Absence of Ovarian Compensatory Hypertrophy After Unilateral Ovariectomy During the Anestrous Season in the Ewe[1] (34766)

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Unilateral ovariectomy performed on some mammals during sexually quiescent periods such as prepuberty (1, 2) or while in the "androgen-sterilized" state (3) results in compensatory hypertrophy of the remaining ovary. The response of animals to this treatment during seasonal anestrus has had little study.

The pituitary–gonadal relations during anestrus are poorly understood. Moore *et al.* (4) concluded that there was a depression in pituitary gonadotropin content during anestrus in ground squirrels. In ewes, Cole and Miller (5) and Warwick (6) failed to show a seasonal difference in gonadotropin content of the pituitary gland. Kammlade *et al.* (7) found that the gonadotropin content of the sheep pituitary gland during the anestrus season was higher than at certain stages of the estrous cycle, but the content of the gland during the later part of the cycle was higher than that in the anestrous season.

Ovaries of ewes during the anestrous season may contain many large follicles which macroscopically appear similar to the follicles present during the breeding season (5, 7). The present experiments were designed to test the hypothesis that the ovaries of the anestrous female are capable of compensatory follicular hypertrophy and that the onset of the breeding season will be advanced after unilateal ovariectomy.

Materials and Methods. Expt. 1 was conducted using 20 mature Targhee ewes. All the ewes were checked with vasectomized rams for 1 month before the start of the experiment and none of the ewes showed heat during that period. Ten ewes chosen randomly were unilaterally ovariectomized and the other 10 were "sham-operated" as controls (June 5–6, 1968). None of the ewes was found to have CL at laparotomy. Fifteen days after unilateral ovariectomy or control surgery all animals again underwent laparotomy; follicles above 5 mm in diameter were counted and measured at that time. Ewes were laparotomized a third time 25–30 days after the second laparotomy and the same measurements were taken. Animals were observed for heat daily from the time of original surgery until they were killed at the third estrus of the breeding season. At autospy, the ovaries were removed. The number of CL from the last previous estrus was counted and the number of follicles above 5 mm in diameter was recorded. Total follicular fluid weight was estimated as a difference by weighing the ovaries and then chopping them into bits, blotting the free fluid and weighing the remaining tissue. An index of the total surface area of the follicles above 5 mm in diameter was calculated as a summation of the squares of the follicle diameters.

Expt. 2 was conducted to confirm the failure to produce compensatory hypertrophy observed during anestrus in Expt. 1; but with the ovaries being subjected to more cirtical examination. Eighteen Targhee ewes which had not shown estrus during a minimum period of 1 month were randomly assigned to groups treated as follows and surgery per-

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TABLE I. Effect of Unilateral Ovariectomy During the Anestrous Season (June 5–6) in Ewes.

Treatment	Ovary studied	No. of ewes	No. of follicles above 5 mm in diam[a]		Surface area index of follicles above 5 mm in diam[b]	
			1st laparotomy (June 20–21)	2nd laparotomy (July 20–21)	1st laparotomy (June 20–21)	2nd laparotomy (July 20–21)
Left ovary removed	Right	6	1.5	2.25	68[c]	125[de]
Control	Right	6	1.5	2.00	66[c]	118[de]
Right ovary removed	Left	4	1.84	2.17	97[cdc]	107[de]
Control	Left	4	1.67	1.87	72[cd]	98[cde]

[a] Only insignificant differences were shown among numbers of follicles above 5 mm in diameter at either laparotomy.

[b] Values in the two columns with the same superscript are not significantly different.

formed on them on May 9–10, 1969: (i) unilaterally-ovariectomized and killed 10 days later; (ii) sham-operated and killed 10 days later; (iii) unilaterally-ovariectomized and killed 30 days later; and (iv) sham-operated and killed 30 days later. None of the ewes was found to have CL at surgery. At the time of autopsy ovaries were collected and total follicular fluid weight was estimated as above; the number of follicles above 3 mm diameter was also counted. Data were analysed by analysis of variance and by Tukey's "W" procedure (8).

Results and Discussion. Since there is an inequality of size and function between the right and left ovaries in the ewe (9) comparisons were made of the right-remaining ovaries of the treated ewes with the right-ovaries of the controls, and the left-remaining ovaries with the left of the controls.

No significant effect of unilateral ovariectomy on the number of follicles above 5 mm in diameter was shown in Expt. 1 at either 15 days or 40–45 days (Table I). The surface area of follicles above 5 mm in diameter also did not differ significantly between comparable ovaries but did differ from one laparotomy stage to the next.

The average date of onset of estrus for unilaterally ovariectomized ewes was September 6 as against September 4 for control ewes. The mean lengths of the first and second estrous cycles in treated ewes were not different from those of the control ewes (16.6 and 17.0 vs. 16.2 and 17.1 days).

When the ewes were killed at the third estrus of their breeding season there were highly significant differences between treated and control groups, in weight of the ovary, weight of follicular fluid, average number of CL and surface area of follicles above 5 mm in diameter (Table II).

TABLE II. Effect of Unilateral Ovariectomy During Anestrus When Ewes Were Killed at the Third Estrus of the Breeding Season.

Treatment	Ovary studied	No. of ewes	Wt of ovary (g)	Wt of stroma (g) ovarian tissue less CL	Wt of follicular fluid (mg)	Av no. of CL	Index of surface area of follicles above 5-mm diam
Left ovary removed	Right[a]	5	3.002	1.498	701	2	280.2
Control	Right	6	1.913	1.201	431	1	120.8
Right ovary removed	Left	4	2.959	1.548	745	1.8	320.6
Control	Left	4	1.935	1.262	530	0.67	115.2

[a] One cystic ovary 6.551 g in weight and containing 2.546 g of follicular fluid was not included in the data; if it were, the means would have been much higher.

Thus ewes which failed to show compensatory hypertrophy during anestrus did respond during the breeding season.

Ovary weight did not differ significantly between treatments (ovariectomy vs. sham), sides (left vs. right) or different intervals from surgery to autopsy. Follicular fluid weights and numbers of follicles above 3 mm in diameter were not different between comparable ovaries of unilaterally-ovariectomized and control ewes, but there was significantly more follicular fluid and a greater number of follicles in the 30-day group than in the 10-day group (Table III).

When unilateral ovariectomy was performed during anestrus, the remaining ovary did not show compensatory hypertrophy. Sundaram and Stob (10) showed compensatory changes in anestrous ewes after unilateral ovariectomy but many of their ewes had corpora lutea so they could not be considered in a stage of anestrus with minimal ovarian activity.

Donovan (11) ovariectomized ferrets unilaterally during the anestrous stage and interpreted the results as showing compensatory changes. His observations were complicated by the fact that the weights of control ovaries were always recorded earlier in anestrus than retained ovaries. Any change in ovarian weight due to the animal going into a different phase of anestrus would be confounded with possible compensatory hypertrophy.

Although there was no evidence of compensatory hypertrophy in our sheep studies there were significant changes in ovarian follicular activity from one stage to another during anestrus (first laparotomy stage to the second in Expt. 1 and first stage at slaughter to the second stage in Expt. 2). The difference could be due to the fact that the ewes were passing through different phases or degrees of anestrus at different times of the year.

It has been observed in sheep that the phenomenon of anestrus has different "depths" (12, 13) as judged by percentage of the flock showing the condition. In the months of May and June anestrus seems to be very deep. Probably the ewe needs cyclic changes in gonadotropic secretion to show compensatory hypertrophy.

The mechanism of compensatory hypertrophy has been explained (14) on the basis of there being an increased release of gonadotropin due to decrease in blood estrogen levels following hemi-castration. The failure of the anestrous ewes to show compensatory hypertrophy may be explainable on the basis of anestrous ovaries secreting too small an amount of estrogen to affect the hypothalamic centers and the removal of one ovary might not make any difference in the response of the hypothalamus to that small change. Alternatively, the hypothalamic centers might be insensitive to changes in estrogen levels during anestrus. It has been shown in rats with lesions in the hypothalamus that the ovaries lose the capacity to compensate after hemi-castration although the ovaries are morphologically normal in appearance (15).

Another frequently proposed explanation for compensatory hypertrophy is that double the amount of gonadotropin is available to

TABLE III. Effect of Unilateral Ovariectomy During Anestrus on Ovary Weights (mg) Total Follicular Fluid Weight (mg) and on Number of Follicles above 3 mm Diameter.[a]

Stage of killing	Measurement	Lt. ovary removed— rt. studied	Control: rt. ovary studied	Rt. ovary removed— lt. studied	Control: lt. ovary studied
10 days after unilateral ovariectomy	Ovarian wt	1619	1589	1588	1435
	Total follicular fluid wt	480	509	431	403
	No. of follicles	4.0	4.33	3.33	3.67
30 days after unilateral ovariectomy	Ovarian wt	1537	1503	1528	1389
	Total follicular fluid wt	311	345	273	244
	No. of follicles	3.23	3.67	2.67	4.33

[a] Ten-day follicular fluid weights different from 30 day ($p < .01$).

the remaining ovary after unilateral ovariectomy and consequently there is an increased follicular development (16). It might be judged from the observed amount of follicular development in the present experiment that there was gonadotropin in circulation. In this case, however, doubling the amount presumed to be available by removing one ovary did not result in compensatory hypertrophy.

Seasonally anestrous phenomena have been compared to those of other anovulatory states like prepuberty (17) and the sterile condition induced by exposure to excessive light or androgen in early life (18). Seasonal anestrus in the ewe seems to be different from the above states in its effect on the response to unilateral ovariectomy.

Summary. Unilateral ovariectomy was performed on ewes during the anestrous season and the effect was determined on the remaining ovary during that anestrus and during the following breeding season. Experiments were performed in both 1968 and 1969. In Expt. 1, observations at laparotomy on control and unilaterally ovariectomized ewes were made at 15 days, and 40–45 days after original surgery. No significant differences were noticed in the number of follicles above 5 mm in diameter/ovary or in their calculated total surface area. However, when these same animals were killed at the third heat period of the breeding season, the unilaterally ovariectomized ewes showed a significantly greater number and total surface area of follicles above 5 mm in diameter, a greater number of ovulations, and greater total follicular fluid weight per ovary.

In Expt. 2, ewes were killed during anestrus at 10 and 30 days after experimental surgery. No significant differences in individual ovaries were noted for number of follicles above 3-mm diameter, total follicular fluid weight, or weight of ovary between unilaterally ovariectomized and control ewes.

1. Arai, H., Amer. J. Anat. **28**, 59 (1920).

2. Dailey, R. A., Peters, J. B., First, N. L., Chapman, A. B., and Casida, R. E., J. Anim. Sci. **28**, 59 (1920).

3. Gorski, R. A., and Barraclough, C. A., Proc. Soc. Exp. Biol. Med. **110**, 298 (1962).

4. Moore, C. R., Simmons, G. F., Wells, L. J., Zalisky, M., and Nelson, W. O., Anat. Rec. **60**, 279 (1934).

5. Cole, H. H., and Miller, R. F., Amer. J. Anat. **57**, 39 (1935).

6. Warwick, E. J., Proc. Soc. Exp. Biol. Med. **63**, 530-533 (1946).

7. Kammlade, W. G., Jr., Welch, J. A., Nalbandor, A. V., and Norton, H. W., J. Anim. Sci. **11**, 646 (1952).

8. Steele, R. G. D., and Torrie, J. H., "Principles and Procedures of Statistics." McGraw-Hill, New York (1960).

9. McKenzie, F. F., and Terrill, C. A., Mo. Agr. Sta., Res. Bull. 88 pp. **264**, (1937).

10. Sundaram, S. K., and Stob, M., J. Anim. Sci. **26**, 374 (1967).

11. Donovan, B. T., J. Endocrinol. **38**, 173 (1967).

12. Hafez, E. S. E., J. Agr. Sci. **42**, 189 (1952).

13. Mallampati, R. S., unpublished data.

14. Greenwald, G. S., Endocrinology **82**, 591 (1968).

15. Flerko', B., and Bárdos, V., Acta. Endocrinol. **36**, 180 (1961).

16. McLaren, A., Proc. Roy. Soc. Ser. B. **166**, 316 (1966).

17. Everett, J. W., "Sex and Internal Secretions" (W. C. Young, ed.). Williams & Wilkins, Baltimore (1961).

18. Rothchild, I., *in* "Proceedings of Easter School Foundation of Agricultural Science" (Lammings and Amoroso, eds.), Butterworths, London (1967).

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