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Effect of ovine conceptus secretory proteins and purified ovine trophoblast protein-1 on interoestrous interval and plasma concentrations of prostaglandins F-2α and E and of 13,14-dihydro-15-keto prostaglandin F-2α in cyclic ewes*

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Summary. Conceptus secretory proteins (oCSP) were obtained from medium in which sheep conceptuses, collected on Day 16 of pregnancy, were cultured for 30 h. A portion of the culture medium (500 ml) was prepared for intrauterine infusion by concentrating the proteins by Amicon ultrafiltration (M_r 500 cutoff). A second portion (500 ml medium) was used to purify sheep trophoblast protein one (oTP-1). Proteins remaining after oTP-1 purification were concentrated and then passed through an anti-oTP-1 sepharose CL-4B affinity column to remove any remaining oTP-1 (oCSP − oTP-1). Serum proteins (oSP) were collected from a Day-16 pregnant ewe and diluted for infusion. Catheters were placed in the uterus of cyclic (Day 10) ewes. The following combinations of proteins were infused: 0·75 mg oCSP + 0·75 mg oSP (5 ewes), 0·75 mg oCSP − oTP-1 + 0·75 mg oSP (4 ewes), 0·05 mg oTP-1 + 1·45 mg oSP (5 ewes) and 1·5 mg oSP only (5 ewes). Infusions were twice daily on Days 12 and 13 (08:00 and 17:00 h) and once on Day 14 (08:00 h). On Day 14, ewes were injected intravenously at 08:00 h with 0·5 mg oestradiol-17β. Blood sampling began 30 min before oestradiol injection and continued every 30 min for 10 h. On Day 15 ewes received 10 i.u. oxytocin intravenously (08:00 h). Blood samples were collected 10 min before oxytocin and every 10 min for 1 h after oxytocin injection. Concentrations of prostaglandin (PG) F, PGE-2/PGE-1 (PGE) and 13,14-dihydro-15-keto-PGF-2α (PGFM) were measured by specific radioimmunoassay. Ewes treated with oTP-1 and oCSP had longer ($P < 0·05$) interoestrous intervals (27 and 25 days, respectively) compared to ewes treated with oSP and oCSP − oTP-1 (19 and 19 days, respectively) (s.e.m. = 1·56 days). These results indicate that oTP-1 alone is as potent as total conceptus secretory proteins in extending luteal maintenance. Ewes treated with oTP-1 and oCSP had no increase in PGF after oestradiol injection while production of PGF did increase 6–10 h after oestradiol in ewes treated with oSP and oCSP − oTP-1. PGFM was correlated with PGF concentrations ($r = 0·57$, $P < 0·01$) although presence or absence of increases in production of PGFM for the treatment groups were not the same as those for PGF. No effects of treatment on PGE were detected. Ewes treated with oTP-1 had lower ($P < 0·05$) PGF and PGFM values after oxytocin injection than did ewes in the other 3 treatment groups ($P < 0·05$). PGFM and PGF values were correlated ($r = 0·69$, $P < 0·01$). No differences in PGE values were detected between the 4 treatment groups

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after oxytocin injection. We conclude that oTP-1 alone prevents luteal regression during early pregnancy in ewes by inhibiting uterine production of luteolytic amounts of PGF in response to oestradiol and oxytocin.

Keywords: prostaglandin F-2α; prostaglandin E-2; conceptus; pregnancy; proteins; sheep

Introduction

Establishment of pregnancy in the ewe requires maintenance of a functional corpus luteum (CL) until at least Day 50 of pregnancy (Denamur & Martinet, 1955; Wiltbank & Casida, 1956). A conceptus must be present in the uterus by Day 12 or 13 of pregnancy or the CL regresses at the normal time (Day 15–16) and the ewe returns to oestrus (Moor & Rowson, 1966). Rowson & Moor (1967) and Martal *et al.* (1979) demonstrated that homogenates of Day-14–16 conceptuses prolonged CL maintenance when infused into the uterine lumen, while homogenates of Day-24 conceptuses were ineffective. The bioactive material was assumed to be a protein and was called trophoblastin.

The major secretory proteins produced by the sheep conceptus *in vitro* were first described by Wilson *et al.* (1978) and later characterized by Godkin *et al.* (1982). The major protein produced is an acidic protein of M_r 19 000 named ovine trophoblast protein one (oTP-1) by Godkin *et al.* (1984a) or ovine trophoblast protein B (Martal *et al.*, 1984). Purified oTP-1 or the total array of sheep conceptus secretory proteins (oCSP) extended luteal maintenance when infused into the uterine lumen of cyclic ewes: oTP-1 extended the cycle for only 4–5 days, while ewes receiving oCSP maintained CL for at least 25 days (Godkin *et al.*, 1984a). This result suggested that: (1) other proteins in oCSP might also be important in luteal maintenance; (2) oTP-1 was altered during purification; or (3) oTP-1, when infused alone, underwent rapid proteolytic degradation.

The uterine luteolysin in the ewe is assumed to be prostaglandin (PG) F (Goding, 1974). The use of exogenous oestradiol and oxytocin to test for potential inhibition of luteolysis due to an alteration of PGF secretion is based on the hypothesis proposed by McCracken (1980) whereby oestrogen from developing follicles increases uterine endometrial oxytocin receptors. The uterine endometrium then becomes responsive to oxytocin, released from the neurohypophysis and/or CL, which induces synthesis and release of PGF. Evidence supporting this hypothesis indicates that: (1) destruction of follicles causes a delay in luteal regression (Karsch *et al.*, 1970); (2) immunization against oxytocin (Sheldrick *et al.*, 1980; Schams *et al.*, 1983) increases cycle length; and (3) 97% of all episodes of PGF production by the uterus coincide with peaks of oxytocin in the blood (Hooper *et al.*, 1987).

Total oCSP or serum protein was infused into uteri of cyclic ewes on Days 12, 13 and 14 of the oestrous cycle and effects on uterine production of PGF were tested by injecting oestradiol or oxytocin and measuring peripheral plasma concentrations of 13,14-dihydro-15-keto-PGF-2α (PGFM) (Fincher *et al.*, 1986), a stable metabolite of PGF correlated with production of PGF from the uterus (Lewis *et al.*, 1977; Louis *et al.*, 1977). Intrauterine infusion of oCSP inhibited uterine production of PGF in response to exogenous oestradiol and oxytocin (Fincher *et al.*, 1986). Similarly, uterine production of PGF in response to exogenous oestradiol (Fincher *et al.*, 1986) and oxytocin (Fairclough *et al.*, 1984) is inhibited during pregnancy. These results suggest that oCSP secreted by the conceptus during pregnancy directly or indirectly inhibits uterine production of PGF and, therefore, luteolysis.

Estimates of uterine production of PGF in pregnant and non-pregnant ewes have been inconsistent. Some authors reported that pregnancy decreased uterine production of PGF (Thorburn *et al.*, 1975; Peterson *et al.*, 1976), others found that pregnancy increased secretion of PGF from the uterus (Wilson *et al.*, 1972; Lewis *et al.*, 1977), while others found no change (Pexton *et al.*, 1975). Those who reported no alteration in PGF production during pregnancy implicated PGE-2 in luteal maintenance since PGE-2 is capable of temporarily inhibiting luteolysis caused by PGF

(Henderson *et al.*, 1977; Pratt *et al.*, 1977). Silvia *et al.* (1984) reported that concentrations of PGE-2 in uterine venous plasma increased between Days 13 and 15 of pregnancy. Conceptus proteins may, therefore, affect prostaglandin synthesis and metabolism to favour PGE-2. This could account for the decrease in PGF reported by Fincher *et al.* (1986).

The objectives of the present experiment were to determine whether: (1) oTP-1 alone in oCSP is responsible for extension of the interoestrous interval, (2) oTP-1 and oCSP inhibit uterine production of PGF and (3) oTP-1 or oCSP alter metabolism of PGs to favour production of PGE-2.

Materials and Methods

Protein preparation. Ewes of primarily Rambouillet breeding were observed daily for oestrus using a vasectomized ram and mated with intact rams at 12-h intervals until the end of oestrus. Ewes were laparotomized on Day 16 of pregnancy and conceptuses collected by flushing the uterus with 20 ml sterile Minimal Essential Medium (MEM, Gibco, Grand Island, New York, 14072, U.S.A). The conceptuses were cultured for 30 h in MEM according to the method of Godkin *et al.* (1982). Medium was collected, pooled and stored at −20°C until further processing. One conceptus was cultured for 30 h in MEM lacking leucine and with the addition of 50 μCi [4,5-^{3}H]leucine (sp. act. 120–190 Ci/mmol: Amersham, Arlington Heights, IL). This medium was used as a radiolabelled tracer for purification of oTP-1 from pools of radioinert oCSP.

Pooled oCSP from 70 cultures was dialysed against 4 litres 10 mM-Tris–HCl, pH 8·2, changed three times, and then partitioned for processing to obtain proteins for infusion. About 500 ml MEM containing oCSP were concentrated to one-tenth the original volume using an Amicon ultrafilter (Amicon Co., Danvers, MA) with a cutoff of M_r 500. The concentrated oCSP was dialysed against 0·9% (w/v) NaCl and protein concentration was determined (Lowry *et al.*, 1951) using bovine serum albumin as the standard. The oCSP was then diluted to 0·75 mg/ml with saline and stored as samples of 1 ml for infusion.

Purification of oTP-1 from 500 ml medium was by the method of Godkin *et al.* (1982). The oTP-1 was examined by sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and Coomassie brilliant blue staining. The oTP-1 was dialysed against 0·9% (w/v) NaCl and protein concentration was determined (Lowry *et al.*, 1951). The oTP-1 was then diluted to 50 μg/ml and samples of 1 ml were used for infusion.

The oCSP which remained after oTP-1 purification was pooled and concentrated to one-tenth the original volume by Amicon ultrafiltration (M_r 500 cutoff) and remaining oTP-1 was removed by affinity chromatography. Immunoglobulin-G (IgG) (90 mg) was purified from oTP-1 specific rabbit antiserum by using Protein A coupled to Sepharose Cl-4B (Sigma Chemical Company, St Louis, MO). The IgG was linked to CNBr-activated Sepharose CL-4B (Sigma Chemical Co.) and anti-oTP-1 Sepharose CL-4B was poured into a 10 × 1·5 cm column. Concentrated oCSP lacking oTP-1 (oCSP − oTP-1) was dialysed against 0·1 M-phosphate buffered saline (pH 7·4) and then repeatedly passed through the anti-oTP-1 affinity column. The oTP-1 was eluted from the affinity column with 50 mM-glycine, 200 mM-NaCl, pH 2·3, after each pass. After each pass, the oCSP − oTP-1 (100 μg) was examined by SDS-PAGE and silver staining to determine whether all of the oTP-1 had been removed. When no oTP-1 could be detected by silver staining, oCSP − oTP-1 was considered free of oTP-1. The oCSP − oTP-1 was dialysed against saline, protein concentration was measured (Lowry *et al.*, 1951) and the samples were diluted to 0·75 mg/ml and used as 1-ml volumes for infusion.

Blood was collected from the jugular vein of a Day-16 pregnant ewe and allowed to clot at room temperature (1 h) and then at 4°C (overnight). Serum for infusion was collected, dialysed against saline and the protein concentration was determined (Lowry *et al.*, 1951). Serum proteins (oSP) were diluted to 1·45 mg/ml or 0·75 mg/ml depending on the dilution required for infusion and stored as 1 ml samples at −20°C until used.

Intrauterine infusions. On Day 10 of the oestrous cycle 19 ewes of primarily Rambouillet breeding were laparotomized. A catheter (V6 tubing, Bolab, Lake Havasu City, AZ) was placed into each uterine horn via the oviduct and extended into the uterine lumen to about 3 cm below the utero-tubal junction. A catheter was also placed into the inferior vena cava via the femoral vein with the tip of the catheter between the point of entry of the utero-ovarian vein into the inferior vena cava and the liver. Catheter placement was confirmed by palpation. The CL present at Day 10 were marked with India ink. All catheters were exteriorized through the left flank and maintained, wrapped in a Betadine-soaked gauze sponge, in a bag stitched to the skin of the ewe. The venous catheter was flushed daily with 2 ml heparinized saline (200 i.u./ml).

Proteins were infused twice on Days 12 and 13 (08:00 and 17:00 h) and once on Day 14 (08:00 h). Protein infusions per uterine horn contained: (1) 0·75 mg oCSP + 0·75 mg oSP (oCSP, 5 ewes); (2) 0·75 mg oCSP − oTP-1 + 0·75 mg oSP (oCSP − oTP-1, 4 ewes); (3) 0·05 mg oTP-1 + 1·45 mg oSP (oTP-1, 5 ewes) and (4) 1·5 mg oSP (oSP, 5 ewes). All infusions contained 50 mg ampicillin to prevent uterine infection. Each infusion was followed by 1 ml 0·9% (w/v) sterile NaCl to clear the catheter. Total infusion volume was 3·2 ml/horn.

Each ewe received an intravenous injection of 0·5 mg oestradiol on Day 14 (08:00 h) and an intravenous injection of 10 i.u. oxytocin on Day 15 (08:00 h). Blood samples (10 ml) were collected every 30 min beginning at 07:30 h on Day 14 and for 10 h after oestradiol injection. On Day 15 blood samples were collected using a Harvard withdrawal pump (Harvard Apparatus Co., Dover, MA) every 10 min from 10 min before oxytocin injection to 1 h after the oxytocin injection. Blood samples were acidified immediately after collection with 1 ml 0·1 M-HCl (Lewis *et al.*, 1977).

Heparinized saline (1 ml, 200 i.u./ml) present in the catheter prevented samples from clotting. After each blood sample the catheter was flushed with 2 ml heparinized saline. Blood samples were centrifuged at 1200 *g*, plasma was collected and stored at −20°C until assayed for PGs.

After blood collection on Day 15 each ewe was checked daily for oestrus by using a vasectomized ram. On Day 25, ewes were returned to surgery to confirm catheter placement, remove catheters and examine marked CL. The interoestrous interval was recorded for each ewe. Ewes not returning to oestrus were not included in the analysis of interoestrous interval data.

Radioimmunoassay of plasma samples. All blood samples were assayed for PGF, PGE and PGFM using specific radioimmunoassays. The PGFM assay has been validated previously (Fincher *et al.*, 1986). Cross-reactivity of the antibody with other PGs was reported by Guilbault *et al.* (1984). Two antibodies were used for measuring PGF as one antibody became unavailable. Cross-reactivity of the first and second antibodies with other PGs have been reported elsewhere by Knickerbocker *et al.* (1986) and Kennedy (1985), respectively. Both assays were validated for sheep plasma. Values in volumes of 20–200 μl of a random sample were parallel to the standard curve. Addition of 50–5000 pg PGF to aliquants of a random sample gave a regression of amount added (x = pg) to amount measured (y = pg) of $y = 102 + 0{\cdot}954x$ ($R^2 = 0{\cdot}98$, $P < 0{\cdot}01$). For the second assay, addition of 50–1000 pg PGF to aliquants of a random sample gave a regression of amount added on amount measured of $y = -11{\cdot}0 + 0{\cdot}95x$ ($R^2 = 0{\cdot}95$, $P < 0{\cdot}01$). Cross-reactivity of the PGE antibody has been reported by Lewis *et al.* (1978). The antibody has 20% cross-reactivity with PGE-1, and so the results are presented as PGE. To validate the assay for sheep plasma, 20–200 μl volumes of a random plasma sample were assayed and parallelism to the standard curve was demonstrated. Addition of 50–1000 pg PGE to aliquants of a random plasma sample gave a regression of amount added (x) to amount measured (y) of $y = -10{\cdot}1 + 0{\cdot}94x$ ($R^2 = 0{\cdot}96$, $P < 0{\cdot}01$). Due to haemolysis, some samples were acidified with 20% 1 M-HCl and then extracted with 4 ml ethyl acetate. The ethyl acetate was dried under nitrogen and then the PGE was redissolved in plasma collected from a wether which had received two injections at 12-h intervals of banamine (100 mg/injection; Schering Veterinary, Kenilworth, NJ) to inhibit synthesis of prostaglandins. Samples were then assayed as described for the other samples. The assay requiring extraction was also validated. Samples of 100–1000 μl plasma demonstrated parallelism to the standard curve. Addition of 50–2500 pg of PGE to aliquants of a random sample gave a regression of amount of PGE added (x) on amount PGE measured (y) of $y = 190 + 1{\cdot}06x$ ($R^2 = 0{\cdot}92$, $P < 0{\cdot}01$).

Interassay and intra-assay coefficients of variation for the radioimmunoassays were 16·8% and 7·7% for the first PGF antibody, 18·4% and 9·3% for the second PGF antibody, 29·0% and 15·7% for the PGE assay without extraction and 11·33% and 5·36% for the PGFM assay, respectively. Samples to be extracted (PGE) were measured in a single assay with an intra-assay coefficient of variation of 20·1%. Sensitivities of the PGF, PGFM and PGE assays were all 50 pg/ml.

Statistical analyses. Data were analysed by the method of least squares analysis of variance using the general linear models procedures of the Statistical Analysis System (SAS Institute Inc., Cary, NC). Tests of homogeneity of regression were performed to detect differences in patterns of secretion of PGs over the sampling period for the 4 treatment groups. Data were analysed untransformed and then log transformed to alleviate possible heterogeneity of regression errors. Residual variances for each of the 4 treatment groups, in a mathematical model which included effects of ewes and time, following oestradiol and oxytocin injections, were examined to determine possible treatment effects on variances and to determine whether log transformation was effective in controlling heterogeneity.

Results

Protein preparation

A representative SDS-PAGE gel of purified oTP-1 used in this experiment is presented in Fig. 1 and indicates a single protein of M_r 19 000. A silver-stained SDS-PAGE gel of oCSP − oTP-1 (Lane 2) and oCSP (Lane 3) used in this experiment is presented in Fig. 2 to demonstrate that there was no detectable oTP-1 in the oCSP − oTP-1.

Interoestrous interval

Ewes infused with oTP-1 and oCSP had interoestrous intervals of 27·0 and 25·3 days, respectively, which did not differ from each other, but were longer ($P < 0{\cdot}05$) than for ewes treated with oCSP − oTP-1 (19·3 days) or oSP (19·3 days). The pooled standard error of the mean from analysis of variance for interoestrous interval was 1·56 days.

Concentrations of PGs after oestradiol

Least squares means for PGF, PGFM and PGE in plasma after injection of oestradiol for each of the treatment groups are presented in Fig. 3. Significant differences in amounts of PGs secreted

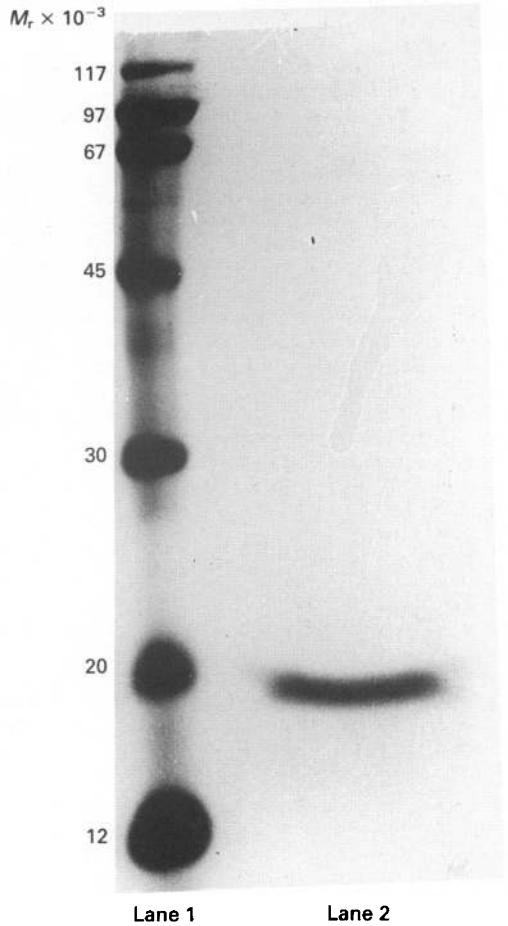

Fig. 1. An SDS-PAGE gel stained with Coomassie brilliant blue showing the purified oTP-1 used in this experiment. Molecular weight markers are shown in Lane 1. oTP-1 (20 μg) is shown in Lane 2. It consisted of a single band of M_r 19 000.

and variation between treatment groups were considered physiologically important. Therefore, mean responses and time trends for each of the PGs were analysed with untransformed and log-transformed values and differences in variances (ratios of residual variances of log-transformed concentrations) among treatments were evaluated. Means and standard errors of untransformed responses are presented in the figures so that variability between treatments can be examined.

Concentrations of PGF after oestradiol were not different for ewes treated with oTP-1 and oCSP and there were no increases in concentrations of PGF (Fig. 3). However, responses of ewes treated with oCSP − oTP-1 or oSP to oestradiol were different ($P < 0{\cdot}01$) from each other and, as a group, from the responses of the oTP-1 and oCSP group. Log transformation of PGF concentrations decreased heterogeneity of variances between treatments; however, variances between oTP-1 and oCSP *versus* oCSP − oTP-1 and oSP remained different ($P < 0{\cdot}01$). Treatment with oTP-1 or oCSP reduced uterine production of PGF in response to oestradiol and reduced the variation in concentrations of PGF.

Concentrations of PGFM in plasma (Fig. 3) were positively correlated with PGF produced by the uterus ($r = 0{\cdot}57$, $P < 0{\cdot}01$). The following differences in PGFM response to oestradiol were

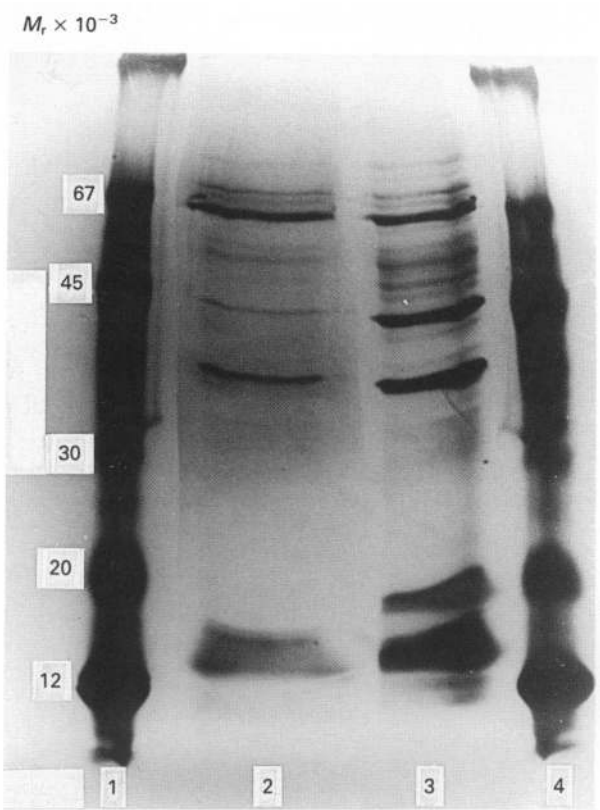

Fig. 2. An SDS-PAGE gel stained with silver showing the oCSP and oCSP − oTP-1 used in this experiment. Lanes 1 and 4, molecular weight markers; Lane 2, 100 μg oCSP − oTP-1; Lane 3, 200 μg oCSP. Note absence of M_r 19 000 band in Lane 2 which is oTP-1.

detected: oTP-1 *versus* oCSP ($P < 0{\cdot}05$), oSP *versus* oCSP − oTP-1 ($P < 0{\cdot}01$) and the combination of oTP-1 and oCSP *versus* oCSP − oTP-1 and oSP ($P < 0{\cdot}01$). The oCSP-treated ewes had a greater PGFM response than did oTP-1 treated ewes and oSP-treated ewes failed to have a peak in PGFM that was coincident with the peak in PGF. This accounts for the differences between the PGF and PGFM data. Residual variances of log transformed data were different between the oTP-1 and oCSP groups, between the oSP and oCSP − oTP-1 groups and the combination of oTP-1 and oCSP *versus* oCSP − oTP-1 and oSP ($P < 0{\cdot}01$, Table 1). Overall, treatment groups lacking oTP-1 had more variable responses to oestradiol in uterine production of PGF measured as PGFM.

Concentrations of PGE in plasma after oestradiol injection were not different between the oTP-1 and oCSP *versus* oCSP − oTP-1 and oSP groups (Fig. 3). A difference ($P < 0{\cdot}01$) between the oSP and oCSP − oTP-1 groups was due to a single very high PGE value for a single ewe 30 min after oestradiol. This value was considered to be an outlier. Analysis of residual variances of log-transformed data indicated no differences between oTP-1 and oCSP groups or the combined oTP-1 and oCSP treated groups *versus* oCSP − oTP-1 and oSP groups, but differences between oSP and oCSP − oTP-1 ($P < 0{\cdot}01$) were detected, probably due to the outlier. Overall, uterine production of PGE in response to oestradiol did not differ among treatment groups.

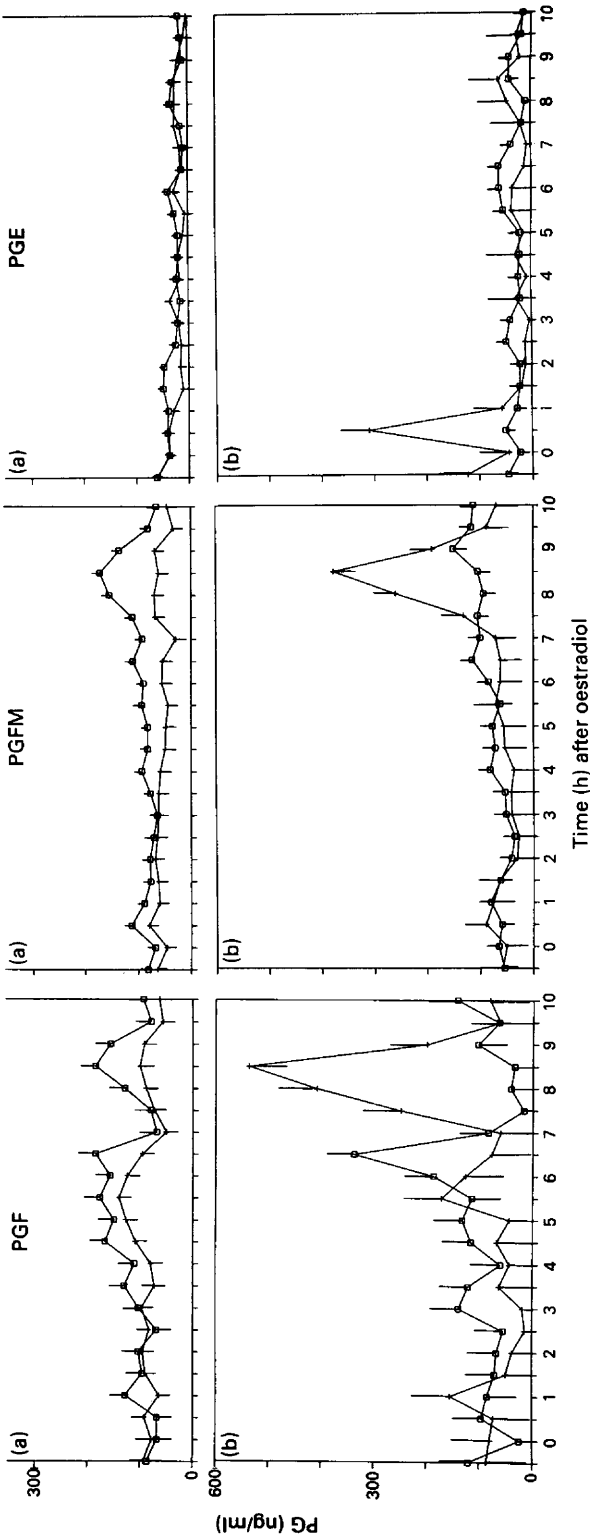

Fig. 3. Least square means for plasma PGF, PGFM and PGE after oestradiol injection of sheep treated with: (a) oCSP (□) (N = 5) and oTP-1 (+) (N = 5); (b) oCSP – oTP-1 (+) (N = 4) and oSP (□) (N = 5). Bars are standard error of mean from analysis of variance from each treatment separately to indicate heterogeneity.

Table 1. Residual error variances from analysis of variance of each treatment separately for PGF, PGFM and PGE plasma concentration data after oestradiol and oxytocin injections and after log transformation

Treatment	Prostaglandin	After oestradiol	After oxytocin
oTP-1	PGF	0·218	0·260
oCSP		0·417**	0·304
oSP		0·448	0·399
oCSP − oTP-1		0·514	0·121**
oTP-1 + oCSP combined		0·324	0·293
oSP + oCSP − oTP-1 combined		0·543**	0·275
oTP-1	PGFM	0·242	0·024
oCSP		0·036**	0·023
oSP		0·109	0·164
oCSP − oTP-1		0·299**	0·038**
oTP-1 + oCSP combined		0·142	0·031
oSP + oCSP − oTP-1 combined		0·218**	0·094**
oTP-1	PGE	0·296	0·137
oCSP		0·246	0·130
oSP		0·124	0·128
oCSP − oTP-1		0·385**	0·141
oTP-1 + oCSP combined		0·271	0·163
oSP + oCSP − oTP-1 combined		0·247	0·135

**Effects of treatment were detected ($P < 0·01$) compared to the previous treatment.

Concentrations of PGs after oxytocin

Least squares means for concentrations of PGF, PGFM and PGE for each treatment group in response to oxytocin injection are presented in Fig. 4. Analyses of untransformed data indicated that oxytocin-induced uterine production of PGF was lower ($P < 0·05$) for the oTP-1 group compared to the other 3 treatment groups. Examination of residual variance for log transformed data indicated that variances were not different between oTP-1 and oCSP groups, the combination of oTP-1 and oCSP *versus* oCSP − oTP-1 and oSP, but were different between the oSP and oCSP − oTP-1 ($P < 0·01$).

Concentrations of PGFM and PGF in plasma after oxytocin injection were correlated (Fig. 4, $r = 0·69$, $P < 0·01$). The PGFM response to oxytocin was lower for the oTP-1 group than for oCSP group ($P < 0·01$) and the combination of oTP-1 and oCSP was lower than for oCSP − oTP-1 and oSP groups ($P < 0·01$). Variances of log-transformed data were not different between oTP-1 and oCSP, but were different ($P < 0·01$) between oSP and oCSP − oTP-1 and the combination of oTP-1 and oCSP *versus* oCSP − oTP-1 and oSP. For ewes receiving intrauterine infusions of oTP-1, mean concentrations of PGFM and variation in concentrations of PGFM were reduced after oxytocin injection.

In contrast to treatment effects on uterine responses measured as PGF and PGFM, no differences in time trends or in residual variances of log-transformed concentrations of PGE were detected (Fig. 4).

Discussion

The present results clearly indicate that oTP-1 alone in oCSP is capable of extending luteal maintenance. Godkin *et al.* (1984a) reported that oTP-1 alone did not cause luteal extension to the same degree as oCSP, but data from the present study demonstrate that extension of the interoestrous interval is equivalent for purified oTP-1 alone (27·0 days) and oCSP (25·3 days). Differences

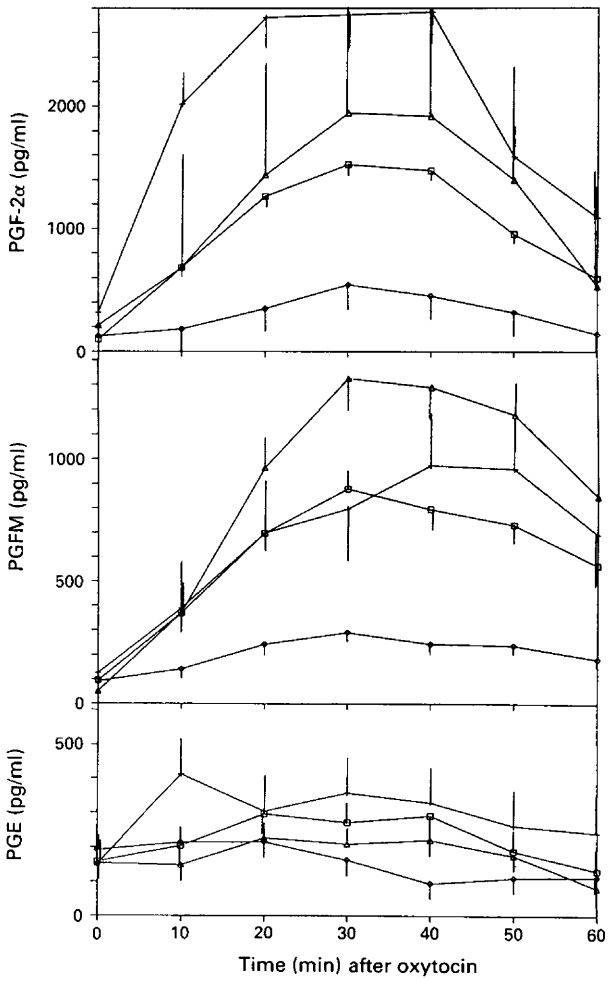

Fig. 4. Least square means for plasma PGF, PGFM and PGE after oxytocin injection of sheep treated with oCSP (□) (N = 5), oTP-1 (◇) (N = 4), oCSP − oTP-1 (△) (N = 4) or oSP (+) (N = 3). Bars are standard error of mean from analysis of variance of each treatment separately to illustrate heterogeneity of variance.

between the two experiments may have been due to differences in activity of the purified oTP-1. Alternatively, inclusion of serum proteins in the present study may have protected oTP-1 from degradation in the uterus whereas Godkin *et al.* (1984a) infused oTP-1 without carrier protein.

Comparisons of concentrations of PGF in plasma of the vena cava in ewes from each treatment, after either oestradiol or oxytocin injection, indicated that the effect of oTP-1 on luteal maintenance is mediated by inhibition of uterine secretion of PGF in response to both oestradiol and oxytocin. oTP-1 also decreases the variability in uterine secretion of PGF. The oCSP appeared to be less effective in inhibiting PGF secretion, suggesting that purified oTP-1 may be more potent. The effect of oTP-1 is antiluteolytic with respect to the hypothesis of McCracken (1980) which states that oestradiol and oxytocin control secretion of luteolytic amounts of PGF by the uterus during the oestrous cycle. No differences in concentrations of PGE in plasma were detected between the 4 treatment groups after oestradiol or oxytocin injections. Therefore, the hypothesis that oTP-1 causes a shift in PG metabolism towards increased production of PGE was not

supported. Results from the present study support the hypothesis that inhibition of luteolysis during early pregnancy is accomplished by a decrease in uterine production of PGF and agree with earlier results indicating that oCSP inhibits uterine production of PGF (Fincher *et al.*, 1986). These data do not support the hypothesis that PGE is responsible for inhibition of luteolysis. It is likely that the increase in PGE secretion in early pregnancy (Ellinwood *et al.*, 1979; Silvia *et al.*, 1984) is from the conceptus which does produce large amounts of PGE (Marcus, 1981). Also, other conceptus signals, independent of oTP-1, may cause increased secretion of PGE from the uterus. Contributions of conceptus or endometrial PGE to inhibition of luteolysis remain to be elucidated. Pratt *et al.* (1979) were able to extend the oestrous cycle by only 2 days with infusions of PGE.

Concentrations of PGFM were correlated with uterine production of PGF in the present study which agrees with results from earlier experiments (Lewis *et al.*, 1977; Louis *et al.*, 1977). In general, conclusions reached following analyses of PGF data were similar to those reached using PGFM data.

The mechanism by which oTP-1 inhibits PGF production by the uterus is not known. Considering the model for control of luteolysis proposed by McCracken (1980), there are several possibilities: (1) inhibition of synthesis of the oxytocin receptor; (2) inhibition of binding of oxytocin to its receptor; (3) inhibition of synthesis of oestrogen receptors; (4) inhibition of binding of oestradiol to its receptor or (5) inhibition of the cyclo-oxygenase pathway enzymes necessary for the synthesis of prostaglandins. Available evidence indicates that oTP-1 does not compete with oxytocin for binding to the oxytocin receptor (A. P. F. Flint, J. L. Vallet & F. W. Bazer, unpublished data). Hooper *et al.* (1986) reported that pulsatile release of oxytocin was similar for cyclic and pregnant ewes, but pregnant ewes have fewer coincident peaks of PGF production. McCracken *et al.* (1984) reported that oxytocin receptors were lower in endometrium from pregnant than cyclic ewes. oTP-1 may cause loss of receptors by inhibiting synthesis of new oxytocin receptors or degradation of those present. Flint *et al.* (1986) reported that oxytocin increased endometrial phosphatidylinositol turnover. Thus, oTP-1 may alter the oxytocin receptor and/or modify phosphatidylinositol turnover to inhibit phospholipase C or inhibit inositol recycling. Findlay *et al.* (1982) reported that endometrial oestradiol receptors were lower in pregnant than non-pregnant ewes due to a local effect of the conceptus. oTP-1 could decrease synthesis of oestradiol receptors or inhibit oestrogen binding by modification of the oestradiol receptor. The presence of oTP-1 receptors on endometrial cells necessary to mediate these proposed effects has been demonstrated (Godkin *et al.*, 1984b). The second messenger system for the oTP-1 receptor is yet to be elucidated. Endometrial cyclic 3′,5′-adenosine monophosphate is decreased and cyclic 3′,5′-guanosine monophosphate is unaffected by oTP-1 (Vallet *et al.*, 1988). Also, oTP-1 alters endometrial protein secretion, increasing secretion of some proteins while decreasing secretion of others. Those proteins affected by oTP-1 may also have effects proposed previously for oTP-1.

oTP-1 has recently been shown to have a degree of amino acid sequence homology with alpha interferons, especially bovine interferon alpha II (Imakawa *et al.*, 1987; Stewart *et al.*, 1987; Charpigny *et al.*, 1988). In addition to the antiluteolytic activity of oTP-1 described in this paper, oTP-1 also has potent antiviral activity (Pontzer *et al.*, 1988) which may be important during early pregnancy.

We conclude from the present study that: (1) oTP-1 is the only antiluteolytic protein produced by the sheep conceptus on Day 16 of pregnancy which is capable of extending luteal maintenance; (2) oTP-1 inhibits uterine production of PGF in response to exogenous oestradiol and oxytocin and also decreases the variability of PGF production; and (3) oTP-1 does not favour production of PGE over PGF by the uterus in response to oestrogen or oxytocin administration.

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