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Prostaglandin E_1 (PGE_1), but not prostaglandin E_2 (PGE_2), alters luteal and endometrial luteinizing hormone (LH) occupied and unoccupied LH receptors and mRNA for LH receptors in ovine luteal tissue to prevent luteolysis

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ABSTRACT

Loss of luteal progesterone secretion at the end of the ovine estrous cycle is via uterine $PGF_2\alpha$ secretion. However, uterine $PGF_2\alpha$ secretion is not decreased during early pregnancy in ewes. Instead, the embryo imparts a resistance to $PGF_2\alpha$. Prostaglandins E (PGE; $PGE_1 + PGE_2$) are increased in endometrium and uterine venous blood during early pregnancy in ewes to prevent luteolysis. Chronic intrauterine infusion of PGE_1 or PGE_2 prevents spontaneous or IUD, estradiol-17β, or $PGF_2\alpha$-induced premature luteolysis in nonbred ewes. The objective was to determine whether chronic intrauterine infusion of PGE_1 or PGE_2 affected mRNA for LH receptors, occupied and unoccupied receptors for LH in luteal and caruncular endometrium, and luteal function. Ewes received Vehicle, PGE_1, or PGE_2 every 4 h from days 10 to 16 of the estrous cycle via a cathether installed in the uterine lumen ipsilateral to the luteal-containing ovary.

Jugular venous blood was collected daily for analysis of progesterone and uterine venous blood was collected on day-16 for analysis of $PGF_2\alpha$ and PGE. Corpora lutea and caruncular endometrium were collected from day-10 preluteolytic control ewes and day-16 ewes treated with Vehicle, PGE_1 or PGE_2 for analysis of the mRNA for LH receptors and occupied and unoccupied receptors for LH. Luteal weights on day-16 in ewes treated with PGE_1 or PGE_2 and day-10 control ewes were similar ($P \geq 0.05$), but were greater ($P \leq 0.05$) than in day-16 Vehicle-treated ewes. Progesterone profiles on days 10–16 differed ($P \leq 0.05$) among treatment groups: $PGE_1 > PGE_2 >$ Vehicle-treated ewes. Concentrations of $PGF_2\alpha$ and PGE in uterine venous plasma on day-16 were similar ($P \geq 0.05$) in the three treatment groups. Luteal mRNA for LH receptors and unoccupied and occupied LH receptors were similar ($P \geq 0.05$) in day-10 control ewes and day-16 ewes treated with PGE_2 and were lower ($P \leq 0.05$) in day-16 Vehicle-treated ewes. PGE_2 prevented loss ($P \leq 0.05$) of day-16 luteal mRNA for LH receptors and occupied and unoccupied LH receptors. Luteal and caruncular tissue mRNA for LH receptors and occupied and unoccupied LH receptors were greater ($P \leq 0.05$) on day-16 of PGE_1-treated ewes than any treatment group. mRNA for LH receptors and occupied and unoccupied receptors for LH in caruncules were greater ($P \leq 0.05$) in day-16 Vehicle or PGE_2-treated ewes than in day-10 control ewes. It is concluded that PGE_1 and PGE_2 share some common mechanisms to prevent luteolysis; however, only PGE_1 increased luteal and endometrial mRNA for LH receptors and occupied and unoccupied LH receptors. PGE_2 prevents a decrease in luteal mRNA for LH receptors and occupied and unoccupied receptors for LH without altering endometrial mRNA for LH receptors or occupied and unoccupied receptors for LH.

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1. Introduction

Approximately one-third of ovine and bovine embryos are lost during the first third of pregnancy [1–5]. Additional losses of 6–8% occur after the first third of pregnancy in ewes [6]. These losses may be due to deficiencies in luteal progesterone secretion, since progesterone is required throughout gestation to maintain pregnancy [7–10]. The corpus luteum is the source of progesterone during early pregnancy in ewes [7–10]. The corpus luteum contains both small luteal (SLC) and large luteal (LLC) steroidogenic cells [11,21], both of which secrete progesterone, and both SLC and LLC have membrane bound LH receptors [22,23]. Basal progesterone secretion by LLC is many fold greater than by SLC [23]. Progesterone secretion by SLC is stimulated by LH, which increases adenylate

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cyclase activity to increase cAMP; 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 to progesterone by steroidogenic enzymes in the smooth endoplasmic reticulum [24–29]. Despite the presence of LH receptors on LLC, LH does not stimulate cAMP production and the subsequent cascade leading to increased progesterone secretion. Thus, progesterone secretion in LLC occurs via a constitutively activated PKA [29,30].

The reduction in progesterone secretion by the corpus luteum at the end of the estrous cycle occurs due to uterine secretion of $PGF_2\alpha$, which is delivered locally from the uterine vein to the adjacent ovarian artery [7–10]. $PGF_2\alpha$ initiates luteolysis via $PGF_2\alpha$ binding to $PGF_2\alpha$ receptors on LLC to increase protein kinase C (PKC; [31,32]); this begins around day-13 in ewes and day-17 in cows [7–10]. $PGF_2\alpha$ is a vasoconstrictor and decreases ovarian blood flow causing ischaemia of the corpus luteum, which leads to decreased adenylate cyclase activity, reduced PKA activity, increased LLC PKC, decreased LLC progesterone secretion, and decreased circulating progesterone [11–21,27,29–35]. When $PGF_2\alpha$ binds to its receptors on LLC increased PKC mediates luteolysis by increasing calcium uptake and oxytocin release [23,35]. Oxytocin binding to oxytocin receptors on SLC increases calcium ion and decreases progesterone secretion by SLC followed by increases in endonucleases for apoptosis for physiologic and morphologic regression of the corpus luteum [18]. LH receptors in SLC decrease approximately 22.5 h after the onset of luteolysis [33,34]. Prevention of luteolysis during early pregnancy in ewes is not via inhibition of endometrial $PGF_2\alpha$ secretion. During early pregnancy in ewes, concentrations of $PGF_2\alpha$ in endometrium [36], endometrial $PGF_2\alpha$ secretion *in vitro* [37], uterine [38–46] and ovarian [39,40] venous $PGF_2\alpha$, $PGF_2\alpha$ in luteal tissue [40], binding of $PGF_2\alpha$ to luteal membranes [47], or transport of $PGF_2\alpha$ locally from the uterine vein to the adjacent ovarian artery [48] are not decreased. Instead, the corpus luteum is more resistant to $PGF_2\alpha$, since it requires more $PGF_2\alpha$ to decrease circulating progesterone during early pregnancy than in nonpregnant ewes [49–51]. This resistance is probably mediated via increased PGE_1 and PGE_2 in ovine endometrium on day-13 postbreeding [36,52–54], leading to increased PGE in uterine venous blood during early pregnancy [41–46]. PGE continues to increase as pregnancy progresses [10,55]. This resistance to $PGF_2\alpha$ is not by alterations in biological inhibitors of PKC [56]. PGE_1 and PGE_2 increase luteal cAMP and progesterone secretion *in vitro* and progesterone secretion *in vivo* [57–59]. Furthermore, PGE_1 [7–10,60] or PGE_2 [6–10,61,62] infused chronically into the ovine uterine horn adjacent to the luteal-containing ovary prevents spontaneous or premature luteolysis induced by estradiol-17β [63,64],), an IUD [65,66], or $PGF_2\alpha$ [67]. Acute treatment with PGE_1 at the ovine luteal-containing ovary increases circulating progesterone for a longer duration than treatment with PGE_2 [68]. In addition, a single intramuscular injection of PGE_1 increased progesterone for the duration of the 72-h sampling period in cows [69].

Endometrial LH receptors may play a role in luteolysis through increases in uterine $PGF_2\alpha$ secretion during the estrous cycle. Endometrial LH receptors increase the last few days of the estrous cycle and LH increased secretion of $PGF_2\alpha$ or its catabolite (PGFM) *in vitro* and *in vivo* late in the estrous cycle of cows and thus may enhance the luteolytic process [70–72]. However, LH increased ovine caruncular PGE secretion *in vitro* when an embryo was present, but increased only $PGF_2\alpha$ secretion by endometrium from nonbred ewes [55]. Thus, PGE is the major direct luteotropic or antiluteolytic signal from the uterine horn to the adjacent luteal-containing ovary to prevent luteolysis during early pregnancy [7–10]. PGE_1 and PGE_2 are vasodilators [73,74] and increase luteal progesterone secretion *in vitro* [57–59] and *in vivo* [67,69], while $PGF_2\alpha$ is a vasoconstrictor [33,34,73,74]. Ovine luteal tissue of pregnancy secretes PGE, and PGE_1 or PGE_2 is the luteotropin in ewes by day-50 [10,75–77]. This appears to be the reason that LH is not required to maintain pregnancy after day-50 in ewes [78,79]. PGE_2 [80], nitric oxide (NO; [81–84]), estradiol-17β and progesterone [83] are also obligatory for implantation in rodents. In addition, chronic treatment of ewes with an NO donor at the luteal-containing ovary [85] or in the ipsilateral uterine horn prevents luteolysis by altering the PGE:$PGF_2\alpha$ ratio [86], presumably via inhibition of 9-keto-reductase to reduce conversion of PGE_2 to $PGF_2\alpha$ [86,87]. Therefore, the objective of this experiment was to determine whether PGE_1 or PGE_2 infused chronically into the uterine horn adjacent to the luteal-containing ovary affected luteal and endometrial mRNA for LH receptors, occupied and unoccupied LH receptors, and luteal function during the ovine estrous cycle.

2. Materials and methods

2.1. Animals and sample collection

The experimental protocol was approved by the University of Hawaii IACUC. Mature Merino crossbred ewes were checked twice daily (0800 and 1700 h) for estrus with brisket-painted vasectomized rams. Estrus was designated as day-0 of the estrous cycle. Only ewes with two consecutive interestrous intervals of 16–17 days were used. Ewes were fasted for 12 h before surgery and received 1cc (0.54 mg) atropine sulfate (Phoenix Pharmaceuticals, Inc., St. Joseph, MO) intramuscularly as a preanesthetic. Ewes were laparotomized under pentobarbital sodium anesthesia (Anthony Products Co., Arcadia, CA). A polyvinyl catheter (Cole Parmer, Chicago, IL) was installed in the jugular vein and into the uterine horn adjacent to the ovary containing the corpus luteum. The uterine cathether was exteriorized through the flank to administer treatments. Treatments were: Vehicle ($N = 5$), PGE_1 (Cayman Chemical Co. Inc., Ann Arbor, MI; $N = 6$), or PGE_2 (Cayman Chemical Co. Inc., Ann Arbor, MI; $N = 6$). Samples of jugular venous blood were collected via heparinized syringes starting on day-10 postestrus at 0, 4, 16, 28, 40, 52, 64, 76, 88, 100, 112, 124, 136, 148, and 160 h relative to initiation of treatments for analysis of plasma progesterone by RIA [88]. Uterine venous blood was collected via heparinized syringes at 1800 h on day-16 for analysis of PGE and $PGF_2\alpha$ by RIA [89]. Samples of uterine blood received 0.1 ml 0.1 N HCL per ml of blood collected to inhibit platelet prostanoid production prior to centrifugation to collect plasma [39]. Plasma was stored at −20 °C until analysis of PGE and $PGF_2\alpha$. Corpora lutea were collected at 1800 h on day-16, weighed, split in half, halves were weighed, and frozen and stored in liquid nitrogen until assay of occupied and unoccupied receptors for LH, and for mRNA for LH receptor and β actin. Day-16 caruncles were collected from the anterior third of the uterine horn adjacent to the luteal-containing ovary. Caruncles were weighed, frozen, and stored in liquid nitrogen until assay for mRNA for LH receptors and β actin, and occupied and unoccupied receptors for LH. Day-10 corpora lutea and caruncules were also collected and processed as above to serve as preluteolytic controls.

2.2. Real-time PCR for mRNA LH receptor analyses

Total RNA was extracted from ovine corpora lutea using the TRI reagent (Sigma, Saint Louis, MO, USA). Individual RNA samples were DNAse digested and cleaned by incubation with RNase-Free DNase kit (Qiagen, Valencia, CA, USA) in RNeasy spin columns (RNeasy Midi Kit, Qiagen, Valencia, CA, USA) according to manufacturer's instructions. Concentrations of RNA were determined by spectrophotometry (Nano Drop ND-100, Technologies, Inc., Wilmington, DE, USA) and integrity of RNA was verified by denaturing