

Full Length Research Paper

Effects of 8-epi-prostaglandin F2 α and prostaglandin F2 α on serum progesterone concentration and corpus luteum size in ewes

D. T. Yates*, L. J. Yates, D. M. Hallford and T. T. Ross

Department of Animal and Range Sciences, New Mexico State University, Las Cruces, NM 88003-003, New Mexico
Agricultural Experiment Station, Mexico.

Accepted 29 September, 2010

Prostaglandin F2 α (PGF2 α) is luteolytic in sheep. This study assessed luteolytic ability of an isomer, 8-epi-PGF2 α . Ten ewes were estrus-synchronized and blood serum was collected for 12 days and analyzed for progesterone concentration. Ewes were laparoscopically administered 200 μ g 8-epi-PGF2 α or PGF2 α , or placebo in each ovary on day 8 and ovariectomized on day 13. Corpora lutea (CL) were weighed. Serum progesterone was reduced ($P < 0.041$) by PGF2 α on day 9, but recovered to control levels ($P = 0.646$) by day 10. Interestingly, 8-epi-PGF2 α increased ($P < 0.050$) serum progesterone on days 9, 10, and 12. However, area under curve for serum progesterone between days 8 and 12 did not differ ($P = 0.165$) among treatments. In ewes administered PGF2 α , serum progesterone decreased sharply ($P < 0.001$) after treatment, but recovered ($P = 0.217$) to pre-treatment levels by day 11, indicating limited luteolysis after one-time dosage. Mean CL weight did not differ ($P = 0.836$) among treatments, but weight of largest CL was reduced by 8-epi-PGF2 α ($P = 0.032$) and PGF2 α ($P = 0.015$). One-time, intra-ovarian administration of 8-epi-PGF2 α at this dosage did not cause luteolysis. Additionally, serum progesterone is capable of recovery from one-time, intra-ovarian administration of PGF2 α .

Key words: 8-epi-prostaglandin F2 α , luteolysis, prostaglandin F2 α .

INTRODUCTION

Prostaglandin F2 α (PGF2 α) is known to be the natural luteolysin in domestic ruminants (Goding, 1974). However, during periods of oxidative stress, production of 8-epi-PGF2 α , a structural isomer of PGF2 α , is increased (Kinsella, 2001). This isoprostane is produced from arachidonic acid by a seemingly unregulated cyclooxygenase-independent pathway involving reactive oxygen species as pseudo-enzymes (Klein et al., 1997; Yin et al., 2008). Previous research has shown 8-epi-PGF2 α to be a vasoconstrictive compound, similar to PGF2 α (Takahashi et al., 1992), although, it is not known if the mechanism by which vasoconstriction occurs is common between the two compounds (Unmack et al., 2001; Comporti et al., 2008). Prostaglandin F2 α has been shown to reduce serum progesterone concentration within 24 h in sheep when administered directly into the ovary, while prostaglandin E actually increased

progesterone concentration (Inskeep et al., 1975). Despite structural similarities, it is unclear if 8-epi-PGF2 α possesses the same luteolytic ability as PGF2 α or if the former functions as a luteotropin similarly to prostaglandin E. Previous research also indicates that PGF2 α -induced reduction of progesterone concentration and luteal cell death are temporally, and possibly mechanistically, discrete events (Pate, 1995; Griffeth et al., 2002), and may also require different dosage (Juengel et al., 2000). The objective of the current study was to evaluate the abilities of 8-epi-PGF2 α and PGF2 α to elicit functional luteolysis, as indicated by reduced serum progesterone concentration, and structural luteolysis, as indicated by corpora lutea (CL) weights, when administered intra-ovarian on day 8 of the estrous cycle in sheep.

MATERIALS AND METHODS

Estrus synchronization and animal care

All procedures were approved by the New Mexico State University

*Corresponding author. E-mail: dty4888@yahoo.com. Tel: 1(520)626-1454. Fax: 1(520)626-1283.

Institutional Animal Care and Use Committee. Mature, cyclic Rambouillet ewes were weighed (70.0 ± 2.6 kg) and examined for health. Animals were allowed free access to shelter, shade, and water and were fed chopped alfalfa hay (approximately 2 kg/ewe) once daily at 0700, unless otherwise stated. On day -14 (day 1 = estrus), all ewes received a progesterone-impregnated vaginal insert (EAZI-BREED CIDR, 0.3 g progesterone; Pharmacia and Upjohn, Co., Hamilton, New Zealand) to synchronize estrus. All CIDR were removed on day 0 and a mature vasectomized ram equipped with a marking harness was introduced into the pen to detect estrus. Nine of the 10 ewes were marked within 24 h of CIDR removal, while the remaining ewe responded within 48 h.

Experimental procedure

Ten ewes were randomly assigned to 1 of 3 treatments: 200 μ g 8-epi-PGF2 α per ovary (in 0.1 ml absolute ethanol; BioMol International, LP, Plymouth Meeting, PA; Cat # PG-049; $n = 4$), 200 μ g PGF2 α per ovary (in 0.1 ml absolute ethanol per ovary; Lutalyse, Pfizer, Inc., New York, NY; $n = 3$), or 0.1 ml ethanol carrier (control; $n = 3$) per ovary. Blood samples were collected once daily at 1300 from day 0 to 12 to assess functional luteolysis. On day 8, treatment was injected into the interstitial tissue of both ovaries in each ewe through laparoscopy. On day 13, ovariectomies were performed on all ewes. Corpora lutea were removed from each ovary and weighed as an indication of structural luteolysis.

Serum collection and progesterone analysis

Blood was collected through jugular venipuncture (Corvac serum separator tubes). Samples were kept at room temperature for 30 to 60 min and then centrifuged ($1,500 \times g$ at 4°C for 15 min). After centrifugation, serum was stored in plastic vials at -80°C until ready for assay. Radioimmunoassay (Coat-A-Count, Siemens Medical Solutions Diagnostics, Los Angeles, CA) was used to quantify progesterone concentration in all serum samples with modifications described by Schneider and Hallford (1996). All samples were analyzed in a single assay with an intra-assay CV of 8.7%.

Surgical procedures

Ewes were held off feed and water for 18 h before laparoscopic procedures. To begin the procedure, general anesthesia (Ketamine, 1 ml, i.v.; Vedco Inc., St. Joseph, MO) was administered and the abdominal area was washed and shorn. Ewes were placed in a supine position on a wheeled laparoscopy gurney. Lidocaine (5 ml, s.c.; Vedco Inc., St. Joseph, MO) was administered in the abdomen approximately 3 cm to either side of the mid-line and 2 cm anterior to the mammary gland. A 1 cm incision was made through the skin and a trocar was used to insert a 6 mm laparoscopic cannula (Karl Storz Veterinary, Goleta, CA) through the abdominal wall and into the abdominal cavity at the site of the right-side incision. Carbon dioxide gas was slowly pumped through the cannula to moderately inflate the abdominal cavity. After inflation, a second trocar was used to insert a 10 mm cannula at the site of the left incision, and the laparoscopic scope (GYRUS XLS-300, ACMI Corp., Southborough, MA) was inserted into the second cannula to locate the ovaries. A needle (18 gauge, 60 cm) was inserted through the first cannula and into the center of the interstitial tissue of the ovary. An assistant then pushed the prescribed treatment through the needle and into the ovary using a 1 ml syringe. Injected fluid was followed by a predetermined amount of air to displace any fluid that may have remained in the needle. Treatment administration was immediately repeated for the second ovary, regardless of appearance or absence of a CL on either or both ovaries. Animals were then returned to their original pens and closely monitored for

24 h.

Feed and water was restricted for 24 h before ovariectomy. Ewes were anesthetized with Ketamine (1 ml, i.v.), surgical sites were washed and shorn, and ewes were placed in a supine position on a wheeled surgical gurney, restrained, and transported to the surgical room, where ovaries were removed by trained technicians using an IACUC-approved protocol under sustained general anesthesia (Ketamine, 1 ml, i.v., approximately once every 15 minutes). After removal of the ovaries, animals were given penicillin (5 mL, s.c.), flunixin meglumine (2.5 mL, i.m.; Vetco Inc., St. Joseph, MO), and topical antimicrobial and insecticide treatments, and were offered one-fourth alfalfa rations and *ad libitum* water for 3 days.

Statistical analysis

Ewe was considered the experimental unit. Serum progesterone data were treated as a split plot with treatment in the main plot and day and treatment \times day interaction in the sub plot. These data were analyzed by the mixed procedure of SAS (SAS Inst. Inc., Cary, NC) with repeated measures function. When treatments \times day interactions were observed, treatment effects were evaluated within each day. Luteal weights and serum progesterone area under the curve (trapezoidal summation method) were treated as a completely randomized design and analyzed with the GLM procedure of SAS. Correlation comparisons were made using the correlation procedure of SAS.

RESULTS

A treatment \times day interaction was observed ($P = 0.002$) for serum progesterone concentration, therefore treatment effects were examined within each day and day effects were examined within each treatment. As expected, serum progesterone concentration (Figure 1) did not differ ($P > 0.652$) among treatment groups on any day before treatment administration. On day 9, the first day after treatment, serum progesterone concentration was sharply reduced ($P < 0.041$) in ewes receiving PGF2 α compared to day 9 levels in control ewes as well as their own day 8 levels. However, serum progesterone concentration in these ewes had recovered to control levels ($P > 0.217$) by day 10 and to their own pre-treatment levels by day 11. Surprisingly, serum progesterone concentration was actually greater ($P < 0.007$) in ewes administered 8-epi-PGF2 α compared to control ewes on days 9, 10, and 12. Despite progesterone concentration differences among treatments on individual days, total area under curve between days 8 and 12 for serum progesterone did not differ ($P = 0.165$) among treatments, suggesting that treatment effects on total progesterone output was mild. Interestingly, serum progesterone total area under curve for this period did not correlate strongly to total CL weight ($r = 0.36$; $P = 0.308$), average CL weight ($r = 0.44$; $P = 0.209$), or body weight ($r = 0.12$; $P = 0.774$). Mean CL weight (Table 1) did not differ ($P = 0.836$) among treatments, but weight of largest CL was reduced in both 8-epi-PGF2 α ($P = 0.032$) and PGF2 α ($P = 0.015$) groups compared to controls, although, physiological significance

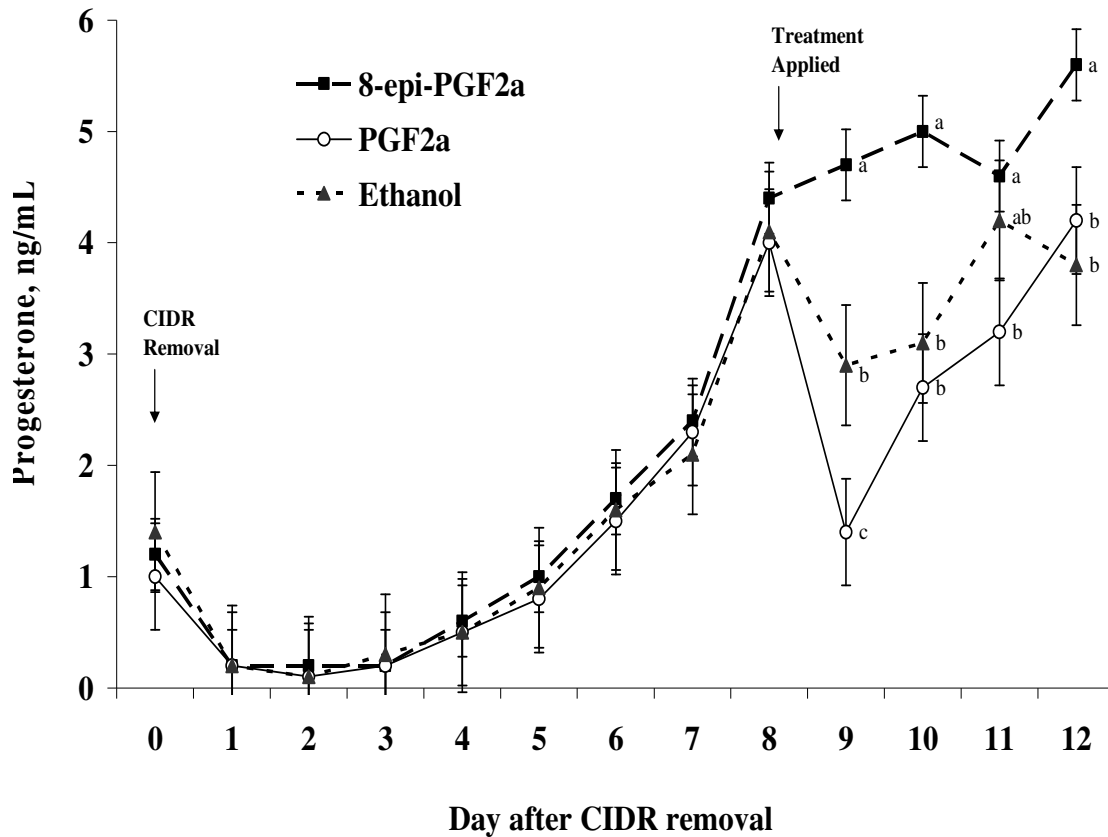


Figure 1. Progesterone concentrations in Rambouillet ewes administered 200 μ g 8-epi-PGF2 α , 200 μ g PGF2 α , or ethanol placebo laparoscopically into interstitial tissue of each ovary on day 8 of estrous cycle (CIDR removal on day 0). Treatment \times day interaction, $P = 0.002$. Differing superscripts indicate differing ($P < 0.050$) means for treatment effect within day.

Table 1. Corpus luteum weights (g) at day 13 in Rambouillet ewes administered 200 μ g 8-epi-PGF2 α , 200 μ g PGF2 α , or ethanol placebo laparoscopically into interstitial tissue of each ovary on day 8 of estrous cycle (CIDR removal on day 0).

Item	Ethanol ¹	8-epi-PGF2 α ²	PGF2 α ²	SE ³	P - value
Average CL weight	0.62	0.59	0.54	0.044	0.458
Largest CL weight ⁴	0.76 ^a	0.61 ^b	0.57 ^b	0.042	0.033

¹Pure ethanol (0.1 ml), ²Suspended in 0.1 mL pure ethanol, ³Standard error (n = 3, 4, and 3 for ethanol, 8-epi-PGF2 α and PGF2 α groups, respectively), ⁴Different superscripts indicate differing means ($P < 0.050$).

of the latter is unclear. Although, ewes exhibited anywhere from 1 to 3 CL, number of CL/ewe did not affect mean CL weight ($P = 0.811$) or weight of the largest CL ($P = 0.124$).

DISCUSSION

On the first day after treatment, serum progesterone concentration was sharply reduced in ewes receiving PGF2 α compared to control ewes, as well as their own

levels measured just before treatment. This observation is consistent with previous findings of reduced progesterone concentrations in response to similar dosage of PGF2 α administered intra-ovarian (Inskeep et al., 1975). However, serum progesterone concentration in these ewes had recovered within days of the initial reduction. Although, recovery of steroidogenesis does not occur during natural luteolysis, Towle et al. (2002) reported that progesterone depression associated with sub-luteolytic amounts of exogenous PGF2 α may indeed be temporary. Other research indicates that pulsatility of

release patterns may be necessary for complete luteolysis (Zarco et al., 1988) and that one-time administration of PGF 2α may not result in full luteolysis at certain dosages (Goodman, 1994; Niswender et al., 2000; Rubianes et al., 2003). These data may indicate the need for multiple administrations of intra-ovarian PGF 2α in the current dosage for complete and irreversible functional luteolysis. Surprisingly, serum progesterone concentration was actually greater in ewes administered 8-epi-PGF 2α compared to control ewes after treatment. Although, little data exist that suggest 8-epi-PGF 2α is either luteolytic or luteotropic, previous research has indicated that 8-epi-PGF 2α and PGF 2α may act through different receptors, even when inducing similar responses, despite their structural similarities. Kiriya et al. (1997) found that, although 8-epi-PGF 2α does bind to the prostaglandin F receptor, the former actually possesses greater affinity for the prostaglandin E 3 receptor, as well as some binding capability to prostaglandin E 2 receptor and thromboxane A 2 receptor. Kinsella (2001) and Comporti et al. (2008) also reported an ability of 8-epi-PGF 2α to act through the thromboxane A 2 receptor, though not exclusively, while Elmhurst et al. (1997) and Unmack et al. (2001) found additional evidence of binding to prostaglandin E receptors. Binding to prostaglandin E receptors may help explain increased serum progesterone concentration in ewes receiving 8-epi-PGF 2α in the current study, as prostaglandin E is known to promote steroidogenesis (Speroff and Ramwell, 1970; Kim et al., 2001). However, thromboxane A 2 is thought to antagonize steroidogenesis (Wang et al., 2008). Despite progesterone concentration differences among treatments on individual days, total area under curve for serum progesterone after treatment did not differ among treatments, suggesting that treatment effects on total progesterone output was mild. Interestingly, serum progesterone total area under curve did not correlate strongly to total or average CL weight or body weight. This observation does not reflect previous findings in goats (Jarrell and Dziuk, 1991), but does support findings in gilts (Wetteman et al., 1980). Although, mean CL weight did not differ among treatments, weight of largest CL was reduced in both 8-epi-PGF 2α and PGF 2α groups compared to controls, indicating only mild or partial structural luteolysis. Absence of substantial indication of structural luteolysis in the presence of altered serum progesterone concentration is understandable after considering timing of luteolytic events. Pate (1995) and Neill (2006) reported that depression of progesterone precedes apoptosis and structural demise of luteal cells, while Griffeth et al. (2002) described the possibility for different mechanisms by which PGF 2α elicits these events. Additionally, Juengel et al. (2000) found that progesterone can be diminished by PGF 2α at dosages that do not result in luteal cell death.

Data from the current study indicated that 8-epi-PGF 2α

administered intra-ovarian increased serum progesterone concentration despite structural similarities between 8-epi-PGF 2α and PGF 2α , a known luteolysin. Prostaglandin F 2α caused an initial decrease in serum progesterone concentration, but these concentrations fully recovered within 2 days, indicating that dosage used was sub-luteolytic. Despite effects of 8-epi-PGF 2α and PGF 2α on serum progesterone concentration and size of largest CL, neither compound elicited an effect on mean corpus luteum weight. Data from this and other studies indicate the possibility of more conclusive results from either increased dosage or repeated administration of compounds.

REFERENCES

- Comporti M, Signorini C, Arezzini B, Vecchio D, Monaco B, Gardi C (2008). F 2 -isoprostanones are not just markers of oxidative stress. *Free Radic. Boil. Med.*, 44: 247-256.
- Elmhurst JL, Betti PA, Rangachari PK (1997). Intestinal effects of isoprostanones: Evidence for the involvement of prostanoid EP and TP receptors. *J. Pharmacol. Exp. Ther.*, 282:1198-1205.
- Goding JR (1974). The demonstration that PGF 2α is the uterine luteolysin in the ewe. *J. Reprod. Fertil.*, 38:261-271.
- Goodman RL (1994). Neuroendocrine control of the ovine estrous cycle. In: *The Physiology of Reproduction* (Knobil E, Neill JD eds.). Raven Press, New York, 2: 659-710.
- Griffeth RJ, Nett RM, Burns PD, Escudero JM, Inskeep EK, Niswender GD (2002). Is luteal production of PGF 2α required for luteolysis? *Biol. Reprod.*, 66(1): 287 (Abstr.).
- Inskeep EK, Smutny WJ, Butcher RL, Pexton JE (1975). Effects of intrafollicular injections of prostaglandins in non-pregnant and pregnant ewes. *J. Anim. Sci.*, 41: 1098-1104.
- Jarrell VL, Dziuk PJ (1991). Effect of number of corpora lutea and fetuses on concentrations of progesterone in blood of goats. *J. Anim. Sci.*, 69: 770-773.
- Juengel JL, Haworth JD, Rollyson MK, Silva PJ, Sawyer HR, Niswender GD (2000). Effect of dose of prostaglandin F 2α on steroidogenic components and oligonucleosomes in ovine luteal tissue. *Biol. Reprod.*, 62: 1047-1051.
- Kim L, Weems YS, Bridges PJ, LeaMaster BR, Ching L, Vincent DL, Weems CW (2001). Effects of indomethacin, luteinizing hormone (LH), prostaglandin E 2 (PGE 2), trilostane, mifepristone, ethamoxytriphetol (MER-25) on secretion of prostaglandin E (PGE), prostaglandin F 2α (PGF 2α) and progesterone by ovine corpora lutea of pregnancy or the estrous cycle. *Prostaglandins and other lipid mediators*, 63: 189-203.
- Kinsella BT (2001). Thromboxane A 2 signalling in humans: a 'tail' of two receptors. *Biochem. Soc. Transactions*, 29: 641-654.
- Kiriya M, Ushikubi F, Kobayashi T, Hirata M, Sugimoto Y, Narumiya S (1997). Ligand binding specificities of the eight types and subtypes of the mouse prostanoid receptors expressed in Chinese hamster ovary cells. *Br. J. Pharmacol.*, 122: 217-224.
- Klein T, Reutter F, Schweer H, Seyberth HW, Nusing RM (1997). Generation of the isoprostanone 8-epi-prostaglandin F 2α in vitro and in vivo via the cyclooxygenases. *J. Pharmacol. Exp. Ther.*, 282: 1658-1665.
- Neill JD (2006). Control of ovine estrous cycle. In: *Knobil and Neill's Physiology of Reproduction*, 3rd ed. Ed. Neill JD. Academic Press, St. Louis, MO, pp. 2389-2447.
- Niswender GD, Juengel JL, Silva PJ, Rollyson MK, McIntush EW (2000). Mechanisms controlling the function and life span of the corpus luteum. *Physiol. Rev.*, 80: 1-29.
- Pate JL (1995). Involvement of immune cells in regulation of ovarian function. *J. Reprod. Fertil. Suppl.*, 49: 365-377.
- Rubianes E, Menchaca A, Carvajal B (2003). Response of the 1-5 day-aged ovine corpus luteum to prostaglandin F 2α . *Anim. Reprod.*

- Sci., 78: 47-55.
- Schneider FA, Hallford D. (1996). Use of a rapid progesterone radioimmunoassay to predict pregnancy and fetal numbers in ewes. *Sheep Goat Res. J.*, 12: 33-38.
- Speroff L, Ramwell PW (1970). Prostaglandin stimulation of in vitro progesterone synthesis. *J. Clin. Endocrinol. Metab.*, 30: 345-350.
- Takahashi K, Nammour TM, Fukunaga M, Ebert J, Morrow JD, Roberts LJ, Hoover RL, Badr KF (1992). Glomerular actions of a free radical-generated novel prostaglandin, 8-epi-prostaglandin F₂α, in the rat. Evidence for interaction with thromboxane A₂ receptors. *J. Clin. Invest.*, 90: 136-141.
- Towle TA, Tsang PC, Milvae RA, Newbury MK, McCracken JA (2002). Dynamic in vivo changes in tissue inhibitors of metalloproteinases 1 and 2, and matrix metalloproteinases 2 and 9, during prostaglandin F(2α)-induced luteolysis in sheep. *Biol. Reprod.*, 66: 1515-1521.
- Unmack MA, Rangachari PK, Skadhauge E (2001). Effects of isoprostanes and prostanoids on porcine small intestine. *J. Pharmacol. Exp. Ther.*, 296: 434-441.
- Wang XJ, Yin X, Schiffer RB, King SR, Stocco DM, Grammas P (2008). Inhibition of thromboxane A synthase activity enhances steroidogenesis and steroidogenic acute regulatory gene expression in MA-10 mouse leydig cells. *Endocrinol.*, 149: 851-857.
- Wetteman RP, Johnson RK, Omtvedt IT (1980). The relationships between plasma progesterone and LH and the numbers of corpora lutea and embryos in purebred and crossbred gilts. *J. Anim. Sci.*, 51: 1352-1357.
- Yin K, Halushka PV, Yan YT, Wong PY (2008). Antiaggregatory activity of 8-epi-prostaglandin F₂ alpha and other F-series prostanoids and their binding to thromboxane A₂/prostaglandin H₂ receptors in human platelets. *J. Pharmacol. Exp. Ther.*, 270: 1192-1196.
- Zarco L, Stabenfeldt GH, Quirke JF, Kindahl H, Bradford GE (1988). Release of prostaglandin F-2 alpha and the timing of events associated with luteolysis in ewes with oestrous cycles of different lengths. *J. Reprod. Fertil.*, 83: 517-526.