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Effect of Coitus on Serum levels of Testosterone and LH in Male and Female Rabbits[1] (38945)

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In the reflexly ovulating female rabbit, the coital stimulus initiates a preovulatory surge of luteinizing hormone (LH). Elevations in plasma LH stimulate the synthesis and release of ovarian steroids, and increased blood levels of progestin, estrogen, and testosterone can be measured coincident with the preovulatory rise in circulating LH (1–3). Although the hormonal events which transpire in the male rabbit as a result of sexual excitement are less well understood, striking increases in the blood levels of testicular androgens have been reported by Endröczi and Lissák (4), Saginor and Horton (5), and Haltmeyer and Eik-Nes (6) following exposure to females, ejaculation, or injection of human chorionic gonadotropin. LH was not assayed by these investigators, but the results have suggested that a reflex discharge of LH might likewise occur in the male rabbit.

In a previous study (3) which introduced a radioimmunoassay procedure for rabbit LH using guinea pig anti-ovine LH, we failed to support this concept of reflex LH discharge in the male. Whereas in a series of preliminary tests, serum LH levels in female rabbits increased significantly within 1 hr after mating, those in the male remained low and essentially unchanged. The present paper reports further investigations on the effects of copulation on LH discharge and T secretion in male and female rabbits.

Materials and Methods. Sexually mature, male and female New Zealand White rabbits (4–6 kg) were caged individually and maintained on Ace-Hi special rabbit pellets. Blood samples were collected by heart puncture from restrained, unanesthetized animals of both sexes. Serum hormone levels were measured by radioimmunoassays using a different aliquot of the same sample for each hormone. Column preparation and elution methods for T and estradiol have been previously described (2). LH was determined by the procedure of Scaramuzzi *et al.* (3), but instead of an antiserum to ovine LH, an anti-rabbit-LH serum generated in a guinea pig was used. Although this homologous antiserum at a dilution of 1/15,000 gave a 13% cross reaction with a rabbit TSH preparation (Parlow N-561-C), we did not consider this a serious problem since we had previously demonstrated the inability of endogenous TSH to interfere with LH radioimmunoassay in the rabbit (3). Moreover, the changes in serum LH obtained following mating in male and female rabbits in the present study fully confirmed those of our former study in which a specific anti-ovine-LH was used.

Purified rabbit LH (Papkoff Ex 1306 B) was iodinated as described by Scaramuzzi *et al.* (3). The accuracy of the assay was determined by the recoveries obtained when LH standard was added to hypophysectomized rabbit serum. Recoveries in picograms of the added LH were, respectively, as follows: 300:380; 450:440; 600:600; 750:700; and 900:850. Evaluation of the duplicate samples in the same assay showed an intraassay coefficient of variation of 5% (n = 32) and the interassay coefficient of variation (36 pool samples in 12 assays performed over a period of several months) was 16.5%.

Results. In male rabbits, basal serum levels of T varied widely, ranging from 131 to 12,149 pg/ml, and no consistent changes following coitus could be demonstrated (Fig. 1). The marked fall in serum T occurring at 5 hr after mating was attributed to the stress

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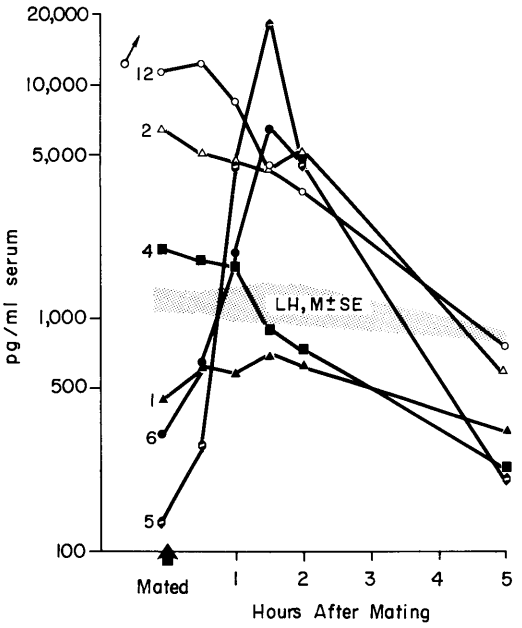

FIG. 1. Serum testosterone (T) and LH levels in mated male rabbits. Lines represent T levels of individual animals. Shaded area shows mean ± SE of LH values for the group in pg/ml serum. Note the logarithmic scale.

of the bleeding procedure since a consistent drop in serum T concentration occurred in eight isolated control males not exposed to females but bled at the same time intervals as the mated males. On the other hand, basal serum levels of LH in male rabbits were fairly constant, ranging from 888 to 1850 pg/ml (mean ± SE, 1221 ± 168) and no significant elevation was measurable in blood samples collected consecutively at .5, 1, 1.5, 2, and 5 hr postcoitum (M ± SE shown by stippled band in Fig. 1).

Because anestrous rabbits can be induced to mate and ovulate, and chronically ovariectomized rabbits can be brought into estrous behavior by estrogen priming (7), the effect of a similar estrogen-priming regimen was tested in male rabbits. In the six bucks tested, a priming dose of either 25 or 50 μg of estradiol benzoate (EB) was followed within 24 hr by a marked drop in serum T and ⅔ males which received the 50 μg doses of EB on Days 1 and 2 refused to mate on Day 3. Figure 2 shows the effects of coitus on serum T levels of the four estrogen-primed males which did mate with estrous females on Day 3, 24 hr after receiving a second priming dose of EB.

Figure 3 compares the concentrations of serum LH in mated untreated and mated estrogen-primed males with those of females. Although a slight rise in serum LH concentration could be detected during the first 2 hr after copulation in the estrogen-primed males, it was in no way comparable to the reflex LH discharge which occurs in female rabbits in response to the coital stimulus. In 12 intact does which ovulated as a consequence of mating, basal serum LH levels ranged between 1150 and 3300 pg/ml with a mean ± SE of 2352 ± 234, slightly higher than basal LH levels assayed in males (1993 ± 152). By ½ hr after mating, however, serum levels of LH in females, in marked contrast to those in males, had risen to a mean ± SE of 12,469 ± 1592 pg/ml and by 2 hr had peaked at 55,688 ± 7825 pg/ml, after which they gradually subsided to approach basal levels by 6 hr (mean ± SE, 2925 ± 825 pg/ml). On the other hand, in three does in which apparent copulation failed to induce ovulation, the mean level of serum LH (1825 ± 468 pg/ml) was similar to that of males and, as in males, remained essentially unchanged during the subsequent 6-hr period of blood sampling. Ten does included in this study had been ovariectomized 1–5 mo and were brought into estrous behavior by two priming doses of EB. Although the basal serum LH level (mean ± SE, 1685 ± 981 pg/ml) was comparable to that of the estrogen-primed intact group, postcoital elevations in serum LH were consistently lower and appeared to be less well sustained than those of intact mated does.

Blood T levels in mated female rabbits were reported earlier (2). In does, peripheral serum T concentrations (mean ± SE, 66 ± 17 pg/ml) were lower and less variable than those of bucks (mean ± SE, 3210 ± 2290 pg/ml), and they tended to increase three- to fourfold in response to the dramatic elevations in circulating LH levels which occurred following coitus.

Discussion. In our study, basal serum levels of T in male rabbits were found to vary widely from animal to animal, and

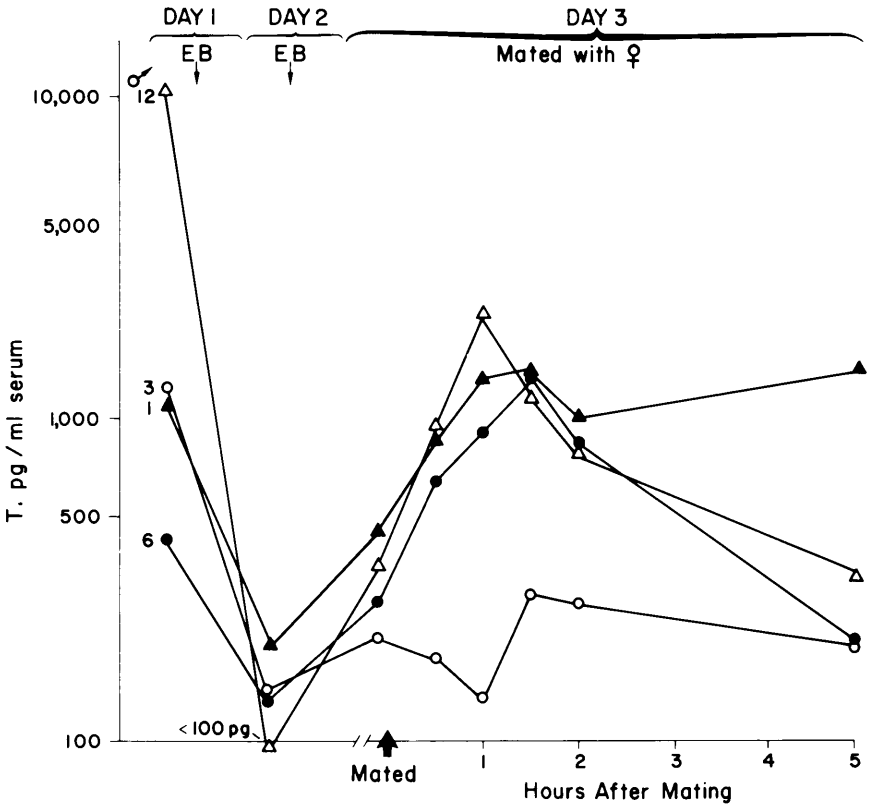

FIG. 2. Effect of two priming doses of estradiol benzoate (EB) on serum testosterone (T) levels in male rabbits. Males 1, 3, and 6 received 25 μg and male 12 received 50 μg EB sc on Days 1 and 2. All mated with estrous females on Day 3. Lines show T levels of individual animals. Note the logarithmic scale. Blood samples for assay were drawn at 9 AM immediately before EB injection on Days 1 and 2. The initial sample on Day 3 was likewise obtained at approximately 9 AM immediately before mating. Thus, there was a 24-hr interval between EB injection and the succeeding blood sample.

changes following copulation were inconsistent and appeared to be unrelated to measurable alterations in peripheral LH concentration. Similar wide variations in the resting blood levels of T in male rabbits were reported by Moor and Younglai (8) and these investigators, who were using a homologous LH radioimmunoassay comparable to ours, observed fluctuations in circulating LH values which appeared to precede or coincide with changes in plasma T. In a more recent study, Younglai, Moor, and Dimond (9) measured the LH and T levels of male rabbits in plasma collected from a central ear artery via an indwelling catheter. In general, exposure to females or coitus led to increases in T which were often preceded by a pulsatile rise in LH. However, since their results were not always predictable and because sampling from males in the absence of females also showed a pulsatile release of LH and T, they were unable to conclude that sexual excitement was responsible.

The spectacular fall in serum T which occurred in male rabbits within 24 hr after sc injection of EB without a significant change in serum LH (Figs. 2, 3) suggests that estrogen may act directly at the testicular level to suppress T synthesis. Chowdhury (10) noted a conspicuous decline in the levels of both pituitary LH and serum T in rats within 24 hr after a single 50 μg injection of EB, but was unable to detect significant changes in serum LH even after 4 days of treatment. Gomes and co-workers (11) recently reported that antispermatogenic agents which reduce testis weight and T secretion in rats do not influence serum LH

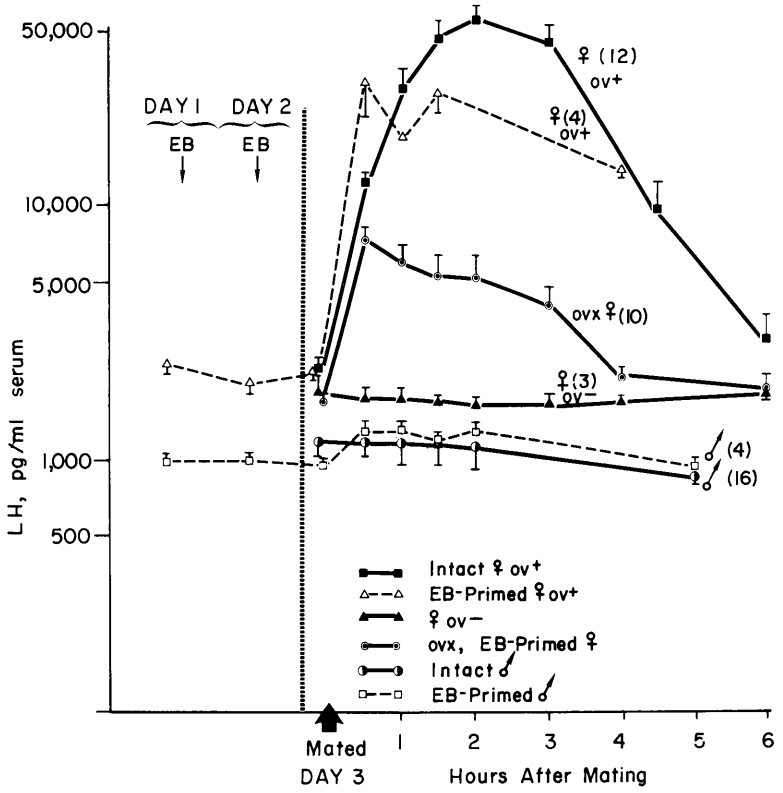

FIG. 3. Serum LH levels (mean ± SE) in mated male and female rabbits. Effect of estrogen priming in males and in intact and ovariectomized females. Number of animals/group shown in parentheses. The LH values of females which failed to ovulate following *apparent* coitus did not show postcoital rises. Note the logarithmic scale.

levels. The T surges occurring in three of the four mated estrogen-primed males (Fig. 2) appear to follow the same pattern as in Fig. 1; namely, that low basal levels of T tend to increase most following sexual excitement. This would suggest that initially high T levels may inhibit the response of the testes to further stimulation. Similar findings have been reported recently by other investigators (9, 12).

Our findings in male rabbits contrast sharply with the pronounced elevations in serum T and LH of female rabbits following coitus (2). In the doe the ovaries are not required for reflex LH discharge, although the lesser amounts of LH released by the mated, estrogen-primed ovariectomized does suggest that ovarian steroids may exert a modifying influence on LH synthesis and discharge (Fig. 3). In males this modifying effect of gonadal steroids is less apparent and the possibility that the dissimilarity in LH discharge between the sexes might be explained by a negative feedback action of T or a positive feedback effect of estrogen was not supported by the results obtained in estrogen-primed males in which circulating levels of T were drastically reduced (Figs. 2, 3). This could not be attributed to an unresponsive male pituitary, since injections of LH-RH (300 ng/kg iv) into either intact or orchidectomized male rabbits induce rapid elevations in pituitary LH discharge (unpublished).

Summary. Levels of testosterone (T) and LH in the peripheral serum of male and female rabbits were measured and compared following coitus. Blood was collected by heart puncture from restrained, unanesthetized animals of both sexes. In male rabbits, basal serum T levels were highly variable, ranging from 131 to 12,149 pg/ml, and if

low preceding coitus they tended to rise; whereas, if high, they usually dropped as they did in nonmated males subjected to repeated heart punctures. In contrast, basal serum LH levels in males were quite constant (mean ± SE, 1993 ± 152 pg/ml) and were not significantly altered after coitus unless blood T levels had been drastically lowered by two priming doses of estradiol benzoate. In intact does, on the other hand, copulation which resulted in ovulation induced an approximately 20-fold increase in serum LH concentration which was sustained for about 4 hr. Postcoital elevations in serum LH also occurred in estrogen-primed intact and estrogen-primed ovariectomized does. Under the conditions of our experiments, the parallel elevations in serum LH and T observed postcoitally in the female rabbit could not be demonstrated in the male.

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