Production of interferon by the conceptus in red deer *Cervus elaphus*

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A type I interferon secreted by early sheep and cow conceptuses is responsible for the maternal recognition of pregnancy in these species. Interferon-tau (IFN τ) acts locally on the maternal endometrium to prevent luteolysis and prolong progesterone secretion. The production of IFN τ was investigated in early pregnancy in red deer, *Cervus elaphus*. The oestrous cycles of 14 hinds were synchronized using intra-vaginal controlled internal progesterone-releasing devices. Hinds were run with a fertile stag, then slaughtered on either day 20 ($n = 10$) or day 22 after withdrawal of progesterone ($n = 11$). Conceptuses were recovered after uterine excision and flushing with sterile saline. Conceptus RNA was reverse transcribed and amplified by PCR using primers designed from highly conserved regions of ovine and bovine IFN τ genes. The resulting PCR products were cloned and fully sequenced. Sequence comparisons indicate that the transcript characterized is closely related to the IFN τ and interferon-omega genes of bovids and giraffe, showing > 85% nucleotide sequence homology and > 74% predicted amino acid similarity with previously cloned genes. Northern blot analysis of total conceptus RNA using a homologous IFN τ probe confirmed the high expression of IFN τ which is encoded by a transcript of approximately 1 kilobase. Anti-viral activity was measured in uterine flushes from pregnant hinds using a cytopathic effect inhibition assay ($4.3 \times 10^3 \pm 0.78 \times 10^3$ iu ml⁻¹; $n = 14$), but was not detectable in flushes from non-pregnant hinds ($n = 7$), confirming that preimplantation red deer conceptuses release interferons. This is the first demonstration of IFN τ expression in a cervid conceptus and provides evidence that IFN τ may be involved in the maternal recognition of pregnancy in red deer.

Introduction

Many species of deer are rare, endangered or threatened in their natural habitats (IUCN, 1996) and this, together with the increasing importance of commercial deer farming, has led to an interest in applying techniques such as artificial insemination, *in vitro* fertilization and embryo transfer to deer (Waldhalm *et al.*, 1989; Fennessy *et al.*, 1990, 1994; Wildt 1992; Berg *et al.*, 1995). However, embryo transfer experiments have not always led to a high percentage of live births (Waldhalm *et al.*, 1989; Fennessy *et al.*, 1994), indicating a need for further knowledge of the physiological processes underlying early pregnancy in deer.

As is the case in sheep and cattle, the red deer blastocyst remains unattached to the endometrium for a relatively long period after conception. The conceptus must communicate with the mother to prevent regression of the corpus luteum at the end of the luteal cycle, well before the time of blastocyst attachment to the maternal endometrium. In the

domestic bovids, a type I interferon (IFN) secreted by the trophoblast of the developing blastocyst has been identified as the embryonic signal responsible for preventing luteolysis (for review, see Roberts *et al.*, 1992). This trophoblast interferon, interferon-tau (IFN τ), is most closely related to the 172 amino acid IFN-omega (IFN ω) subclass of interferons (Imakawa *et al.*, 1987), and has anti-viral and anti-proliferative properties (Roberts, 1989).

In sheep, the corpus luteum regresses as the result of release of the luteolytic hormone, prostaglandin F_{2 α} (PGF_{2 α}), from the uterine endometrium late in the oestrous cycle (McCracken *et al.*, 1972). A mixture of isoforms of IFN τ is secreted by the blastocyst into the uterine lumen (Godkin *et al.*, 1982, 1984) which suppresses the normal pattern of pulsatile release of PGF_{2 α} (Fincher *et al.*, 1986) through inhibition of expression of oxytocin receptors in the uterine endometrium (Flint *et al.*, 1991). This inhibition results in the prevention of luteolysis, ensuring the continued luteal secretion of progesterone required for the maintenance of pregnancy (Flint *et al.*, 1994).

Characteristics of early conceptus development and endocrinology are not well established for red deer, *Cervus elaphus*. The hormonal basis of maternal recognition of

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pregnancy remains unclear in cervids, although pregnancy is known to be dependent on maintenance of the corpus luteum (Asher *et al.*, 1996) but interferons may be involved in the establishment of early pregnancy (Flint 1995). Systemic administration of exogenous IFN α delays luteal regression in red deer hinds (Bainbridge *et al.*, 1996), as it does in sheep and cattle (Stewart *et al.*, 1989a; Plante *et al.*, 1991). Furthermore, uterine flushings from pregnant red deer hinds contain anti-viral activity, suggesting the presence of IFN (Flint, 1995; Flint *et al.*, 1997).

The aim of the present study was to determine whether IFN τ is expressed by preimplantation red deer blastocysts and to determine, by sequencing of cDNA, whether this protein is similar to that expressed in the Bovidae.

Materials and Methods

Animals

The study was conducted at ADAS Rosemaund Research Centre, Hereford (52°10'N) in autumn 1995. A total of 21 mature (4–7 years of age) red deer hinds (*Cervus elaphus*) with a mean ($\pm$ SEM) live weight of 91.4 ± 2.3 kg were kept in fenced grass paddocks.

Experimental protocol

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, New Zealand). 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, after which time hinds were run with a fertile stag. Animals were slaughtered on either day 20 ($n = 10$) or day 22 ($n = 11$), with the average live-weight of hinds slaughtered on each day being 91–92 kg. CIDR devices were inserted on 3 October 1995 (animals slaughtered on day 20) or 12 October 1995 (animals slaughtered on day 22).

Collection of embryos

The uterus was dissected from the surrounding tissue and the ovaries were checked for evidence of recent ovulation. The uterus was bisected at the cervical end and the two horns were separated. Sterile saline solution (10–20 ml) was injected into the tip of each uterine horn through a blunt needle inserted into the uterine lumen. The flushing solution containing the embryo was collected into a sterile container from the cervical end. The embryo was removed and the saline was frozen immediately on dry ice and kept at -70°C until needed for assay.

PCR and cloning of deer trophoblast interferon

Total RNA (5 μg) extracted (Chomczynski and Saachi, 1987) from blastocysts was primed with random hexamer

primers (3 $\mu\text{g ml}^{-1}$, Gibco BRL, Uxbridge, Middlesex) and transcribed with Superscript RNase H reverse transcriptase (Gibco). Single-stranded cDNA (one-twentieth of total) was amplified by PCR, using the following primers designed from conserved areas of previously sequenced ovine and bovine IFN τ genes: IFN τ sense primer: 5'-GAG AAT TCT CAG YCA GCC CRG CAG C-3' (Y = either C or T, R = G or A); IFN τ anti-sense primer: 5'-CGA AGC TTC AAG GTG AGT TCA GAT CTC C-3'. The expected PCR product size using the IFN τ primers was 635 base pairs (bp). A further sense primer, oTP-1 cDNA 5' primer (after Nephew *et al.*, 1993; 5'-CCT GTC TGC AGG ACA GAA AAG ACT T-3') was used in combination with the IFN τ anti-sense primer, to give an expected PCR product of 444 bp. Primer location relative to a potential cap site of IFN τ cDNA (Nephew *et al.*, 1993) was 30–54 for the IFN τ sense primer, 637–664 for the IFN τ anti-sense primer and 221–245 for the oTP-1 sense primer. PCR was carried out in 25 μl final volume using 0.625 U *Taq* DNA polymerase (Gibco), 2.5 μl 10 $\times$ buffer, 5 pM each primer, 1.5 mmol $\text{MgCl}_2 \text{ l}^{-1}$ and 0.2 mmol l^{-1} of each of the four dNTPs. The following profile was used in the Omnigene Temperature Cycler (Hybaid, Teddington, Middlesex): 40 cycles of 94°C for 30 s, 60°C for 30 s and 72°C for 45 s. PCR products were purified after excision from a 1% low-melting point gel using the US Bioclean MP kit (United States Biochemical, Cleveland, OH). PCR products were cloned into the p-GEM-T Easy Vector (Promega Ltd, Southampton) or pBluescript II KS (Stratagene Ltd, Cambridge) and amplified in JM109 cells (Promega). Plasmid DNA was extracted using the Wizard mini-prep system (Promega) and both strands were fully sequenced using an automatic DNA sequencer using both plasmid and internal primers (ABI 373A, Perkin-Elmer, Applied Biosystems, Foster City, CA). Blastocyst RNA was checked for DNA contamination by PCR under the conditions detailed above without reverse transcription. The Lasergene Software Package was used for sequence manipulation including contig alignment, multiple sequence alignment and sequence comparisons using the Clustal Algorithm (DNASTar Inc., Madison, WI).

Northern blot analysis of trophoblast RNA

Total RNA (20 μg) was separated on a 1% agarose-formaldehyde gel with an RNA marker (Promega) and transferred to Hybond N membrane (Amersham International Plc, Amersham, Bucks) by capillary blotting (Sambrook *et al.*, 1989). A 635 bp PCR product raised from day 20 cDNA using IFN τ sense and anti-sense primers (see above) was labelled using the Megaprime DNA labelling system and [α - ^{32}P]dCTP (Amersham). Pre-hybridization was for 1 h and was followed by hybridization overnight at 42°C in solution containing 50% formamide, 5 $\times$ SSPE (20 $\times$ SSPE is 3.6 mol NaCl l^{-1} , 0.2 mol sodium phosphate l^{-1} , 0.02 mol EDTA l^{-1} , pH 7.4), 5 $\times$ Denhardt's (100 $\times$ Denhardt's is 2% BSA, 2% (w/v) Ficoll and 2% (w/v) polyvinylpyrrolidone), 0.5% (w/v) SDS and 100 $\mu\text{g ml}^{-1}$ denatured salmon sperm DNA (Sigma, Poole). Membranes were washed for 2 $\times$ 10 min at room temperature in 2 $\times$ SSC (0.3% mol NaCl l^{-1} containing 0.03% mol sodium citrate l^{-1}); 0.1% (w/v) SDS; for 1 $\times$ 15 min

at 65°C in 1 × SSC; 0.1% SDS; for 1 × 10 min at 65°C in 0.1 × SSC; 0.1% SDS, and then autoradiographed for 48 h. The RNA blot was washed in boiling 0.1% SDS to remove the probe and was re-probed with an 18S riboprobe (Promega) labelled with [α -³²P]dUTP (Amersham), with hybridization and washing to a stringency of 0.1 × SSC; 0.1% SDS at 65°C and autoradiographed for 12 h.

Anti-viral assay

Uterine flushings were analysed for anti-viral activity using a cytopathic effect inhibition assay (Meager 1987; Abayasekara *et al.*, 1995). Briefly, cultures of Madin–Darby bovine kidney (MDBK) cells were exposed to dilutions of IFN standards or unknown samples before addition of Semliki Forest virus (SFV); the inhibitory effect of IFN on the cytopathic action of SFV was determined by measuring cell density. Each concentration of unknown was assayed in duplicate. All additions were made in culture medium containing 10% (v/v) fetal calf serum, 50 iu penicillin ml⁻¹, 50 µg streptomycin ml⁻¹ and 2 mmol L-glutamine l⁻¹ in minimal essential medium with Earle's salts (Gibco); cultures were at 37°C in 5% CO₂ in air. The lowest concentration of SFV required to cause 100% cell death was initially determined by serial dilutions and culture under assay conditions, and this concentration was used throughout. Concentrations of bovine recombinant interferon- α ₁ (laboratory standard, Ciba-Geigy Ltd, Basel) were standardized against the First International Standard (1987), containing human recombinant IFN α ₁, obtained from the National Institute of Biological Standards and Control (Potters Bar, Herts). The potency of the laboratory standard was 1.57 iu ng⁻¹. Wells containing only cells (no virus controls) and cells plus virus (no IFN controls) were run on each plate and the mid-point between these values was used to calculate concentrations of IFN causing 50% inhibition of cytopathic effect. These end points were then expressed in terms of the International Standard and were corrected for flushing volume. The sensitivity of the assay was 50 iu ml⁻¹.

Statistical analyses

Results are expressed as means $\pm$ SEM. Comparison between amounts of anti-viral activity, measured in flushings from either day 20 or day 22 pregnant deer, was carried out using Student's *t* test. Densitometric scans of northern blots probed with either IFN τ or 18S were analysed by *t* test.

Results

Sequencing

Filamentous blastocysts were collected from five hinds on day 20 and nine hinds on day 22. RNA was extracted from four day 20 blastocysts and three day 22 blastocysts, and cDNA was prepared by reverse transcription. All samples showed amplification of the expected 635 bp product after

PCR with the IFN τ primers, and of the expected 444 bp product using oTP-1 sense and IFN τ anti-sense primer (data not shown). The 635 bp PCR product from a day 20 cDNA sample was cloned into p-GEM-T, and the 444 bp PCR product from a different day 20 cDNA sample was cloned into pBluescript. Both clones were fully sequenced in both directions and the 444 bp sequence showed 100% nucleotide homology with a section of the 635 bp sequence. The blastocyst RNA samples used did not produce amplified products without earlier reverse transcription, indicating that the PCR products were not amplified from contaminating genomic DNA in the samples. The cDNA sequence and deduced amino acid sequence for *Cervus elaphus* trophoblast interferon (cvIFN τ) obtained from the 635 bp clone are shown (Fig. 1). The sequence indicates that cvIFN τ has a 585 nucleotide open reading frame and encodes a putative 23 amino acid signal sequence and a 172 amino acid mature protein that are characteristic of both IFN τ and IFN ω .

Northern blot analysis

Northern blot analysis of total RNA from day 20 and day 22 blastocysts demonstrated the presence of an mRNA species of approximately 1 kilobase (kb) that hybridizes strongly to the [α -³²P]dCTP-labelled IFN τ cDNA (Fig. 2). The relative amount of total RNA in each sample is illustrated by hybridization to the 18S riboprobe. Densitometric scanning showed that the IFN:18S RNA ratio decreased between days 20 and 22 (mean ratio: day 20 = 0.49; day 22 = 0.20), suggesting that IFN mRNA decreased relative to total RNA at this time ($P < 0.05$).

Anti-viral activity

The anti-viral activity measured in uterine flushes is illustrated (Table 1). Uterine flushes from pregnant hinds show significant amounts of anti-viral activity ($4.3 \times 10^3 \pm 0.78 \times 10^3$ iu ml⁻¹; $n = 14$), with those from non-pregnant hinds showing undetectable amounts (< 50 iu ml⁻¹, $n = 7$). No significant difference in anti-viral activity was observed in the uterine flushings collected from day 20 and day 22 pregnant hinds ($P > 0.1$).

Discussion

The presence of significant anti-viral activity in uterine flushes from pregnant hinds indicates that red deer blastocysts release interferons, with amounts reported in the present study being comparable with those measured by Flint *et al.* (1997) from pregnant red deer hinds culled on the Isle of Rum. In domestic ruminants, the presence of IFN τ is reflected in anti-viral activity detected in uterine flushes (Pontzer *et al.*, 1988; Roberts *et al.*, 1989). However, amounts of anti-viral activity in red deer are lower than those measured in bovids, in which concentrations may reach 10⁶ units ml⁻¹ (Flint *et al.*, 1994). Lower anti-viral activity may be consistent with high endometrial sensitivity to IFN τ .

(a)															
30	<u>GAGAATCCTCAGCCAGCCCGGCAGCAGCCTCATCTTCCCC</u>	ATG	GCC	TTC	GTG	CTC									
		Met	Ala	Phe	Val	Leu									S5
85	TCT CTA CTG ATG GCC CTG GTG CTG GCC AGC TAC AGC CCA GGA GGG														
	Ser Leu Leu Met Ala Leu Val Leu Ala Ser Tyr Ser Pro Gly Gly														S20
130	TCC CTG GGC TGT GAC CTG TCT CAG AAC CAT GTG CTG CTT GGC AGG														
	Ser Leu Gly Cys Asp Leu Ser Gln Asn His Val Leu Leu Gly Arg														12
175	CAG AAC CTC AAG CTC CTG GGC CAA ATG ACG AGA CTC TCC CCT CGC														
	Gln Asn Leu Lys Leu Leu Gly Gln Met Thr Arg Leu Ser Pro Arg														27
(b)															
220	<u>TTC TGT CTG CAG GAC AGA AAA GAC TTC GGT CTT CCC CAG GAG ATG</u>														
	Phe Cys Leu Gln Asp Arg Lys Asp Phe Gly Leu Pro Gln Glu Met														42
265	GTG GAG GGC GGC CAG CTT CAG AAG GAC CAG GCC ATC TCT GTG CTC														
	Val Glu Gly Gly Gln Leu Gln Lys Asp Gln Ala Ile Ser Val Leu														57
310	CAC GAG ATG CTC CAG CAA TGC TTC AAC CTC TTC CAC ACA GAG CGC														
	His Glu Met Leu Gln Gln Cys Phe Asn Leu Phe His Thr Glu Arg														72
355	TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC CGC ACT														
	Ser Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Gln Leu Arg Thr														87
400	GGA CTC CAT CAG CAG CTG GAC GAC CTG GAT GCC TGC CTG GGG CAG														
	Gly Leu His Gln Gln Leu Asp Asp Leu Asp Ala Cys Leu Gly Gln														102
445	GTG ATG GGA AAG GAA GAC TCT GAC CTG AGA AGG ATG GGC CCC ACT														
	Val Met Gly Lys Glu Asp Ser Asp Leu Arg Arg Met Gly Pro Thr														117
490	CTG ACC GTG AAG AAG TAC TTC CAG GGA ATC CAC GTC TAC CTG CAA														
	Leu Thr Val Lys Lys Tyr Phe Gln Gly Ile His Val Tyr Leu Gln														132
535	GAG AAG GAA TAT AGC GAC TGC GCC TGG GAA ATC GTC CAA GTG GAG														
	Glu Lys Glu Tyr Ser Asp Cys Ala Trp Glu Ile Val Gln Val Glu														147
580	ATG ATG AGA GCT CTC TCT TCA ATA TCC AGG TTG CAA AAA AGG TTA														
	Met Met Arg Ala Leu Ser Ser Ile Ser Arg Leu Gln Lys Arg Leu														162
(c)															
625	<u>AGA AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA AGCTTCG</u>														
	Arg Lys Met Gly Gly Asp Leu Asn Ser Pro End														173

Fig. 1. The nucleotide sequence of cervine interferon-tau (IFN τ) (numbered at left, after Nephew *et al.*, 1993), showing the deduced amino acid sequence of the polypeptide (numbered at right). The position of a putative signal peptide, numbered S1–S23, is illustrated using bold type. Nucleotide sequences for primers used in PCR are underlined and labelled: (a) IFN τ sense, (b) oTP-1 cDNA 5' and (c) IFN τ anti-sense.

The presence of anti-viral activity in uterine flushings does not prove that a previously uninvestigated species produces blastocyst interferons that act as anti-luteolysins. Production of low concentrations of IFN by early conceptuses (usually detected as anti-viral activity) has been reported in a range of non-ruminant species (Roberts *et al.*, 1990). This finding indicates that IFN production during pregnancy may be a fairly general phenomenon (Leaman and Roberts, 1992) with IFNs playing a role in the viral protection of conceptuses at the time of implantation (D'Andrea *et al.*, 1994). The pig

conceptus spontaneously and intensively secretes IFN between day 12 and day 20 of pregnancy (Cross and Roberts 1989; Miranda *et al.*, 1990). However, in pigs, there is no homologous IFN τ mRNA expression in the conceptus (Charlier *et al.*, 1989), as has been shown in deer. The size of the cvIFN τ mRNA (about 1 kb), as measured by northern blotting, is consistent with the size of IFN τ mRNA in sheep and cattle (Charlier *et al.*, 1989; Stewart *et al.*, 1989b). Amounts of mRNA encoding cvIFN τ are lower in day 22 than day 20 blastocysts, which is in agreement with data

Fig. 2. Northern blot analysis of total RNA from day 20 and day 22 red deer blastocysts probed with (a) IFN τ cDNA probe and (b) 18S riboprobe. Lanes 1 and 2 contain total RNA from day 20 blastocysts, and lanes 3 and 4 contain RNA from day 22 blastocysts. The positions of ribosomal RNA are given as size indicators.

Table 1. Summary of results showing anti-viral activity of uterine flushes (iu ml⁻¹) on day 20 and day 22 after removal of controlled internal drug-releasing devices in pregnant and non-pregnant red deer hinds

	Pregnant	Non-pregnant
Day 20	$3.8 \times 10^3 \pm 8.9 \times 10^2$ (n = 5)	nd (n = 5)
Day 22	$4.7 \times 10^3 \pm 1.1 \times 10^3$ (n = 9)	nd (n = 2)

n, number of hinds; nd, not detectable (anti-viral activity below sensitivity of assay).

from sheep in which amounts of mRNA are maximal on days 14–15 and decrease by day 21 of pregnancy (Hansen *et al.*, 1988; Charlier *et al.*, 1989).

Further evidence that IFN τ functions in the maternal recognition of pregnancy in red deer is provided by the high expression of IFN τ mRNA in the blastocyst and by the similarity between the IFN sequenced here and previously sequenced IFN τ and IFN ω genes from other species. The coding region of the nucleotide sequence of cvIFN τ is > 85% homologous and the predicted amino acid sequence is > 74% homologous with IFN τ from sheep, cows, goats, musk ox and giraffe (Table 2). CvIFN τ also shows high nucleotide sequence similarity with previously sequenced ovine and bovine IFN ω genes in the coding region. The possibility that the IFN sequenced here was derived from a genomic IFN ω

Table 2. Comparison of sequence similarities of IFN τ and IFN ω in Ruminantia

	CvIFN τ	BoIFN τ	OvIFN τ	CaIFN τ	OmIFN τ	GiIFN τ	BoIFN ω	OvIFN ω	Accession number
CvIFN τ		87.4	85.5	85.4	85.4	85.9	87.4	88.8	AJ000638
^a BoIFN τ	81.1		88.8	88.3	88.3	89.5	99.0	84.5	M31556
^b OvIFN τ	78.1	81.6		96.3	93.2	85.5	88.8	82.5	M88773
^c CaIFN τ	74.0	80.6	90.8		94.0	85.0	88.3	82.1	M73243
^c OmIFN τ	75.5	80.6	87.8	88.3		84.7	88.4	83.0	M73244
^d GiIFN τ	78.6	85.2	77.0	75.5	75.5		89.5	82.1	U55050
^e BoIFN ω	81.1	98.0	82.1	81.1	81.1	85.2		84.7	M60913
^b OvIFN ω	80.6	75.0	70.9	68.4	69.4	70.9	74.5		X59067

Percentage nucleotide sequence (upper right) and amino acid sequence (lower left) similarities between each pair of IFN in the coding region are shown. Where multiple clones of IFN τ have been isolated from a single species, the clone that has highest sequence similarity to deer IFN τ was chosen for this comparison. Cv, *Cervus elaphus*; Bo, *Bos taurus*; Ov, *Ovis aries*; Ca, *Capra hircus*; Om, *Ovis moschatus*; Gi, *Giraffa camelopardalis*. Sequence data are from: ^aImakawa *et al.*, 1989, ^bNephew *et al.*, 1993, ^cLeaman and Roberts, 1992, ^dLiu *et al.*, 1996 and ^eHansen *et al.*, 1991.

gene rather than from IFN τ mRNA is unlikely, as genomic DNA contamination of the RNA used was ruled out. However, it is possible that the high amount of mRNA detected in the blastocyst represents an IFN ω also expressed in the adult. Owing to the high expression of IFN mRNA in the red deer blastocyst, and in view of the sequence similarity between IFN τ and IFN ω , the term IFN τ has been used to describe the transcript isolated here.

The similarity of cvIFN τ to ovine and bovine IFN ω may explain why Leaman and Roberts (1992) were unable to isolate an IFN τ gene from white-tailed deer (*Odocoileus virginianus*) genomic DNA by PCR, despite Southern blotting indicating that this species was likely to possess such a gene. The primers used were designed to reduce the probability of amplifying IFN ω genes instead of IFN τ genes.

The predicted primary sequence of cvIFN τ includes cysteine residues at positions 1, 29, 99 and 139. The cysteines at these four sites are conserved in all other mammalian IFN α , IFN ω and IFN τ , except for giraffe IFN τ which lacks Cys99 (Liu *et al.*, 1996), and they form two specific disulphide bridges (Cys1–Cys99 and Cys29–Cys139) in human IFN α (Wetzel, 1981). The cysteine residues at positions 29 and 139 are essential for anti-viral activity (Shafferman *et al.*, 1987). Most IFN τ have a fifth Cys at position 86 which is absent from the cvIFN τ reported here. However, the musk ox IFN τ also lacks this Cys (Leaman and Roberts 1992). In both the musk ox sequence and the sequence reported here, this Cys is replaced by an Arg residue, as it is in sheep IFN ω (Nephew *et al.*, 1993); in bovine IFN ω , there is a Leu at this position (Hansen *et al.*, 1991).

The translation initiation sequence TCCCC is identical to that of bovine IFN α , and the sequence of the signal peptide differs by only two amino acids (Val⁵¹⁴→Ala; Gly⁵¹⁷→Ser) when compared with ovine and bovine IFN τ genes. A peptidase recognition site (Ser-Leu-Gly) is present at the junction between the signal peptide and the mature protein, as in all other IFN τ and IFN ω . CvIFN τ does not contain a potential site for N-glycosylation as do bovine and certain ovine IFN τ (Klemann *et al.*, 1990).

Bovine IFN τ genes are approximately 30, 50 and 75% identical in primary amino acid sequence to the IFN β , IFN α and IFN ω , respectively, within the same species (Roberts *et al.*, 1992). However, whereas the genes for the IFN ω are widely distributed among mammalian species, those for IFN τ seem to be restricted to the Ruminantia suborder of the Artiodactyla and probably evolved from the IFN ω between 30 and 65 million years ago (Leaman and Roberts, 1992). The IFN τ share a high degree of amino acid and nucleotide sequence identity and differ from the IFN ω in the 3' non-coding regions of the genes (Roberts *et al.*, 1991). It remains to be elucidated whether the 3' end of cvIFN τ is more similar to the IFN ω or IFN τ of the Bovidae; and whether IFN τ is expressed only in the trophoblast. An IFN τ gene has been isolated from genomic DNA of giraffe (*Giraffa camelopardalis*), a non-bovid species (Liu *et al.*, 1996). This gene shows > 85% deduced amino acid sequence identity with bovine IFN τ and > 78% with cvIFN τ . Bovids (antelope and domestic species such as sheep, cows and goats), cervids (deer, pronghorns and moschids) and the Giraffidae (giraffe and okapi) are all members of the infra-order Pecora of the Ruminantia (Young, 1981). The presence of

IFN τ in all these groups suggests the evolution of this form of pregnancy signalling before the radiation of the separate families. It would be of interest to know whether the chevrotains express IFN τ ; if they did, this would imply that all Pecoran families have these characteristics.

The oestrous cycle of red deer shows many similarities to that of domestic ruminants, being characterized by well delineated follicular, luteal and luteolytic phases (Asher and Fisher, 1991). The non-pregnant red deer is seasonally polyoestrous, exhibiting regular 18 day cycles (Guinness *et al.*, 1971), with the onset of the breeding season occurring during the autumn. In sheep, oxytocin secreted by the corpus luteum acts at uterine oxytocin receptors to stimulate pulses of endometrial PGF_{2 α} secretion. The PGF_{2 α} in turn acts on the corpus luteum to release further oxytocin in a utero-ovarian positive feedback interaction that culminates in luteolysis (for review, see Flint *et al.*, 1992). The time at which luteal regression occurs is influenced by the time at which oxytocin receptors are generated in the uterus (Sheldrick and Flint, 1985). Spontaneous luteolysis in red deer has been shown to involve synchronous pulsatile secretion of oxytocin and PGF_{2 α} and may be initiated by a similar positive feedback interaction between the uterus and the corpus luteum (Bainbridge and Jabbour, 1997). It is not yet known how IFN acts to prevent luteal regression in red deer, although IFN α treatment suppresses the pre-luteolytic increase in endometrial oxytocin sensitivity (Bainbridge *et al.*, 1996; Bainbridge and Jabbour, 1997). Oxytocin receptor concentrations in the caruncular endometrium of red deer hinds are lower in pregnant than in non-pregnant hinds (Flint *et al.*, 1997). IFN τ in red deer may block the stimulatory effect of circulating oxytocin on secretion of PGF_{2 α} by inhibition of oxytocin receptor gene expression in the endometrium, as occurs in sheep (Mirando *et al.*, 1993) and, as a result, the corpus luteum formed after ovulation becomes the corpus luteum of pregnancy.

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