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Full Length Research Paper

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

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Prostaglandin F2 (PGF2) is luteolytic in sheep. This study assessed luteolytic ability of an isomer, 8-epi-PGF2 . Ten ewes were estrus-synchronized and blood serum was collected for 12 days and analyzed for progesterone concentration. Ewes were laparoscopically administered 200 µg 8-epi -PGF2 or PGF2 , 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 PGF2α on day 9, but recovered to control levels (P = 0.646) by day 10. Interestingly, 8-epi-PGF2α 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 PGF2α, 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-PGF2α (P = 0.032) and PGF2α (P = 0.015). One-time, intra-ovarian administration of 8-epi-PGF2α at this dosage did not cause luteolysis. Additionally, serum progesterone is capable of recovery from one-time, intra-ovarian administration of PGF2α.

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

INTRODUCTION

Prostaglandin F2 (PGF2) is known to be the natural luteolysin in domestic ruminants (Goding, 1974). However, during periods of oxidative stress, production of 8-epi-PGF2 , a structural isomer of PGF2 , is increased (Kinsella, 2001). This isoprostane is produced from arachodonic 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 to be a vasoconstrictive compound, similar to PGF2 (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 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 possesses the same luteolytic ability as PGF2 or if the former functions as a luteotropin similarly to prostaglandin E. Previous research also indicates that PGF2 -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 and PGF2 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.

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MATERIALS AND METHODS

Estrus synchronization and animal care

All procedures were approved by the New Mexico State University

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 µg 8-epi-PGF2 per ovary (in 0.1 ml absolute ethanol; BioMol International, LP, Plymouth Meeting, PA; Cat # PG- 049; n = 4), 200 µg PGF2 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 x 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.), flunixine 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 x 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 x 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 x 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 PGF2α 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-PGF2α ($P = 0.032$) and PGF2α ($P = 0.015$) groups compared to controls, although, physiological significance

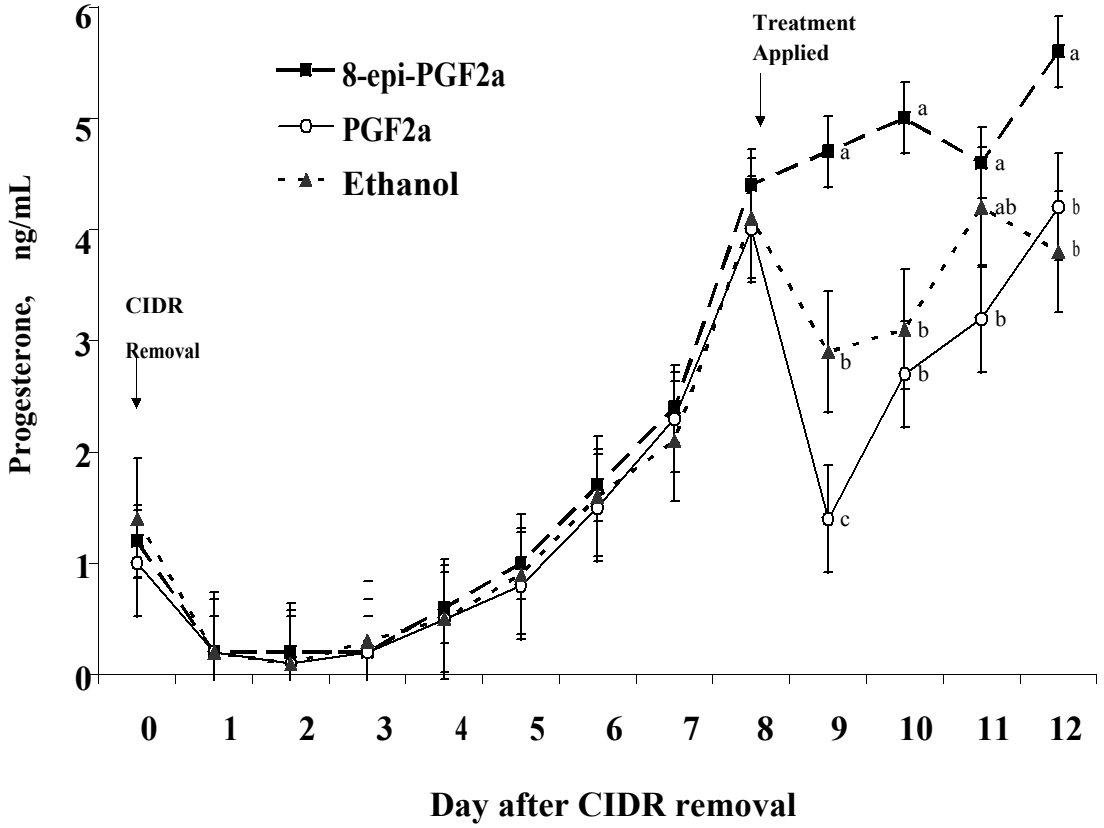

Figure 1. Progesterone concentrations in Rambouillet ewes administered 200 µg 8-epi-PGF2 , 200 µg PGF2 , or ethanol placebo laparoscopically into interstitial tissue of each ovary on day 8 of estrous cycle (CIDR removal on day 0). Treatment x day interaction, P = 0.002. Differing superscripts indicate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

Table 1. Corpus luteum weights (g) at day 13 in Rambouillet ewes administered 200 µg 8-epi-PGF2 , 200 µg PGF2 , 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 [2]	PGF2 [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

[1]Pure ethanol (0.1 ml), [2]Suspended in 0.1 mL pure ethanol, [3]Standard error (n = 3, 4, and 3 for ethanol, 8-epi-PGF2α and PGF2α groups, respectively), [4]Different 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 PGF2α 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 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 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 PGF2 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 PGF2 in the current dosage for complete and irreversible functional luteolysis. Surprisingly, serum progesterone concentration was actually greater in ewes administered 8-epi-PGF2 α compared to control ewes after treatment. Although, little data exist that suggest 8-epi-PGF2 is either luteolytic or luteotropic, previous research has indicated that 8-epi- PGF2 and PGF2 may act through different receptors, even when inducing similar responses, despite their structural similarities. Kiriyama et al. (1997) found that, although 8-epi- PGF2 does bind to the prostaglandin F receptor, the former actually possesses greater affinity for the prostaglandin E3 receptor, as well as some binding capability to prostaglandin E2 receptor and thromboxane A2 receptor. Kinsella (2001) and Comporti et al. (2008) also reported an ability of 8-epi-PGF2 to act through the thromboxane A2 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-PGF2 in the current study, as prostaglandin E is known to promote steroidogenesis (Speroff and Ramwell, 1970; Kim et al., 2001). However, thromboxane A2 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-PGF2 α and PGF2α 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 PGF2 elicits these events. Additionally, Juengel et al. (2000) found that progesterone can be diminished by PGF2 at dosages that do not result in luteal cell death.

Data from the current study indicated that 8-epi-PGF2 administered intra-ovarian increased serum progesterone concentration despite structural similarities between 8-epi-PGF2 and PGF2 , a known luteolysin. Prostaglandin F2 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-PGF2 and PGF2 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.

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