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Arachidonic Acid and Bovine Corpus Luteum Function[1] (38514)

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Hansel *et al.* (1) isolated arachidonic acid from bovine endometrium and demonstrated its luteolytic effect when injected into the ovarian bursae of the pseudopregnant hysterectomized hamsters at levels of 100 μg or more. These workers estimated that 0.5–1.0 mg arachidonic acid per g dried tissue is present in the bovine endometrium during the luteal phase of the estrous cycle. The luteolytic activity appeared to be specific, since similar compounds with different degrees of unsaturation were unable to cause a similar effect. Hoffman (2) has recently reported that intraperitoneal injections of arachidonic acid (12–25 mg) were luteolytic in the pseudopregnant rabbit. The fact that arachidonic acid, the essential fatty acid precursor of prostaglandins $E_{2\alpha}$ and $F_{2\alpha}$ ($PGF_{2\alpha}$) exists in high concentration in bovine endometrial tissue focused attention on the possibility that the bovine endometrium might provide the corpus luteum with arachidonic acid, which is converted into prostaglandins by the luteal tissue. Wilks *et al.* (3) have provided evidence that both interstitial and luteal tissues of the rabbit can synthesize $PGF_{2\alpha}$. Emphasis in this study has been placed on the role of arachidonic acid in causing regression of the bovine corpus luteum and on the presence of an enzyme system capable of generating PGF in the bovine ovary.

Materials and Methods. Eight normal Holstein heifers at the 12th or 13th day of the estrous cycle were used in this study. A total of 3 mg of arachidonic acid in 1 ml of a 0.1 *M* Tham [Tris (hydroxy-methyl) aminomethane] buffer at pH 7.0 was administered to four animals; four others treated in a similar fashion with the buffer served as controls. Flank laparotomies were made in all animals under local anesthesia, and the uterine branch of the utero-ovarian vein was cannulated so that frequent ovarian venous samples could be collected. At the same time, a catheter was placed into the jugular vein, and frequent blood collections (Fig. 1) from both veins were made during a 75-hr period. The arachidonic acid (1.5 mg) was injected directly into the luteal tissue at 0 and again at 4.5 hr (Fig. 1). Blood was immediately centrifuged at 4° and plasma was frozen in aliquots for prostaglandin, progesterone, luteinizing hormone and estrogen determinations. At 72 hr, the corpora lutea were removed through an incision in the anterior wall of the vagina.

Plasma extraction of prostaglandins F. Inactive lipids were removed from 1 ml plasma samples by extraction on a mechanical shaker (5 min) with 3 vol of light petroleum. An internal standard [3000 cpm of (^{3}H)-$PGF_{2\alpha}$, 100 Ci/m*M*, New England Nuclear Corporation] was added to each sample before extraction. After removal of the light petroleum and addition of 2 drops of 5 *N* HCl, the samples were extracted for prostaglandins with 10 ml of chloroform by shaking for 1 hr. The organic phase was dried under nitrogen and the residue dissolved in 1 ml of 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. $PGF_{2\alpha}$ standards (5–200 pg), tissue and plasma 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 ^{3}H-$PGF_{2\alpha}$ (S.A. 100 Ci/m*M*, New England Nuclear Corporation). After shaking, 0.1 ml of diluted PGF antibody specific for prostaglandins of the F series (kindly supplied by K. Kirton, The Upjohn Company) was

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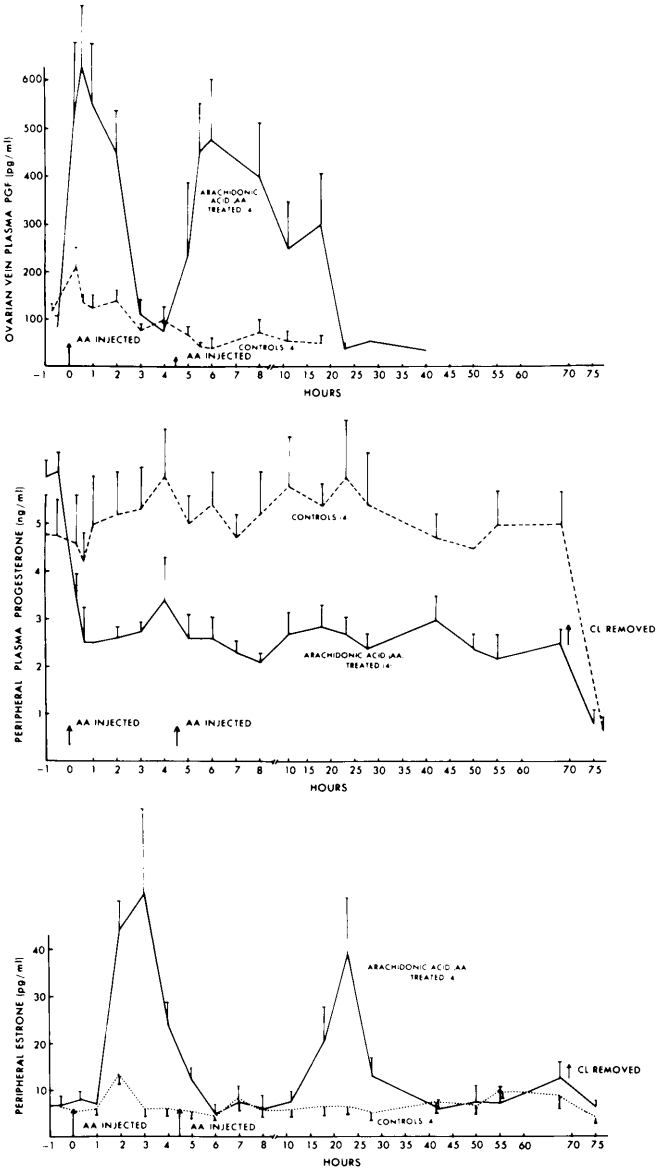

FIG. 1. Concurrent changes in jugular plasma levels of progesterone and estrone and ovarian venous plasma levels of prostaglandin F before and after injections of arachidonic acid (1.5 mg at 1 and 4.5 hr) or vehicle buffer into normal Holstein heifers at the 12th or 13th day of the estrous cycle. Vertical bars represent standard errors of the mean.

added. The tubes were 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, 25 mg Norite A, 25 mg Dextran T-80, and 100 mg gelatin in 100 ml of 0.1 *M* phosphate buffer pH 7.0. After shaking, the tubes were 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. Addition of arachidonic acid in amounts up to 5.0 μg to the incubation mixture did not interfere in the assay.

Reliability of the PGF assay. Seven samples of pooled jugular plasma (1 ml each) collected 2 days after estrus, were analyzed by the method described and found to contain 20 ± 3 pg/ml. When $PGF_{2\alpha}$ was added to these samples (20–200 pg/ml), 95 ± 8.5% (SEM) was recovered (18 determinations). These recoveries were based on values corrected for exogenous PGF and for losses during extraction, as indicated by recoveries of the labeled internal standard. The average within assay coefficient of variation (CV) was 7.6% and the between-assay CV was 8.9%. These data compare favorably with those obtained in previous validation studies for the assay using unextracted plasma (4).

Progesterone radioimmunoassay. The method of Shemesh, Lindner and Ayalon (5) for extraction and separation was employed. A specific progesterone antiserum was used, and dextran-coated charcoal was employed for separation of free from antibody bound progesterone, as previously described (4).

Estrogen radioimmunoassay. The method of Echternkamp and Hansel (6) was used for estrogen determinations. Addition of arachidonic acid in amounts up to 5.0 μg/ml to the incubation mixture had no effect on the assay of estrogens.

LH radioimmunoassay. The solid phase radioimmunoassay method, as described by Hobson and Hansel (7) for bovine LH, was used.

Results and Discussion. The results of this experiment are shown in Fig. 1, from which it may be seen that PGF levels in ovarian venous blood rose to extremely high levels immediately following each injection of arachidonic acid into the corpora lutea. Peripheral plasma progesterone levels declined significantly ($P < 0.05$) from 6.0 ng/ml to an average of 2.5 ng/ml, but did not decline further until the corpus luteum was removed at 72 hr. Large peripheral plasma estrone peaks were associated with the PGF peaks in each treated animal. In contrast, neither PGF levels in ovarian venous blood, nor estrone nor progesterone levels in peripheral blood changed appreciably in the control cows. PGF levels were significantly higher in the treated animals than in the controls at 0.5 hr ($P < 0.005$) and at 6 hr ($P < 0.005$). Estrone levels were significantly elevated in the treated animals at 3 hr ($P < 0.05$) and 16 hr ($P < 0.05$). It should be noted that a small rise in ovarian venous PGF and a concurrent small rise in jugular venous estrone occurred immediately after the first injection of the buffer in the control animals. This small peak in PGF possibly represents a release in response to manipulating the tissue. The levels of estradiol-17β in jugular vein plasma paralleled the estrone levels in all cases and were slightly lower.

These results clearly show that the bovine ovary has the ability to convert arachidonic acid to PGF, and they further suggest that the large amounts of PGF produced in this way stimulated estrogen secretion and eventually caused significantly ($P < 0.05$) decreased progesterone levels. However, complete luteal regression did not occur, as indicated by the further decline in plasma progesterone noted after removal of the corpus luteum at 72 hr. The corpora lutea removed at 72 hr from the arachidonic acid treated animals weighed 4.3 ± 1.4 g, as compared to 6.3 ± 0.5 for the corpora lutea of the control animals ($P < 0.10$).

The ovarian compartments responsible for the elevated PGF and estrogen levels remain to be determined. Although the arachidonic acid was placed within the corpus luteum, rapid diffusion to other parts of the ovary may have occurred. It has been recently reported (8) that ovarian homogenates from pregnant rats are capable of synthesizing prostaglandin-like compounds from labelled arachidonic acid; synthesis was suppressed by treatment of the rats with anti-LH prior to sacrifice. Most studies indicate that the bovine corpus luteum cannot synthesize estrogens (9), which suggests that follicular elements of the ovary were responsible for the increased estrogen levels we have observed following injection of arachidonic acid into the corpus luteum. However, in view of these results, the possibility that the bovine corpus luteum can produce estrogens needs further study.

These data also indicate that the increased estrogen levels observed were not due to release of pituitary LH. Plasma LH levels

were low (0.5–1.9 ng/m) in both treated and control animals throughout the experiment; there was no evidence of an increase or decrease after treatment. Carlson *et al.* (10) reported elevated plasma LH and estradiol-17β levels in the ewe after infusions of 6 μg/hr, or more of $PGF_{2\alpha}$ directly into the carotid artery. However, this effect was not observed after infusion of $PGF_{2\alpha}$ into either the carotid arteries or jugular veins of ovariectomized animals (11, 12).

Summary. Injections of arachidonic acid directly into the corpora lutea of normal cattle at the 12th or 13th day of the estrous cycle resulted in (a) a decline in jugular vein plasma progesterone levels from an average of 6 ng/ml to 2.5 ng/ml ($P < 0.05$), (b) an immediate rise in PGF levels in ovarian venous plasma, and (c) a sharp rise in jugular vein plasma estrogen levels. Jugular plasma estrogen peaks were associated with each ovarian vein PGF peak in each animal; jugular plasma LH levels did not change. These results show that the bovine ovary can convert arachidonic acid to PGF and that arachidonic acid administered directly into the corpus luteum results in partial regression of the corpus luteum. PGF appears to stimulate estrogen production by the ovary in the absence of measurable changes of plasma LH.

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1. Hansel, W., Shemesh, M., Hixon, J., and Lukaszewska, J., Biol. Reprod. (in press) (1974).
2. Hoffman, L. H., J. Reprod. Fert. **36,** 401 (1974).
3. Wilks, J. W., Hunter, K. K., and Norland, J. F., Biol. Reprod. **7,** 105 (1972).
4. Hixon, J. E., Nadaraja, R., Schechter, R. J., and Hansel, W., Prostaglandins **4,** 679 (1973).
5. Shemesh, M., Lindner, H. R., and Ayalon, N., J. Reprod. Fert. **26,** 167 (1971).
6. Echternkamp, S. E., and Hansel, W., J. Animal Sci. **37,** 1362 (1973).
7. Hobson, W. C., and Hansel, W., Endocrinology **91,** 195 (1972).
8. Chaslow, F. L., and Pharriss, B. B., Prostaglandins **1,** 107 (1972).
9. Savard, K., Marsh, J. M., and Rice, R. F., Rec. Prog. Horm. Res. **21,** 285 (1965).
10. Carlson, J. D., Barcikowski, B., and McCracken, J. A., J. Reprod. Fert. **34,** 357 (1973).
11. Cerini, M. E. D., Chamley, W. A., Findley, J. K., and Goding, J. R., Prostaglandins **2,** 433 (1972).
12. Harms, P. G., Ojeda, S. R., and McCann, S. M., Endocrinology **94,** 1459 (1974).

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