

## Progesterin and Sterol Synthesis by Rabbit Ovarian Interstitial Tissue in Response to LH *in Vivo* and *in Vitro*<sup>1</sup> (35384)

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Hilliard *et al.* (1, 2) demonstrated that 20 $\alpha$ -hydroxypregn-4-en-3-one (20 $\alpha$ -ol) in rabbit ovarian venous blood increases from undetectable levels before mating or before LH injection to levels, at 2 hr later, greater than during pregnancy, and diminished to undetectable levels within 10 hr (about the time of ovulation). Coincidentally, cholesterol content of ovarian interstitial tissue is depleted (3). Later, they proposed that the 20 $\alpha$ -ol secretion after mating may act to heighten LH release in the rabbit (4); possibly explaining the observation that pituitary content of LH is continuously reduced for as long as 7 hr (5) and that gonadotropic potency of peripheral blood remains elevated for as long as 6 hr (2).

Based upon progesterin content of ovarian venous blood, Hilliard *et al.* (1) found 20 $\alpha$ -ol was produced by estrous rabbits in quantities about 10-fold greater than progesterone, while Endo *et al.* (6) concluded that ovarian interstitial tissue in pregnant rabbits produced only 20 $\alpha$ -ol. To our knowledge, progesterin secretion in estrous rabbits has not been evaluated during incubation of ovarian interstitial tissue *in vitro*, as Solod *et al.* (7) reported for ovaries from pseudopregnant rabbits. Using similar methods, Armstrong and Price (8) concluded that LH depletes esterified cholesterol by inhibiting synthesis of cholesterol and by inhibiting esterification of cholesterol.

The present experiments were designed to assess ovarian synthesis of progesterins and

sterols by two methods during an 8-hr interval following injections of LH into estrous rabbits. The two methods were ovarian interstitial tissue content of progesterins and sterols after intravenous injection of LH, and synthesis of progesterins and sterols in interstitial tissue incubated with LH *in vitro*. Another objective was to determine whether the progesterin synthetic response of interstitial tissue to LH *in vitro* was additive to that of LH previously injected *in vivo*.

**Materials and Methods.** Mature female rabbits were isolated for at least 30 days at 18° with a 14-hr photoperiod daily. Three controls received no treatment. Nine rabbits were given 100  $\mu$ g of LH<sup>3</sup> intravenously; three were killed within 5 min of LH injection; three, 2 hr later; and three, 8 hr later. The ovaries were removed immediately and chilled in iced saline. None had evidence of corpora lutea. After the visible follicles were removed, each pair of ovaries was cut into 1-mm slices and these slices of ovarian interstitial tissue were randomized into three parts. Each part was weighed and placed in a 25-ml incubation flask, containing 5 ml of Krebs-Ringer-bicarbonate buffer with 1 mg/ml of glucose, and gassed with 95% O<sub>2</sub>-5% CO<sub>2</sub>. One flask was frozen (-79°) immediately to determine initial content of progesterins and sterols. Another flask was incubated for 2 hr at 37° to determine *in vitro* synthesis of progesterins and sterols. The remaining flask was incubated similarly but with 2  $\mu$ g of LH<sup>3</sup>/ml of incubation medium.

To calculate extraction losses, <sup>14</sup>C-progesterone, <sup>14</sup>C-20 $\alpha$ -ol, <sup>14</sup>C-cholesterol, and <sup>14</sup>C-cholesteryl palmitate were added to each incubation flask. The content of each flask

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<sup>3</sup> NIH-LH-B5, supplied by The Endocrinology Study Section.

TABLE I. Progesterin and Sterol Content of Rabbit Ovarian Interstitial Tissue After Injection of LH.

Progesterin or sterol	Time after injection of LH <i>in vivo</i>			
	0	5 min	2 hr	8 hr
Progesterone ( $\mu\text{g/g}$ )	42*	68	157	66
20 $\alpha$ -ol ( $\mu\text{g/g}$ )	38	88	117	41
Cholesterol (mg/g)	1.93	2.83	2.66	1.05
Esterified chol (mg/g)	21.09	27.04	16.01	4.83

\* Each value is the average for three rabbits. Pooled standard errors were: progesterone, 31; 20 $\alpha$ -ol, 29; cholesterol, 0.56; and esterified chol, 1.1.

was homogenized and extracted three times with diethyl ether. After evaporation of the ether, the residues were transferred to thin-layer silica gel chromatography plates for isolation of progesterone, 20 $\alpha$ -ol, cholesterol, and esterified cholesterol. Progesterins were quantified by spectrophotometric absorption at 240  $m\mu$  and sterols were quantified by the Lieberman-Burchard reaction. These procedures were described and validated by Armstrong *et al.* (9).

*Results.* Based upon progesterins and sterols in fresh-frozen tissues, the interstitial tissue content of 20 $\alpha$ -ol more than doubled (Table I) and progesterone increased 60% within 5 min after LH injection. By 2 hr after LH injection, progesterone and 20 $\alpha$ -ol levels increased nearly 4- and 3-fold, respectively. Levels of both progesterins at 8 hr after LH were similar to those in uninjected controls. Ovarian interstitial tissue from these estrous rabbits contained nearly 10 times more esterified cholesterol than cholesterol (Table I). Within 5 min after injection of LH, esterified cholesterol increased about 25% and chole-

sterol increased more than 40%. But, a marked reduction of the ovarian content of both sterols occurred during the following 8 hr.

Net synthesis *in vitro* of progesterins and sterols was calculated by subtracting the fresh-frozen content of a progesterin or sterol from that present after a 2-hr incubation. Changes in net synthesis of progesterins by ovarian interstitial tissue during the 8-hr period after LH injection (Table II) were similar to changes in initial progesterin content. Synthetic capacity for both progesterins increased within 5 min after LH injection. Progesterone and 20 $\alpha$ -ol syntheses at 2 hr after LH injection were more than 4- and 10-fold greater than control values. By 8 hr after LH injection, progesterin synthetic capacity was similar to control ovaries. We found little net cholesterol synthesis *in vitro* in ovaries taken from control rabbits, and a significant quantity of esterified cholesterol was lost from the ovarian interstitial tissue from control rabbits during the 2-hr incubations. However, at 2 and 8 hr after LH

TABLE II. Net Progesterin and Sterol Synthesis in Rabbit Ovarian Interstitial Tissue Incubated *in Vitro* After *in Vivo* LH Injection.

Progesterin or sterol	Time after injection of LH <i>in vivo</i>			
	0	5 min	2 hr	8 hr
Progesterone ( $\mu\text{g/g/2 hr}$ )	32*	66	140	26
20 $\alpha$ -ol ( $\mu\text{g/g/2 hr}$ )	10	24	118	1
Cholesterol (mg/g/2 hr)	0.07	0.77	1.49	0.35
Esterified chol (mg/g/2 hr)	-3.40	-4.68	3.01	1.08

\* Each value is the average of three rabbits. Pooled standard errors were: progesterone, 61; 20 $\alpha$ -ol, 44; cholesterol, 0.72; and esterified chol, 2.47.

TABLE III. *In Vitro* Synthesis of Progesterin and Sterol Attributed to LH in Incubation Medium.

Progesterin or sterol	Time after injection of LH <i>in vivo</i>			
	0	5 min	2 hr	8 hr
Progesterone ( $\mu\text{g/g/2 hr}$ )	105 <sup>a</sup>	33	-43	-4
20 $\alpha$ -ol ( $\mu\text{g/g/2 hr}$ )	151	118	-10	-10
Cholesterol ( $\text{mg/g/2 hr}$ )	1.68	0.11	-0.83	-0.18
Esterified chol ( $\text{mg/g/2 hr}$ )	8.05	3.55	-4.44	0.14

<sup>a</sup> Each value is the average of three rabbits. Pooled standard errors were: progesterone, 27; 20 $\alpha$ -ol, 36; cholesterol, 0.59; and esterified chol, 3.70.

injections, significant quantities of both sterols accumulated during incubations *in vitro*.

Net synthesis *in vitro* of progestins and sterols attributed to LH in the incubation medium was calculated by subtracting the quantity in a flask without LH after 2-hr incubation from that in a flask with LH. Net synthesis of progesterone and of 20 $\alpha$ -ol attributed to LH added to the incubation medium was greatest in ovaries from rabbits that were not given LH intravenously (Table III). This response to LH *in vitro* was reduced within 5 min after intravenous injection of LH, and abolished within 2 hr. When rabbits were injected with LH 2 or 8 hr before they were killed, the ovaries did not respond with added progestin synthesis to LH in the incubation medium. Ovaries from control rabbits responded to LH in the incubation medium with significant sterol synthesis (Table III), but this influence had virtually disappeared within 5 min after intravenous injection of LH. At 2 hr after LH injections, LH in the medium caused a net loss of sterols during the 2-hr incubation.

**Discussion.** In agreement with data on progestin content of ovarian venous blood (1-3), our results reveal maximal content and maximal *in vitro* synthesis of progestin about 2 hr following LH injection. Levels of 20 $\alpha$ -ol are much greater than levels of progesterone in ovarian venous blood following LH injections into estrous rabbits (1). Although the 20 $\alpha$ -ol secreted in response to mating or LH is derived principally from interstitial tissue, we found roughly equivalent quantities of these two progestins in ovarian interstitial tissue from estrous rabbits. Fur-

ther, the interstitial tissue synthesized roughly equivalent quantities of progesterone and 20 $\alpha$ -ol *in vitro*.

Behrman and Armstrong (10) reported increased activity of cholesterol esterase in rat lutein tissue in response to LH. A similar response in rabbit ovarian interstitial tissue is suggested by the marked loss of esterified cholesterol 2 and 8 hr after LH injections (Table I). Although a significant quantity of esterified cholesterol accumulated in response to added LH during 2-hr incubation of ovaries from control rabbits (Table III), we found a net loss of esterified cholesterol in response to added LH during *in vitro* incubation of ovaries removed 2 hr following LH injections. The parallel loss of cholesterol in response to LH *in vitro* agrees with the observation (8) that LH in the incubation medium inhibits incorporation of acetate into cholesterol, both free and esterified in ovarian interstitial tissue from estrous rabbits. Similarly, intravenous injection of LH, 2 hr before ovaries were recovered, obliterated any progestin secretion response to LH added to the *in vitro* incubation medium.

This interference of injected LH with steroidogenic response of interstitial tissue to LH *in vitro* was detected within 5 min following intravenous injection of LH, suggesting that injected LH may have been bound to interstitial tissue *in vivo* and thereby affected steroidogenesis *in vitro*. Alternatively, that both interstitial tissue content and *in vitro* (without LH) progestin synthesis were stimulated within 5 min of injection of LH suggests that LH may have affected steroidogenic enzymes within this period.

**Summary.** Progesterin and sterol secretory

capacity of rabbit ovarian interstitial tissue was investigated with and without LH. Based upon ovarian content and upon synthesis *in vitro*, progesterone and 20 $\alpha$ -ol secretion increased within 5 min after intravenous injection of LH. At 2 hr after LH injection, interstitial tissue content of progesterone and 20 $\alpha$ -ol were about 3 and 4 times larger and *in vitro* syntheses were 4 and 12 times larger than controls. By 8 hr after LH injection, progestin synthesis returned to near control levels and stores of cholesterol and esterified cholesterol were depleted to 60 and 23% of control levels. Synthesis by ovarian interstitial tissue of progestins and sterols *in vitro* was enhanced by LH in the incubation medium, especially among the rabbits not injected with LH. This steroidogenic response to LH *in vitro* was reduced within 5 min and abolished within 2 hr after intravenous injection of LH.

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