BIOLOGY OF REPRODUCTION 21, 845–856 (1979)

Maintenance of the Corpus Luteum of Early Pregnancy in the Ewe. II. Prostaglandin Secretion by the Endometrium in vitro and in vivo

W. E. ELLINWOOD,[2] T. M. NETT and G. D. NISWENDER

Department of Physiology and Biophysics,
Colorado State University,
Fort Collins, Colorado 80523

ABSTRACT

To determine if the synthesis and/or secretion of prostaglandins $PGF_{2\alpha}$ and PGE_2 differed during the late luteal phase of the cycle and early pregnancy, a combination of in vivo and in vitro parameters were studied. Synthesis of $PGF_{2\alpha}$ and PGE_2 by ovine endometrial tissue in vitro was studied on Days 13, 15 and 17 of the estrous cycle and pregnancy. The prostaglandins were quantified by radioimmunoassay. There was no difference in the quantity of $PGF_{2\alpha}$ synthesized by tissue collected on Day 13 of either the cycle or pregnancy. Synthesis of $PGF_{2\alpha}$ was greater on Day 15 ($P<0.01$) and tended to be greater on Day 17 ($P<0.10$) of pregnancy than on Day 15 or 17 of the estrous cycle. Synthesis of PGE_2 was also significantly greater on Days 15 ($P<.001$) and 17 ($P<0.05$) of pregnancy than on the same days of the estrous cycle. Synthesis rates of both PGs in vitro tended to decrease from Days 13–17 in tissue collected from cycling ewes, while increasing from Days 13–15, then falling again on Day 17 of pregnancy. Concentrations of $PGF_{2\alpha}$ and PGE_2 in endometrial tissue followed the same pattern as in vitro secretion, suggesting that in vitro secretion accurately reflects the in vivo capacity of endometrial tissue to synthesize PGs. Concentrations of $PGF_{2\alpha}$ in uteroovarian venous serum were greater on Day 13 ($P<0.05$) and not different on Days 15 and 17 of pregnancy compared with the same days of the estrous cycle. However, concentrations of PGE_2 in uteroovarian venous serum were greater on Days 15 ($P<0.01$) and 17 ($P<0.05$) of pregnancy than on the same days of the estrous cycle. Prostaglandin $F_{2\alpha}$ was present in uterine flushings in extremely small quantities (0.3–0.6 ng/ml) and PGE_2 was nondetectable (<10 pg/ml) on Days 13, 15 and 17 of the estrous cycle. In contrast, concentrations of $PGF_{2\alpha}$ and PGE_2 were relatively high on all days of pregnancy examined and on Day 15 were at least 50 times greater than on Day 15 of the estrous cycle.

These data indicate that the capacity of endometrial tissue to secrete $PGF_{2\alpha}$ is increased during early pregnancy. The finding that PGE_2 is also secreted at a greater rate during early pregnancy lends support to the hypothesis that PGE_2 may be the factor responsible for maintenance of the corpus luteum of early pregnancy.

INTRODUCTION

There is good evidence that prostaglandin $(PG)F_{2\alpha}$ of uterine origin is the agent responsible for regression of the corpus luteum at the end of the estrous cycle of the ewe (for reviews see Goding, 1974; Horton and Poyser, 1977). Maintenance of early pregnancy in the ewe requires extension of the lifespan of the corpus luteum (Denamur and Martinet, 1977), but the effect of pregnancy on the luteolytic process is poorly understood. The embryo must be present in the uterus by the 13th day postestrus to prevent luteolysis (Moor and Rowson, 1966a,b) even though attachment of the trophoblast to the uterine endometrium does not occur until after Day 18 postestrus (Amoroso, 1951) and interdigitation of embryonic and maternal tissues does not occur until the 4th week of gestation (Boshier, 1969). Thus, the embryo somehow overcomes the luteolytic effects of $PGF_{2\alpha}$ 4–5 days prior to attachment.

The hypothesis that the embryo reduces synthesis and/or secretion of $PGF_{2\alpha}$ from endometrial tissue has received considerable attention but results have been contradictory. Thorburn et al. (1973) and Barcikowski et al. (1974) reported that PGF concentrations in uteroovarian venous plasma were lower during early pregnancy than during luteolysis in cycling ewes. In contrast, concentrations of

Accepted July 23, 1979.
Received May 16, 1979.
[1]Supported by a grant from Colorado State University Experiment Station.
[2]Present address: Oregon Regional Primate Research Center, Beaverton, OR 97005.

$PGF_{2\alpha}$ in uterine venous plasma of pregnant ewes were reported to be greater (Wilson et al., 1972a) or not different (Pexton et al., 1975a,b; Cerini et al., 1976; Nett et al., 1976; Lewis et al., 1977) than concentrations in uteroovarian venous plasma of cycling ewes on Days 13–17 postestrus. Nett et al. (1976) suggested that the frequency of peak concentrations of $PGF_{2\alpha}$ in uteroovarian venous plasma rather than the mean concentrations may be important in luteolysis. More research is needed before this issue can be resolved.

Inskeep et al. (1975) found that intrafollicular injection of $PGF_{2\alpha}$ in doses that were luteolytic in nonpregnant ewes were not luteolytic in pregnant ewes, suggesting that luteolysis was prevented at the ovarian level by a factor produced during early pregnancy. In a series of elegant experiments involving vascular cross-anastomoses, Mapletoft and coworkers confirmed that a factor from the gravid uterine horn prevents luteolysis at the ovarian level and that this effect is exerted through a local uteroovarian venoarterial pathway (Mapletoft et al., 1975; Mapletoft et al., 1976). Conflicting data have been obtained in vitro which suggests that $PGF_{2\alpha}$ has a slight stimulatory effect on progesterone secretion from dispersed bovine luteal cells (Hixon and Hansel, 1977).

Prostaglandin E_2 has been identified in extracts of endometrial tissue of ewes (Wilson et al., 1972b) and has the potential to be the factor that maintains the corpus luteum. It is a potent vasodilator and could overcome the vasoconstrictive effects of $PGF_{2\alpha}$ (Bergstrom et al., 1968; Dunham et al., 1974). PGE_2 has been shown to stimulate cAMP synthesis and progesterone production by luteal tissue in vitro (Speroff and Ramwell, 1970; Marsh, 1971; Kuehl et al., 1972; Maeyama et al., 1976) and will prevent $PGF_{2\alpha}$-induced reduction in progesterone secretion when both hormones are infused simultaneously into the ovarian artery of cycling ewes (Henderson et al., 1977). Pratt et al. (1977) found that intrauterine administration of PGE_2 lengthened the estrous cycle of ewes by ∿2 days; more recently, intrauterine administration of large doses of PGE_2 have been reported to prevent both natural and estradiol-induced luteolysis in cycling ewes (Magnes et al., 1978; Colcord et al., 1978). Since $PGF_{2\alpha}$ has been shown to be transferred from uteroovarian vein to ovarian artery in the ewe (McCracken et al., 1972; Land et al., 1976) and cow (Hixon and Hansel, 1974), it seems likely that PGE_2 could also reach the ovary via this pathway.

Bazer et al. (1977) hypothesized that maintenance of the corpus luteum of pregnancy in the pig is due to a shift in direction of secretion of $PGF_{2\alpha}$ towards the uterine lumen and away from the vascular system. In nonpregnant pigs, $PGF_{2\alpha}$ is secreted primarily into the vasculature, whereas during pregnancy it is secreted primarily into the uterine lumen where it appears to be sequestered.

The present study was designed to investigate 3 possibilities whereby the luteotropic effects of the ovine embryo are mediated via the uterus: 1) the embryo prevents production and/or alters secretion of the uterine luteolytic factor; 2) the embryo stimulates the uterus to synthesize and secrete a factor which maintains the corpus luteum at the level of the ovary; and 3) both mechanisms occur simultaneously. When embryos are removed from the uterine lumen after Day 12 of pregnancy, the corpus luteum is maintained until at least Day 25 (Moor and Rowson, 1966a; Ellinwood et al., 1979). Thus, the effect of the embryo is relatively long lasting and does not require continuous presence of the embryo in the uterine lumen. To test the hypothesis that the embryo affects endometrial synthesis and/or secretion of prostaglandins, the capacity of endometrial tissue to synthesize $PGF_{2\alpha}$ and PGE_2 in vitro on Days 13, 15 and 17 of the estrous cycle and pregnancy was examined. Concentrations of $PGF_{2\alpha}$ and PGE_2 were also measured in endometrial tissue, uteroovarian venous serum and uterine luminal flushings on Days 13, 15 and 17 of the estrous cycle and pregnancy.

MATERIALS AND METHODS

Animals and Experimental Protocol

Western range ewes were checked visually for estrous behavior twice daily using vasectomized rams. The first day a ewe stood to be mounted was designated as Day 0 of the estrous cycle. Only ewes that had 2 consecutive normal estrous cycles (16–18 days) were used. To obtain pregnant ewes, estrous ewes were penned with 2 fertile rams. Thirty ewes, 15 nonpregnant and 15 pregnant, were assigned randomly for sample collection on Days 13, 15 and 17 postestrus (n = 5/group). Ewes were anesthetized with sodium pentobarbital and the reproductive tract exposed via a midventral laparotomy. Blood samples (50 ml) were taken simultaneously from the uteroovarian vein on the side ipsilateral to the ovary bearing the corpus luteum and from the jugular vein. To minimize trauma-

induced prostaglandin synthesis, care was taken to handle the reproductive tract by holding the mesometrium rather than the uterus itself. The uterine horn ipsilateral to the ovary bearing the corpus luteum was flushed with 25 ml sterile isotonic saline (Ellinwood et al., 1979). If the ewe was from a pregnant group, pregnancy was verified by identifying an embryo in the flush. Flushings were transferred to Nalgene vials and snap-frozen in a dry ice-acetone bath. The uterus was quickly removed and placed on ice. The endometrium was dissected free and placed in 50 ml ice cold tissue culture Medium 199 containing 25 mM HEPES (N-2-hydroxyethylpiperazine-N'-2-ethanosulfonic acid, pH 7.4). An aliquot of endometrial tissue (1–2 g) was snap-frozen (elapsed time 30 seconds) in a dry ice-acetone bath for later determination of concentrations of PG in tissue. Only endometrial tissue from the uterine horn ipsilateral to the ovary bearing the corpus luteum was taken. In each pregnant ewe, the embryo was recovered from the uterine horn ipsilateral to the ovary bearing the corpus luteum.

Eight slices of endometrial tissue, ∼200 mg each, were taken at random from each uterus and washed 3 times in separate beakers containing fresh, ice cold Medium 199. Slices were incubated individually at 37°C in a 15 ml glass vial containing 10 ml Medium 199. Two of the 8 slices from each uterus were treated with indomethacin (10^{-5} M), an inhibitor of PG synthesis (Vane, 1971). Samples (2 ml) were taken from each vial at 20, 80 and 160 min and frozen in a dry ice-acetone bath for later analysis of PG. At the end of the incubation period, slices were blotted dry and weighed. Each sample of supernatant was assayed in duplicate for $PGF_{2\alpha}$ and PGE_2. Little synthesis of either PG occurred between 80 and 160 min; therefore, data are expressed as pg PG secreted/mg wet tissue weight/80 min. Data were corrected by subtracting the amount of PG produced by the indomethacin treated slices from the total PG synthesized. Indomethacin (10^{-5} M) inhibited synthesis of $PGF_{2\alpha}$ and PGE_2 by 92% and 91%, respectively. Net synthesis of each PG by each slice (pg/mg/80 min) was calculated and the average rate for 6 slices (corrected for the amount of PGs produced by the 2 indomethacin treated slices) yielded a single value for each animal.

To quantify PG in endometrial tissue, each sample (stored frozen at −20°C) was thawed just enough so that the frozen pellet of tissue would slide out of the tube. The pellet was weighed and homogenized for 60 seconds at one-half maximal speed in a Polytron homogenizer (Brinkman Instruments, Inc.) in absolute ethanol (5 ml/g) containing 10^{-5} M indomethacin to abolish synthesis of PG during homogenization. After homogenization, 3000 cpm [^{3}H]-$PGF_{2\alpha}$ (104 pg) and 5000 cpm [^{3}H]-PGE_2 (12 pg) were added to monitor losses during the remainder of the procedure. Homogenates were centrifuged for 10 min at 2250 × g and supernatants were transferred to 50 ml extraction tubes. Each pellet was washed with 5 ml absolute ethanol, centrifuged and supernatants were decanted. Combined ethanolic extracts were dried under a stream of nitrogen at 45°C and reconstituted in 5 ml phosphate buffered saline (pH 7.0). Contaminating neutral lipids were extracted with 10 ml petroleum ether, which was discarded. After the pH of the saline solution was adjusted to 4.0 with citric acid, PGs were extracted twice with 2 volumes ethyl acetate. The combined organic phases were dried under nitrogen at 45°C and silicic acid column chromatography was performed as described by Caldwell et al. (1971). Average recoveries of [^{3}H]-PGE_2 and [^{3}H]-$PGF_{2\alpha}$ through the entire procedure were 50% and 60%, respectively.

Jugular and uteroovarian venous blood was allowed to clot overnight at 4°C and serum was separated by centrifugation. Prostaglandins are not formed during the clotting process in sheep (Nett, unpublished observation). Uterine flushings were centrifuged at 2250 × g for 1 h to remove uterine and embryonic debris. After acidification to pH 4.0 with citric acid, aliquots of uteroovarian venous serum, uterine flushings or tissue culture medium ($PGF_{2\alpha}$ assay) were extracted twice in 10 volumes ethyl acetate. Each extract was washed with 1 ml deionized water, which was also acidified to pH 4.0 with citric acid. Combined organic phases were dried under nitrogen at 45°C and reconstituted in phosphate buffered saline containing 0.1% gelatin for radioimmunoassay. Recovery of [^{3}H]-PG (both $F_{2\alpha}$ and E_2) from uterine flushings and tissue culture medium was 95–100% while recovery from serum was 85–90%. Concentration of protein in uterine flushings was determined by the method of Lowry et al. (1951).

Radioimmunoassay

Glass-distilled organic solvents from Burdick and Jackson Laboratories (Muskegon, MI) were used without further purification. [^{3}H]-Prostaglandin E_2 (130 Ci/mM) and [^{3}H]-$PGE_{2\alpha}$ (9.2 Ci/mM) were obtained from New England Nuclear Corp. (Boston, MA). All nonradioactive PGs, except PGB_2 were gifts from the Upjohn Co. (Kalamazoo, MI). Prostaglandin B_2 and arachidonic acid were obtained from Sigma Chemical Co. (St. Louis, MO). High specific activity carrier-free Na-^{125}I was obtained from Amersham-Searle Corp. (Arlington Heights, IL). Tissue culture Medium 199 was obtained from Grand Island Biological Co. (Santa Clara, CA).

For thin-layer chromatography (TLC), silica gel thin-layer plates (Eastman no. 6061) were sprayed to saturation with 10% $AgNO_3$ in 86% aqueous ethanol, dried with a hair dryer and activated in an oven for 3 h at 60°C (Willis, 1970). Plates were stored in a sealed opaque envelope and were used within 2 weeks. Samples were applied in absolute ethanol and plates were developed in ethyl acetate:H_2O:iso-octane:acetic acid (11:10:5:2, equilibrated 2 h) to a distance of 17 cm (Kingston and Greaves, 1976). Marker prostaglandins were visualized with anisaldehyde reagent (Kiefer et al., 1975). After TLC, the desired zones of the plate were scraped onto glossy paper, transferred to extraction tubes and vortexed with 3 ml 2% NaCl to precipitate Ag^{++} ions (Willis, 1970). The solution was then adjusted to pH 4.0 with citric acid and extracted twice with 3 ml ethyl acetate. The organic phase from each extraction was washed twice in separate tubes containing 1 ml water which had been acidified to pH 4.0 with citric acid. Double backwashing in water was necessary to remove excess NaCl and ensure low blanks in the radioimmunoassay. The combined organic phases were dried under a stream of nitrogen at 45°C and reconstituted in 0.05 M phosphate buffered saline containing 0.1% gelatin for

radioimmunoassay. The ability of [^{3}H]-$PGF_{2\alpha}$ to bind to anti-$PGF_{2\alpha}$ serum after chromatography on silica gel was reduced by 27.9 ± 3.3% (mean ± SEM). This could not be attributed to blanks associated with extraction of $PGF_{2\alpha}$ from the silica gel and appeared to be caused by alteration of the PG on the TLC plate. Final values after TLC were corrected for the loss in bindability, which was monitored on every plate. Binding of [^{3}H]-PGE_2 to anti-PGE_2 serum was unaffected by TLC. Separation of PG of the E and F series was performed on extracts of endometrial tissue using silicic acid mini-columns (0.5 × 7 cm) as described by Caldwell et al. (1971).

An equilibrium double-antibody radioimmunoassay procedure (Niswender and Midgley, 1972) was used for quantification of $PGF_{2\alpha}$ and PGE_2. Antiserum to PGE_2 was raised in rabbits against a PGE_2-bovine serum albumin conjugate (Hwang et al., 1975). Radioactive antigen used in the PGE_2 assay was PGE_2-5,6,8,11,12,14,15-[^{3}H] (specific activity = 130 Ci/mM) obtained from New England Nuclear Corp. (Boston, MA). Approximately 30% of the radioactive antigen was bound to antibody at a final dilution of 1:20,000 in the absence of unlabeled hormone. Antiserum to $PGF_{2\alpha}$ was a gift from Dr. Kenneth Kirton of the Upjohn Company (Kalamazoo, MI). Radioactive antigen in the $PGF_{2\alpha}$ assay was an $PGF_{2\alpha}$-[^{125}I]-tyrosine methyl ester ($PGF_{2\alpha}$-TME-[^{125}I]) conjugate (Nett et al., 1976) radioiodinated by the method of Niswender et al. (1969). Approximately 45% of the radioactive antigen was bound to antibody at a final dilution of 1:15,000 in the absence of unlabeled hormone.

Relative potencies of several PGs and PG metabolites in the $PGF_{2\alpha}$ and PGE_2 assays are listed in Table 1. Prostaglandin E_1 had the greatest crossreactivity (20.36%) of any of the compounds tested in the PGE_2 assay system. Prostaglandin A_2 and $PGF_{2\alpha}$ exhibited about 1% of the activity of PGE_2 and all other compounds tested had less than 0.5% crossreactivity. Prostaglandin $F_{1\alpha}$ cross reacted in the $PGF_{2\alpha}$ assay in nonparallel fashion, with relative potency ranging from 2.5% to greater than 100% (Fig. 2). This phenomenon has been observed in other $PGF_{2\alpha}$ radioimmunoassay systems (Kirton et al., 1972; Stylos et al., 1972). Relative potencies of all other compounds tested in the $PGF_{2\alpha}$ assay system were less than 0.5% of $PGF_{2\alpha}$.

Values obtained by radioimmunoassay of culture medium, uteroovarian venous serum and uterine flushings before and after extraction and TLC are shown in Table 2. Varying quantities of nonextracted samples of uterine venous serum and uterine flushings produced inhibition curves that were not parallel to the standard curve. There were no differences ($P>0.05$) between extracted and chromatographed values for any test substances in either assay system as determined by one-way analysis of variance and Tukey's hsd procedure. There was no difference ($P>0.05$) in concentrations of PGE_2 determined on nonextracted, extracted, or chromatographed tissue culture medium. Therefore, measurements of both prostaglandins were performed directly on extracted serum, culture medium ($PGF_{2\alpha}$) and uterine flushings without chromatography. Radioimmunoassay of PGE_2 in tissue culture medium was performed without extraction.

Since $PGF_{1\alpha}$ cross reacts in the $PGF_{2\alpha}$ assay in a nonparallel fashion, significant contaminating quantities of $PGF_{1\alpha}$ would result in nonparallelism between unknown and standard inhibition curves. This was not the case. Inhibition curves generated with varying quantities of extracts of uteroovarian venous serum, uterine flushings and tissue culture medium were parallel to standard curves in both assay systems (Figs. 1, 2). Prostaglandin $F_{2\alpha}$ (78 pg–20 ng/ml) added to serum [regression analysis gave a correlation coefficient (r) of 0.99 between the $PGF_{2\alpha}$ added and that measured with a slope (b) of 1.03], or added to tissue culture medium ($r = 0.99$, $b = 1.00$) could be quantitatively recovered. Similarly PGE_2 (78 pg–20 ng/ml) added to serum prior to extraction ($r = 0.99$, $b = 1.12$) or to tissue culture medium ($r = 0.99$, $b = 1.09$) could be quantitatively recovered. Intraassay coefficients of variation at 25 and 70% inhibition in the $PGF_{2\alpha}$ assays were 13.7 and 7.7%, respectively. Corresponding interassay coefficients of variation were 18.3 and 11.8%. Intraassay coefficients of variation at 15 and 70% inhibition in the PGE_2 assays were 21.5 and 7.9%, respectively. Corresponding interassay coefficients of variation were 28.2 and 10.6%.

Concentrations of progesterone in jugular and

TABLE 1. Relative potency of selected prostaglandins in $PGF_{2\alpha}$ and PGE_2 radioimmunoassay systems.

Compound	PGE_2 assay	$PGF_{2\alpha}$ assay
PGE_2	100.0	0.03
$PGF_{2\alpha}$	1.04	100.00
PGE_1	20.36	0.05
$PGF_{1\alpha}$	0.32	2.5–100[a]
PGA_1	0.19	<0.01
PGA_2	1.12	<0.01
PGB_2	<0.01	<0.01
13,14-Dihydro-15-keto-$PGF_{2\alpha}$	<0.01	0.23
15-keto-$PGF_{2\alpha}$	<0.01	0.34
13,14-Dihydro-15-keto-PGE_2	0.04	0.04
PGI (6-keto-$PGF_{1\alpha}$)	0.24	0.21
Arachidonic acid	<0.01	<0.01

[a]See Fig. 2.

TABLE 2. Comparison of concentrations of PGE_2 and $PGF_{2\alpha}$ in uteroovarian venous serum, uterine flushings and tissue culture medium after no extraction, extraction or thin-layer chromatography.[a]

	Nonextracted (ng/ml)		Extracted (ng/ml)		TLC (ng/ml)	
	Value (n = 5)	Parallel	Value (n = 5)	Parallel	Value (n = 5)	Parallel
PGE_2						
Vein serum[b]	0.67 ± 0.04	No	0.67 ± 0.04	Yes	.43 ± 0.09	Yes
Ut. flush[c]	22.8 ± 2.0	No	14.5 ± 2.2	Yes	14.5 ± 1.8	Yes
M-199[d]	4.79 ± 0.12	Yes	4.44 ± 0.05	Yes	5.11 ± 0.6	Yes
$PGF_{2\alpha}$						
Vein serum	4.4 ± 0.6	No	1.4 ± 0.2	Yes	1.6 ± 0.1	Yes
Ut. flush	25.6 ± 0.7	No	6.6 ± 0.8	Yes	5.7 ± 0.8	Yes
M-199	34.5 ± 1.6	Yes	21.3 ± 0.6	Yes	19.9 ± 0.7	Yes

[a]Mean ± SEM.
[b]Vein serum = pooled from Day 15 of the cycle.
[c]Ut. flush = pool taken from ewes on Days 14 and 15 of pregnancy.
[d]M-199 = pool of in vitro supernatant representing all 3 days of pregnancy and cycle.

uteroovarian venous serum were quantified by radioimmunoassay according to the method of Niswender (1973).

Data were analyzed by analysis of variance. If nonhomogeneity of variance was indicated, data were transformed by taking the log of 1 plus the value and analysis was performed on transformed data. Significance of differences between treatment means was tested using the LSD procedure (Steel and Torrie, 1960).

RESULTS

Data regarding the net synthesis of $PGF_{2\alpha}$ in vitro and PGE_2 by endometrial tissue collected on Days 13, 15 and 17 of the estrous cycle and pregnancy are shown in Table 3. Synthesis of PGE_2 was greater on Days 15 ($P<0.01$) and 17 ($P<0.05$) of pregnancy than on the same days of the estrous cycle. Synthesis of $PGF_{2\alpha}$ was greater on Day 15 of pregnancy ($P<0.01$) than on Day 15 of the estrous cycle and tended to be greater on Day 17 ($P<0.10$). Net synthesis of both PGs tended to decrease from Days 13–17 in tissue collected from cycling ewes, while increasing from Days 13–15 and then falling again on Day 17 in tissue collected from pregnant ewes.

Concentrations of $PGF_{2\alpha}$ and PGE_2 in endometrial tissue followed the same pattern as synthesis in vitro on Days 13, 15 and 17 of the estrous cycle and pregnancy (Table 4). Concentrations of PGE_2 in endometrial tissue were greater on Days 15 ($P<0.01$) and 17 ($P<0.05$) of pregnancy than on the corresponding days of the estrous cycle. Concentrations of $PGF_{2\alpha}$ were greater on Day 15 ($P<0.01$) of pregnancy than on Day 15 of the estrous cycle. There was no difference in endometrial concentrations of $PGF_{2\alpha}$ between pregnant and cycling ewes on Days 13 and 17 postestrus.

Prostaglandin $F_{2\alpha}$ concentration in the uteroovarian vein was greater on Day 13 of pregnancy than on Day 13 of the estrous cycle ($P<0.05$), but there were no other differences (Table 5). Prostaglandin E_2 concentrations in uteroovarian venous serum were greater on Days 15 ($P<0.01$) and 17 ($P<0.05$) of pregnancy than on the same days of the estrous cycle (Table 5).

Prostaglandin $F_{2\alpha}$ was present in the uterine lumen in extremely small quantities and PGE_2 was nondetectable on Days 13, 15 and 17 of the estrous cycle (Table 6). Concentrations of $PGF_{2\alpha}$ and PGE_2 were greater ($P<0.01$) on Days 13, 15 and 17 of pregnancy than on corresponding days of the estrous cycle; on Day

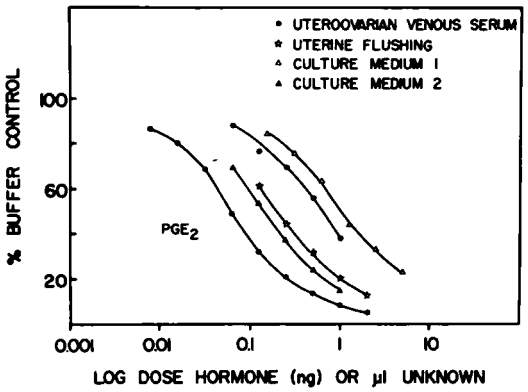

FIG. 1. Inhibition curves generated in the radioimmunoassay for PGE_2 by increasing quantities of PGE_2 (●), uteroovarian venous serum (○), uterine flushings (★), culture medium 1 after incubation of endometrial slices from cyclic ewes (△) and culture medium 2 after incubation of endometrial slices from pregnant ewes (▲). Each point represents the mean of 3 determinations.

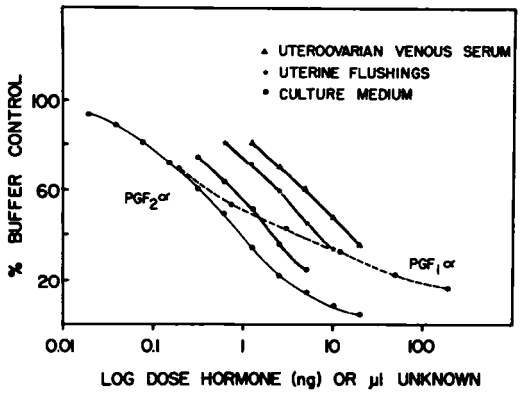

FIG. 2. Inhibition curves generated in the radioimmunoassay for $PGF_{2\alpha}$ by increasing quantities of $PGF_{2\alpha}$ (●—●), $PGF_{1\alpha}$ (○—○) and serial dilutions of extracts of uteroovarian venous serum (▲), uterine flushings (★) and culture medium (○). Each point represents the mean of 3 determinations.

17 of pregnancy they reached levels that were ∿50 times greater than on Day 15 of the estrous cycle. Protein concentration in uterine flushings was greater on all days of pregnancy than on corresponding days of the estrous cycle and increased from Days 13–17 postestrus (Table 7). There were no differences in protein concentrations of uterine flushings taken during the estrous cycle.

Concentration of progesterone in jugular vein serum was greater on Days 15 and 17 of pregnancy ($P<0.01$) than on the same days of the estrous cycle. In nonpregnant ewes, progesterone concentrations had begun to fall by Day 15 and reached baseline levels by Day 17 postestrus. These data indicated that these ewes were behaving normally in terms of timing and onset of luteal regression and that luteal function was maintained and normal through Day 17 in all pregnant ewes.

DISCUSSION

Both $PGF_{2\alpha}$ and PGE_2 were synthesized and secreted in vitro by endometrial tissue collected on Days 13, 15 and 17 of the estrous cycle and pregnancy. Presence of an embryo appears to stimulate synthesis and secretion of both $PGF_{2\alpha}$ and PGE_2 and this is most apparent on Day 15 postestrus. The fact that concentrations of $PGF_{2\alpha}$ and PGE_2 in endometrial tissue followed the same pattern as in vitro secretion strongly suggests that the secretion rates determined are a reflection of the capacity of endometrial tissue to synthesize PGs. These data indicate that the capacity of the endometrium to synthesize $PGF_{2\alpha}$ during early pregnancy is not decreased, but is actually increased. The findings agree with those of Wilson et al. (1972a) and Lewis et al. (1977).

Since $PGF_{2\alpha}$ is secreted from the uterus is a pulsatile manner (Thorburn et al., 1973; Nett et al., 1976), it is difficult to make valid conclusions about concentrations in uteroovarian venous serum when a single sample was taken from each ewe. However, there was no evidence in these studies that concentrations of $PGF_{2\alpha}$ in uteroovarian venous serum were reduced during early pregnancy. This is in variance with the conclusions of Thorburn et al. (1973) and Barcikowski et al. (1974). Thorburn et al. (1973) reported that PGF concentrations in uteroovarian venous plasma were lower during early pregnancy than during luteolysis in cycling ewes. However, pregnancy could be verified in only 1 of the 3 ewes presumed pregnant. The profile of concentrations of PGF in the uteroovarian vein depicted for this animal does not appear to be different than the profiles depicted for 2 nonpregnant ewes in which luteolysis was shown to occur. In another study, Barcikowski et al. (1974) reported a difference in secretion of PGF between cycling and pregnant sheep. However, the comparison was made between 1 cycling ewe which had a uteroovarian autotransplant and 1 pregnant ewe

TABLE 3. Secretion of $PGF_{2\alpha}$ and PGE_2 by endometrial tissue in vitro (pg/mg/80 min ± SEM).[a]

	$PGF_{2\alpha}$			PGE_2		
	Cycle		Pregnancy	Cycle		Pregnancy
Day 13	424.3 ± 95.0	N	506.3 ± 13.0	96.6 ± 22.6	N	123.8 ± 37.5
	N		*	N		*
Day 15	232.5 ± 81.0	**	720.8 ± 37.4	51.8 ± 19.3	**	205.6 ± 20.1
	N		**	N		**
Day 17	110.5 ± 42.0	N	223.3 ± 28.1	24.3 ± 11.4	*	98.1 ± 15.8

[a]Compare means in adjacent boxes; n = 5.

*P<0.05; **P<0.01; N = no difference (P>0.05).

TABLE 4. Concentrations of $PGF_{2\alpha}$ and PGE_2 in endometrial tissue on Days 13, 15 and 17 of the estrous cycle and pregnancy.[a]

	$PGF_{2\alpha}$ (ng/g)			PGE_2 (ng/g)		
	Cycle		Pregnancy	Cycle		Pregnancy
Day 13	175.6 ± 35.7	N	148.3 ± 11.9	28.0 ± 6.1	N	30.8 ± 9.6
	N		*	N		*
Day 15	115.7 ± 35.3	**	250.7 ± 27.0	21.1 ± 6.7	**	72.4 ± 22.0
	N		N	N		N
Day 17	116.4 ± 16.8	N	148.8 ± 22.5	9.1 ± 1.1	*	40.9 ± 13.5

[a]Mean ± SEM; n = 5. Compare means in adjacent boxes.

*P<0.05; **P<0.01: N = no difference (P>0.05).

TABLE 5. Concentrations of $PGF_{2\alpha}$ and PGE_2 in uteroovarian venous serum on Days 13, 15 and 17 of the estrous cycle and pregnancy.[a]

	$PGF_{2\alpha}$ (ng/ml)			PGE_2 (pg/ml)		
	Cycle		Pregnancy	Cycle		Pregnancy
Day 13	4.2 ± 1.2	*	9.2 ± 2.5	478 ± 81	N	694 ± 53
	N		N	N		*
Day 15	4.8 ± 1.6	N	7.7 ± 1.2	453 ± 71	**	1071 ± 161
	N		N	N		*
Day 17	2.3 ± 0.5	N	6.9 ± 1.6	329 ± 56	*	674 ± 158

[a]Mean ± SEM; n = 5. Compare means in adjacent boxes.

*P<0.05; **P<0.01; N = no difference (P>0.05).

TABLE 6. Concentrations of $PGF_{2\alpha}$ and PGE_2 in uterine flushings on Days 13, 15 and 17 of the estrous cycle and pregnancy.[a]

	$PGF_{2\alpha}$ (ng/ml)			PGE_2 (pg/ml)		
	Cycle		Pregnancy	Cycle		Pregnancy
Day 13	0.5 ± 0.2	**	1.9 ± 0.4	<10	**	136 ± 63
	N		**	N		**
Day 15	0.6 ± 0.2	**	22.3 ± 5.6	<10	**	4210 ± 90
	N		N	N		N
Day 17	0.3 ± 0.2	**	21.6 ± 3.9	<10	**	2520 ± 150

[a]Mean ± SEM; n = 5. Compare means in adjacent boxes.

*P<0.05; **P<0.01; N = no difference (P>0.05).

TABLE 7. Protein recovered from uterine lumen on Days 13, 15 and 17 of estrous cycle and pregnancy (μg/ml 25 ml flush ± SEM).[a]

	Cycle		Pregnancy
Day 13	114.8 ± 30	*	283.6 ± 53
	N		N
Day 15	154.4 ± 64	*	366.0 ± 84
	N		**
Day 17	123.3 ± 54	**	594.0 ± 28

[a]Mean ± SEM; n = 5. Compare means in adjacent boxes.

*P<0.05; **P<0.01; N = no difference (P>0.05).

with the reproductive tract in situ. In contrast, concentrations of $PGF_{2\alpha}$ in uterine or uteroovarian venous plasma of pregnant ewes have been found to be greater (Wilson et al., 1972a) or not different (Pexton et al., 1975a,b; Cerini et al., 1976; Nett et al., 1976; Lewis et al., 1977) than concentrations of uterine or uteroovarian venous plasma of cycling ewes on Days 13–17 postestrus.

The data from the present study indicate that PGs are somehow sequestered into the uterine lumen during early pregnancy. The concentrations of protein recovered from the uterine lumen were greater on Days 13, 15 and 17 of pregnancy than on the corresponding days of the estrous cycle. Some of the protein detected in uterine flushings may have been due to solubilization of embryonic proteins. However, total protein recovered on Day 13 of pregnancy was greater ($P<0.05$) than on Day 13 of the cycle, when contribution of embryonic protein to total protein was negligible. On Day 13 of pregnancy, the embryo is a small spherical or slightly ovate blastocyst no more than 2–3 mm in diameter, yet an average of 7.1 mg (283.6 μg/ml × 25 ml) total protein was recovered from the uterine lumen of the 5 pregnant ewes, compared with 2.9 mg from the 5 cycling ewes. This suggests that the endometrium may secrete pregnancy-specific proteins as early as Day 13 postcoitum in the ewe. Some of these proteins might sequester $PGF_{2\alpha}$ (Ellinwood et al., unpublished observation). Roberts et al. (1974) detected a pregnancy-specific protein in the uterine lumen of the pregnant cow on Day 16 of pregnancy. Preliminary observations in sheep suggest the presence of a similar protein on Day 13 of pregnancy (Roberts et al., 1976).

It is possible that the embryo may synthesize the PGs found in the uterine lumen during early pregnancy. Shemesh et al. (1978) have demonstrated that gonads from preimplantation bovine embryos are capable of secreting both PGEs and PGFs. In addition, Dickmann and Spilman (1975) have identified PGs in blastoceolic fluid of the preimplantation rabbit blastocyst. However, it is not known whether these PGs were synthesized by the blastocyst or were sequestered from the intrauterine environment. There is considerable evidence that molecules as large as ferritin (mw 480,000) can be transported into the blastoceolic fluid (Hastings and Enders, 1974; Borland et al., 1976; Borland et al., 1977). The possibility that the embryo may induce a shift in the direction of secretion of $PGF_{2\alpha}$ from lamina propria to uterine lumen where it can be sequestered by the blastocyst or a uterine luminal protein, as has been hypothesized in the pig (Bazer et al., 1977), must not be overlooked.

The fact that endometrial tissue collected from pregnant animals had a greater capacity to synthesize PGE_2 on Days 15 and 17 of pregnancy than on corresponding days of the estrous cycle may be significant in terms of maintenance of the corpus luteum. Prostaglandin E_2 can counteract the luteolytic effects of $PGF_{2\alpha}$ when both hormones are given simultaneously into the ovarian artery of cycling ewes (Henderson et al., 1977). In addition, intrauterine administration of PGE_2 to cycling ewes will overcome both natural and estradiol-induced luteolysis (Magness et al., 1978; Colcord et al., 1978). The antiluteolytic effect of PGE_2 could be due to its vasodilator properties (Bergstrom et al., 1968; Dunham et al., 1974) or its ability to stimulate synthesis of cAMP and progesterone in luteal tissue (Speroff and Ramwell, 1970; Marsh, 1971; Kuehl et al.,

1972; Maeyama et al., 1976). In addition to the fact that in vitro synthesis and endometrial tissue concentrations of PGE_2 were greater on Days 15 and 17 of pregnancy than on Days 15 and 17 of the estrous cycle, concentrations of PGE_2 in uteroovarian venous serum were also greater on Days 15 and 17 of pregnancy than on corresponding days of the estrous cycle. These findings support the hypothesis that PGE_2 is the factor responsible for maintenance of the corpus luteum of early pregnancy in the ewe, but definitive testing of this hypothesis awaits further research, particularly since in a similar study in which samples were collected from cannulated uterine veins Lewis et al. (1978) did not find higher levels of PGE_2 in uterine venous plasma of pregnant animals on Day 15 compared with values from nonpregnant ewes.

It is not clear why the major differences in $PGF_{2\alpha}$ and PGE_2 synthesis and concentrations in endometrial tissue, uterine lumen and uteroovarian venous serum were most apparent on Day 15 of pregnancy. The endometrium was more active in terms of synthesis of both $PGF_{2\alpha}$ and PGE_2 on Day 15 of pregnancy, yet the signal for maintenance of the corpus luteum occurs on Day 13 postestrus (Moor and Rowson, 1966a,b). One explanation is that the process which results in maintenance of the corpus luteum begins on Day 13 and is not maximal until Day 15. This is supported by the observation that rates of synthesis and endometrial tissue concentrations of PGE_2 tend to be greater on Day 13 of pregnancy than on Day 13 of the estrous cycle, but are not statistically greater until Day 15. The magnitude of the increase in synthesis of PGE_2 may be small enough not to be detected by the present methodology yet large enough to overcome luteolysis. The large increase in secretion of PGE_2 observed on Day 15 may be necessary to ensure that the corpus luteum is maintained. Another explanation for the apparent discrepancy may be that the "signal" for maintenance of the corpus luteum does not occur on Day 13. The fate of the corpus luteum may not be irreversibly determined by Day 13 postestrus since luteolysis can be arrested by hysterectomy on Day 15 of the estrous cycle (Moor et al., 1970). However, from histological examination it appeared that hysterectomy did not prevent death of cells that had already begun to degenerate at the time of surgery. This suggests that either an antiluteolytic or a luteotropic signal is indeed necessary before Day 15 to prevent the onset of luteolysis.

In summary, the capacity of endometrial tissue to synthesis $PGF_{2\alpha}$ is not decreased, but is increased during early pregnancy in the ewe. This is in direct opposition to the hypothesis that the embryo reduces synthesis and/or secretion of $PGF_{2\alpha}$ during early pregnancy. Both $PGF_{2\alpha}$ and PGE_2 are sequestered in the uterine lumen during early pregnancy. Whether this is due to accumulation of PGs in blastoceolic fluid, binding of PGs to embryonic or uterine proteins, or de novo synthesis of PGs by the embryo can not be determined from these data. However, the observation that the amount of protein recovered in uterine flushings on Days 13, 15 and 17 of pregnancy was greater than on the same days of the estrous cycle suggests that pregnancy-specific proteins may be present in the uterine lumen as early as Day 13 postcoitum. Some of these proteins could be PG binding proteins. Finally, the fact that the capacity of endometrial tissue to secrete PGE_2, which has antiluteolytic properties, is also increased during early pregnancy lends support to the hypothesis that PGE_2 may be the factor responsible for maintenance of the corpus luteum of early pregnancy in the ewe.

ACKNOWLEDGMENTS

The authors wish to thank Vaughn Cook for his expert assistance during the course of these studies.

REFERENCES

Amoroso, E. C. (1951). The interaction of the trophoblast and endometrium at the time of implantation in the sheep. J. Anat. 85, 428–431.

Barcikowski, B., Carlson, J. C., Wilson, L. and McCracken, J. M. (1974). The effect of endogenous and exogenous estradiol-17β on the release of prostaglandin $F_2\alpha$ from the ovine uterus. Endocrinology 95, 1340–1349.

Bazer, F. W. and Thatcher, W. W. (1977). Theory of maternal recognition of pregnancy in swine based on estrogen controlled endocrine versus exocrine secretion of prostaglandin $F_2\alpha$ by the uterine endometrium. Prostaglandins 14, 397–401.

Bergstrom, S., Carlson, L. A. and Weeks, J. R. (1968). The prostaglandins: A family of biologically active lipids. Pharmacol. Rev. 20, 1–48.

Borland, R. M., Biggers, J. D. and Lechene, C. P. (1976). Kinetic aspects of rabbit blastoceole fluid accumulation: An application of electron probe microanalysis. Dev. Biol. 50, 201–211.

Borland, R. M., Erickson, G. F. and Ducibella, T. (1977). Accumulation of steroids in rabbit preimplantation blastocysts. J. Reprod. Fert. 49, 219–224.

Boshier, D. P. (1969). A histological and histochemical examination of implantation and early placentome formation in sheep. J. Reprod. Fert. 19, 51–61.

Caldwell, B. V., Burstein, S., Brock, W. A. and Speroff, L. (1971). Radioimmunoassay of F prostaglandins. J. Clin. Endocrinol. Metab. 33, 171–175.

Cerini, J. C., Cerini, M. E., Cumming, I. A., Findlay, J. K. and Lawson, R.A.S. (1976). Prostaglandin F secretion during early pregnancy in sheep. J. Reprod. Fert. 46, 533 Abstr.

Colcord, M. L., Hoyer, G. L. and Weems, C. W. (1978). Effect of prostaglandin E_2 (PGE_2) as an antiluteolysin in estrogen-induced luteolysis in ewes. J. An. Sci. 46 (Suppl. 1), 352, Abstr. 344.

Denamur, R. and Martinet, J. (1955). Effets de l'ovariectomie chez la brebis pendant la gestation. C.R. Seanc. Soc. Biol. 149, 2105–2107.

Dickmann, Z. and Spilman, C. H. (1975). Prostaglandins in rabbit blastocysts. Science 190, 997–998.

Dunham, E. W., Haddox, M. K. and Goldbert, N. D. (1974). Alterations of vein cyclic 3′,5′-nucleotide concentrations during changes in contractility. Proc. Soc. Nat. Acad. Sci. USA 71, 815–819.

Ellinwood, W. E., Nett, T. M. and Niswender, G. D. (1979). Maintenance of the corpus luteum of early pregnancy in the ewe. I. Luteotropic properties of embryonic homogenates. Biol. Reprod. 21, 281–288.

Goding, J. R. (1974). The demonstration that $PGF_2\alpha$ is the uterine luteolysin in the ewe. J. Reprod. Fert. 38, 261–271.

Hastings, II, R. A. and Enders, A. C. (1974). Uptake of exogenous protein by the preimplantation rabbit blastocyst. Anat. Rec. 179, 311–330.

Henderson, K. M., Scaramuzzi, R. J. and Baird, D. T. (1977). Simultaneous infusion of prostaglandin E antagonizes the luteolytic action of prostaglandin $F_2\alpha$ *in vivo*. J. Endocrinol. 73, 379–383.

Hixon, J. E. and Hansel, W. (1974). Evidence for preferential transfer of prostaglandin $F_2\alpha$ to the ovarian artery following intrauterine administration in cattle. Biol. Reprod. 11, 543–552.

Hixon, J. E. and Hansel, W. (1977). Luteotropic effects of prostaglandin F_2 with bovine luteal cell preparations. Proc. 69th Annual Meeting Am. Soc. An. Sci., 427 Abstr.

Horton, E. W. and Poyser, N. L. (1977). Uterine luteolytic hormone: A physiological role for prostaglandin $F_2\alpha$. Physiol. Rev. 56, 595–651.

Hwang, D. H., Mathias, M. M., Dupont, J. and Myer, D. L. (1975). Linoleate enrichment of diet and prostaglandin metabolism in rats. J. Nutr. 105, 995–1002.

Inskeep, E. K., Smutny, W. J., Butcher, R. L. and Pexton, J. E. (1975). Effects of intrafollicular injections of prostaglandins in non-pregnant and pregnant ewes. J. An. Sci. 41, 1098–1104.

Kiefer, H. C., Johnson, C. R. and Arora, K. L. (1975). Colorimetric identification of prostaglandins in subnanomole amounts. Anal. Biochem. 68, 336–340.

Kingston, W. P. and Greaves, M. W. (1976). Factors affecting prostaglandin synthesis by rat skin microsomes. Prostaglandins 12, 51–59.

Kirton, K. T., Cornette, J. C. and Barr, K. C. (1972). Characterization of antibody to prostaglandin $F_2\alpha$. Biochem. Biophys. Res. Commun. 47, 903–909.

Kuehl, Jr., F. A., Humes, J. L., Cirillo, V. J. and Ham, E. M. (1972). Cyclic AMP and prostaglandins in hormone action. In: Advances in Cyclic Nucleotide Research. Vol. 1. (P. Greengard, G. A. Robison and R. Paoletti, eds.). Raven Press, New York. pp. 493–502.

Land, R. B., Baird, D. T. and Scaramuzzi, R. J. (1976). Dynamic studies of prostaglandin $F_2\alpha$ in the uteroovarian circulation of the sheep. J. Reprod. Fert. 47, 209–214.

Lewis, G. S., Jenkins, P. E., Fogwell, R. L. and Inskeep, E. K. (1978). Concentrations of prostaglandins E_2 and $F_2\alpha$ and their relationship to luteal function in early pregnant ewes. J. An. Sci. 47, 1314–1323.

Lewis, G. S., Wilson, Jr., 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. An. Sci. 45, 320–327.

Lowry, O. H., Rosenbrough, N. J., Fair, A. L. and Randall, R. J. (1951). Protein measurement with the folin phenol reagent. J. Biol. Chem. 193, 265–275.

Maeyama, M., Saita, B., Ichihara, K., Munemura, M. and Mori, N. (1976). Effect of prostaglandin E_2 on adenosine-3′,5′-monophosphate accumulation and progesterone synthesis in human corpora lutea *in vitro*. J. Steroid Biochem. 7, 295–300.

Magness, R. R., Huie, J. M. and Weems, C. W. (1978). Effect of contralateral and ipsilateral intrauterine infusion of prostaglandin E_2. J. An. Sci. 46 (Suppl. 1), 376, Abstr. 401.

Mapletoft, R. J., Del Campo, M. R. and Ginther, O. J. (1975). Unilateral luteotropic effect of uterine venous effluent of gravid uterine horn in sheep. Proc. Soc. Exp. Biol. Med. 150, 129–133.

Mapletoft, R. J., Lapin, D. R. and Ginther, O. J. (1976). The ovarian artery as the final component of the local luteotropic pathway between a gravid uterine horn and ovary in ewes. Biol. Reprod. 15, 414–421.

Marsh, J. (1971). The effect of prostaglandins on the adenyl cyclase of the ovine corpus luteum. In: Prostaglandins. (P. Rammwell and J. E. Show, eds.). Ann. N.Y. Acad. Sci. 180, 416–425.

McCracken, J. A., Carlson, J. C., Glew, M. E., Goding, J. R., Baird, D. T., Green, K. and Samuelsson, B. (1972). Prostaglandin $F_2\alpha$ identified as a luteolytic hormone in sheep. Nature New Biol. 238, 129–134.

Moor, R. M. and Rowson, L.E.A. (1966a). The corpus luteum of the sheep: Effect of the removal of embryos on luteal function. J. Endocrinol. 34, 497–502.

Moor, R. M. and Rowson, L.E.A. (1966b). The corpus luteum of the sheep: Functional relationship between the embryo and the corpus luteum. J. Endocrinol. 34, 233–239.

Moor, R. M., Hay, M. F., Short, R. V. and Rowson, L.E.A. (1970). The corpus luteum of the sheep:

Effect of uterine removal during luteal regression. J. Reprod. Fert. 21, 319–326.

Nett, T. M., Staigmiller, R. B., Akbar, A. M., Diekman, M. A., Ellinwood, W. E. and Niswender, G. D. (1976). Secretion of prostaglandin $F_2\alpha$ in pregnant and cycling ewes. J. An. Sci. 42, 876–880.

Niswender, G. D. (1973). Influence of the site of conjugation on the specificity of antibodies to progesterone. Steroids 22, 413–424.

Niswender, G. D. and Midgley, Jr., A. R. (1970). Hapten-radioimmunoassay for steroid hormones. In: Immunologic Methods in Steroid Determination. (F. G. Caldwell and B. V. Caldwell, eds.). Appleton-Century-Crofts, New York. p. 149.

Niswender, G. D., Reichert, Jr., L. E., Midgley, Jr., A. R. and Nalbandov, A. V. (1969). Radioimmunoassay for bovine and ovine luteinizing hormone. Endocrinology 84, 1166–1173.

Pexton, J. E., Weems, C. W. and Inskeep, E. K. (1975a). Prostaglandin F in uterine venous plasma, ovarian arterial and venous plasma and in ovarian and luteal tissue of pregnant and non-pregnant ewes. J. An. Sci. 41, 154–159.

Pexton, J. E., Weems, C. W. and Inskeep, E. K. (1975b). Prostaglandins F in uterine and ovarian venous plasma form non-pregnant and pregnant ewes collected by cannulation. Prostaglandins 9, 501–509.

Pratt, B. R., Butcher, R. L. and Inskeep, E. K. (1977). Antiluteolytic effect of the conceptus and PGE_2 in ewes. J. An. Sci. 46, 784–791.

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

Roberts, G. P., Parker, J. M. and Symons, H. W. (1976). Macromolecular components of genital tract fluids from the sheep. J. Reprod. Fert. 48, 99–107.

Shemesh, M., Ailenberg, M., Milaguir, F., Ayalon, N. and Hansel, W. (1978). Hormone secretion by cultured bovine pre- and postimplantation gonads. Biol. Reprod. 19, 761–767.

Speroff, L. and Ramwell, P. W. (1970). Prostaglandin stimulation of *in vitro* progesterone synthesis. J. Clin. Endocrinol. Metab. 30, 345–350.

Steel, R.G.D. and Torrie, J. H. (1960). Principles and Procedures of Statistics. McGraw Hill Book Co., New York.

Stylos, W., Burstein, S., Rivetz, B., Gunsalus, P. and Skarnes, R. C. (1972). The production of anti-G prostaglandin serum and its use in radioimmunoassay. Intra-Sci. Chem. Rep. 6, 71.

Thorburn, G. D., Cox, R. I., Currie, W. B., Restall, B. J. and Schneider, W. (1973). Prostaglandin F and progesterone concentrations in the uteroovarian venous plasma of the ewe during the estrous cycle and early pregnancy. J. Reprod. Fert. Suppl. 18, 151–158.

Vane, J.R. (1971). Inhibition of prostaglandin synthesis as a mechanism of action of aspirin-like drugs. Nature New Biol. 231, 232–235.

Willis, A. L. (1970). Simplified thin-layer chromatography of prostaglandins in biological extracts. Proc. Brit. Pharm. Soc., 583p–584p.

Wilson, Jr., L., Butcher, R. L. and Inskeep, E. K. (1972). Prostaglandin $F_2\alpha$ in the uterus of ewes during early pregnancy. Prostaglandins 1, 479–482.

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