Effects of Prostaglandins E_2 and $F_{2\alpha}$ (PGE_2; $PGF_{2\alpha}$), Trilostane, Mifepristone, Palmitic Acid (PA), Indomethacin (INDO), Ethamoxytriphetol (MER-25), PGE_2 + PA, or $PGF_{2\alpha}$ + PA on PGE_2, $PGF_{2\alpha}$, and Progesterone Secretion by Bovine Corpora Lutea of Mid-Pregnancy in Vitro

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Abstract

The effects of PGE_2, $PGF_{2\alpha}$, trilostane, RU-486, PA, INDO, MER-25, PGE_2, or $PGF_{2\alpha}$ + PA on secretion of progesterone, PGE_2, or $PGF_{2\alpha}$ by bovine corpora lutea (CL) of mid-pregnancy in vitro for 4 and 8 hr was examined. Secretion of PGE_2 and $PGF_{2\alpha}$ increased with time in culture ($P \leq 0.05$). PGE_2 and PGE_2 + PA increased ($P \leq 0.05$) secretion of progesterone at 4 and 8 h, progesterone secretion was increased ($P \leq 0.05$) at 4 h; but not at 8 h ($P \geq 0.05$) by trilostane, mifepristone, $PGF_{2\alpha}$ and $PGF_{2\alpha}$ + PA, and was decreased at 8 h by $PGF_{2\alpha}$ and $PGF_{2\alpha}$ + PA. Indomethacin decreased ($P \leq 0.05$) secretion of PGE_2, $PGF_{2\alpha}$, and progesterone at 4 and 8 h. Trilostane, PA, $PGF_{2\alpha}$, RU-486 and $PGF_{2\alpha}$ + PA increased ($P \leq 0.05$) PGE_2 at 4 h only. Palmitic acid decreased ($P \leq 0.05$) $PGF_{2\alpha}$ at 4 h, while trilostane, RU-486, or MER-25 did not affect ($P \leq 0.05$) PGE_2 of $PGF_{2\alpha}$ secretion. It is concluded that PGE_2 of luteal tissue origin is the luteotropin at mid-pregnancy in cows. Also, it is suggested that PA may alter progesterone secretion by affecting the inter conversion of PGE_2 and $PGF_{2\alpha}$.

Key words: cow, PGE_2, $PGF_{2\alpha}$, progesterone, RU-486, palmitic acid, indomethacin, MER-25, corpus luteum, pregnancy.

Introduction

Conflicting results have been reported for regulation and function of CL of cows during pregnancy. Concentrations of progesterone in jugular venous plasma have benn reported to increase two fold from day 12 to 18 postbreeding in cows (11, 41, 43) and not to change from days 20 - 280 (43). Others report that jugular venous progesterone increases through day 85 of pregnancy in cows and does not increase until day 230 before increasing on day 240 followed by decreases at parturition (14, 51, 52). Weights of CL are similar throughout pregnancy in cattle (6, 14, 15). Content and concentration of progesterone in the CL peak between 37 - 87 days postbreeding, decline through day 185, increase 100% by day 240, and decrease 10 days prior to parturition (14, 51, 52). The 20α and 20β metabolites of progesterone do not change during gestation in cows (51). The placenta of cows (7), unlike sheep (64),

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Received: August 22, 2002; Revised: October 24, 2002; Accepted: October 25, 2002.

does not secrete progesterone unless the CL regresses. Since progesterone declines to 0.5 μg/ml in 72 hr after lutectomy of cattle after mid-pregnancy, cows are vulnerable to abortion (18), unless the placenta compensates for loss of progesterone from the CL during pregnancy (3, 16, 18, 49, 63).

Secretion of progesterone in vitro by bovine luteal tissue of the estrous cycle is stimulated by luteinizing hormone (LH, 21) and luteal tissue regresses *in vivo* when cows are given antisera to LH (50, 58). However, the in vitro-stimulated secretion of progesterone by bovine CL at mid-pregnancy is reduced markedly when compared to the *in vitro* response of mature CL of the estrous cycle (67). In addition, pituitary LH content (33), concentrations of LH in blood (34), and LH pulse amplitude and pulse frequency (14, 28, 48) decrease as pregnancy progresses. PGE_2, not LH, has been reported to stimulate luteal secretion of progesterone *in vitro* by CL at mid-pregnancy in sheep (26, 66) and cows (67). In addition, secretion of PGE_2 and $PGF_{2\alpha}$ by bovine CL of the estrous cycle or pregnancy has been reported to increase linearly with time in culture (9, 68). This does not occur in CL of the estrous cycle or mid-pregnancy in sheep (26, 66).

The conflicting data regarding progesterone secretion during pregnancy in cows suggest that regulation or changes in luteal function may occur at different stages of pregnancy. Progesterone (12, 13, 37, 42, 56, 72), estradiol-17β (1, 6, 10, 19, 27, 30, 55), PGE_2 (23, 24, 29, 30, 44, 45, 57-63), oxytocin (32), or biogenic amines (46, 47) may all be autocrine regulators of bovine CL secretion of progesterone. Trilostane, a competitive inhibitor of 3β-hydroxysteroid dehydrogenase (3β-HSD; 25, 41), increased serum pregnenolone and reduced serum progesterone in Rhesus monkeys during the mid-luteal phase of the menstrual cycle (12, 13). Trilostane decreased placental progesterone secretion in 90-day lutectomized (70) or ovariectomized (69) pregnant ewes or ewes at parturition (25), increased inferior vena caval $PGF_{2\alpha}$ (69), and caused abortion (69, 70). Since 3β-HSD also catalyzes the conversion of dehydroepiandrosterone (DHEA) to estradiol-17β, it was expected that estradiol-17β secretion would be suppressed by trilostane (69, 70). However, in Rhesus monkeys during the mid-luteal phase of the menstrual cycle (12) and in lutectomized (70) or ovariectomized (69) 90-day pregnant ewes, trilostane increased circulating estradiol-17β. In the pregnant rat, RU-486, a progesterone receptor antagonist (38) both increased and decreased progesterone secretion (54). In ovariectomized 90-day pregnant ewes, RU-486 induced abortion and increased circulating $PGF_{2\alpha}$ before abortion occurred (71). Estradiol-$17_{2\alpha}$ receptors may be involved in regulating CL progesterone secretion (10, 19, 55), since infusion of exogenous estradiol-17β during the estrous cycle in sheep can prolong luteal function (10) or promote luteolysis (58, 62, 63). In the ovine CL, estradiol-17β receptors on large steroidogenic cells are greater than in small luteal cells (19). MER-25, a nonsteroidal estrogen receptor antagonist (26), decreased circulating progesterone in pregnant baboons (1, 6). In addition, PA has been reported to inhibit the enzyme PGE_2-9-ketoreductase (20, 36), which inhibits the interconversion of PGE_2 and $PGF_{2\alpha}$ (2).

The objective of this experiment was to: 1. determine whether indomethacin decreases luteal PGE_2 and progesterone secretion, 2. determine whether PA affects luteal secretion of PGE_2, $PGF_{2\alpha}$, and progesterone, and 3. whether RU-486, MER-25, and trilostane affects luteal secretion of PGE_2, $PGF_{2\alpha}$, and progesterone *in vitro* by bovine CL collected at mid-pregnancy.

Materials and Methods

*Bos indicus (*BI*)* and *Bos Taurus (*BT*)* cows at 186-195 (3, BI; 3 BT) and 234-243 (3BI; 3BT) days of pregnancy were used. Corpora lutea were collected by a flank laparotomy in a squeeze chute from cows using 2% Lidocaine (The Butler Co., Columbus, Ohio) as a local anesthetic given subcutaneously. The protocol for this experiment was approved by the Texas A & M University IACUC. Ten slices from each CL were weighed, randomized to treatments within each animal, minced, and incubated at 39°C, pH 7.2 under 95% O_2/5% CO_2, in 5 ml of M-199 containing 25 mM Hepes buffer and Earle's Salts (GIBCO, Grand Island, NY), 0.1% bovine fatty acid-free serum albumin (Sigma Chemical Co., St. Louis, MO), and 100 IU/ml penicillin G, 0.2 mg/ml streptomycin sulfate (Sigma Chemical Co., St. Louis, MO, U.S.A.), and 20 μg/ml 25-hydroxy-cholesterol (Sigma Chemical Co.). Tissues were incubated for 1 h without treatments and for 4 and 8 h with treatments (26, 67, 68). Treatments were: vehicle, indomethacin, (20 μM; Cayman chemical Co., Ann Arbor, MI), palmitic acid (100 ng/ml; Sigma Chemical Co.), PGE_2 (100 ng/ml; Cayman Chemical Co., Ann Arbor, MI, U.S.A.), MER-25 (100 ng/ml; Merrell National Laboratories), trilostane (100 mg/ml; Forrest McKesson, NY, U.S.A.), mifepristone (RU-486, 100 mg/ml; Academia Sinica, Beijing, PR CHINA), PGE_2 + PA, and $PGF_{2\alpha}$ + PA. Media were collected at 4 and 8 h, acidified with 0.1 ml of 0.1 N HCL per ml of medium to inhibit prostaglandin-metabolizing enzymes (65), and medium with fresh treatments was replaced at 4 h. Media were frozen at -20°C until assay for progesterone (64), PGE_2 (65), and $PGF_{2\alpha}$ (65) by RIA. Inter and intraassay coefficients of

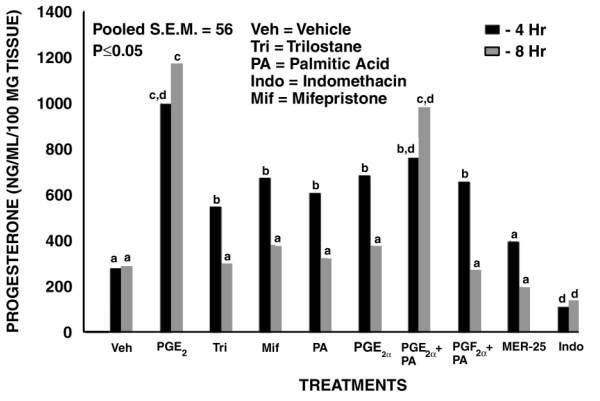

Fig. 1. Secretion of progesterone by bovine corpora lutea of mid-pregnancy in vitro treated with vehicle, trilostane, palmitic acid (PA), mifepristone, MER-25, indomethacin, $PGF_{2\alpha}$, PGE_2, $PGF_{2\alpha}$ + PA, or PGE_2 + PA. Means ± SEM with different superscripts are significant at $P < 0.05$.

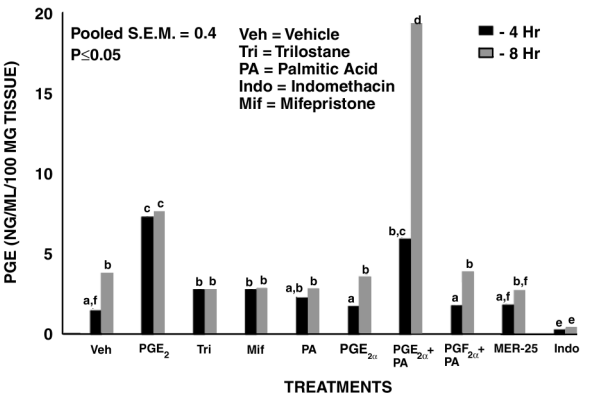

Fig. 2. Secretion of prostaglandin E (PGE) by corpora lutea of mid-pregnancy in vitro treated with vehicle, trilostane, palmitic acid (PA), mifepristone, MER-25, indomethacin, $PGF_{2\alpha}$, PGE_2, $PGF_{2\alpha}$\ + PA, or PGE_2 + PA. Means ± SEM with different superscripts are significant at $P < 0.05$.

variation for progesterone, PGE_2, and $PGF_{2\alpha}$ were, respectively: 6, 4; 9, 6; and 8, 7%. Data for breed type or stage of pregnancy did not differ ($P \geq 0.05$; 53) and were pooled. Data were analyzed for homogeneity of variance by Bartlett's Box F test (53). Data for treatment effects were analyzed by a 2×10 Factorial Design for Analysis of Variance (53). When differences were detected, means were compared by a Student Newman Kuehl's Test (53).

Results

Progesterone secretion was increased ($P \leq 0.05$) at 4 and 8 h by PGE_2 and PGE_2 + PA; at 4 h by trilostane, mifepristone, and $PGF_{2\alpha}$; and was decreased ($P \leq 0.05$) at 4 and 8 h by indomethacin (Fig. 1). Secretion of PGE increased from 4 to 8 h in the vehicle control group; at 8 h in the PGE_2 + $PGF_{2\alpha}$, $PGF_{2\alpha}$ + PA, and MER-25; and was decreased ($P \leq 0.05$) at 4 and 8 h by treatment with indomethacin (Fig. 2). Secretion of $PGF_{2\alpha}$ increased ($P \leq 0.05$) from 4 to 8 h in the vehicle control group (Fig. 3). Concentrations of $PGF_{2\alpha}$ were increased at 4 and 8 h in the $PGF_{2\alpha}$ and $PGF_{2\alpha}$ + PA treatment groups and at 8 h in the PGE_2, trilostane, mifepristone, PGE_2 + PA, and MER-25 treatment groups, but was decreased ($P \leq 0.05$) at 8 h by PA and at 4 and 8 h by indomethacin (Fig. 3).

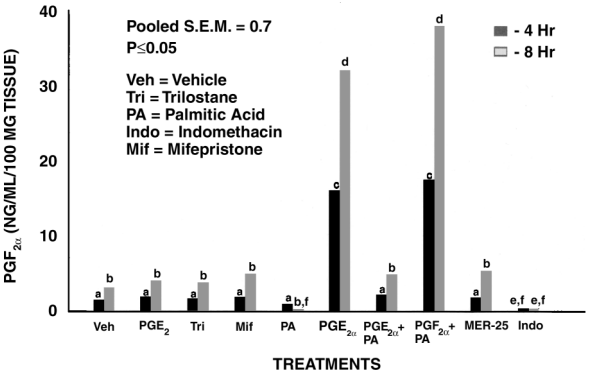

Fig. 3. Secretion of prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$) by bovine corpora lutea of mid-pregnancy in vitro treated with vehicle, trilostane, palmitic acid (PA), mifepristone, MER-25, indomethacin, $PGF_{2\alpha}$, PGE_2, $PGF_{2\alpha}$ + PA, or PGE_2 + PA. Means ± SEM with different superscripts are significant at $P < 0.05$.

Discussion

The increase in PGE_2 with time in culture and PGE_2-stimulated secretion of progesterone at 4 and 8 h by bovine CL at mid-pregnancy *in vitro* confirms previous data (67). While PGE_2 stimulated bovine luteal tissue secretion progesterone of the estrous cycle (67) or mid-pregnancy (67), LH only stimulated luteal secretion of progesterone by bovine luteal tissue of the estrous cycle (67). The indomethacin-induced decreases in PGE_2 and progesterone by bovine luteal tissue at mid-pregnancy provides further evidence that PGE_2 is the luteotropin at mid-pregnancy in cattle, since pituitary LH (33), LH in blood (34), and LH pulse frequency and peak height (28, 48) decreases as pregnancy progresses. Similar data exits in sheep where PGE_2-stimulated progesterone secretion by luteal tissue from the estrous cycle or mid-pregnancy, while LH only stimulated progesterone by luteal tissue of the estrous cycle (26, 66). In addition, indomethacin decreased PGE_2 (5) and progesterone (4) in jugular venous blood at mid-pregnancy in sheep and decreased PGE_2 and progesterone secretion by luteal tissue from mid-pregnant sheep(26). This indomethacin-

induced decrease in PGE_2 and progesterone secretion by luteal tissue from mid-pregnant sheep *in vitro* was reversed by treatment with PGE_2 (26).

The trilostane, mifepristone, and MER-25-induced increases in progesterone at 4 but not at 8 h, without affecting PGE_2 or $PGF_{2\alpha}$ substantially were unexpected. Trilostane, mifepristone, or MER-25 did not alter secretion of progesterone by CL slices *in vitro* from sheep at 4 or 8 h during the estrous cycle or mid-pregnancy (26). This does not support previous data where lowering luteal progesterone secretion or blocking progesterone receptor activity affected progesterone secretion (20, 37, 54, 56). Mifepristone does not alter circulating placental progesterone, estradiol-17β, or PGE secretion, but increases $PGF_{2\alpha}$ secretion and causes abortion in 90-day ovariectomized pregnant ewes (71). Trilostane lowers placental secretion of progesterone, increases estradiol-17β, and $PGF_{2\alpha}$ secretion, and does not affect PGE secretion, but aborts 90-day lutectomized (70) or ovariectomized (69) pregnant ewes.

The $PGF_{2\alpha}$-induced secretion of progesterone at 4 h followed by a decrease in progesterone at 8 h by bovine luteal slices *in vitro* at mid-pregnancy confirms similar results by dissociated bovine luteal tissue from the estrous cycle *in vitro* (22). Presumably, $PGF_{2\alpha}$ was first luteotropic *in vitro*, then luteolytic as seen *in vitro* in cattle (23). $PGF_{2\alpha}$ could have been converted to PGE_2 by PGE_2-9-ketoreductase (2). Palmitic acid has been reported to be an inhibitor of PGE_2-9-ketoreductase (20, 36). However, PA alone had only a small effect on basal secretion of PGE_2 and $PGF_{2\alpha}$ by bovine CL slices at mid-pregnancy *in vitro* presented here. Increases in $PGF_{2\alpha}$ and PGE_2 with time in culture confirms previous data, by bovine CL of the estrous cycle (9, 68) or mid-pregnancy (68), although progesterone secretion does not change based on data presented here and reported previously (9, 67). The lack of an effect on progesterone secretion as PGE_2 and $PGF_{2\alpha}$ secretion increases with time in culture is probably because the PGE_2:$PGF_{2\alpha}$ ratio remains the same. In summary, these data support the concept that PGE_2 is the luteotropin at mid-pregnancy in cows.

Acknowledgments

The authors gratefully acknowledge the assistance of Mr. Don Neuendorf, Texas Agricultural Experiment Station, Overton, Texas, for care, estrous detection, and mating of cows used in this experiment. The antisera for the progesterone assay was kindly supplied by Dr. R.L. Butcher, West Virginia University, WV; antisera for $PGF_{2\alpha}$ was kindly provided by Dr. L. Levine, Brandeis University, Waltham, MA; and antiserum for PGE assays was kindly supplied by Dr. N. Mason, Lily Research Laboratories, Indianapolis, IN. The authors want to acknowledge the support of Mr. Bruce Robinson, Niihau Ranch; Summer Erdman, Ulupalakua Ranch; and Eric Johnson, Hawaii Meg-Cor, Honolulu, HI for providing surgical supplies. The authors want to thank Mr. Wayne Toma for assistance with statistical analysis and computer graphics and Ms. Beverly Chang for typing of the manuscript.

This paper is Journal Series No. 4630 of the Hawaii Institute of Tropical Agriculture and Human Resources. This research was supported in part by USDA-CSREES Special Grant No. CSREES 98-34135-6783 administered by the Pacific Basin Advisory Group, Hatch Project 259 to CWW (USDA Regional Research Project W-112), and Hatch Project 3128 to RDR (USDA Regional Research Project W-112).

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