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Ovine Prostaglandin $F_{2\alpha}$ Receptor

Steroid Influence on Steady-State Levels of Luteal mRNA

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Expression of the receptor for prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is decreased in the ovine corpus luteum during regression and increased in early pregnancy. This study was designed to evaluate the influence of progesterone and/or 17β-estradiol (E_2) on this regulation. Circulating progesterone (functional regression) and luteal PGF receptor mRNA decreased ($p < 0.05$) within 8 h of $PGF_{2\alpha}$-induced luteal regression in midluteal phase (day 10; d 10) ewes; however, internucleosomal DNA fragmentation (structural regression) was not yet increased. Additionally, luteal PGF receptor mRNA and circulating progesterone were greater ($p < 0.05$) in pregnant than in nonpregnant ewes on d 14, but not on d 12. Twelve hours following injection of d 10 ewes with E_2, steady-state levels of mRNA for PGF receptor were decreased ($p < 0.05$), although circulating progesterone and DNA laddering were unchanged. Conversely, luteal mRNA for PGF receptor was increased ($p < 0.05$) by E_2 treatment in hysterectomized ewes. These results provide evidence that (1) luteal PGF receptor expression parallels circulating progesterone levels during functional regression and in early pregnancy, but (2) expression of PGF receptor can be dissociated from alterations in circulating progesterone by injection with E_2. Additionally, decreased PGF receptor expression initiated by E_2 is uterine-dependent, whereas the direct luteal effect (hysterectomized ewes) of E_2 is a stimulation of PGF receptor expression. These results collectively support the belief that the apparent downregulation of PGF receptor during luteal regression is associated with uterine-derived $PGF_{2\alpha}$ and its intracellular effects rather than with alterations in ovarian steroid production.

Key Words: Ovine corpus luteum; FP; luteal regression; progesterone; 17β-estradiol.

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Introduction

Hormonal regulation of development, function, and regression of the ovine corpus luteum is complex. Luteolysis in the nonpregnant ewe begins on approximately day 14 (d 0 = estrus), and progresses rapidly through d 16–17 *(1)*. The corpus luteum is required for maintenance of progesterone production and implantation in the early stages of pregnancy. Therefore, in early pregnancy, specific cellular events must be instituted during the midluteal phase to prevent luteolysis. Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) is the physiological initiator of luteal regression in the ewe *(2,3)*. $PGF_{2\alpha}$ is produced in the uterus and transported to the ovary, where it causes a reduction in secretion of progesterone (functional regression) and cellular demise (structural regression, luteolysis; *4–6*). Additionally, luteolysis can be induced earlier than normal in the luteal phase with the administration of exogenous $PGF_{2\alpha}$ or its analogs *(2,6,7)*. Structural regression in the ovine corpus luteum is associated with internucleosomal DNA fragmentation, one of many indicators of apoptosis *(8,9)*. During luteal regression in several species, including the ewe, decreased secretion of progesterone precedes DNA fragmentation, and functional and structural regression of the corpus luteum appear to be distinct events *(9–12)*. It has been suggested that functional regression is directly regulated by $PGF_{2\alpha}$, but structural regression is mediated by other factors *(13)*.

Binding studies have identified specific receptors for $PGF_{2\alpha}$ in the ovine corpus luteum *(14,15)*. Full-length cDNA encoding the membrane receptor for $PGF_{2\alpha}$ (PGF receptor, designated FP) has also been cloned from an ovine luteal cell cDNA library *(16)*. Downregulation of mRNA encoding this receptor during luteal regression was demonstrated by a marked reduction in relative amounts of mRNA for PGF receptor in corpora lutea collected from nonpregnant ewes during natural luteal regression (d 16; *17*). Steady-state levels of mRNA for PGF receptor on d 14 were variable among animals, and there was a strong association in individual ewes among decreased serum progesterone levels, decreased luteal mRNA for the PGF receptor, and increased internucleosomal DNA fragmentation in corpora

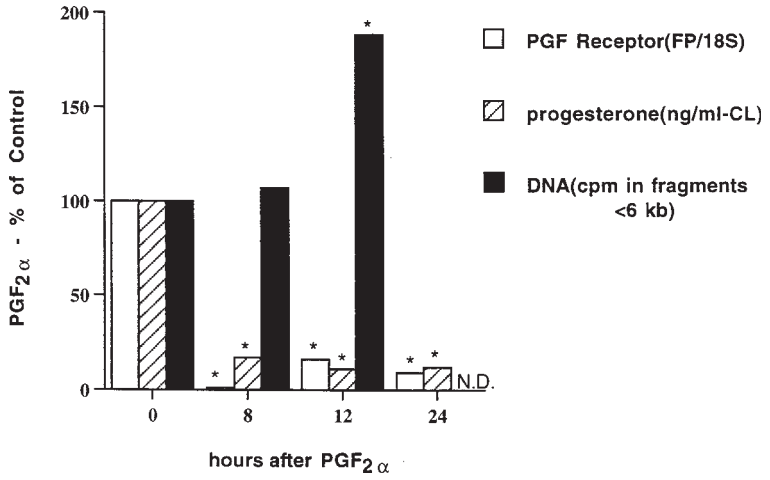

Fig. 1. Time-course of luteal response to $PGF_{2\alpha}$ injection. Midluteal-phase ewes (d 10) were injected with $PGF_{2\alpha}$ (10 mg, im). Blood and corpora lutea (CL) were collected 8, 12, or 24 h after injection, and analyzed for circulating progesterone (hatched bars, normalized to number of CL) and steady-state luteal content of mRNA encoding PGF receptor (FP, open bars) or low-mol-wt fragmentation (<6 kbp; solid bars) of genomic DNA as described in Materials and Methods. Values are expressed as a mean ratio of time-point (treatment)/time 0 (control). $n \geq 4$, $^*p < 0.05$ different from time 0 when analyzed as mean values. N.D. = not determined

lutea (indicative of apoptosis; *17*). Conversely, luteal levels of mRNA for PGF receptor were significantly greater in ewes on d 16 of pregnancy, when compared with nonpregnant animals *(17)*. Therefore, it appears that expression of PGF receptor is increased in corpora lutea in early pregnancy and reduced during luteolysis. These alterations in expression of PGF receptor mRNA are in agreement with reported changes in receptor binding measurements during the estrous cycle and pregnancy *(15)*. Because expression of mRNA for PGF receptor is decreased during luteolysis, this could be associated with the functional or the structural phase of luteal regression. If it occurs during functional regression, the cause of this downregulation could be the result of decreased progesterone. Alternatively, decreased expression may be a response to $PGF_{2\alpha}$ binding to the receptor (homologous downregulation).

In addition to $PGF_{2\alpha}$, exogenous 17β-estradiol has been shown to promote luteolysis, whereas removal of endogenous estradiol prolongs the luteal life-span *(18,19)*. In the ewe, estradiol-induced luteolysis is uterine-dependent *(18)*. Furthermore, E_2 stimulates release of $PGF_{2\alpha}$ from the ovine uterus *(20,21)*. Therefore, estradiol-induced luteolysis could be indirectly mediated by $PGF_{2\alpha}$. Thus, estradiol may also play a role in downregulation of PGF receptor during regression.

The present study was designed to investigate whether the expression of mRNA encoding PGF receptor is directly associated with alterations in progesterone secretion in functional regression and early pregnancy. The potential role of E_2 was also investigated.

Results

Circulating progesterone and steady-state levels of luteal mRNA encoding PGF receptor were reduced ($p < 0.05$) 8 h following injection with $PGF_{2\alpha}$ on d 10 of the estrous cycle (Fig. 1). Circulating progesterone and PGF receptor mRNA remained reduced ($p < 0.05$) 12 and 24 h after $PGF_{2\alpha}$. No visible internucleosomal fragmentation (laddering) of luteal genomic DNA on agarose gels was observed in samples taken from control ewes. Eight hours following injection with $PGF_{2\alpha}$, DNA laddering was faintly observed in some animals; however, a distinct "laddering" pattern appeared in all animals at 12 h (as shown in Fig. 2). Similarly, increased ($p < 0.05$) luteal DNA fragmentation was not measured until 12 h following injection (Fig. 1).

When compared with nonpregnant animals on d 16 of the estrous cycle, amounts of mRNA encoding PGF receptor were greater ($p < 0.05$) in corpora lutea collected from ewes on d 16 of pregnancy (18.3 ± 6.1-fold, pregnant vs nonpregnant; $n = 4$; $p < 0.05$). To determine whether this change in steady-state expression of mRNA encoding PGF receptor also accompanied changes in progesterone secretion, earlier times in pregnancy were also investigated. Figure 3 demonstrates the association between circulating progesterone levels and steady-state mRNA encoding PGF receptor in corpora lutea on d 12 and d 14 of pregnancy. The levels of progesterone and amounts of PGF receptor mRNA between pregnant and nonpregnant animals were similar on d 12; however, these values were greater ($p < 0.05$) in pregnant ewes on d 14 compared to d 14 of the estrous cycle. It was previously reported that no laddering of genomic DNA low-mol-wt fragments was observed on d 16 of pregnancy. Thus, genomic DNA was not evaluated in this experiment *(8)*.

The effect of E_2 injection was investigated to determine whether alterations in expression of mRNA for PGF receptor might be dissociated from alterations in circulating progesterone levels. The concentration of estradiol given

Fig. 2. Autoradiograph of an agarose gel showing the effect of $PGF_{2\alpha}$ injection on internucleosomal fragmentation of luteal genomic DNA. Corpora lutea were collected from d 10 ewes in an uninjected control **(A)** and 12 h following injection with $PGF_{2\alpha}$ **(B)**. Genomic DNA was prepared, end-labeled with [α^{32}P] dideoxy-ATP, and visualized by agarose-gel electrophoresis and autoradiography as described in Materials and Methods. A mol wt of 6kb indicated.

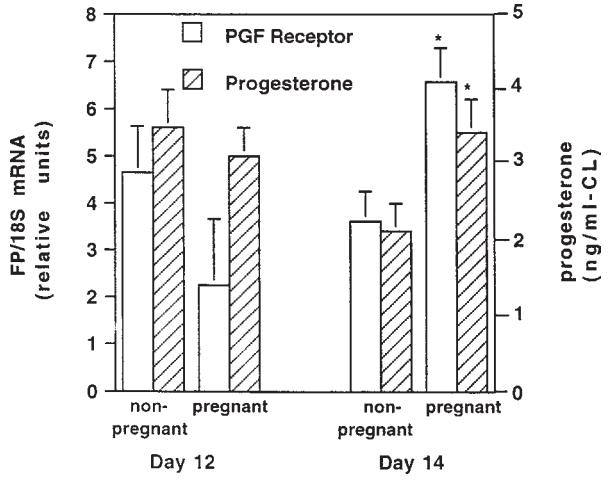

Fig. 3. Comparison in corpora lutea of early pregnancy between circulating progesterone and luteal expression of PGF receptor. Blood and corpora lutea (CL) were collected from nonpregnant and pregnant ewes on d 12 and d 14, and analyzed for circulating progesterone (hatched bars) and steady-state luteal content of mRNA encoding PGF receptor (FP, open bars) as described in Materials and Methods. Values are mean ± SEM progesterone (ng/mL, normalized to number of CL) or mRNA (FP/18S ribosomal RNA). $n \geq 4$; $^*p < 0.05$ pregnant different from nonpregnant.

(25 μg, iv; plus 37.5 μg, im) was sufficient to produce visual changes in uterine vasculature, but did not significantly ($p > 0.05$) alter circulating E_2 levels 12 h following injection (data not shown). Steady-state amounts of luteal mRNA for PGF receptor were reduced ($p < 0.05$) by injection, although circulating levels of progesterone had not changed, and DNA fragmentation was not different from controls (Fig. 4). Similar to that previously observed (Fig. 1), 12 h following injection with $PGF_{2\alpha}$, circulating progesterone and luteal PGF receptor mRNA were reduced, and luteal internucleosomal DNA fragmentation was increased, whether or not E_2 was also given.

E_2 is known to stimulate $PGF_{2\alpha}$ secretion in the ovine uterus *(20,21)*. Therefore, the effect of estradiol injection on luteal expression of PGF receptor in hysterectomized ewes was evaluated. Circulating levels of progesterone were not significantly altered 12 or 24 h following hysterectomy in d 10 ewes (prehysterectomy, 1.72 ± 0.31 ng/mL; posthysterectomy, 12 h = 0.95 ± 0.22 ng/mL, 24 h = 1.22 ± 0.13 ng/mL). Circulating progesterone was also not different between hysterectomized control ewes and those given E_2 (Fig. 5). However, unlike intact animals (Fig. 4), mRNA encoding PGF receptor was greater ($p < 0.05$) in hysterectomized ewes injected with estradiol compared with uninjected controls (Fig. 5). Laddering of genomic DNA was not observed by agarose-gel electrophoresis in any sample from hysterectomized ewes (data not shown).

Discussion

Steady-state levels of luteal mRNA encoding PGF receptor have been shown to decrease during natural or $PGF_{2\alpha}$-induced regression *(17,22)*. $PGF_{2\alpha}$ has been associated with directly initiating intracellular events associated with functional regression (decreased progesterone production) in the ovine corpus luteum *(21–23)*. Expression of PGF receptor mRNA was reduced in bovine luteinized granulosa cells incubated with $PGF_{2\alpha}$ in vitro *(23)*. Thus, whether the decreased expression of PGF receptor during luteal regression is the result of homologous downregulation by $PGF_{2\alpha}$–PGF receptor interactions or decreased progesterone production is not known. Decreased expression of PGF receptor in the late luteal phase was observed in ewes in which circulating progesterone levels had

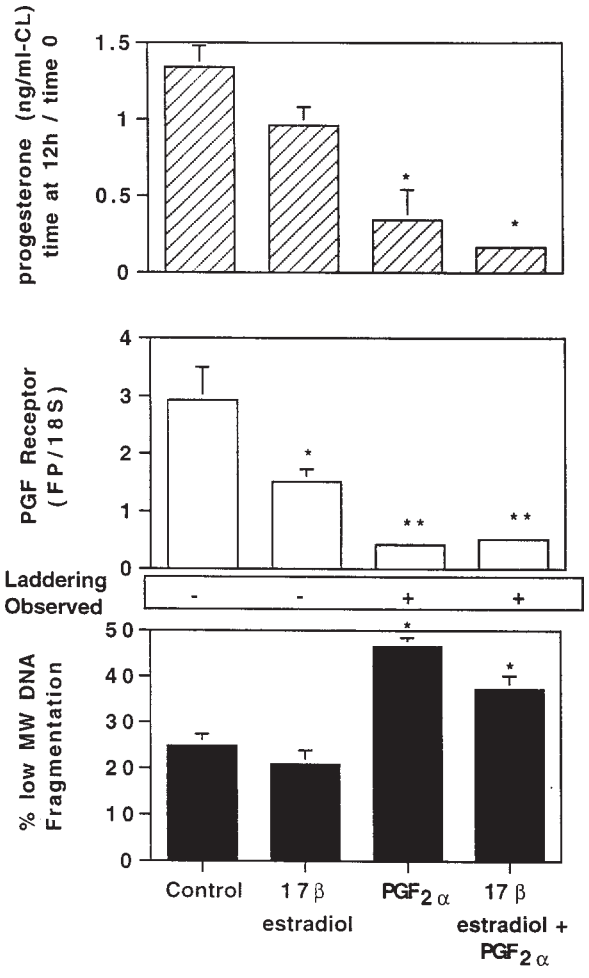

Fig. 4. Effect of E_2 and/or $PGF_{2\alpha}$ on luteal expression of PGF receptor in intact ewes. Day 10 ewes were injected with E_2 (25 μg iv, 37.5 μg im), $PGF_{2\alpha}$ (10 mg im), or both. Twelve hours later, blood and corpora lutea (CL) were collected from treated and uninjected (control) ewes, and analyzed for circulating progesterone (hatched bars), steady-state luteal content of mRNA encoding PGF receptor (FP, open bars), or low-mol-wt fragmentation (<6 kbp; solid bars) as described in Materials and Methods. Presence (+) or absence (–) of visible laddering of DNA is indicated. Values are expressed as a ratio of mean progesterone (ng/mL, normalized to number of CL) at time of collection (12 h) vs time of injection (time 0), and mRNA for PGF receptor (FP/18S ribosomal RNA) or % genomic DNA appearing as low-mol-wt fragments at time of collection. $n = 3$; $^*p < 0.05$ different from control.

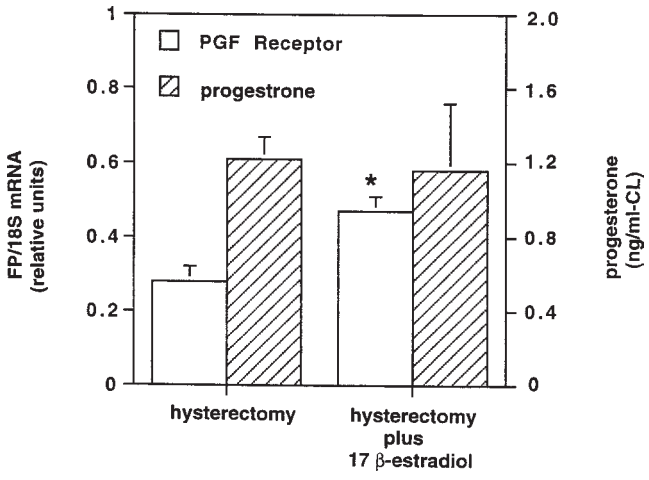

Fig. 5. Effect of E_2 on luteal expression of PGF receptor in hysterectomized ewes. Day 10 ewes were hysterectomized. Twelve hours later, ewes were injected with E_2 (25 μg iv, 37.5 μg, im). Twenty-four hours after hysterectomy, blood and corpora lutea (CL) were collected from hysterectomized control or treated ewes, and analyzed for circulating progesterone (hatched bars) and steady-state luteal content of mRNA encoding PGF receptor (open bars) as described in Materials and Methods. Values are the mean ± SEM progesterone (ng/mL, normalized to number of CL) and mRNA (FP/18S ribosomal RNA). $n = 4$; $^*p < 0.05$ different from hysterectomized controls.

dropped (functional regression; *17*). Additionally, this decrease was also accompanied by increased internucleosomal DNA fragmentation (laddering), an indicator of irreversible cell damage (structural regression). Therefore, expression of PGF receptor may be altered during functional and/or structural luteal regression. Juengel et al. *(22,24)* reported no differences in steady-state luteal mRNA encoding PGF receptor in cyclic ewes between d 10 and d 15 of the estrous cycle. However, there was no indication in that report that circulating progesterone levels had dropped by d 15. In the present study, steady state levels of mRNA for PGF receptor were decreased within 8 h of injection of ewes with $PGF_{2\alpha}$, a time at which circulating progesterone levels were also reduced, and DNA fragmentation was not yet increased. Therefore, these results demonstrate that regulation of PGF receptor expression is associated with functional luteal regression, which occurs prior to the onset of substantial DNA fragmentation.

In a previous study, steady-state levels of mRNA for PGF receptor were increased in corpora lutea collected on d 16 of pregnancy in ewes, when compared with d 10 or d 16 in nonpregnant animals *(17)*. Messenger RNA encoding PGF receptor in ovine corpora lutea was increased following injection of ewes with luteinizing hormone LH *(22)*. Additionally, mRNA encoding PGF receptor was increased in cultured bovine luteinized granulosa cells incubated with forskolin, although progesterone production was not measured in those cells *(23)*. Therefore, luteotropic support (working via increased cAMP) appears to stimulate expression of PGF receptor. Recent evidence in monkeys has provided support for the previous hypothesis proposed by Rothchild (25) that progesterone is the primary luteotropin in the corpus luteum *(26)*. Thus, whether increased luteal expression of PGF receptor is owing to increases in cAMP and/or progesterone is not clear. The results presented here demonstrate that luteal mRNA encoding PGF receptor is greater in corpora lutea of pregnant ewes than those in nonpregnant ewes on d 14, but not on d 12. The greater expression of PGF receptor is accompanied by greater circulating progesterone levels. Therefore, these findings pro-

vide a further link between expression of PGF receptor and progesterone production.

In addition to $PGF_{2\alpha}$, E_2 has been proposed as a mediator of luteolysis in ewes. Luteolysis was induced by administration of estradiol during the mid- *(18,20)* or late-luteal phase *(27)*. Furthermore, removal of endogenous estradiol by destruction of ovarian follicles prolonged the life-span of the corpus luteum *(19)*. At the end of the ovine estrous cycle, circulating concentrations of progesterone decline in the absence of pregnancy. This decline is associated with a concurrent rise in circulating levels of E_2 from developing preovulatory follicles *(1)*. In cyclic heifers on d 19 of the estrous cycle, circulating ratios of estradiol to progesterone were elevated in animals with regressing corpora lutea when compared with animals with nonregressing corpora lutea *(28)*. Thus, at the time of regression, the relative abundance of the steroids shifts, and estradiol levels become dominant during luteolysis. High-affinity receptors for estradiol in the ovine corpus luteum were reported to be elevated at the end of the estrous cycle and to be most highly concentrated in large luteal cells *(29)*. Because circulating levels of E_2 are increased in ewes during the late-luteal phase, estrogen may have a direct effect on luteal function during this time, and this effect could be directly on large cells that are known to be responsive to a luteolytic signal from $PGF_{2\alpha}$ *(30–32)*.

It is likely that there are a variety of physiological responses to estradiol injection in intact ewes. The results presented here show that one such response is a reduction in steady-state amounts of luteal mRNA for PGF receptor. This response was seen even though circulating progesterone levels had not yet decreased. Thus, these results provide evidence for a dissociation between regulation of PGF receptor expression and progesterone production. In another study, however, 16 h following implantation of estradiol in midluteal-phase ewes, there was no effect on mRNA encoding PGF receptor *(24)*. The reason for the discrepancy between those values and the values we report here 12 h following injection is unclear. However, estradiol levels given by injection in this study were not high enough to alter significantly circulating E_2 levels after 12 h, whereas they were substantially elevated in the other study *(24)*. The differences in method of estradiol administration are also likely to have played a role.

In the ewe, the presence of the uterus was required to demonstrate estradiol-induced luteal regression *(18,33)*. Uterine-derived $PGF_{2\alpha}$ in ewes is responsible for the onset of regression *(5)*. Estradiol is known to stimulate uterine production of $PGF_{2\alpha}$ via upregulation of oxytocin receptors in the endometrium *(27)*. In one study by Zhang et al. *(21)*, E_2 was administered as implants in ewes in which ovarian follicles had been destroyed by X-irradiation on d 8. Estradiol shortened the time to luteolysis and increased the frequency in peaking of circulating $PGF_{2\alpha}$ metabolite, suggesting estradiol-induced luteolysis may be via uterine stimulation of $PGF_{2\alpha}$ release. Spencer et al. *(27)* demonstrated that intrauterine injections of interferon-τ protected against luteolysis in ewes treated with a pharmacological dose of estradiol. This was associated with inhibition of expression of the genes encoding uterine estrogen and oxytocin receptors, an event that would reduce uterine secretion of $PGF_{2\alpha}$. This further establishes the uterine involvement in estrogen-induced luteolysis, and supports that luteal effects are indirectly mediated by uterine-derived $PGF_{2\alpha}$. Therefore, the response seen here in intact ewes could result indirectly from stimulation of $PGF_{2\alpha}$ secretion by the uterus. The administration of estradiol in the studies reported here (62.5 μg) was similar to that given to ovariectomized ewes to mimic the preovulatory estradiol peak *(34)*. This dose was also substantially below that given by others to induce luteolysis (750 μg; *20,27,35*). In the studies reported here, estradiol inhibited expression of PGF receptor in corpora lutea collected from intact ewes, but stimulated expression in hysterectomized ewes. Thus, it appears that in luteal PGF receptor expression, the net physiological response to estradiol injection in intact ewes requires the presence of a uterus. This also occurred in the absence of increased circulating progesterone levels, thereby providing further dissociation of PGF receptor expression from progesterone production.

In summary, the results presented here have demonstrated that decreased steady-state expression of PGF receptor during luteolysis is associated with functional regression and occurs prior to DNA changes during structural regression. Additionally, it has been shown that the downregulation of PGF receptor expression in the ovine corpus luteum is not the direct result of decreased progesterone production. This suggests that $PGF_{2\alpha}$ may produce homologous downregulation by a direct interaction with the PGF receptor. This finding is consistent with a recent report in which $PGF_{2\alpha}$ caused a reduction of PGF receptor mRNA expression in isolated ovine large luteal cells during in vitro incubations *(36)*. Finally, a direct luteal role for estradiol in stimulating or supporting expression of PGF receptor has been shown. The specific interactions between these regulators of luteal function and expression of PGF receptor remain to be further elucidated.

Materials and Methods

Animals and Tissue Collection

The estrous cycles of Western range ewes (2–5 yr of age) were synchronized by injection with $PGF_{2\alpha}$ (Lutalyse; Upjohn Company, Kalamazoo, MI, 10 mg im) on d –12 and d –3 (0 being synchronous estrus). Follicular development was stimulated with an sc injection of pregnant mare's serum gonadotropin (750 IU, sc) on d –3 followed on d 0 with an injection of human chorionic gonadotropin (750 IU iv; Sigma Chemical Co., St. Louis, MO). On d 10, a luteolytic dose of $PGF_{2\alpha}$ (10 mg im) and/or a nonluteolytic

dose of E_2 (25 μg iv plus 37.5 μg im) was given by injection, and tissue samples were collected on d 10 and d 11 depending on treatment and time. **Note:** The dose used was determined from a reported study in which estradiol was administered (25 μg, im and 25 μg, iv) to mimic more closely the physiological concentrations found in the preovulatory estradiol peak *(34)*. This resulted in a nonsignificant change in mean levels of all animals in each group 12 h following injection. It is likely that increased estradiol levels peaked earlier than that time, although samples between preinjection and the 12-h time-point were not taken.

To obtain corpora lutea from pregnant animals, ewes were exposed to fertile rams, and breeding dates were recorded. Pregnancy was confirmed by uterine flushing at the time of tissue collection on d 12 or d 14. Corpora lutea were collected at the indicated times, and quick-frozen in liquid nitrogen for subsequent quantification of mRNA for PGF receptor and evaluation of genomic DNA. Blood samples were collected prior to injections and at the time of tissue collection by jugular venipuncture for determination of circulating progesterone and E_2 levels. For hysterectomy, the reproductive tract was exteriorized. The uteroovarian vasculature was clamped and dissected away from the uterus, ligated between the cervix and ovaries, and removed. All animal procedures were approved by and performed in accordance with the University of Arizona Animal Care and Use Committee.

Steroid Analysis

Progesterone and E_2 content of blood were determined by radioimmunoassay (RIA) as previously described *(32)*. The progesterone antibody was kindly contributed by Gordon Niswender. The assay sensitivity was 0.56 pg/tube, and inter- and intraassay coefficients of variation were 9.1 and 7.0%, respectively. The E_2 antibody was purchased from Sigma Chemical Company. The assay sensitivity was 2.88 pg/tube, and inter- and intraassay coefficients of variation were 6.1 and 3.1%, respectively.

Northern Blot Analysis

Corpora lutea were thawed and immediately dissected into small fragments. Total cellular RNA was extracted by the guanidinium thiocyanate-phenol-chloroform extraction procedure. The purity and quantity of RNA were estimated based on optical density (A_{260}/A_{280}).

Isolated RNA was analyzed by Northern blot analysis as previously described *(17)* using cDNA for PGF receptor (FP) that was 752 bp in length (639–1390 bp; *16*) and labeled by random priming using [α^{32}P]deoxy-CTP (3000 Ci/mmol; Amersham, Arlington Heights, IL). Labeled cDNA was purified by column chromatography (Nuc Trap Push column, Stratagene, La Jolla, CA). Following hybridization analysis, the blots were rehybridized with radiolabeled 18S ribosomal RNA riboprobe that was synthesized by in vitro transcription from linearized plasmid templates using RNA polymerase, [α^{32}P]-CTP (3000 Ci/mmol; Amersham), and Gemini II Riboprobe Core System (Promega, Madison, WI).

For Northern blot analysis, 5 μg of total RNA from each sample were separated through a 1% denaturing gel and transferred to nylon membranes (Stratagene). Membranes were prehybridized for 2 h at 65° (18S) or 4 h at 42°C (FP), followed by overnight hybridization with cDNA or cRNA probes. Membranes were washed at room temperature with 2X SSC–0.1% SDS, followed by 1–2 consecutive 15-min washes with 0.5X SSC–0.1% SDS. Membranes were then exposed to Kodak X-Omat films and quantified by Instant Imager Electronic Autoradiograph Analyzer (Packard Instrument Co., Meridan, CT). Changes in PGF receptor mRNA levels were normalized to 18S ribosomal RNA levels in each sample.

DNA Analysis

Genomic DNA from luteal pieces was prepared as previously described *(8)*. The purity and quantity were estimated by optical density (A_{260}/A_{280}). An equivalent amount of genomic DNA from each sample was radiolabeled on 3'-ends with [α^{32}P]dideoxy-ATP (3000 Ci/mmol; Amersham) using 25 U terminal transferase (Boehringer-Mannheim, Indianapolis, IN). DNA samples were separated by electrophoresis through 2% agarose gels (500 ng DNA/sample). Gels were dried without heat in a slab dryer and exposed to film (Kodak X-Omat film at –70°C) for autoradiographic analysis. Quantification of low-mol-wt fragmentation was done using Instant Imager Electronic Autoradiograph Analyzer (Packard Instrument Co.).

Statistical Analysis

Values from luteal tissue and blood samples were from individual ewes. All data were analyzed by one-way analysis of variance, followed where appropriated by Fisher PLSD multiple-range testing. Significance was assigned at the 0.05 level.

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References

1. Hauger, R. L., Karsch, F. J., and Foster, D. L. (1977). *Endocrinology* **101,** 807–817.
2. McCracken, J. A., Glew, M. E., and Scaramuzzi, R. J. (1970). *J. Clin. Endocrinol. Metab.* **30,** 544–546.
3. McCracken, J. A., Carlson, J. C., Glew, M. E., Goding, J. R., Baird, D. T., Green, K., et al. (1972). *Nature New Biol.* **83,** 527–536.
4. Del Campo, C. H. and Ginther, O. J. (1973). *Am. J. Vet. Res.* **34,** 300–316,
5. Heap, R. B., Fleet, I. R., and Hamon, M. (1985). *J. Reprod. Fertil.* **74,** 645–656.

6. Nett, T. M. and Niswender, G. D. (1981). *Acta Vet. Scand.* **77(Suppl),** 117–130.
7. Braden, T. D., Gamboni, F., and Niswender G. D. (1988). *Biol. Reprod.* **39,** 245–253.
8. Rueda, B. R., Wegner, J. A., Marion, S. L., Wahlen, D. D., and Hoyer P. B. (1995). *Biol. Reprod.* **52,** 305–312.
9. McGuire, W. J., Juengel, J. L., and Niswender G. D. (1994). *Biol. Reprod.* **51,** 800–806.
10. Fraser, H. M., Abbott, M., Laird, N. C., McNeilly, A. S., Nestor, J. J., Jr., and Vickery, B. H. (1985). *J. Endocrinol.* **111,** 83–90.
11. Pate, J. L. and Nephew, K. P. (1988). *Biol. Reprod.* **38,** 568–576.
12. Juengel, J. L., Gaverick, H. A., Johnson, A. L., Youngquist, R. S., and Smith, M. F. (1993). *Endocrinology* **132,** 249–254.
13. Hoyer, P. B. (1998). *J. Soc. Gynecol. Invest.* **5,** 49–57.
14. Balapure, A. K., Caicedo, I. C., Kawada, K., Watt, D. S., Rexroad, C. E., Jr., and Fitz, T. A. (1989). *Biol. Reprod.* **41,** 385–392.
15. Wiepz, G. J., Wiltbank, M. C., Nett, T. M., and Niswender, G. D. (1992). *Biol. Reprod.* **47,** 984–991.
16. Graves, P. E., Bailey, T. J., Pierce, K. L, Rueda, B. R., Gil, D. W., Woodard, D. F., et al. (1995). *Endocrinology* **136,** 3430–3436.
17. Rueda, B. R., Botros, I. W., Pierce, K. L., Regan, J. W., and Hoyer, P. B. (1995). *Endocrine* **3,** 781–787.
18. Stormshak, F., Kelley, H. E., and Hawk, H. W. (1969). *J. Anim. Sci.* **29,** 476–478.
19. Karsch, F. J., Noveroske, J. W., Roche, J. F., Norton, H. W., and Nalbandov, A. V. (1970). *Endocrinology* **87,** 1228–1236.
20. Hixon, J. E. and Flint, A. P. F. (1987). *J. Reprod. Fertil.* **79,** 457–467.
21. Zhang, J., Weston, P. G., and Hixon, J. E. (1991). *Biol. Reprod.* **45,** 395–402.
22. Juengel, J. L., Wiltbank, M. C., Meberg, B. M., and Niswender, G. D. (1996). *Biol. Reprod.* **54,** 1096–1102.
23. Mamluk, R., Chen, D., Greber, Y., Davis, J. S., and Meidan, R. (1998). *Biol. Reprod.* **58,** 849–856.
24. Juengel, J. L., Melner, M. H., Clapper, J. A., Turzillo, A. M., Moss, G. E., Nett, T. M., et al. (1998). *J. Reprod. Fertil.* **113,** 299–305.
25. Rothchild, I. (1981). *Rec. Prog. Horm. Res.* **37,** 183–298.
26. Duffy, D. M., Hess, D. L., and Stouffer, R. L. (1994). *J. Clin. Endocrinol. Met.* **79,** 1587–1594.
27. Spencer, T. E., Becker, W. C., George, P., Mirando, M. A., Ogle, T. F., and Bazer, F. W. (1995). *Endocrinology* **136,** 4932–4944.
28. Mirando, M. A., Becker, W. C., and Whiteaker, S. S. (1993). *Biol. Reprod.* **48,** 874–882.
29. Glass, J. D., Fitz, T. A., and Niswender, G. D. (1984). *Biol. Reprod.* **31,** 967–974.
30. Wegner, J. A., Martinez-Zaguilan, R., Gillies, R. J., and Hoyer P. B. (1991). *Endocrinology* **128,** 929–936.
31. Wiltbank, M. C., Knickerbocker, J. J., and Niswender, G. D. (1989). *Biol. Reprod.* **40,** 1194–1200.
32. Hoyer, P. B. and Marion, S. L. (1989). *J. Reprod. Fertil.* **86,** 445–455.
33. Bolt, D. J. and Hawk, H. W. (1975). *J. Anim. Sci.* **40,** 687–690.
34. Caraty, A., Locatelli, A., and Martin, G. B. (1989). *J. Endocrinol.* **123,** 375–382.
35. Hawk, H. W. and Bolt, D. J. (1970). *Biol. Reprod.* **2,** 275–278.
36. Tsai, S., Anderson, L. E., Juengel, J., Niswender, G. D., and Wiltbank, M. C. (1998). *J. Reprod. Fertil.* **114,** 69–75.