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Effects of the Estrous Cycle and Early Pregnancy on Bovine Uterine, Luteal, and Follicular Responses[1]

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ABSTRACT

Uterine, luteal, and follicular responses associated with the estrous cycle and early pregnancy in cattle were examined. Dairy (n = 19) and beef (n = 19) cattle were slaughtered either on Day 4, 8, 12, 14, 16, or 19 postestrus (estrus = Day o). Corpus luteum (CL) weight, specific $PGF_{2\alpha}$ binding by the luteal particulate fraction (100,000 × g pellet), in vitro estradiol (E_2) production by the two largest follicles, total recoverable uterine luminal protein (TP), total recoverable immunoreactive uterine luminal PGF (TPGF), and peripheral plasma steroids were evaluated. In a parallel study, beef cattle (n = 22) were slaughtered either on Day 8, 12, 14, 16, or 19 of pregnancy for measurements of TP, TPGF, and plasma steroids. Uterine luminal proteins, from cyclic and pregnant cattle (Day 19), were subjected to sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) for determination of protein molecular weights and protein profile characterization.

In cyclic cattle, CL regression was not completed by Day 19. Follicle E_2 secretion varied among animals within day ($\bar{x}$ = 4.0–24.0 ng E_2/follicle/3.5 h; $P<0.01$), but not among days. Total $PGF_{2\alpha}$ binding (fmole/CL) for Days 4, 8, 12, 14, 16, and 19 was 14.29, 145.79, 177.34, 111.82, 174.51, and 199.17 ($P<0.01$). TPGF (ng) varied among cycle Days 4 (14.4), 8 (13.9), 12 (19.7), 14 (47.7), 16 (17.4), and 19 (111.0 ng: $P<0.01$). In contrast, TPGF (ng) from pregnant cattle was 481.6 and 1187.8 ng on Day 16 (n = 5) and 19 (n = 6). TP (mg) in cyclic cattle varied ($P<0.05$) among Days 4 (7.34), 8 (7.03), 12 (4.14), 14 (15.92), 16 (5.88), and 19 (11.35). Mean TP (mg) for pregnant cattle ranged from 2.73 to 12.09. Thirty-two protein categories were identified in cyclic cattle uterine flushings by SDS-PAGE (M_T range × 10^{-3} = 18.7 to 292.0). Proteins appeared with greater frequency later in the cycle than earlier (Days 14, 16, 19: 60% vs Days 4, 8, 12: 45%). Composite SDS-PAGE profiles from Days 8–12 and Days 14–16 differed ($P<0.10$) suggesting luteal phase stimulation of protein secretion. Protein profiles (SDS-PAGE) from Day 19 of pregnancy differed from Day 19 of the estrous cycle, resembled those of midluteal phase, and revealed four protein constituents possibly unique to early pregnancy.

In summary, uterine luminal PGF increased with luteal phase of the estrous cycle at a time when there appeared to be a stable population of specific $PGF_{2\alpha}$ binding sites in the CL. Higher PGF in utero at Days 16 and 19 of pregnancy and changes in TP and SDS-PAGE protein profiles may reflect responsiveness of endometrium to changes in ovarian status (cyclic) and/or conceptus activity (pregnancy comparisons).

INTRODUCTION

Intercommunication between the ovary and uterus is essential for normal reproduction in cattle. The integrative action of ovarian steroids causes cyclic changes in numerous uterine responses including blood flow (Ford et al., 1979), endometrial histology and cytology (Priedkalns, 1976; Marinov and Lovell, 1968), and uterine fluid protein profiles (Roberts and Parker, 1974a,b, 1976). Such changes occur because of the biological requirement to establish an intrauterine environment capable of sustaining the bovine conceptus, a concept supported by the stringent requirements for synchrony between the endometrium and

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conceptus in successful embryo transfer (Sreenan, 1978). In the absence of pregnancy, resumption of cyclicity depends initially upon successful luteolysis. In cattle this process is thought to occur in response to the action of prostaglandin (PG) $F_{2\alpha}$ of uterine endometrial origin, for which specific receptors have been identified in bovine luteal cell membranes (Kimball and Lauderdale, 1975; Lin and Rao, 1977,1978; Rao et al., 1979). Endometrial production of $PGF_{2\alpha}$ may be coupled to protein synthetic mechanisms, the activity of which depends upon the array and temporal pattern of appearance of ovarian progestins and estrogens (French and Casida, 1973; Huslig et al., 1979; Bartol et al., 1981). Presence of a viable conceptus in utero prior to Day 16 postestrus (estrus = Day o) results in luteal maintenance (Sreenan, 1978). Hence, any alterations in the internal uterine milieu unique to early pregnancy must reflect, in part, contributions of or alterations induced by the conceptus (Eley et al., 1979b; Lewis et al., 1979 a,b) prerequisite to maternal recognition of pregnency (luteal maintenance).

Objectives of the present study were: 1) to describe peripheral plasma estrogen and progesterone profiles in normal cyclic and early pregnant cattle from which uterine flushings were obtained; 2) to characterize changes in corpora lutea weights and contents and concentrations of specific $PGF_{2\alpha}$ receptors in luteal tissue from cyclic cattle; 3) to determine if the two largest ovarian follicles from each cyclic animal could produce estradiol in vitro, and whether differences in such production existed among days of the estrous cycle; 4) to characterize levels of immunoreactive PGF in uterine flushings of both cyclic and early pregnant cattle; and 5) to examine both qualitative and quantitative changes in the protein components of uterine flushings obtained from cyclic and early pregnant cattle.

MATERIALS AND METHODS

Experimental Animals

Reproductive tract tissues, uterine flushings, and blood were collected from 38 cyclic and 22 pregnant beef and dairy cattle. All cattle were nonlactating and had produced at least one calf. To condense the period of time during which experimental material was collected from cyclic animals, 19 dairy and 19 crossbred beef cattle were synchronized to estrus with $PGF_{2\alpha}$. All animals were treated twice, 12 days apart, with 33.5 mg $PGF_{2\alpha}$ THAM salt (i.m.) dissolved in 5 ml of 0.9% saline. Following the second $PGF_{2\alpha}$ injection, animals were observed twice daily for signs of estrous behavior. Cattle were slaughtered either on Day 4, 8, 12, 14, 16, or 19 following onset of estrus (day of onset of estrus = Day o). All cattle had at least two estrous cycles of normal length (19 to 24 days) prior to synchronization. Similarly, 22 crossbred beef cattle were slaughtered on either Day 8, 12, 14, 16, or 19 postcoitum following a natural service. Pregnancy was confirmed by visual identification of a conceptus in uterine flushings.

At slaughter, samples of peripheral blood from each animal were collected in Erlenmeyer flasks containing heparin. Reproductive tracts were excised and placed on ice within 5 min of slaughter. All tissues collected, except follicles from cyclic cattle, were frozen in liquid nitrogen and placed in storage at −70°C within 30 min of slaughter.

Ovarian Tissue

Corpora Lutea. Corpora lutea (CL) were dissected from ovaries and trimmed of surrounding connective tissue. To inhibit further prostaglandin synthesis, each CL was sliced in half with a razor blade and rinsed thoroughly in a fresh ice-cold 0.9% saline solution containing indomethacin (10.0 μg/ml), and wet weights were determined. Corpora lutea then were frozen in liquid nitrogen and stored at −70°C.

Follicles. The two largest follicles from each cyclic animal, ranging in size from 5 to 20 mm, were dissected from the ovaries and trimmed free of surrounding connective tissue. Follicular fluid was aspirated with a 22 gauge needle, and wet weights of the individual collapsed follicles were recorded. Follicles were incubated in vitro according to methods described by Moor (1973) for ovine follicles.

Collection of Uterine Flushings

Uterine flushings were collected from cyclic cattle using the postslaughter procedure described by Bazer et al. (1978). Twenty milliliters of sterile, 0.33 M saline were introduced into each horn via a polyvinyl catheter attached to a 50 ml sterile syringe. The uterine horn being flushed was massaged gently but thoroughly over its entire length, with the catheter in place, before the saline flushing was withdrawn into the syringe and recovery volume recorded. This system of collection minimized increases in uterine luminal content of PGF associated with in vivo collection procedures (Bartol et al., 1981).

Uteri of pregnant cattle were flushed in precisely the same manner described for cyclic cattle. However, flushings were recovered by cutting off the tip of the uterine horn and allowing fluid to drain into a clean specimen dish on ice. Day 19 uteri were flushed in a similar manner without clamping each uterine horn. Pregnancy was confirmed by visual identification of the conceptus in uterine flushings. Uterine flushings from both cyclic and pregnant cattle were centrifuged at 4°C for 20 min at 12,000 × g. Supernatant was filtered through a 0.45 um filter to remove any remaining cellular debris and/or bacteria. This filtrate was transferred into sterile serum vials and stored at −20°C until analyses were performed.

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Steroid Radioimmunoassays for Plasma

After collection from 38 cyclic (19 dairy and 19 crossbred beef) and 22 pregnant cattle, heparinized blood samples were centrifuged in a refrigerated centrifuge (4°C) for 20 min at 12,000 × g. Plasma was obtained and stored at −20°C until analyzed for progestins, estrone, and estradiol concentration by radioimmunoassay (RIA).

Progestin concentrations in peripheral plasma were determined by RIA procedure described by Abraham et al. (1971). Description and precision of the assay were reported by Chenault et al. (1975). Validations of the progestin isolation and assay procedures were reported by Knight et al. (1977). Antiserum for this assay was a gift of J. L. Fleeger of Texas A&M University. Antiserum was prepared in a rabbit against 11-α-hydroxy-progesterone hemisuccinate conjugated to bovine serum albumin. Since this progesterone antiserum was highly specific, the RIA essentially measured progesterone (pregn-4-ene-3,20 dione; P_4). Sensitivity of this assay with respect to the standard curve was 25 pg. Recovery was 81.2 ± 2.0%, and the interassay and intraassay coefficients of variation were 18.1% and 15.5%, respectively.

Estrone (E_1) and estradiol (E_2) concentrations in plasma and in follicle incubation medium were measured by RIA after extraction from these fluids with diethyl ether and isolation by Sephadex LH-20 column chromatography (Chenault et al., 1975,1976; Eley et al., 1979a; Eley et al., 1981). The intraassay coefficients of variation were 19.9% and 23.7% for E_1 and E_2, respectively.

Specific $PGF_{2\alpha}$-Binding Assay for Corpora Lutea

Corpora lutea from all cyclic cattle (dairy and beef) were shipped, on dry ice, to Kalamazoo, MI (The Upjohn Co.), and maintained at −70°C prior to determination of specific $PGF_{2\alpha}$ binding by the 100,000 × g luteal particulate fraction according to previously described methods (Kimball and Lauderdale, 1975).

In vitro Incubation of Bovine Follicles

The two largest follicles from each cyclic animal were obtained according to methods described previously. A total of 66 follicles obtained from 36 cattle were incubated. Follicles ranged in size from 5 to 20 mm. Each follicle was submerged in an ice-cold bath of 50% saline and 50% incubation medium while surrounding ovarian connective tissue was removed.

Incubation medium consisted of eight parts Medium 199 with Hanks salts and glutamine (Difco Labs., Detroit, MI) and two parts fetal calf serum (Gibco, Grand Island, NY). To each liter of incubation medium, 56 mg ascorbic acid and 18 mg gentamicin sulfate (Schering Corp., Bloomfield, NJ) were added. Medium of this type was used successfully for culture of whole sheep follicles (Moor, 1973).

Follicles were incubated individually and free floating in 5 ml of incubation medium in 25 ml Erlenmeyer flasks. Incubations were carried out under a gaseous atmosphere of 50% N_2:45% O_2, and 5% CO_2 in a Dubnoff shaker at 37°C for 3.5 h with agitation at one stroke per second. Incubation medium was changed at 0.5 h and then hourly at 1.5, 2.5, and 3.5 h of incubation. Samples of incubation medium from each exchange period were stored separately at −20°C until analyzed for estradiol by RIA. Medium samples were extracted twice with two volumes of fresh diethyl ether. Isolation of estradiol from other estrogens in the pooled ether extracts was accomplished by Sephadex LH-20 column chromatography as described by Chenault et al. (1975).

Radioimmunoassay for PGF in Uterine Flushings

Concentrations of unextracted prostaglandin F (PGF) in bovine uterine flushings were determined with the double-antibody, radioimmunoassay system described by Cornette et al. (1972). First antibody, donated by Dr. K. T. Kirton of the Upjohn Co. (Kalamazoo, MI), was generated in rabbits against $PGF_{2\alpha}$ conjugated, at the C1 carbon, to bovine serum albumin. Second antibody (goat anti-rabbit IgG, Cappel Labs., Inc., Downington, PA) and radiolabeled $PGF_{2\alpha}$ (^{3}H-9; specific activity 10.9 Ci/mmole from New England Nuclear, Boston, MA) were used. Cross reactivity of the first antibody with $PGF_{1\alpha}$ was 8.0%. Interassay and intraassay coefficients of variation were 7.58% and 13.64%, respectively. Details of PGF assay procedure and validation in bovine uterine flushings have been reported (Bartol et al., 1981). Results were expressed as immunoreactive PGF, not $PGF_{2\alpha}$. Total recoverable immunoreactive uterine luminal PGF (TPGF) was taken as the product of uterine flushing PGF concentrations (pg/ml) and flushing volume. When actual flushing volume recovered was less than 20.0 ml per horn (40.0 ml/uterus), a value of 20.0 ml was used as the recovery volume, but when the volume recovered was greater than 20.0 ml, the actual volume collected was used for calculations.

Analytical Techniques for Proteins in Uterine Flushings

Total protein determination. Protein concentrations of uterine flushings from both cyclic and pregnant cattle were estimated according to procedures described by Lowry et al. (1951) using purified bovine serum albumin (BSA; fraction V, Sigma Chemical Co., St. Louis, MO) as a standard. Each uterine flushing sample was assayed in triplicate. Total protein content (TP) was calculated on a per horn basis by multiplying the sample protein concentration (mg/ml) by the sample recovery volume (ml). Total recoverable uterine luminal protein (TP; mg/40 ml) was calculated by adding together the TP for each horn.

Preparation for polyacrylamide gel electrophoresis (PAGE). Following determination of TP, uterine flushing samples were prepared for polyacrylamide gel electrophoresis (PAGE). Samples from each uterine horn were pooled for each cow and concentrated by ultrafiltration through a membrane with a pore size M_r<500 (UM05; Amicon Ultrafiltration unit, Lexington, MA). Ultrafiltration was performed at 4°C and 50 to 65 psi. Effluents from each sample were frozen and stored separately. The remaining concentrated sample was aspirated from the surface of the ultrafiltration membrane and the membrane carefully flushed several times with 0.5 to 1.0 ml of 0.05 M

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phosphate buffer. Protein concentrations (mg/ml) then were determined on each sample according to the method of Lowry et al. (1951) as described above.

Sodium dodecyl sulfate-PAGE (SDS-PAGE). To examine the temporal pattern of appearance and array of proteins in uterine flushings, proteins from each animal were subjected to sodium dodecyl sulfate polyacrylamide (10%) gel electrophoresis (SDS-PAGE) performed according to methods described by Laemmli 1970).

Protein standards used for molecular weight ($M_r \times 10^{-3}$) determinations included β-lactoglobulin (17.5), aldolase (40.0), ovalbumin (45.0), catalase (58.0), and transferrin (80.0). Additionally, a cross-linked bovine albumin molecular weight marker preparation was obtained (Sigma Chemical Co., St. Louis, MO) for higher molecular weight estimates (BSAI, $M_r \times 10^{-3}$ = 66.0; BSAII = 132.0; BSAIII = 198.0; BSAIV = 254.0; and BSAV = 330.0). This standard was processed according to procedures described by Davies and Stark (1970).

After electrophoresis, proteins were fixed in the gels with 7.5% acetic acid in 40% ethanol overnight at room temperature. Gels were stained for 3 h in a 0.125% solution of Coomassie brilliant blue R-250 (Sigma Chemical Co., St. Louis, MO) in 7.5% acetic acid (v/v) with 40% ethanol. The gels were diffusion-destained at 37°C with repeated changes of 7% acetic acid in 10% ethanol, and stored in 7% acetic acid.

Identification of protein bands, molecular weight estimation, and quantitative analysis. Destained standard protein and sample gels were spectrophotometrically scanned at 620 nm and recorded graphically. Individual bands were identified at their peak on a scan, and scans were compared visually to the gels from which they were obtained. Bands within each gel were referenced to a protein, which was coelectrophoretic with the BSA standard (Ra = 1.0; M_r = 66,000) and which was the major band in all gels of uterine flushing proteins. After electrophoresis, standard proteins with estimated molecular weights greater than 66,000 (BSA, Ra = 1.0) had Ra values less than 1.0 (towards cathode), and proteins with estimated molecular weights less than 66,000 had Ra values greater than 1.0 (towards anode).

Standard curves for estimation of molecular weights of proteins in uterine flushings were derived by linear regression of the Ra for each standard protein, as calculated from the scans, on the logarithm of their respective molecular weights. Separate standard curves were generated for high molecular weight standards ($y = 7.515 - 1.351x$; $66{,}000 < X < 320{,}000$) and low molecular weight standards ($y = 17.685 - 3.477x$; $17{,}500 < X < 66{,}000$, where y = Ra and x = log M_r). These equations were used to estimate the molecular weights of all uterine flushing protein bands identified on scans of the SDS-PAGE gels. Proteins on all gels were grouped by molecular weight category, and the frequency of appearance of protein bands was determined on an among cow-day basis.

The relative proportion of protein (mg) in each of the identified molecular weight categories was determined by calculating the area on the scan under each protein-band peak by trigonometric procedures, and expressed as a percentage of the total peak area for each gel scan. The percentage area for each peak multiplied by the total protein content for its corresponding uterine flushing was taken as an estimate of the proportion of total protein (mg) in that flushing represented by proteins of the various molecular weight categories present.

Statistical Analyses

The method of least squares analysis of variance (Harvey, 1972) was used to evaluate effects of status (S; dairy vs beef), day of estrous cycle (D), location of CL, animal (for follicle data), and higher order interactions for cyclic cattle variables including peripheral plasma steroids (E_1, E_2, and P_4), CL weights, specific $PGF_{2\alpha}$ binding by the 100,000 × g luteal particulate fraction (fmole/CL, fmole/mg protein), total recoverable uterine luminal protein (TP), total recoverable immunoreactive uterine luminal PGF (TPGF), and in vitro estradiol production by the two largest follicles (ng/follicle/5 ml medium; pg/mg tissue). Peripheral plasma steroid (E_1, E_2, and P_4) and TP data from pregnant cattle were analyzed using the General Linear Models (GLM) programs of the Statistical Analysis System (Barr et al., 1979) considering variability due to day of the estrous cycle and location of uterine horn relative to CL (for TP).

The frequency distribution (presence or absence of bands) of protein bands determined on a within cow-day basis for all cow gels was subjected to Brandt's factorial Chi-square analysis of attribute data (Batson, 1956). Orthogonal comparisons were made of frequency distributions of protein bands both among cycle days and among five molecular weight classes. These classes were chosen based upon previous reports of Sephadex G-200 chromatography of equine (Zavy, 1977) and bovine (Mills, 1975) uterine flushing proteins. Class ranges ($M_r \times 10^{-3}$) were I, > 150; II, 150–91; III, 90–61; IV, 60–31; V, 30–15.

The amount of protein (mg) estimated to be in the various molecular weight categories (n = 32) identified in cyclic cattle uterine flushings was subjected to least squares analysis of variance which considered variability due to day of cycle, status (dairy vs beef), animal, and interactions. Protein molecular weight was considered as a continuous independent variable to generate least squares regression curves of uterine luminal protein mass (mg) across all molecular weight categories identified. These curves were examined on an overall and within-cycle day basis.

RESULTS

Peripheral Plasma Steroids

Least squares means for peripheral plasma progesterone (P_4), estradiol (E_2), and estrone (E_1) for cyclic cattle are listed in Table 1. Plasma P_4 was affected significantly ($P<0.05$) by cycle day, and generally increased after Day 4. Both plasma E_1 and E_2 concentrations were affected ($P<0.01$ and $P<0.10$, respectively) by day of cycle and appeared to be elevated on Days 4 (E_1, 10.1 pg/ml; E_2, 23.0 pg/ml) and 16 (E_1, 28.1 pg/ml; E_2, 13.5 pg/ml). Means for Day 19 plasma P_4 (10.0 ng/ml) and estrogen

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TABLE 1. Least squares means (± SEM) for peripheral plasma steroid concentrations in cyclic and pregnant cattle.

Day[a]	Cattle		Progesterone (ng/ml) $\bar{x}$ ± SEM		Estradiol (pg/ml) $\bar{x}$ ± SEM		Estrone (pg/ml) $\bar{x}$ ± SEM	
	Cyclic	Pregnant	Cyclic[b]	Pregnant[c]	Cyclic[b]	Pregnant[c]	Cyclic[b]	Pregnant
4	6	...[d]	2.7 ± 1.0	...[d]	23.1 ± 3.2	...[d]	10.1 ± 2.0	...[d]
8	7	3	5.4 ± 1.2	10.2 ± 2.2	12.8 ± 1.6	7.5 ± 1.2	2.6 ± 1.0	7.6 ± 1.2
12	6	4	10.4 ± 2.0	9.4 ± 1.6	15.0 ± 2.2	5.6 ± 1.1	6.7 ± 1.1	5.9 ± 1.0
14	6	4	7.4 ± 1.9	9.3 ± 2.2	9.4 ± 2.1	5.2 ± 1.1	9.0 ± 2.0	3.7 ± 1.0
16	7	5	9.4 ± 1.1	6.9 ± 2.2	13.5 ± 2.6	7.3 ± 1.0	28.1 ± 3.1	3.6 ± 0.9
19	6	6	10.0 ± 1.2	10.1 ± 1.8	8.0 ± 1.4	6.1 ± 1.4	5.9 ± 1.4	7.7 ± 1.0
Total	38	22						
$\bar{x}$			7.4 ± 0.9	9.3 ± 0.8	13.4 ± 1.5	6.3 ± 0.5	10.5 ± 1.3	5.5 ± 0.6

[a]Day = day postestrus (day of estrus = Day 0).

[b]Significant day effects: P_4, $P<0.05$; E_2, $P<0.10$; E_1, $P<0.01$.

[c]No significant day effects or trends detected.

[d]No measurements made on Day 4 of pregnancy.

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concentrations (E_1 = 5.9; E_2 = 8.0 pg/ml) suggested that luteal regression had not occurred.

Listed also in Table 1 are the least squares means of peripheral plasma P_4, E_1, and E_2 for pregnant cattle (Days 8, 12, 14, 16, and 19). No significant day effects or day trends were detected for plasma P_4 ($\bar{x}$ ± SEM = 9.3 ± 0.8 ng/ml) or E_2 (6.3 ± 0.5 pg/ml). However, a day trend (P<0.01) was detected for plasma E_1 with levels highest on Days 8 and 19. Indirect evidence of CL maintenance and pregnancy was reflected in the mean plasma P_4 level (9.3 ± 0.8 ng/ml). This P_4 concentration was maintained through Day 19 of pregnancy and was similar to peripheral plasma P_4 concentrations of cyclic cattle from Days 12 to 19.

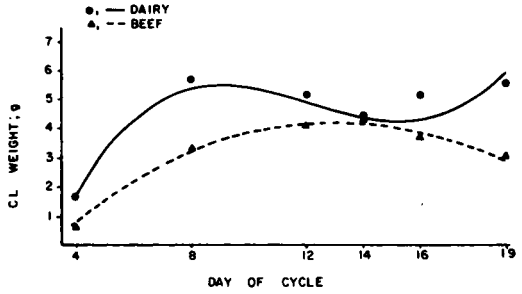

Fig. 1. Corpora lutea weights (g; y = CL weight, x = day of estrous cycle). Solid line ($y = -10.2384 + 4.2931x - 0.3734x^2 + 0.0101x^3$) represents significant (P<0.01) cubic regression for dairy cattle. Broken line ($y = -2.989 + 1.0812x - 0.0405x^2$) represents significant (P<0.01) quadratic regression for beef cattle. Status X Day interaction significant at P<0.01. Symbols represent least squares means for days.

CL Weights

Corpora lutea weights of cyclic cattle (dairy and beef) were affected (P<0.01) by cycle day. Least squares means of CL weights (g) for Days 4, 8, 12, 14, 16, and 19 were 1.1, 4.5, 4.4, 3.6, 4.7, and 4.2, respectively. Corpora lutea weights were higher (P<0.01) for dairy (4.5 g) than for beef cattle (3.2 g). Individual day trends for both dairy and beef cattle are presented in Fig. 1. A Status (dairy vs beef) by Day interaction was detected (P<0.05). It is possible that the dairy CL developed more quickly than beef CL early in the cycle between Days 4 and 8. This is reflected by the steeper slope of the dairy CL-weight regression line (Fig. 1). However, no similar interactions were detected with respect to peripheral plasma progesterone. Corpora lutea weights were positively correlated with peripheral plasma P_4 (r = 0.37; P<0.02) and negatively correlated with peripheral plasma E_2 (r = -0.31; P<0.06). Relationships between CL weights, P_4, and E_2 on Day 19 indicated that in this sample of 38 cyclic cattle luteal regression was not completed by Day 19 (n = 6) of the estrous cycle.

Specific Binding of $PGF_{2\alpha}$

Specific binding of $PGF_{2\alpha}$ to the 100,000 X g luteal particulate fraction of CL obtained throughout the estrous cycle was expressed both as specific $PGF_{2\alpha}$ binding site concentration (fmole/mg CL protein) and total $PGF_{2\alpha}$

TABLE 2. Specific binding of $PGF_{2\alpha}$ to the 100,000 X g bovine luteal particulate fraction.

Day	$PGF_{2\alpha}$ concentration (fmole/mg)	Total $PGF_{2\alpha}$ bound (fmole/CL)		
	Overall[ab]	Overall[a]	Dairy[b]	Beef[b]
4	1.02	14.29	25.05	3.52
8	1.87	145.79	212.82	78.77
12	1.83	177.34	221.61	133.07
14	1.55	111.82	102.56	121.08
16	2.08	274.51	418.97	130.04
19	1.67	199.17	291.43	106.91
$\bar{x}$	1.72 ± 0.8	157.82 ± 8.68	212.07[c]	95.57[c]

[a]Significant day effect (P<0.01).

[b]Status X Day interaction (P<0.01).

[c]Dairy>beef (P<0.01).

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binding (fmole/CL). The 100,000 x g luteal particulate fraction included both plasma membranes and disrupted subcellular membranes. Since no attempt was made to dissociate bound receptors or to measure occupied $PGF_{2\alpha}$ binding sites in the particulate fraction, the assay system effectively measured the concentration (fmole/mg CL protein) of $PGF_{2\alpha}$ bound to available (free) sites.

Results of the binding analyses are listed in Table 2. Specific $PGF_{2\alpha}$ binding site concentration (fmole/mg) was affected significantly ($P<0.01$) by cycle day. Mean binding site concentrations were lowest on Day 4 (1.02 fmole/mg), increased significantly by Day 8 (1.87 fmole/mg), and were highest on Day 16 (2.08 fmole/mg) following a generally increasing day trend ($P<0.01$). Fluctuations in specific $PGF_{2\alpha}$ binding site concentrations were positively correlated with CL weight ($r = 0.52$; $P<0.0001$), and elevations or depressions in mean binding site concentrations reflected similar changes in CL weight (Fig. 1).

Total $PGF_{2\alpha}$ binding by the 100,000 x g luteal particulate fraction (fmole/CL; Table 2) also was affected by estrous cycle stage ($P<0.01$), and was higher in dairy (212.07 fmole/CL) than in beef cattle (95.57 fmole/CL; $P<0.01$). Overall and for each status (dairy and beef), total binding increased markedly from Day 4 (14.29 fmole/CL) to Day 8 (145.79 fmole/CL; Table 2). As with concentration, $PGF_{2\alpha}$ binding site content was highest overall on Day 16 (274.51 fmole/CL). A Status by Day interaction was detected ($P<0.01$) and attributed to CL weight, which obviously influenced the total $PGF_{2\alpha}$ binding estimate. Corpus luteum weight and total $PGF_{2\alpha}$ binding were highly positively correlated ($r = 0.81$; $P<0.01$).

In vitro Follicle Estradiol Secretion

In vitro E_2 production by the two largest bovine follicles (both pg E_2/mg tissue/h or 3.5 h and ng E_2/follicle/h or 3.5 h) varied significantly ($P<0.01$) among animals within the same day of the estrous cycle (Table 3). Differences among days were not significant. Plasma concentrations of E_2 among cows were not correlated with in vitro follicular secretion of estradiol. In vitro production of E_2 by the largest and next largest follicle was not different when expressed on a per milligram of follicle

TABLE 3. Least squares means of collapsed follicle weights and in vitro estradiol production by the two largest follicles.

Day[a]	Cattle	Follicles	Follicle weight (mg)[b] $\bar{x}$ ± SEM	ng/5 ml Media/follicle[c] Per 1 h	ng/5 ml Media/follicle[c] Per 3.5 h	pg/mg Tissue[c] Per 1 h	pg/mg Tissue[c] Per 3.5 h
4	6	11	137.1 ± 41.3	4.29	15.07	45.7	159.9
8	7	10	140.4 ± 44.4	6.89	24.13	45.2	158.2
12	6	11	133.9 ± 41.3	1.73	6.04	14.0	48.9
14	6	12	90.3 ± 38.2	3.36	11.75	35.4	123.9
16	7	10	95.5 ± 41.9	1.32	4.61	16.0	55.9
19	6	12	221.6 ± 38.2	6.31	22.10	34.2	119.5
Total	38	66					
$\bar{x}$			136.5 ± 22.1	3.98	13.90	31.7	111.1

[a]Day = day postestrus (estrus = Day 0).
[b]Follicle weights not significantly different among days ($P>0.10$).
[c]Significant effect of cow (Day), $P<0.01$; no significant day effects for E_2 production.

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tissue basis. However, the largest follicle produced 33.4% more E_2 ($P<0.05$) during the 3.5 h incubation than did the next largest follicle within each animal (14.53 ng E_2/follicle/3.5 h vs 10.89 ng E_2/follicle/3.5 h).

Mean E_2 production for the entire 3.5 h incubation period (ng/follicle) ranged from 4.61 to 24.13. Estradiol production by all follicles during the incubation period (3.5 h) followed a curvilinear (quadratic) time trend as shown in Fig. 2. Data are expressed as pg E_2 per 5 ml of medium per hour per entire follicle. During the first 0.5 h of incubation, follicles released ∿2729.2 pg E_2 into the medium. Over the remaining 3 h, ∿13,207.8 pg of E_2 were released into the medium. Response for the last 3 h of incubation indicated that in vitro E_2 production rate was 2201.2 pg/0.5 h. This was ∿528 pg E_2/0.5 h less than the production rate during the first 30 min period (2729.2 pg E_2/0.5 h vs 2201.2 pg E_2/0.5 h). Actual in vitro follicular production of E_2 was nearly constant for the 4 h incubation. Results were interpreted as indicating active in vitro follicular synthesis and release of E_2. Using an identical incubation system, Chenault (1977) found that whole bovine follicles secreted significantly ($P<0.01$) more E_2 into medium (64.3 pg/mg follicle tissue) than was extracted from homogenized tissues of whole incubated frozen follicles (16.1 pg/mg tissue). Thus E_2 was synthesized during the incubation (Chenault, 1977).

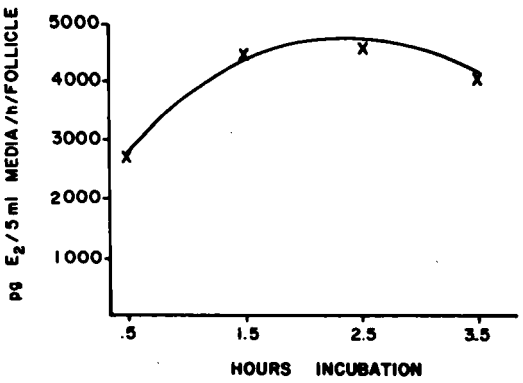

FIG. 2. In vitro estradiol secretion by the two largest follicles (pg/5 ml medium/h/follicle;y axis). Significant least squares regression ($y = 4666.0 + 43.5032x - 5.4543x^2$; $P<0.01$). Symbols represent least squares means for hours (x axis).

Uterine Luminal PGF

The amount of immunoreactive PGF recovered in uterine flushings of cyclic and pregnant cattle is summarized in Table 4. Amounts of total recoverable immunoreactive uterine luminal PGF (TPGF), from both cyclic and pregnant cattle, were affected significantly by Day ($P<0.01$ and $P<0.05$). Contents (ng/40 ml) of immunoreactive PGF in uterine flushings of cyclic cattle were lowest on Days 4, 8, and 12 (14.4, 13.9, and 19.7), but increased markedly thereafter, reaching a peak of 111.0 ng/40 ml on Day 19. Based upon previous

TABLE 4. Quantity[a] of immunoreactive PGF recovered from the uterine lumena of cyclic and early pregnant cattle.

Day postestrus	Prostaglandin F (ng/40 ml flush)	
	Estrous cycle[ab]	Pregnancy[ab]
4	14.4 (6)[c]	
8	13.9 (7)	17.6 (3)[c]
12	19.7 (6)	ND[d] (4)
14	47.7 (6)	48.0 (4)
16	27.4 (7)	482.6 (5)
19	111.0 (6)	1188.7 (6)

[a]Represent least squares mean.

[b]$P<0.05$ for day effects. Mean square errors for cyclic and pregnant cattle were 17,661 and 141,576, respectively.

[c]Number of animals in parentheses.

[d]Not detectable.

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observations (of plasma P_4 and CL weights) this Day 19 peak in uterine flushing PGF content occurred prior to luteolysis. However, the increase in TPGF occurred at a time when both concentration and content of available $PGF_{2\alpha}$ binding sites in the 100,000 x g luteal particulate fractions obtained from the Day 19 cattle were relatively stable at near peak levels.

Appreciably more PGF was detected in uterine flushings from pregnant than from cyclic cattle on Days 16 and 19 postestrus (Table 4). Uterine luminal PGF in pregnant cattle followed a pattern similar to that of cyclic cattle from Days 8 to 14, but increased dramatically from Day 16 (482.6 ng) to a peak on Day 19 of 1188.7 ng.

Total Recoverable Uterine Luminal Protein (Cyclic Cattle)

The amount of total recoverable uterine luminal protein (TP; mg/40 ml) varied ($P<0.05$) with day postestrus in cyclic cattle (Table 5). Levels of TP were higher ($P<0.01$) in beef (10.50 mg/40 ml) than in dairy animals (6.34 mg/40 ml), and a Status (dairy vs beef) by Day interaction was detected ($P<0.01$). This interaction was most apparent on cycle Days 8 and 14 when TP levels (mg/40 ml) for beef cattle were markedly elevated (Day 8, 12.11 vs 3.60, and Day 14, 22.87 vs 5.50). Although differences between dairy and beef TP were striking, there was no evidence to suggest that the Status by Day interaction occurred as a result of blood contamination in the uterine flushings. Both overall and for each status, TP appeared to be elevated later in the cycle (Days 14, 16, and 19). This increasing pattern reflected the increasing trend found for peripheral plasma progesterone, although no significant correlation was found between TP and plasma concentrations of P_4. Maximal and consistent stimulation of intrauterine protein production and accumulation within the uterine lumen appeared to occur during periods associated with P_4 exposure.

SDS-PAGE (Cyclic Cattle)

Protein molecular weight (PMW) categories. To make among- and within-day comparisons of individual cow SDS-PAGE protein profiles, it was necessary to assign each protein band to a category based upon molecular weight. First, each protein band identified on a gel scan was assigned a molecular weight. All protein bands then were ranked by molecular weight in ascending order. Neighboring protein bands in the ranking with molecular weights ($M_r \times 10^{-3}$) less than 33, between 33 and 70, and greater than 70 were considered different and placed in separate molecular weight categories if their molecular weights differed by ($\times 10^{-3}$) 2.0, 1.5, or 3.0, respectively. For example, Day 8 uterine flushings from six cattle contained a similar protein with a mean molecular weight of 36,055 (n = 6; 36,391, 36,812, 35,983, 36,745, 35,597, and 34,905). Proteins with molecular weight estimates on either side of this included one of 33,059 (n = 2; 33,040 and 33,078), and

TABLE 5. Total recoverable uterine luminal protein (mg).

Day	Cyclic cattle[a] (mg/40 ml)			Pregnant cattle (mg/40 ml)
	Overall	Dairy[b] (n)[c]	Beef[b] (n)	Overall[d] (n)
4	7.34	8.76 (3)	5.93 (3)	...
8	7.03	3.60 (4)	12.11 (3)	4.93 (3)
12	4.14	3.28 (3)	5.00 (3)	3.08 (4)
14	15.92	5.50 (3)	22.87 (3)	5.50 (4)
16	5.88	4.92 (3)	6.35 (4)	2.73 (5)
19	11.35	11.99 (3)	10.71 (3)	12.64 (6)
$\bar{X}$	8.61 ± 1.01	6.34 (19)	10.50 (19)	6.30 ± 1.89 (22)

[a] Day effects ($P<0.05$).

[b] Status, dairy<beef ($P<0.05$); Status x Day ($P<0.01$).

[c] n = Number of animals.

[d] No significant day effects detected.

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TABLE 6. Number and frequency of appearance of uterine luminal proteins during the estrous cycle.

Day	(n)[a]	No. different proteins	Mean no. proteins/cow	No. possible proteins	No. observed[b] proteins	Frequency	Mean[c] frequency
4	(3)	9	4 ± 1	27 (3 × 9)	13	0.48	
8	(7)	24	11 ± 1	168 (7 × 24)	78	0.46	0.45
12	(5)	22	9 ± 2	110 (5 × 22)	47	0.43	
14	(4)	21	12 ± 1	84 (4 × 21)	47	0.55	
16	(5)	19	11 ± 1	95 (5 × 19)	58	0.61	0.60[d]
19	(2)	11	8 ± 1	22 (2 × 11)	16	0.72	

[a] Number animals.

[b] Number observed proteins greater than number of different proteins because cows had many of the same proteins.

[c] Single degree of freedom orthogonal comparisons were 4 vs 8, 12, 14, 16, 19 (χ^2 = 0.018, P>0.99); 8, 12, 14, 16 vs 19 (χ^2 = 3.579, P<0.05); 12 vs 14, 16 (χ^2 = 6.020, P<0.025); 8 vs 12 (χ^2 = 0.194, P>0.75); and 14 vs 16 (χ^2 = 0.077, P>0.97). Overall χ^2 = 9.888 (5 df, P<0.10).

[d] Frequency of bands on Days 14, 16, 19>4, 8, 12 (P<0.025).

one at 39,160 (n = 7; 38,690, 38,922, 38,150, 39,400, 39,673, 39,673, and 40,616). Using the M_r 1,500 division, none of the three groups overlapped. Unequal molecular weight range limits (× 10^{-3}) of 2.0, 1.5, and 3.0 were chosen because the SDS-PAGE system used did not separate proteins by molecular weight in a perfectly linear fashion over the entire molecular weight range of 18.7 to 292.0 (× 10^{-3}). This combination of range limits resulted in no overlap between molecular weight categories.

Proteins identified in uterine flushings of cyclic cattle (Days 4, 8, 12, 14, 16, and 19) by SDS-PAGE in 10% polyacrylamide gels fell into 32 protein molecular weight categories (P1-P32). Proteins with molecular weights less than bovine serum albumin (BSA; M_r × 10^{-3} = 66.0, Ra = 1.0) ranged from 18.7 to 56.9. Molecular weight categories greater than 66.0 (P14-P32) ranged from 77.1 to 292.0. Since the SDS-PAGE system involved dissociation and denaturation of proteins prior to electrophoresis, some of the PMW categories identified may represent subunits of larger complex proteins.

Distribution of protein bands. Comparisons of frequency of appearance of protein bands in all molecular weight categories (P1-P32) among all estrous cycle days (Days 4, 8, 12, 14, 16, and 19) indicated that the frequency with which protein bands could be expected to appear was greater on Days 14, 16, 19 (60%), than on Days 4, 8, and 12 (45%) of the estrous cycle (Table 6). Orthogonal comparisons of frequency data for protein bands among five molecular weight range classes (M_r × 10^{-3}, I > 150; II = 150–91; III = 90–61; IV = 60–31; V = 30–15) indicated that proteins appeared with equal frequency across all ranges of molecular weights examined. Additionally, the number of different proteins observed appeared to be greatest during the luteal phase (Day 8, n = 24; Day 12, n = 21; and Day 16, n = 19; Table 6). Fewer proteins were present early in the cycle (Day 4, n = 9) than late in cycle (Day 19, n = 11); however, the proteins observed on Day 19 appeared with much greater frequency than did those on Day 4 (72% vs 48%). The statistical analysis indicated that the greater number and frequency of protein bands in the luteal phase was likely due to progesterone stimulation. However, induction of protein synthesis was not preferential within certain molecular weight classes. It occurred across all molecular weight classes as measured by the presence or absence

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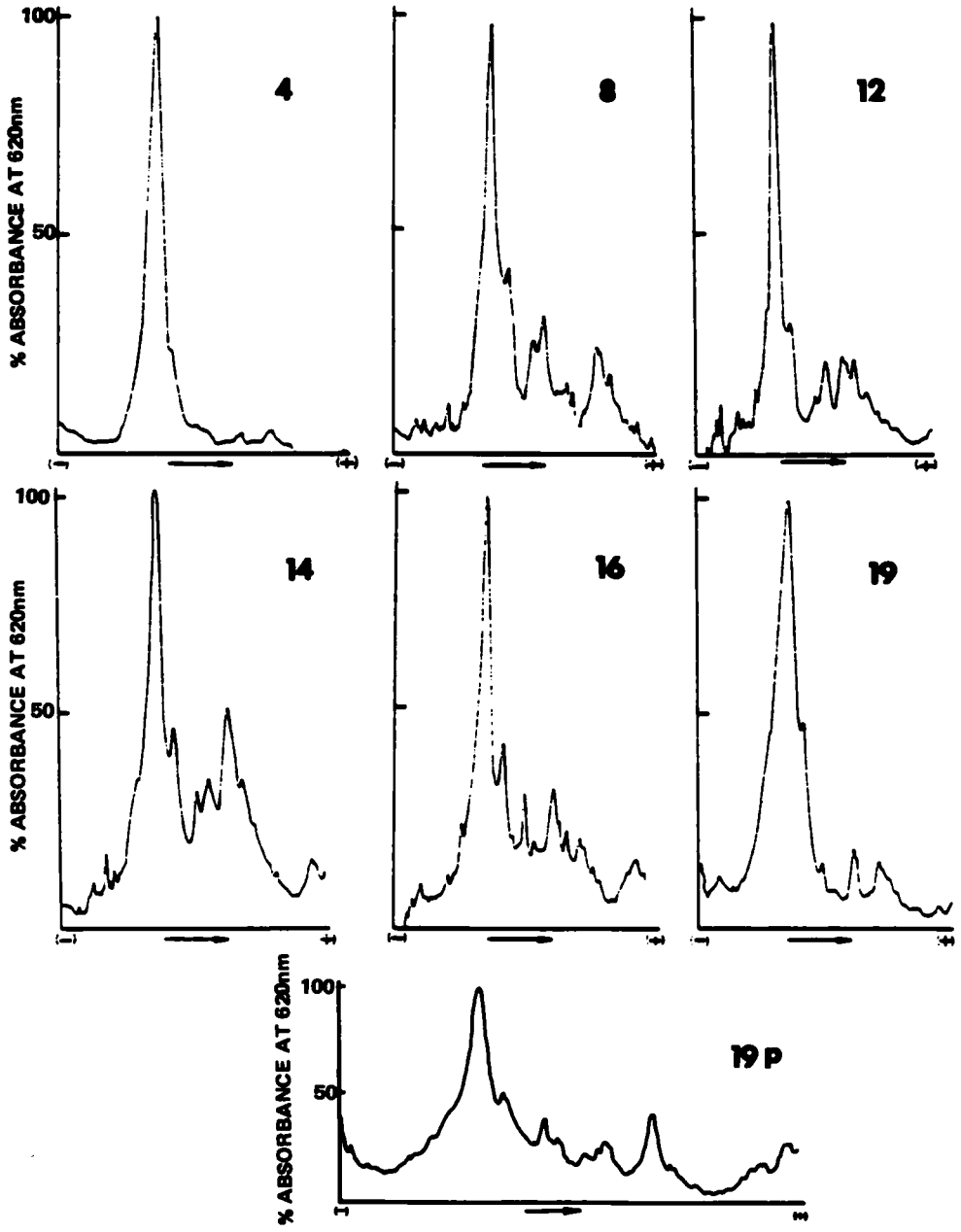

Fig. 3. Representative spectrophotometric scans of individual SDS-PAGE gels of uterine flushing proteins from estrous cycle Days 4, 8, 12, 14, 16, and 19, and pregnancy Day 19. Y axis is percent absorbance at 620 nm. Direction of electrophoretic migration indicated by arrow on x axis (cathode to anode).

of protein bands in SDS-PAGE gels.

Qualitative and Quantitative Analyses of SDS-PAGE Profiles. Representative scans of individual cow SDS-PAGE gels from Days 4, 8, 12, 14, 16, and 19 are presented in Fig. 3. Visual appraisal of all SDS-PAGE profiles suggested that the amount of protein present in the different bands was affected by cycle day.

The major band seen in all scans was co-electrophoretic with the BSA standard ($M_r \times 10^{-3} = 66.0$) and was considered to be BSA. Individual protein bands to the left of this peak (BSA) have molecular weights ($\times 10^{-3}$) greater than 66.0, while those to the right have weights

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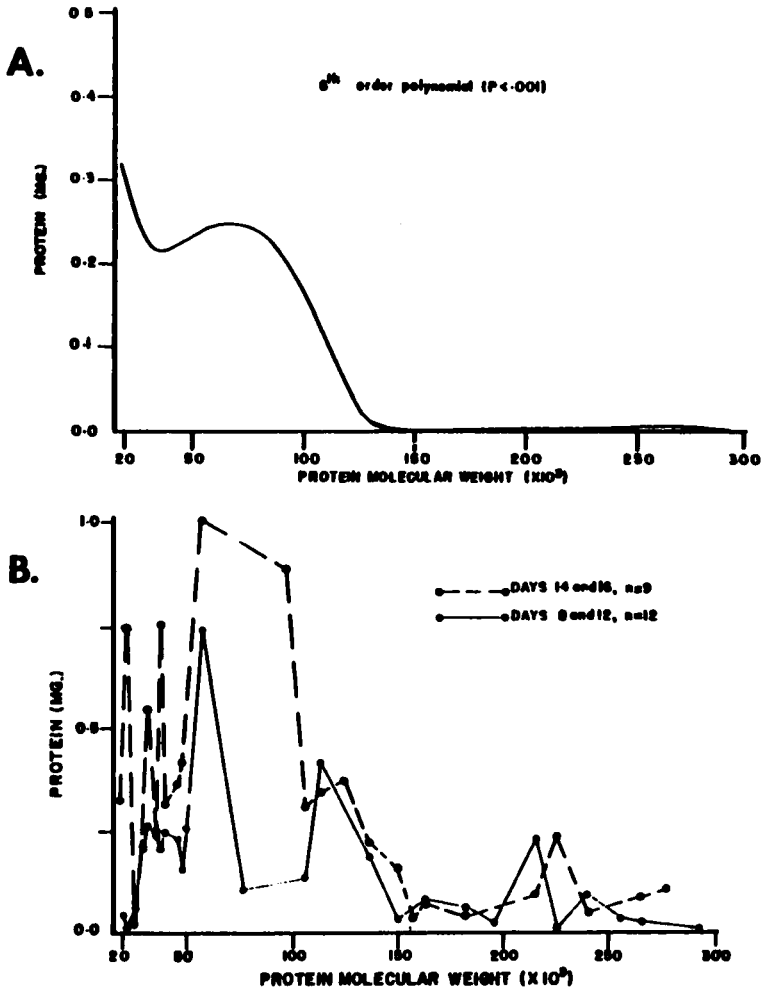

FIG. 4. A) Overall distribution of uterine luminal proteins (mg; y axis) across molecular weight categories (x axis) in cyclic cattle. Sixth order polynomial least squares regression ($y = 0.86076 - 0.49857x + 0.13961x^2 - 0.1765x^3 + 0.00107x^4 - 0.00003x^5 + 0.0000003x^6$; $P<0.001$).

B) Estimates of uterine luminal protein recovery (mg) by molecular weight classes in cyclic cattle. Points represent pooled arithmetic means of protein (mg) estimated to represent each PMW category for Days 8 to 12 (pooled; closed circles) and Days 14 to 16 (pooled; open circles). Patterns shown are significantly different ($P<0.10$).

less than 66.0. The representative scans shown in Fig. 3 reaffirm results obtained by analysis of frequencies in that proteins can be seen across the entire range of molecular weights. Number of peaks (proteins) clearly increased from Day 4 to Day 14, and decreased from Day 16 to Day 19. Additionally, percent absorbance of proteins other than BSA increased from less than 10% on Day 4 to 25–50% on Day 14, and began to return to more basal levels on Day 19. Since percent absorbance is an indirect measure of protein mass, these observations suggested that proteins of all molecular weight categories not only appeared in utero with greater frequency during the luteal phase, but also increased in quantity.

A statistical approach, designed to examine the quantitative pattern of protein distribution in bovine uterine flushings during the estrous cycle, was undertaken to examine objectively the hypothesis that the amount of protein (mg) estimated to be in the different molecular weight categories was affected by stage of cycle. The initial overall analysis, considering all protein bands (P1-P32) among all cycle days examined, failed to reveal significant effects of

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day. Bovine serum albumin ($M_r \times 10^{-3} = 66.0$) represented more than 60% of the total protein in all flushings, and its inclusion in the analysis masked or weighted any trends present with respect to all other proteins present in lesser proportions. Therefore, subsequent analyses excluded BSA (P13) to examine any differences in the quantitative pattern of protein distribution that might exist.

When data were analyzed in this manner, a significant ($P<0.025$) effect of cycle day was detected on amount of protein (mg) present in different PMW categories. The distribution of protein mass (mg) across all PMW categories for all days examined was represented best by a sixth order polynomial shown in Fig. 4A. This analysis indicated that proteins with molecular weights ($\times 10^{-3}$) in a range from ~18.0 to 135.0 were present in the greatest quantity throughout the estrous cycle.

To determine objectively if the quantitative protein profile changed during the estrous cycle, patterns similar to the overall regression shown in Fig. 4A were generated for each individual cycle day or for pooled day data. Mathematical models were identical to that of the overall analysis. Tests for heterogeneity of regression were performed on individual and pooled day data based upon identical orthogonal contrasts used for comparison of the frequency of appearance of proteins during the estrous cycle (Table 6). Significant heterogeneity ($P<0.10$) was found only for the comparison of sixth order curves generated from pooled Day 8 to 12 vs pooled Day 14 to 16 data, indicating that the quantitative protein patterns generated from these two cycle stages differed. This quantitative difference is presented graphically in Fig. 4B and appeared to be primarily a result of differences in protein mass (mg) in the molecular weight categories ($\times 10^{-3}$) less than 150. In this range, 12 proteins which were identified on both Days 8–12 and Days 14–16 were present in greater quantity on Days 14–16. Individual spectrophotometric scans from Days 8, 12, 14, and 16 (Fig. 3) further illustrate this point. When percent absorbance at 620 nm is used as a relative estimate of protein mass (mg), the average peak height of proteins in the lower molecular weight ranges (peaks to the right of BSA) on Days 8 and 12 are comparatively less than those on Days 14 and 16 (~20% vs 35%). These results complement the analyses of frequencies indicating that, in addition to an increase in the number and frequency of appearance of proteins in bovine uterine flushings during the later luteal phase (Days 14, 16, and 19), quantitative protein profiles differ during the estrous cycle with maximal stimulation occurring on Days 14–16. Although luteal stimulation was associated with increases in numbers and frequencies of proteins across all PMW classes, quantitative differences in protein mass were associated primarily with proteins of less than 150,000 molecular weight.

Total Recoverable Uterine Luminal Protein (Pregnant Cattle)

Total recoverable uterine luminal protein levels (mg/40 ml) for pregnant cattle are also presented in Table 5. In contrast to cyclic cattle, TP was not significantly affected by day postestrus (Days 8, 12, 14, 16, and 19). It should be noted, however, that pregnancy Day 4 was not evaluated and that lack of data from this day might have contributed to nondetection of day effects. Overall mean TP for pregnant cattle was 6.30 ± 0.89 mg/ml. Mean TP levels for each day of pregnancy appeared to be somewhat lower than corresponding levels for cyclic cattle on Days 8, 12, 14, and 16 (4.93, 3.08, 5.50, and 2.73 vs 7.03, 4.14, 15.92, and 5.88). However, Day 19 TP levels for cyclic and pregnant cattle were quite similar (11.35 vs 12.64).

SDS-PAGE (Pregnant Cattle)

Proteins identified by SDS-PAGE in uterine flushings of Day 19 pregnant cattle (n = 3) fell into 18 molecular weight categories. Fourteen of these 18 proteins correspond to PMW categories described for cyclic cattle. Eight of these 14 proteins were absent from SDS-PAGE protein profiles of estrous cycle Day 4 and Day 19 uterine flushings, but present on other cycle days (Days 8, 12, 14, or 16). The remaining six proteins were common to uterine flushings from cycle Day 4 or 19 but absent on other cycle days (Days 8, 12, 14, or 16), with the exception of BSA which was present in all flushings. Four proteins (MW $\times 10^{-3}$ = 15.2, 306.8, 322.2, 342.8) were identified in bovine uterine flushings from Day 19 of pregnancy which were not found in uterine flushings collected at any stage of the estrous cycle.

Proteins in uterine flushings from Day 19 of pregnancy appeared with a frequency essentially equal to those of Day 19 of the estrous cycle

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(68% vs 72%). However, appreciably more protein bands were seen in flushings from Day 19 of pregnancy than from Day 19 of the cycle (19 vs 11; 64% increase). Comparison of the SDS-PAGE profiles (Fig. 3) for Day 19 of pregnancy and the estrous cycle, respectively, suggested that the quantitative protein profiles for these two days differed, with more protein present in flushings from Day 19 of pregnancy across all molecular weight categories. Collectively, data suggested that the internal uterine protein milieu on Day 19 of pregnancy differed from that of estrous cycle Day 19 and resembled more closely an intrauterine protein milieu from the midluteal phase.

DISCUSSION

In the present study peripheral plasma E_1, E_2, and P_4 profiles for cyclic and pregnant cattle fell within normal ranges (Hansel et al., 1973; Ayalon, 1978).

Consistent with the report of Lukaszewska and Hansel (1980), CL weight and peripheral plasma concentrations of P_4 in cyclic cattle were positively correlated ($r = 0.37$; $P<0.02$). It was apparent in cattle assigned to represent the estrous cycle that luteal regression had not occurred or was not complete on or before Day 19 in agreement with observations of Lukaszewska and Hansel (1980). Since no group of cyclic cattle was represented in which CL regression occurred, responses characterized likely represented factors or conditions associated with stimulation and development of mid- to late-luteal phase uterine and ovarian conditions prerequisite to luteolysis.

Changes in $PGF_{2\alpha}$-specific binding sites (concentration and content) in the 100,000 × g luteal particulate fraction of CL obtained from cyclic cattle were related to growth and development of the CL. The period of CL growth after Day 4 (Fig. 1) appeared to be the time during which $PGF_{2\alpha}$ binding sites were most abundant. Twice daily injection of $PGF_{2\alpha}$ (25 mg, i.m.) on Days 3 and 4 postestrus were sufficient to reduce estrous cycle length and peripheral plasma P_4 concentrations in normal cyclic Holstein heifers (Beal et al., 1980). However, treatment with $PGF_{2\alpha}$ once per day on Days 2, 3, and 4, Days 2 and 3, or Days 3 and 4, or twice per day on Days 2 and 3 or Day 4 alone did not alter cycle length or severely depress peripheral plasma P_4 (Beal et al., 1980). These data suggest maturational changes necessary for normal luteal development. The refractory nature of the bovine CL to normally luteolytic doses of exogenous $PGF_{2\alpha}$ administered prior to Day 4 postestrus (Lauderdale, 1972; Beal et al., 1980) may represent the response of immature CL which have not yet synthesized sufficient numbers of high affinity $PGF_{2\alpha}$-specific binding sites.

Patterns of change in both concentration and content of $PGF_{2\alpha}$-specific binding sites reported in the present study (Table 2) were similar to those reported by Vernon (1979) for cyclic mares and Rao et al. (1975) for cyclic cattle. Additionally, Rao et al. (1978) reported that the affinity of bovine luteal receptors for $PGF_{2\alpha}$ increased over 200-fold between Days 13 (K_d 8.5×10^{-4}) and 20 (K_d 17.0×10^{-6}). Data suggest that the total number of available $PGF_{2\alpha}$-specific binding sites, present in bovine luteal tissue, increases from Day 4 of the estrous cycle as the CL matures and its biosynthetic processes become more active. During the mid- to late-luteal phase, the CL contains a stable population of $PGF_{2\alpha}$-specific binding sites with high affinity and specificity for $PGF_{2\alpha}$. Such a system would create optimum conditions for recognition of the luteolysin and CL regression.

In vitro estradiol production by the two largest ovarian follicles from each animal varied ($P<0.01$) among animals within the same cycle day, but differences among days were not significant (Table 3). All follicles were removed from an early- to mid-luteal phase endocrine environment apparently without being exposed, in vivo, to a preovulatory surge of luteinizing hormone (LH). Several studies involving ovine follicles indicated that the ability of a follicle to produce estrogens in vitro depended upon the endocrine (gonadotropic) environment to which it was exposed prior to excision (Hay and Moor, 1975; Moor, 1973; Seamark et al., 1974). Since, in the present study, all follicles were removed from appreciably similar in vivo endocrine environments prior to luteolysis, it perhaps is not surprising that no significant effects of cycle day were detected for in vitro follicle E_2 secretion. Results of the present follicle incubation study indicated that there were ovarian follicles present on all days of the estrous cycle examined and that these follicles were capable of synthesizing estradiol in vitro.

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The pattern of increase in TPGF for cyclic cattle (Table 4) was similar to those found in cyclic mares (Zavy, 1977) and gilts (Frank et al., 1977). The increase in uterine luminal PGF content in cyclic cattle after Day 16 to peak levels on Day 19 (Table 4) was consistent with earlier reports that bovine endometrial arachidonic acid concentrations were elevated between Days 10 and 14 postestrus (Hansel et al., 1975) and that endometrial PGF concentrations were higher after Day 15 than between Days 10 and 14 (Shemesh and Hansel, 1975). The positive correlations detected betweeen peripheral plasma P_4 and TPGF in cyclic cattle ($r = 0.37$, $P<0.05$) suggested that progesterone priming may be a requirement for prostaglandin synthesis. Work with several species, including guinea pigs (Blatchley et al., 1971; Naylor and Poyser, 1975), ewes (Ford et al., 1975; Louis et al., 1977), pigs (Frank et al., 1978), mares (Vernon, 1979), and cattle (Bartol et al., 1981), supports the concept that estrogen, acting on a progesterone-dominated uterus, stimulates increased synthesis and/or release of PGF as measured either in endometrial tissue, uterine venous plasma, or uterine flushings.

Observations in the present study, that uterine luminal PGF content was more than 10-fold higher in pregnant than in cyclic cattle on Days 16 and 19 postestrus (Table 4), were similar to findings by Lewis et al. (1977) and Wilson et al. (1972a,b) in which concentrations and contents of $PGF_{2\alpha}$ were higher in endometrium obtained from pregnant than from nonpregnant ewes. Elevation in TPGF seen in pregnant cattle after Day 14 (Table 4) may have resulted in part from production of PGF by the conceptus or conceptus-endometrial interactions which enhanced uterine synthesis/release and/or pooling of PGF within the uterine lumen.

Lewis et al. (1979b) demonstrated that Day 19 bovine blastocysts produce significant amounts of prostaglandins in vitro. Prostaglandin synthesis by the conceptus was greater at Day 19 than Day 16, and the pattern of production was markedly different from that of maternal endometrial tissue. Whatever the source of uterine luminal PGF during early pregnancy in cattle, questions remain as to the role of this substance in utero at this time and the mechanisms associated with conceptus and/or uterine products (Thatcher et al., 1980) which may prevent luteolysis in the face of such elevated levels of PGF.

Analyses of uterine luminal proteins obtained postmortem from cyclic cattle agreed with earlier observations in cattle (Mills, 1975; Roberts and Parker, 1974a,b), sheep (Heap, 1962; Roberts and Parker, 1976), mares (Zavy et al., 1979), and pigs (Bazer, 1975) that maximum stimulation of uterine protein synthesis and/or secretion occurs in response to progestational stimulation. Values determined for total recovered uterine luminal protein (TP) from cyclic and pregnant cattle in the present study were consonant with values reported by Mills (1975) and Roberts and Parker (1974a) for bovine uterine flushings collected postmortem. In cyclic cattle, although TP levels were higher in beef than in dairy cattle ($P<0.01$, 10.5 mg/40 ml vs 6.34 mg/40 ml), TP tended to be elevated during the late luteal phase (Days 14, 16, and 19; Table 5). Although no significant correlations were found between TP and peripheral plasma progesterone, the general pattern of change in TP reflected the linear increasing trend detected for progesterone in cyclic cattle.

Results of SDS-PAGE analyses of uterine luminal proteins from cyclic cattle indicated that the array of proteins present within the bovine uterus changed quantitatively and qualitatively during the estrous cycle. Maximum stimulation occurred during the luteal phase. This agreed with results of Murray et al. (1972) for cyclic pigs, and Zavy (1977) for cyclic mares, which indicated that normal luteal phase stimulation was associated with increases in the number and/or quantity of uterine luminal proteins primarily in categories of less than M_r 90,000. Results support the contention that maximum stimulation of uterine protein synthesis or uptake and luminally directed secretion occurs in response to progestational stimulation. However, absence of a significant correlation between peripheral plasma progesterone and total uterine luminal protein suggested that progesterone may play a permissive rather than a directly stimulatory role in enchancement of uterine protein synthesis and luminal release.

Cyclic changes in uterine luminal proteins and TPGF appeared to be related if not coupled phenomena. The two response variables were positively correlated in both cyclic ($r = 0.37$, $P<0.02$) and pregnant ($r = 0.42$, $P<0.05$) cattle.

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Bartol et al., (1981) reported that estradiol-17β (3 mg, i.v.), administered to cyclic cattle on Day 14 or 15 postestrus, caused increases in both TP and TPGF as measured in uterine flushings obtained postmortem. French and Casida (1973) and Huslig et al. (1979) indicated that pharmacological inhibition of protein synthesis blocked the luteolytic actions of estrogen and suggested that responses such as those reported by Bartol et al. (1981) might reflect increases in cellular activity associated with production of enzymes and other proteinaceous cellular products involved in prostaglandin synthesis. Hence, the increase in number, frequency of appearance, and mass of uterine luminal proteins from cyclic cattle from Days 8 to 12 and Days 14 to 16 (Fig. 4B), and the parallel pattern of change in TPGF (Table 4), may reflect increased uterine metabolic activity associated with synthesis and release of the luteolysin.

The concept of protein production by the bovine conceptus is supported by results of the present SDS-PAGE analyses of uterine luminal proteins from cattle on Day 19 of pregnancy. The quantitative and qualitative array of proteins found in utero on Day 19 of pregnancy differed markedly from that found on Day 19 of the estrous cycle. In general, the uterine protein milieu described for pregnancy Day 19 more nearly resembled a midluteal phase condition. Furthermore, four proteins were identified in uterine flushings from Day 19 of pregnancy that were apparently absent from the uterine flushings of cyclic cattle. These data suggest that the intrauterine protein milieu changes dynamically in response to presence and contributions of the conceptus. Such changes undoubtedly serve to create an intrauterine environment unique to the condition of pregnancy which maximizes the potential for continued development of the conceptus. Embryo transfers in cattle have been most successful if completed before Day 14 or 15 (Sreenan, 1978), and hysterectomy later than estrous cycle Day 16 will not prevent regression of the bovine CL (Anderson et al., 1969; Wiltbank and Casida, 1956). Quantitative and qualitative alterations in the bovine intrauterine environment, noted to occur primarily after Day 14, may reflect, in part, conceptus-mediated changes associated with prevention of luteolysis.

It is apparent from the present studies that normal patterns of development and secretion of steroids by the bovine ovary induce or are associated with dynamic quantitative and/or qualitative changes in the array of proteins and amount of prostaglandin F present in utero. The temporal pattern of appearance and ratio of ovarian steroids (estrogens and progesterone) acting on the uterus no doubt are critical factors in either establishing an intrauterine environment capable of maintaining the early bovine conceptus or in stimulation of production of the luteolysin (PGF) as a prerequisite to luteal regression. The bovine conceptus may initiate radical alterations in composition of the intrauterine milieu, especially after Days 12 to 14, which reflect processes associated with maternal recognition of pregnancy. The nature of response and ability of the uterus to respond to physiological cues of either ovarian or conceptus origin would appear, therefore, to represent a major control point in efficient reproduction.

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REFERENCES

Abraham, G. E., Swerdloff, R., Tulchinsky, D. and Odell, W. D. (1971). Radioimmunoassay of plasma progestin. J. Clin. Endocrinol. 32, 619–624.

Anderson, L. L., Bland, K. P. and Melampy, R. M. (1969). Comparative aspects of uterine luteal relationships. Rec. Prog. Horm. Res. 25, 57–104.

Ayalon, M. (1978). A review of embryonic mortality in cattle. J. Reprod. Fertil. 54, Suppl. 12, 483–493.

Barr, A. J., Goodnight, J. H., Sall, J. P., Blair, W. H. and Chilko, D. M. (1979). SAS User's Guide. SAS Institute Inc., Raleigh, NC.

Bartol, F. F., Thatcher, W. W., Lewis, G. S., Bliss, E. L., Drost, M. and Bazer, F. W. (1981). Effect of estradiol-17β on PGF and total protein content in bovine uterine flushings and peripheral plasma concentration of 13, 14-dihydro-15-keto-$PGF_{2\alpha}$. Theriogenology 15, 345–358.

Batson, J. (1956). Application of factorial χ^2 analysis to experiments in chemistry. Transactions of the 10th Annual Meeting Am. Soc. Quant. Chem. p. 9.

Bazer, F. W. (1975). Uterine protein secretions: Relationship to development of the conceptus. J. Anim. Sci. 41, 1376– 1382.

Bazer, F. W., Roberts, R. M. and Sharp, D. C. (1978). Collection and analysis of female genital tract secretions. In: Methods in Mammalian Reproduction. (J. C. Daniel, ed.). Academic Press, NY. pp. 503–528.

Beal, W. E., Milvae, R. A. and Hansel, W. (1980). Oestrous cycle length and plasma progesterone concentrations following administration of prostaglandin F-2α in the bovine estrous cycle. J. Reprod. Fertil. 59, 393–396.

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Blatchley, F. R., Donovan, B. T., Poyser, N. L., Horton, E. W., Thoman, C. J. and Los, M. (1971). Identification of $PGF_{2\alpha}$ in utero-ovarian blood of guinea pig after treatment with estrogen. Nature 230, 243–244.

Chenault, J. R. (1977). In vivo and in vitro responses of cattle to prostaglandin $F_{2\alpha}$. Doctoral Dissertation, University of Florida, Gainesville.

Chenault, J. R., Thatcher, W. W., Kalra, P. S., Abrams, R. M. and Wilcox, C. J. (1975). Transitory changes in plasma progestins, estradiol, and luteinizing hormone approaching ovulation in the bovine. J. Dairy Sci. 58, 709–717.

Chenault, J. R., Thatcher, W. W., Kalra, P. S., Abrams, R. M. and Wilcox, C. J. (1976). Plasma progestins, estradiol and luteinizing hormone following prostaglandin $F_{2\alpha}$ injection. J. Dairy Sci. 59, 1342–1346.

Cornette, J. C., Kirton, K. T., Barr, K. L. and Forbes, A. D. (1972). Radioimmunoassay of prostaglandins. J. Reprod. Med. 9, 355–360.

Davies, G. E. and Stark, G. R. (1970). Use of dimethyl suberimidate, a cross-linking reagent, in studying the subunit structure of oligomeric proteins. Proc. Natl. Acad. Sci. 66, 651–656.

Eley, D. S., Thatcher, W. W., Head, H. H., Collier, R. J. and Wilcox. C. J. (1981). Periparturient endocrine changes of conceptus and maternal units in Jersey cows bred for milk yield. J. Dairy Sci. 64, 296–311.

Eley, R. M., Thatcher, W. W. and Bazer, F. W. (1979a). Hormonal and physical changes associated with bovine conceptus development. J. Reprod. Fertil. 55, 181–190.

Eley, R. M., Thatcher, W. W., Bazer, F. W. and Fields, M. J. (1979b). Metabolism of progesterone and androstenedione in vitro by bovine endometrium and conceptus. J. Anim. Sci. 49, Suppl. 1, 294 Abstr.

Ford, S. P., Chenault, J. R. and Echternkamp, S. E. (1979). Uterine blood flow of cows during the oestrous cycle and early pregnancy: Effect of the conceptus on the uterine blood supply. J. Reprod. Fertil. 56, 53–62.

Ford, S. P., Weems, C. W., Pitts, R. E., Pexton, J. E., Butcher, R. L. and Inskeep, E. K. (1975). Effects of estradiol-17β and progesterone on prostaglandin F in sheep uteri and uterine venous plasma. J. Anim. Sci. 41, 1407–1413.

Frank, M., Bazer, F. W., Thatcher, W. W. and Wilcox, C. J. (1977). A study of prostaglandin $F_{2\alpha}$ as the the luteolysin in swine. III. Effects of estradiol valerate on prostaglandin F, progestins, estrone and estradiol concentrations in the utero-ovarian vein of nonpregnant gilts. Prostaglandins 14, 1183– 1196.

Frank, M., Bazer, F. W., Thatcher, W. W. and Wilcox, C. J. (1978). A study of prostaglandin $F_{2\alpha}$ as the luteolysin in swine. IV. An explanation for the luteotrophic effect of estradiol. Prostaglandins 15, 151–159.

French, L. R. and Casida, L. E. (1973). Effect of actinomycin on corpus luteum regression in ewes. J. Anim. Sci. 37, 1218–1221.

Hansel, W., Concannon, P. W. and Lukaszewska, J. H. (1973). Corpora lutea of large domestic animals. Biol. Reprod. 8, 222–245.

Hansel, W. and Echternkamp, S. E. (1972). Control of ovarian functions in domestic animals. Am. Zool. 12, 225–243.

Hansel, W., Shemesh, M., Hixon, J. and Lukaszewska, J. (1975). Extraction, isolation and identification of a luteolytic substance from bovine endometrium. Biol. Reprod. 13, 30–37.

Harvey, W. R. (1972). Instructions for Use of Least-Squares and Maximum Likelihood General Purpose Program 252K Mixed Model Version. Ohio State University, Columbus.

Hay, M. F. and Moor, R. M. (1975). Functional and structural relationship in the Graafian follicle population of the sheep ovary. J. Reprod. Fertil. 45, 583–593.

Heap, R. B. (1962). Some chemical constituents of uterine washings: A method of analysis with results from various species. J. Endocrinol. 24, 367–378.

Huslig, R. L., Fogwell, R. L. and Smith, W. L. (1979). The prostaglandin forming cyclooxygenase of ovine uterus: Relationship to luteal function. Biol. Reprod. 21, 589–600.

Kimball, F. A. and Lauderdale, J. W. (1975). Prostaglandin E_1 and $F_{2\alpha}$ specific binding in bovine CL: Comparison with luteolytic effects. Prostaglandins 10, 313–331.

Knight, J. W., Bazer, F. W., Thatcher, W. W., Franke, D. E. and Wallace, H. D. (1977). Conceptus development in intact and unilaterally hysterectomized-ovariectomized gilts: Interrelations among hormonal status, placental development, fetal fluids and fetal growth. J. Anim. Sci. 44, 620–637.

Laemmli, U. K. (1970). Cleavage of structural proteins during the assembly of the head of bacteriophage T_4. Nature (Lond.) 227, 680–685.

Lauderdale, J. W. (1972). Effects of $PGF_{2\alpha}$ on pregnancy and estrous cycle of cattle. J. Anim. Sci. 35, 246 Abstr.

Lewis, G. S., Basha, S.M.M., Bazer, F. W., Roberts, R. M. and Thatcher, W. W. (1979a). Proteins originating from bovine and porcine blastocysts. J. Anim. Sci. 49, Suppl. 1, 313 Abstr.

Lewis, G. S., Thatcher, W. W., Bazer, F. W., Roberts, R. M. and Williams, W. F. (1979b). Metabolism of arachidonic acid by bovine blastocysts and endometrium. J. Anim. Sci. 49, Suppl. 1, 313 Abstr.

Lewis, G. S., Wilson, L., Wilks, J. W., Pexton, J. E., Fogwell, R. L., Ford, S. P., Butcher, R. L., Thayne, W. V. and Inskeep, E. K. (1977). $PGF_{2\alpha}$ and its metabolites in uterine and jugular venous plasma and endometrium of ewes during early pregnancy. J. Anim. Sci. 45, 320–327.

Lin, M. T. and Rao, C. V. (1977). (^{3}H) prostaglandins binding to dispersed bovine luteal cells: Evidence for discrete prostaglandin receptors. Biochem. Biophys. Res. Commun. 78, 510–516.

Lin, M. T. and Rao, C. V. (1978). Properties of [^{3}H] prostaglandin $F_{2\alpha}$ binding to dispersed bovine luteal cells. Life Sci. 22, 303–312.

Louis, T. M., Parry, D. M., Robinson, J. S., Thorburn, G. D. and Challis, J.R.G. (1977). Effects of exogenous progesterone and oestradiol on prostaglandin F and 13, 14-dihydro-15-oxo prostaglandin $F_{2\alpha}$ concentrations in uteri and

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plasma of ovariectomized ewes. J. Endocrinol. 73, 427–439.

Lowry, O. H., Rosebrough, N. C., Farr, A. L. and Randall, R. J. (1951). Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Lukaszewska, J. and Hansel, W. (1980). Corpus luteum maintenance during early pregnancy in the cow. J. Reprod. Fertil. 59, 485–493.

Marinov, U. and Lovell, J. E. (1968). Cytology of the bovine uterine epithelium during the estrous cycle. Am. J. Vet. Res. 19, 13–29.

Mills, A. C. (1975). Cyclic nature of bovine uterine luminal proteins and their relationship to peripheral plasma progesterone and estrogen levels. Doctoral Dissertation, University of Florida, Gainesville.

Moor, R. M. (1973). Oestrogen production by individual follicles explanted from ovaries of sheep. J. Reprod. Fertil. 32, 545–548.

Murray, F. A., Bazer, F. W., Wallace, H. D. and Warnick, A. C. (1972). Quantitative and qualitative variation in the secretion of protein by the porcine uterus during the estrous cycle. Biol. Reprod. 7, 314–320.

Naylor, B. and Poyser, N. L. (1975). Effect of estradiol and progesterone on the in vitro production of $PGF_{2\alpha}$ by the guinea pig uterus. Br. J. Pharmacol. 55, 229–232.

Priedkalns, J. (1976). Female reproductive system. In: Textbook of Veterinary Histology. (H. D. Dellmann and E. M. Brown, eds.). Lea and Febiger, Philadelphia. pp. 319–349.

Priedkalns, J. and Weber, A. F. (1968). Ultrastructural studies of the bovine Graafian follicle and corpus luteum. Z. Zellforsch. Mikrosk. Anat. 91, 554.

Rao, C. V. (1975). The presence of discrete receptors for prostaglandin $F_{2\alpha}$ in the cell membranes of bovine corpora lutea. Biochem. Biophys. Res. Commun. 64, 416–424.

Rao, C. V., Estergreen, V. L., Carman, F. R., Moss, G. E. and Frandle, K. A. (1978). Gonadotropin and prostaglandin ($PGF_{2\alpha}$) receptors in bovine corpora lutea (CL) of early, mid and late luteal phase. 61st Annual Meeting Federation of American Society of Experimental Biologists. Abstr. 724.

Rao, C. V., Mitra, S. and Carman, F. R. (1979). Lysosomal (LY) prostaglandin (PG) $F_{2\alpha}$ receptors: Comparison of its properties with plasma membrane (PM) receptors. Biol. Reprod. 20, Suppl. 1, 111A.

Roberts, G. P. and Parker, J. M. (1974a). Macromolecular components of the luminal fluid from the bovine uterus. J. Reprod. Fertil. 40, 291–303.

Roberts, G. P. and Parker, J. M. (1974b). An investigation of enzymes and hormone binding proteins in the luminal fluid of the bovine uterus. J. Reprod. Fertil. 40, 305–313.

Roberts, G. P. and Parker, J. M. (1976). Fractionation and comparison of proteins from bovine uterine fluid and bovine allantoic fluid. Biochem. Biophys. Acta 446, 69–76.

Seamark, R. F., Moor, R. M. and McIntosh, J.E.A. (1974). Steroid hormone production by sheep ovarian follicles cultured in vitro. J. Reprod. Fertil. 41, 143–158.

Shemesh, M. and Hansel, W. (1975). Levels of PGF in bovine endometrium, uterine venous, ovarian artery and jugular plasma during the estrous cycle. Proc. Soc. Exp. Biol. Med. 148, 123–126.

Sreenan, J. M. (1978). Non-surgical embryo transfer in cattle. Theriogenology 9, 69–83.

Thatcher, W. W., Lewis, G. S., Eley, R. M., Bazer, F. W., Fields, M. J., Williams, W. F. and Wilcox, C. J. (1980). Contribution of the bovine conceptus to the endocrinological phenomenon existing at implantation, during gestation and around parturition. 9th International Congress of Animal Reproduction and Artificial Insemination. 1, 9–22.

Vernon, M. W. (1979). The role of prostaglandins in the utero-ovarian axis of the cycling and early pregnant mare. Doctoral Dissertation. University of Florida, Gainesville.

Wilson, L., Jr., Butcher, R. L. and Inskeep, E. K. (1972a). Prostaglandin $F_{2\alpha}$ in the uterus of ewes during early pregnancy. Prostaglandins 1, 479–482.

Wilson, L., Jr., Cenedella, R. J., Butcher, R. L. and Inskeep, E. K. (1972b). Levels of prostaglandins in the uterine endometrium during the ovine estrous cycle. J. Anim. Sci. 34, 93–99.

Wiltbank, J. N. and Casida, L. E. (1956). Alteration of ovarian activity by hysterectomy. J. Anim. Sci. 15, 134–140.

Zavy, M. T. (1977). Uterine luminal secretions in the cycling mare. M. S. Thesis, University of Florida, Gainesville.

Zavy, M. T., Mayer, R. E., Vernon, M. W., Bazer, F. W. and Sharp, D. C. (1979). An investigation of the uterine luminal environment of nonpregnant and pregnant pony mares. J. Reprod. Fertil. Suppl 27, 403–411.

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