Stimulation of 2',5'-oligoadenylate synthetase activity in sheep endometrium during pregnancy, by intrauterine infusion of ovine trophoblast protein-1, and by intramuscular administration of recombinant bovine interferon- α_1

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Summary. In Expt 1, activity of 2',5'-oligoadenylate (2',5'-A) synthetase in endometrium collected on Day 16 (oestrus is Day 0) from the uterine horn ipsilateral to the corpus luteum was greater ($P < 0.001$) for pregnant (135.5 ± 1.72 nmol/mg protein/h) than for cyclic ewes (58.5 ± 0.99 nmol/mg protein/h). In pregnant ewes, activity of 2',5'-A synthetase in endometrium collected from the contralateral uterine horn (119.5 ± 1.72 nmol/mg protein/h) did not differ from that of the ipsilateral horn. In Expt 2, three ovariectomized ewes were treated with progesterone for 10 days and then with oestrogen for 2 days. Activity of 2',5'-A synthetase on Day 13 was 18% greater ($P < 0.10$) in endometrium collected from the uterine horn receiving infusions of 30 μ g ovine trophoblast protein-1 (oTP-1) twice a day on Days 10, 11 and 12 (57.7 ± 0.22 nmol/mg protein/h) than from the uterine horn receiving control infusions of serum protein (SP; 48.8 ± 0.22 nmol/mg protein/h). In Expt 3, activity of 2',5'-A synthetase on Day 15 was not significantly greater in endometrium collected from the uterine horn of cyclic ewes receiving infusions of 30 μ g oTP-1 twice a day on Days 12, 13 and 14 (46.5 ± 0.37 nmol/mg protein/h) than in endometrium from the uterine horn receiving infusions of SP (38.2 ± 0.37 nmol/mg protein/h). When results of Expt 2 and Expt 3 were combined, intrauterine infusion of oTP-1 increased ($P < 0.05$) activity of 2',5'-A synthetase in endometrium by 20%. In Expt 4, activity of 2',5'-A synthetase in endometrium collected from cyclic ewes on Day 16 was increased ($P < 0.001$) by i.m. injection of 1 mg recombinant bovine interferon- α_1 twice a day on Days 12, 13, 14 and 15 (993.8 ± 85.9 nmol/mg protein/h) compared with endometrium from ewes similarly treated with placebo (162.1 ± 85.9 nmol/mg protein/h).

These results indicate that activity of 2',5'-A synthetase in endometrium of ewes was increased during early pregnancy, probably by the action of the antiluteolytic conceptus interferon oTP-1. Ewes treated with recombinant bovine interferon- α_1 also had increased endometrial 2',5'-A synthetase activity, indicating that systemic administration of this interferon may directly affect the uterus and enhance fertility rate in ewes.

Keywords: pregnancy; endometrium; 2',5'-oligoadenylate synthetase; ovine trophoblast protein-1; interferon; sheep

Introduction

Ovine trophoblast protein-1 (oTP-1) and bovine trophoblast protein-1 (bTP-1), major polypeptides secreted by peri-implantation conceptuses, are involved in establishment of pregnancy

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(Martal *et al.*, 1979; for review see Bazer *et al.*, 1989, 1991; Thatcher *et al.*, 1989, 1990). These proteins have a high degree of amino acid sequence homology with interferons of the α_{II} subclass (Imakawa *et al.*, 1987, 1989; Stewart *et al.*, 1987, 1989; Charpigny *et al.*, 1988; Charlier *et al.*, 1989); oTP-1 and bTP-1 have potent antiviral activity (Martal *et al.*, 1988; Pontzer *et al.*, 1988; Lifsey *et al.*, 1989; Roberts *et al.*, 1989; Plante *et al.*, 1990; Short *et al.*, 1991) and oTP-1 possesses immunosuppressive (Newton *et al.*, 1989; Fillion *et al.*, 1991) and antiproliferative (Roberts *et al.*, 1989) biological activities characteristic of interferons.

Preparations of bovine conceptus secretory proteins enriched in bTP-1 stimulate cellular 2',5'-oligoadenylate (2',5'-A) synthetase activity *in vitro* (Short *et al.*, 1991). The activity of 2',5'-A synthetase is also greater in endometrium of cows during early pregnancy when conceptus bTP-1 secretion is high (Short *et al.*, 1991). Cellular activity of 2',5'-A synthetase is stimulated by interferons which catalyse increased production of 2',5'-A which, in turn, activates a 2',5'-A-dependent endoribonuclease. The 2',5'-A-dependent endoribonuclease cleaves both viral and cellular RNAs, thereby inhibiting protein synthesis and viral replication within the host cell (see Johnston & Torrence, 1984; Taylor & Grossberg, 1990). However, it is not known whether the antileuteolytic effects of oTP-1 and bTP-1 involve activation of the 2',5'-A synthetase system.

Systemic administration of recombinant bovine interferon- α_1 (rbIFN- α_1) has been reported to increase fertility in ewes (Davis *et al.*, 1990; Nephew *et al.*, 1990; Schalue-Francis *et al.*, 1991), but the mechanism responsible is not known.

The present experiments were conducted to determine whether endometrial 2',5'-A synthetase activity increases during early pregnancy or after intrauterine infusion of oTP-1 in ewes. The possibility that reported increases in fertility resulting from intramuscular injections of recombinant bovine interferon- α_1 (rbIFN- α_1) may involve direct action on the uterus (i.e. increased activity of 2',5'-A synthetase) was also examined.

Materials and Methods

Animals. Ewes primarily of Rambouillet breeding were observed daily for oestrous behaviour using a vasectomized ram; day of onset of oestrus was designated Day 0. Ewes assigned to pregnant status for collection of conceptuses and for Expt 1 were mated to intact rams at detection of oestrus and 10 and 24 h later. Ewes assigned to cyclic status for Expts 1, 3 and 4 were mated to vasectomized rams at detection of oestrus only. Ewes assigned to Expt 2 were ovariectomized for longer than 30 days. Surgeries were performed with ewes under halothane-induced general anaesthesia (Fincher *et al.*, 1986).

Experiment 1. Endometrium was collected from ten cyclic and six pregnant ewes at ovariectomy-hysterectomy on Day 16 during two different breeding seasons. Pregnancy was confirmed by the presence of an apparently normal conceptus after flushing each uterus with 20 ml MEM. Uteri of cyclic ewes were similarly flushed. Endometrium was collected from the uterine horn ipsilateral to the corpus luteum of cyclic ewes and from the ipsilateral and contralateral uterine horns of pregnant ewes.

Preparation of proteins for Experiments 2 and 3. Pregnant ewes were laparotomized on Day 16 after oestrus and conceptuses were collected by flushing the uterus with 20 ml modified Eagle's minimum essential medium (MEM). Conceptuses were cultured as described by Godkin *et al.* (1982). After 30 h, medium was collected, pooled and stored at -20°C . Medium was thawed and oTP-1 was purified as previously described (Vallet *et al.*, 1988). The concentration of oTP-1 was determined by the method of Lowry *et al.* (1951), using bovine serum albumin as standard. OTP-1 was then diluted with 0.9% (w/v) NaCl to an oTP-1 concentration of 30 $\mu\text{g/ml}$ and 1.0 ml aliquants were stored at -20°C .

Blood was collected from the jugular vein of a pregnant ewe on Day 16 and allowed to clot at room temperature (1 h) and then at 4°C overnight. Serum was collected, pooled and dialysed and the protein concentration was determined on pooled, dialysed serum. Serum protein (SP) was then diluted with 0.9% NaCl to 750 or 1470 $\mu\text{g/ml}$, divided into 2.0- or 1.0-ml aliquants, respectively, and stored at -20°C .

Experiment 2. Three ewes, ovariectomized for more than 30 days, received i.m. injections of 50 mg progesterone/day in 1 ml maize oil for 10 days and then 100 μg oestradiol-17 β in 2 ml maize oil for 2 days. On Day 8 after initiation of steroid treatment, ewes were laparotomized and catheters were placed into each uterine horn (Vallet *et al.*, 1988). Each uterine horn was separated with a double ligature placed just above the internal bifurcation. On Days 10, 11 and 12, each ewe received infusions of 30 μg oTP-1 (1 ml) plus 1470 μg SP (1 ml) twice a day into one uterine horn and 1500 μg SP (2 ml) into the other at 08:00 and 17:00 h. Each infusion of protein contained 50 mg ampicillin and was

followed by 1 ml 0.9% NaCl to clear the catheter. Endometrium was collected from both uterine horns of each ewe at hysterectomy on Day 13.

Experiment 3. Three cyclic ewes were laparotomized on Day 10, catheters were placed into each uterine horn and uterine horns were separated with ligatures as previously described. On Days 12, 13 and 14, each ewe received infusions of 30 µg oTP-1 plus 1470 µg SP twice a day into one uterine horn and 1500 µg SP into the other uterine horn as described for Expt 2. Endometrium was collected from both uterine horns of each ewe at hysterectomy on Day 15.

Experiment 4. Twelve cyclic ewes received i.m. injections of 1 mg rIFN- α 1 or vehicle placebo, obtained from Ciba Geigy Limited (Basle, Switzerland), twice a day on Days 12, 13, 14 and 15 during two different breeding seasons (three ewes per treatment per season). Endometrium was collected from both uterine horns of each ewe at hysterectomy on Day 16.

Endometrial activity of 2',5'-A synthetase. Endometrium collected at hysterectomy was stored at -70°C until assayed for endometrial 2',5'-A synthetase activity. Endometrial samples were prepared for analysis by adding minced 0.5 g portions of frozen endometrium to 4 ml of ice-cold buffer containing 20 mmol HEPES/l, 5 mmol MgCl_2 /l, 120 mmol KCl/l, 7 mmol dithiothreitol/l and 10% glycerol (pH 7.5). Samples were homogenized with a polytron homogenizer for 15 s. After homogenization, an additional 4 ml of buffer containing 1% Nonidet P-40 was added. The suspension was mixed and subjected to one freeze-thaw cycle. Supernatant obtained after centrifugation at $12\,000 \times g$ for 5 min was used to assay the enzyme which catalyses conversion of [^{14}C]ATP to [^{14}C]2',5'-oligoadenylate products (Short & Fulton, 1987).

Activity of 2',5'-A synthetase in endometrium collected during the second breeding season in Expt 4 was quantified after further modification of the assay. Nonidet (0.5%, v/v) was included in the homogenizing buffer and samples (2.5 ml homogenizing buffer and 250 µg tissue) were homogenized at a power setting of 40 for 40 s using a Virtishear homogenizer (The Virtis Co., Inc., Gardiner, NY, USA) with a microfine generator. Filter paper discs (Whatman 1) were placed on top of columns of Dowex-1 (Sigma Chemical Co., St Louis, MO, USA) used to separate nucleotide from nucleosides. After application of samples, columns were eluted with three 1-ml portions of water and two 1-ml portions of 0.01M-HCl:1M-NaCl. Radioactivity of each eluate was measured, and counts were normalized to 30 000 dpm per assay vessel.

For each endometrial sample, duplicate determinations were performed in each of two different assays. Intra- and interassay coefficients of variation for samples from ewes in Expts 1–3 and the first season of Expt 4 were 10.3% and 16.9%, respectively. Intra- and interassay coefficients of variation for samples from ewes during the second season of Expt 4 were 13.3% and 16.1%, respectively. Concentration of protein in the supernatant assayed was determined (Lowry *et al.*, 1951) and activity of 2',5'-A synthetase was expressed as nmol of 2',5'-A produced/mg protein/h.

Statistical analyses. Data were subjected to least-squares analysis of variance (ANOVA) using the General Linear Models procedure of the Statistical Analysis System (SAS, 1982). For Expt 1, ANOVA for a randomized block design was performed for a 2×2 factorial arrangement. Reproductive status and uterine horn were main effects and season was the block effect. Reproductive status and uterine horn were confounded because endometrium was collected from only the ipsilateral horn of cyclic ewes, but from both horns of pregnant ewes. For Expts 2 and 3, one-way ANOVA was performed for a randomized block design with protein treatment as the main effect and ewe as the block effect. Data from Expts 2 and 3 were then combined (Snedecor & Cochran, 1980) and a two-way ANOVA was performed; protein treatment and experiment were main effects and the ewe was nested within experiment. For Expt 4, ANOVA was performed for a randomized block design with treatment as the main effect and season as the block effect. All tests of hypotheses were made using the appropriate error terms according to the expectations of the mean squares (Snedecor & Cochran, 1980).

Results

Experiment 1

Mean activity of 2',5'-A synthetase in endometrium collected on Day 16 from the uterine horn ipsilateral to the corpus luteum was 132% greater ($P < 0.001$) for pregnant ewes than for cyclic ewes (Table 1). Activity of 2',5'-A synthetase did not differ between endometrium collected from the ipsilateral and contralateral uterine horns of pregnant ewes.

Experiments 2 and 3

For ovariectomized ewes in Expt 2, mean activity of 2',5'-A synthetase in endometrium collected on Day 13 from the uterine horn infused with oTP-1 tended to be greater ($P < 0.10$) than in endometrium from the SP-infused uterine horn (Table 2). For intact ewes in Expt 3, mean activity of 2',5'-A synthetase in endometrium collected on Day 15 from the uterine horn infused with oTP-1 was greater than in endometrium from the SP-infused uterine horn (Table 2), but this

Table 1. Activity of 2',5'-oligoadenylate synthetase (nmol/mg protein/h) in endometrium from cyclic and pregnant ewes on Day 16 after oestrus (Expt 1)

	Cyclic	Pregnant
Ipsilateral horn	58.5 ± 9.9 ^a	135.5 ± 17.2 ^b
Contralateral horn	—	119.5 ± 17.2 ^b

^{a,b}Mean activity was greater ($P < 0.001$) for pregnant than for cyclic ewes but did not differ between horns of pregnant ewes.

effect was not statistically significant because variation was large and degrees of freedom were few. Across both experiments, intrauterine infusion of oTP-1 increased ($P < 0.05$) activity of 2',5'-A synthetase in endometrium compared with infusion of SP (Table 2).

Table 2. Mean activity of 2',5'-oligoadenylate synthetase (nmol/mg protein/h) in endometrium from ewes infused with serum protein (SP) or ovine trophoblast protein-1 (oTP-1) (Expts 2 and 3)

	SP	oTP-1	% increase	Significance
Ovariectomized ewes (Expt 2) ^a	48.8 ± 2.2	57.7 ± 2.2	18	$P < 0.10$
Intact ewes (Expt 3) ^b	38.2 ± 3.7	46.5 ± 3.7	22	n.s.
All ewes (Expts 2 and 3)	43.5 ± 2.1	52.1 ± 2.1	20	$P < 0.05$

^aThree ovariectomized ewes were treated with progesterone for 10 days and then with oestradiol for 2 days. SP was infused into one uterine horn and oTP-1 was infused into the other uterine horn of each ewe on the last 3 days of steroid treatment. Endometrium was collected at hysterectomy on the day after the last infusions.

^bThree intact ewes received infusions of SP into one uterine horn and oTP-1 into the other uterine horn on Days 12, 13 and 14 after oestrus. Endometrium was collected at hysterectomy on Day 15.

Experiment 4

Mean activity of 2',5'-A synthetase in endometrium collected on Day 16 was greater ($P < 0.001$) for ewes injected with rbIFN- α_1 than for placebo-injected ewes across both years (Table 3). For both treatment groups, greater ($P < 0.001$) 2',5'-A synthetase activity was detected in endometrium collected during the second season than in the first season. This probably resulted primarily from modifications to the 2',5'-A synthetase assay. However, ewes treated with rbIFN- α_1 had a greater increase in endometrial 2',5'-A synthetase activity than placebo-treated ewes during the second season than during the first, resulting in a treatment $\times$ season interaction ($P < 0.001$).

Discussion

The results of the present study indicate that activity of 2',5'-A synthetase in endometrium increased during the peri-implantation period of pregnancy in sheep in association with secretion of

Table 3. Mean activity of 2',5'-oligoadenylate synthetase (nmol/mg protein/h) in endometrium from cyclic ewes^a treated with placebo or recombinant bovine interferon- α_1 (rbIFN- α_1) (Expt 4)

Breeding season	Placebo ^b	rbIFN ^b
1	79.9	115.3
2	244.4	1872.4

^aMean activity was affected by treatment ($P < 0.001$), season ($P < 0.001$) and treatment $\times$ season ($P < 0.001$); s.e.m. = 121.5.

^bEwes received i.m. injections of placebo or 1 mg rbIFN- α_1 twice a day on Days 12, 13, 14 and 15 after oestrus and endometrium was collected at hysterectomy on Day 16.

oTP-1 and inhibition of luteolysis. In cows, activity of 2',5'-A synthetase in endometrium also increases between Days 15 and 18 of pregnancy (Short *et al.*, 1991), when maternal recognition of pregnancy occurs (Northey & French, 1980; Humblot & Dalla Porta, 1984). Increased activity of 2',5'-A synthetase in endometrium of ewes and cows during early pregnancy is probably due to the action of the conceptus interferons, oTP-1 and bTP-1, respectively, because interferons are known to stimulate the 2',5'-A synthetase system (see Taylor & Grossberg, 1990). Further, intrauterine infusion of oTP-1 increased endometrial activity of 2',5'-A synthetase in the present study, and treatment with bTP-1 *in vitro* increased activity of 2',5'-A synthetase in monolayers of cow endometrial stromal and epithelial cells (Short *et al.*, 1991).

Peri-implantation pig conceptuses also secrete proteins which have antiviral activity (Cross & Roberts, 1989; Groothuis *et al.*, 1990; Mirando *et al.*, 1990a) and are capable of stimulating 2',5'-A synthetase activity in Madin Darby bovine kidney cells and bovine endometrial explants (E. C. Short & R. D. Geisert, unpublished observations). During early pregnancy, however, these pig conceptus interferons do not stimulate pig endometrial 2',5'-A synthetase activity (Groothuis *et al.*, 1990; E. C. Short & R. D. Geisert, unpublished observations) and do not appear to be involved in corpus luteum maintenance in pigs (Harney & Bazer, 1989). These results suggest that establishment of pregnancy in pigs does not involve increased endometrial 2',5'-A synthetase activity. Whether increased activity of 2',5'-A synthetase in endometrium of sheep and cattle is involved in establishment of pregnancy in these species remains to be determined.

Systemic administration of rbIFN to cyclic ewes increases endometrial 2',5'-A synthetase activity (present study), but does not extend inter-oestrous interval in sheep when given on Days 12–15 at a dose of 2.0 mg/day (Schalue-Francis *et al.*, 1991; M. A. Mirando, M. A. Davis & F. W. Bazer, unpublished observations) or 2.5 mg/day (Flint *et al.*, 1991). These results suggest that either the 2',5'-A system is not involved in luteolysis or that systemically administered rbIFN- α_1 acts more on different cells (e.g. stromal cells) than does oTP-1 within the uterine lumen (e.g. epithelial cells). In this context, it is noteworthy that treatment with bovine interferons *in vitro* induces greater 2',5'-A synthetase activity in stromal cells than in epithelial cells (Short *et al.*, 1991).

The role of the endometrial 2',5'-A system in preventing CL regression during early pregnancy in ruminants is not known. An intracellular 2',5'-A-dependent endoribonuclease is involved in cleaving viral and cellular RNAs, thereby preventing synthesis of proteins required for replication of viruses within host cells as well as cellular proteins (see Johnston & Torrence, 1984; Taylor & Grossberg, 1990). Increased endometrial activity of 2',5'-A synthetase induced in sheep and cattle by conceptus interferons during early pregnancy may be involved in the transition of endometrial protein synthesis from that of the oestrous cycle to that of early pregnancy (Findlay *et al.*, 1981) via alteration in degradation of mRNA. OTP-1 induces alterations in endometrial protein synthesis (Godkin *et al.*, 1984; Vallet *et al.*, 1987; Salamonsen *et al.*, 1988) and prevents CL regression

by inhibiting oxytocin-induced endometrial phosphoinositide hydrolysis and prostaglandin F-2 α secretion (Vallet *et al.*, 1988; Mirando *et al.*, 1990b). It is possible that changes in endometrial protein synthesis are required during early pregnancy in ruminants to reduce oxytocin-induced prostaglandin F-2 α secretion and prevent luteolysis.

Intrauterine infusion of oTP-1 increases endometrial 2',5'-A synthetase activity (present study), providing additional evidence that oTP-1 is an interferon; oTP-1 has extensive amino acid sequence homology with several known α -interferons as well as the biological activities characteristic of interferons (see Introduction).

Endometrial 2',5'-A synthetase activity is increased more during pregnancy than the increase caused by intrauterine infusion of oTP-1. This may be due to the constant exposure of endometrium to oTP-1 during pregnancy, whereas infusion of oTP-1 ~13–14 h prior to hysterectomy is unlikely to provide continued stimulation of the endometrium during that entire period. Day 15–16 sheep conceptuses are capable of secreting >100 μ g oTP-1/day in culture (Godkin *et al.*, 1982; Ashworth & Bazer, 1989), which may be indicative of synthesis *in vivo*, but only 60 μ g oTP-1 was infused on each day during the present study. Alternatively, conceptus secretory products other than oTP-1 may also increase endometrial 2',5'-A synthetase activity during early pregnancy in sheep.

Activity of 2',5'-A synthetase in endometrium of pregnant ewes did not differ between the uterine horns ipsilateral and contralateral to the corpus luteum. This is in contrast to results from cows which indicated that 2',5'-A synthetase increased between Days 15 and 18 of pregnancy in endometrium from the ipsilateral horn, but was not increased in endometrium from the contralateral horn (Short *et al.*, 1991). Increased endometrial 2',5'-A synthetase activity in both uterine horns of pregnant ewes may be due to elongation of conceptuses into the contralateral uterine horn by Day 16 (Chang & Rowson, 1965) and apparent secretion of adequate oTP-1 into that uterine horn to stimulate 2',5'-A synthetase activity. In cows, few conceptuses elongate into the contralateral uterine horn by Day 18 (Chang, 1952; Geisert *et al.*, 1988; Short *et al.*, 1991; see Bazer & First, 1983). Therefore, sufficient bTP-1 may not have been secreted into the contralateral horn to increase endometrial 2',5'-A synthetase activity significantly (Short *et al.*, 1991).

The reason for the presence of 2',5'-A synthetase activity in endometrium of cyclic ewes is not known, but its presence is not unusual, because 2',5'-A synthetase activity was also detected in endometrium of cyclic cows (Short *et al.*, 1991) and in a variety of cells and tissues which were not challenged with virus, double-stranded RNA or interferon (Hearl & Johnston, 1985; Laurence *et al.*, 1985; Short *et al.*, 1991). Endometrial 2',5'-A synthetase activity in cyclic ewes and cows may be induced by circulating interferons detected in blood from the uterine artery of cyclic ewes (M. A. Mirando & F. W. Bazer, unpublished observations) and cyclic gilts (Mirando *et al.*, 1990a).

The mechanism whereby systemic administration of rbIFN- α_1 enhanced fertility rates in previous studies (Davis *et al.*, 1990; Nephew *et al.*, 1990; Schalue-Francis *et al.*, 1991) is not known, but it does not appear to be identical to that by which oTP-1, secreted by the conceptus or infused into the uterus, inhibits corpus luteum regression, because systemic administration of rbIFN- α_1 to cyclic ewes does not extend interoestrous intervals (Flint *et al.*, 1991; Schalue-Francis *et al.*, 1991; M. A. Mirando, M. A. Davis & F. W. Bazer, unpublished observations) or result in detectable antiviral activity within the uterine lumen (Schalue-Francis *et al.*, 1991) when given on Days 12–15 and at a dose (2.0–2.5 mg/day) which increased fertility in ewes (Davis *et al.*, 1990; Nephew *et al.*, 1990; Schalue-Francis *et al.*, 1991). Alternative mechanisms for increased fertility in ewes treated with rbIFN- α_1 include (i) inhibition of luteolysis indirectly by acting on follicular development or directly by acting on the corpus luteum; (ii) increased conceptus development and/or oTP-1 secretion; or (iii) enhanced action of conceptus-secreted oTP-1 on the endometrium. The mechanism does not appear to involve stimulation of conceptus secretion of oTP-1 (Davis *et al.*, 1990). Systemic administration of rbIFN- α_1 may enhance the antiluteolytic action of oTP-1 because interoestrous intervals are increased (Nephew *et al.*, 1990; Davis *et al.*, 1990), and plasma

progesterone concentrations are maintained (M. A. Davis & F. W. Bazer, unpublished observations) in bred-nonpregnant rbIFN- α_1 -treated ewes, but not in bred-nonpregnant placebo-treated ewes. Systemic administration of rbIFN- α_1 did extend interoestrous intervals and maintain concentrations of progesterone in plasma of cyclic cows (Plante *et al.*, 1989). The reason for the apparent antiluteolytic effect of rbIFN- α_1 in cows is not known, but may be related to the degree of sequence homology, which may be slightly greater with bTP-1 than with oTP-1 (Stewart *et al.*, 1990; Flint *et al.*, 1991). However, administration of 2.5 mg rbIFN- α_1 /day to cyclic ewes on Days 9–15 extended interoestrous intervals by ~ 1.5 days (Flint *et al.*, 1991).

Results of the present study indicate the activity of 2',5'-A synthetase in endometrium of ewes is increased during early pregnancy and by intrauterine infusion of oTP-1. These results also indicate that systemic administration of rbIFN- α_1 to ewes stimulates endometrial 2',5'-A synthetase activity. Whether increased endometrial 2',5'-A synthetase activity is involved in establishment of pregnancy in sheep remains to be determined.

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