Some Factors Affecting Gonadotropin Levels in Sheep.* (30810)

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With the development of specific and quantitative bioassay methods for gonadotropic hormones, considerable data have been accumulated on blood and pituitary levels in a number of mammalian species(1-4). These reports indicate the existence of marked differences in concentration and ratio of LH and FSH among those species studied and emphasize the importance of additional comparative data from other mammals. Further, it is essential to determine those conditions which may alter gonadotropin levels before experimental data may be properly evaluated in terms of factors regulating pituitary-gonadal activity. The present investigation was undertaken to determine the influence of gonadectomy, hypothalamic lesions and anestrus on blood and pituitary content of FSH and LH in sheep.

Materials and methods. Donor animals. Intact and gonadectomized male and female sheep of mixed breeding were used to provide cavernous sinus serum by the method of McFarland, Clegg and Ganong(5). Between 50 and 250 ml of blood were collected in non-sterile citrated syringes from unanesthetized animals. All donor animals provided several aliquots of blood throughout the experiment. In the case of blood for FSH determinations, serum from each of 2 donor ewes was pooled to provide sufficient material for injection into 5 rats per pooled sample. After clotting at 4°C the blood was centrifuged at 6-8,000 rpm for 10 minutes at 0°C, the serum aspirated and stored in the deep freeze until assayed for LH and FSH. Pituitaries were taken at slaughter, the posterior lobe removed and the gland trimmed of excess connective tissue. After weighing to the nearest 1.0 mg they were immediately placed on dry ice. All pituitary glands were lyophylized and stored in a desiccator until assayed.

Bioassays. FSH was determined by the HCG augmentation method of Steelman and Pohley(6). Twenty-one- or 22-day-old immature female rats of the Sprague-Dawley strain were injected twice daily for 3 days and autopsied 12 hours ± 1 hour after the last injection. Forty IU of HCG were used as the augmenting dose. A minimum of 5 rats per group was used throughout. In each assay a standard preparation of FSH (NIH FSH S-1) was tested at 3 dose levels (50, 100 and 150 μg) for serum or 4 dose levels (40, 80, 160 and 320 μg) for pituitaries. The unknowns were tested at a single dose level, either 36 ml of serum or 20 mg of pituitary powder homogenized in 3.0 ml physiological saline.

LH was assayed using immature female Sprague-Dawley rats by the ovarian ascorbic acid depletion (OAAD) method of Parlow (7). A minimum of 5 rats per treatment group was used throughout. The test substance, either 2.0 ml serum or 0.1 mg pituitary powder in 0.5 ml physiological saline, was injected over a 45 to 60 second period into the lateral tail vein of the lightly anesthetized rat. Four hours ± 10 minutes later the left ovary only was removed and weighed on a torsion balance to the nearest 0.2 mg. The use of only one ovary eliminates dilution of the phosphoric acid homogenate and gives better agreement between duplicate readings. Ascorbic acid was determined by the method of Mindlin and Butler(8). Each assay used 2 dose levels of a reference preparation (NIH LH S-7) with a 4-fold interval between successive doses. The levels used were 0.8 and 3.2 μg/100 g body weight of the assay animal.

Results. Amounts of FSH ranging from approximately 1.0 to 1.5 μg equivalents NIH LH S-1 per ml of serum were present in the serum of gonadectomized sheep. No activity could be detected in 36 ml of serum from intact animals (Fig. 1). The difference in FSH activity between ovariectomized females

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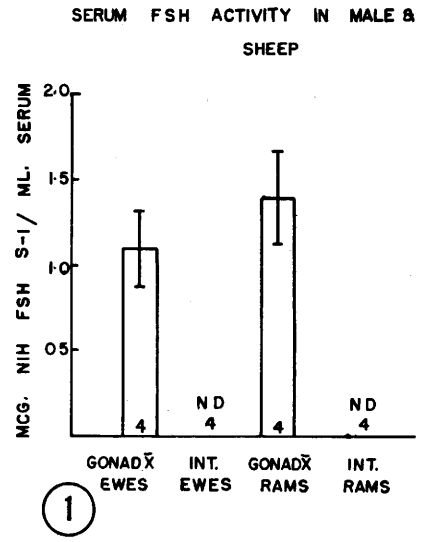
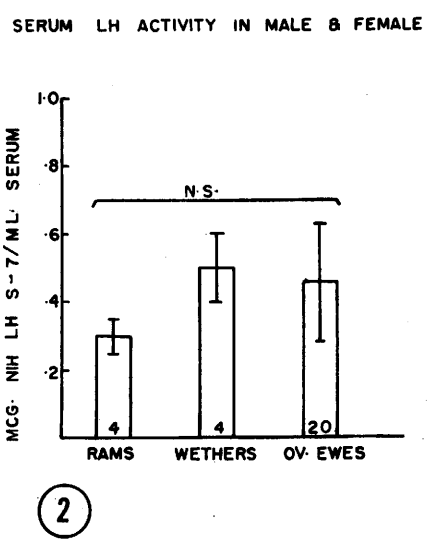
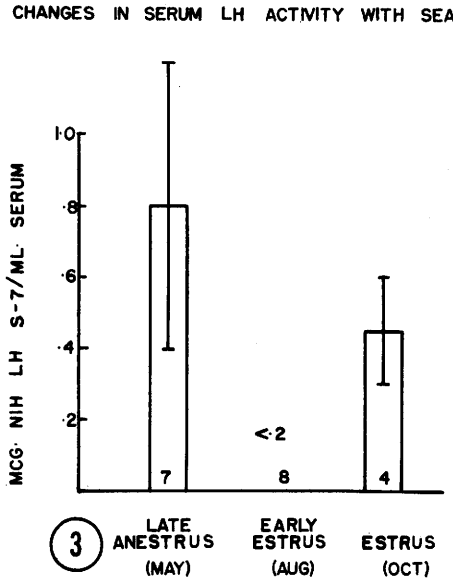
and orchidectomized males (wethers) was not significant.

Serum LH activity in intact and gonadectomized male sheep and ovariectomized ewes is shown in Fig. 2. The ram and wether samples were collected during anestrus (March). Blood samples from the ovariectomized ewes were collected at different times of the year (March, August and October). Since, in these samples, no seasonal trends in serum LH levels were evident, these data were pooled. There were no significant differences in serum







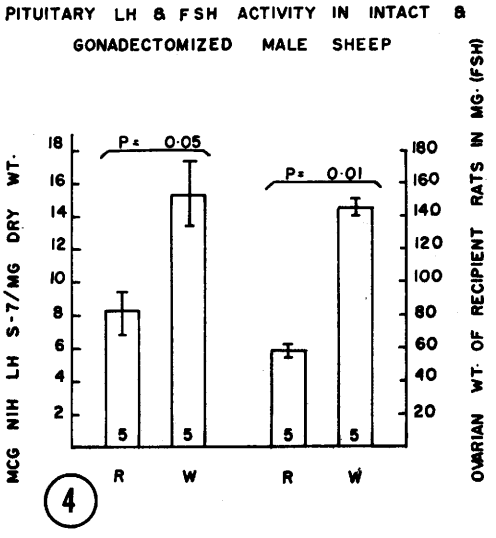


FIG. 1. Bars represent means and vertical lines standard errors. Numbers at base of bars indicate number of donor sheep. N.D. = non-detectable; Wethers = gonadectomized male sheep.

FIG. 2. Bars represent means and vertical lines standard errors. Numbers at base of bars indicate number of donor sheep. Wethers = gonadectomized male sheep; N. S. = not significant.

FIG. 3. Bars represent means and standard errors. Numbers at base of bars indicate number of donor sheep.

FIG. 4. Bars represent means and standard errors. Numbers at base of each bar represent number of donor sheep. R = rams; W = wethers.

TABLE I. Pituitary Weight, LH and FSH Concentration in Intact and Ovariectomized Ewes with Hypothalamic Lesions.

Ewe No.	Position of lesion	Pituitary dry wt (mg)	LH* (μg/mg)	FSH† (μg/mg)	LH/FSH ratio
Intact					
113	Unilateral M.E.	96	7.6	7.6	.75
317	"	46	2.1	—	—
047	Bilateral O.C.	127	6.3	3.6	1.75
097	M.E.	90	5.6	4.1	1.37
315	M.E.	117	5.6	4.1	1.37
131	M.E.	108	8.2	2.7	3.04
Mean ± S.E.		97.3 (11.7)	5.6 (.8)	4.4 (.8)	1.66 (.38)
Ovariectomized					
70	Unilateral M.E.	222	19.5	37.0	.53
54	M.E.	283	8.3	30.0	.28
011	M.E.	247	10.7	10.9	.98
231	Bilateral O.C.	139	8.2	55.0	.15
267	M.E.-O.C.	117	8.1	37.0	.22
73	O.C.	290	6.7	25.0	.27
Mean ± S.E.		216.3 (29.8)‡	8.4 (1.9) n.s.	31.6 (5.9)‡	.26 (.13)‡

* μg equivalents NIH LH S-7.
† μg equivalents NIH FSH S-1.
‡ P = <0.01.
n.s. = not significant.
M.E. = Median eminence.
O.C. = Optic chiasm.

LH activity among the 3 groups of sheep.

Seasonal changes in serum LH activity in intact ewes are shown in Fig. 3. The samples were collected on the same day of the month at each of the times shown. Serum LH activity in all 8 ewes during the early breeding season (August) was below the sensitivity of the assay. The levels observed during May ranged from 0.4 μg equivalents NIH LH S-7 to 1.2 μg equivalents NIH LH S-7 per ml serum and were higher than those in October (0.3 μg equivalents NIH LH S-7 to 0.6 μg equivalents NIH LH S-7 per ml serum). These differences were not significant due to the large variations between sheep. In contrast to FSH, significant differences in LH between intact and ovariectomized ewes were not observed.

Table I shows pituitary gonadotropin data from ewes with hypothalamic lesions. Inspection of the values for both LH and FSH indicates that the position of the lesion did not affect the concentration of hormone in the pituitary gland. Pituitary content of ewes bearing unilateral and optic chiasm lesions were not different from those with bilateral median eminence lesions. These data are in agreement with previous work on the ewe from this laboratory(9) in which, in the absence of gonadal atrophy, hypothalamic lesions did not affect the pituitary concentration of gonadotropins. Furthermore, serum LH data and ovarian histology on the intact ewes (McDonald and Clegg, unpublished observations) indicated normal levels of circulating LH and FSH.

Ovariectomy in the ewe leads to an increase in size of the pituitary gland and to a highly significant increase in concentration of pituitary FSH. However, there was no significant difference in pituitary LH concentration. The marked increase in pituitary FSH led to a significant change in the LH:FSH ratio in the pituitary gland.

Fig. 4 shows pituitary LH and FSH data obtained from intact and gonadectomized male sheep. Since the data for pituitary FSH could not be quantitated in terms of NIH FSH standard owing to the poor slope of the standard curve for this assay only the ovarian weight response is shown. There was a significant rise in both pituitary LH and FSH following castration; the increase was significant at the 5 and 1% levels, respectively. These data do suggest that, in contrast to the female, pituitary content of LH is increased following gonadectomy in male sheep.

Discussion. The rise in serum FSH activity (Fig. 1) consequent to gonadectomy in the sheep has also been noted in male and female rats(3), female mice(1) and post-menopausal

women(10). However, the increase in the ewe appears to be quantitatively smaller compared to that in the rat and mouse(1,3).

The response of circulating LH to removal of the gonads appears to be less consistent than that of FSH. Increases have been found to occur in rats(3) and humans(10). However, the mouse does not respond to ovariectomy with increasing serum LH levels(1); in this respect it appears to be similar to gonadectomized male and female sheep.

The significance of the changes in serum LH activity in the intact ewe with the time of year is not fully understood. Based on total gonadotropin assays of pituitary material, other work(11,12) also indicated a slightly higher potency during the non-breeding or anestrus part of the year.

An increase in gonadotropic potency of the pituitary gland following gonadectomy is known to occur in a number of species. Early work(13,14) however, did not differentiate between the LH and FSH principles. Parlow (15) has clearly shown that, in the rat, the increase in potency noted following gonadectomy can be solely due to an increase in one gonadotropin, a similar observation has also been made in the mouse(1). In the ewe it would appear that the increase in potency of the pituitary gland following ovariectomy is due almost entirely to an increase in FSH concentration, whereas in the male both the LH and FSH concentrations increase. Not all species appear to react to gonadectomy identically. Recent work in the sow(4) indicates that increases in both LH and FSH occur following ovariectomy. As data from the rat, mouse, sow and sheep were all obtained using the same assay methods and quantitated with NIH standard hormone preparations, the results are directly comparable. Only in the case of the sheep, however, has it been possible to quantitate the results with a standard preparation from the same animal. The apparent difference in the effect of gonadectomy among species on gonadotropin levels is difficult to interpret. One explanation could be species differences in gonadotropins reflected in the response of the assay animal.

Summary. LH and FSH activities have been determined in blood and pituitaries obtained from intact and gonadectomized male and female sheep. Serum LH activity is not altered in this species by either castration or ovariectomy. FSH activity in the blood increases following either operation. The increase in potency of the ewe pituitary following ovariectomy is due almost entirely to an increase in FSH concentration. In the male both LH and FSH levels increase. Changes in serum LH activity occur in the intact ewe which appear to be related to the time of year and particularly to the onset of cyclic activity.

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