. Furthermore, the future potential of AI is enormous, particularly in relation to the establishment of genetic improvement schemes (eg. group breeding schemes, sire reference programmes). 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 also provides a cheaper means of importing or exporting genetic material, as frozen semen is considerably less expensive to transport than live animals.

There are three major components to AI; (a) oestrous synchronisation, (b) semen collection, processing and storage and (c) insemination. Each component will be discussed separately.

Oestrous synchronisation: 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 dependant on the time of year the treatments are administered. Generally, results are most consistent after the onset of the natural breeding season in autumn, although this could be modified by using melatonin implants to advance the onset of breeding activity.

A common method of oestrous synchronisation in domestic livestock is the use of progestagens (progesterone or its synthetic analogues) to override natural oestrus/ovulation by simulating or prolonging an oestrous cycle. Progestagen-releasing devices are inserted intravaginally or subcutaneously for periods of 12-16 days. The sudden drop in blood progestagen levels that occurs upon removal of the device stimulates oestrus/ovulation. Therefore, synchrony is achieved by synchronous withdrawal of the devices.

While a wide range of progestagen-releasing devices has yet to be tested for efficacy of oestrous synchronisation in fallow deer, a number of studies have been conducted on the use of the intravaginal CIDR device ("Controlled Internal Drug Release"; NZ Dairy Board, Hamilton, NZ). The CIDR - type G device contains 0.3 g (9%) progesterone and is inserted intravaginally for 14 days in fallow does. 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 1).

Clearance of exogenous progesterone from the blood stream following CIDR device removal is very rapid and occurs within the first 2 hours (Figure 2). This stimulates an increase in LH secretion from the pituitary gland, which culminates in the onset of the massive "pre-ovulatory" LH surge (which eventually leads to ovulation) between 40-55 hours (Figure 2).

The use of pregnant mare serum gonadotrophin (PMSG) at or near CIDR device withdrawal is contra-indicated for oestrous synchronisation in fallow deer. Even low doses of PMSG appear to reduce fertility and increase embryonic mortality in this species (Asher & Smith, 1987).

Another form of oestrous synchronisation commonly used in cattle involves promoting the premature regression of the corpus luteum by injecting the powerful luteolytic hormone, prostaglandin F2a (or one of its analogues). This hormone is normally produced by the uterus of the non-pregnant female on days 19 to 21 of the oestrous cycle and terminates the progesterone secretory activity of the corpus luteum. Exogenous prostaglandin will also terminate the secretory life of the corpus luteum if injected from about day 10 of the cycle (Figure 1). However, the corpus luteum appears to be insensitive (refractory) to prostaglandin before day 10. Therefore, the common treatment regimen for cattle is to administer two injections, 10-12 days apart. If a young corpus luteum is refractory at the first

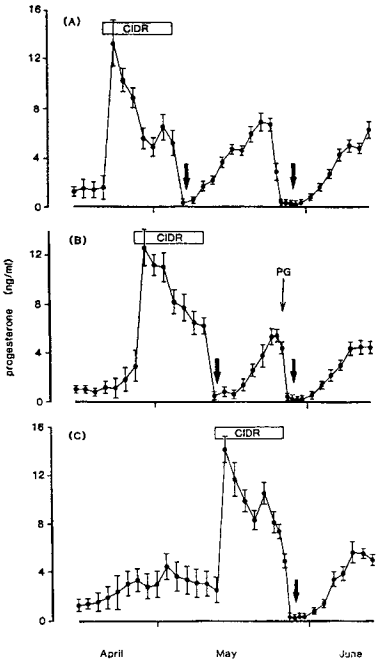

Figure 1: Mean (± s.e.m.) plasma progesterone concentrations of fallow does (n=5 per group) during three treatment regimens, each designed to synchronise oestrus on 28 May. (A) initial 14-day CIDR device treatment followed by a natural 21-day oestrous cycle, (B) initial 14-day CIDR device treatment followed by an injection of Estrumate (prostaglandin analogue) on day 14 of the subsequent cycle and (C) 14-day CIDR device treatment alone. Arrows indicate oestrus. (Asher & Thompson, 1989)

injection, it will certainly succumb to the second injection.

It must be stressed, however, that prostaglandin synchronisation regimes can only be effective during the breeding season as there is a requirement for the presence of active luteal tissue.

Recent studies on fallow does have shown that a single injection of prostaglandin analogue (2 ml 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 high endogenous blood progesterone levels over a 12-hour period (ie. a more gradual clearance than for CIDR device removal), as shown in Figure 3 The onset of oestrus and the "pre-

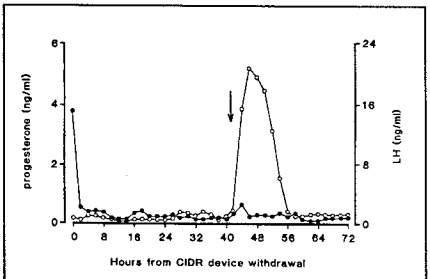

Figure 2.: Profiles of plasma progesterone (•) and LH (o) concentrations following CIDR device removal from a fallow doe. The arrow indicates the onset of oestrus (Asher & Thompson, 1989).

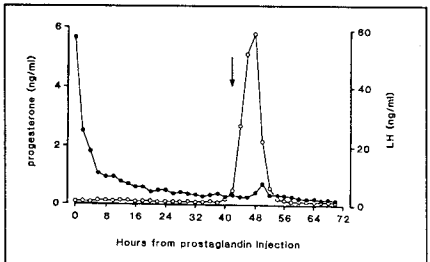

Figure 3: Profiles of plasma progesterone (•) and LH (o) concentrations following administration of prostaglandin analogue on day 13 of the oestrous cycle of a fallow doe. The arrow indicates the onset of oestrus (Asher & Thompson, 1989).

ovulatory" LH surge occur between 40 and 56 hours from prostaglandin injection.

Semen collection, processing and storage: As fallow bucks are not fertile throughout the year, 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 by electro-ejaculation, a process involving passing a mild electric current through the rectum to stimulate contraction of the seminal vesicles. The bucks are heavily sedated with an i.m. injection of an aqueous mixture of 5 mg ketamine hydrochloride (Ketalar; Parke Davis Pty Ltd, USA) and 2.5 mg xylazine hydrochloride (Rompun; Bayer Leverkusen, Germany) per kg liveweight. They are then electro-ejaculated by using a rectal probe (2.5 cm in diam-

eter and 25 cm in length, with 3 lateral electrodes) powered by a mains-charged battery. The probe is liberally coated with lubricating gel and inserted about 15 cm into the rectum. A single ejaculate is collected into pre-warmed glass semen vials after 2 or 3 electric stimulations (~5 seconds duration each) about 5 seconds apart. The voltage seldom exceeds 5 V. The ejaculate is immediately transferred to a water bath at 37 °C (98.6°F).

Studies on ejaculates collected at 2-monthly intervals from 2-3 year old fallow bucks clearly showed marked seasonal variation in the occurrence and concentration of viable spermatozoa. Ejaculates were completely devoid of spermatozoa in summer. However, moderate concentrations were present in early autumn and high concentrations (up to 4.5 billion/ml in some ejaculates) occurred through to the end of the breeding season in spring (Asher *et al.*, 1987).

The effective season for semen collection from fallow bucks in New Zealand extends from late March through to September . However, it may be possible to advance the onset of this season, or even extend the season, by using melatonin implants.

Collection of semen by natural ejaculation is presently being investigated. This involves using ovariectomised does induced into oestrus with pharmacological doses of oestradiol benzoate. The does are fitted with internally worn artificial vaginas (AV's) for collection of ejaculates after natural mating. Should an effective procedural protocol be established, the number and quality of ejaculates collected from a buck will probably increase dramatically. Furthermore, there will be less risk to the buck.

While semen collection from fallow bucks presents some problems, cryopreservation of fallow semen is very simple and effective. In fact, fallow spermatozoa are remarkably robust and post-thaw recovery rates (percentage of spermatozoa remaining alive following thawing) are often in excess of 85%, even after 8-9 years of storage.

The method of fallow semen processing used at the Ruakura Agricultural Centre 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 then diluted to a concentration of 100 million live spermatozoa/ml in 2.9% sodium citrate-20% egg yolk extender; made up as follows:

72 ml of 2.9% sodium citrate solution

1.25 g of fructose

20 ml of egg yolk

8 ml of glycerol

0.1 g of streptomycin

The extended semen is then loaded into 0.25 ml straws (ie. 25 million spermatozoa/straw). The semen is then frozen in nitrogen vapour to -125°C in a programmable freezer (6°C per minute reduction) and later transferred to liquid nitrogen until required for insemination.

With the present techniques of semen collection by electro-ejaculation it has been possible to obtain up to four hundred 0.25 ml straws of semen per ejaculate. Most ejaculates average about 80 straws. For some bucks, we have collected over 2000 straws per season. The use of AV technology may further improve on this figure.

Insemination: Preliminary studies on fallow deer AI were simultaneously conducted on the Ruakura Agricultural Centre (NZ) and Camden Deer Unit (Australia) in 1986. This pooled together several years of work on oestrous synchronisation and semen collection. The outcome was very pleasing and suggested that commercial application of AI in fallow deer would not be far away (Asher *et al.*, 1988a; Mulley *et al.*, 1988).

Considerable progress has been made since these early studies, and the techniques developed are now used routinely in New Zealand, Australia, USA and Canada. The use of cryopreserved semen necessitates the application of laparoscopic intra-uterine insemination techniques. However, the recent use of fresh semen in New Zealand has allowed the development of a low cost intracervical insemination procedure.

Laparoscopic intra-uterine AI: This is presently the preferred method of AI in fallow deer (Asher *et al.*, 1990a; Mylrea *et al.*, 1991) as it allows precise placement of relatively small quantities of semen close to the site of fertilisation. Early studies involving intra-uterine deposition of 85×10^6 motile frozen-thawed spermatozoa 56-58 hours after CIDR device withdrawal resulted in a rather disappointing 42% fawning rate (Asher *et al.*, 1988a). It was postulated that the inseminations were conducted too early relative to ovulation (Asher *et al.*, 1990b) and more recent intra-uterine inseminations performed with 20-40 $\times 10^6$ motile frozen-thawed spermatozoa 65-70 hours after CIDR device with-

drawal resulted in an overall 68% conception rate (Asher *et al.*, 1990a); this being a considerable improvement over the earlier studies.

The most recent on-farm studies on laparoscopic intra-uterine insemination of 547 fallow deer, conducted during the 1990 breeding season in NZ (ie. April/May) investigated variables such as insemination timing (60 vs 65 vs 70 hours after CIDR device removal), type of CIDR device (type-S vs type G), CIDR device vs prostaglandin synchronisation, presence or absence of vasectomised bucks during synchronisation, and numbers of spermatozoa per inseminate (50, 25 or 10 x 10^6 spermatozoa per inseminate). The control regime was similar to that established by Asher *et al.* (1990a) and, on the basis of ultrasonography, resulted in an overall 67.5% conception rate. The results (Table1) indicate a degree of flexibility in timing of insemination relative to CIDR device withdrawal, CIDR device synchronisation is more effective than prostaglandin synchronisation early in the breeding season, buck presence is not essential during synchronisation treatment, there is little difference in efficacy of the two types of CIDR device (the type-S device is now obsolete), and numbers of motile frozen-thawed spermatozoa required for respectable conception rates (60-70%) are lower than previously used commercially (Asher *et al.*, 1992).

Recent experimental use of fresh semen at doses of between 20 x 10^6 and 2 x 10^6 motile spermatozoa per intra-uterine inseminate has resulted in conception rates of 70-80%, indicating considerable potential in AI programmes (Asher *et al.*, 1992). Further studies are required to extend the shelf-life of fresh semen from <48 hours to >7 days.

For laparoscopic intra-uterine inseminations, fallow deer does are individually sedated with an intravenous injection of ketamine hydrochloride (2.0 mg/kg) and xylazine hydrochloride (1.0 mg/kg). Once recumbent, the does are placed on their back in laparoscopy trolleys, tilted head-down at 30°, and have their posterior abdomen shaved and swabbed (70% ethanol and 1% sodium iodide solution). The abdomen is inflated with carbon dioxide gas, and two trocars, one either side of the midline, are inserted about 10 cm (4 inches) anterior to the mammary gland. The laparoscope, a rigid fibre-optic light scope, and the insemination pipette containing semen are inserted through the trocars, and semen is injected into the lumen of both uterine horns located with the laparoscope. Following insemination and removal of trocars, long-acting antibiotics are administered by intramuscular injection. Sedation is reversed with an intravenous injection of yohimbine hydrochloride (0.4 mg/kg) and the does are returned to pasture. The total procedure, from administration of sedatives to complete reversal, lasts 7-12 minutes per doe.

This form of artificial insemination obviously requires skilled inseminators. Furthermore, the minimum requirements for handling facilities include mains supply electric power, reticulated water supplies, a crush/cradle for animal restraint and a suitable floor surface (preferably concrete) for the

Table 1: Treatment protocols and conception rates for trials involving laparoscopic intra-uterine AI with frozen-thawed semen (Asher *et al.*, 1992).

	Farm					
Treatment	A	B	C	D	F	Standardised conception rate (± se)
Control.*	66%	68%	61%	71%	70%	67 ± 3%
60 hours	67%	-	-	-	-	67 ± 9%
70 hours	73%	-	-	-	-	73 ± 8%
Buck absent	-	62%	-	-	-	61 ± 9%
25x10^6 sperm	-	-	76%	-	-	80 ± 8%
10x10^6 sperm	-	-	69%	-	-	74 ± 9%
Type-S device	-	-	-	66%	-	62 ± 10%
Prostaglandin	-	-	-	-	53%	49 ± 10%

* The control treatment regimen involved inserting CIDR-type G devices intravaginally for 14 days, intra-uterine insemination with 50x10^6 frozen-thawed spermatozoa 65 hours after CIDR device withdrawal, and the continuous presence of vasectomised bucks.

AI team. All other gear relevant to the AI is usually supplied by the insemination team.

Despite the supposed impenetrability of the cervix of fallow deer, transcervical intra-uterine insemination (ie. *per vaginum*) has been successfully attempted on anaesthetised does by exteriorising the *os cervix* (Asher *et al.*, 1990a). Conception rates following this technique have been in excess of 70% using fresh or frozen-thawed semen. However, transcervical techniques require prolonged periods of anaesthesia (20-25 minutes) but do demonstrate an alternative to the more invasive technique of laparoscopic insemination and warrant further study.

Intracervical AI: Attempts at intracervical insemination with inseminates containing 20-40 x 10^6 frozen-thawed spermatozoa have yielded highly variable results amongst commercial inseminators, ranging from 38% to 80% conception rate. Recent studies indicate that the success rate to intracervical insemination may be dependent on the method of oestrous synchronisation, the timing of insemination and the number/type of spermatozoa per inseminate. Intracervical deposition of 140 x 10^6 motile frozen-thawed spermatozoa 12 hours after the median onset of oestrus (ie. 60 hours after CIDR device withdrawal) resulted in conception rates ranging from 84.5% to 40.7% depending on the form of synchronisation (Table 2). However, such large numbers of spermatozoa are unacceptable commercially.

In the latest on-farm study conducted in NZ in 1991, intracervical insemination of does at 60 hours with low doses (50, 25 or 12x10^6 spermatozoa) of fresh semen (ie. within 10 hours of collection) resulted in conception rates of between 73 and 80%. This is a very exciting result, as it combines the efficiency of low spermatozoa dose with a very low cost technique.

Intracervical AI of fallow does is performed in the crush/cradle without resorting to anaesthetic drugs. The cervix is located with the aid of a speculum inserted into the vagina. The tip of the insemination pipette is guided into the cervical opening and the semen deposited 1-2 cm inside the cervix. The total procedure can be completed in under one minute per doe.

Pregnancy diagnosis following AI: A number of methods of early pregnancy diagnoses have been used to obtain an early indication of the outcome of AI programmes in fallow deer. Early studies 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 (Asher *et al.*, 1988a). More recently studies on AI of fallow deer have utilised ultrasonography to visualise foetal development between days 40 and 50 post-insemination (Asher *et al.*, 1990a; Asher *et al.*, 1992). Foetal age estimation, given that there is usually a minimum disparity of 10 days between conceptions to AI and those to return oestrus at fertile buck introduction, are based on previous ultrasonographic studies in the species (Mulley *et al.*, 1987). Recent studies show a high correlation between ultrasound results and birth data, indicating only low levels of embryonic mortality following AI (Asher *et al.* 1992).

Table 2: Conception rates of fallow deer does following intracervical insemination with 140x10^6 motile frozen spermatozoa 12 hours after the median time to onset of oestrus (Jabbour *et al.*, 1991).

Synchronisation treatment	No. of does inseminated	No. of does pregnant (Day 42)	Conception rate (%)
CIDR device	26	22	84.5
CIDR device + 50 iu PMSG	26	16	61.5
Prostaglandin	27	11	40.7
Prostaglandin + 50 i.u. PMSG	26	17	65.4
TOTAL	105	66	62.9

Embryo transfer

It will be a number of years before embryo transfer (ET) will be employed routinely within the fallow deer farming industry, as only a limited amount of research has been conducted on establishing successful protocols. However, it is probable that fallow does of high genetic merit will eventually be identified within some herds. ET will then be a valuable tool to maximise the number of progeny obtained from these does (or "donors").

The various components of ET technology include (1) induction of superovulation, (2) ensuring successful fertilisation, (3) embryo recovery, (4) embryo cryopreservation, (5) recipient synchronisation and (6) embryo transfer into the recipient doe.

Superovulation: Initial studies at the Ruakura Agricultural Centre in 1987 investigated superovulation regimes and surgical embryo recovery. Donor responses to the various superovulation regimes (CIDR + PMSG; CIDR + FSH; CIDR + PMSG + FSH) were promising (up to 30 ovulations/doe) but fertilisation rates were disappointingly low (~10%). Subsequent studies showed that the most consistent superovulatory response (~ 9 ovulations/donor) was achieved following administration of 200 i.u.. PMSG (Folligon; Intervet, Australia) and 0.5 units ovine FSH (Ovagen; ICP, Auckland, NZ) towards the later stages of treatment with intravaginal CIDR devices (Table 3).

Table 3: Mean (±s.e.m.) ovulatory response to 200 i.u. PMSG and variable does of ovine FSH (Jabbour & Asher 1992).

FSH units	Corpora lutea	Total stimulation points
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

However, fertilization rates were still disappointingly low following natural mating and/or intravaginal AI. These studies have indicated that spermatozoa passage through the cervix of superovulated does was severely impaired, possibly due to high oestradiol secretion from multiple ovarian follicles.

Furthermore, ovulations appeared to be non-synchronous, leading to variable ova/embryo ages at the time of collection (Day 6 from inseminations). Further studies are required to improve ova fertilization rates (possible by using laparoscopic intrauterine insemination) and to promote ovulation synchrony within donors.

Embryo cryopreservation: There are no published accounts describing embryo freezing in fallow deer. However, red deer embryos have been frozen routinely using standard techniques for mammalian embryos. For this species, conception rates following transfer of frozen-thawed embryos are similar to those obtained for fresh embryos. There is no reason to believe that fallow deer embryos would act differently to cryopreservation.

Embryo transfer: Media reports of a commercial operation in NZ have indicated recent sucess in transferring fresh embryos from part Mesopotamian donors into European recipients. However details of success rates have not been forthcoming. Given the high fertility of does undergoing the standard oestrous synchronisation procedure, it is reasonable to assume that transfer of single embryos of monovulated does is likely to acheive acceptable pregnancy rates.

Future of ET: ET is a promising tool for fallow deer farmers and scientists concerned with propagation of endangered cervid species. Studies need to concentrate on increasing the success of embryo recovery from superovulated donors. Once this is achieved, however, the techniques are likely to be used to increase the numbers of progency from purebred genotypes. In particular, it would be gratifying to see the application of ET technology to increasing the number of pure Mesopotamian fallow deer by instigation of an inter-subspecies surrogancy programme. This would establish a model for other endangered cervid species/subspecies.

Out-of-season breeding

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 Australasian 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 to late summer.

Early attempts to advance oestrus and ovulation in fallow does using intravaginal CIDR devices in conjunction with an exogenous gonadotrophin (eg. PMSG) or gonadotrophin-releasing hormone (ie. GnRH) were successful in inducing oestrus/ovulation up to 6 weeks earlier than normal (see Asher & Macmillan, 1986; Asher & Smith, 1987). However, conception rates were generally low and there was the additional problem of some does conceiving twins following PMSG treatment. It is probable that buck fertility was suboptimal at the time of induced oestrus/ovulation.

More recent studies have centred on the administration of the pineal hormone, melatonin, as its mode of action is common to both sexes. Blood melatonin levels are elevated naturally only during darkness. During summer the total duration of elevation within each 24 hour period is short. However, this elevation increases as days become shorter (nights longer). 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 with exogenous melatonin; thus inducing a physiological "autumn" or "short-day" state.

The first attempts at such supplementation involved feeding bucks with melatonin-laced pellets at 3.00 pm every day during part of summer (Asher *et al.*,1987). This was designed to elevate blood melatonin levels several hours before they would be elevated 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. For example, treated bucks exhibited increased neck muscle development during and immediately after the treatment period. More encouraging, however, was the observation that treated bucks exhibited an earlier attainment of fertility, as determined by the presence of viable spermatozoa in ejaculates in mid summer.

More recently, subcutaneous melatonin implants have become in vogue. These work by constantly releasing melatonin into the blood stream, resulting in a perpetual elevation of blood melatonin concentrations throughout the day. The effect is actually additive at night, with exogenous levels superimposed on natural endogenous levels (Figure 4). Swamping the system with exogenous melatonin is registered by the deer as a short-day state.

Recent studies involving administration of small subcutaneous melatonin implants (Schering Pty Ltd., Alexandria, NSW, Australia) to fallow does and bucks in summer produced very spectacular results (Asher *et al.,* 1988). A total of 18 does, comprising 6 pubertal, 6 non-pregnant adult and 6 pregnant does, each received single Regulin implants on four occasions at 30 day intervals from 10 November 1986. A further 18 non-treated does (same distribution of age/pregnancy status) served as herd-mate controls. Two adult bucks were also implanted under the same regimen and 4 further bucks served as controls.

The rut of the treated deer occurred about 7-8 weeks earlier than for the controls. It is significant that, not only did the treated does exhibit oestrus early (Figure 5), the treated sire bucks exhibited a marked advancement in reproductive development and expressed full "rutting" behaviour in response to the early oestrous activity. Furthermore, the vast majority of treated does (94%) conceived to their first oestrus; the remaining 6% conceiving to a return oestrus 21 days later.

The melatonin-treated does fawned 7-8 weeks before the control does. Three of the early-born fawns died during severe storms, highlighting the desirability of providing adequate shelter at this time of year.

While all three groups of does (ie. pubertal, non-pregnant adult and pregnant does) appear to have responded similarly to melatonin treatment, 4 of the 6 does that were treated while still pregnant (late gestation) failed to lactate following the 1986 fawning and subsequently lost their 1986-born fawns. It is probable that the initiation of lactation was affected by melatonin treatment; suggesting a contra-indication to the use of melatonin in pregnant does.

Melatonin implants are presently commercially available in NZ and Australia. In NZ, however, their application to female deer is restricted to pubertal animals due to the potential conflict with lactogenesis. Public discussions over the use of melatonin implants to increase the productivity of red deer have tended to focus on two issues: firstly on cost-benefit analysis due to the high cost of treatment relative to the value of extra product obtained. This argument presently applies to fallow deer, as cost of treatment seems to outweigh the value of increased performance. Secondly, while melatonin is a naturally occuring hormone in all mammals, the use of exogenous hormones to increase carcass productivity (whether used directly or indirectly on the meat animal) does not find favour in some markets,

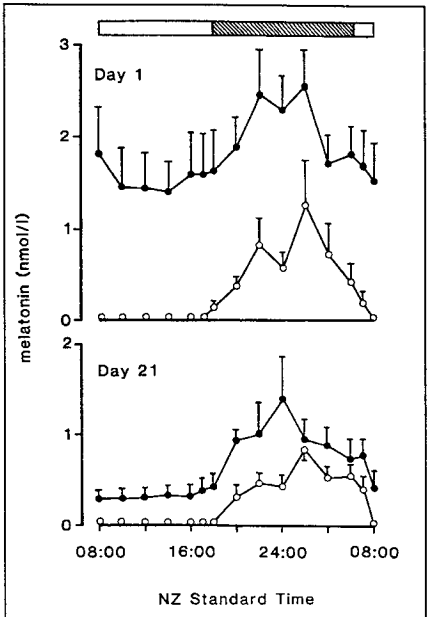

Figure 4: Circadian profiles of mean (± s.e.m.) plasma melatonin concentrations of 4 fallow does each receiving a single s.c. melatonin implant (Regulin) in the ear (●—●) and 4 control does (o—o). The profiles are from Day 1 and Day 21 of implant insertion. The shaded horizontal bar represents darkness (Asher *et al.*, 1988b).

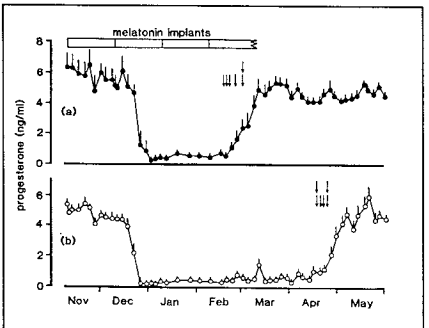

Figure 5: Profiles of mean (± s.e.m.) plasma progesterone levels of (a) 6 melatonin-treated ●—● and (b) 6 control (o—o) fallow does from fawning (Dec) through the onset of breeding activity. Arrows indicate the occurrence of first oestrus (Asher *et al.*, 1988b)

particularly in Europe. Market forces may well mitigate against future hormonal manipulations in meat-producing animals.

Induction of twinning

Fallow does normally give birth to a singleton fawn following conception to a single ovulation. The incidence of natural twinning in NZ herds is probably less than 1:500 births. Overseas reports of high twinning rates (~30%) are largely unsubstantiated and may represent spurious observations of several fawns following single does.

Induction of twinning in fallow deer is possible by increasing the ovulation rates of each animal (eg. CIDR device + PMSG treatment; see Asher & Smith, 1987). However, it must be stressed that of all induced twinnings recorded on the Ruakura Agricultural Centre (n=11), none has resulted in a single live fawn at weaning. Typically, the fawns are born non-viable due to excessively low birth weights (<2.0 kg each). It would appear that the total birth weight of twins (~4.0 kg) roughly equals the normal birth weight of a singleton. Thus, it is probable that embryonic competition, due to the fallow deer placentation system, exacts a high price on foetal growth.

On the basis of work done to date, it would appear that induction of twinning in fallow deer is counter-productive.

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Catherine Morrow, BSc MSc (Hons)
Catherine has been a member of Ruakura's Deer Reproduction and Physiology Programme since 1988, following completion of a BSc degree at Waikato University. She continued her university studies while at Ruakura and received her MSc (Hons) degree with first class honours in 1992. Her thesis was titled 'Studies on oestrous synchronisation of farmed fallow deer (*Dama dama*)'. Catherine has a strong interest in the application of assisted reproductive technologies in conservation biology, with particular emphasis on propagation of endangered cervids and bovids.

Felicia Veldhuizen, BAgSc
Felicia joined the Ruakura deer programme in 1989 following completion of a BAgSc degree at Lincoln University, Canterbury. She is particularly skilled in gamete cryopreservation and is presently completing an MSc study on semen freezing for red deer and fallow deer.

Both Catherine and Felicia have been closely involved in commercial artificial insemination of fallow deer, travelling to USA and Canada on several occasions to conduct AI services. Both are highly skilled in laparoscopic intra-uterine insemination techniques.