Theriogenology 82 (2014) 1224–1230

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Effects of intraluteal implants of prostaglandin E1 or E2 on angiogenic growth factors in luteal tissue of Angus and Brahman cows

Yoshie S. Weems [a], Yan Ma [b], Stephen P. Ford [b], Terry M. Nett [c], Rhonda C. Vann [d], Andrew W. Lewis [e], Don A. Neuendorff [e], Thomas H. Welsh Jr [f], Ronald D. Randel [e], Charles W. Weems [a,*]

[a] Department of HNFAS, University of Hawaii, Honolulu, Hawaii, USA
[b] Department of Animal Science, University of Wyoming, Laramie, Wyoming, USA
[c] College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado, USA
[d] Department of Animal Science, Mississippi State University, Brown Loam, Mississippi, USA
[e] Texas AgriLife Research, Texas A&M University System, Overton, Texas, USA
[f] Department of Animal Science, Texas A&M University, College Station, Texas, USA

ARTICLE INFO

Article history:
Received 24 January 2014
Received in revised form 29 July 2014
Accepted 29 July 2014

Keywords:
Prostaglandin E_1
Prostaglandin E_2
Vascular endothelial growth factor
Fibroblast growth factor-2
Angiopoietin-1
Angiopoietin-2

ABSTRACT

Previously, it was reported that intraluteal implants containing prostaglandin E_1 or E_2 (PGE_1 and PGE_2) in Angus or Brahman cows prevented luteolysis by preventing loss of mRNA expression for luteal LH receptors and luteal unoccupied and occupied LH receptors. In addition, intraluteal implants containing PGE_1 or PGE_2 upregulated mRNA expression for FP prostanoid receptors and downregulated mRNA expression for EP_2 and EP_4 prostanoid receptors. Luteal weight during the estrous cycle of Brahman cows was reported to be lesser than that of Angus cows but not during pregnancy. The objective of this experiment was to determine whether intraluteal implants containing PGE_1 or PGE_2 alter vascular endothelial growth factor (VEGF), fibroblast growth factor-2 (FGF-2), angiopoietin-1 (ANG-1), and angiopoietin-2 (ANG-2) protein in Brahman or Angus cows. On Day 13 of the estrous cycle, Angus cows received no intraluteal implant and corpora lutea were retrieved, or Angus and Brahman cows received intraluteal silastic implants containing vehicle, PGE_1, or PGE_2 on Day 13 and corpora lutea were retrieved on Day 19. Corpora lutea slices were analyzed for VEGF, FGF-2, ANG-1, and ANG-2 angiogenic proteins *via* Western blot. Day-13 Angus cow luteal tissue served as preluteolytic controls. Data for VEGF were not affected ($P > 0.05$) by day, breed, or treatment. PGE_1 or PGE_2 increased ($P < 0.05$) FGF-2 in luteal tissue of Angus cows compared with Day-13 and Day-19 Angus controls but decreased ($P < 0.05$) FGF-2 in luteal tissue of Brahman cows when compared w Day-13 or Day-19 Angus controls. There was no effect ($P > 0.05$) of PGE_1 or PGE_2 on ANG-1 in Angus luteal tissue when compared with Day-13 or Day-19 controls, but ANG-1 was decreased ($P < 0.05$) by PGE_1 or PGE_2 in Brahman cows when compared with Day-19 Brahman controls. ANG-2 was increased ($P < 0.05$) on Day 19 in Angus Vehicle controls when compared with Day-13 Angus controls, which was prevented ($P < 0.05$) by PGE_1 but not by PGE_2 in Angus cows. There was no effect ($P > 0.05$) of PGE_1

* Corresponding author. Tel.: 808 388 1344; fax: 808 956 7095.
E-mail address: weems@hawaii.edu (C.W. Weems).

0093-691X/$ – see front matter Published by Elsevier Inc.
http://dx.doi.org/10.1016/j.theriogenology.2014.07.039

or PGE_2 on ANG-2 in Brahman cows. PGE_1 or PGE_2 may alter cow luteal FGF-2, ANG-1, or ANG-2 but not VEGF to prevent luteolysis; however, species or breed differences may exist.

Published by Elsevier Inc.

1. Introduction

The corpus luteum is the source of progesterone during the estrous cycle [1,2]. Luteal weights are similar from the middle of the estrous cycle and throughout pregnancy in *Bos taurus* cows [3,4]. Concentrations of circulating progesterone in *Bos taurus* cows increase twofold from Day 12 to Day 18 after breeding and do not change from Day 20 to Day 260 of pregnancy [4]. However, luteal weights are lighter in *Bos indicus* cows on Day 15 of the estrous cycle than in *Bos taurus* cows, but luteal weights are similar during mid to late pregnancy in *Bos indicus* and *Bos taurus* cows. Luteal secretion of progesterone and prostaglandin E per unit mass *in vitro* on Day 15 of the estrous cycle and Day 200 of pregnancy are similar in *Bos indicus* and *Bos taurus* cows [5,6,9]. The placentas of cows do not secrete progesterone when the corpus luteum is functional, which is important to maintain luteal function throughout pregnancy [10].

Loss of progesterone secretion at the end of the estrous cycle in ewes or cows is *via* uterine $PGF_{2\alpha}$ secretion [1,2]. In a single sample of uterine venous blood of cows on Day 18 of pregnancy, $PGF_{2\alpha}$ is decreased but not in ovarian arterial or venous blood or in luteal tissue [7]. In the ewe, $PGF_{2\alpha}$ in the endometrium, uterine or ovarian venous blood, luteal tissue, binding of $PGF_{2\alpha}$ to luteal membranes, or transport of $PGF_{2\alpha}$ from the uterine vein to the adjacent ovarian artery of the luteal-containing ovary are not decreased to explain prevention of luteolysis during early pregnancy [1,2,8]. The embryo–endometrial interaction provides resistance to $PGF_{2\alpha}$-induced luteolysis during early pregnancy [2]. This resistance to $PGF_{2\alpha}$ is due to increases in endometrial PGE_1 and PGE_2 and PGE ($PGE_1 + PGE_2$) during early pregnancy [1,2].

In cows, *Bos taurus* and *Bos indicus* luteal tissue during the estrous cycle or pregnancy secretes much PGE_1 or PGE_2 *in vitro*, which increases with time in culture [6,9]. PGE_1 or PGE_2 stimulates bovine luteal progesterone secretion *via* cAMP *in vitro* [11]. In cows, PGE_1 given intramuscularly increased circulating progesterone for the duration of the 72-hour sampling period [12]. In Angus and Brahman cows, intraluteal silastic implants containing PGE_1 or PGE_2 on Day 13 to Day 19 prevented the decline in luteal weight, circulating progesterone, luteal mRNA for LH receptors, and unoccupied and occupied receptors for LH in Angus or Brahman cows [13]. However, the FP prostanoid receptor was decreased on Day 19 in both Angus and Brahman vehicle controls, whereas PGE_1 or PGE_2 on Day 19 in these same cows increased mRNA for the prostanoid FP and EP3B receptors and decreased mRNA for prostanoid EP2 and EP4 receptors [14]. An EP3 prostanoid receptor agonist *in vivo* is as effective as $PGF_{2\alpha}$ in causing luteolysis [15]. Data in ewes also support PGE_1 or PGE_2 as antiluteolysins to prevent luteolysis. Concentrations of PGE ($PGE_1 + PGE_2$) are increased in uterine venous blood during early pregnancy [1,2]. Chronic intrauterine infusions of PGE_1 or PGE_2 adjacent to the corpus luteum–containing ovary prevent a spontaneous or induced luteolysis [1,2]). The common antiluteolytic mechanism in the cow or ewe is that both PGE_1 or PGE_2 prevent loss of luteal mRNA for LH receptors and prevent loss of luteal unoccupied and occupied receptors for LH [13,16].

The angiogenic factors VEGF, FGF-2, ANG-1, and ANG-2 have been associated with growth or demise of luteal tissue potentially through alterations in vascularity [17–22]. VEGF was higher in developing corpora lutea than at mid- or-late luteal stages of the estrous cycle; FGF-2 was elevated throughout the estrous cycle and has been reported to be involved in both maintenance and demise of the corpus luteum, whereas ANG-1 has been reported to facilitate luteal vascular growth and ANG-2 was reported to increase in luteal tissue during luteal regression [17–22]. Studies on changes in luteal angiogenic growth factors have used primarily $PGF_{2\alpha}$-induced luteolysis. The decline in progesterone in jugular venous blood of cows decreases to less than 1 ng/mL within 4 hours after lutectomy, which is similar to an exogenous dose of $PGF_{2\alpha}$-induced luteolysis at midcycle [23]. This indicates that $PGF_{2\alpha}$-induced luteolysis is a pharmacologic effect, because progesterone decreases slowly over 3 to 4 days during a natural luteolysis [24]. Furthermore, synchronization of estrus in cows alters the response to agonists by bovine luteal or endometrial tissue *in vitro* [25,26].

The objective of this experiment was to determine whether intraluteal implants containing PGE_1 or PGE_2 affect the luteal angiogenic factors VEGF, FGF-2, ANG-1, and ANG-2 in cows when estrus is not synchronized.

2. Materials and methods

2.1. Cows and surgery

This experiment was approved by Texas A&M University Animal Care and Use Committee (TAMU AUC 2008–129). Cows were checked twice daily (05:00 and 17:00 hours) for estrus during September to November. Sixteen nonlactating multiparous Angus cows and 12 nonlactating multiparous Brahman cows aged from 5 to 8 years were maintained on pasture and feed, and water was withdrawn 12 hours before surgery. Before surgery, the flank of the cow was clipped, washed with soap and water, scrubbed with Betadine (Purdue Frederick, Stamford, CT, USA), and Lidocaine (Hospira Inc., Lake Forest, IL, USA) was administered subcutaneously into the flank as a local anesthesia. Ten milliliters of penicillin was given around the incision and intramuscularly (Bimeda Inc., Le Sueur, MN) after surgery [13,14].

2.2. Intraluteal implants and collection of samples

One-half of Dow 382 silastic elastomer (Dow Corning Chemical Co, Midland, MI) was mixed in a sterile tube with