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Levels of Prostaglandin F (PGF) in Bovine Endometrium, Uterine Venous, Ovarian Arterial and Jugular Plasma during the Estrous Cycle[1] (38489)

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The exact mechanism for termination of function of the bovine corpus luteum is unknown. In some species, including guinea pigs (1), sheep (2), cattle (3), pseudopregnant rats (4), and hamsters (5) unilateral hysterectomy prolongs the life span of corpora lutea in the adjacent ovary. Exogenous $PGF_{2\alpha}$ has a luteolytic action in the rat (6), rabbit (7), guinea pig (8), hamster (9), ewe (10), cow (11), and monkey (12). In humans, hysterectomy does not cause maintenance of the corpus luteum (13). Prostaglandins may not play a role in luteolysis in humans, since exogenous $PGF_{2\alpha}$ administered during the luteal phase of the menstrual cycle had no effect on progesterone output or length of the cycle (14).

The purpose of the present experiment was to determine how much PGF is present in the bovine uterine vein blood and endometrium compared with that present in jugular venous and ovarian arterial blood, and to determine if the presence of PGF in ovarian arterial blood might account for the luteolytic action of the uterus during the estrous cycle.

Materials and Methods. Animals. Sixteen Holstein heifers at known stages of the estrous cycle were completely anesthetized with halothane and subjected to mid-ventral laparotomies. A small branch of the ovarian artery in the hilus of the ovary was severed and an ovarian arterial blood sample was collected into a citrated tube. The uterine vein was then isolated, severed and a venous sample was collected into a citrated tube. The blood samples were cooled rapidly, the plasma was separated by centrifugation and the samples were immediately frozen and stored at −20° until analyzed. The uteri were then removed, placed on ice, and the endometria dissected. The endometrial tissue was frozen on dry ice as soon as it was dissected, then lyophilized and stored at −20°.

Extraction of prostaglandin F from endometrial tissue and plasma. Finely ground lyophilized endometrial tissue (30 mg) was extracted with 10 ml of 85% ethanol. The ethanolic extract was reextracted with 10 ml light petroleum to remove inactive lipids. An internal standard of ^3H-$PGF_{2\alpha}$ was included in each sample. The ethanolic extract was dried and aliquots amounting to 0.1 of the total were used for radioimmunoassay and recovery determinations.

Plasma samples (1 ml) were also extracted for 5 min with 3 vol light petroleum to remove inactive lipids. An internal standard (3000 cpm (^3H)-$PGF_{2\alpha}$, 100 cm*M*, New England Nuclear Corp) was added to each sample before extraction. After removal of the light petroleum and addition of two drops of 5 NHCl, the samples were extracted for prostaglandins with 10 ml chloroform by shaking for 1 hr. The organic phase was dried under nitrogen and the residue dissolved in 1 ml ethanol. One tenth of each sample was used for determining the recovery of the internal standard and the remainder for radioimmunoassay. Each sample was assayed in triplicate and extracts of the water blank were included in each assay.

Radioimmunoassay of PGF and progesterone. $PGF_{2\alpha}$ standards (5–200 pg), plasma samples and tissue extracts were dried under nitrogen at 45° in 70 × 12 mm test tubes and taken up in 0.1 ml phosphate buffer (pH 7) containing 3500 cpm of ^3H-$PGF_{2\alpha}$ (S.A. 100 c/m*M*, New England Nuclear Corporation). After shaking, 0.1 ml of diluted PGF specific antibody was added to each tube. The tubes were again shaken gently and incubated overnight at 4°. One ml of a suspension of Dextran-coated charcoal was then added to each tube. The dextran-charcoal consisted of 250 mg charcoal (Norite A), 25 mg Dextran T-80 and

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100 mg gelatin in 100 ml of 0.1 *M* phosphate buffer, pH 7.0. The tubes were again shaken, allowed to stand at 4° for 5 min, and centrifuged for 10 min at 9500 *g*. The supernatant was decanted into scintillation vials and counted in a liquid scintillation spectrometer. Standard curves and unknown samples were run in triplicate. Plasma progesterone was determined by previously described methods (15).

Results. Seven samples of pooled endometrial tissue were analysed for PGF by the method described. When $PGF_{2\alpha}$ (0.2–1.0 ng) was added to these pooled samples, 92 ± 5.2% (SEM) was recovered (21 determinations). These recoveries were based on values corrected for endogenous PGF and for losses during extraction, as determined by recovery of the labelled internal standard. The coefficient of variation (CV) for the entire assay procedure was 9.0% and the between assay CV was 12.3%.

TABLE I. LEVELS OF PROSTAGLANDIN F IN THE BOVINE ENDOMETRIUM DURING THE ESTROUS CYCLE.

Day of cycle	No. of animals	Concentration (ng/g) adjacent to CL*	Concentration (ng/g) opposite to CL*
1–5	4	54 ± 15[a]	63 ± 13[c]
10–14	4	42 ± 9[a]	34 ± 19[c]
15–17	4	128 ± 8.5[b]	100
20–0	4	135 ± 10.5[b]	130 ± 13[d]

* Means ± S.E./g dry tissue. [a–d] Means designated by the same superscripts are not significantly different ($P > 0.05$).

PGF was found in the bovine endometrium at all stages of the estrous cycle (Table I). No differences in levels of PGF were detected ($P > 0.5$) between uterine horns adjacent or opposite to the functional corpus luteum (Table I). The concentrations (ng/g dry tissue) of PGF in the endometrium on days 15–17 and 20–0 were significantly ($P < 0.001$) elevated over levels found at days 1–5 and 10–14. However, no significant differences were found between days 1 and 5 and between 10 and 14, or between days 15 and 17 and 20 to 0 ($P > 0.5$).

The levels of prostaglandin F measured in uterine venous blood from the same animals are shown in Table II. Low levels were found on days 1–14 (0.162 ± 0.044; m ± S.E.), whereas on days 15 until the day of estrus the levels measured ranged from 1.5 to 3.0 ng/ml. Uterine venous blood levels increased significantly ($P < 0.001$) at days 15–17 and remained elevated until day 20. However, the uterine vein levels began to decline before the onset of estrus and were significantly lower ($P < 0.001$) at days 20–0 than at days 15–17.

In contrast, the concentration of the PGF in the ovarian artery and in the jugular vein remained low throughout the whole cycle (Table II), and no significant differences were found ($P < 0.05$). Uterine venous plasma levels of PGF were significantly ($P < 0.001$) higher than either ovarian arterial or jugular venous levels at days 15–17 and 20–0.

Discussion. The results of this investigation show that PGF levels in the endometrium and uterine venous blood rise at

TABLE II. PGF AND PROGESTERONE LEVELS IN BOVINE UTERINE VEIN, OVARIAN ARTERY AND JUGULAR VEIN PLASMA AT DIFFERENT STAGES OF THE ESTROUS CYCLE.

Day of cycle	Jugular progesterone (ng/ml)*	PGF concentration (ng/ml) Uterine vein*	Ovarian artery*	Jugular vein*
1–5	0.9 ± 0.24[a]	0.138 ± 0.031[a]	0.079 ± 0.024[f]	0.084 ± 0.020[g]
10–14	3.0 ± 0.23[b]	0.187 ± 0.057[c]	0.076 ± 0.020[f]	0.053 ± 0.01[g]
15–17	2.5 ± 0.38[b]	2.85 ± 0.220[d]	0.093 ± 0.034[f]	0.048 ± 0.017[g]
20–0	0.9 ± 0.23[a]	1.47 ± 0.103[e]	0.113 ± 0.049[f]	0.055 ± 0.01[g]

* Means ± S.E. [a–g] Means designated by the same superscripts are not significantly different ($P > 0.05$). In addition, uterine vein levels of PGF were significantly ($P < 0.001$) elevated over both ovarian arterial and jugular venous levels at days 15–17 and 20–0 (estrus).

the time the corpus luteum begins to regress and plasma progesterone levels fall. However by the time the first signs of estrous behaviour were detected the PGF level had already begun to decline. This rise and fall in PGF before the onset of estrus corresponds with the elevation and decline in peripheral plasma testosterone (16) and estradiol (11), which occur during the 3 days preceding estrus. The increased plasma testosterone levels probably reflect the role of testosterone as an estrogen precursor. However, testosterone may also play a role in inducing behavioral estrus (16).

Other reports have shown an effect of $PGF_{2\alpha}$ on estrogen secretion. Hixon *et al.* (15) showed that intra-ovarian injections of 300 μg of $PGF_{2\alpha}$ were followed by significant elevations in plasma estrogens, even though these amounts of $PGF_{2\alpha}$ were insufficient to cause luteolysis. Liggins *et al.* (17) observed an increase in the plasma concentration of estradiol-17β in maternal inferior vena caval plasma of one pregnant ewe following $PGF_{2\alpha}$ infusion.

The possibility that PGF reaches the ovary without entering the systemic circulation by passing locally from the utero-ovarian vein to the closely associated ovarian artery (11) by way of a "counter current" process was examined, and the data obtained did not support this idea (Table II). PGF levels in the ovarian artery were not significantly higher than those of the peripheral blood, even at times when very high levels of PGF were found in the uterine vein; nor did PGF levels in the ovarian artery rise significantly at any time during the cycle. Thus, the role of a local preferential transfer of endometrial $PGF_{2\alpha}$ as a luteolytic mechanism in the normal cow remains in doubt, despite the fact that in other studies (18) we have obtained clear evidence for such a preferential transfer after placing 6 mg of $PGF_{2\alpha}$ into the uterine lumen. Perhaps, more frequent sampling and measurement of PGF levels in ovarian arterial blood of normal animals prior to the onset of luteal regression will be necessary to provide a final answer to this question. It is also possible that a part of the luteolytic activity is transferred from the uterus to the ovary by way of the lymphatics or by transperitoneal diffusion.

Summary. Prostaglandins F (PGF) were measured in uterine vein, ovarian artery, and jugular vein plasma and in the endometrial tissues at various times during the bovine estrous cycle, and were compared to peripheral plasma progesterone levels. Four groups of heifers at days 1–5, 10–14, 15–17 and 20–0 of the estrous cycle were studied. Low levels of PGF (48 $\pm$ 12 ng/g dry tissue were measured in the endometrium on days 1–14 of the cycle. Higher values (131 $\pm$ 9.0) were found at days 15 until the day of estrus ($P < 0.001$). Similarly, very low levels of PGF were observed in the uterine vein plasma at days 1–14 (0.162 $\pm$ 0.044) ng/ml M $\pm$ S.E.), whereas on days 15 until the day of estrus the levels ranged from 1.5 to 3.0 ng/ml. The increases in uterine vein PGF on day 15 occured even while peripheral plasma progesterone levels were still high. However, PGF was not elevated in either the ovarian artery or the jugular vein at any time during the cycle, even when uterine vein levels were greatly elevated. No differences in PGF content were detected in endometrial tissue from uterine horns adjacent or opposite to the functional corpus luteum.

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