Production of interferon by red deer (Cervus elaphus) conceptuses and the effects of roIFN-τ on the timing of luteolysis and the success of asynchronous embryo transfer

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The role of interferon in early pregnancy in red deer was investigated by (a) measuring production of interferon by the conceptus, (b) testing the anti-luteolytic effect of recombinant interferon-τ in non-pregnant hinds, and (c) treatment of hinds with interferon after asynchronous embryo transfer. Blastocysts were collected from 34 hinds by uterine flushing 14 (n = 2), 16 (n = 2), 18 (n = 8), 20 (n = 13) or 22 (n = 9) days after synchronization of oestrus with progesterone withdrawal. Interferon anti-viral activity was detectable in uterine flushings from day 16 to day 22, and increased with duration of gestation (P < 0.01) and developmental stage (P < 0.01). When interferon-τ was administered daily between day 14 and day 20 to non-pregnant hinds to mimic natural blastocyst production, luteolysis was delayed by a dose of 0.2 mg day⁻¹ (27.3 ± 1.3 days after synchronization, n = 4 versus 21 ± 0 days in control hinds, n = 3; P < 0.05). Interferon-τ was administered to hinds after asynchronous embryo transfer to determine whether it protects the conceptus against early pregnancy loss. Embryos (n = 24) collected on day 6 from naturally mated, superovulated donors (n = 15) were transferred into synchronized recipients on day 10 or day 11. Interferon-τ treatment (0.2 mg daily from day 14 to 20) increased calving rate from 0 to 64% in all recipients (0/11 versus 7/11, P < 0.005), and from 0 to 67% in day 10 recipients (0/8 versus 6/9, P < 0.01). The increased success rate of asynchronous embryo transfer after interferon-τ treatment in cervids may be of benefit where mismatched embryo–maternal signalling leads to failure in the establishment of pregnancy.

Introduction

Many deer species are rare, endangered or threatened in their natural habitats (IUCN, 1996; Jabbour et al., 1997). Assisted reproductive techniques developed in farm animals, such as artificial insemination, embryo transfer (ET) and in vitro embryo production, could be applied to increase population growth and reduce inbreeding in captive populations of various cervids. Embryo transfer has been applied in various endangered animals (Pitra et al., 1991), and has been used, with varying success, in several species of deer (white-tailed deer: Magyar et al., 1988; Waldhalm et al., 1989; Père David’s deer: Argo et al., 1994; red deer: Fennessy et al., 1994; fallow deer: Jabbour et al., 1994; Morrow et al., 1994). However, success rates after both intra- and interspecies ET in ruminants have often been disappointing, and the reasons for failure difficult to ascertain (Jabbour et al., 1997). One factor that may limit the success rates of ET programmes is oestrous asynchrony between the donor and recipient animals. In sheep, optimal embryonic survival is achieved if the donor and recipient are in oestrus on the same day (Moore and Shelton, 1964; Rowson and Moor, 1966a; Ashworth and Bazer, 1989). Rowson and Moor (1966a) showed that the maximum asynchrony with the uterine environment that ovine embryos can tolerate is 2 days. When embryos were transferred into recipient ewes that were in oestrus 3 days before their respective donors, no pregnancies resulted.

In non-pregnant hinds, the regression of the corpus luteum at the end of the oestrous cycle leads to an abrupt decrease in the circulating concentration of progesterone (white-tailed deer: Plotka et al., 1980; red deer: Adam et al., 1985; fallow deer: Asher et al., 1986). This maternal luteolysis represents an initial obstacle that the embryo needs to surmount, both during normal pregnancy and after ET. During early pregnancy in the Bovidae, the trophoderm of the developing conceptus secretes into the uterine lumen an interferon, interferon τ (IFN-τ), which acts as the anti-luteolytic in these species (Roberts et al., 1992). Through interactions with endometrial receptors (Kaluz et al., 1996),
IFN-τ inhibits the pulsatile secretion of the luteolytic hormone prostaglandin F$_2$α (PGF$_2$α) by the uterus (Fincher et al., 1986; Flint et al., 1991; Parkinson et al., 1992), delaying regression of the corpus luteum, and ensuring continued luteal secretion of progesterone required for the maintenance of pregnancy. The failure of this embryo-maternal signal would result in early pregnancy loss, and may be an important factor in the failure to establish pregnancy that follows ET. However, the physiological events involved in early pregnancy establishment in the Cervidae are poorly understood and further research in this area could lead to improvements in conception rates after both inter- and intraspecies ET programmes.

Pre-implantation red deer embryos produce IFN-τ, which is closely related to those interferons secreted by the Bovidae (Demmers et al., 1999). Interferon-τ may also act as the anti-luteolysin in red deer, as a closely related molecule, IFN-α, delays luteolysis when administered during the oestrous cycle (Bainbridge et al., 1996). The aim of the present study was to investigate the use of interferon to improve the success of controlled breeding programmes in cervids. The time of interferon production by the red deer blastocyst was determined, and then whether exogenous recombinant ovine IFN-τ (roIFN-τ) administered at this time would delay luteolysis was assessed in non-pregnant hinds. Finally, the effect of administering roIFN-τ on the success of asynchronous multiple-ovulation and embryo transfer (MOET) was determined. In addition, the developmental stages of early red deer embryos are reported in detail for the first time.

**Materials and Methods**

**Source and standardization of interferon**

Recombinant ovine IFN-τ prepared in yeast from ovine cDNA by the Lincoln Fermentation Facility (University of Nebraska), was filter-sterilized and supplied at a concentration of 1 mg protein ml$^{-1}$ in buffer containing 20 mmol Tris–HCl l$^{-1}$, pH 7.5 and 150 mmol sodium chloride l$^{-1}$. The anti-viral activity of the roIFN-τ, measured by the anti-viral assay described below, was 2.83 $\times$ 10$^8$ U ml$^{-1}$.

**Experiment 1: timing of interferon production by red deer blastocysts**

**Animals and treatments.** Early red deer blastocysts were collected by uterine flushing at different stages of gestation to determine the timing of interferon production. The experiment used 34 mature (aged 3–12 years) red deer hinds (Cervus elaphus) with a mean live weight of 87.8 ± 1.6 kg, maintained in fenced grass paddocks at the Institute of Zoology, Whipsnade Wild Animal Park, Bedfordshire (51°50′N) and at the ADAS Rosemaund Research Centre, Hereford (52°10′N). Oestrus was synchronized in each hind by administration of progesterone by intravaginal pessary (Controlled Internal Drug-Releasing (CIDR) device, type G, 0.3 g progesterone per device; CHH Plastic Products Group Ltd, Hamilton, NZ). The first CIDR device was inserted for 8 days and was replaced with a second device for 4 days. The time of withdrawal of the second device was taken as day 0. The CIDR devices were inserted between 3 and 12 October 1995 (n = 14, Rosemaund); between 9 and 20 November 1996 (n = 13, Whipsnade) and between 2 and 7 December 1996 (n = 7, Rosemaund). After removal of CIDR devices, hinds were run with fertile mature stags (at a ratio of four hinds per stag).

**Embryo collection.** Blastocysts were collected either by laparotomy or at slaughter 14 (n = 2), 16 (n = 2), 18 (n = 8), 20 (n = 13) or 22 (n = 9) days after CIDR removal. Before laparotomy, animals were fasted overnight and then anaesthetized using xylazine hydrochloride (1–2 mg kg$^{-1}$ liveweight, i.v.; Rompun, Bayer Plc. Vet Group, Bury St Edmonds) followed by sodium thiopentone (10–20 ml, 2.5% (v/v), i.v.; May and Baker Ltd, Dagenham). Animals were transferred to a cradle, intubated and maintained under anaesthesia using halothane (Zeneca Ltd, Macclesfield) during surgery. The reproductive tract was exteriorized after mid-ventral laparotomy and the ovaries checked for evidence of recent ovulation. Embryos were collected by injecting 20 ml of Dulbecco’s PBS (Gibco BRL, Uxbridge) at 38°C into the oviduct of each horn in turn through a blunt needle inserted into the uterine lumen. The flushing medium, including the embryo, was collected via a catheter inserted at the cervical end of each horn into a sterile tube. Uterine incisions were closed with purse string sutures and the abdominal wall was closed in three layers. Long-acting antibiotic (5 ml Duphasen, i.m.; Solvay Duphar Veterinary, Southampton), Bupivacaine (10 ml, 0.5% (v/v), s.c.; Antigen Pharmaceutical, Roscrea) and non-steroidal analgesic (4 ml of 200 mg Finadyne, i.m.; Schering Plough Animal Health, Welwyn Garden City) were administered to the wound site. Sedation was reversed with yohimbine (0.4 mg kg$^{-1}$, i.v.; Sigma, Poole). Synthetic prostaglandin (500 mg, Estrumate, Coopers Animal Health Ltd, Crewe) was administered i.m. to ensure a return to natural cyclicity. Collection of embryos after animals had been killed was as described by Demmers et al. (1999). Dulbecco’s PBS (20 ml) was used as the flushing medium. Conceptuses were removed from the flushing medium under a dissecting microscope and categorized into four different stages of development depending on size and shape: (1) hatched blastocyst, not yet expanding; (2) expanding blastocyst, ovoid or tubular in shape; (3) early filamentous, long and thin (± approximately 1 mm), and (4) late filamentous, long and wide (approximately 2–5 mm). Flushing medium (4 ml) was transferred into a tube containing 1 ml 5% BSA (Sigma), immediately frozen on dry ice and stored at −70°C for anti-viral assay.

**Interferon anti-viral assay.** Uterine flushings were analysed for anti-viral activity using a cytopathic effect inhibition assay as described by Demmers et al. (1999). Concentrations of the laboratory standard interferon (roIFN-τ, see above) were standardized against the reference reagent for recombinant human interferon-omega (rHuIFN-ω, 87/622) obtained from the National Institute for Biological Standards and Control (Potters Bar). The potency of the laboratory
standard was 283 U ml\(^{-1}\). Wells containing cells without virus (cell controls) and cells plus virus (virus controls) were run on each plate, and the mid-point between these values was used to calculate concentrations of interferon causing a 50% inhibition of the cytopathic effect. These end points were then expressed in terms of the International Standard and corrected for flushing volume. The sensitivity of the assay was 33 U ml\(^{-1}\) (calculated as \(2 \pm SD\) below mean cell controls).

**Experiment 2: administration of roIFN-\(\tau\) to non-pregnant hinds during the luteal phase of the oestrous cycle**

**Animals and treatments.** Two different doses were administered to non-pregnant hinds to mimic embryonic interferon production and determine the effects of roIFN-\(\tau\) on the time of luteolysis. The experiment used 15 mature (2–5 years of age) red deer hinds with a mean live weight of 101.3 ± 4.7 kg, maintained in fenced grass paddocks at ADAS Rosemaund. Oestrus was synchronized in each hind by treatment with CIDR devices as detailed above, inserted on 29 September 1997. The hinds were assigned randomly to one of three groups adjusted for liveweight. Six of the hinds, which failed to ovulate after synchronization of oestrus (assessed on the basis of progesterone profiles), were excluded from further analysis. From day 14 to day 20, hinds were injected i.m. with either 0 (group 1, \(n = 3\)); 0.2 (group 2, \(n = 4\)) or 0.5 mg (group 3, \(n = 2\)) of roIFN-\(\tau\) in saline each day. Injections were given twice a day at 08:00 and 16:00 h. The rectal temperature of hinds in all three groups was measured on day 14 every 2 h for 8 h from the time of the first injection, and at the time of each injection thereafter for the remainder of the treatment period, to confirm the lack of any adverse effects of the treatment. A prolonged increase in temperature would have been taken as an indication of cytokine toxicity.

**Progesterone profile determination.** Blood samples (5 ml) were collected three times a week from the time of CIDR withdrawal until day 34 for subsequent progesterone concentration determination. Hinds were restrained manually in a drop-floor crush for blood sampling by jugular venepuncture into heparinized tubes. Blood was centrifuged immediately at 1500 g for 10 min and the plasma was stored at –20°C until assay. Plasma concentrations of progesterone were determined by solvent-extracted radioimmunoassay validated for deer by Loudon et al. (1990). The sensitivity of the assay was 0.05 ng ml\(^{-1}\) (calculated as \(2 \pm SD\) below mean total binding); the intra-assay coefficient of variation was 11.45% and the interassay coefficients of variation were 5.5 and 6.5% at 1.06 ng ml\(^{-1}\) and 4.07 ng ml\(^{-1}\), respectively.

**Experiment 3: effects of roIFN-\(\tau\) treatment on the success rates of asynchronous embryo transfer**

**Animals and treatments.** Recombinant ovine IFN-\(\tau\) was administered to recipient hinds at the time of embryonic interferon production to determine whether interferon improves success rates after ET. A 4–5 day asynchrony in donor-recipient oestrous cycles was used in the ET experiment, with embryos collected on day 6 from superovulated hinds and transferred on the same day into recipients on days 10 or 11 after synchronization. Embryo transfer experiments were conducted at ADAS Rosemaund over 3 days. Oestrus was synchronized in each hind by treatment with CIDR devices as detailed above. As it was suspected from previous experiments that hinds that had been transferred to the research centre recently were less likely to ovulate normally after CIDR removal, all hinds were acclimatized to their housing conditions for at least 3 months before use.

**Donor hinds.** The CIDR devices were inserted into 15 mature (2–6 years of age) red deer hinds with a mean live weight of 99.9 ± 12.1 kg between 21 and 23 October 1997. Superovulation was induced using a combination of 7.2 mg FSH (with low LH contamination; Ovagen; ICP, Auckland) and 200 IU PMSG (pregnant mares' serum gonadotrophin; Intervet, Cambridge). FSH was given i.m. over eight equal doses, twice daily for 4 days from 72 h before to 12 h after CIDR removal. PMSG was administered i.m. at the time of the last FSH injection, after which hinds were run with a fertile stag. On day 6, embryos were collected from hinds by laparotomy as described for Expt 1. Clenbuterol hydrochloride (0.075 mg, i.v.; Ventipulmin, Boehringer-Ingehielm Ltd, Bracknell) was administered before surgery to ease manipulation of the reproductive tract. The numbers of corpora lutea and large follicles were recorded at the time of embryo recovery. Embryos were flushed from the uterus using Encare flushing medium (20 ml; ICP) at 38°C. Embryos were identified in the flushing medium, immediately transferred into fresh medium (38°C) and held at room temperature. Embryos were assessed microscopically using standard quality assessment techniques for ET (Robertson and Nelson 1998), and the stage of development (number of cells) of the embryo was recorded. Single embryos were transferred into recipient hinds on the same day.

**Recipient hinds.** The CIDR devices were inserted into 30 hinds (3–9 years of age) with a mean live weight of 100.1 ± 1.2 kg between 17 and 19 October 1997. Each hind was given 200 IU PMSG i.m. at the time of CIDR removal to increase ovulation rate (Asher et al., 1992). Embryos were transferred by intra-uterine laparoscopy (McMillan and Hall, 1994) on day 10 or 11 after CIDR withdrawal once it had been established that the hind had recently ovulated. Animals were sedated using xylazine hydrochloride (0.075 mg; Ventipulmin) and ketamine hydrochloride (0.8–1.6 mg kg\(^{-1}\); Ketacet, Fort Dodge Animal Health Ltd, Southampton). Clenbuterol hydrochloride (0.075 mg; Ventipulmin) was administered i.m. before surgery to ease manipulation of the reproductive tract. Abdominal puncture sites were injected s.c. with local anaesthetic (2% (v/v) Lignocaine; C-Vet Veterinary products, Leyland). Embryos were transferred in flushing medium to the uterine horn ipsilateral to the corpus luteum of fasted recipients once it was established that the hind had recently ovulated. Antibiotics (5–10 ml Duphapen, i.m.) and analgesics (3 ml Finadyne, i.m.) were administered postoperatively to the wound site. Sedation was reversed using yohimbine (0.4 mg kg\(^{-1}\), i.v.).
After transfer of day 6 embryos into recipient hinds on day 10 or 11, the recipients were assigned randomly to one of two groups, adjusted for liveweight and balanced for day of transfer into recipients (day 10 or day 11). From day 14 to day 20, hinds were injected i.m. with either 0 mg (n = 12) or 0.2 mg (n = 12) of rolFN-τ in saline each day. Injections were given twice a day at 08:00 and 16:00 h. From 2 weeks after transfer, recipient hinds were run with fertile mature stags to enable hinds to mate naturally during their subsequent oestrous cycles. Hinds were scanned by transrectal ultrasonography on days 42–44, and calving dates were assessed (hinds were observed daily) to determine whether the pregnancy was maintained after ET.

Statistical analyses

Data are presented as mean ± SEM unless otherwise stated. Differences between treatment groups in Expt 2 were analysed by two-way repeated measures ANOVA (progesterone profiles and body temperatures in the first 8 h after the first interferon injection), and one-way ANOVA (average body temperature in each hind over the remainder of the treatment period). Fisher’s exact test was used to analyse calving rates in interferon- or placebo-treated recipient hinds (Expt 3), and Student’s t test was used to compare anti-viral concentrations in flushings collected during different breeding seasons (Expt 1). Data with non-equal variances was compared using Mann–Whitney U test (time of completion of luteal regression in Expt 2), and Kruskal–Wallis one-way ANOVA on ranks (differences in anti-viral activity in uterine flushings with duration of gestation or developmental stage in Expt 1). The time of completion of luteal regression (Expt 2) was defined as the first day after the plasma progesterone concentration decreased to < 1 ng ml⁻¹. All analyses were performed using SigmaStat statistical software (Jandel Scientific, Erkrath).

Results

Experiment 1: timing of interferon production by red deer blastocysts

Embryo development. Single blastocysts were collected from 34 red deer hinds by uterine flushing on days 14 (n = 2), 16 (n = 2), 18 (n = 8), 20 (n = 13) or 22 (n = 9) after CIDR withdrawal. On day 14 after CIDR withdrawal, the two conceptuses collected were spherical hatched blastocysts, with mesoderm visibly differentiating from the inner cell mass (category 1, Fig. 1a). By day 16, blastocysts had commenced the process of expansion and were enlarged and distended, with a prominent embryonic disc (category 2, Fig. 1b). In the latest stages, the blastocyst appeared wrinkled and slightly collapsed (Fig. 1c). By day 18, a proportion of conceptuses had commenced a phase of rapid elongation, as has been described in other domestic ruminants (Rowson and Moor, 1966b). Blastodermic vesicles showed considerable variation in size and shape: four of the eight conceptuses were still expanding blastocysts (category 2, Fig. 1d), but the remainder was early filamentous (category 3, Fig. 1g). By day 20, most of the blastocysts collected were either early or late filamentous (categories 3 or 4) with the embryonic disc visible as a translucent oval shape (Fig. 1e). By day 22, all conceptuses were filamentous and were frequently adhered to the uterus, indicating that the first stage of embryo attachment or implantation had begun. Larger conceptuses were frequently damaged during the flushing process (Fig. 1h).

Anti-viral activity. The production of interferon anti-viral activity by red deer blastocysts is illustrated (Fig. 2). There was no significant difference in the amount of anti-viral activity measured in samples collected in the two breeding seasons, 1995 and 1996 (P > 0.05) and, therefore, data were pooled. Anti-viral activity was first detectable in the uterine flush from a single pregnant hind on day 16 after CIDR removal (2.2 × 10³ U per flush), but was not detectable in flushings from another day 16 hind or from day 14 hinds. By day 18, anti-viral activity was detectable in flushings from seven out of eight samples (3.6 ± 0.7 × 10³ U per flush), and was present in all samples collected on day 20 (10.1 ± 1.8 × 10³ U per flush, n = 13) and day 22 (10.5 ± 2.1 × 10³ U per flush, n = 9). The amount of anti-viral activity increased significantly with duration of gestation (P < 0.01, Fig. 2a), although there was substantial variation at each stage of gestation, especially in later stages. The first appearance of measurable anti-viral activity in uterine flushings coincided with the onset of expansion of the blastocyst (Fig. 2b). Anti-viral activity was not detected in the flushings of hinds pregnant with unexpanded blastocysts (n = 2) but was present in low amounts in six out of eight flushings containing expanding blastocysts (3.4 ± 0.9 × 10³ U per flush, n = 8). Anti-viral activity increased significantly (P < 0.01) with each developmental stage, from expanding to early filamentous (7.6 ± 1.5 × 10³ U per flush, n = 14) and late filamentous (12.4 ± 2.0 × 10³ U per flush, n = 10).

Experiment 2: administration of rolFN-τ to non-pregnant hinds during the luteal phase of the oestrous cycle

Progesterone profiles of non-pregnant hinds treated with 0 (n = 3), 0.2 (n = 4) or 0.5 mg (n = 2) of rolFN-τ daily over the period of blastocyst interferon production (days 14–20) are illustrated (Fig. 3). The time of completion of luteal regression in hinds was significantly delayed by administration of 0.2 mg interferon day⁻¹ (27.3 ± 1.3 days) compared with the controls (21 ± 0 days; P < 0.05). This treatment regimen (0.2 mg interferon day⁻¹ administered from day 14 to day 20) was therefore used in Expt 3. A higher dose of interferon (0.5 mg interferon day⁻¹) did not lead to a significant delay in the time of completion of luteolysis in two hinds (23.5 ± 2.5 days), and was not considered in further analysis of progesterone concentrations. The progesterone profiles of hinds under the two different treatment regimens (0 or 0.2 mg interferon day⁻¹) did not differ from day 5 to day 18, after which time plasma concentrations in the control hinds decreased sharply to < 1 ng ml⁻¹. Progesterone concentrations in hinds on days 20
Fig. 1. Photographs of red deer conceptuses illustrating different stages of development: (a), hatched blastocyst (category 1) collected on day 14 after removal of progesterone-releasing controlled internal drug-releasing (CIDR) devices; (b) and (c) ovoid expanding blastocysts (category 2) collected on days 16 and 20, respectively; (d) tubular expanding blastocyst (category 2) collected on day 18; (e–g) early filamentous blastocysts (category 3) collected on day 20 (e,f) and day 18 (g); and (h) late filamentous blastocyst (category 4) collected on day 20 damaged during the flushing process. Scale bars represent (a) 0.5 mm, (b,c) 1 mm, (d) 2 mm (e–h) 5 mm.
and 23 were significantly higher in interferon-treated hinds than in control hinds \( (P < 0.05) \).

A significant increase in rectal temperature was noted in the 8 h after administration of the first interferon injection in both interferon-treated groups \( (P < 0.05, \text{ data not shown}) \). The temperatures reached peak values 4 h after the first injection in hinds treated with 0.2 mg roIFN-\( \tau \) day\(^{-1} \) \( (39.3 \pm 0.4^\circ \text{C versus 38.4 } \pm 0.2^\circ \text{C in controls}) \), and 8 h after the first injection in hinds treated with 0.5 mg roIFN-\( \tau \) day\(^{-1} \) \( (39.6 \pm 0.3^\circ \text{C versus 38.6 } \pm 0.1^\circ \text{C in controls}) \). Temperatures decreased to normal in both interferon groups 24 h after the first injection \( (39.0 \pm 0.5^\circ \text{C in the 0 mg; 38.9 } \pm 0.2^\circ \text{C in the 0.2 mg and 38.6 } \pm 0.2^\circ \text{C in the 0.5 mg IFN day}^{-1} \text{ group}) \), confirming the absence of any long-term adverse effects of treatment. Rectal temperatures did not vary significantly between the groups for the remainder of the treatment period \( (P > 0.05) \).

**Experiment 3: the effects of roIFN-\( \tau \) treatment on the success rates of asynchronous embryo transfer**

**Embryo recovery and transfer.** All 15 donor hinds showed evidence of recent ovulation, although there was considerable variability in the number of corpora lutea recorded in each hind (mean 6.4 ± 1.43, range 1–20). Viable embryos were recovered from seven of the 15 hinds, with up to 16 transferable embryos collected from a single hind (mean 1.96 ± 1.06 embryos per superovulated hind). The overall embryo recovery rate (the number of embryos collected relative to the total number of corpora lutea) for the experiment was 30.2%. Embryos ranged from the two-cell
stage (n = 1; not transferred) to the 16-cell stage, and 24 were transferred into recipient hinds. Of the recipient hinds, 19 were at day 10 after CIDR withdrawal and 5 at day 11. The hinds were allocated to the two treatment groups (12 animals per group). Two of the day 10 recipient hinds (one from each group) died over the winter months of unrelated causes and were excluded from the study.

**Pregnancy maintenance.** Interferon treatment (0.2 mg roIFN-τ administered daily from day 14 to day 20) significantly increased the calving rate from 0 to 64% in all gestation ranged from 238 to 245 days (mean 240.6 day 10 recipients (0/8 versus 6/9, indicating that these hinds were fertile. They gave birth to live young from a subsequent oestrus cycle, maintained to term, only two (one from each group) failed to give birth to live young from a subsequent oestrous cycle, indicating that these hinds were fertile.

**Discussion**

Analysis of anti-viral activity in uterine flushings from pregnant hinds indicates that the conceptus produces interferon from day 16 to day 22 after CIDR removal. This period corresponds to approximately days 14–20 of gestation, as mating occurs at about 40 h after CIDR removal in red deer (Argo and Loudon, 1992). Anti-viral activity is not detectable in flushings from non-pregnant hinds (Demmers et al., 1999). The production of interferon between day 14 and day 20 of pregnancy in red deer is comparable with interferon production by the conceptuses of other domestic ruminants. In sheep, IFN-τ is produced between day 13 and day 21 of pregnancy (Godkin et al., 1982; Hansen et al., 1985) and in cows between day 16 and day 24 (Bartol et al., 1985). The onset of interferon production coincides with expansion of the blastocyst and maximal production at the late filamentous stage. A large increase in interferon production at about day 18 of pregnancy corresponds to the morphological transition of the red deer blastocyst from a spherical to a filamentous form, as seen in sheep and cows (Farin et al., 1989, 1990, 1991). Thus, the conceptus begins producing interferon approximately 5 days before the time of luteal regression.

Fetal growth in red deer hinds of known gestational age has been described by McMahon et al. (1997) between day 27 and day 55, and by Wendam et al. (1986) and Adam et al. (1988a,b) between day 72 and day 224 of pregnancy. However, until now, conceptus development before day 27 has not been described in red deer of known gestational age. The stages of development of the early red deer conceptus between day 12 and day 20 of gestation are similar to those described for the sheep conceptus (Rowson and Moor 1966b), although delayed by approximately 4 days.

Administration of roIFN-τ to non-pregnant hinds at the time of conceptus interferon production during pregnancy leads to a significant (P < 0.05) delay in luteolysis by approximately 6 days. This is in agreement with previous studies by Bainbridge et al. (1996), in which administration of a related compound, IFN-α,1, between day 13 and day 21 after CIDR removal delayed luteal regression by approximately 10 days. The greater delay in luteal regression in that experiment may be a reflection of the different dose regimens or interferon preparation used. In the present study, administration of roIFN-τ led to transient pyrexia in treated hinds, an effect also seen with administration of IFN-α (Bainbridge et al., 1996). Raised body temperature after administration of IFN-α to cattle has been associated with a decrease in circulating progesterone concentrations (Newton et al., 1990) and reduced fertility (Barros et al., 1992). Although such an effect is not apparent in deer, it is clear that the dose of roIFN-τ used in such experiments is important, as deer may be unusually sensitive to the preparation. Administration of higher doses of roIFN-τ (1–2 mg daily) to red deer in a similar experiment resulted in side-effects, including lethargy and diarrhoea (K. Demmers, unpublished).

Administration of roIFN-τ to recipient hinds after asynchronous ET led to a significant increase (P < 0.005) in calving rate from 0% in placebo-treated hinds to 64% in interferon-treated hinds. The overall calving rate after ET in this study (32%) is low when compared with success rates in commercial red deer ET operations, reflecting the deliberate asynchrony between oestrous cycles in donor and recipient hinds. The asynchrony may have been further increased by the superovulation of donors, as the administration of gonadotrophins is associated with a reduction in the interval between CIDR removal and oestrus (Asher et al., 1992). No pregnancies survived to term after ET into placebo-treated recipients that were synchronized 4–5 days before their respective donors. This result is in agreement with previous experiments in sheep, in which the maximum asynchrony with the uterine environment ovine embryos could tolerate was 2 days (Rowson and Moor, 1966a).

The marked effect of interferon treatment on pregnancy rates in the present study may reflect the deliberate asynchrony in the ET regimen used. Despite the small number of pregnancies carried to term in the ET experiment overall, administration of roIFN-τ ‘rescued’ embryos that would otherwise fail to establish pregnancy. This finding may be due to differences in the time of signal production between when the mother needs to receive the signal to prevent luteolysis, and the period during which it is produced. In this case, the immature transplanted embryos would not produce sufficient interferon to be detected by the mother during the critical period needed for the maternal recognition of pregnancy (Short, 1969). Administered interferon overcomes the shortfall in endogenous embryo production until such time as natural production is sufficient. It is doubtful whether any significant effect of interferon administration would be seen if it was given to recipients after synchronous ET in red deer, as success rates in terms of surrogate pregnancies per donor in commercial programmes are high (Fennessey et al., 1994). Furthermore, the rate of early embryonic loss in red deer is low compared with that in sheep and cows (Peterson et al., 1997).

Previous studies comparing the effects of interferon administration on subsequent pregnancy rate in other ruminants have produced conflicting results. Martinod et al. (1991) found that IFN-α treatment increased pregnancy rates.
in recipient ewes after synchronous ET, but this effect was not significant. Both Nephew et al. (1990) and Schalwe-Francis et al. (1991) found that the lambing rate after natural mating increased with IFN-α treatment but, in the study of Schalwe-Francis et al. (1991), the effect could not be repeated the next season with a group of animals of more normal fertility. However, in heifers, systemic IFN-α treatment led to decreased conception rates (Barros et al., 1992).

In conclusion, the present study has shown that the administration of IFN-τ to recipient hinds after ET increases the success rate of a cervid MOET programme. This shows the potential benefit of interferon treatment where a mismatch between embryo and maternal signalling leads to failure in establishment of pregnancy. Despite the difficulties in extrapolating established techniques to exotic animals due to the variability in physiology of even closely related species, this research has implications not just for cervids, but also for other ruminants (for example, bovids) in which interferon is involved in the maternal recognition of pregnancy. In ET procedures in endangered species, interferon could be administered after the transfer of a lower quality or frozen embryo, or to overcome differences between donor and recipient cycles after transfer between closely related species. Recent work on slow-release mechanisms (L’Haridon et al., 1995) raises the possibility that IFN-τ could be more conveniently introduced into the uterus at the time of ET, thus reducing handling and stress to recipients. In this way, natural interferon production by the conceptus could be augmented until other factors involved in the establishment of pregnancy become important.

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