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RECENT ADVANCES IN ARTIFICIAL INSEMINATION AND EMBRYO TRANSFER IN FARMED FALLOW DEER

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Summary

There has been considerable international interest in the artificial manipulation of farmed fallow deer to increase genetic performance. Artificial insemination has been applied commercially for rapid dissemination and international exchange of desirable genetic material. Conception rates of ~ 70% have been obtained for fixed-time (65 - 70 hours have post-CIDR device withdrawal) laparoscopic, intra-uterine, insemination of frozen-thawed semen (20-40 million live spermatozoa per inseminate). Semen is collected primarily by electro-ejaculation but artificial vagina techniques are being developed. Multiple ovulation-embryo transfer (MOET) technology for fallow deer is still in the experimental stages. Average rates of 7 - 20 ovulations/doe have been obtained following treatment of does with progesterone (14 - day CIDR device), PMSG and FSH preparations. However, fertilisation and embryo recovery rates are generally low (< 30%) and further studies are required to determine causal factors. Cryopreservation and embryo transfer have yet to be attempted for this species.

INTRODUCTION

Artificial insemination and embryo transfer are tools for genetic improvement within herds of domestic animals. These technologies are not substitutes for normal reproductive management aimed at optimizing environmental performance, but rather allow farmers to gain access to genetic material that will eventually increase the genetic potential for on-farm performance. In recent years there has been international recognition of the genetic gains that can arise in farmed fallow deer herds from the appropriate application of artificial insemination (AI) and embryo transfer (ET) programmes. This is perhaps highlighted by the recent introduction of subspecies hybridization of European fallow deer (Dama dama dama) with the very rare Mesopotamian fallow deer (Dama dama mesopotamica), for which presently only three Mesopotamian fallow bucks are effectively producing progeny, largely by AI.

ARTIFICIAL INSEMINATION

The application of AI technology within the fallow deer farming industry is still in its infancy. However, the future potential of AI is enormous, particularly in relation to the establishment of genetic improvement schemes. AI allows for a more rapid dissemination of desirable genetic material than would be remotely possible by natural mating. This is particularly important when considering such rare genotypes as the Mesopotamian fallow deer. Moreover, AI provides a safer and cheaper means of international exchange of genetic material by way of semen. There is also the important possibility of employing AI to identify genetically superior sires (e.g. sire-refencing schemes).

Estrous Synchronisation

Natural oestrous detection in fallow does can be performed by using bucks fitted with ram mating harnesses. However, this is very time consuming and somewhat impractical for AI. Fixed-time = AI following estrous synchronisation is more practical and cost-effective. The response of the does to the synchronisation treatment, as indicated by the proportion of does displaying estrus and the degree of synchrony of estrus, is most consistent after the onset of the natural breeding season (rut). Synchronisation of estrus/ovulation can be achieved either by simulating the activity of the ovarian corpus luteum through the administration of progesterone (e.g. CIDR device) or by shortening the luteal phase of the estrus cycle by injecting prostaglandin between Days 12 and 15 of the estrus cycle.

The intravaginal CIDR [Controlled Internal Drug Release] device has been tested comprehensively for its efficacy in synchronising estrus in fallow does. THe retention rate of the device is very high (98 - 100%) and during insertion it elevates blood progesterone concentrations to levels comparable to those observed during the mid-estrous cycle. Following CIDR device removal after 14 days, progesterone is cleared rapidly from the blood stream within two hours. This stimulates an increase in pituitary LH secretion, culminating in the onset of estrus and the pre-ovulatory LH surge 40-55 hours later. Ovulation occurs about 24 hours after the onset of estrus.

Induction of premature regression of the ovaran corpus luteum by injecting prostaglandin analogue results in very tight synchrony of oestrus in fallow does. However, the corpus luteum appears to be refactory to prostaglandin before Day 10 of the cycle. This necessitates the administration of prostaglandin either as a single injection between Days 12 and 15 of the cycle (necessitating prior synchronisation with CIDR devices) or two injections 10-12 days apart. If administered at the correct stage of the oestrous cycle, prostaglandin injection will cause clearance of endogenous progesterone over a 12-14 hour period and result in estrus being displayed 40-50 hours after injection.

There are two very important considerations with respect to estrous synchronisation in fallow does. First, non-parous (pubertal) does are generally unsuitable for AI programmes as they do not appear to exhibit a suitable degree of estrous synchrony following CIDR device withdrawal or prostaglandin injection. Second, the use of PMSG (Pregnant Mare Serum Gonadotrophin) at or near CIDR device withdrawal/prostaglandin injection is contraindicated due to an unacceptably high incidence of multiple ovulations, which are associated with lower conception rates and higher embryonic mortality in fallow deer.

Semen Collection and Processing

Semen collection from fallow bucks is highly seasonal due to the annual pattern of spermatogenesis is the species. To-date, semen from bucks has been collected primarily by electro-ejaculation, necessitating heavy sedation and electrical stimulation per rectum. This method limits the frequency of semen collection and may reduce the quality of the harvested semen.

On account of the disadvantages of electro-ejaculation, an alternative method of semen collection is presently being investigated at Ruakua. Recently we have developed a prototype internal artificial vagina (AV). For semen collection with the AV, ovariectomised does are treated with CIDR devices for six days and 0.05 mg estradiol injection; and exposed to the bucks. Following mating, the AV is removed and the semen aspirated off for processing.

Cryopreservation of fallow deer semen is comparatively simple and effective. For the international commercial supply of semen, a standard protocol has been adopted by the Ruakua Deer Artificial Breeding Centre. After the ejaculate volume and concentration/motility of spermatozoa is established, the semen is diluted to a concentration of 200 million live cells per millilitre in sodium citrate - egg yolk - glycerol diluent. 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. This often results in semen with post-thaw recovery rates in excess of 75%.

Insemination Techniques

The two methods of AI practiced on fallow deer in New Zealand, Australia and USA are intravaginal/intracervical (per vaginum) and laparoscopic intra-uterine insemination. Intravaginal insemination is the simplest method but requires large quantities of viable spermatozoa (> 100 million) for reasonable success rates. As this form of insemination is analogous to natural mating, semen placement is timed to coincide with the mean time to onset of estrus, being approximately 48 hours after CIDR device withdrawal/prostaglandin injection.

Intracervical insemination is likely to be less wasteful of spermatozoa than intravaginal insemination. Although optimum timing of insemination has yet to be determined, present studies indicate that at least 40-50 million live spermatozoa are required. Success rates of such inseminations performed 48 hours after CIDR device withdrawal range from 40-65%.

Laproscopic intra-uterine insemination allows for a precise placement of relatively small quantities of semen close to the site of fertilisation. Early studies involving intra-uterine deposition of 85 million live spermatozoa 56-58 hours after CIDR device withdrawal resulted in a disappointing 42% fawning rate. More recently, intra-uterine inseminations performed with 20-40 million live spermatozoa at 65-70 hours after CIDR device withdrawal resulted in 60-70% conception rates. This suggests that in the former study inseminations were conducted too early relative to CIDR device withdrawal or, more relevantly, relative to the time of ovulation. Additional studies are warranted to establish the interaction effect between the time of insemination and the dose of spermatozoa on conception rates.

Ultrasound Determination of Pregnancy to AI

Ultrasonographic pregnancy diagnosis is a useful tool for the management of fallow deer. Recent studies at Ruakura revealed that foetal age can be estimated to within 5-10 days within the first 90 days of gestation, by use of a rectal probe. This renders the determination of conception to AI, as opposed to the return estrus (21-22 days later), a simple process.

Present Usage of AI in Fallow Deer

Commercial application of AI of farmed fallow deer, based largely on predicted and actual semen sales from the Ruakura Deer Artificial Breeding Centre (which supplies > 90% of all commercially available fallow deer semen within the world), is in the order of 1,000 inseminations in New Zealand., 1,000 inseminations in Australia and 1,500 inseminations in USA/Canada in the 1990 year. This represents a 400% increase from the previous year.

EMBRYO TRANSFER

Multiple ovulation - embryo transfer (MOET) technology in fallow deer is still in the experimental stages. The interest in the technology is based on the possibility of using a proportion of the base herd of European fallow deer as recipients for multiple embryos derived from Mesopotamian fallow deer. Future applications is also important within genetic improvement programmes and international exchange of superior genetic material.

Superovulation/Embryo Recovery

Recent studies at Ruakura have aimed at determining an exogenous hormone regimen that will stimulate a good superovulatory response and high embryo recovery/fertilization rates. In two major studies, treatment with exogenous gonadotrophins (FSH; Follicle Stimulating Hormone: PMSG; Pregnant Mare Serum Gonadotrophin) induced a range of ovulation rates from 0-30 depending on relative proportions of the two hormones and actual rates delivered (see Table 1 for 1989 data). Generally, however, ova fertilization and recovery rates were very low (0 - 30%) even though ample spermatozoa were present from natural mating and AI.

Present indications are that gamete transport, and hence fertilisation, are adversely affected by high follicular secretion of estradiol. Further studies are needed to define the optimal site and time of semen deposition to improve fertility of superovulated fallow does. Considerable research is required in this area if embryo transfer is to become established commercially.

Embryo Cryopreservation/Transfer

There are no published accounts describing embryo freezing and transfer in fallow deer. It is unlikely, however, that these steps will be limiting in MOET programmes for this species.

TABLE 1: Mean (± s.e.m.) ovulatory response of fallow does to 200 i.u. PMSG and variable does of ovine FSH

FSH UNITS ADMINISTERED	NUMBER OF OVULATIONS/DOE
0.00	1.1 ± 0.4
0.25	7.2 ± 1.7
0.50	9.5 ± 2.5
0.75	8.6 ± 2.4
1.00	7.4 ± 2.1