ADVANCED REPRODUCTION TECHNIQUES FOR FARMED FALLOW DEER

G.W. Asher, H.N. Jabbour, C.J. Morrow, F. Veldhuizen

Ruakura Agricultural Centre, MAFTechnology, Private Bag, Hamilton

Introduction

The fallow deer farming industry is now of an age to implement manipulative control of livestock reproduction to improve environmental and genetic performance. Such technologies include the control of seasonal breeding patterns, artificial insemination, embryo transfer, induction of twinning, IVM/IVF, embryo splitting, cloning and sperm/embryo sexing. Some of these technologies have already been implemented commercially, some may prove counter-productive for this species, and others are still scientific frontiers. This paper will examine recent advances in controlled reproduction of fallow deer and speculate on future technologies.

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 many 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. 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. Consequently, 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 (24-h) period is short. However, this secretory period increases as days become shorter. 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 "short-day" state.

Subcutaneous melatonin implants (Regulin; Young's Animals Health NZ Ltd) 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.

Recent studies involving administration of Regulin implants to fallow does and bucks in summer produced spectacular results. Treatment was applied to pubertal, non-pregnant adult and pregnant adult does, as well as 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, the treated sire bucks also exhibited a marked advancement in reproductive development 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 attributable primarily 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 probably that the initiation 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 (AI)

(i) Justification: 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 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. However, the procedures are time consuming and, for the purposes of AI, fixed-time insemination following oestrous synchronisation is more practical than following natural detected oestrus. 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; Alex Harvey Holt Ltd, Hamilton, NZ) 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.

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. 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. Clearance of exogenous progesterone from the blood stream following CIDR removal is very rapid and occurs within two hours. This stimulates an increase in luteinizing hormone (LH) secretion from the pituitary gland, culminating in the onset of oestrus and the pre-ovulatory LH surge between 40-55 hours. Ovulation occurs about 24 hours after the onset of oestrus.

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. 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 cycle (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. The onset of oestrus and the pre-ovulatory LH surge occur between 48 and 56 hours from prostaglandin injection. As with CIDR withdrawal, ovulation occurs about 24 hours after the onset of oestrus. Further 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 (21 days). Therefore, it is possible to obtain a high degree of synchrony of a return oestrus following synchronisation of the first oestrus. 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. 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. Furthermore, PMSG undoubtedly alters the temporal relationship between synchronisation treatment, the onset of oestrus and ovulation, requiring different timing schedules for AI.

(iii) Semen collection and processing: 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 has been 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 80%. 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. The extended semen is then loaded into 0.25 ml straws (50 million cells per straw) and frozen in nitrogen vapour to -125^{o}C in a programmable freezer (6^{o}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. 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.

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 that 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 withdrawal only produced 42% fawning rate. 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 in NZ and USA). 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.

(v) Ultrasound determination of pregnancy rate: Ultrasonographic pregnancy diagnosis is a useful tool for management of fallow deer. Recent studies at Ruakura 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, 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 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 1).

Table 1 : 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. Eight does receiving FSH alone failed to respond (TS <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) 11 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 2).

Table 2 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.

Induction of twinning

(i) Justification: The main criterion of on-farm performance on most deer farms is the number of fawns weaned relative to the number of does of reproductive age. As the incidence of multiple births amongst fallow deer is less than 1 per 500 births, maximum weaning rates can seldom exceed 100%. An increase in the incidence of twinning could, in theory, increase the overall weaning rate.

(ii) Hormonal induction of twinning: Induction of twinning in fallow deer is possible by increasing the ovulation rates of each doe with CIDR + PMSG treatment. Intramuscular administration of 100-500 i.u. PMSG per doe at CIDR withdrawal will increase number of multiple ovulations within a herd. However, of all induced twinnings recorded at Ruakura (n=12 as of December 1989) 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 corresponds to the normal birth weight of a singleton. Thus, it is probable that embryonic competition, due to the fallow placentation system, exacts a high price on foetal growth.

On the basis of observations to date, it appears that the induction of twinning in fallow deer is completely counter-productive. Furthermore, the use of PMSG, even in low doses (100 i.u.), for oestrous synchronisation also leads to an increased incidence of multiple ovulation, low fertilization rates, increased embryonic mortality and twinning in fallow deer. This has undoubtedly been the partial cause of some AI failures in NZ and Australia during the 1989 season.

IVM and IVF

In vitro (oocyte) *maturation* (IVM) and *In vitro fertilization* (IVF) are presently being researched, at Ruakura. Basically, the procedures involve (a) the recovery of immature eggs (oocytes) from ovaries of slaughtered does, (b) maturation of oocytes within a test-tube containing various stimulatory hormones and nutrients, (c) fertilisation of the matured ovum within the test-tube, (d) culture of the fertilised ovum to the 8 cell-blastocyst stages and (e) transfer of the embryo into a recipient doe or (f) cryopreservation pending transfer.

When the fallow deer industry reaches the stage whereby cull does are worth meat value only, IVM and IVF technologies may prove useful. At any stage of the reproductive cycle, the ovaries of mature females contain numerous

developing oocytes. Normally, only one of these oocytes will receive the appropriate hormonal stimulus to develop further into a mature ovum within an ovulatory follicle, once or maybe twice a year. However, numerous oocytes can be recovered from the ovaries of slaughtered does and matured to the stage of capability of fertilisation. This is a very valuable resource otherwise wasted. Even if the cull doe has little genetic value (and this may not always be the case), the genotype of the sperm donor may be exceptionally valuable and justify the effort. The techniques may also allow for the recovery of valuable genetic material from genetically superior does whose demise is imminent or accidental. The techniques would also provide a reliable supply of ova/embryos for future studies on embryo splitting, cloning, embryo sexing and gene transfer.

The rate at which studies on IVM and IVF progress at Ruakura will depend upon the availability of ovarian material from the DSP's.

Embryo splitting

Splitting of 8 cell-morula embryos has been successfully applied to a number of domestic mammal species. The techniques are used to produce genetically identical offspring. It is only a matter of time before splitting of deer embryos is performed. Except in the case of very rare or valuable animals, it is unlikely that embryo splitting will be applied commercially in the near future (i.e. 5-10 years). However, the technology has the potential of producing genetically identical pairs of individuals for research purposes (i.e. classical identical twin studies).

Cloning

Present technology for cloning mammals involves the transfer of nuclei (cells) from later stage embryos (up to early blastocyst stage) to an enucleated oocyte. The oocyte and transferred cell containing the nucleus are fused by electrofusion and then transferred to recipients. Cloning takes embryo splitting one stage further, as it involves the production of numerous genetically identical individuals from a single embryo. The technology is very novel and is reputed to be successful for sheep and cattle. It may not be long before such technology is developed for farmed deer. Its potential impact within all livestock industries is enormous.

Sperm and embryo sexing

Sexed semen would have major benefits in any livestock industry. However, despite many claims of success there is no convincing method for separating X- and Y-chromosome bearing sperm without killing them. Some techniques show some promise (e.g. separation on protein columns) but it is unlikely that a successful technique will arise in the short term.

It is possible to sex embryos by several techniques (sex chromatin; karyotyping; H-Y antigen; DNA probes and metabolic activity). All techniques have problem areas and the most promising commercial is probably the use of DNA probes. For this test the embryo must be biopsied (usually one half or one quarter embryo is sacrificed), the result is objective, accurate, known in 3 hours, and most embryos are sexable. The test is currently being assessed commercially for cattle in Australia. Development of the technology for deer will be dependant on commercial developments in embryo transfer.