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Serum LH and FSH Levels in the Pregnant Rabbit (40703)

KEVIN G. OSTEEN AND THOMAS M. MILLS

Department of Endocrinology, Medical College of Georgia, Augusta, Georgia 30912

Introduction. Early work in the rabbit, an induced ovulator, demonstrated that coital stimulation triggers a release of gonadotropin from the pituitary (1, 2). More recently, workers have shown that a sharp rise in serum luteinizing hormone (LH) concentration takes place as early as 15 min after mating with peak values by 2 hr and subsequently returns to precoital levels by 4 to 6 hr (3–7). Dufy-Barbe *et al.* (4) reported no concomitant increase in serum levels of follicle stimulating hormone (FSH) through the first 4 hr postcoitum. In contrast, Goodman and Neill (6) did report a significant rise in serum FSH by 2 to 3 hr after mating with a slightly elevated FSH value at 5 hr. Differences in assay sensitivity may possibly account for this discrepancy since the first group of workers (4) used a heterologous gonadotropin assay system (ovine-LH as standard), while the latter workers (6) utilized a system designed specifically for determination of rabbit gonadotropin levels. Despite these published studies of gonadotropin levels in the preovulatory period, little has been reported concerning the levels of these hormones during pregnancy in the rabbit. Scaramuzzi *et al.* (3) measured serum LH in pregnant rabbits at selected time periods and found that serum LH levels were not different from those found in the precoital period. Simultaneous measurements of both LH and FSH in the serum of rabbits throughout the entire period of pregnancy have not been reported.

As a part of ongoing studies from this laboratory on the function of the rabbit ovary, we have examined the levels of serum LH and FSH through pregnancy. We sought to define the gonadotropic support available to the ovary, especially over the first 5 postcoital days. During this time, the ovary is transformed from an estrogen-producing organ dominated by large follicles to a structure containing numerous corpora lutea synthesizing principally progesterone.

Materials and Methods. Animals. Sexually mature male and female white rabbits of the New Zealand strain (3.5–4.5 kg) were obtained from a local supplier and kept isolated from one another. Rabbits were maintained at constant temperature on a 14-hr light, 10-hr dark schedule with pressed food and water *ad libitum.*

Mating was accomplished by placing the doe in the cage of a male rabbit. The success of matings was confirmed by microscopic examination of vaginal washings for the presence of sperm.

Blood Samples. Sequential peripheral venous blood samples were drawn from the marginal ear vein of each rabbit immediately before and 1, 2, 3, 4, and 12 hr after mating. Additional samples were collected on Day 1 (24 hr after mating) and Days 2, 3, 4, 5, 8, 11, 14, 18, 21, 24, 27, and 31. These samples were allowed to clot on ice and serum collected centrifugally at 4°C. All serum samples were stored at −20°C until assayed for gonadotropin.

Gonadotropin Assay. Serum LH and FSH were measured in each serum sample by means of specific homologous double-antibody radioimmunoassays utilizing NIAMDD kits for rabbit gonadotropin. These kits included purified gonadotropin (NIAMDD-LH-AFP-559-B and NIAMDD-FSH-AFP-538-C) which were used both as standards and for iodination.

Radioiodination procedure. The purified LH and FSH preparations were radiolabeled with ^{131}I according to the chloramine-T procedure of Greenwood *et al.* (8). Labeled hormone was separated from unbound iodine on a column of Bio-Gel P-60. The radioactive hormone was diluted in 0.1% bovine serum albumin phosphate-buffered saline (BSA–PBS) at a concentration of 2 to 3 ng/100 μl containing 30,000 to 40,000 cpm.

Antisera. Antibodies against rabbit LH (AFP-8-1-28) and against rabbit FSH (AFP-2-7-1), had been raised in guinea pigs and were supplied with the kits. The anti-LH was

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diluted to 1:600,000 for use in the assay while the anti-FSH was diluted to 1:60,000. In both assays, the hormone–antibody complex was precipitated with a 1:200 dilution of anti-guinea pig γ-globulin raised in goats.

Assay procedure. The assays were performed over a 7-day period at 4°C. Duplicates of 200-μl serum samples were diluted to 700 μl with 1% BSA–PBS and then incubated for 24 hr in the presence of the specific antisera. The respective labeled hormones were then added in 100 μl of 0.1% BSA–PBS followed by another 24-hr incubation. The second antibody was then introduced in a volume of 200 μl and the incubation continued for an additional 4 days. After centrifugation, the supernatant fractions were discarded and the ^{131}I content of the precipitates counted in a Beckman gamma counter (model 310). Standard lines ran from 0.0625 to 4 ng for LH and from 0.0625 to 5 ng for FSH.

The average intraassay coefficient of variation was 6.8% for the duplicate samples. Statistical analyses were performed using Duncan's multirange test (9).

Results. Five mated rabbits delivered normal litters (average litter size 10.2) from 31 to 32 days after mating. All does prepared satisfactory nests and exhibited normal nursing behavior.

The pattern of LH in serum through the first 5 days of pregnancy is shown in Fig. 1. A statistically significant increase in serum LH was seen at 1 hr after mating with peak values by 2 hr ($P < 0.05$). The serum LH then dropped continuously, returning to estrous levels by 12 hr postcoitum. No further increase in serum LH was seen through the next 5 days of pregnancy in any of the does.

The pattern of FSH in serum is shown through the first 5 days of pregnancy in Fig. 2. Though considerable animal variation was evident, a significant increase in serum FSH was observed by 1 hr after mating ($P < 0.05$). FSH values reached a maximum at 2 to 3 hr postcoitum and then declined to precoital levels by 12 hr. In contrast to serum LH, a second statistically significant surge of FSH occurred ($P < 0.05$), reached a maximum at 24 and 48 hr postcoitum, and then declined to precoital levels by Day 3 of pregnancy. This second serum peak of FSH showed considerably less animal variation than the first FSH surge and was exhibited by all five pregnant animals.

The serum LH and FSH levels at various days through the duration of pregnancy are shown in Table I. No significant increase in the serum concentrations of either gonadotropin was seen from Day 8 of pregnancy to delivery.

Discussion. Many species, including the rabbit, are dependent upon pituitary gonadotropin support of the ovary throughout pregnancy. We have used homologous kits for rabbit gonadotropins supplied by the NIAMDD to measure serum LH and FSH throughout the duration of pregnancy in the species.

Serum LH rose from an estrous value of 0.6 ± 0.14 to 12.6 ± 1.8 ng/ml by 2 hr

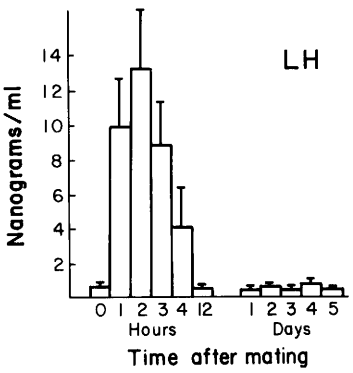

FIG. 1. Serum LH levels before and at various times after mating. Each bar represents the mean of duplicate determinations performed on sera from five different does. Brackets represent one SEM.

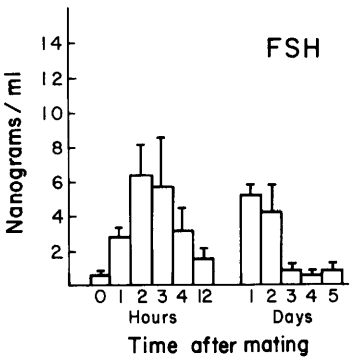

FIG. 2. Serum FSH levels before and at various times after mating. Each bar represents the mean of duplicate determinations performed on sera from five different does. Brackets represent one SEM.

TABLE I. SERUM LEVELS OF LH AND FSH AT VARIOUS DAYS THROUGH PREGNANCY IN THE RABBIT.[a]

Day of pregnancy	LH (ng/ml)	FSH (ng/ml)
8	0.63 ± 0.12	1.06 ± 0.08
11	0.74 ± 0.14	1.27 ± 0.08
14	0.45 ± 0.04	1.25 ± 0.10
18	0.41 ± 0.02	0.82 ± 0.08
21	0.42 ± 0.03	0.82 ± 0.07
24[b]	0.52 ± 0.08	1.4 ± 0.1
27	0.45 ± 0.04	1.4 ± 0.11
31[c]	0.42 ± 0.01	0.83 ± 0.16

[a] Values reported are mean ± SEM for sera from five animals.
[b] Represents sera from two animals.
[c] Represents sera from three animals.

postcoitum and gradually returned to precoital levels by 12 hr. This general pattern of LH release with mating has been reported by several investigators (3–7). Each of the pregnant animals in this study exhibited a similar rapid rise in serum LH through the preovulatory period. Measurements of serum LH at various days of pregnancy showed no further significant increases above the 12-hr level. This finding is in agreement with the study of Scaramuzzi *et al.* (3) in which pregnant rabbits at 14–15 or 24–25 days of pregnancy showed no elevated serum LH levels. Therefore, only tonic, but measurable levels of LH appear to be necessary for the maintenance of pregnancy in the rabbit.

Serum FSH values also exhibited a preovulatory surge in three of the five animals studied, rising from an estrous value of 0.6 ± 0.07 to 6.1 ± 1.4 ng/ml at 2–3 hr postcoitum. Serum FSH values then decreased to nearly precoital values at 12 hr after mating. The magnitude, duration, and animal variability seen in this FSH peak is similar to that reported by Goodman and Neill (6), who used a similar homologous FSH assay system. Following the preovulatory FSH peak, a second surge of FSH occurred at a period when serum LH was not elevated. A similar second peak of FSH, distinct from LH, has been shown to occur in rodents. Most recently, Chappel *et al.* (10), working with hamsters, demonstrated this rise of FSH on the first day of estrus after the proestrus surges of FSH and LH had returned to basal concentrations.

The significance of the postovulatory FSH surge in the rabbit may lie in the control of follicular development during this critical period of pregnancy or pseudopregnancy. Postovulatory follicle development in the rabbit is of special interest since the corpus luteum in this species is an estrogen-dependent tissue (11, 12). Specific receptors for estradiol-17β have been reported to be present in 3- to 4-day-old corpora lutea (13) with total estrogen dependence 1–2 days thereafter (14). The source of the luteotropic estrogen appears to be the large follicles present during this time (12). The growth and development of these "luteotropic follicles" to replace those which have ovulated may be the target of the postovulatory FSH surge.

Summary. Serum levels of LH and FSH were measured in the pregnant rabbit utilizing homologous assays for rabbit gonadotropins. Results show that following mating, serum LH rose to peak levels by 2 hr postcoitum, returned to estrous levels by 12 hr, and remained at low levels through the duration of pregnancy. Serum FSH also exhibited a preovulatory surge at 2–3 hr after mating, returning to precoital levels by 12 hr. In contrast to serum LH, however, a second surge of serum FSH occurred at 1–2 days of pregnancy at a time when serum LH was not elevated. Following the second peak, serum FSH also remained low through the remainder of pregnancy.

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