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Blood Flow: A Mediator of Ovarian Function[1]

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Historically, hormones from the adenohypophysis have been shown to be important in regulating the corpus luteum of mammals. However, complete understanding of the interactions between the adenohypophyseal hormones and other endocrinological and physiological factors which control luteal function has remained elusive. The relationships between the gonadotropic hormones and the physiological activity of the ovaries have been the subject of several recent reviews (Ross et al., 1970; Vande Wiele et al., 1970; Niswender et al., 1972; Hansel et al., 1973; Midgley et al., 1973; and Nalbandov, 1973). In all of these reviews LH has been implicated as the endocrine factor primarily responsible for the control of luteal function. Yet, when endogenous levels of LH and progesterone are quantified throughout the reproductive cycle in women (Midgley and Jaffe, 1966; Ross et al., 1967), monkeys (Neill et al., 1967; Niswender and Spies, 1973), rats (Gay et al., 1970), sheep (Niswender et al., 1968; Niswender, 1974), pigs (Niswender et al., 1970; Henricks et al., 1972; Wilfinger et al., 1973) or cattle (Henricks et al., 1970; Swanson and Hafs, 1970; Hansel and Echternkamp, 1972) levels of LH are at their nadir when secretion of progesterone is maximal. These findings are difficult to interpret if LH is the hormone responsible for regulation of luteal function.

The situation is even more confusing when one considers that LH stimulates the synthesis of progesterone when incubated with slices of human (Savard et al., 1965; Marsh and LeMaire, 1974), bovine (Savard et al., 1965; Marsh et al., 1966; Armstrong and Black, 1966), ovine (Kaltenbach et al., 1967) or porcine (Cook et al., 1967) luteal tissue *in vitro*. Additionally, LH has been shown to promote luteinization of monkey (Channing, 1970a), human (Channing, 1969) and porcine (Channing, 1970b) granulosa cells in culture and appears necessary for maintenance of luteal cells in a functional and differentiated state (Gospodarowicz and Gospodarowicz, 1975).

Several hypotheses could explain the low levels of LH in serum during the period of maximal secretion of progesterone by the corpus luteum. 1) Progesterone could be exerting an inhibitory feedback on secretion of LH. However, this does not appear to be the case in monkeys (Karsch et al., 1973; Resko et al., 1974) or sheep (Pelletier and Signoret, 1969; Scaramuzzi et al., 1971; Diekman and Malven, 1973). 2) The ovary could be "utilizing" LH for maximal secretion of progesterone resulting in low circulating concentrations of LH in serum. Although evidence obtained in women supported this hypothesis (Naftolin et al., 1968; Llerena et al., 1969) data from more rigidly designed experiments in sheep have failed to demonstrate arterial-venous differences in levels of LH across the ovary (Scaramuzzi et al., 1970; Cicmanec and Niswender, 1973). Metabolic clearance rates of LH were constant at different stages of the reproductive cycle in women (Kohler et al., 1968) and sheep (Abkar et al., 1974) which also argues against ovarian utilization of large quantities of LH during the luteal phase of the cycle. 3) A dramatic increase in the number of receptors for LH on the luteal cell during the development of the corpus luteum could allow small quantities of LH to be effective for the stimulation of steroidogenesis. Evidence is available to support this hypothesis (Channing and Kammerman, 1973; Zeleznik et al., 1974) and this phenomenon is probably of major importance in the regulation of luteal function. 4) A final hypothesis is that blood flow to the ovary increases during the luteal phase of the estrous

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cycle. Even though the concentrations of LH in serum remains low, the total quantity of tropic hormones reaching the corpus luteum would be elevated due to increased flow of blood to this organ. It has been well documented that blood flow is important in regulation of steroid secretion from the adrenal cortex (Porter and Klaiber, 1965; Urquhart, 1965; L'Age et al., 1970) and the testis (Eik-Nes, 1964). Therefore, it seems likely that flow of blood to the ovary may be important in regulating the function of the corpus luteum. The quantification and physiological regulation of ovarian blood flow is the subject of the remainder of this communication.

Flow of Blood to the Ovaries During the Estrous Cycle

The sheep was used for these studies because of the ease with which vascular surgery can be performed, the large blood volume which allows frequent sampling and the wealth of information regarding regulation of luteal function in this species. Western range ewes of mixed breeding (4 to 8 years of age) weighing between 40 and 70 kg were checked for estrus twice daily using vasectomized rams. The first day a ewe stood to be mounted by a ram was designated as Day 0 of the cycle. Only ewes which had exhibited at least two consecutive, normal estrous cycles (14–18 days) were used in the following experiments.

Doppler ultrasonic blood flow transducers were used to measure the velocity of the blood flowing in the ovarian artery throughout the estrous cycle (Niswender et al., 1975). Blood flow transducers were implanted around the ovarian arteries in four ewes on Day three or four of the cycle. In three ewes one to three corpora lutea were present on one ovary (luteal ovary) with no corpora lutea on the opposite ovary (non-luteal ovary). Two arteries supplied the left ovary of the fourth ewe so this ovary was removed and the blood flow transducer was implanted on the artery leading to the right ovary which contained a corpus luteum. Data were collected beginning on Day seven or nine of the cycle. Blood flow recordings and blood samples from indwelling jugular cannulas were obtained at 6-h intervals for three days, at 4-h intervals for two days, at 2-h intervals for six days and again at 6-h intervals for three days. This schedule was designed so that samples were obtained most frequently around estrus. All samples were quantified for LH (Niswender et al., 1969) FSH (L'Hermite et al., 1972), prolactin (Davis et al., 1971) and progesterone (Niswender, 1973). At each sampling period the output from the Doppler velocity meter was recorded on magnetic tape for 2.5 min from each transducer. The frequency data for each 2.5 min period were averaged using a Beckman Universal Eput Meter and Timer.

At the termination of the experiment, the diameter of the ovarian artery was determined as described previously (Niswender et al., 1975). The average diameter of the arteries which supplied the luteal ovary was 0.52 ± 0.13

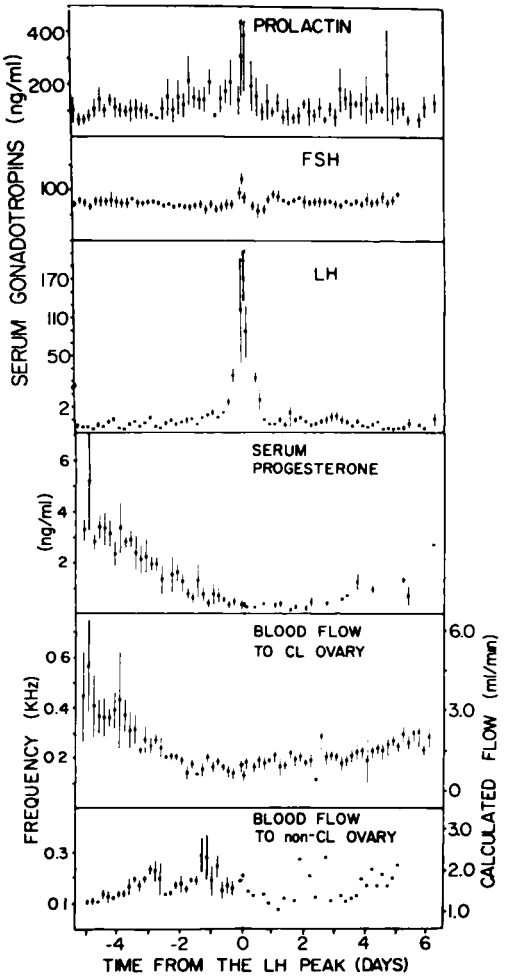

FIG. 1. Concentrations of gonadotropic hormones and progesterone in serum and blood flow to the ovaries throughout the estrous cycle in ewes. All data are normalized to the preovulatory peak of LH. Each point represents the mean ± one standard error (n = 4).

TABLE 1. Multiple correlations between serum levels of reproductive hormones and blood flow to the ovary bearing the corpus luteum.

Parameter	FSH	Prolactin	Progesterone	Ovarian blood flow[a]
LH	0.2772* (388)[b]	0.2366* (390)	-0.1124 (199)	-0.1363 (386)
FSH		0.4316** (389)	0.0827 (199)	0.1053 (384)
Prolactin			-0.0545 (200)	-0.1337 (396)
Progesterone				0.2543* (197)

*P<0.05.

**P<0.01.

[a]Only the data obtained during the 5-day period preceeding the preovulatory peak of LH were used to determine the correlation between progesterone and blood flow to the luteal ovary (see Fig. 1).

[b]Numbers in parentheses = degrees of freedom.

mm (mean ± S.E.). The average diameter of the arteries which supplied the nonluteal ovary was 0.38 ± 0.13 mm. Blood flow was calculated as velocity times cross-sectional area of the artery.

Levels of reproductive hormones in serum and the flow of blood to each ovary throughout an estrous cycle are depicted in Fig. 1. Peak levels of all three gonadotropic hormones occurred at estrus, and levels of LH and FSH ($P<0.05$), LH and prolactin ($P<0.05$) and FSH and prolactin ($P<0.01$) were correlated (Table 1). Levels of progesterone in serum and blood flow to the ovaries with corpora lutea were also correlated ($P<0.05$). In fact, most of the outlying values for progesterone were associated with outlying values for blood flow to luteal ovaries.

In subsequent studies, we have confirmed the data regarding decreased flow of blood to the luteal ovary during the late luteal phase of the estrous cycle (Niswender et al., 1975). In four additional ewes mean blood flow was 4.16, 3.96 and 2.71 ml/min on Days 12, 14 and 16 of the cycle, respectively. These data are quite similar to those obtained 1, 3 and 5 days prior to the preovulatory peak of LH in the present study.

In a second series of experiments radioactive microspheres (Wagner et al., 1969; Rosenfeld et al., 1973) were used to estimate the flow of blood to the luteal and extraluteal components of the ovary. Two sizes of microspheres (50 μ or 15 μ in diameter) were infused so that arteriole-venule shunting of blood within the ovarian tissues could also be evaluated. The 50 ± 10 μ microspheres contained either ^{46}Sc (12.7 mCi/g) or ^{51}Cr (73.6 mCi/g) whereas the 15 ± 5 μ microspheres contained either ^{85}Sr (9.6 mCi/g) or ^{141}Ce (7.5 mCi/g). The ewes were administered 20 μCi of ^{46}Sc (24,000 microspheres), 40 μCi of ^{51}Cr (12,000 microspheres), 20 μCi of ^{85}Sr (1,760,000 microspheres) or 20 μCi of ^{141}Ce (1,760,000 microspheres). These doses were calculated to yield approximately equal counting rates. Infusions were performed as outlined in Table 2. Indwelling polyvinyl cannulas were inserted into the dorsal aorta via the right femoral artery in nine ewes. The tip of the cannula was positioned at the level of the diaphragm. Another cannula was placed into the left femoral artery and the tip was positioned at the origin of the iliac artery. The appropriate 50 μ and 15 μ microspheres were premixed and infused via the cannula into the dorsal aorta in 4 ml of 20 percent dextran at 2 ml/min with a Sage Model 375 Constant Flow Pump. Blood was collected from the left iliac artery at a rate of 2 ml/min beginning 30 sec prior to the start of the microsphere infusion and continuing for 5 min. Ten ml of distilled water were added to each sample to lyse the red blood cells, and the tubes were centrifuged at 800 × g for 10 min. The fluid was aspirated and the samples were stored to be counted with the tissue samples from the same ewe. Triplicate samples equal to 1 percent of the dose were taken at the time of infusions to quantify the number of microspheres infused. All ewes were killed on Day 11 of the cycle and the ovaries, samples of gracilis muscle from each side (approximately 2 g), and lung (three samples approximately 1 g each) were removed, weighed and radioactivity was quantified (± 1 percent counting error or for a

TABLE 2. Experimental design for infusions of radioactive microspheres[a] on days 2, 6 and 10 of the estrous cycle.

		Day of cycle					
		2		6		10	
Size of microspheres		50 μ	15 μ	50 μ	15 μ	50 μ	15 μ
Ewe no.	1	^{46}Sc	^{85}Sr	^{51}Cr	^{141}Ce		
	2	^{51}Cr	^{141}Ce	^{46}Sc	^{85}Sr		
	3	^{46}Sc	^{85}Sr	^{51}Cr	^{141}Ce		
	4	^{46}Sc	^{141}Ce			^{51}Cr	^{85}Sr
	5	^{51}Cr	^{85}Sr			^{46}Sc	^{141}Ce
	6	^{51}Cr	^{141}Ce			^{51}Sc	^{85}Sr
	7			^{46}Sc	^{141}Ce	^{51}Cr	^{85}Sr
	8			^{51}Cr	^{85}Sr	^{46}Sc	^{141}Ce
	9			^{46}Sc	^{85}Sr	^{51}Cr	^{141}Ce

[a]Twenty μCi of ^{46}Sc, ^{85}Sr, and ^{141}Ce were infused into the dorsal aorta. Forty μCi of ^{51}Cr were infused.

maximum of 20 min) in a Nuclear Chicago Model 1185 Automatic Gamma Spectrometer. The data were corrected for background and channel overlap. To convert the data to percent uptake, cpm/g wet weight of tissue was divided by 0.01 times cpm infused.

Data regarding the uptake of radioactive microspheres by the ovaries are depicted in Fig. 2. The majority of microspheres entrapped by the ovary were within the corpus luteum with only small numbers of microspheres entrapped by the extraluteal components of the luteal or the non-luteal ovary. There was a significant linear increase ($P<0.01$) in the number of microspheres entrapped by the corpus luteum on Days 2, 6 and 10 of the estrous cycle. There was no difference in the uptake of 50 μ vs. 15 μ microspheres in any of the ovarian tissues. The uptake of microspheres by gracilis muscle was not different on Days 2, 6 and 10 of the estrous cycle, nor was there a difference in uptake between the right and left sides. There were no microspheres detectable in any samples of lung tissue. Using the cpm in the iliac blood sample collected at the time of each infusion and the cpm/g of tissue it was possible to calculate flow of blood in terms of ml/min/g of tissue (Rosenfeld et al., 1973). However, when this calculation was made the estimates were highly variable and were influenced by the size of microspheres infused. Therefore, this calculation did not seem appropriate and all data were expressed as percent uptake of injected dose.

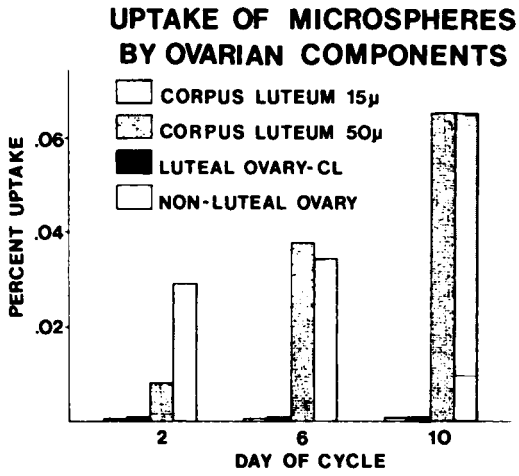

FIG. 2. The uptake of radioactive microspheres by the ovaries and corpora lutea of ewes on Days 2, 6 and 10 of the estrous cycle. The data plotted for the nonluteal ovary and the luteal ovary minus the corpus luteum represents the mean uptake of 50 μ and 15 μ microspheres. There was a significant ($P<0.05$) linear increase in the entrapment of both 50 μ and 15 μ microspheres by the corpus luteum between Days 2 and 10 of the estrous cycle.

In another experiment, 16 ewes were fitted with aortal cannulas. Radioactive microspheres were infused on Days 12, 14 and 16 of the estrous cycle in a manner similar to that described for the previous experiment. However, blood samples from the iliac artery were not collected, and the tip of the aortal cannula was positioned 5 cm cranial to the origin of the ovarian arteries to increase the number of microspheres entering the ovarian circulation.

As depicted in Fig. 3, there was a progressive decrease ($P<0.01$) from Days 12 to 16 in the number of microspheres entrapped by the corpus luteum. In addition, there were significantly fewer ($P<0.05$) 15 μ than 50 μ micro-

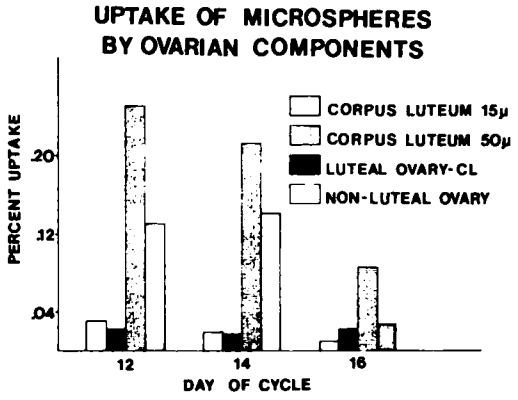

FIG. 3. The uptake of radioactive microspheres by the ovaries and corpora lutea of ewes on Days 12, 14 and 16 of the estrous cycle. The data plotted for the nonluteal ovary and the luteal ovary minus the corpus luteum represents the mean uptake of 50 μ and 15 μ microspheres. There was a significant linear decrease ($P<0.01$) in the uptake of 50 μ microspheres by the corpus luteum between Days 12 and 16 of the estrous cycle. The numbers of 50 μ and 15 μ microspheres entrapped on Day 16 was less ($P<0.05$) than on Days 12 or 14. There were fewer ($P<0.05$) 15 μ than 50 μ microspheres entrapped within the corpus luteum on Day 16.

spheres entrapped by the corpus luteum on Day 16. There were no differences noted among days in the small numbers of microspheres entrapped by the extraluteal component of the luteal ovary or the nonluteal ovary. Likewise, there was no difference in the numbers of 15 μ vs. 50 μ microspheres entrapped in the nonluteal ovarian tissues. The uptake of microspheres in gracilis muscle was not different on Days 12, 14 and 16 of the cycle, nor was there a difference in the uptake of 15 vs. 50 μ microspheres. Likewise, there was no difference in the number of microspheres observed in samples of gracilis muscle obtained from the left and right sides. There were no detectable microspheres in any of the samples of lung.

It was not surprising that blood flow was very low on Day 2 of the estrous cycle since morphological data indicates that the corpus luteum is not vascularized until approximately 96 h after ovulation (McClellan et al., 1975). Invasion of the corpus luteum by capillaries coincided with decreased tissue levels of progesterone and increased serum levels of progesterone. Flow of blood to the ovary containing the corpus luteum appeared to increase three to seven fold during the luteal phase of the estrous cycle and then decreases dramatically as the corpus luteum regresses. This observation, and the fact that greater than 90 percent of the radioactive microspheres in the luteal ovary were entrapped by the corpus luteum (see Fig. 2 and 3; Thorburn and Hales, 1972), indicated that this organ was receiving increased quantities of gonadotropic hormones during the luteal phase of the cycle, even though concentrations of these hormones in the circulation did not increase. If blood flow to the corpus luteum is increased during the luteal phase of the cycle then the quantities of oxygen, glucose, acetate, cholesterol, etc., as well as the quantities of gonadotropic hormones, which reach this organ will also be elevated. An increase in the flow of blood may be an important mechanism whereby the corpus luteum is stimulated to secrete maximal levels of progesterone.

Other investigators have reported that blood flow to the corpus luteum or the luteal ovary decreases during periods of luteal regression in sheep (Mattner et al., 1972), pigs (Rathmacher and Anderson, 1968) and rabbits (Abdul-Karim and Bruce, 1973). The increased flow of blood during the luteal phase of the cycle was specific for the ovary containing the corpus luteum and did not occur in the non-luteal ovary.

In these studies the estimates of blood flow to the luteal ovary (0.3 to 6 ml/min) were somewhat lower than the 10 to 20 ml/min reported by McCracken et al. (1971) but agree with the 6 to 8 ml/min observed by Cook et al. (1969), the 1.5 to 12 ml/min reported by Domanski et al. (1967) and the 2 to 9 ml/min noted by Mattner and Thorburn (1969). Since McCracken et al. (1971) used the ovarian transplant model for their studies and collected blood from the constricted jugular vein, it was possible that a significant amount of blood was contributed by tissues other than the ovaries.

Changes in blood flow to the ovary bearing the corpus luteum could not be explained merely by changes in the weight of the corpus luteum. If blood flow was adjusted for the weight of the corpus luteum at different stages of the estrous cycle (Karsch, 1970), the changes (expressed as ml/min/g ovary) would be slightly less dramatic than shown in Fig. 2, but the patterns would be similar. This is not surprising since the weight of the corpus luteum during the midluteal phase of the cycle is 450 to 800 mg while the remainder of the ovary weighs 1 to 2 g.

A deficiency of the present study is the lack of information regarding levels of estrogen.

Estradiol increased blood flow to the uterus (Rosenfeld et al., 1973), but the effects of this hormone on blood flow to the ovary are unknown. However, it seems unlikely that estradiol was responsible for the changes in blood flow to the ovary noted in this study. Estradiol tends to be low during the luteal phase of the cycle and increases dramatically 24 h prior to the preovulatory LH peak (Scaramuzzi et al., 1970; Cox et al., 1971). Blood flow to the ovary bearing the corpus luteum had reached its nadir when levels of estradiol were presumably at their highest.

In the present studies there was excellent agreement between the pattern of blood flow quantified with the Doppler procedure and the uptake of radioactive microspheres by the ovary throughout the estrous cycle. By precisely determining the diameter of the ovarian artery, the Doppler ultrasonic procedure could be made quantitative. Measurements of blood flow using the Doppler procedure agreed with estimates obtained using electromagnetic flow procedures (Vatner et al., 1970; Reneman et al., 1973). The major advantage of the Doppler method is that frequent measurements can be made over long periods of time. The major disadvantage of the Doppler procedure are that it is difficult to measure precisely the diameter of the ovarian artery, and it is limited to the quantification of blood flowing in an individual vessel. In addition, the Doppler method measures the velocity of blood flowing through the vessel; therefore, if the diameter of the vessel is changing, velocity by itself is not a meaningful estimate of blood flow. It is unlikely that this was a problem in the present study due to the method used for implantation of the transducers (Niswender et al., 1975). The fact that serum levels of LH, FSH, prolactin and progesterone obtained in this study were identical to those observed previously in this laboratory (Niswender, 1974) suggests that implantation of the blood flow transducers did not impair ovarian function.

The use of radioactive microspheres potentially allows estimation of the distribution of blood to individual compartments within the ovary. However, the number of determinations in an individual animal is limited to the number of isotopes which can be counted differentially. The microsphere procedure as employed in these experiments reflected only relative changes in flow of blood to the ovaries. Another problem associated with the microsphere procedure is adequate mixing of the microspheres within the blood. In the present study samples of gracilis muscle were obtained from each hindquarter to evaluate the uniformity of distribution of microspheres. Entrapment of microspheres was not different between left and right sides suggesting that the microspheres were uniformly distributed. This technique can also be criticized because it depends upon blockage of blood vessels by the microspheres. In practice this is usually not a problem since only a small number of microspheres of high specific activity are injected, resulting in blockage of an insignificant number of capillaries. The fact that the uptake of microspheres by the corpus luteum continued to increase on Days 6 and 10 of the cycle in these studies suggests that only a small proportion of capillaries was blocked.

It seems likely that the major difficulty in using the microsphere method for estimating blood flow to the ovaries of sheep is the unique relationship between the ovarian arteries and the dorsal aorta. Each artery originates directly from the dorsal aorta at an angle of 90°. Therefore, due to the small size of these arteries and the potential for the relatively large microspheres to localize in that portion of the aorta where velocity is greatest (laminar flow), the uptake of microspheres may not reliably estimate flow of blood to the ovary. This situation is unique for the ovary. The blood supply to most tissues is via large arterial branches, and therefore, the microspheres tend to be distributed more uniformly.

We have reported previously a high correlation between blood flow data using the Doppler procedure and the entrapment of radioactive microspheres by the ovary (Niswender et al., 1975). Because the techniques are based upon totally different principles this correlation was encouraging. In the present studies the pattern of blood flow throughout the cycle was very similar to the pattern observed for the entrapment of microspheres by the ovary. Both techniques indicated that blood flow to the luteal ovary increased until Day 10 or 12 and then decreased dramatically during the late luteal phase of the cycle. In addition, both procedures indicated that blood flow to the nonluteal ovary did not change significantly throughout the cycle. In the absence of a corpus luteum blood flow to both ovaries was the same.

The dramatic decrease in flow of blood to

the luteal ovary during the latter stages of the estrous cycle suggested a change in the microcirculation of the corpus luteum. Therefore, a morphometric procedure was used to determine if the decrease in blood flow could be correlated with a change in the capillary network of the corpus luteum. Corpora lutea were removed surgically from 24 normal cycling ewes on Days 9, 10, 12, 13, 14, 15, 16 and 17 of the estrous cycle. A slice (approximately 1 mm) was taken from the center of each corpus luteum and immersed in 4 percent glutaraldehyde buffered with 0.1M cacodylate (pH 7.4) and containing 7 percent sucrose. Tissue samples were postfixed in 2 percent osmium tetroxide buffered with 0.1M cacodylate (pH 7.2) at 4°C overnight, stained *en bloc* with 0.5 percent uranyl acetate, dehydrated in ethanol and embedded in epon 812. Thick (1 μ) sections were stained with toluidine blue for light microscopy. Cellular composition of each corpus luteum was determined by the "hit" technique described by Chalkley (1943). A total of 625 hits was recorded for each section. The components of the corpus luteum were classified as granulosa-lutein cells, theca-lutein cells, capillaries or connective tissue. Granulosa-lutein cells were distinguished by their large size (50 μ to 70 μ in diameter), spherical nucleus with a large nucleolus and their pale, granular cytoplasm. Theca-lutein cells were classified on the basis of their smaller size (25 to 40 μ in diameter), ovoid nucleus and large lipid droplets in the cytoplasm. Hits in the endothelial cells, blood cells or lumens of capillaries were recorded as capillaries. All cellular components not identified as granulosa-lutein cells, theca-lutein cells or capillaries were defined as connective tissue.

The volume of each type of cell comprising the corpus luteum, except capillary volume, did not change from Day 10 to Day 17 of the estrous cycle. The volume of the corpus luteum occupied by capillaries decreased linearly ($P<0.01$) during the latter stages of the cycle (Table 3).

It was surprising that the relative volume occupied by the granulosa-lutein, thecal-lutein and connective tissue cells did not change from Days 10 to 17 of the estrous cycle, since the average weight of the corpora lutea declined by approximately 80 percent (Karsch, 1970). This finding implies that either these cell types disappear from the corpus luteum at the same rate or that all of these cells decrease in size in a similar fashion. There was no evidence of an 80 percent reduction in the size of any of these cell types. The decrease in luteal volume occupied by the vasculature appeared due to a reduction (approximately 50 percent) in the number of hits in endothelial cells and capillary lumens. There was an increase in the number of hits recorded in leucocytes and in intra-capillary debris. The observed decrease in luteal volume occupied by capillaries coincided with the decrease in blood flow to the corpus luteum noted previously.

Effects of LH and Prolactin on Ovarian Blood Flow

There has been considerable controversy regarding the roles of LH and prolactin in the regulation of luteal function in ewes (Denamur and Mauleon, 1968; Kaltenbach et al., 1968; Karsch et al., 1971a, b). Much of the controversy was apparently resolved when Schroff et al. (1971) and Denamur et al. (1973) reported that both LH and prolactin were necessary for normal luteal function in hypophysectomized ewes. However, prolactin does not appear to be steroidogenotropic in sheep (Kaltenbach et al., 1967). Recent data reported by Burd et al.

TABLE 3. Cellular composition of the ovine corpus luteum[a].

Cell type	Days of cycle			
	10	13	15	17
Granulosa-lutein	33.8 ± 2.5	35.1 ± 1.7	38.1 ± 3.3	35.6 ± 4.0
Theca-lutein	16.5 ± 1.6	16.7 ± 1.8	13.2 ± 1.4	18.5 ± 0.7
Connective tissue	35.7 ± 1.3	36.4 ± 2.7	36.6 ± 2.1	37.6 ± 1.9
Capillary**	14.4 ± 0.6	11.9 ± 0.8	12.0 ± 1.2	8.3 ± 1.7

[a]Values listed represent the percent volumes of the corpus luteum occupied by each cell type.

**$P<0.01$ for a linear decrease in capillary volume with time.

(1975) suggests that prolactin may increase blood flow to one of its target organs. These authors reported a simultaneous increase in concentrations of prolactin in serum and blood flow to the mammary gland just prior to parturition in ewes. Treatment of ewes with 2-Br-α-ergocryptine (CB-154) prevent the elevation in both prolactin and mammary blood flow but did not interfere with parturition. Prolactin increased arterial blood pressure (Horrobin et al., 1973) and facilitated the effect of noradrenaline and angiotensin on aortic and arteriolar smooth muscle preparations (Manku et al., 1973). Therefore, an experiment was designed to evaluate the effect of a reduction in the levels of prolactin in serum on ovarian blood flow and the secretion of progesterone.

Doppler ultrasonic blood flow transducers were surgically placed around the ovarian artery approximately 3 cm from the ovary bearing the corpus luteum in nine ewes on Day 5 of the estrous cycle. Corpora lutea were marked with carbon black for subsequent identification. Beginning at 0600 on Day 8, five ewes received im injections of 1 mg of CB-154 in 1 ml of 95 percent ethanol every 12 h until 0600 h on Day 11. Four control ewes received injections of 95 percent ethanol. Starting at 0600 h on Day 10, three of the ewes treated with CB-154 were given iv injections of 25 ml of rabbit anti-prolactin serum to reduce further systemic levels of prolactin. The antiserum, which was prepared by pooling serum obtained at weekly intervals from 3 rabbits immunized with NIH-P-S8, was injected at 6-h intervals until 0600 h on Day 11. The remaining two ewes, treated with CB-154, received 25 ml of normal rabbit serum (NRS) every 6 h. Data on ovarian blood flow were recorded and samples of blood from the jugular vein were drawn every 6 h from 1200 h on Day 7 to 2400 h on Day 11 and at 0800 h on Day 12. The blood was allowed to clot and serum was collected by centrifugation. The samples were stored at −20°C until assayed for progesterone. The relative concentration of prolactin antibody was also determined in each sample collected during the period of treatment with antiserum or NRS. Approximately 50,000 cpm (circa 150 pg) of prolactin-^{125}I were incubated with 0.01 ml of serum and 0.49 ml of phosphate-buffered saline, containing 0.1 percent gelatin, for 6 h at 37°C. The antigen-antibody complexes (and free antibody) were precipitated by incubation at 4°C for 30 min with anti-rabbit gamma globulin covalently linked to micro-crystalline cellulose (Sigma Chemical Co., St. Louis, MO). The tubes were centrifuged at 2000 × g for 5 min, the supernatant was decanted and the radioactivity in the pellets was determined.

Neither CB-154 alone, nor a combination of CB-154 and anti-prolactin serum influenced the flow of blood to the ovary or levels of progesterone in jugular venous serum (Fig. 4, 5). There was sufficient antibody in 0.01 ml of serum collected from anti-prolactin-treated ewes to bind 50–100 percent of the radioiodinated prolactin added *in vitro*. Serum collected from NRS-treated ewes did not bind prolactin-^{125}I.

Results obtained in this study support the hypothesis that prolactin is not required for normal luteal function in the ewe (Karsch et al., 1971a). However, this conclusion must be tempered because the hormone-neutralizing capability of this antiserum was not proven. The failure of treatment with CB-154 and/or anti-prolactin serum to influence luteal function in the present study was similar to observations reported previously in the ewe (Niswender, 1974), and the cow (Hoffman et al., 1974). In these studies, small quantities of prolactin (<1 ng/ml) may have been sufficient to support luteal function, a point which was not investigated rigorously. However, in the present study it was demonstrated that sufficient antibody was present in 0.01 ml of serum

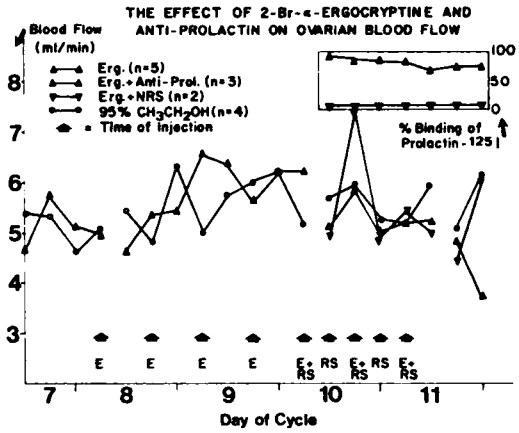

FIG. 4. Blood flow to the ovaries of ewes treated with 2-Br-α-ergocryptine and anti-prolactin serum on Days 8 to 11 of the estrous cycle. Injections of ergocryptine or ethanol (E), and normal rabbit serum or anti-prolactin serum (RS) are indicated by the arrows. There was no significant effect of treatment on blood flow to the luteal ovary.

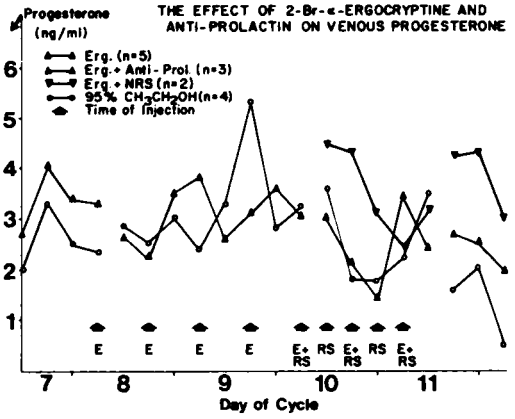

FIG. 5. Levels of progesterone in jugular serum of ewes treated with 2-Br-α-ergocryptine and anti-prolactin serum on Days 8 to 11 of the estrous cycle. Injections of ergocryptine or ethanol (E), and normal rabbit serum of anti-prolactin serum (RS) are indicated by the arrows. There was no significant effect of treatment on levels of progesterone in serum.

obtained throughout the treatment period to bind essentially all of the prolactin-^{125}I added *in vitro*. Thus, there was excess antibody present in the serum and it is very likely that all endogenous prolactin was bound to antibody. However, it was not certain whether binding to antibody prevented the prolactin from exerting its full biological activity on the ovary. It is possible for antibodies to potentiate the effects of the hormones *in vivo* (Cole, 1973).

Several investigators have reported that active or passive neutralization of LH inhibits luteal function. Therefore, a second experiment was designed to ascertain the effects of anti-LH on blood flow to the luteal ovary and secretion of progesterone. Blood flow transducers were implanted into nine ewes on Day seven of the cycle. At 1200, 1800 and 2400 h on Day 10 and at 0600 h on Day 11, five ewes were administered iv 25 ml of ammonium sulfate precipitated anti-LH serum. Blood flow data were recorded and samples of jugular blood were drawn every hour between 1100 and 1800 h on Day 10, every 2 h from 2000 h on Day 10 to 1800 h on Day 11 and every 6 h from 2400 h on Day 11 to 1200 h on Day 12. Four control ewes were given injections of 25 ml of ammonium sulfate-precipitated NRS. At the end of the experiment corpora lutea were removed and weighed.

Treatment of ewes with anti-LH serum resulted in significant decreases ($P<0.01$) in both systemic levels of progesterone and blood flow to the luteal ovary (Fig. 6). The decreases were linear throughout the treatment period and the two parameters were correlated ($P<0.01$). Corpora lutea collected from control ewes weighed 813 ± 71 mg while those obtained from ewes treated with anti-LH serum weighed 286 ± 87 mg ($P<0.01$). Other investigators have shown that treatment of pigs (Spies et al., 1967), cows (Snook et al., 1969; Hoffman et al., 1974) or sheep (Fuller and Hansel, 1970) with anti-LH serum reduced luteal function. However, the mechanism whereby this treatment exerted its effect was not investigated. Our data suggest that one of the effects of anti-LH serum is to decrease blood flow to the luteal ovary. McCracken et al. (1971) reported that infusions of LH increased blood flow to the autotransplanted ovaries of ewes. However, anti-LH serum produced a similar effect in their studies.

The use of specific antisera, as opposed to hypophysectomy, has several advantages for studying the regulation of reproductive phenomena. Selective neutralization of gonadotropins is possible using hormone-specific antisera. Therefore, replacement of several pituitary hormones to a level and pattern similar to those seen in intact animals is not necessary. In addition, many procedures for hypophysectomy are extremely traumatic and are not compatible with normal physiological function. However, there are also problems associated

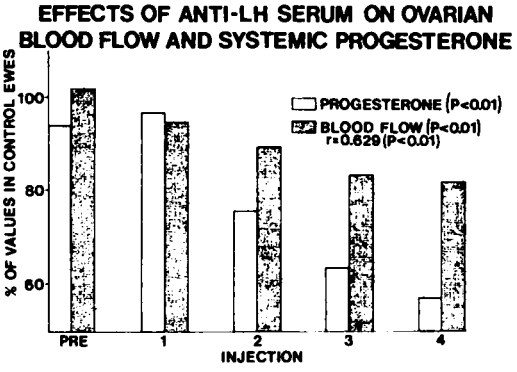

FIG. 6. Levels of progesterone in serum and flow of blood to the luteal ovary following injections of anti-LH serum (n = 5) beginning at 1200 h on Day 10 of the estrous cycle. There was a significant ($P<0.01$) linear decrease in serum levels of progesterone and blood flow to the luteal ovary. Levels of progesterone and blood flow to the luteal ovary were highly correlated ($P<0.01$).

with the use of antisera for the neutralization of hormones *in vivo*. It is difficult to quantify residual levels of the hormone due to the excess antibody present in serum. Although several methods were used in these studies to remove the antigen-antibody complexes and the excess antibody, none proved efficacious. It is easy to demonstrate that there are excess quantities of antibody present in the serum of treated animals and that these antibodies are capable of binding the hormone. However, these observations do not prove that the hormone has been "neutralized." In fact, the binding of a hormone to antibody can result in increased biological activity of the hormone due to a decreased rate of clearance from the circulation (Cole, 1973). It is also possible that a hormone molecule could bind to its receptor even though it is bound to an antibody. Therefore, it would be helpful if the "neutralizing" activity of the antisera was assessed before a biological experiment was performed. For example, in the present study we still cannot rule out the possibility that sufficient quantities of biologically active prolactin were available to maintain normal luteal function. This problem remained even after the demonstration of excess antibody in the serum of treated ewes and the ability of the antibody to bind prolactin.

Since treatment of cyclic ewes with anti-LH serum resulted in a dramatic decrease in luteal function, a final experiment was conducted to determine if infusion of LH would increase blood flow to the ovaries and/or secretion of progesterone. Blood flow transducers were placed around the ovarian artery on the side of the corpus luteum in six ewes and each ewe was fitted with an indwelling jugular cannula. On Day 9 of the cycle, three ewes received: 1) an iv injection of 4 μg of NIH-LH-S16 followed by an iv infusion of LH at 8 μg/h from 1330 to 1430 h; 2) an injection of 40 μg and an infusion of 80 μg/h from 1430 to 1530 h; and 3) an injection of 400 μg and an infusion of 800 μg/h from 1530 to 1630 h. Three control ewes were treated similarly with bovine serum albumin. Blood flow data and samples for progesterone and LH analyses were obtained at 15-min intervals from 1300 to 1630 h.

The results obtained in this study are shown in Fig. 7. The LH treatments produced levels of LH ranging from 0.3 to 115 ng/ml. There was a dramatic, dose related increase ($P<0.01$) in systematic levels of progesterone as a result of the treatment. Blood flow was also significantly elevated by treatment with LH ($P<0.05$) but the increase was not dramatic. In contrast, data obtained in rats suggests that LH causes a dramatic increase in blood flow to the ovaries within 20 min of administration (Wurtman, 1964). This response was so consistent in the prepubertal rat that an increase in ovarian hyperemia has been used as a bioassay for LH (Ellis, 1961).

The mechanism whereby LH stimulated ovarian blood flow in the present study is not known. Administration of HCG is followed by an increase in cardiac output in rabbits (Flickinger et al., personal communication). In addition, Szego and Gitin (1961) postulated that a primary action of LH on the ovary was to increase release of histamine and increase blood flow. Piacsek and Huth (1971) obtained data to support this hypothesis. The data from our studies are also compatable with this hypothesis. It seems likely that the major influence of LH on steroidogenesis is mediated by membrane receptors. However, the increase in blood flow to the ovary could also be an extremely important mechanism of action.

EFFECTS OF LH ON OVARIAN BLOOD FLOW AND SYSTEMIC PROGESTERONE

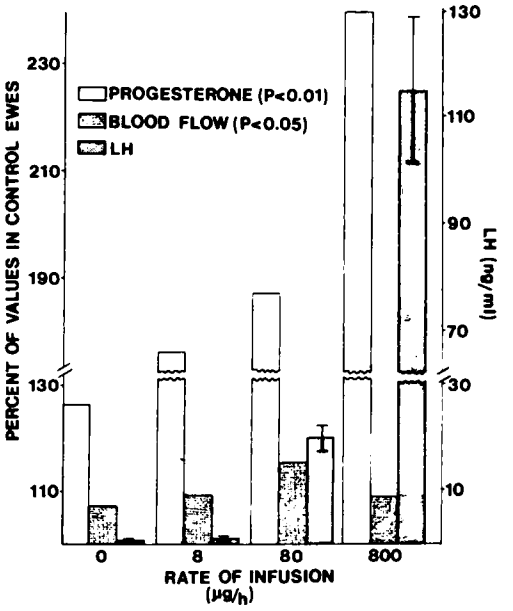

FIG. 7. Levels of progesterone in serum and blood flow to the luteal ovary following the administration of LH. Levels of progesterone ($P<0.01$) and blood flow to the luteal ovary ($P<0.05$) were increased following administration of increasing levels of LH.

Effects of Prostaglandin (PG) $F_2\alpha$ on Ovarian Blood Flow

Results from these studies in sheep and reports by others in sheep (Mattner et al., 1972; Brown et al., 1974) and rabbits (Novy and Cook, 1973) indicated that systemic levels of progesterone and blood flow to the ovary bearing the corpus luteum were correlated. There were no cyclic changes in blood flow to ovaries without corpora lutea, however, at the end of the luteal phase of the cycle both systemic levels of progesterone and blood flow to the ovary with the corpus luteum fell precipitously. These observations raise the question as to what physiological factor(s) are responsible for this decrease in blood flow to the ovary. It has been suggested that $PGF_2\alpha$ may initiate a drastic reduction in luteal blood flow during induced luteal regression in the autotransplanted ovary of the ewe (Baird, 1974). Therefore, experiments were performed to determine the effects of exogenous $PGF_2\alpha$ on blood flow to the ovary and systemic levels of progesterone.

Blood flow transducers were placed on both ovarian arteries of five ewes on Day 5 of the estrous cycle and an indwelling polyvinyl cannula was inserted into the uterine horn ipsilateral to the corpus luteum. The cannula was exteriorized via a high lumbar incision. On Day 9 indwelling cannulas were inserted and blood flow recordings and blood samples were collected at −2 h, −1 h, −½ h and 0 h. At 0 h and +4 h, 5 mg of $PGF_2\alpha$ were injected into the uterus via the cannula. Blood flow recordings and jugular samples for progesterone analyses were obtained at hourly intervals until +17 h. The results are depicted in Fig. 8. Blood flow to the luteal ovary decreased ($P<0.05$) within 4 h and systemic concentrations of progesterone declined ($P<0.05$) within 6 h. Both remained low for the duration of the experiment. There was no change in blood flow to the non-luteal ovary. These results have been confirmed in 6 ewes treated with 25 mg $PGF_2\alpha$ administered im, 5 ewes administered 50 μg of ICI-81008 im and 5 ewes administered 25 μg of ICI-79939 im. In all cases, administration of $PGF_2\alpha$ or one of its analogs, resulted in a decrease in systemic levels of progesterone and blood flow to the luteal ovary. In eleven uninjected control ewes in which blood flow recordings and jugular blood samples were taken at hourly intervals for 12 h, concentrations of progesterone and blood flow to the luteal ovary did not change.

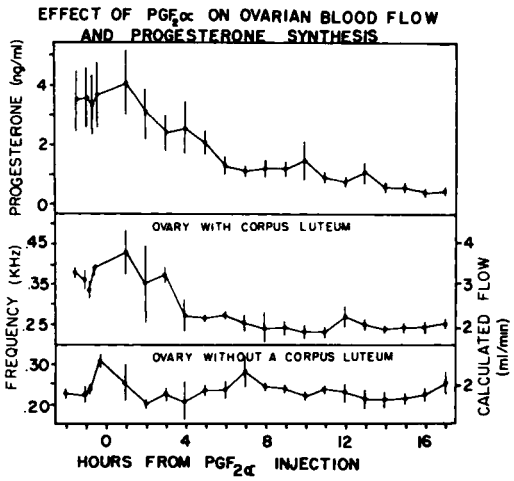

FIG. 8. Levels of progesterone and blood flow to the ovaries following administration of $PGF_2\alpha$ to five ewes on Day 9 of the estrous cycle. Intrauterine injections of $PGF_2\alpha$ (5 mg) were given at 0 and 4 h. Blood flow to the luteal ovary and levels of progesterone decreased within 6 h ($P<0.05$). Blood flow to the non-luteal ovary did not change.

These data suggest that treatment of ewes with $PGF_2\alpha$ during the mid-luteal phase of the estrous cycle results in hemodynamic changes in the luteal ovary which are similar to those seen during the late luteal phase of the cycle when regression of the corpus luteum occurs normally. These findings are in agreement with those obtained in rabbits (Pharriss et al., 1970; Bruce and Hillier, 1974) and with those of Thorburn and Hales (1972) in sheep but conflict with those obtained in sheep by Baird (1974). To date we do not have good evidence suggesting $PGF_2\alpha$ can induce arteriole-venule shunting within the corpus luteum similar to that noted during the late luteal phase of the cycle.

In all of the studies we have conducted there has been a high degree of correlation between blood flow to the luteal ovary and systemic levels of progesterone. Blood flow to the luteal ovary increases as the corpus luteum develops, is maintained when secretion of progesterone is maximal and declines as systemic levels of progesterone decline. Treatment of ewes during the midluteal phase of the estrous cycle with either $PGF_2\alpha$ or anti-LH serum results in decreased circulating levels of progesterone and a decline in blood flow to the luteal ovary. Administration of LH resulted in increased

blood flow to the luteal ovary and systemic levels of progesterone. However, the close association between these two parameters does not necessarily imply a cause and effect relationship. That is, blood flow to the luteal ovary may influence the secretion of progesterone or progesterone may influence blood flow. However, it is also possible that both of these phenomena are influenced in the same manner by other factors, such as LH, prostaglandins, estradiol, etc.

The site chosen for collection of blood samples for progesterone analyses may also have influenced the data obtained in these studies. A higher correlation between blood flow to the luteal ovary and progesterone secretion may have been apparent if the samples for progesterone quantification had been obtained from the ovarian vein. Obviously, jugular concentrations of progesterone reflect secretion of progesterone from the ovary, but in a dampened fashion.

The results obtained in these studies of ovarian function are similar to those reported for other steroid-secreting organs. Increased arterial blood flow to the testis increased the ability of this organ to secrete testosterone when stimulated with human chorionic gonadotropin (Eik-Nes, 1964). Porter and Klaiber (1965) reported that the secretion of corticosterone in hypophysectomized rats was significantly increased with either increased ACTH input or increased adrenal perfusion rates maintaining a constant μg/min of ACTH. Urquhart (1965) observed that "the rate of ACTH presentation to the adrenal gland was better correlated with the steady-state rate of cortisol secretion than was blood ACTH concentration" in dogs. Blood flow to the adrenal gland had the greatest influence when levels of ACTH were insufficient to promote maximal secretion of steroids (Yates et al., 1969; L'Age et al., 1970). Thus, it is clear that blood flow to a steroid-producing endocrine gland must be considered along with the concentrations of tropic hormone in blood if the regulation of these glands is to be understood completely.

It is interesting to speculate as to the factors which might be involved in the increased ovarian steroid output coincident with the increases in ovarian blood flow. First, since blood serves as the primary source of all metabolic requirements for cells, it is possible that some blood-borne substances (i.e., oxygen, acetate, glucose, cholesterol, etc.) necessary for maximal secretion of steroids is limiting. Increased blood flow to the ovary would increase the quantities of these substances reaching the ovary and could result in a rise in steroid output. There is no evidence that any blood-borne substance necessary for secretion of steroids is limiting. A second possibility is that the corpus luteum contains some substance which is inhibitory to steroid secretion and that increased perfusion of this organ by blood reduces the levels of the inhibitory factor. We have obtained evidence that progesterone inhibits the activity of 3-β-hydroxy-steroid dehydrogenase, the enzyme complex necessary for conversion of pregnenolone to progesterone (Caffrey et al., unpublished data). However, it has not been demonstrated that this inhibition is important in the regulation of progesterone synthesis *in vivo*. A third possibility is that only a fraction of the steroid secreting cells within the corpus luteum are functioning at any given time. If this was true then increased perfusion of this structure with blood could result in increased steroid production. Heterogeneity of function of cells within the corpus luteum has been demonstrated and will be discussed in the next section.

Relationship between Intra-luteal Blood Flow and Binding of HCG

There is accumulating evidence which suggests that there is heterogeneity of function within the corpus luteum. The concentration of progesterone in different sections of bovine corpora lutea was highly variable (Estergreen et al., 1968). In addition, if one looks at the autoradiographic localization of HCG-^{125}I 70 min after the infusion of this hormone into the ovarian artery, there are areas where considerable HCG is bound and the other areas where little or no HCG can be demonstrated (Fig. 9). There were no apparent morphological differences between these areas. The specificity of the binding of HCG was demonstrated by lack of radioactivity in tissue collected from ewes pretreated with LH or treated with BSA-^{125}I. Therefore, a series of experiments were conducted to determine if regional differences in binding of HCG within the corpus luteum were due to regional differences in blood flow.

In the first experiment, 17 corpora lutea were collected from four superovulated ewes which had been anesthetized and given infusions of 200 μCi of HCG-^{125}I into the ovarian

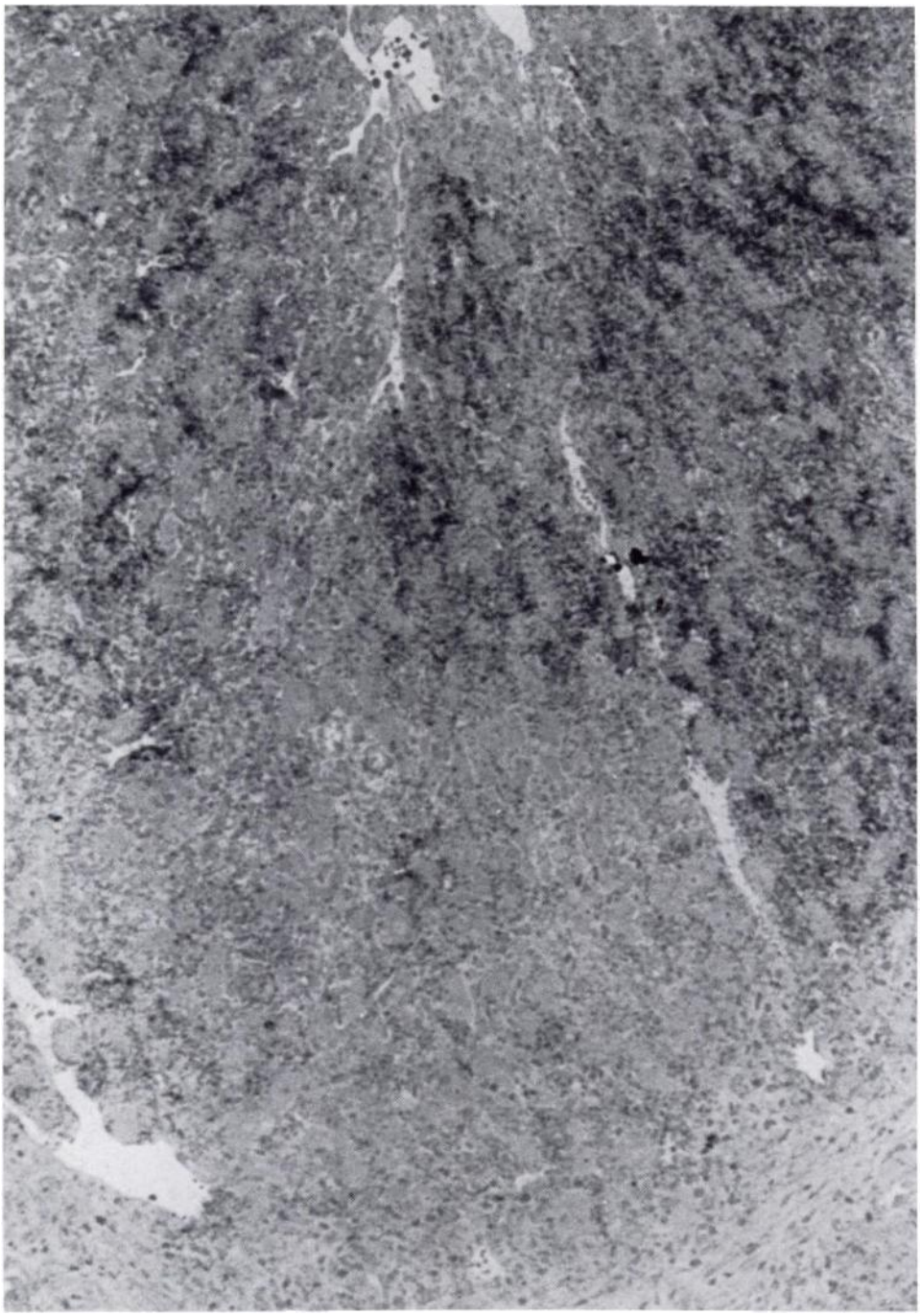

FIG. 9. Autoradiographic localization of HCG-^{125}I in the corpus luteum (100×).

artery over a 2-min period. This treatment was followed by an infusion of 2 μCi of ^{85}Sr microspheres (15 ± 5 μ) over a 2-min period. At either 5, 20 or 40 min after the end of the infusion corpora lutea were removed and sliced at 0.5 mm. Each slice was weighed and counted for ^{125}I and ^{85}Sr. The data were corrected for background and channel overlap and expressed as cpm/mg tissue. Since the ^{125}I to ^{85}Sr ratio did not change significantly with time the data

TABLE 4. Correlation between binding of HCG and entrapment of microspheres in luteal slices.

r	No. of CL's	Significance
0.21 – 0.50	4	N.S.
0.61 – 0.71	4	0.05
0.71 – 0.80	3	0.05
0.81 – 0.90	3	0.01
0.91 – 0.96	3	0.01

from all corpora lutea were pooled and analyzed to determine if the binding of HCG (cpm-^{125}I) and entrapment of microspheres (cpm-^{85}Sr) were correlated (Table 4). In four corpora lutea the two parameters were not correlated while in the remaining 13 corpora lutea significant correlations ($P<0.05$ and $P<0.01$) were observed. Since the variation in the ratio of ^{125}I to ^{85}Sr in this study were considerable, both within and between the corpora lutea, the experiment was repeated in three ewes.

In the second experiment infusions were made in a manner similar to those described above. However, all corpora lutea were removed 30 min following the infusion of microspheres and sectioned at 0.5 mm. Each slice was further divided into 16 pieces of approximately equal size. Each piece was weighed immediately (circa 5 mg), placed in a 5 ml disposable test tube and 2 ml petroleum ether were added. The tube was stoppered and the ^{125}I and ^{85}Sr content determined. The tissue was then homogenized with five to seven strokes of a glass rod and the tubes were vortexed for 30 sec. The petroleum ether was decanted, taken to dryness under nitrogen and the progesterone content of the residue was determined. The data from this experiment were calculated as cpm-^{125}I/mg, cpm-^{85}Sr/mg and ng progesterone/mg tissue and analyzed by multiple correlation (Table 5). There was a highly significant ($P<0.01$) correlation between the binding of HCG and the entrapment of microspheres in all corpora lutea. Binding of HCG and concentrations of progesterone were correlated in two corpora lutea, whereas microsphere entrapment and concentration of progesterone were correlated in one corpus luteum. However, if one calculates the coefficient of determination for each of the significant correlation coefficients, the values range from 61 to 75 percent for microspheres and HCG, 6 to 16 percent for HCG and progesterone concentration, and 4 percent for microspheres and progesterone concentration. These data suggest that much of the variation in binding of HCG can be attributed to the variation in blood flow. However, very little of the progesterone content in the corpus luteum can be attributed to either blood flow or binding of HCG. It seems likely that factors such as capillary permeability, saturation of receptors with endogenous gonadotropins, dynamics of lymph flow, etc., could influence the binding of gonadotropins to the luteal cell. When more is known about each of these parameters, it will be possible to determine if heterogeneity of function within the corpus luteum is related to local differences in blood flow.

There are several hypotheses which could have explained the heterogeneity of binding of HCG within a corpus luteum noted by autoradiography. However, the high degree of correlation noted between the binding of HCG and the entrapment of microspheres suggests that local differences in intraluteal blood flow determines

TABLE 5. Correlations among entrapment of microspheres, HCG binding and progesterone.

	df	MS[a] – HCG[b]	HCG – Prog[c]	MS – Prog
CL-1	85	0.79**	0.39**	0.20
CL-2	119	0.87**	0.25*	0.21*
CL-3	57	0.79**	0.20	0.23

[a]Entrapment of radioactive microspheres (cpm/mg tissue).

[b]Binding of HCG (cpm/mg tissue).

[c]Progesterone concentration (ng/mg tissue).

*$P<0.05$.

**$P<0.01$.

the availability of HCG to its receptor. It remains to be determined whether the areas of increased blood flow within the corpus luteum change with time. Since there was no apparent morphological difference between areas of high binding and those with little binding it seems likely that these areas do change continually. The mechanisms responsible for regulation of the distribution of blood flow within the corpus luteum remain to be identified.

The lack of a consistent, high correlation between concentrations of progesterone and either the binding of HCG or the entrapment of microspheres could result from several factors. Had the tissues been collected immediately after the infusion of HCG and microspheres, the concentration of progesterone may have been more highly correlated with these parameters. It is also possible that low concentrations of progesterone in tissue reflect a high degree of secretory activity. However, a negative correlation was not observed.

SUMMARY AND CONCLUSIONS

Data from several studies are presented which suggest that blood flow to the corpus luteum may be important in the regulation of this gland. Blood flow to the luteal ovary increases from less than 1 ml/min to 3 to 7 ml/min as the corpus luteum develops and is maintained. During regression, blood flow to the luteal ovary declines sharply. Data obtained with radioactive microspheres indicates that the changes in blood flow to the luteal ovary can be attributed to changes in flow to the corpus luteum, which receives the majority of the blood. In addition, the entrapment of 15 μ microspheres was less than that for 50 μ microspheres during the late luteal phase of the cycle suggesting that arteriole-venule shunting of blood occurs. As a result of the decline in total ovarian blood flow and the shunting of blood within the corpus luteum, there appears to be a severe restriction in the quantity of blood available to the luteal cell during regression. Morphological data obtained during this period indicate that there is even a decrease in the relative volume of the capillary network within the corpus luteum.

Treatment of ewes with a combination of CB-154 and anti-prolactin serum did not influence either blood flow to the luteal ovary or systemic levels of progesterone. It was demonstrated in this study that CB-154 treatment was followed by a dramatic decline in serum levels of prolactin. In addition, an excess of antibodies against prolactin was demonstrated in sera collected from treated ewes. It was also demonstrated that the antibodies in serum were capable of binding prolactin. Treatment of ewes during mid-cycle with anti-LH serum resulted in a rapid decline in blood flow to the luteal ovary and in circulating levels of progesterone. Infusion of exogenous LH resulted in a dramatic increase in serum levels of progesterone associated with a less dramatic increase in blood flow to the luteal ovary.

When ewes were administered $PGF_2\alpha$ or analogs of $PGF_2\alpha$ serum levels of progesterone and blood flow to the luteal ovary declined to basal levels within 6 h. The hemodynamic changes associated with luteal regression in $PGF_2\alpha$-treated ewes were similar to those observed in cycling ewes.

Finally, regional blood flow within the corpus luteum was studied using 15 μ radioactive microspheres. When small pieces of tissue were taken following infusions of HCG-^{125}I and ^{85}Sr microspheres into the ovarian artery the cpm/mg tissue of the two isotopes were highly correlated. These data suggest that the specific binding of HCG to luteal cells is influenced by the flow of blood to these cells. From these studies it appears that blood flow may be an important factor in regulating the activity of the gonadotropic hormones at the luteal cell level. It further appears that a secondary mechanism of action of LH may be to increase blood flow to the corpus luteum.

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