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In vivo intra-luteal implants of prostaglandin (PG) E_1 or E_2 (PGE_1, PGE_2) prevent luteolysis in cows. I. Luteal weight, circulating progesterone, mRNA for luteal luteinizing hormone (LH) receptor, and occupied and unoccupied luteal receptors for LH

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ABSTRACT

Previously, it was reported that chronic intra-uterine infusion of PGE_1 or PGE_2 every four hours inhibited luteolysis in ewes. However, estradiol-17β or PGE_2 given intra-uterine every 8 h did not inhibit luteolysis in heifers, but infusion of estradiol + PGE_2 inhibited luteolysis in heifers. The objective of this experiment was to determine whether and how intra-luteal implants containing PGE_1 or PGE_2 prevent luteolysis in Angus or Brahman cows. On day-13 post-estrus, Angus cows received no intra-luteal implant and corpora lutea were retrieved or Angus and Brahman cows received intra-luteal silastic implants containing Vehicle, PGE_1, or PGE_2 and corpora lutea were retrieved on day-19. Coccygeal blood was collected daily for analysis for progesterone. Breed did not influence the effect of PGE_1 or PGE_2 on luteal mRNA for LH receptors or unoccupied or occupied luteal LH receptors did not differ ($P > 0.05$) so the data were pooled. Luteal weights of Vehicle-treated Angus or Brahman cows from days-13–19 were lower ($P < 0.05$) than those treated with intra-luteal implants containing PGE_1 or PGE_2. Day-13 Angus luteal weights were heavier ($P < 0.05$) than Vehicle-treated Angus cows on day-19 and luteal weights of day-13 corpora lutea were similar ($P > 0.05$) to Angus cows on day-19 treated with intra-luteal implants containing PGE_1 or PGE_2.

Profiles of circulating progesterone in Angus or Brahman cows treated with intra-luteal implants containing PGE_1 or PGE_2 differed ($P < 0.05$) from controls, but profiles of progesterone did not differ ($P > 0.05$) between breeds or between cows treated with intra-luteal implants containing PGE_1 or PGE_2. Intra-luteal implants containing PGE_1 or PGE_2 prevented ($P < 0.05$) loss of luteal mRNA for LH receptors and unoccupied or occupied receptors for LH compared to controls. It is concluded that PGE_1 or PGE_2 alone delays luteolysis regardless of breed. We also conclude that either PGE_1 or PGE_2 prevented luteolysis in cows by up-regulating expression of mRNA for LH receptors and by preventing loss of unoccupied and occupied LH receptors in luteal tissue.

1. Introduction

Approximately one-third of ovine and bovine embryos are lost during the first third of pregnancy [1–11]. Additional losses of 6–8% occur after the first third of pregnancy in ewes [12]. These losses may be due to deficiencies in luteal progesterone secretion, since progesterone is required throughout gestation to maintain pregnancy [13–23]. The corpus luteum is the source of progesterone during the estrous cycle [13–20]. Sources of progesterone during pregnancy differ in cows and ewes [13–16,26]. In cows, concentrations of circulating progesterone increase two fold from day-12 to 18 post-breeding in cows and do not change from days-20 to

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280 [24]. The placenta of cows does not secrete progesterone when the corpus luteum is functional [25]. In ewes, circulating progesterone increased after day-50 and until day-130 of pregnancy, and then decreased by day-135 when luteal steroidogenic enzymes decreased [28]. Half of the progesterone circulating at day-90 is from the ovine corpus luteum and half is from the placenta [16,27].

Both luteinizing hormone, PGE_1, or PGE_2 increase progesterone secretion by increasing cAMP in luteal tissue of ewes or cows *in vitro* [29–32]. The corpus luteum contains both small luteal (SLC) and large luteal (LLC) steroidogenic cells to secrete progesterone [33,34]. Both SLC and LLC have luteinizing hormone (LH) receptors in their cell membrane [35,36]. Basal progesterone secretion by LLC is greater than by SLC [36,37]. Progesterone secretion by SLC is regulated by LH, which increases adenylate cyclase activity to increase cAMP [36–43]. cAMP activates protein kinase A (PKA) to activate cholesterol transport by steroid regulatory protein (StAR) to the mitochondria where cytochrome P450 side chain cleavage enzyme (SSC) produces pregnenolone for SLC to convert pregnenolone to progesterone by 3-β-hydroxy-steroid dehydrogenase in the smooth endoplasmic reticulum [23,44]. LH does not stimulate LLC cAMP to increase PKA activity and progesterone secretion, but LLC progesterone secretion is via a constitutively active PKA [41,44].

Secretion of progesterone by bovine and ovine luteal tissue during the estrous cycle is regulated by LH [16–23,45]. Bovine luteal tissue regresses when cows are given antisera to LH *in vivo* [46]. However, ovine or bovine luteal tissue does not secrete progesterone in response to LH after day-50 of pregnancy or at day-200 of pregnancy, respectively [16,45,47,48]. However, PGE_1 or PGE_2, not LH, does stimulate luteal secretion of progesterone by after day-50 of pregnancy in ewes or at day-200 of pregnancy in cows [16,45,47,48]. Luteal tissue of ewes secrete little detectable PGE or $PGF_{2\alpha}$ during the estrous cycle *in vitro*, but ovine luteal tissue secretes much PGE after day-50 of pregnancy [47]. Indomethacin decreased day-90 ovine progesterone and PGE secretion *in vivo* or *in vitro* [47,54], which can be restored by PGE_2 *in vitro*, but not LH [47]. Ovine luteal progesterone secretion is regulated by LH until day-50 of pregnancy and PGE regulates luteal and placental progesterone secretion after day 50 [16,54–57]. Pregnancy specific protein B (PSPB) regulates ovine luteal and placental tissue PGE secretion after day-50 of pregnancy, day-200 bovine luteal and placental PGE secretion, and PGE regulates ovine luteal and placental progesterone secretion in ewes [16,54–57]. These data fit changes in LH observed during pregnancy in ruminants where pituitary LH content [49], concentrations of LH in blood [50], and LH pulse amplitude and pulse frequency in ewes or cows decreased as pregnancy progresses [51,52].

Progesterone secretion by the corpus luteum of ruminants at the end of the estrous cycle is terminated via uterine secretion of $PGF_{2\alpha}$ [58–61], which is delivered locally from the uterine vein to the adjacent ovarian artery [16,62–64]. Uterine secretion of $PGF_{2\alpha}$ initiates luteolysis via binding to its cognate receptors on LLC [65] beginning around days-12–13 in ewes and days-16–17 in cows [62–64]. $PGF_{2\alpha}$ is a vasoconstrictor and first decreases ovarian blood flow [45–47] causing ischaemia of the corpus luteum followed by decreases in circulating progesterone [66–71]. Binding of $PGF_{2\alpha}$ to its receptor on LLC [65] increased protein kinase C (PKC, 24, 36, 73] to mediate luteolysis by increasing intracellular calcium [23,73,74] and release of oxytocin in LLC [23]. Oxytocin decreases SLC progesterone secretion by binding to oxytocin receptors on SLC to increase intracellular calcium and decrease SLC progesterone secretion [23]. Prostaglandin E3 (EP3) receptor may be involved in loss of ability of SLC to secrete progesterone. Sulprostone, an EP3 receptor agonist, decreased ovine circulating progesterone *in vivo*, decreased luteal mRNA for LH receptors, and decreased occupied and unoccupied LH receptors as effectively as $PGF_{2\alpha}$ and within the same time frame as $PGF_{2\alpha}$ [75]. EP3 receptor mechanisms of activation are via increases in cytosolic calcium and decreases in adenylate cyclase activity [76–79]. Completion of luteolysis is accompanied by decreases in LH receptors approximately 22.5 h after $PGF_{2\alpha}$ and is followed by increases in endonucleases for apoptosis of luteal cells [80–82].

Prevention of luteolysis during early pregnancy in ewes is not via inhibition of uterine endometrial $PGF_{2\alpha}$ secretion. During early pregnancy in ewes, concentrations of $PGF_{2\alpha}$ in uterine endometrium [16,83–86], endometrial $PGF_{2\alpha}$ secretion [87], $PGF_2\alpha$ transporter to deliver $PGF_{2\alpha}$ from the uterine vein to the ovarian artery of the luteal-containing ovary [95], concentrations of uterine venous $PGF_{2\alpha}$ [88–93], concentrations of $PGF_{2\alpha}$ in ovarian venous or arterial blood [61], concentration of $PGF_{2\alpha}$ in luteal tissue [61], binding of $PGF_{2\alpha}$ to luteal membranes [94] are not decreased. In addition, $PGF_{2\alpha}$ is not decreased in ovarian arterial blood or luteal tissue during early pregnancy in cows [96]. Instead, the embryo imparts luteal resistance to $PGF_{2\alpha}$, since it requires more $PGF_{2\alpha}$ to decrease circulating progesterone during early pregnancy than in nonpregnant ewes [97–99]. This resistance appears to be due to the two-fold increase in PGE_1 and PGE_2 in ovine endometrium on day-13 post-breeding [16,83–86], increased endometrial PGE secretion [87], and increases in PGE in uterine venous blood during early pregnancy [88–94], which increases as pregnancy progresses [16,55,56] and not by decreases in inhibitors of PKC [100]. PGE_1 or PGE_2 increased luteal progesterone secretion *in vitro* in ewes or cows and *in vivo* in ewes [44,29–32,101,102]. Furthermore, PGE_1 or PGE_2 infused chronically into the ovine uterine horn adjacent to the luteal-containing ovary prevented a natural [103,104] or a premature luteolysis induced by estradiol-17β [106,107], $PGF_{2\alpha}$ [105,108], or an intrauterine device (IUD) [109,110]. Acute treatment with PGE_1 into the interstitial tissue of the ovarian vascular pedicle of the sheep ovary containing a corpus luteum increased circulating progesterone longer than treatment with PGE_2 indicating similar, but also different mechanisms of action [101]. Chronic intrauterine infusion of PGE_1 in ewes increased luteal and endometrial mRNA for LH receptors, unoccupied and occupied receptors for LH, and the profile of circulating progesterone [102]. In contrast, PGE_2 only prevented loss of luteal progesterone secretion by preventing loss of luteal mRNA for LH receptors and unoccupied and occupied receptors for LH [102]. Thus, PGE is the major direct luteotropic/antiluteolytic signal delivered from the uterine horn containing the conceptus to the adjacent luteal-containing ovary during early pregnancy of ewes to prevent luteolysis [13–16]. Endometrial LH receptors that appear late in the luteal phase of the estrous cycle were reported to increase uterine $PGF_{2\alpha}$ secretion and to play a role in luteolysis [111–113]. However, the increase in endometrial LH receptors late in the estrous cycle may be to regulate PGE secretion if an embryo is present [56]. *In vitro* secretion of $PGF_{2\alpha}$ by ovine caruncular endometrium collected on day-15 of the estrous cycle was stimulated by LH; likewise *in vitro* secretion of PGE by caruncular tissue was stimulated by LH from days 13–50 of pregnancy and by PSPB after day-50 [56], Thus, an increase in endometrial LH receptors late in the luteal phase may play a role in the establishment of pregnancy by stimulating PGE secretion if an embryo is present [56]. Endometrial stroma secretes PGE, which may also be regulated by EGF and INF-τ, while the luminal epithelium secretes predominantly $PGF_{2\alpha}$ [114–119]. Both PGE_1 and PGE_2 are vasodilators and increase luteal progesterone secretion, which is in contrast to the vasoconstrictor action of $PGF_{2\alpha}$ [29–32,70,71].

Data that PGE_1 or PGE_2 act as the direct signal to prevent luteolysis during early pregnancy in the cow are less clear. *In vitro*, bovine luteal tissue during the estrous cycle or pregnancy secretes both PGE and $PGF_{2\alpha}$ [120,121]. Moreover, indomethacin decreased progesterone secretion during the bovine estrous cycle *in vivo* [53] and PGE and progesterone secretion from luteal tissue