Corpus Luteum Action on Ovarian Follicular Development After Destruction of Macroscopically Visible Follicles in Ewes[1] (35922)

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The corpus luteum (CL) has a possible stimulatory effect on follicular development and ovulation. Robinson (1) demonstrated that the presence of a previously formed CL increased the efficacy of pregnant mare serum gonadotropin (PMSG) in inducing ovulation in sheep. Rowson (2) also gave evidence that the presence of a CL greatly facilitated the ovulatory response to PMSG in cows if given in the presence of the CL rather than after enucleation of the CL. In humans Bloch (3) observed that the greatest number of small follicles was present at the time of maximal development of the CL and that the largest of these follicles was in the immediate vicinty of the CL.

The present experiment was designed to determine the effects of presence or absence of the CL and of destruction or intactness of macroscopically visible ovarian surface follicles on further follicular growth.

Materials and Methods. Thirty-two mature Targhee ewes were assigned randomly to four treatment groups: (i) sham operated; (ii) CL removed; (iii) follicles destroyed; (iv) CL removed plus follicles destroyed. At laparotomy, the location and number of CL were noted and each animal within each group was classified as a single or twin ovulator. Both unilaterally and bilaterally ovulating ewes were included in the study of the interval from CL removal until the next estrus; only unilaterally ovulating ewes were included in the rest of the study. Among the 32 ewes assigned to the experiment, 24 were usable as unilateral ovulators.

All surgery was done on day 8 of the estrous cycle (first day of estrus = day 1) while the animals were under general anesthesia. The ovaries and uterus were exposed by midventral laparotomy. Removal of CL was performed (groups 2 and 4) by exerting pressure with the fingers around the base of the CL. Hemorrhage was controlled by electrocoagulation. All macroscopically visible follicles were destroyed (groups 3 and 4) by cauterization with an electrosurgical unit. The tip of the cauterizer was activated and then introduced into the follicle. Sham operation consisted of midventral laparotomy and manipulation of ovaries. The number of CL present at the time of surgery was recorded for all groups. Following surgery the ewes were separated from the flock and checked for estrus twice daily using vasectomized rams.

Whether or not the ewes showed estrus, all were autopsied 4 days following surgery. Data recorded for each ovary were the number of follicles $\geq$4 mm in diameter, the diameter of each follicle $\geq$4 mm, and total follicular fluid weight (FFW) for each ovary. To estimate FFW the ovarian stroma was weighed after slicing and blotting with toweling paper and this weight plus the weight of the CL were subtracted from the gross weight of the ovary. When new ovulations were found at autopsy the follicular count and follicular fluid volume were adjusted by considering each new ovulation as an 8 mm

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TABLE I. Effect of CL Removal in Ewes with Destroyed Follicles.[a]

Corpora lutea	No. of CL	No. of ewes	CL ovary FFW (mg)	CL ovary Diam[b] largest follicle (mm)	CL ovary No. of follicles ≧4 mm	Non-CL ovary FFW (mg)	Non-CL ovary Diam[b] largest follicle (mm)	Non-CL ovary No. of follicles ≧4 mm
Intact	1	3	477	5.98	1.66	271	4.86	1.02
	>1	3	656	5.76	2.00	280	3.00	.00
Removed	1	3	263	4.00	0.34	222	4.44	.34
	>1	4	437	6.74	1.00	360	4.50	.76

[a] All macroscopically visible surface follicles were cauterized on day 8 of estrous cycle and ewes were autopsied 4 days later.

[b] Ovaries having no follicles 4 mm or greater were, for the purpose of analysis of diameter of largest follicle, credited with a 3 mm follicle.

follicle.

The following factors were studied statistically by the least squares method: CL intact (CL) vs CL removed (CLr); 1 CL (1 CL) vs more than 1 CL (>1 CL); CL ovary (CL-O) vs non-CL ovary (NCL-O). Each factor was studied within each of the two kinds of ewes: "follicles intact" and "follicles destroyed."

Results. Occurrence of estrus and ovulation. In the group with CL removed and follicles intact, 6 of 8 ewes showed estrus at an average interval of 2.3 days after surgery; whereas in the group with CL removed and follicles destroyed, 5 of 8 ewes showed estrus at an average interval of 3.6 days (3.6 vs 2.3, $p<.05$; error mean square 0.76). In the former group, all ewes which showed estrus ovulated; whereas none of the ewes in the latter group ovulated. None of the 16 ewes in the groups with CL intact, whether follicles were intact or destroyed, showed estrus or ovulation before autopsy on day 12 of their estrous cycle.

Follicular development in ewes with intact follicles. Only one ewe allotted to the CL-removed group had 2 CL. Consequently she was omitted and only the 1 CL subgroup was studied (3 ewes) and within those ewes a comparison was made between CL-O and NCL-O. The difference in FFW between the two types of ovaries within that small number of ewes was not significant (CL-O, 606 mg; NCL-O, 467 mg). A 2 × 2 analysis of variance was made within CL-intact ewes for 1 CL (4 ewes) vs >1 CL (3 ewes) and CL-O vs NCL-O. The CL-O had more FFW than the NCL-O as averaged over 1 CL and >1 CL (578 mg vs 400 mg; $p<.01$; error mean square 7400). No significant differences were found in ewes with intact follicles for the diameter of the largest follicle or for the number of follicles ≧4 mm.

Follicular development in ewes with destroyed follicles. In the group in which follicles were destroyed, FFW, diameter of largest follicle, and number of follicles ≧4 mm were significantly greater in the CL-O than in the NCL-O (Tables I and II). The difference for FFW was greater, however, in the CL intact than in the CL removed groups (290.8 vs 59.2, $p<.001$) as it was also for the number of follicles ≧4 mm in diameter (1.32 vs 0.12, $p<.01$). Greater FFW was present in the ewes with >1 CL than in the ewes with 1 CL (433.4 vs 308.3, $p<.05$). The difference between the CL-O and the NCL-O was greater in the >1 CL ewes than in the ewes with 1 CL for the diameter of the largest follicle (2.50 vs 0.34, $p<.05$) and number of follicles ≧4 mm (1.12 vs 0.32, $p<.05$).

Discussion. The average interval from removal of CL (follicles intact) and return to estrus is consistent with the observations by Lang (4), Mallampati and Casida (5), and Dufour *et al.* (6). When CL were removed and surface follicles were destroyed, the interval from surgery to estrus was longer than when follicles were left intact. Only the folli-

TABLE II. Analysis of Variance of Ovarian Measurements for Ewes with Destroyed Follicles.

Source of variation	*df*	Mean squares			Error term used for each character
		FFW	Diam of largest follicle	No. of follicles ≥4 mm	
CL vs CLr	1	64,668	0.00	2.02[a]	S
CL-O vs NCL-O	1	196,047[c]	12.92[b]	3.40[b]	S × O
1 CL vs >1 CL	1	100,234[a]	0.23	0.07	S
CL vs CLr × CL-O vs NCL-O	1	85,846[c]	1.68	2.34[b]	S × O
CL vs CLr × 1 CL vs >1 CL	1	6142	9.54	1.22	S
CL-O vs NCL-O × 1 CL vs >1 CL	1	16,988	7.57[a]	1.00[a]	S × O
CL vs CLr × CL-O vs NCL-O × 1 CL vs >1 CL	1	7389	0.41	0.47	S × O
Sheep (S)	9	15,549	3.46	0.34	
Sheep × ovary (S × O)	9	4576	1.26	0.18	

[a] $p < .05$.
[b] $p < .01$.
[c] $p < .001$.

cles which were grossly visible at the surface of the ovary were cauterized directly. Presumably, many small vesicular follicles beneath the ovarian surface were not destroyed. The delay in return to estrus probably represents the time needed for the small, intact follicles to grow and produce estrogen sufficient to bring about estrous behavior. This explanation holds also for the difference between the proportion of ewes ovulating after exhibiting estrus.

A local intraovarian association of the CL with follicular development was found. Comparison of the follicular diameter and FFW 4 days after the larger follicles had been destroyed indicated that the difference between the CL-O and the NCL-O was greater with the CL intact than when removed. Growth of follicles appeared to be greater in an ovary that contained >1 CL than in an ovary which contained 1 CL. In addition in ewes with CL and follicles intact the CL-bearing ovary had significantly more FFW than the non-CL ovary. The local intraovarian association of the CL with follicular development is consistent with certain observations of other workers. Falck (7) showed that estrogen secretion occurred only when either granulosa or luteal tissue was in contact with theca interna or interstitial tissues in the rat. Ryan *et al.* (8) using isolated granulosa and thecal cells from human ovarian follicles, demonstrated that either granulosa or thecal cells were able to synthesize estrone but only thecal cells were able to produce estradiol. Bloch (3) observed that larger follicles were in the immediate vicinity of the CL.

Although the results of the present experiment demonstrate an intraovarian relationship between the CL and the follicles, it is likely that a systemic mechanism involving the pituitary gonadotropins was also involved. The importance of an interaction between luteinizing hormone (LH) and follicle stimulating hormone (FSH) on follicular development was pointed out by Lostroh and Johnson (9). They demonstrated that LH and FSH given simultaneously caused formation of larger follicles and consequent uterine growth in immature hypophysectomized rats; whereas neither FSH nor LH alone stimulated follicular development or uterine growth. Greep *et al.* (10) observed some follicular growth in hypophysectomized rats treated with FSH; but when LH was given in association with FSH, uterine growth was increased disproportionately to the greater follicular stimulation.

It is assumed that interaction between the two gonadotropic hormones brings about follicular development during the estrous cycle. The level of circulating estrogen assumed to be the main feedback agent regulating chronic release of FSH, was likely altered in the present experiment because of destruction of follicles. The level of circulating progesterone

assumed to regulate the acute release of FSH and LH, was likely altered by removal of CL.

The demonstrated stimulatory effect of the CL on follicular maturation after PMSG injection (1, 2) could have involved either a systemic action of the CL via the general circulation, and the pituitary-hypothalamic area, or a local action within the ovary on the follicles. A period of treatment with progesterone in anestrous goats (11) and with synthetic progestogens in cows (12), preceding a treatment with PMSG led also to a more uniform induction of ovulation than occurred with PMSG treatment given alone. Greep (13) showed that HCG treatment of hypophysectomized rats led to estrogen secretion but only if CL were present before surgery.

The question might be raised whether destruction of small follicles during the process of CL removal might have been responsible for the decreased follicular development in that ovary. This seems unlikely, however, since there was greater FFW 4 days after removal of 2 CL than after removal of 1 CL. It is recognized that the method of estimating FFW would possibly include other fluids freed by the chopping procedure such as those that might result from trauma. It should be noted, however, that differences were found not only in FFW, but also in numbers and diameters of follicles.

The results of the present study are consistent with the hypothesis that the CL favors follicular development, in part, by means of a local intraovarian mechanism. Other approaches for testing this hypothesis are to be sought.

Summary. In unilateral ovulating ewes with follicles intact, the follicular fluid weight (FFW) at day 12 of the estrous cycle was greater ($p<.05$) in the ovary bearing the corpus luteum (CL-O) than in the ovary not bearing the corpus luteum (NCL-O) as averaged over the presence of 1 corpus luteum (1 CL) and more than one corpus luteum (>1 CL). However, when the CL were removed on day 8 of the estrous cycle, the difference in FFW between the CL-O and the NCL-O at day 12 was not significant. When all visible surface follicles were destroyed by cautery the difference between the CL-O and the NCL-O for the FFW and the number of follicles $\geq$4 mm was greater if the CL was left intact than when removed. The difference in diameter of the largest follicle between the CL-O and the NCL-O was greater in >1 CL than in 1 CL animals. The results of the present study are consistent with the hypothesis that the CL favors follicular development, in part, by means of a local intraovarian mechanism.

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