nuclear volumes. This could be due to: (a) factors which affect the sizing sensitivity of the instrument, or (b) the fact that nuclear size is not completely related to polyploidy, but can be partially changed by other nuclear constituents such as proteins (unpublished data Muramatsu).

Summary. A procedure is described that allows rapid counting and sizing of liver nuclei. A good correlation exists between the degree of liver polyploidy and nuclear size. Both of these parameters increase as a function of age.

1. Waterlow, J. C., Cavito, J., and Stehen, J. M. L., Advan. Protein Chem. **15**, 131 (1960).

2. Jacob, M., Mandel, L., and Mandel, P., Experientia **10**, 218 (1954).

3. Kompmann, M., Paddags, I., and Sandritter, W., Arch. Pathol. **82**, 303 (1966).

4. Lapam, L. W., Science **159**, 310 (1968).

5. Epstein, C. J., Proc. Natl. Acad. Sci. U.S. **57**, 327 (1967).

6. Blobel, G. and Potter, V. R., Science **154**, 1662 (1966).

7. Allison, J. B., Wannemacher, R. W., Jr., Banks, W. L., Jr., and Wunner, W. H., J. Nutr. **84**, 383 (1964).

8. Wannemacher, R. W., Jr., Cooper, W. K., and Yatvin, M. B., Biochem. J. **107**, 615 (1968).

9. Brecher, G., Jakobek, E. F., Schneiderman, M. A., Williams, G. Z., and Schmidt, P. J., Ann. N. Y. Acad. Sci. **99**, 242 (1962).

10. Enesco, M. and Leblond, G. P., J. Embryol. Exptl. Morphol. **10**, 531 (1962).

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Radioimmunoassay of Serum Levels of Luteinizing Hormone during the Cycle and Early Pregnancy in Ewes* (33454)

GORDON D. NISWENDER[1], JAMES F. ROCHE, DOUGLAS L. FOSTER, AND A. REES MIDGLEY, JR.[2]

Department of Pathology, The University of Michigan, Ann Arbor, Michigan 48104; and Department of Animal Science—Genetics, University of Illinois, Urbana, Illinois 61801

Studies concerning the role of luteinizing hormone (LH) in different reproductive processes in sheep have been limited by the inability to measure accurately this hormone in small aliquots of blood. The recent development of a specific and highly sensitive radioimmunoassay for ovine LH (1) made it possible to determine circulating blood levels of this hormone. This should lead to a better understanding of the endocrinology of the estrous cycle and of early gestation. The present study was conducted (a) to determine the circulating levels of LH during these two periods, and (b) to critically define the time relationships between the onset of estrus and the peak of LH presumably responsible for ovulation.

Materials and Methods. A preliminary experiment was conducted to determine what method was best to obtain maximal yields of serum with a high LH content. Jugular blood was collected from three ovariectomized ewes and 15-ml aliquots were allowed to stand either at room temperature or at 4° for different periods of time. The serum was then obtained by centrifugation, frozen, and stored for subsequent LH assay.

In a second experiment, 10 ewes, which had exhibited at least one normal estrous cycle, were bled daily for 20 days. In addition, 10 ewes which had their estrous cycles synchronized with progestogen impregnated silicone implants (2) were mated at the second estrus following implant removal and were bled once daily for 20 days beginning the day after estrus. Six of these ewes were confirmed to be pregnant by laparotomy and

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TABLE I. Luteinizing Hormone Content of Blood Allowed to Stand at Room Temperature or at 4° for Different Time Intervals.

Ewe no.	Stored (hr)	Room temp	4°
1	3	—[a]	2.3[a]
2	3	12.0	9.4
3	3	7.0	6.4
1	6	1.9	2.3
2	6	9.8	9.9
3	6	6.4	6.5
1	23	2.0	1.7
2	23	9.3	9.4
3	23	6.6	6.2

[a] mμg of NIH-LH-S12 per ml of serum.

examination of the reproductive tract 22 days postmating.

All ewes in this experiment were penned daily between 1 and 2 pm and were checked for estrus using vasectomized rams fitted with marking harnesses. Fresh paint on the rump of a ewe or observed mating were the criteria used. Fifteen to 20 ml of blood were obtained daily from the jugular vein of each ewe. The blood was allowed to clot, serum was obtained by centrifugation, frozen, and stored at —20° until assayed for LH.

In the third experiment, estrus was induced in 13 anestrous ewes with a progestogen implant and pregnant mare serum gonadotropin. Blood was withdrawn at 4-hr intervals for a period of 24 hr beginning 22 hr after implant removal. Estrus was checked in these ewes every hour during the bleeding period. Serum was obtained as described in the second experiment.

The methods used for radioimmunoassay have been described in detail (1). NIH-LH-S12 was used as a standard and all results are expressed in terms of this preparation.

Results and Discussion. Results of the first experiment are shown in Table I. Neither the temperature, nor the time for which the blood was allowed to stand before centrifugation, appeared to influence the level of LH detected by radioimmunoassay.

Results of the second experiment are depicted graphically in Fig. 1. Of the 10 ewes which exhibited estrus during the period of daily bleedings, 7 had elevated serum levels of LH (mean 28.6 mμg NIH-LH-S12/ml; range 6.0–82.5) within 24 hr of the time estrus was first observed. Increased levels of LH were not observed in three ewes which had shown estrus. In the other 193 serum samples collected from these 10 ewes, 54 contained LH levels between 0.5 and 2.0 mμg/ml and LH was not detectable (<0.5 mμg/ml) in the remaining samples.

In the 6 pregnant ewes LH levels did not differ, within the limits of sensitivity of this assay, from the basal level detected in cyclic ewes during the 20-day bleeding period. Only 24 of 120 serum samples collected had LH levels greater than 0.5 mμg/ml with the highest observed level being 6.6 mμg/ml on the day after estrus in one ewe.

Some evidence suggests that LH plays a major role in maintenance of luteal function in the ewe. Continuous infusion of LH into ewes hypophysectomized on day 12 (3) or into intact ewes (4) extended the life span of the corpus luteum to days 20 and 30, respectively. LH has also been shown to stimulate the synthesis of progesterone in ovine luteal tissue incubated *in vitro* (5). Thus, it might be expected that the pregnant ewe would have detectable blood levels of LH. However, our finding that LH levels are very low in the serum of pregnant ewes does not appear to support this supposition. This study provided no conclusive evidence regarding the role of LH in the regulation of the corpus luteum. Further studies regarding how LH is metabolized and utilized, or both will be needed to ascertain how this hormone influences the life-span and secretory function of the corpus luteum.

Figure 2 contains the results of the third experiment which was designed to characterize the LH peak in relation to the onset of estrus. Six ewes exhibited estrus following implant removal. An LH peak was observed in all of these ewes within 12 hr after the onset of estrus. The mean peak value for these 6 ewes was 47 mμg/ml (range 25–73 mμg/ml). The duration of elevated serum levels of LH did not last more than 12 hr. This finding is in agreement with previous data (6–8) which indicated that a major decrease in pituitary

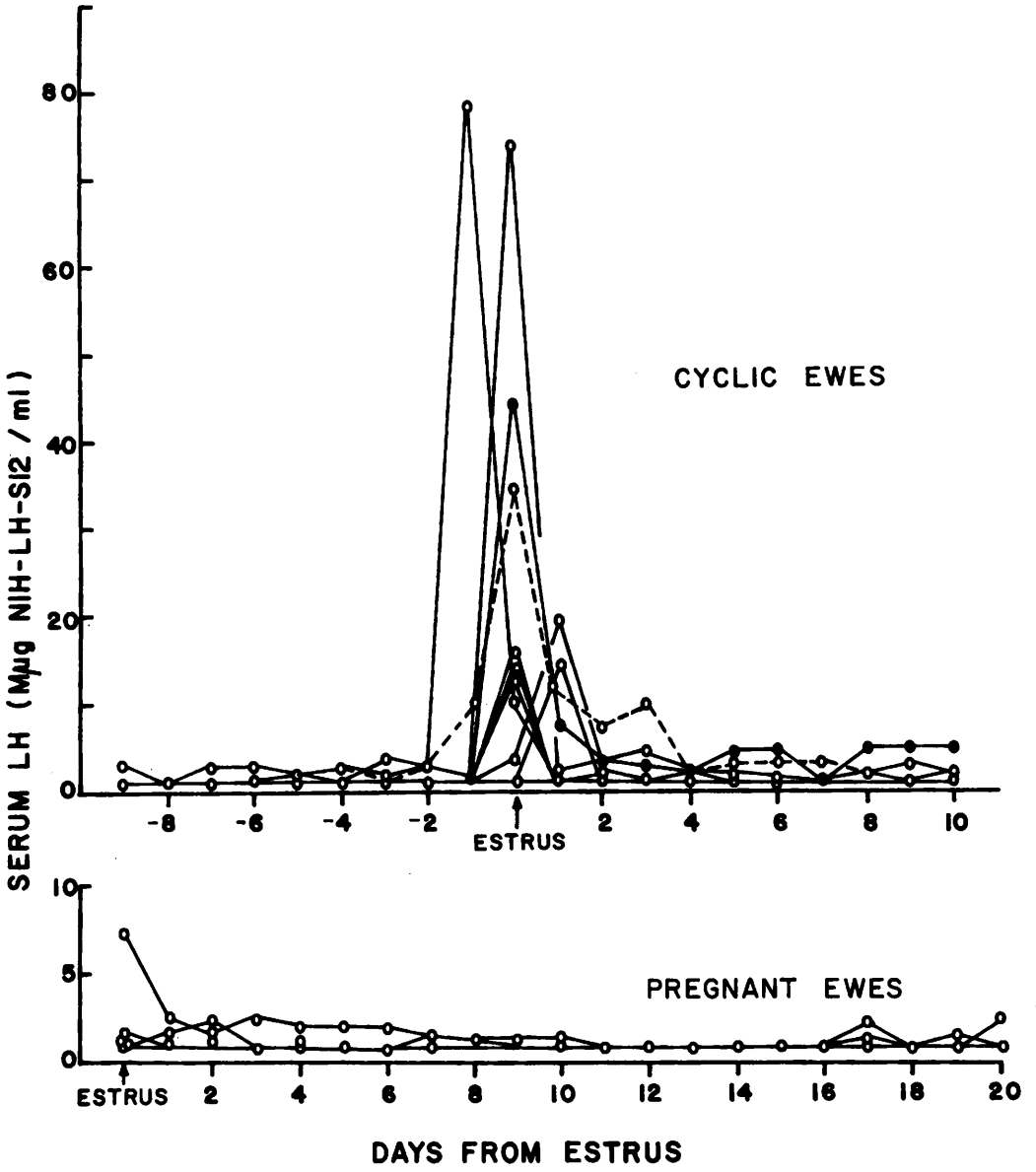

FIG. 1. Serum levels of luteinizing hormone during the cycle and the first 20 days of gestation in sheep.

LH content occurred from 4 to 36 hr after the onset of estrus. The latter workers (8) also suggested that a concomitant rise in serum levels took place between 5 and 8 hr after the first acceptance of the ram. The duration of the LH peak may explain why elevated LH levels were not observed in all ewes when they were bled once a day. When estrus was checked each hour, the LH peaks closely coincided with the onset of estrus, again suggesting that daily heat checks (as for Expt. 2) are not adequate for critical characterization of the LH release.

During these experiments serum samples were also collected from castrate females, castrate males, intact males, females 40 days pregnant, and hypophysectomized females. The results are shown in Table II. The LH

TABLE II. Luteinizing Hormone Content of Serum from Sheep in Various Endocrinologic States.

Endocrinologic state	No. of sheep	Av LH content[a]	Range[a]
Female (castrate)	4	4.8	(3.1– 5.7)
Male (castrate)	4	20.9	(10.5–31.5)
Male (intact)	2	2.0	(1.9– 2.1)
Female (intact, day 10)	10	<0.8	(<0.5– 1.2)
Female (6 weeks pregnant)	3	<0.6	(<0.5– 1.2)
Female (hypophysectomized)	2	<0.7	(<0.5– 1.3)

[a] mμg of NIH-LH-S12 per ml of serum.

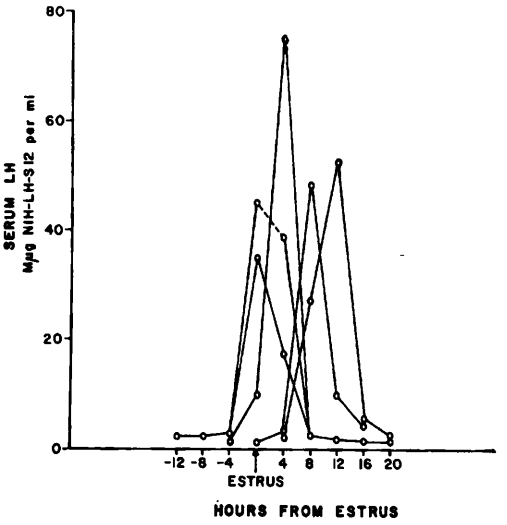

FIG. 2. Serum levels of luteinizing hormone at the time of estrus in sheep.

content of serum from castrate males was higher than in castrate females, although both were significantly elevated. Intact males had low levels of LH similar to those in intact females at times other than at estrus. LH was not detectable in serum from 2 of 3 ewes which were 40 days pregnant. These data are similar to those observed during the first 20 days of gestation. LH was not detectable in serum from one hypophysectomized ewe and a low level was observed in another.

Summary. Development of a radioimmunoassay for ovine luteinizing hormone (LH) has allowed determination of levels of LH in small sequential aliquots of serum obtained from ewes throughout the estrous cycle and during the first 20 days of gestation. Elevated levels of LH were noted on the day of estrus in cyclic ewes. When ewes were checked for estrus at hourly intervals and blood was collected every 4 hr, the LH peak (mean = 47 mμg/ml) was observed from 0–12 hr after the onset of estrus. The LH levels at other stages of the cycle were very low or nondetectable (<0.5 mμg/ml). The levels in ewes during the first 20 days and at day 40 of gestation were also very low or nondetectable. This finding was discussed in light of existing data suggesting that LH is responsible for maintenance of the corpus luteum of gestation. Serum content of LH of both males and females in various endocrinologic states was also reported.

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1. Niswender, G. D., Reichert, L. E., Jr., Midgley, A. R., Jr., and Nalbandov, A. V., Endocrinology, in press.

2. Dziuk, P. J. and Cook, B., Endocrinology 78, 208 (1966).

3. Kaltenbach, C. C., Graber, J. W., Niswender, G. D., and Nalbandov, A. V., Endocrinology 82, 818 (1968).

4. Nalbandov, A. V. and Karsch, F. J., Proc. Inter. Cong. Endocrinol., 3rd, Mexico City (1968) in press.

5. Kaltenbach, C. C., Cook, B., Niswender, G. D., and Nalbandov, A. V., Endocrinology 81, 1407 (1967).

6. Santolucito, J. A., Clegg, M. T., and Cole, H. H., Endocrinology 66, 273 (1960).

7. Robertson, H. A. and Hutchinson, J. S. M., J. Endocrinol. 24, 143 (1962).

8. Dierschke, D. J. and Clegg, M. T., J. Reprod. Fertility 15, 321 (1968).

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