Arterial–Venous Differences in Gonadotropin Concentration Across the Ovary of Sheep During Different Reproductive States[1] (37535)

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(Introduced by L. C. Faulkner)

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The importance of pituitary hormones for maintenance of normal function of the corpus luteum in sheep has been well established. Continuous infusions of luteinizing hormone (LH), but not prolactin, extended the life span of the corpus luteum in cycling or pregnant ewes which were hypophysectomized (1) or in intact cycling ewes (2). In contrast, Denamur and Mauleon (3) reported that injections of prolactin, but not LH, maintained the corpus luteum in hypophysectomized, hysterectomized ewes. More recently, results obtained by Shroff *et al.* (4) strongly suggested that both LH and prolactin are necessary for normal function of the corpus luteum in cyclic or pregnant ewes which were hypophysectomized. However, the mechanisms by which these gonadotropic hormones influence luteal function remain unexplained.

Serum concentrations of LH and prolactin are not elevated during the luteal phase of the cycle or during early pregnancy (5, 6). However, it is possible that there is an increased "utilization" of gonadotropic hormones by the corpus luteum during these periods. In fact, levels of LH in the effluent from the ovary bearing the corpus luteum were significantly lower than peripheral venous levels during the luteal phase of the cycle in women (7, 8). However, Scaramuzzi, Caldwell and Moor (9) found no differences between peripheral and ovarian venous concentrations of LH in cyclic sheep when samples were obtained from anesthetized ewes. The present study was conducted to determine if the "utilization" of gonadotropins by the ovary of the unanesthetized ewe varies during different functional states of the corpus luteum. To assess "utilization," arterial–venous differences in the concentration of gonadotropins across the ovaries were determined at frequent intervals in chronically cannulated ewes.

Materials and Methods. Mature ewes of mixed breeding were checked for estrus twice daily using vasectomized, raddled rams. The day following estrus was assigned as Day 1 of the cycle. A total of seven ewes were used in three treatment groups. Ewes on Day 7 of the estrous cycle (two ewes) and Day 14 of pregnancy (two ewes) were studied because the corpus luteum is active during these periods. Ewes on Day 14 of the cycle (three ewes) were studied because regression of the corpus luteum usually begins about this time.

Prior to surgery, water was withheld for 24 hr and feed was withheld for 48 hr. All ewes received 0.01 mg atropine sulfate/kg body weight and were anesthetized with 20–25 ml sodium pentobarbital (65 mg/ml) administered intravenously. Surgical anesthesia was maintained by a closed-system inhalation procedure using nitrous oxide, halothane, and oxygen. A midventral incision was made and indwelling polyvinyl cannulas (0.034 in. i.d., 0.058 in. o.d.) were placed in both ovarian veins by aseptic surgical technique. Only ewes with a single corpus luteum on one ovary

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were used. Cannulas were inserted through a small incision in the ovarian vein 6–10 cm from the ovary and were held in place with a single, purse-string suture of 5-0 cardiovascular silk. Immediately following placement of the ovarian venous cannulas 20,000 units of sodium heparin were injected. The cannulas were directed through the lateral abdominal wall and tunneled subcutaneously to the dorsal midline. Adjacent to the point where the cannulas passed through the skin they were directed into a closed Plexiglas box for protection. Following closure of the abdominal cavity, polyvinyl cannulas (0.060 in. i.d., 0.078 in. o.d.) were positioned in the abdominal aorta and posterior vena cava by deep insertion via the femoral artery or femoral vein.

Upon recovery from anesthesia, the ewes were stanchioned in individual pens to facilitate collection of blood samples with minimal restraint. Heparin (1000–3000 units) was administered subcutaneously every 6 hr during the period of sample collection, and cannulas were flushed at 30-min intervals with heparinized saline (300 units/ml) containing procaine penicillin G (3000 units/ml).

Blood samples (6–10 ml) were collected from the arterial cannulas at 0.5- or 1-hr intervals while venous samples were collected at either 1- or 2-hr intervals for periods ranging from 18 to 52 hr. Blood was allowed to flow from all cannulas by normal vascular pressure. Due to the longer times required to obtained samples from ovarian veins, these collections were begun first and aortic and caval samples were collected midway through the ovarian venous collections. Hematocrit and serum protein determinations were made on all ewes throughout periods of sample collection to monitor the effects of collecting numerous samples.

Plasma concentrations of LH, follicle-stimulating hormone (FSH), and prolactin were determined by radioimmunoassay using double antibody techniques previously described (10, 11, 6). NIH-LH-S12, NIH-FSH-S4 and NIH-P-S8 were used as standards in the respective radioimmunoassays. To insure accurate measurement of the low concentrations of LH the sensitivity of the radioimmunoassay was increased by dilution of the anti-ovine LH serum (GDN-15) to 1:160,000 and by increasing the incubation time that the standard or unknown samples reacted with antibody from 24 to 48 hr. The incubation time following the addition of ovine LH-^{131}I was also increased from 24 to 48 hr. With these modifications the sensitivity, defined as that amount of hormone required to give a mean amount of activity which did not overlap with the mean activity less one standard deviation of the buffer control tubes (12), of the assay was increased approximately tenfold so that it was possible to detect 8–10 pg of LH/assay tube or 40–50 pg of LH/ml of plasma. The limit of sensitivity of the FSH and prolactin radioimmunoassays was 4.26 and 0.58 ng/assay tube, respectively. All estimates of FSH and prolactin were made at points between 20 and 80% on the standard inhibition curve. Plasma samples were assayed in duplicate, using 200 μl/assay tube for LH and FSH and at 5–50 μl/assay tube for prolactin. Due to the marked fluctuation in levels of LH, samples were assayed in duplicate in two separate assays to confirm the observed changes.

Progesterone concentrations were determined by radioimmunoassay (12). In brief, this method involved extraction of 1.0 ml of plasma with petroleum ether and purification of the extract by single dimension thin-layer chromatography on silica gel impregnated glass fiber sheets (Gelman Instrument Co., Ann Arbor, MI) using cyclohexane:ethyl acetate (9:1). Progesterone was quantitated using an antiserum to progesterone-11-bovine serum albumin and progesterone-11-tyrosine methyl ester ^{131}I as the radioactive form of the steroid in a double antibody radioimmunoassay (10). Progesterone-1,2-^{3}H was added to each plasma sample to allow the values to be corrected for procedural losses.

Comparisons of LH, FSH and prolactin concentrations in samples taken simultaneously from the four cannulas were made to determine arterial–venous (A–V) differences across the ovaries and in the systemic circulation. If the concentration of the hormone in either sample was below the sensitivity of the assay both values were discarded. The differences between the arterial and each

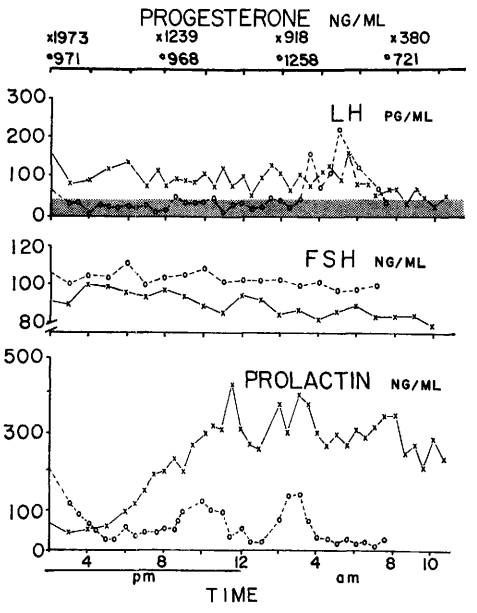

FIG. 1. Plasma concentrations of gonadotropic hormones in 2 ewes beginning on Day 7 of the estrous cycle. Ovarian venous progesterone concentrations from the ovaries containing the corpus luteum were determined at less frequent intervals. The shaded portion of the LH graph indicates those concentrations below the limit of sensitivity of the assay. (O - -) Ewe 178-3; (X —) Ewe 178-4.

venous level of each gonadotropin at each collection was statistically analyzed using a "paired *t*" test for each animal. When no differences were found in data from individual animals the data from all animals in each group were pooled and analyzed in the same manner.

Results. The levels of progesterone in selected samples collected from the ovarian vein on the side of the corpus luteum are shown in the upper portions of Figs. 1, 2, and 3. Concentrations of progesterone remained high throughout the sampling period in all four ewes in which sampling was begun on either Day 7 of the cycle (Fig. 1) or Day 14 of pregnancy (Fig. 2). The concentration of progesterone fell to below 1 ng/ml during the sampling period in two of three ewes in which sampling was initiated on Day 14 of the cycle (Fig. 3) while in the remaining ewe progesterone levels remained high throughout the collection period.

The within assay error, expressed as the coefficient of variation for the eight different radioimmunoassays in which LH was determined varied from 4.8 to 8.2% based on an average of 227 replicates/assay. The data regarding differences in LH concentrations between arterial and venous samples are presented in Table I. There was considerable variation in the levels of LH although they tended to be higher in venous than in arterial samples on Day 7 of the cycle, while on Day 14 of pregnancy the venous concentrations of LH tended to be lower than those in the artery. The only statistically significant A–V difference was the increased ($p < .05$) level of LH noted in the vein leading from the ovary which did not contain the corpus luteum on Day 7 of the cycle.

The within assay error in the four radioimmunoassays for FSH varied from 5.1 to 7.8%. Data summarizing the differences between arterial and venous concentrations of

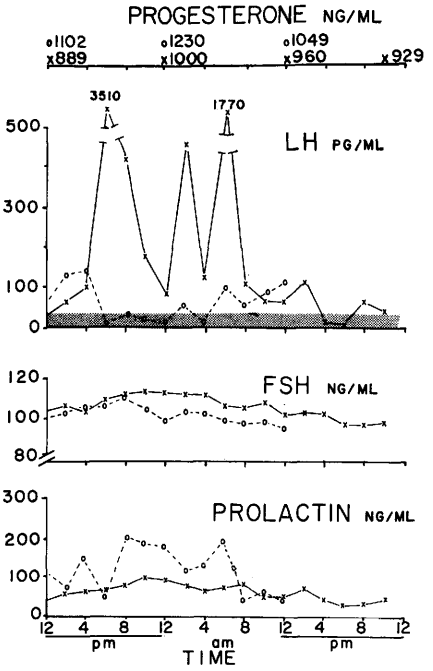

FIG. 2. Plasma concentrations of gonadotropic hormones in 2 ewes beginning on Day 14 of pregnancy. Ovarian venous progesterone concentrations from the ovaries containing the corpus luteum were determined at less frequent intervals. The shaded portion of the LH graph indicates those concentrations below the limit of sensitivity of the assay. (O - -) Ewe 178-8; (X —) Ewe 178-9.

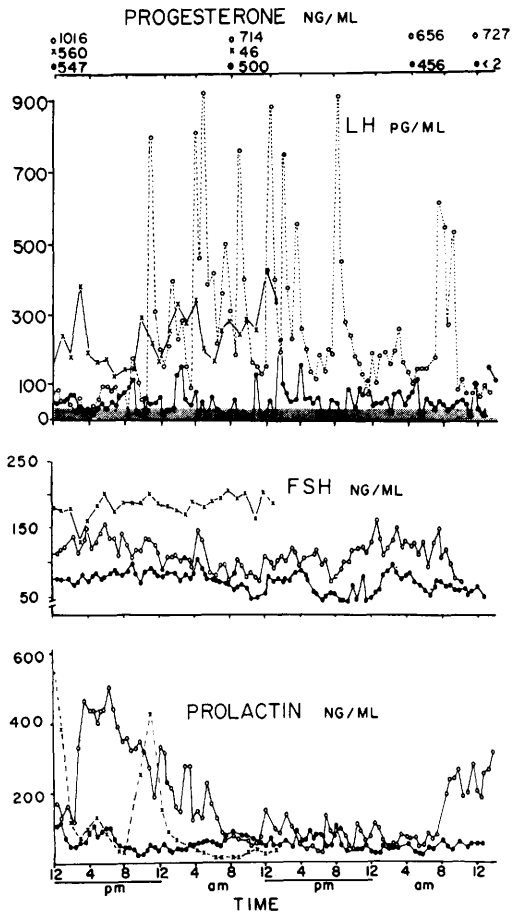

FIG. 3. Plasma concentrations of gonadotropic hormones on 3 ewes beginning on Day 14 of the estrous cycle. Ovarian venous progesterone concentrations from the ovaries containing the corpus luteum were determined at less frequent intervals. The shaded portion of the LH graph indicates those concentrations below the limit of sensitivity of the assay. (O - -) Ewe 178-7; (× —) Ewe 178-6; (0—) Ewe 178-5.

FSH are shown in Table II. There were no significant A–V differences in FSH concentrations for the sites sampled in any of the groups studied. The within assay error in the prolactin radioimmunoassays varied from 5.9 to 6.4%. Data summarizing the difference between arterial and venous levels of prolactin are shown in Table III. The only significant A–V difference noted was the comparison between the arterial levels and levels in the vein leading from the ovary without the corpus luteum in ewes on Day 14 of the estrous cycle.

TABLE I. Comparison of Arterial and Venous Concentrations of Luteinizing Hormone in Ewes.[a]

Comparison	No. of pairs	% Difference (mean ± SE)
Day 7 of the estrous cycle		
DA to CL	26	+17.0 ± 10.2
DA to NOCL	10	+43.0 ± 6.4[b]
DA to PVC	31	+14.1 ± 11.0
Day 14 of the estrous cycle		
DA to CL	51	+1.2 ± 3.8
DA to NOCL	33	−4.6 ± 9.5
DA to PVC	49	+1.2 ± 0.4
Day 14 of pregnancy		
DA to CL	8	−23.8 ± 14.8
DA to PVC	11	−12.5 ± 8.8

[a] DA to CL: paired dorsal aorta concn compared to venous concn from the ovary bearing the corpus luteum. DA to NOCL: paired dorsal aorta concn compared to venous concn from the ovary *not* bearing the corpus luteum. DA to PVC: paired dorsal aorta concn compared to posterior vena cava concn.

[b] Significant at the $p < 0.05$ level.

The concentrations of LH, FSH, and prolactin in arterial samples collected at frequent intervals from ewes beginning on Day 7 of the cycle are presented in Fig. 1. There was considerable variation in the concentrations of LH and prolactin, while a more stable pattern was noted for FSH. Similar data are illustrated in Fig. 2 for ewes beginning on Day 14 of the cycle. Again both LH and prolactin levels were extremely variable. Considerable fluctuation in the levels of FSH was also noted in these ewes, although in no case was there more than a twofold difference throughout the sampling period. The mean serum levels of gonadotropins with their 95% confidence limits for ewe 178-7 from 7 AM to 12:30 PM on Day 15 of the cycle are shown in Table IV. This time interval includes three apparent releases of LH; in most cases the confidence limits associated with sequential samples do not overlap. The arterial levels of LH, FSH and prolactin in ewes beginning on Day 14 of pregnancy are shown in Fig. 3. The levels of LH in both ewes and prolactin in one ewe tended to fluctuate markedly, while levels of prolactin in the other ewe and FSH in both ewes were relatively stable.

TABLE II. Comparison of Arterial and Venous Concentrations of Follicle Stimulating Hormone in Ewes.[a]

Comparison	No. of pairs	% Difference (mean ± SE)
Day 7 of the estrous cycle		
DA to CL	33	+0.9 ± 1.5
DA to NOCL	18	+0.7 ± 2.9
DA to PVC	36	+0.3 ± 1.4
Day 14 of the estrous cycle		
DA to CL	104	+3.2 ± 1.5
DA to NOCL	66	+1.1 ± 1.5
DA to PVC	103	+3.4 ± 1.2
Day 14 of pregnancy		
DA to CL	31	+0.7 ± 0.8
DA to PVC	31	+0.5 ± 0.7

[a] DA to CL: paired dorsal aorta concn compared to venous concn from the ovary bearing the corpus luteum. DA to NOCL: paired dorsal aorta concn compared to venous concn from the ovary *not* bearing the corpus luteum. DA to PVC: paired dorsal aorta concn compared to posterior vena cava concn.

Discussion. Llerena *et al.* (8) and Naftolin *et al.* (7) have previously reported that ovarian venous levels of LH from the ovary with the corpus luteum were significantly lower than peripheral levels in women during the midluteal phase of the cycle. Both LH and prolactin play a role in maintenance of the corpus luteum in the sheep (4). This experiment was designed to determine if A–V differences across the ovary containing the corpus luteum could be demonstrated and if so whether this A–V difference changed with varying functional states of this gland.

The data regarding concentrations of progesterone in ovarian venous blood clearly indicate that the corpus luteum was functional throughout the sampling period in ewes in which sampling was begun on Day 7 of the cycle or Day 14 of pregnancy. Initially, the corpus luteum was also actively secreting progesterone in the three ewes in which sampling was begun on Day 14 of the cycle, however, in two of the ewes the luteal tissue was apparently nonfunctional by the end of the sampling period since progesterone concentrations declined to less than 2 ng/ml.

No data were obtained which could be interpreted as "utilization" of LH or prolactin by the corpus luteum or any other ovarian structure. Likewise, there was no evidence of ovarian "utilization" of FSH even in ewes sampled from Days 14 to 17 of the cycle when follicular growth is active. The only significant A–V difference in LH was across the ovary without the corpus luteum on Day 7 of the cycle and this difference represented an increase (+43%) in the ovarian vein above arterial levels and was not significantly different from the increase (+22%) in the posterior vena cava. The only significant A–V difference in prolactin concentrations was a decreased level (−12%) across the ovary without the corpus luteum for ewes beginning Day 14 of the cycle.

Several factors limit the interpretations of the data obtained in this study or in similar studies conducted by others (7, 8). The present experiment could not have been performed without sensitive radioimmunoassay procedures, however this technique may have limited usefulness for this type of study. Since antibodies to gonadotropic hormones recognize only limited portions of the molecule, and not the entire molecule it is possible that the hormone could be modified in some

TABLE III. Comparison of Arterial and Venous Concentrations of Prolactin in Ewes.[a]

Comparison	No. of pairs	% Difference (mean ± SE)
Day 7 of the estrous cycle		
DA to CL	33	−0.4 ± 3.1
DA to NOCL	17	−1.6 ± 2.9
DA to PVC	35	−6.9 ± 4.1
Day 14 of the estrous cycle		
DA to CL	102	−6.3 ± 4.0
DA to NOCL	63	−11.9 ± 5.5[b]
DA to PVC	99	−3.3 ± 2.8
Day 14 of pregnancy		
DA to CL	31	−3.1 ± 3.9
DA to NOCL	3	−9.0 ± 10.1
DA to PVC	31	−4.0 ± 2.8

[a] DA to CL: paired dorsal aorta concn compared to venous concn from the ovary bearing the corpus luteum. DA to NOCL: paired dorsal aorta concn compared to venous concn from the ovary *not* bearing the corpus luteum. DA to PVC: paired dorsal aorta concn compared to posterior vena cava concn.

[b] Significant at the $p < 0.05$ level.

TABLE IV. Serum Levels (Mean and 95% Confidence Limit) of LH, FSH, and Prolactin in Ewe 178-7 Beginning at 7 AM on Day 15 of the Cycle.

	LH		FSH (ng/ml)		Prolactin (ng/ml)	
Time sample	Mean level	95% Confidence limit	Mean level	95% Confidence limit	Mean level	95% Confidence limit
7:00	353	296–422	83	74– 95	86	70–106
7:30	504	440–578	95	83–108	66	54– 81
8:00	311	255–380	94	83–107	81	67–100
8:30	179	129–249	79	70– 90	59	49– 73
9:00	766	690–852	87	78–100	61	51– 75
9:30	401	342–471	106	93–121	42	35– 51
10:00	286	232–354	96	84–111	45	37– 55
10:30	167	117–240	80	72– 92	72	60– 89
11:00	155	106–228	86	76– 98	151	121–190
11:30	127	80–205	83	74–205		
12:00	156	107–229	79	70– 91	96	79–119
12:30	878	795–969	109	95–126	78	64– 96

way by the ovary, but that the antibody did not detect the change. In fact, it seems possible that if the ovary degrades the gonadotropin molecule in such a way as to produce pieces, the radioimmunoassay may actually recognize each piece as an intact molecule. This might explain the significantly elevated levels of LH found in the ovarian vein in ewes on Day 7 of of cycle. There has been excellent agreement between bioassay and radioimmunoassay estimates of the LH, FSH and prolactin contents of ovine pituitary preparations, except in cases where the preparations have been subjected to various oxidative or enzymatic treatments; however, agreement between the two assay procedures has not been shown for serum, primarily because of the insensitivity of the bioassay procedures.

The sequential samples collected during this study yielded information which previous studies did not provide. The concentrations of gonadotropins changed rapidly. This was particularly true for LH on Days 14 and 15 of the cycle when LH changed as much as 3%/min. Similar observations have been made in women (13) particularly during the luteal phase of the cycle. Therefore, paired samples must be collected over exactly the same time intervals if accurate comparisons are to be made. However, the flow rates of blood from cannulas in vessels of different sizes varies markedly. To overcome this problem samples from the dorsal aorta and posterior vena cava were collected in a 20–30-sec interval exactly midway through the ovarian vein collection which required 3–7 min.

Although many of the periodic elevations in LH levels were 2 to 10 times greater than levels in preceding or subsequent samples, these elevations were very low when compared to peak levels of LH during estrus (80–200 ng/ml). Since sampling was performed at 30-min or 1-hr intervals it was not possible to determine if the fluctuations were time related or concentration dependent due to the rapid disappearance of LH from blood. The increased concentrations of LH are probably the result of limited periods of release of the hormone followed by periods of little or no release based on the rate of disappearance of the hormone.

For long-term collection of ovarian venous samples it is necessary that the cannula does not alter blood flow to the organ. In our studies the small polyvinyl cannulae used occupied less than 25% of the lumen of the ovarian veins and no evidence of venous stasis was observed at the time of cannula placement or upon examination of the cannulation site at the termination of the experiment.

Previous studies in rats have indicated that anesthetics can influence gonadotropin concentrations and/or events associated with the reproductive cycle (14). Naftolin *et al.* (7) tested the effect of anesthesia on LH con-

centration in a group of men who were sampled the day prior to, the day of, and the day following anesthesia. They concluded that the LH concentration did not vary greatly between samples collected in anesthetized or unanesthetized subjects. However, the study did not deal with acute effects of anesthesia. In the present study all but the initial set of samples were collected in unanesthetized animals. Comparison of gonadotropin concentrations in anesthetized ewes with those determined during the 15–48-hr period following recovery from anesthesia did not suggest any obvious effect of anesthesia. The values for prolactin in this study were 2–20 times higher than those previously reported in sheep during similar reproductive states (6). Although the exact reasons for these elevated prolactin levels cannot be ascertained from this study it seems likely that the prolactin levels were being influenced by the treatment of the ewes. The ewes were restrained in small pens, they were bled at frequent intervals and the lights were turned on and off at frequent intervals during the night time hours.

Summary. Concentrations of gonadotropins in arterial, systemic venous, and ovarian venous plasma were determined by radioimmunoassay in order to assess the role of "utilization" of these hormones in the regulation of ovarian function. Samples were collected at frequent intervals (0.5–2 hr) for periods ranging from 18 to 52 hr from ewes beginning on Days 7 and 14 of the cycle and Day 14 of pregnancy. The functional state of the corpus luteum was assessed by concentrations of progesterone in ovarian venous blood and by histology. There was no evidence that the corpus luteum "utilizes" any gonadotropin during periods of active luteal function nor was there evidence of "utilization" of any gonadotropin during periods of active follicular growth. The data did suggest that serum levels of LH and possibly prolactin and FSH are regulated by periodic episodic releases of these hormones. Episodic "bursts" of LH were particularly apparent in ewes in which sampling was begun on Day 14 of the cycle.

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