Effects of ovine conceptus secretory proteins and progesterone on oxytocin-stimulated endometrial production of prostaglandin and turnover of inositol phosphate in ovariectomized ewes

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Summary. This study examined the effects of progesterone and intrauterine injection of ovine conceptus secretory proteins (oCSP) on endometrial responsiveness to oxytocin. Twelve ewes were ovariectomized on day 4 of the cycle (oestrus = day 0) and assigned in a 2×2 factorial arrangement, to receive either 1.5 mg ovine serum proteins (SP) or oCSP containing 25 µg ovine trophoblast protein 1 (oTP-1) (by radioimmunoassay) in 1.5 mg total protein into each uterine horn, via catheters, twice a day on days 11, 12, 13 and 14. Ewes received 200 mg progesterone per day (i.m.) from day 4 to day 10 or 15. Oxytocin-induced prostaglandin $F_{2\alpha}$ was measured as 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) on days 11, 12, 13 and 14 in plasma from three integrated, 10 min (10 ml) blood samples (0–10, 10–20, 20–30 min) obtained after intravenous injection of 20 iu oxytocin, and in a pre-oxytocin (– 10 to 0 min) sample collected via an indwelling jugular catheter. The pre-oxytocin samples were also assayed for progesterone. Oxytocin-induced turnover of inositol phosphate was determined in endometrium on day 15 after hysterectomy. In ewes receiving progesterone to day 10, plasma progesterone decreased from about 12 to 2 ng ml⁻¹ (SEM $\pm$ 2.6) during the treatment period (days 11–14), but remained high (12–20 $\pm$ 2.6 ng ml⁻¹) in ewes that received progesterone to day 15. Intrauterine injection of oCSP resulted in high basal concentrations of PGFM on days 12 and 13 compared with SP-treated ewes ($P < 0.01$). Treatments with progesterone did not affect basal PGFM concentrations. Treatment with oCSP abolished oxytocin-induced endometrial secretion of prostaglandin only if progesterone was maintained to day 15 ($P < 0.01$); in ewes receiving such treatment, oCSP inhibited ($P < 0.01$), but SP did not inhibit, oxytocin-induced endometrial turnover of inositol phosphate ($P < 0.06$), which was greater in ewes treated with progesterone to day 10 than in those treated to day 15 ($P < 0.05$). Ewes that responded to oxytocin with increased PGFM exhibited increased oxytocin-stimulated turnover of inositol phosphate on day 15. These results indicate that the antiluteolytic action oTP-1 exerts on the endometrium requires progesterone and that this mechanism involves inhibition of oxytocin-stimulated turnover of inositol phosphate.

Keywords: sheep; conceptus; prostaglandin $F_{2\alpha}$; progesterone; oxytocin

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Introduction

Establishment of pregnancy in ewes requires the presence of a conceptus in the uterus by days 12–13 of the oestrous cycle (Moor & Rowson, 1966a, b). A conceptus secretory protein, initially identified as trophoblastin (Martal *et al.*, 1979), later characterized and named ovine trophoblast protein-1 (oTP-1; Godkin *et al.*, 1982, 1984), is thought to be primarily responsible for the anti-luteolytic effects of the ovine conceptus (see Bazer *et al.*, 1989, 1991). oTP-1 is a type I trophoblast interferon secreted by sheep conceptuses between days 10 and 21 of pregnancy (Bazer *et al.*, 1986; Imakawa *et al.*, 1987; Stewart *et al.*, 1987). Vallet *et al.* (1988b) demonstrated that purified oTP-1 or oCSP extend the interoestrus interval to a similar extent when injected into the uterine lumen on days 12–14 of the oestrous cycle. However, oCSP from which oTP-1 was removed did not extend the interoestrus interval when compared with control ewes treated with serum protein. The mechanism by which oTP-1 extends corpus luteum function remains to be defined.

The endometrium of cyclic ewes releases pulses of prostaglandin $F_{2\alpha}$ (PGF $_{2\alpha}$) into the uterine vein on days 14–17 (McCracken *et al.*, 1971; Thorburn *et al.*, 1972; Zarco *et al.*, 1988). Pulsatile secretion of uterine PGF $_{2\alpha}$ is thought to be responsible for luteolysis, since continuous infusion of, or immunization against, PGF $_{2\alpha}$ results in extension of the cycle (Scaramuzzi & Baird, 1976; Fairclough *et al.*, 1981). Pulsatile release of oxytocin from the ovary, neurohypophysis or from both is thought to be responsible for pulsatile secretion of PGF $_{2\alpha}$ from the endometrium, since >95% of all pulses of PGF $_{2\alpha}$ occur at the same time as pulses of oxytocin (Flint & Sheldrick, 1986; Hooper *et al.*, 1986). Secretion of oxytocin is similar in cyclic and pregnant ewes during maternal recognition of pregnancy and parallels the rise and fall of progesterone secretion by the corpus luteum (Flint & Sheldrick, 1986; Hooper *et al.*, 1986; Rhodes & Nathanielsz, 1990). Concentrations of endometrial oxytocin receptor are high during luteolysis, but are greatly reduced in pregnant ewes (Roberts *et al.*, 1976; McCracken *et al.*, 1984; Sheldrick & Flint, 1985). Pulsatile secretion of PGF $_{2\alpha}$ by the uterus is also reduced during the period of maternal recognition of pregnancy (Hooper *et al.*, 1986; Zarco *et al.*, 1988). Oxytocin-stimulated endometrial secretion of PGF $_{2\alpha}$ is thought to be mediated by the inositol trisphosphate and diacylglycerol second-messenger system (Flint *et al.*, 1986; Hixon & Flint, 1987; Mirando *et al.*, 1990).

Progesterone downregulates oestrogen and progesterone receptors (Evans & Leavitt, 1980; Okulicz *et al.*, 1981; Leavitt *et al.*, 1985) and is thought to keep the endometrium unresponsive to oestrogen during dioestrus (McCracken *et al.*, 1984). In pregnant ewes, conceptus secretory proteins are responsible, directly or indirectly, for generating an antiluteolytic signal to inhibit pulsatile release of uterine PGF $_{2\alpha}$, thus ensuring continued progesterone production by the corpus luteum, and for maintaining uterine responsiveness to progesterone. This study examined the interaction between oCSP and progesterone in development of oxytocin-induced uterine production of prostaglandin and turnover of inositol phosphate in ovariectomized ewes.

Materials and Methods

Animals

Mature ewes of primarily Rambouillet breeding were observed daily for oestrous behaviour using vasectomized rams. Twelve ewes, having exhibited at least two oestrous cycles of normal length, were assigned randomly, at standing oestrus, to a treatment group. On day 4, bilateral uterine catheterization and ovariectomy were performed in supine ewes under halothane anaesthesia (Fincher *et al.*, 1986). Ewes were fitted with indwelling jugular catheters on day 10 and housed individually in crates for the remainder of the experiment.

Preparation of proteins

Pregnant ewes were laparotomized 16 days after mating, and conceptuses were collected and cultured as described by Godkin *et al.* (1982). Concentration of oTP-1 was determined by radioimmunoassay (Vallet *et al.*, 1988a) and

oCSP was diluted with sterile 0.9% NaCl to an oTP-1 concentration of $25 \mu\text{g ml}^{-1}$. Concentration of total protein in oCSP after dilution was 0.75 mg ml^{-1} (Lowry *et al.*, 1951). Aliquants of oCSP (1.0 ml) were stored at -20°C .

For preparation of serum proteins (SP), blood was collected from the jugular vein of a pregnant ewe on day 16 after mating and allowed to clot at room temperature (for 1 h) and then at 4°C (overnight). Serum was diluted with sterile 0.9% NaCl to protein concentrations of either 1.5 or 7.5 mg ml^{-1} , divided into 1.0 or 0.1 ml aliquants, respectively, and stored at -20°C .

Treatments

Twelve ewes were allotted randomly in a 2×2 factorial arrangement to receive intrauterine injections of oCSP or SP on days 11, 12, 13 and 14 and intramuscular injection of progesterone (200 mg day^{-1}) from day 4 to day 10 or 15. A total of 1.5 mg protein was injected into each uterine horn twice a day (07:00 and 17:30 h). Progesterone injections were given after the treatment at 17:30 h each day. Protein treatments were followed by 50 mg ampicillin in 0.1 ml saline and 1.0 ml of 0.9% saline to clear the catheter. Before injection with oCSP, 0.75 mg SP (0.1 ml) was added to bring total protein to 1.5 mg (1.1 ml). The SP (1.5 mg) was in a total volume of 1.0 ml. After protein treatments at 07:00 h on days 11–14, ewes were challenged with 20 iu oxytocin i.v. to stimulate maximally the available oxytocin receptors. Integrated blood samples (10 ml in 10 min) were collected using a Harvard pump apparatus (Harvard Apparatus Co., Dover, MA, USA). Oxytocin was injected into the jugular vein via an indwelling catheter after collection of a pretreatment sample (-10 to 0 min), and three additional samples were collected ($0-10$, $10-20$ and $20-30 \text{ min}$). Approximately 1 ml heparinized (200 iu ml^{-1}) 0.9% NaCl flushed into the catheters between each sample prevented blood from clotting. Samples were stored on ice and centrifuged within 90 min of collection at $1800 g$ and 4°C for 10 min. The plasma collected was stored at -20°C until assayed for PGFM and progesterone.

Turnover of inositol phosphate

Turnover of oxytocin-stimulated inositol phosphate was determined as described by Mirando *et al.* (1990) in duplicate incubations of 100 mg endometrium from each ewe. Care was taken to include similar amounts of caruncular and intercaruncular endometrium in each incubation. Incubations from each ewe were treated with 0 or 100 nmol oxytocin l^{-1} (Mirando *et al.*, 1990).

Progesterone

Concentration of progesterone, in $200 \mu\text{l}$ plasma obtained before the oxytocin challenge (07:00 h) on days 11–14, was determined by radioimmunoassay (Mirando *et al.*, 1990). Average extraction efficiency was 84.8%. Assay sensitivity was 10 pg per tube and the intra- and interassay coefficients of variation were 6.2 and 9.5%, respectively.

Prostaglandin

Oxytocin-stimulated uterine production of $\text{PGF}_{2\alpha}$ was determined by measuring its stable metabolite, 13,14-dihydro-15-keto-prostaglandin $\text{F}_{2\alpha}$ (PGFM) in $200 \mu\text{l}$ plasma collected from the jugular vein (Fincher *et al.*, 1986). Assay sensitivity was 10 pg per tube and intra- and interassay coefficients of variation were 3.1 and 8.4%, respectively. Basal PGFM concentration in plasma was estimated from the pre-oxytocin-challenge blood sample (-10 to 0 min). This value was subtracted from oxytocin-stimulated PGFM concentration for each ewe on each day.

Statistical analyses

Data were subjected to least-squares analysis of variance using the General Linear Models procedure of the Statistical Analysis System (SAS, 1982). For analysis of turnover of inositol phosphate, data were analysed untransformed and then log-transformed to alleviate possible heterogeneity of error variance (Snedecor & Cochran, 1980). Split-plot analyses of variance for a 2×2 factorial arrangement were performed with protein and progesterone treatments as main effects and ewe nested within protein $\times$ progesterone treatment for PGFM, progesterone and turnover of inositol phosphate. Split-plot analysis of variance was performed on basal PGFM concentration with main effects of treatment (oCSP versus SP) and day (11, 12, 13 and 14). Ewes were nested within treatment, and day was cross-classified with treatment. All tests of hypotheses were performed using the appropriate error terms according to expectation of the mean squares (Snedecor & Cochran, 1980). Data presented are least-square means.

Results

All ewes had similar concentrations of progesterone in plasma on the day protein infusions commenced (Fig. 1). In ewes receiving progesterone to day 10, plasma concentrations of progesterone

decreased from day 11 to day 14 ($P < 0.01$) and were not affected by protein treatment ($P > 0.1$). Plasma progesterone remained high and was not affected by protein treatment in ewes receiving progesterone to day 15 ($P = 0.52$).

Fig. 1. Concentrations of progesterone in plasma (mean $\pm$ 2.6 ng ml⁻¹) on days 11–14 of the cycle in ovariectomized ewes receiving 200 mg progesterone (i.m.) from day 4 to day 10 (filled symbols), or from day 4 to day 15 (open symbols), and intrauterine injections twice a day of oCSP (∇ and $\blacktriangledown$) or SP ($\circ$ and $\bullet$) from day 11 to day 14.

No effect of progesterone treatment on basal PGFM concentration was detected ($P > 0.1$); data were therefore pooled within protein treatment (Fig. 2). Across protein treatments, basal PGFM varied from day 11 to day 14 ($P < 0.01$). In addition, a treatment $\times$ day interaction was detected ($P < 0.01$) and reflects the higher basal PGFM for oCSP-treated ewes on days 12 and 13 compared with SP-treated ewes.

Fig. 2. Basal concentrations of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM) (mean $\pm$ SEM) in plasma for ewes treated with serum proteins ($n = 6$, $\square$) or ovine conceptus secretory proteins ($n = 6$, $\boxtimes$) on days 11–14 of the cycle. No effect of progesterone treatment was detected ($P > 0.1$). Ovine conceptus secretory protein treatment resulted in increased basal concentrations of PGFM on days 12 and 13 ($P = 0.07$).

Oxytocin-induced production of $\text{PGF}_{2\alpha}$ (plasma PGFM) is shown for ewes receiving oCSP (Fig. 3) or SP (Fig. 4). One ewe in the SP group receiving progesterone to day 15 was considered an outlier because the endometrium was secreting luteolytic amounts of $\text{PGF}_{2\alpha}$ at the beginning of the period of protein infusion (day 11) and was, therefore, removed from the data set.

Fig. 3. Concentrations of 13,14-dihydro-15-keto-prostaglandin $\text{F}_{2\alpha}$ (PGFM, mean $\pm$ 53.5 pg ml^{-1}) in plasma in response to oxytocin (20 iu) for ewes treated with ovine conceptus secretory proteins and receiving progesterone to (a) day 10 or (b) day 15, on days 11 (○), 12 (●), 13 (▽) and 14 (▼) adjusted for basal PGFM concentrations for each ewe on each day. Effects of progesterone ($P < 0.01$) and protein $\times$ progesterone interaction ($P = 0.07$) were detected.

Intrauterine injection of oCSP abolished oxytocin-induced $\text{PGF}_{2\alpha}$ in ewes receiving progesterone to day 15 ($P < 0.01$), but oCSP did not inhibit oxytocin-induced PGF secretion if progesterone administration was stopped on day 10 ($P > 0.1$). In ewes receiving progesterone to day 10, oCSP treatment resulted in earlier development of oxytocin-induced PGF secretion than in those receiving SP (treatment $\times$ day, $P = 0.07$). Ewes receiving progesterone to day 10 and SP treatment exhibited a pattern of PGF release similar to that expected in cyclic ewes before luteolysis. Ewes that received progesterone to day 15 and SP treatment did not demonstrate oxytocin-induced increases in PGFM by day 14 ($P > 0.1$).

Analysis of log-transformed data for oxytocin-induced turnover of inositol triphosphate and total inositol phosphate (mono, bis and triphosphate) in endometrium yielded similar

Fig. 4. Concentrations of 13,14-dihydro-15-keto-prostaglandin $F_{2\alpha}$ (PGFM, mean) in plasma in response to oxytocin (20 iu) for ewes treated with serum proteins and receiving progesterone to (a) day 10 (SEM = 53.5) or (b) day 15 (SEM = 65.5), on days 11 (○), 12 (●), 13 (▽) and 14 (▼) adjusted for basal PGFM secretion for each ewe on each day. Effects of progesterone ($P < 0.01$) and protein $\times$ progesterone interaction ($P = 0.07$) were detected.

conclusions; therefore, only data for total inositol phosphate are presented (Fig. 5). In ewes receiving progesterone to day 15 and oCSP, turnover was not detected; in ewes receiving progesterone to day 15 and SP, oxytocin treatment on day 15 resulted in an approximate doubling of inositol phosphate turnover compared with controls (Fig. 5; treatment $\times$ oxytocin, $P = 0.08$). Endometrial turnover of inositol phosphate was greater in ewes receiving progesterone to day 10 than in those treated to day 15 ($P = 0.06$), regardless of protein treatment.

Discussion

Maternal recognition of pregnancy in sheep may be accomplished through luteoprotective (Mapletoft *et al.*, 1976; Henderson *et al.*, 1977; Pratt *et al.*, 1977; Ellinwood *et al.*, 1979; Silvia *et al.*, 1984) and antiluteolytic (see Bazer *et al.*, 1989, 1991) effects of the ovine conceptus. This study focused on the antiluteolytic action of the conceptus. The interaction between conceptus secretory proteins and progesterone was examined to determine the local effects of oCSP on development of uterine responsiveness to oxytocin-stimulated $PGF_{2\alpha}$ and turnover of inositol phosphate. This

Fig. 5. Turnover of total inositol phosphate (mono, bis, and triphosphate; d.p.m. g⁻¹ wet weight endometrium) in response to 0 (□) or 100 (▨) nmol oxytocin l⁻¹ *in vitro* on day 15 for ewes treated with ovine conceptus secretory proteins (oCSP) or serum proteins (SP) and receiving progesterone to day 10 (P10) or day 15 (P15) of the cycle. Effects of progesterone ($P < 0.01$) and protein $\times$ oxytocin interaction ($P = 0.08$) were detected.

experiment allowed examination of the effect of conceptus secretory proteins on development of endometrial responsiveness to oxytocin during declining (progesterone to day 10) or maintained (progesterone to day 15) plasma progesterone. The former situation reflects one aspect of pregnancy failure and the latter reflects the physiology of early pregnancy.

These results clearly demonstrate that progesterone in plasma must be maintained at concentrations characteristic of dioestrus for oCSP to inhibit oxytocin-induced turnover of inositol phosphate and production of PGF_{2 α} . Maintaining adequate concentrations of progesterone in plasma in the absence of ovaries in ewes treated with serum protein effectively extended the period during which the endometrium remained unresponsive to oxytocin to day 14. However, by day 15, oxytocin stimulated endometrial turnover of inositol phosphate *in vitro* in these ewes, suggesting that onset of the luteolytic mechanism was delayed by 1–2 days.

Ewes that received progesterone up to day 15 and oCSP treatment did not exhibit increased PGFM in response to oxytocin from day 11 to 14 and remained unresponsive to oxytocin on day 15 (turnover of inositol phosphate). In cyclic ewes, endometrial responsiveness to oxytocin develops between days 12 and 16 (Mirando *et al.*, 1990; Raw *et al.*, 1990), and the mean interval from oestrus to first pulse of PGF_{2 α} is 13.6 days (Zarco *et al.*, 1988). Those results are in close agreement with the present results for ewes that received progesterone up to day 10 and SP. Pregnancy or injection of oCSP on days 12–14 blocks oxytocin-induced turnover of inositol phosphate measured *in vitro* on days 15 or 16, respectively (Mirando *et al.*, 1990); and uterine production of PGF_{2 α} in response to oxytocin *in vivo* is diminished by intrauterine injection of oTP-1 (Vallet *et al.*, 1988b).

In the present study, development of the luteolytic mechanism was monitored as oxytocin-induced endometrial production of PGF_{2 α} on days 11–14 and turnover of inositol phosphate on day 15. These results indicate that endometrial responsiveness to oxytocin developed between days 13 and 14 in SP-treated and between days 12 and 13 in oCSP-treated ewes when plasma progesterone concentrations were declining. Enhanced responsiveness to oxytocin after treatment with purified oTP-1 was demonstrated *in vitro* with endometrium from ovariectomized ewes, and the response was dependent upon the ovariectomized ewes being pretreated with progesterone (Vallet & Bazer, 1989). In addition, oTP-1 was shown to increase endometrial production of PGF_{2 α} when day 15 cyclic endometrium was treated *in vitro* with oTP-1 (Vallet & Bazer, 1989).

The reason for the apparent delay of 1–2 days in development of endometrial responsiveness to oxytocin in ewes receiving intrauterine injections of SP and progesterone to day 15 is unclear. Hixon & Flint (1987) demonstrated that the luteolytic mechanism developed, in response to exogenous oestradiol, over 2–3 days, from a point of basal numbers of oxytocin receptors to coupling the oxytocin receptor to pulsatile secretion of prostaglandin. The present data showed maximal oxytocin-induced turnover of inositol phosphate and oxytocin-induced PGFM on day 13 in ewes in which progesterone was withdrawn from day 10, suggesting that formation of oxytocin receptor occurred soon after progesterone concentrations began to decline. In SP-treated ewes receiving progesterone up to day 15, oxytocin did not stimulate PGFM on day 14 but stimulated turnover of inositol phosphate on day 15, suggesting that oxytocin receptor formation was initiated on day 14. Perhaps the absence of follicular oestrogen or oxytocin, or of both, from a corpus luteum altered the timing of these events. Ovariectomized ewes treated with progesterone to mimic the cyclic variation of this steroid were responsive to oxytocin (plasma PGFM) on day 15, but not on days 5 or 10 of the cycle. Oestrogen treatment potentiated this effect (Homanics & Silvia, 1988). In the present study, using ovariectomized progesterone-treated ewes, plasma PGFM and endometrial turnover of inositol phosphate in response to oxytocin developed in the absence of follicular oestrogen or oxytocin from a corpus luteum. Previous reports also showed that oestrogen may not be necessary for initiating the luteolytic mechanism (Homanics & Silvia, 1988; Vallet & Bazar, 1989; Vallet *et al.*, 1990). However, numerous reports suggest that oestradiol is necessary for completion of luteolysis (Karsch *et al.*, 1970; Baird *et al.*, 1976; Flint & Sheldrick, 1986; Sheldrick & Flint 1990), perhaps by increasing the number of endometrial oxytocin receptors (Hixon & Flint, 1987) or by affecting coupling of the oxytocin receptor to its phosphatidyl inositol second-messenger system (Vallet *et al.*, 1990).

In the present study, basal concentrations of PGFM in plasma were high in ewes receiving oCSP and this effect was not dependent on progesterone treatment. This is consistent with previous reports (Lacroix & Kann, 1983; Fincher *et al.*, 1986; Vallet & Bazer, 1989) that basal production of uterine PGF is raised during early pregnancy. Interestingly, the greatest rise in basal PGFM was evident on day 12 of the cycle, before the endometrium developed oxytocin-induced PGF secretion, suggesting that this may be an event mediated by oCSP, rather than oxytocin. The potential consequences of an oCSP-induced increase in basal PGF production include downregulation of PGF receptors on the corpus luteum, partial depletion of luteal oxytocin before endometrial oxytocin receptors are synthesized or depletion of endometrial phospholipid stores and thus a reduced PGF signal during maternal recognition of pregnancy. The mechanism responsible for raised basal PGFM in pregnant or oCSP-treated ewes was not examined. Establishment of an interferon-mediated antiviral state of mouse L-cells in culture has been demonstrated to involve activation of fatty acid cyclooxygenase and thus PGF production (Pottathil *et al.*, 1980). However, the induction of an antiviral state in Madin Darby bovine kidney cells by oTP-1 occurs in the presence of indomethacin, suggesting that products of the cyclooxygenase pathway (i.e. PGF) may not play a primary role in this response (M. A. Mirando & F. W. Bazer, unpublished observations).

Results from this study are consistent with the hypothesis that the endometrium of ewes exhibits a progesterone-dependent block to the luteolytic effects of oestradiol or oxytocin during dioestrus (McCracken *et al.*, 1984). Ewes receiving progesterone up to day 15 and SP exhibited oxytocin-induced endometrial turnover of inositol phosphate by day 15, which was absent in the oCSP-treated ewes. The uterine luteolytic mechanism developed in the presence of high plasma progesterone concentrations and suggests that chronic progesterone may result in 'down-regulation' of the progesterone receptor (Evans & Leavitt, 1980; Leavitt *et al.*, 1985). In support of this hypothesis, preliminary results indicate that intrauterine injection of oCSP from day 12 to day 14 in ovariectomized, progesterone-treated ewes results in an increase of about 40% in endometrial progesterone receptors when compared with SP infused ewes (M. A. Mirando, R. J. Moffatt, T. L. Ott & F. W. Bazer, unpublished observations). A limited amount of data in cattle (Meyer *et al.*, 1988) and pigs (Deaver & Guthrie, 1980) has demonstrated that the rapid decline in numbers of

progesterone receptors, which occurs as animals approach oestrus, does not occur during a similar stage of pregnancy. These results suggest that conceptus-mediated support (stabilization or up-regulation) of endometrial progesterone receptors may be a common mechanism involved in maternal recognition of pregnancy in these three species.

Results from this study indicate that oCSP requires the presence of progesterone to inhibit oxytocin-induced production of PGF_{2α} and endometrial turnover of inositol phosphate in ovariectomized ewes. Intrauterine injection with oCSP results in raised basal concentrations of PGFM on days 12 and 13 and an earlier onset of oxytocin-induced production of PGF_{2α} than SP-treated control ewes. The mechanism responsible for the interaction between progesterone and conceptus secretory proteins that allows for maternal recognition of pregnancy in ewes remains to be defined.

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