Superovulation in red deer (*Cervus elaphus*) and Père David's deer (*Elaphurus davidianus*), and fertilization rates following artificial insemination with Père David's deer semen

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Two comparative studies were undertaken using adult, female red and Père David's deer to examine the ovulatory response of these animals to a superovulation regimen and fertilization rates following inter- and intraspecific laparoscopic insemination. In Expt 1 six Père David's deer and 12 red deer hinds were treated during the breeding season with an intravaginal progesterone-impregnated controlled internal drug release device (CIDR) for 14 days, with 200 iu pregnant mares' serum gonadotrophin (PMSG) administered 72 h before the device was withdrawn and eight injections of ovine FSH given at 12 h intervals starting at the time of PMSG administration. Oestrous behaviour began one day after CIDR device withdrawal (Père David's deer: 24.00 ± 2.32 h; red deer: 24.60 ± 2.23 h). The duration of oestrus was greater in Père David's deer than in red deer (17.50 ± 1.43 h and 8.25 ± 3.25 h, respectively, $P < 0.001$). The peak LH surge of Père David's deer was 68.65 ± 4.74 ng ml⁻¹ occurring 29.00 ± 2.41 h after removal of the CIDR devices. In comparison, the peak LH surge in red deer was 17.09 ± 3.64 ng ml⁻¹ ($P < 0.001$), occurring 24.00 ± 0.00 h after CIDR device withdrawal. Pre-surge concentrations of LH were also greater ($P < 0.001$) in Père David's deer (1.37 ± 0.11 ng ml⁻¹) than in red deer hinds (0.41 ± 0.02 ng ml⁻¹). Oestradiol concentrations increased steadily during FSH treatment in both species and reached a maximum of 15.60 ± 2.22 pg ml⁻¹ at 21.00 ± 6.20 h after CIDR device withdrawal in Père David's deer and 26.14 ± 6.20 pg ml⁻¹ at 20.40 ± 7.30 h before CIDR device removal in red deer. The mean number of ovulations recorded was 3.83 ± 1.22 (range 1–8) in Père David's deer and 10.80 ± 3.78 (range 4–23) in red deer. In Expt 2, six Père David's deer and six red deer were superovulated with an identical regimen to that described for Expt 1. All hinds were inseminated *in utero* 36 h after CIDR device withdrawal with 25×10^6 frozen-thawed Père David's deer spermatozoa. Embryos were recovered by laparotomy 5, 6 and 7 days after insemination. A total of six embryos (three Père David's deer $\times$ red deer and three Père David's deer $\times$ Père David's deer) were collected. One pregnancy was confirmed following the transfer of all embryos to synchronized red deer recipients. This pregnancy was terminated naturally between days 70 and 78. This is the first report of an interspecific transfer of hybrid embryos in a cervid and of the comparative superovulation response in two related species. The results indicate that treatment with PMSG and ovine FSH can be used in embryo transfer programmes for these species. However, in this study there were low inter-specific fertilization rates and transferred hybrid embryos failed to develop to term.

Introduction

Interest in the application of reproductive technology in cervid species has greatly increased over the past decade. Artificial

insemination with the purpose of rapidly disseminating valuable genetic material from male red and fallow deer has had a widespread application in New Zealand (Kelly and Moore, 1981; Asher *et al.*, 1988a). Similarly, superovulation and subsequent embryo transfer programmes have been attempted in these species for the rapid dissemination of female genetic material (Bringans, 1987; Fennessy *et al.*, 1989; Jabbour *et al.*,

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1992) but the inherent complexities of these protocols have limited their widespread application.

The application of embryo transfer is of importance to conservation biology. Of the 36 species of deer, half are classified as vulnerable or endangered (IUCN, 1988). The application of superovulation, artificial insemination and interspecific transfer of embryos to compatible surrogate recipients may assist in rapidly increasing the numbers of certain vulnerable species.

Attempts at hybridization in deer, whether by artificial insemination or natural mating, have been accompanied by reduced fertility. Pregnancy rates of red deer are markedly lower following insemination with Père David's deer than with red deer semen (Asher *et al.*, 1988b). Similar effects have been noted when red deer have been inseminated with a closely related subspecies, the wapiti (Haigh and Bowen, 1991). The following study was designed to compare the ovarian response of physically distinct but karyotypically similar species, the European red deer and Chinese Père David's deer, to superovulatory treatment with pregnant mares' serum gonadotrophin (PMSG) and ovine FSH (o-FSH). Moreover, the fertilization rates following insemination with inter- or intraspecific semen were determined and pregnancy rates established following embryo transfer to red deer hinds.

Materials and Methods

Animals and collection of blood samples before treatment

In Expt 1, 18 mature (>2 years old) Père David's ($n = 6$) and red deer ($n = 12$) hinds were used to characterize the endocrine, behavioural and ovarian responses to treatment with PMSG and o-FSH. In Expt 2, 12 mature (<2 years old) Père David's deer ($n = 6$) and red deer ($n = 6$) designated as potential embryo donors were treated with the same PMSG/o-FSH regimen as in Expt 1. Mean body weights for Père David's and red deer were 169.5 ± 5.4 (mean $\pm$ SEM, $n = 12$) and 92.14 ± 1.5 kg ($n = 18$, $P < 0.001$), respectively. A further 12 red deer hinds were designated as embryo transfer recipients. All animals were hand-reared, tame and used to repeated handling. Throughout the experiments the hinds grazed at pasture, or were maintained in yards and fed grass silage, and supplemented with barley and soya bean meal. Blood samples were collected twice a week for progesterone determination for 2 months before the experiment to ensure that all animals were undergoing repeated oestrous cycles by the start of the experiment (McLeod *et al.*, 1991; Argo and Loudon, 1992). Samples were collected by jugular venepuncture under manual restraint. Behavioural oestrus was recorded as the response to manual pressure on the hind quarters as previously described (Curlewis *et al.*, 1988; Argo and Loudon, 1992). In both species, hand-reared hinds exhibit very conspicuous oestrous behaviour to human handlers.

Experiment 1: ovarian and endocrine response to PMSG and o-FSH

All hinds were treated with intravaginal controlled internal drug release devices (CIDR; type G, 9% progesterone, AHI

Plastic Moulding Company, Hamilton) on November 11, 1991. On the basis of interspecific weight differences, hinds were fitted with one (red deer) or two (Père David's deer) CIDR devices. At this time both species were reproductively active but the red deer and Père David's deer were in the early and late phases of their respective breeding seasons. CIDR devices were replaced on day 7 and finally withdrawn at 23:00 h on 24 November (Père David's deer) and 02:00 h on 25 November (red deer). The hour of CIDR device withdrawal was designated as time zero (t_0) for each species. Five days before CIDR device withdrawal (19 November) all hinds were treated i.m. with 25 μ g of cloprostenol, a prostaglandin analogue (Estrumate: Cooper's Animal Health, Crewe). Each hind received 200 iu PMSG (Intervet, UK Ltd, Cambridge) 72 h before CIDR device removal. Ovine-FSH (Ovagen: Immunochemical Products Ltd, Auckland) was administered in eight 0.625 mg injections at intervals of 12 h starting at the time of PMSG administration and finishing 12 h after CIDR device removal.

Blood samples were collected from a total of 12 (Père David's deer, $n = 6$; red deer, $n = 6$) hinds at intervals of 12 h for 6 days starting 12 h before PMSG treatment for determination of oestradiol profiles and at intervals of 3 h for 60 h starting from the time of CIDR device withdrawal for determination of LH and progesterone profiles. Blood samples were centrifuged at 2400 g for 10 min. Plasma was aspirated and stored at -20°C until required for analysis. At the time of collection of blood samples, each animal was checked for evidence of behavioural oestrus. The ovarian response of all 18 deer to exogenous gonadotrophin treatment was determined by laparoscopy (Asher *et al.*, 1990) under general anaesthesia: 0.5 mg xylazine hydrochloride kg^{-1} liveweight (Rompun: Bayer Leverkusen) and 0.01 g sodium thiopentone kg^{-1} liveweight (Intraval Sodium: May & Baker Ltd, Dagenham) 108 h after CIDR device removal in each species (i.e. 29 November). Numbers of corpora lutea and large (>3 mm) unruptured follicles were recorded.

Experiment 2: embryo recovery and transfer

All hinds were treated with CIDR devices and a single injection of cloprostenol as described in Expt 1. Five days before CIDR device withdrawal, the animals were randomly allocated to either embryo donor or recipient groups. The hinds in the donor group (Père David's deer, $n = 6$ and red deer, $n = 6$) were treated with the same PMSG/o-FSH regimen as described for Expt 1. CIDR devices were withdrawn at 23:00 h (26 November) and 02:00 h (27 November) from all Père David's and red deer hinds, respectively.

Semen was collected by electroejaculation from a Père David's deer stag during the previous breeding season (Asher *et al.*, 1988b). The ejaculate was extended to a concentration of 100×10^6 spermatozoa ml^{-1} in a diluent containing 2.9% sodium citrate, 20% egg yolk and 8% glycerol, loaded into 0.25 ml straws, frozen in liquid nitrogen vapour and stored in liquid nitrogen until required for insemination. Only semen from straws with a post-thaw motility of >40% were used in the experiment. Hinds in the donor group were inseminated by laparoscopy (Asher *et al.*, 1988b) under general anaesthesia,

Fig. 1. Changes in the mean LH concentrations of (●) red deer (RD) hinds ($n = 4$) and (○) Père David's deer PDD hinds ($n = 6$) during the 15 h following controlled internal drug release (CIDR) device removal. Mean changes in concentration of LH during the LH surge have been normalized around the surge peak. Vertical lines represent the SEM. Horizontal bars represent the duration of behavioural oestrus, normalized with respect to the LH surge peak, for red deer ($n = 5$) and Père David's deer ($n = 6$), respectively. Horizontal lines represent the SEM of the times of onset and termination of oestrous behaviour.

36 h after CIDR device withdrawal. In all animals, a total of 25×10^6 spermatozoa (one straw) was divided equally between both uterine horns. Hinds were housed overnight in their pretreatment groups following insemination.

Embryo recovery was attempted by flushing both uterine horns at laparotomy under general anaesthesia on either days 5, 6 or 7 after artificial insemination as described by Jabbour and Evans (1991). The numbers of corpora lutea and large follicles were recorded at the time of embryo recovery. Embryos and ova were assessed by examination under a stereomicroscope, and numbers and stages of development were recorded. Single embryos were transferred by laparoscopy into the uterine horn ipsilateral to the active corpora lutea of the synchronized recipient hinds. All hinds were group-housed for a week following surgery. Recipient hinds were scanned for pregnancy determination by transrectal ultrasonography 40 and 70 days after embryo transfer.

Radioimmunoassays

Progesterone was measured in a direct radioimmunoassay as previously described for Père David's deer (McLeod

et al., 1991) and red deer (Argo and Loudon, 1992). The antibody was raised in sheep against progesterone 11 α -hemisuccinate-BSA (HP/S/645-111A, NETRIA, St Bartholomew's Hospital, London) and used at a final concentration of 1:18 000. The antisera crossreacted with 11 β -hydroxyprogesterone, 11 α -hydroxyprogesterone, 16 α -hydroxyprogesterone, 5 α -pregnanedione, 5 β -pregnanedione and 20 α -dihydroprogesterone at 208.3, 80.6, 9.0, 8.3, 3.2 and 2.4%, respectively. Assay sensitivity was 0.8 ng ml^{-1} and intra- and interassay coefficients of variation were 7.6% and 5.1% at 0.80 and 1.72 ng ml^{-1} , respectively.

LH was determined using the heterologous ovine double-antibody radioimmunoassay (rabbit, anti-ovine LH serum, R07/6) previously described by Loudon *et al.* (1990). The assay sensitivity was $0.2 \text{ ng NIH-LH-S24 equivalents ml}^{-1}$ plasma, and the intra- and interassay coefficients of variation at 1.12 ng ml^{-1} were 8.9% and 9.4%, respectively.

Oestradiol was determined in an extraction assay as described by Webb *et al.* (1985). The affinity extraction step used sheep, oestradiol antisera (26.9.80). The oestradiol antisera used in the radioimmunoassay was raised in rabbits, against 17 β -oestradiol-11 β -succinyl-BSA (R48, 22.6.83) and used at a final concentration of 1:40 000. The antisera crossreacted with oestrone, oestriol and testosterone at 16, 3 and 1.2%, respectively. The assay sensitivity was 0.95 pg ml^{-1} and intra- and interassay coefficients of variation were 6.3% and 13.1%, at 9.10 and 3.19 pg ml^{-1} , respectively.

Statistical analysis

All data are presented as means $\pm$ SE. Comparisons within and between groups were made with either paired or unpaired Student's *t* tests, respectively, with degrees of freedom adjusted for inequality in the variance of means.

Results

Experiment 1: ovarian and endocrine response to PMSG and o-FSH

After removal of the CIDR devices five of the six red deer hinds displayed oestrous behaviour but only four displayed an LH surge. Each of the six Père David's deer displayed both oestrous behaviour and an LH surge. At laparoscopy, the red deer hind that failed to show oestrous behaviour had very small and inactive ovaries and hence was excluded from further analyses. The mean pre-surge LH concentrations ($1.37 \pm 0.11 \text{ ng ml}^{-1}$ versus $0.41 \pm 0.02 \text{ ng ml}^{-1}$) and mean peak surge LH concentrations ($68.65 \pm 4.74 \text{ ng ml}^{-1}$ versus $17.09 \pm 3.64 \text{ ng ml}^{-1}$) were significantly higher in Père David's deer than in red deer ($t_5 = 16.59$, $P < 0.001$; $t_8 = 7.82$, $P < 0.001$). Although the duration of the LH surge was similar in both species (Père David's deer: 16.75 h ; red deer: 15.80 h), the peak of the LH surge did not occur until 5 h later in the Père David's deer ($29.00 \pm 2.41 \text{ h}$; and in red deer, $24.00 \pm 0.00 \text{ h}$). Hinds of both species displayed oestrous behaviour at the same time relative to CIDR device withdrawal (Père David's deer $24.00 \pm 2.32 \text{ h}$; red deer: $24.60 \pm 2.23 \text{ h}$). The onset of behavioural oestrus coincided with the peak of the LH surge in

Fig. 2. Changes in mean plasma steroid concentrations over the final 4 days of progesterone priming, during which gonadotrophin treatment was given, and the 3 days following controlled internal drug release (CIDR) device withdrawal in (●) red deer (RD) ($n = 5$), and (○) Père David's deer (PDD) ($n = 6$). Vertical lines represent the SEM. Horizontal bars represent the relative mean duration of behavioural oestrus in both species. The double solid arrow indicates the initial combined injection of pregnant mares' serum gonadotrophin (PMSG) and ovine FSH (o-FSH), and the single arrows represent subsequent o-FSH treatments. The open arrow and vertical broken line indicate the time of CIDR device withdrawal.

red deer and occurred 5 h before peak LH values in Père David's deer (Fig. 1). The duration of oestrus was longer in Père David's deer (17.50 ± 1.43 versus 8.25 ± 3.25 h for Père David's deer and red deer, respectively, $t_9 = 3.8$, $P < 0.001$, Fig. 1).

In both species, there were significant changes in plasma concentrations of oestradiol during the period of FSH treatment (Père David's deer, $t_8 = -2.74$, $P < 0.05$; red deer, $t_8 = -3.52$, $P < 0.01$, Fig. 2). Mean oestradiol concentrations increased in Père David's deer from 4.5 ± 2.9 pg ml $^{-1}$ to reach a peak of 14.9 ± 2.5 pg ml $^{-1}$, coincident with the final FSH injection and 10 h after CIDR device withdrawal. In the red deer, mean oestradiol concentrations increased from 2.1 ± 0.5 pg ml $^{-1}$ to 20.2 ± 5.2 pg ml $^{-1}$ 17 h before CIDR device removal.

Plasma progesterone concentrations did not exhibit a significant change during the 60 h following CIDR device removal in Père David's deer hinds ($t_5 = -2.81$, ns). Over the same period, progesterone concentrations in red deer increased significantly from 0.7 ± 0.08 ng ml $^{-1}$ to 3.3 ± 0.98 ng ml $^{-1}$ ($t_5 = -3.38$, $P < 0.05$, Fig. 2).

All 11 red deer hinds and four Père David's deer hinds had multiple ovulations following PMSG/o-FSH treatment (Table 1). In red deer ($n = 11$), 2.55 ± 0.86 large follicles and 9.82 ± 0.09 corpora lutea were observed, giving a total ovarian response (large follicles plus corpora lutea) of 12.37 ± 2.36 . In Père David's deer ($n = 6$) 1.00 ± 0.20 large follicles and 3.83 ± 1.80 corpora lutea were observed with a total ovarian response of 4.83 ± 1.9 . The ovulation rate in both species was 79% of the total ovarian response.

Table 1. The concentration and timing of the preovulatory oestradiol peak, with respect to withdrawal of the controlled internal drug release devices and corresponding ovarian response in red deer and Père David's deer

Deer		Time (h) of peak oestradiol concentration	Oestradiol peak concentration (pg ml ⁻¹)	Ovarian response		
				LF	CL	TOR
Red deer (<i>n</i> = 5)	Mean	-20.4 ^{a*}	26.14 ^c	2.80 ^d	10.80 ^f	13.6 ^h
	SEM	7.30	6.20	0.58	3.73	4.30
	Range	-42-6	10.0-43.0	2-5	4-23	6-28
Père David's deer (<i>n</i> = 6)	Mean	21.0 ^b	15.6 ^c	1.00 ^e	3.83 ^g	4.83 ⁱ
	SEM	6.20	2.22	0.40	1.22	1.30
	Range	-3-45	10-24	0-2	1-8	1-9

*Values with different superscripts are significantly different ($P < 0.05$).

LF: large follicles; CL: corpora lutea; TOR: total ovarian response.

Despite differences in the ovarian response and oestradiol concentrations of individuals, there was little difference in the timing of the LH surge and onset of sexual behaviour within each species. Endocrine profiles for two red deer hinds are shown in Fig. 3. In the case of hind 1 (total ovarian response of 19), oestradiol concentrations were maximal after CIDR withdrawal and progesterone concentrations did not increase for 56 h. This result contrasts with hind 2 (total ovarian response of 28), in which oestradiol concentrations were maximal 24 h before CIDR removal and increasing progesterone concentrations were evident from the time of CIDR withdrawal. In both animals the LH surge and behavioural oestrus occurred at the same time relative to removal of the CIDR device.

Experiment 2: embryo recovery and transfer

Data on the ovarian response and embryo recovery rates following superovulation are shown (Table 2). The mean total ovarian response was 7.00 ± 2.59 and 5.00 ± 1.65 for red deer and Père David's deer, respectively. From the red deer, a total of 11 ova and three hybrid embryos (two-cell, eight-cell and a blastocyst) were recovered from two of the six hinds. In Père David's deer, two ova and three embryos (eight-cell, approximately 32-cell and a blastocyst) were recovered from two out of six hinds. After transfer, one pregnancy was confirmed and maintained until between days 70 and 78, when natural termination of the pregnancy occurred.

Discussion

Treatment with PMSG and o-FSH was successful in inducing multiple ovulations in Père David's deer and red deer. The use of comparatively low doses of both gonadotrophins in combination is reputed to be more suitable than administering either gonadotrophin separately. In sheep (Jabbour and Evans, 1991) and fallow deer (Thompson and Asher, 1988) large doses of PMSG induce a high proportion of large follicles that fail to rupture and FSH alone results in a proportion of animals that fail to respond to treatment. The ovulatory response of red

deer in this study was similar to that shown by Jabbour *et al.* (1992) where the optimum superovulatory response was observed following treatment of red deer hinds with an identical gonadotrophin regimen. There are no comparable data for Père David's deer.

The reasons for the interspecies differences in the ovarian response in this study are unclear. These may be due to variations in body size (the same absolute doses were used) or to the fact that the study was conducted at the early part of the breeding season in red deer and towards the end of the breeding season in Père David's deer. In sheep, there have been suggestions that the response to a given dose of PMSG is affected by the time of the year that PMSG is administered (Braden *et al.*, 1966; Gherardi and Lindsay, 1980). These latter authors observed the greatest response in March and the lowest one in September in the southern hemisphere. Lamond (1962) suggested that this may be due to a change in the sensitivity of the neuroendocrine systems controlling ovarian activity. Although there are no published data on changes in response to gonadotrophins in deer across the breeding season, Curlewis *et al.* (1991) have demonstrated a significant seasonal change in the LH response to exogenous GnRH during the 7-month anoestrous period of Père David's deer.

Notwithstanding the relatively low frequency of sampling (every 12 h), it is clear that oestrogen secretion peaked at or shortly before the time of progesterone withdrawal. The increase in oestrogen concentration in both species during treatment with o-FSH indicates that follicular development is not inhibited by exogenous progesterone. In both species, there was an approximate 24 h delay from progesterone withdrawal to the onset of behavioural oestrus and the LH surge, irrespective of the individual differences in the oestrogen profiles. This finding suggests that in the hind, progesterone inhibits the positive feedback of oestrogen on LH as well as blocking the oestrogen-stimulated expression of oestrous behaviour. In superovulated ewes, there is a dissociation between behavioural oestrus and the pre-ovulatory LH surge (Willadsen, 1979). In contrast, in the study reported here, the period of overt oestrous behaviour occurs at about the same time as the LH surge, as previously shown for natural oestrus

Fig. 3. Endocrine profiles of two superovulated red deer hinds, during the 4 days before and the 3 days after controlled internal drug release (CIDR) device removal, illustrating divergent ovarian responses to identical gonadotrophin treatments. Oestradiol (■), progesterone (△) and LH (●). Horizontal bars represent behavioural oestrus. Solid arrows represent the times of ovine-FSH (o-FSH) treatment. The double solid arrow indicates the initial combined PMSG/o-FSH injection. The open arrow and vertical broken line represent the time of CIDR device removal. TOR: total ovarian response.

in red deer (Argo and Loudon, 1992) and in monovulating and superovulating red deer (Asher *et al.*, 1992).

On the basis of a rapid decline in oestrogen concentration, it is probable that ovulation in some red deer hinds commenced before CIDR device withdrawal. This is substantiated by a rapid increase in the mean progesterone profiles during the 60 h after CIDR device removal, indicating an early onset of luteal function. Ovulations during the progesterone priming period must have occurred in the absence of an endogenous LH surge. LH concentrations remained low during progesterone treatment. These results are consistent with the observations in fallow deer (Jabbour *et al.*, 1993a) and goats (Cameron and Batt, 1991) in which ovulations were observed in animals that failed to exhibit an LH surge following treatment with PMSG. This may be a result of direct action of PMSG at the ovarian level. PMSG has potent LH-like activity (Farmer and Papkoff, 1979)

and has been demonstrated to have a half-life far in excess of endogenous LH in other ruminants (such as goat, Armstrong *et al.*, 1983; and sheep, McIntosh *et al.*, 1975). The later onset of the decline in oestrogen concentration in Père David's deer suggests that ovulation was probably initiated by the endogenous LH surge in this species.

Artificial insemination with frozen-thawed semen has been used for inter-subspecific and inter-specific hybridization of deer (Asher *et al.*, 1988b; Asher *et al.*, 1993; Haigh and Bowen, 1991; Jabbour *et al.*, 1993b), although in interspecific hybridization fertility is very low. For instance, a calving percentage of 7.8 was recorded following intrauterine insemination of red deer hinds with Père David's deer semen (Asher *et al.*, 1988b). In the study reported here, ova recovery rates were low in both species (red deer, 43.15%; Père David's deer, 29.33%). This may be attributed to secretion of high concentrations of

Table 2. The day of uterine flushing after artificial insemination, the ovarian response, ova and embryos collected, and pregnancies arising from the transfer of all embryos in red deer and Père David's deer

Hind No.	Day	LF	CL	Ova	Embryos	Pregnancy
RD21	5	2	1	—	—	—
RD22	5	4	3	2	1 (2 cell)	—
RD53	6	1	1	—	—	—
RD76	6	0	19	7	2 (8 cell) (blastocyst)	— 70-78 days
RD77	7	3	0	—	—	—
RD80	7	0	8	2	—	—
Mean		1.67	5.33	1.80	0.5	
SE		0.67	2.97	1.11	0.34	
PDD2	5	0	4	—	2 (8 cell) (32 cell)	— —
PDD3	5	0	1	—	—	—
PDD7	6	10	1	—	—	—
PDD8	6	3	6	—	—	—
PDD5	7	0	3	—	1 (blastocyst)	—
PDD17	7	0	2	2	—	—
Mean		2.17	2.83	0.33	0.5	
SE		1.64	0.79	0.3		

Group mean and individual results are shown for the red deer (RD, $n = 6$) and Père David's deer (PDD, $n = 6$) inseminated with frozen-thawed Père David's deer semen in Experiment 2.

CL: corpora lutea; LF: large follicles.

oestrogen, which have been shown to disrupt the biochemical maturation of oocytes in ewes (Moor *et al.*, 1985) and also accelerate the transport of gametes in the female reproductive tract. Ova, entering the uterus prematurely, face a hostile environment and may be expelled promptly (Whyman and Moore, 1980). The embryos recovered were at different stages of development, suggesting that the oviductal and uterine environments were not conducive to proper development. The failure of transferred embryos to implant and the natural termination of the one established pregnancy may have been caused by a mismatch between the stage of development of the embryo and the uterine environment of the recipient animals. Problems of genetic incompatibility, implicit in the retarded development of hybrid murine embryos (Frels *et al.*, 1980), inappropriate interactions between trophoblast and endometrium, and immunological involvement (Anderson, 1988) must also be considered as potential causes of embryonic death.

In conclusion, this study has demonstrated that superovulation and embryo transfer technology can be used to establish interspecific pregnancy in cervids. The next step is to understand the mechanisms underlying survival following interspecific embryo transfer.

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