Administration of recombinant bovine interferon- α_1 at the time of maternal recognition of pregnancy inhibits prostaglandin $F_{2\alpha}$ secretion and causes luteal maintenance in cyclic ewes

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Summary. The antiluteolytic protein, ovine trophoblast protein-1, which is secreted by sheep embryos at about the time of the maternal recognition of pregnancy, exhibits significant structural homology with alpha interferons. Experiments were conducted to examine the effects of intra-uterine and systemic administration of a recombinant bovine interferon- α_1 (rboIFN- α_1) upon the interoestrus interval, endometrial oxytocin receptor concentrations and secretion of prostaglandin (PG) $F_{2\alpha}$ in cyclic ewes.

In Expt 1, each ewe had a cannula placed in the tip of a uterine horn ipsilateral to a corpus luteum, 7 days after an induced oestrus. From day 9 after oestrus until day 19, ewes received either 200 ($n = 4$), 667 ($n = 5$) or 2000 ($n = 9$) $\mu\text{g}/24 \text{ h}$ of rboIFN- α_1 , meclofenamic acid ($n = 4$) or vehicle ($n = 11$). Other ewes received 2000 μg rboIFN- $\alpha_1/24 \text{ h}$ ($n = 5$) between days 12 and 15 only. All ewes were killed on day 19. Mean luteal phase, as determined by daily plasma progesterone measurements, was significantly longer ($P < 0.01$) and mean concentrations of 13,14-dihydro-15-keto PGF $_{2\alpha}$ (PGFM) in plasma were lower ($P < 0.05$) in ewes receiving 667 or 2000 μg rboIFN- α_1 between days 9 and 19, or 2000 μg between days 12 and 15, than in animals from other treatment or control groups. A similar protocol was used in Expt 2, in which further ewes received either 2000 μg rboIFN- $\alpha_1/24 \text{ h}$ ($n = 5$) or vehicle ($n = 5$) by bolus infusions twice a day into one uterine horn. Mean luteal phase was significantly ($P < 0.05$) longer in treated than in control animals, but differences in PGFM concentrations were not significant. In Expt 3, after a synchronized oestrus, ewes received either 2.5 mg rboIFN- α_1 by i.m. injection twice a day between days 12 and 15 ($n = 10$), 2.5 mg rboIFN- α_1 by i.m. injection twice a day between days 9 and 15 ($n = 11$), i.m. injection of vehicle alone twice a day ($n = 20$), or continual intra-uterine infusion of 2 mg rboIFN- α_1/day between days 12 and 15 ($n = 7$). The mean luteal phase of ewes receiving rboIFN- α_1 by intrauterine infusion or i.m. injection between days 9 and 15 was significantly longer than for animals from the other two groups ($P < 0.05$). Concentrations of PGFM in plasma and concentrations of endometrial oxytocin receptors were significantly ($P < 0.05$) lower in ewes with prolonged luteal function than in the other animals. These results indicate that, when administered by either the intra-uterine or systemic routes, rboIFN- α_1 can act as an antiluteolysin, apparently by inducing changes analogous to those reported elsewhere for ovine trophoblast protein-1.

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Introduction

The corpus luteum in non-pregnant ewes begins to regress 12–15 days after oestrus, in response to episodic secretion of the uterine luteolysin prostaglandin (PG) $F_{2\alpha}$ (McCracken *et al.*, 1972; Schramm *et al.*, 1983). By contrast, the functional life of the corpus luteum of pregnancy is maintained well beyond the luteal phase of the oestrous cycle, because of an abrogation of episodic secretion of $PGF_{2\alpha}$ (Barcikowski *et al.*, 1974; Zarco *et al.*, 1988). The inhibition of $PGF_{2\alpha}$ secretion is itself dependent upon the production of an antiluteolytic protein by the embryonic trophoblast (Rowson & Moor, 1967; Martal *et al.*, 1979), which has been variously named trophoblast anti-luteolysin (Rowson & Moor, 1967), trophoblastin (Martal *et al.*, 1979) or ovine trophoblast protein-1 (oTP-1; Godkin *et al.*, 1984a). Ovine trophoblast protein-1 inhibits the increase in concentration of uterine oxytocin receptors associated with luteolysis (Sheldrick & Flint, 1985; Vallet *et al.*, 1988) and blocks oxytocin-induced stimulation of endometrial phosphoinositide turnover (Mirando *et al.*, 1990), thereby causing the attenuation of uterine secretion of $PGF_{2\alpha}$ that is characteristic of early pregnancy.

Analysis of the amino acid sequence of oTP-1 has revealed considerable homology with alpha interferons. More than 70% homology is shown to bovine interferon- ω (interferon- α_1) sequences, including regions that are highly conserved throughout the whole interferon group of proteins. Homology to the sequence of bovine interferon- α_1 is $\approx 40\%$ over the *N*-terminal portion and $> 50\%$ overall (Imakawa *et al.*, 1987; Stewart *et al.*, 1987; Charpigny *et al.*, 1988; Charlier *et al.*, 1989). Because of this structural homology, it was of interest to determine whether recombinant bovine interferon- α_1 (rboIFN- α_1) would be able to extend the interoestrous interval of non-pregnant animals, in a manner analogous to that previously reported for oTP-1 (Vallet *et al.*, 1988). Experiments were therefore conducted to examine the effects of intra-uterine and systemic administrations of rboIFN- α_1 upon duration of the luteal phase, $PGF_{2\alpha}$ secretion and concentrations of endometrial oxytocin receptors in cyclic ewes.

Materials and Methods

Experiment 1

Oestrus was synchronized in 38 Dorset Horn ewes by the use of a fluorogesterone-acetate-releasing intravaginal sponge (Chronogest; Intervet, Cambridge, UK), placed for 13 days. On the day of sponge withdrawal, each ewe was given 320 iu pregnant mares' serum gonadotrophin (Intervet, Cambridge, UK) and 250 μ g cloprostenol (Estrumate; Coopers Animal Health, Crewe, UK) by intramuscular injection. Oestrus was detected by raddled, vasectomized rams, which were introduced 24 h after sponge withdrawal. The day of oestrus was designated day 0. On day 7, each ewe was anaesthetized with thiopentone and anaesthesia maintained by N_2O , O_2 and halothane. A cannula (1.2 mm o.d., 0.9 mm i.d.) was inserted into the uterine horn ipsilateral to a corpus luteum. The cannula was inserted for ≈ 2.5 cm, via a stab incision made just below the utero-tubal junction, and was exteriorized through a sublumbar stab incision. At the same time, a further cannula was inserted into one jugular vein. The ewes then received continual intra-uterine infusions of

- (1) 20 ($n = 2$) or 2000 ($n = 2$) μ g meclofenamic acid (MFA)/day, between days 9 and 19, or
- (2) 200 μ g rboIFN- α_1 /day, between days 9 and 19 ($n = 4$), or
- (3) 2000 μ g rboIFN- α_1 /day, between days 9 and 19 ($n = 4$),
all in 9.6 ml sterile physiological saline (0.154 mol NaCl/l);
or
- (4) 2000 μ g rboIFN- α_1 /day, between days 9 and 19 ($n = 5$), or
- (5) 667 μ g rboIFN- α_1 /day, between days 9 and 19 ($n = 5$), or
- (6) 2000 μ g rboIFN- α_1 /day, between days 12 and 15 ($n = 5$),
all in 7.5 ml sterile 5% dextrose solution.

Control animals received:

- (7) dextrose vehicle, between days 9 and 19 ($n = 6$) or
- (8) saline vehicle, between days 9 and 19 ($n = 5$).

Dextrose and saline vehicles were included as, although dextrose is the preferred vehicle for rboIFN- α_1 , saline is preferable for MFA. Groups of ewes were therefore included to control for this difference. The group receiving MFA, a prostaglandin synthetase inhibitor, was included to act as a positive control. Infusions were made using a battery-operated syringe driver (Graesby Medical Ltd, Watford, UK), held by a harness to the ewe's back.

Experiment 2

Oestrus was synchronized and uterine luminal and jugular venous cannulae placed, by the protocol of Expt 1, in a further ten Dorset Horn ewes. These then received either

- (1) 1000 μ g rboIFN- α_1 dissolved in 2.75 ml sterile 5% dextrose solution, given twice a day between days 12 and 15, as bolus infusions into the uterine horn ipsilateral to a corpus luteum ($n = 5$) or
- (2) 2.75 ml dextrose ($n = 5$) twice a day into the uterine horn ipsilateral to a corpus luteum (control).

Experiment 3

Oestrus was synchronized in a further 46 Dorset Horn ewes, as in Expt 1. On the day of oestrus, a cannula was placed in one jugular vein of each ewe, for blood sample collection. Ewes were then allocated to one of four treatment groups:

- (1) i.m. injections of vehicle (sterile 5% dextrose solution; 2 ml) twice a day, between days 9 and 15 after oestrus (controls; $n = 19$),
- (2) i.m. injections of 2500 μ g rboIFN- α_1 in 2 ml sterile 5% dextrose solution twice a day, between days 12 and 15 after oestrus ($n = 10$),
- (3) i.m. injections of 2500 μ g rboIFN- α_1 in 2 ml sterile 5% dextrose solution twice a day, between days 9 and 15 after oestrus ($n = 11$) and
- (4) continual intra-uterine infusion of 2000 μ g rboIFN- α_1 in 7.5 ml sterile 5% dextrose solution per 24 h, between days 12 and 15 (positive control; $n = 7$). Ewes in this group had uterine luminal cannulae placed as in Expt 1.

Blood samples (5 ml) were collected daily from day 8 (Expt 1 and 2) or from the day of oestrus (Expt 3) for plasma progesterone determination. Further samples (10 ml) were collected hourly for 10 h on days 14 and 15 after oestrus for measuring plasma concentrations of the metabolite 13,14-dihydro-15-keto PGF_{2 α} (PGFM). Ewes were killed on day 19. The reproductive tract was recovered and placed on ice within 10 min of stunning. All ovarian structures were described and any corpora lutea dissected and weighed. Endometrial tissue was also collected, as described by Sheldrick & Flint (1985), for measurements of oxytocin receptors. In Expt 3, rectal temperatures were measured before and every 3 h for 15 h after the start of treatments. Measurements then continued every 6 or 9 h until 36 h after the onset of treatments.

Plasma progesterone concentrations were measured by the radioimmunoassay of Hunter *et al.* (1986). Inter- and intra-assay coefficients of variation were 11.4 and 6.9%, respectively; the limit of sensitivity, using samples of 250 μ l, 0.75 pg/ml and the average recovery of [³H]progesterone after extraction 79.3%. Plasma PGFM concentrations were measured by the radioimmunoassay of Kaker *et al.* (1984). Intra and interassay coefficients of variation were 16.9 and 9.8%, respectively; the limit of sensitivity, using samples of 500 μ l, 28 pg/ml and the average recovery of [³H]PGFM after extraction 78%.

Analysis of data

The length of the luteal phase was defined as the interval between the day of oestrus and the first day on which plasma progesterone concentrations fell to <0.3 ng/ml. The convention adopted for ewes whose progesterone concentrations remained >0.3 ng/ml at the time of death was to count the following day for statistical purposes. These data, which had heterogeneous variances between groups, were subjected to Kruskal–Wallis analysis of variance by ranks. Differences between the numbers of animals in each group that exhibited luteal maintenance at the time of death were analysed by χ^2 tests. Mean concentrations of oxytocin receptors on the day they were killed were subjected to analysis of variance with respect to treatment group.

Mean plasma PGFM concentrations, the number of significant episodes of PGFM secretion and the amplitude and area under the curve of each episode were calculated separately for days 14 and 15. Episodes were defined as any increase of >75 pg/ml above the preceding nadir, with peak concentrations of >100 pg/ml (Parkinson *et al.*, 1991). These data were subjected to analysis of variance, using day and treatment groups as factors, in a model that took into account the repeat observations on each sheep.

The maximum increase in rectal temperature after the onset of treatments was calculated and examined by analysis of variance with respect to treatment group. The time between the onset of treatment and the maximum increase in rectal temperature was subjected to Kruskal–Wallis analysis of variance by rank.

Results

Experiment 1

Results from treatment groups 7 and 8 (controls) were similar and were therefore analysed as one group. Similarly, groups 3 and 4 (2000 µg rboIFN- α_1 per 24 h between days 9 and 19) were analysed together. Groups of ewes receiving 667 or 2000 µg rboIFN- α_1 per 24 h between days 9 and 19, or 2000 µg per 24 h between day 12 and 15 had significantly longer mean luteal phases ($P < 0.01$) and significantly more animals with luteal function maintained until the time of death ($P < 0.01$) than other treatment or control groups (Table 1, Fig. 1).

Table 1. Effects of intra-uterine and systemic administration of recombinant bovine interferon- α_1 (rboIFN- α_1) on mean length of the luteal phase and proportion of ewes with luteal function maintained until death

Treatment	Groups	Mean ($\pm$ SEM) length of luteal phase (days)	Ewes with prolonged luteal function Number	(%)
Experiment 1				
Continuous intra-uterine infusion of:				
Vehicle	7 and 8	16.0 $\pm$ 0.51	1/11	9.1
rboIFN- α_1 (µg)				
200	2	16.0 $\pm$ 0.35	0/4	0.0
667 } on days 9–19	5	19.2 $\pm$ 0.33**	2/5	40.0
2000 } on days 12–15	3 and 4	19.7 $\pm$ 0.22**	7/9	77.8
2000 } on days 12–15	6	19.8 $\pm$ 0.18**	4/5	80.0
Meclofenamic acid (20 or 2000 µg) on days 9–19	1	16.8 $\pm$ 0.22	0/4	0.0
Experiment 2				
Twice a day intra-uterine bolus infusions of:				
Vehicle	2	16.8 $\pm$ 0.52	0/5	0.0
1000 µg rboIFN- α_1	1	19.0 $\pm$ 0.49*	0/5	0.0
Experiment 3				
Twice a day intramuscular injections of:				
Vehicle	1	17.3 $\pm$ 0.33	2/19	10.5
rboIFN- α_1 (µg)				
2500 on days 12–15	2	18.2 $\pm$ 0.43	3/10	30.0
2500 on days 9–15	3	18.6 $\pm$ 0.54**	6/11	54.5
Continual intra-uterine infusion of				
2000 µg rboIFN- α_1 /per 24 h on days 12–15	4	19.1 $\pm$ 0.55**	5/7	71.4

*, **Values are significantly different from controls for that experiment ($P < 0.05$, $P < 0.01$).

Plasma PGFM concentrations showed significantly ($P < 0.01$) different patterns of secretion in the different treatment groups (Fig. 2). Mean concentrations were significantly ($P < 0.05$) lower on days 14 and 15 in ewes receiving 2000 µg rboIFN- α_1 (either between days 9 and 19 or between days 12 and 15) than in control animals and were also significantly ($P < 0.05$) lower in MFA-treated ewes on day 14 than in control ewes on the same day. Ewes receiving 200 µg rboIFN- α_1 per 24 h had higher mean concentrations on day 14 than control animals ($P < 0.05$). Differences between episode amplitudes were only significant with respect to treatment; differences between days were not significant. The mean episode amplitude was higher ($P < 0.05$) in ewes receiving 200 µg rboIFN- α_1 (206.6 pg/ml) than in control ewes and in ewes given other doses of rboIFN- α_1 (overall mean 176.5 pg/ml). Areas under the curve of episodes did not differ significantly either between groups or days.

Fig. 1. Mean ($\pm$ SEM) plasma progesterone profiles of ewes in Expt 1 receiving continual intra-uterine infusions (days of treatment indicated by horizontal bars) of (a) saline ($n = 5$) or dextrose ($n = 6$); (b) 200 ($n = 4$), (c) 667 ($n = 5$), (d) 2000 ($n = 9$) and (e) 2000 μ g recombinant bovine interferon- α_1 per 24 h ($n = 5$); and (f) 20 or 2000 μ g meclofenamic acid ($n = 4$); and of ewes in Expt 2, receiving bolus infusions twice a day (arrows) of (g) dextrose (controls; $n = 5$) and (h) 1000 μ g recombinant bovine interferon- α_1 on days 12–15 ($n = 5$).

No significant differences in concentrations of endometrial oxytocin receptors were noted between groups of ewes, except for ewes that had received 200 μ g rboIFN- α_1 per 24 h. These had significantly ($P < 0.01$) higher (1228 ± 524 fmol/mg protein) concentrations than other groups (overall mean 451 ± 185 fmol/mg protein).

Experiment 2

The mean luteal phase was longer in ewes receiving rboIFN- α_1 by intermittent intra-uterine administration than in saline-treated controls ($P < 0.01$; Table 1b, Fig. 1). None of the parameters of PGFM secretion showed significant differences between groups, although the difference in

Fig. 2. Mean ($\pm$ SEM) concentrations of 13,14-dihydro-15-keto prostaglandin F_{2a} (PGFM) in plasma on days 14 ($\square$) and 15 ($\boxtimes$) after oestrus in ewes receiving continual (a, Expt 1) or intermittent (b, Expt 2) intra-uterine administrations of recombinant bovine interferon- α_1 (rboIFN- α_1); n = number of animals in each group. Within each experiment, values marked * are significantly different from those of control animals on the same day, $P < 0.05$. Treatments as in legend for Fig. 1.

mean concentrations between IFN-treated animals and control ewes approached significance ($0.10 > P > 0.05$; Fig. 2). The mean concentration of oxytocin receptors was significantly lower in treated (94.9 ± 28.0 fmol/mg protein) than in control ewes (456.0 ± 237.0 fmol/mg protein; $P < 0.05$).

Experiment 3

The mean luteal phase was longer and proportions of ewes exhibiting maintenance of luteal function at the time of death were greater (Table 1) in ewes receiving systemic rboIFN- α_1 between days 9 and 15 (group 3), or intra-uterine rboIFN- α_1 between days 12 and 15 (group 4), than in control (group 1) ewes; values for ewes receiving systemic rboIFN- α_1 between days 12 and 15 (group 2) were intermediate.

Overall, mean plasma PGFM concentrations on days 14 and 15 were significantly ($P < 0.01$) lower in ewes in groups 3 and 4 (60.4 ± 5.9 , 55.3 ± 4.4 pg/ml) than in groups 1 and 2 (86.8 ± 4.2 , 92.5 ± 11.6 pg/ml). Differences of amplitude and area under the curve of episodes were not significantly. However, when ewes within each group were separated into those which did, or did not, exhibit luteal maintenance at the time of death, low mean plasma PGFM concentrations were recorded only in those ewes in groups 1 and 2 whose luteal function was maintained until the day they were killed. However, in groups 3 and 4, overall mean values were also significantly ($P < 0.05$) lower than those of controls (Fig. 3). Concentrations of endometrial oxytocin receptors were significantly lower in ewes in groups 2 and 3 than in groups 1 and 4 ($P < 0.05$; Fig. 4).

After the onset of treatments, the mean maximum increase in rectal temperature was significantly lower in cyclic control ewes ($0.47 \pm 0.13^\circ\text{C}$) than in ewes receiving systemic ($1.21 \pm 0.20^\circ\text{C}$; $P < 0.01$) or intra-uterine ($1.08 \pm 0.25^\circ\text{C}$; $P < 0.05$) administration of rboIFN- α_1 (Fig. 5). Maximum temperatures were achieved in 3.0 ± 0.0 h in systemically treated ewes, compared with 6.9 ± 0.5 h in ewes receiving intra-uterine interferon. This difference was statistically significant

Fig. 3. Mean ($\pm$ SEM) concentrations of 13,14-dihydro-15-keto prostaglandin $F_{2\alpha}$ (PGFM) in plasma on days 14 ($\square$) and 15 ($\boxtimes$) after oestrus in ewes in Expt 3, receiving systemic (i.m.) or intra-uterine (i.u.) recombinant bovine interferon- α_1 (rboIFN- α_1) or control material. Ewes are divided into those with luteal function maintained at the time of death (extended) and those without such extension (normal); n = number of animals in that group. Means marked * are significantly ($P < 0.05$) different from that of control ewes with non-extended cycles on the same day.

Fig. 4. Mean ($\pm$ SEM) endometrial oxytocin receptor concentrations on day 19 after oestrus, in ewes receiving systemic (i.m.) or intra-uterine (i.u.) recombinant bovine interferon- α_1 (rboIFN- α_1). Treated animals are divided into those with luteal function maintained at the time of death (extended) and those without such extension (normal); n = number of animals in that group. Values marked * are significantly different from controls where indicated, $P < 0.05$.

($P < 0.001$). Increases in rectal temperature occurred only after the onset of treatment; injections after the first produced no further rise in temperature; in ewes receiving intra-uterine rboIFN- α_1 , the rise in temperature was also associated with the onset, rather than the continuation, of treatment (Fig. 5).

Fig. 5. Mean ($\pm$ SEM) rectal temperatures of ewes receiving (a) vehicle, $n = 19$ or (b) recombinant bovine interferon- α_1 by intramuscular injection (arrows, 2.5 mg twice a day, $n = 21$), or (c) continual intra-uterine infusion (horizontal bar, 2.0 mg per 24 h, $n = 7$) or vehicle ($n = 19$).

Discussion

In ewes, maintenance of luteal function in early pregnancy depends upon embryonic secretion of an interferon-like antiluteolytic protein, oTP-1, from the 12th day after oestrus (Rowson & Moor, 1967; Martal *et al.*, 1979; Hansen *et al.*, 1985; Imakawa *et al.*, 1987). The present results indicate that the luteal phase of cyclic ewes can also be extended by systemic or intra-uterine administration of rboIFN- α_1 at a time equivalent to the maternal recognition of pregnancy.

Ewes given rboIFN- α_1 by continual intra-uterine infusion, between either days 9 and 19 (667 or 2000 μg per 24 h) or days 12 and 15 (2000 μg per 24 h), exhibited a significant ($P < 0.01$) extension of the luteal phase, with suppression ($P < 0.05$) of PGF $_{2\alpha}$ secretion (as determined by peripheral plasma PGFM concentrations). Intermittent administration of rboIFN- α_1 between days 12 and 15 was as effective as continual infusion in promoting luteal maintenance. No other comparable studies have been carried out in sheep, although a similar extension of the luteal phase was observed in heifers that received intra-uterine infusions of rboIFN- α_1 between days 14 and 21 (Plante *et al.*, 1989). The antiluteolytic effect of intra-uterine rboIFN- α_1 administration does, however, resemble results obtained by others using intra-uterine infusions of either crude or purified preparations of oTP-1. Intra-uterine infusion of either day 16 ovine conceptus secretory proteins or purified oTP-1 resulted in luteal maintenance in cyclic ewes, while conceptus secretory proteins from which oTP-1 had been removed were ineffective (Godkin *et al.*, 1984b; Vallet *et al.*, 1988). Whether luteal function is extended after administration of rboIFN- α_1 for as long as with administration of conceptus secretory proteins (up to 52 days; Godkin *et al.*, 1984b) is unclear from the present studies, because they terminated on day 19.

The proportion of ewes that had functional corpora lutea at the time of death was lower after systemic administration of rboIFN- α_1 between days 12 and 15 (22.2%; 2/9) than after intra-uterine treatment over the same period (Expt 3, 71.4%, 5/7; Expt 1, 80.0%, 4/5). More ewes responded to systemic interferon treatment when this started earlier, on day 9 (54.5%, 6/11), although, even so, the proportion remained lower than in ewes receiving intra-uterine infusions. Schaule-Francis *et al.* (1991), who administered rboIFN- α_1 to ewes by the systemic route from day 12, also failed to affect the length of the luteal phase significantly, although the effects of earlier onset of treatment were not examined. Nevertheless, in cattle, Plante *et al.* (1989) found that, if rboIFN- α_1 was administered to cyclic animals from day 14 or 15 (i.e. before day 16, the critical time of maternal recognition of pregnancy in that species; Northey & French, 1980), a significant extension of the luteal phase occurred. The present study therefore supports these results by confirming that systemic rboIFN- α_1 treatment needs to be started before the critical time of maternal recognition of pregnancy if it is to be effective in stimulating luteal maintenance.

Previous investigations have reported that fewer episodes of PGF $_{2\alpha}$ release occur in pregnant than in non-pregnant ewes (Zarco *et al.*, 1988) and that the PGFM responses of cyclic ewes to oxytocin or oestradiol administration are reduced after administration of ovine conceptus secretory proteins (Fincher *et al.*, 1984) or oTP-1 (Vallet *et al.*, 1988). The present experiments indicate that PGF $_{2\alpha}$ secretion is also attenuated in animals receiving systemic or intra-uterine rboIFN- α_1 . It was expected that inhibition of PGF $_{2\alpha}$ secretion would, as in pregnancy (McCracken *et al.*, 1981; Flint & Sheldrick, 1986), be accompanied by suppressed endometrial oxytocin receptor concentrations, but no such clear trend occurred. It is probable that, in the present experiments, differences in receptor concentrations that occurred at the time of luteolysis were obscured by the time at which the tissue was collected (day 19). Subsequent studies have found that, on day 15, oxytocin receptor concentrations are lower in ewes receiving intra-uterine rboIFN- α_1 than in control animals (J. L. Vallet & G. E. Lamming, unpublished observations).

These investigations raise several intriguing questions regarding the mode of action of rboIFN- α_1 . Initially, the similarities between the effects of oTP-1 reported in the literature (see references already mentioned) and the effects of intra-uterine rboIFN- α_1 suggested that both act through the same mechanisms. However, although rboIFN- α_1 and oTP-1 compete for the same endometrial receptor (Hansen *et al.*, 1989; Stewart *et al.*, 1989), recent observations indicate that an additional receptor, unique to oTP-1, may also be present (Hansen *et al.*, 1989). The ability of systemically administered interferon to cause luteal maintenance further complicates such questions, for present knowledge indicates that oTP-1 binds solely to receptors on the luminal surface of the endometrium (Godkin *et al.*, 1984a). The suggestion that systemically administered interferon merely diffuses into the uterus and, thus, acts through luminal receptors would explain why it has to be given before the time of the natural embryonic signal. However, in other investigations, when

rboIFN- α_1 was given by intramuscular injection, it did not appear in uterine luminal flushings (Schaule-Francis *et al.*, 1991), implying that the interferon acts through abluminal receptors. If the tissue distribution of systemically-administered rboIFN- α_1 was solely according to proportionate blood flow, <2% of the systemic dose (i.e. $\approx 100 \mu\text{g}$ per 24 h) of interferon would be delivered to the uterus (see Greiss & Anderson, 1970; Clapp, 1978; Reynolds *et al.*, 1984). This would imply that luteal maintenance was achieved by a lower dose of interferon delivered systemically than required by the intra-uterine route, perhaps also implying an action through a different receptor system to oTP-1. Such a hypothesis may also be invoked to explain the apparent stimulation of PGFM and oxytocin receptor concentrations after administration of the lowest dose of rboIFN- α_1 . Unless merely representing an experimental artefact, these results could imply that rboIFN- α_1 , which acts through a general α -interferon receptor, but not through the specific oTP-1 receptor (Hansen *et al.*, 1989), may exert partial antagonistic effects at low concentrations.

The role of leucocytes in the function of ovarian endocrine tissues is, as yet, poorly understood. Nevertheless, leucocytes are present in pre-ovulatory follicles (Hughes *et al.*, 1990), early corpora lutea (Kirsch *et al.*, 1981) and, particularly, regressing corpora lutea (Donaldson & Hansel, 1965). Leucocytes may be required for luteinization (Murdoch & McCormick, 1989) and for normal subsequent luteal function (Alila & Hansel, 1984), while cytokines may directly affect steroidogenesis (Hughes *et al.*, 1990). Thus, systemically administered interferon may act directly upon such intraluteal leucocytes, without use of uterine oTP-1 or interferon receptors, perhaps by rendering the corpus luteum insensitive to PGF_{2 α} (Silvia & Niswender, 1986).

Intra-uterine infusion of MFA failed to result in maintenance of luteal function until the time of death, although slight suppression of plasma PGFM concentrations was noted. As systemic administrations of MFA to goats (Cooke & Knifton, 1981) prevents luteolysis, intra-uterine infusion was expected to be equally effective. MFA treatment was included to compare the effects of a known prostaglandin synthesis inhibitor with those of rboIFN- α_1 . Hence, the failure to demonstrate luteal maintenance following MFA treatment indicates that either insufficient reached the site of action to suppress PGF_{2 α} synthesis adequately or that, like oxytocin (Parkinson *et al.*, 1991), MFA is only active when given by a systemic route.

These results confirm that rboIFN- α_1 can extend the luteal phase of cyclic ewes, by inhibiting secretion of PGF_{2 α} . rboIFN- α_1 is therefore potentially useful in enhancing the signal for the maternal recognition of pregnancy, particularly as it can stimulate luteal maintenance when administered by a systemic route. These results provide additional evidence for functional, as well as structural, similarities between oTP-1 and the alpha interferons.

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