Gonadotropic and Antigonadotropic Activity of Ewe Serum Following Chronic Treatment with Human Chorionic Gonadotropin (HCG)[1] (35526)

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Previous studies have shown that injections of HCG as an antigen into mares resulted not only in high anti-HCG titers but also an increased secretion of follicle-stimulating hormone (FSH) (1). On the contrary, preliminary studies on sera obtained from a ewe following the injection of HCG as an antigen, resulted in an increased secretion of luteinizing hormone (LH), but not FSH (2). LH activity, as judged by both the ventral prostate weight (VPW) and ovarian ascorbic acid depletion (OAAD) bioassays was increased in serum samples obtained from this ewe following immunization. The present study was conducted to further study the effect of anti-HCG on gonadotropin secretion, ovarian morphology, and the occurrences of estrus in ewes.

Methods and Materials. Three ewes less than a year old at the beginning of the experiment received three series of immunizing injections of HCG from Oct. 27, 1965 to Dec. 6, 1965, and from Jul. 11, 1966 to Aug. 19, 1966, and from Nov. 1, 1967 to Dec. 11, 1967. Each ewe was injected subcutaneously with 3160 IU of HCG, once weekly combined with an equal volume of Freund's adjuvant and twice weekly in saline alone. Jugular blood was collected from the three ewes before, during, and for about 10 weeks following immunization with HCG. The blood was allowed to clot overnight at room temperature and was centrifuged the next morning and the serum collected. The serum was stored at 4° until assayed.

Selected serum samples obtained following each series of injections were assayed for anti-HCG activity according to the procedure described by Snook and Cole (3). These same serum samples were also tested for LH activity using the VPW assay described by Greep *et al.* (4). Selected samples obtained following the first two series of injections and all the samples collected before, during and following the third series of injections were assayed for LH activity by radioimmunoassay. The radioimmunoassay procedure, which employs an antiserum to bovine LH, has been described elsewhere (5). Previous studies by Goding *et al.* (6) and ourselves indicate that this antiserum and procedure are equally suitable for measuring bovine and ovine LH levels in serum. NIH-LH-S11 was used as a standard and the results of the radioimmunoassays are expressed in terms of this preparation. Selected samples were also tested for gonadotropic activity in female Long-Evans rats as described by Snook and Cole (1).

The ewes were checked twice daily for the occurence of estrus with a vasectomized ram, beginning on Sept. 15, 6 weeks before the start of the third series of injections and extending until the end of January. All the other ewes in the University flock had ceased to show signs of estrus by the end of January. Ten days following cessation of the third series of injections, the three ewes were laparotomized and the diameters of the largest follicles and copora lutea, if present, were measured on each ovary. The diameter of each uterine horn was also measured.

Results. Antigonadotropic activity. Although anti-HCG activity could be detected 16 days following cessation of the first series of injections, the titers were low, less than 40

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TABLE I. Effect of Various Doses of Serum Collected from the Three Ewes Following Each Immunizing Series of Injections with HCG on Ventral Prostate Weight in Hypophysectomized Sprague-Dawley Rats.

Date serum collected	Time collected after last injection of the series		Dose of serum (ml)	No. of rats/group	Ventral prostate wt (mg)		
	Series	Days			Ewe: 5C95	4H58	4H14
12/22/65	1	16	None	6		7.4	
			8	6	8.4[a]	8.5	9.2[c]
9/19/66	2	31	None	7		6.9	
			2	6	7.4	7.8	6.5
			8	6	10.8[b]	9.5[b]	7.5
12/20/67	3	9	None	6		8.8	8.4
			1	5	12.3[b]	11.9[b]	10.2[a]
			4	6	15.0[c]	17.4[c]	12.0[c]
12/27/67	3	16	None	7		8.4	
			4	7	12.5[c]	12.3[c]	12.8[c]

[a] Compared to noninjected controls: $p < .05$; [b] $p < .01$; and [c] $p < .001$.

rat units[3]/ml for ewes 5C95 and 4H58 and less than 20 rat units/ml for ewe 4H14. Anti-HCG titers of each ewe increased following the second series of injections, but the degree of increase was quite variable. Anti-HCG titers for ewes 5C95 and 4H14 were greater than 400 and 100 rat units/ml, respectively, while the anti HCG titers of ewe 4H58 were greater than 50 but less than 100 rat units/ml. Following the third series of injections, ewes 4H58 and 5C95 had anti-HCG titers greater than 400 but less than 1000 rat units/ml, while ewe 4H14 had anti-HCG titers greater than 200 but less than 400 rat units/ml.

Antisera from the three ewes collected 9 days following the third series of injections were tested for antiovine LH (NIH-LH-S11) activity in the VPW assay and proved negative. Instead, they produced significant increases in ventral prostate and seminal vesicle weights compared to the rats receiving ovine LH alone. Serum collected prior to HCG injection had no effect on weights of the accessory organs.

Gonadotropic activity. Antisera collected from ewes 5C95 and 4H14 following the first series of injections produced slight, but significant, increases in ventral prostate weights (Table I). A greater increase in ventral prostate weight was obtained with antisera collected, from ewes 5C95 and 4H58, 31 days following cessation of the second series of injections than after the first series of injections. All doses of antisera tested following the third series of injections produced significant increases in ventral prostate weights. The degree of ventral prostate stimulation for each ewe was greatest following the third series of injections.

To determine whether the ventral prostate stimulating activity in the antisera was due to LH, serum samples collected from each ewe before, during, and at various times after immunization with HCG were assayed for LH by radioimmunoassay. Serum LH levels were found to increase following each series of injections, in agreement with the results obtained by VPW assay (Table II). Although LH levels were elevated above basal LH levels following the first and second series of injections, they did not reach preovulatory LH levels (>75 ng/ml) until after the third series of injections. Before the start of the third series of injections, LH levels had declined from the levels obtained after

[3] A rat unit of anti-HCG is defined as the reciprocal of the smallest dose of antiserum producing a statistically significant decrease in the uterine weight response to 20 IU of HCG (3).

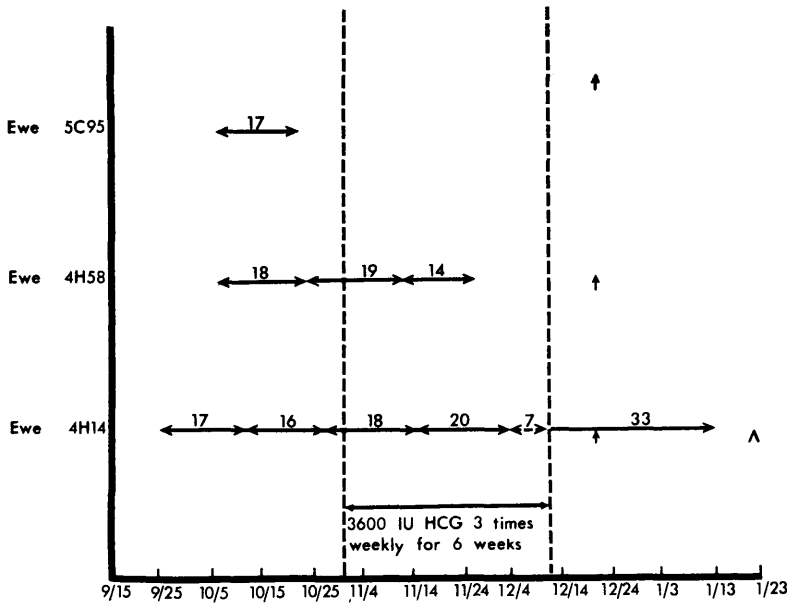

FIG. 1. The occurrence of estrus for each of the three ewes before, during, and after the third series of immunizing injections with HCG: (broken vertical lines) the beginning and the end of the third series of injections; (horizontal arrows) the day each ewe was observed in estrus; (nos. between arrows) length of estrous cycle (days); and [(- -) between two arrows for ewe 4H14] constant estrus. Laparotomies were carried out at times indicated by vertical arrows and mammary development and enlarged vulva were noticed in ewe 4H14 at the time denoted by arrow head.

the first and second series of injections, but were still higher than preinjection levels. LH levels rose during the period of immunization, reaching a peak at either 9 or 16 days following cessation of treatment, and then declined.

One, 2.5, and 5 ml of antisera collected from ewe 5C95, 9 and 23 days following the third series of injections were injected into immature female Long-Evans rats, but only the 1-ml dose level resulted in uterine weights significantly different from the control group. There was no effect on ovarian weight. Serum of ewe 5C95, collected prior to HCG, failed to influence ovarian and uterine weights of rats receiving 5 ml of this serum.

Occurrence of estrus in immunized ewes. Each ewe had at least one cycle of normal length before the start of the third series of injections (Fig. 1). Ewe 5C95 showed no further signs of estrus during the remainder of the experiment, while ewe 4H58 had one cycle of normal length, followed by a slightly shortened cycle, and then stopped cycling.

TABLE II. LH Activity of Serum Samples from Each Ewe Collected Before, During and at Various Times After Immunization with HCG as Determined by Radioimmunoassay.

Date serum collected	LH (ng/ml)[a] Ewe: 5C95	4H58	4H14
10/27/65[b]	2.3	3.1	1.5
1/ 5/66	9.5	11.5	23.5
9/19/66	25.8	26.5	28.5
11/ 1/67	18.0	6.8	10.0
11/15/67	61.0	70.0	37.5
11/29/67	58.0	68.5	68.0
12/13/67	88.0	>160	72.0
12/20/67	125.0	>160	73.5
12/27/67	70.0	>160	85.0
1/ 5/68	70.0	128.0	68.0
1/17/68	61.5	77.0	56.5
1/31/68	62.5	62.5	39.0

[a] Expressed in terms of ng of NIH-LH-S11/ml.
[b] These samples were taken before ewes received any injections.

On the other hand, ewe 4H14 had two normal cycles and was then in constant estrus for a period of seven days; thereafter she did not return to estrus for a period of 33 days.

Ovarian and uterine morphology. No corpora lutea, or signs of recent ovulations, were noted in ewes 5C95 or 4H58; both ewes had one or more follicles 9 mm in diameter plus several smaller follicles greater than 3 mm in diameter when laparotomized 10 days following cessation of the third series of injections. However, ewe 4H14 had two corpora lutea judged to be 8–12 days old on the left ovary, as well as several follicles greater than 3 mm in diameter. The diameter of each uterine horn of ewe 4H14 was larger than those of ewe 5C95, or ewe 4H58. Six weeks following cessation of the third series of injections, ewe 4H14 showed a greatly enlarged vulva and extensive mammary development. The mammary gland was actively secreting milk.

Discussion. Although LH levels (determined by radioimmunoassay prior to HCG injections) were within the range of basal LH levels reported by Geschwind and Dewey (7) and Goding *et al.* (6), LH levels following immunization were considerably higher, as indicated by both VPW and radioimmunoassay. In fact, following the third series of injections, LH levels reached values as high as preovulatory LH levels found in the sheep by other investigators (6–9). The increase in uterine weight obtained in intact immature female rats also indicates that the antisera contained gonadotropic activity. The increased secretion of LH found following immunization of ewes with HCG is in agreement with the preliminary studies of Cole *et al.* (2) and, more recently, the study of Ahren, Cole, Geschwind, and Itze (unpublished data).

The sequence of events leading to the increased secretion of LH was probably different from the events leading to the increased secretion of gonadotropic activity in mares reported by Snook and Cole (1), since no increase in FSH activity was observed in the ewes. The postulated sequence of events leading to the increased secretion of LH in ewes is as follows: The anti-HCG present in the blood blocks the action of LH on its end organ, thereby interfering with normal gonadal hormone secretion. Alteration in gonadal hormone secretion, in turn, leads to an increase in LH. The observation that two ewes failed to return to estrus following immunization and lacked functional corpora lutea, while the other ewe failed to show signs of estrus for 33 days, suggests that gonadal hormone secretion was altered in these ewes. It is very unlikely that the increased secretion of LH was due to the HCG injected as an antigen, since Wheatly and Radford (9) using our antiserum failed to observe any cross-reaction with HCG in their radioimmunoassay for ovine LH.

The increase in ventral prostate weight in the hypophysectomized rat caused by the antiserum indicates that the antiserum does not block the action of LH on its end organ in this animal. This difference in response between the two species may be a reflection of the fact that the concentration of the injected anti-HCG in the blood of the hypophysectomized rat is always much less than it is in the ewe. The enlarged vulva, larger uterine horns, mammary development, and constant estrus, observed in ewe 4H14 having the lowest anti-HCG titers following the third series of injections, suggests that the ability of the antiserum to block the action of LH on its end organ is changed when anti-HCG titers are lower. The anti-HCG titers were not sufficient to block the action of LH on the ovary in this ewe; therefore, LH was able to stimulate estrogen secretion.

The ewe (4H14) with the lowest anti-HCG titers after the third series of injections, also had the lowest level of LH, as determined by radioimmunoassay. It was also observed that when LH levels were lowest, anti-HCG titers were the lowest, and when anti-HCG titers increased, LH levels were also found to increase. Thus, in our experience, there appears to be a direct relationship between anti-HCG titers and LH levels. Snook and Cole (1) found that high gonadotropic levels were not always associated with high anti-HCG titers in mares.

Although all three ewes had high titers of anti-HCG activity following the third series of injections, the antisera from these ewes

lacked antiovine LH activity. This is not surprising since these antisera were found to contain high levels of LH. This would seem to indicate that the blockage of estrus and ovulation in these ewes was was not due to a lack of LH, but probably due to the ability of the antisera to block the action of LH on the ovary, even though it failed to neutralize all the circulating LH. However, it would appear that the antisera were not able to completely block the action of LH on the ovary, since all three ewes had several follicles greater than 3 mm in diameter.

If, as we have postulated, the antiserum neutralizes the action of LH at the ovary by forming an antigen–antibody complex, then peripheral LH levels would probably not represent the effective level of LH within the ovary. Another possible interpretation of these results is that high levels of LH act to inhibit the release of LH-releasing factor (LH-RF) from the hypothalamus through the short feedback loop, thereby preventing the preovulatory LH surge and blocking estrus and ovulation.

Summary. Three ewes were immunized with HCG. All ewes had high anti-HCG titers (>200 rat units/ml) and elevated serum LH levels as determined by ventral prostate weight assays and radioimmunoassays following the third series of immunizing injections. A significant increase in uterine weight was produced by the antisera in intact immature female rats. The antisera failed to neutralize the biological activity of ovine LH in the ventral prostate weight assay, but the antisera were able to block the occurrence of estrus to varying degrees in all ewes and blocked ovulation in two of the ewes as judged by the absence of corpora lutea at the time of laparotomy. One of the ewes showed an enlarged vulva and mammary development 6 weeks following cessation of the third series of injections. It is postulated that the elevated serum LH levels and blockage of estrus and ovulation are due to the ability of the anti-HCG to block the action of endogenous LH on its end organ, thus altering the secretion of the gonadal hormones.

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