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In Vitro Secretion of Progestins: Changes Dependent on the Postovulatory Age of the Ovaries (37908)

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In vitro secretion of adrenal cortical steroids has been studied for many years (1). Channing (2, 3) has studied steroid secretion by the granulosa cells surviving in long-term tissue cultures. However, studies of steroids released during short-term *in vitro* incubations of gonadal tissues are less frequent (4–9). We wanted to establish a dynamic, completely *in vitro* system for quantitating luteal function. In the present report, we have carried out short-term *in vitro* incubations of luteal ovaries obtained on each of Days 1–15 of the pseudopregnancy induced in immature rats by gonadotrophin administration. The progestins released into the incubation medium were quantitated. The hypothesis tested was that the temporal pattern of *in vitro* progesterone secretion should be similar to the temporal pattern of *in vivo* progesterone secretion described by Horikoshi and Wiest (10) for similarly treated rats.

Materials and Methods. Nineteen-day-old female rats were obtained from the Holtzman Laboratory (Madison, Wisconsin) and housed in animal quarters with artificial lights on between 0500 and 1900. At 26 days of age (0900), each rat was given 50 IU of PMS, followed in 56 hr by 25 IU of HCG. Day 0 is the day of HCG injection, Day 1 is the day of estrus, the day on which fresh ova can be found in the Fallopian tube, and the first day on which there are corpora lutea in the ovary. Ovaries were obtained at 0900 on each of Days 1–15 of pseudopregnancy. After decapitation, between 0900 and 1000, the ovaries were excised, chilled, trimmed, and weighed to the nearest 0.2 mg. Each ovary was then quartered and the eight pieces comprising a pair of ovaries from a single animal were placed into 5.0 ml of ice-chilled Krebs–Ringer phosphate buffer, pH 7.4, fortified with glucose to a final concentration of 2 mg/ml. Incubations were carried out at 37° in a Dubnoff Metabolic shaking incubator for exactly 120 min with continuous 100% oxygen as the gaseous phase. Aliquots of the incubation buffer were analyzed for the content of progesterone and 20α-hydroxypregn-4-en-3-one (20α-OHP). After column chromatography on Sephadex LH-20, the steroids were quantitated by a competitive protein-binding radioassay. The details of our method of steroid analyses are published elsewhere (11).

Results and Discussion. Essentially no progestins are released during *in vitro* incubation of luteinized ovaries at 4°, suggesting an "active" secretory process. The *in vitro* release of progesterone and 20α-OHP observed on each of Days 1–15 of pseudopregnancy is presented in Fig. 1. The total progestins, obtained by the sum of progesterone (P) plus 20α-OHP and the ratio: P/20α-OHP, are also presented in Fig. 1. The data are expressed as (mean ± SE) nanograms of steroid released per pair of luteal ovaries per 120-min incubation time.

The *in vitro* progesterone secretion rate was observed to increase gradually between Days 1 and 3 of pseudopregnancy; to in-

[1] A preliminary report of this work was presented at the IV International Congress of Endocrinology, Washington, D. C., June, 1972.

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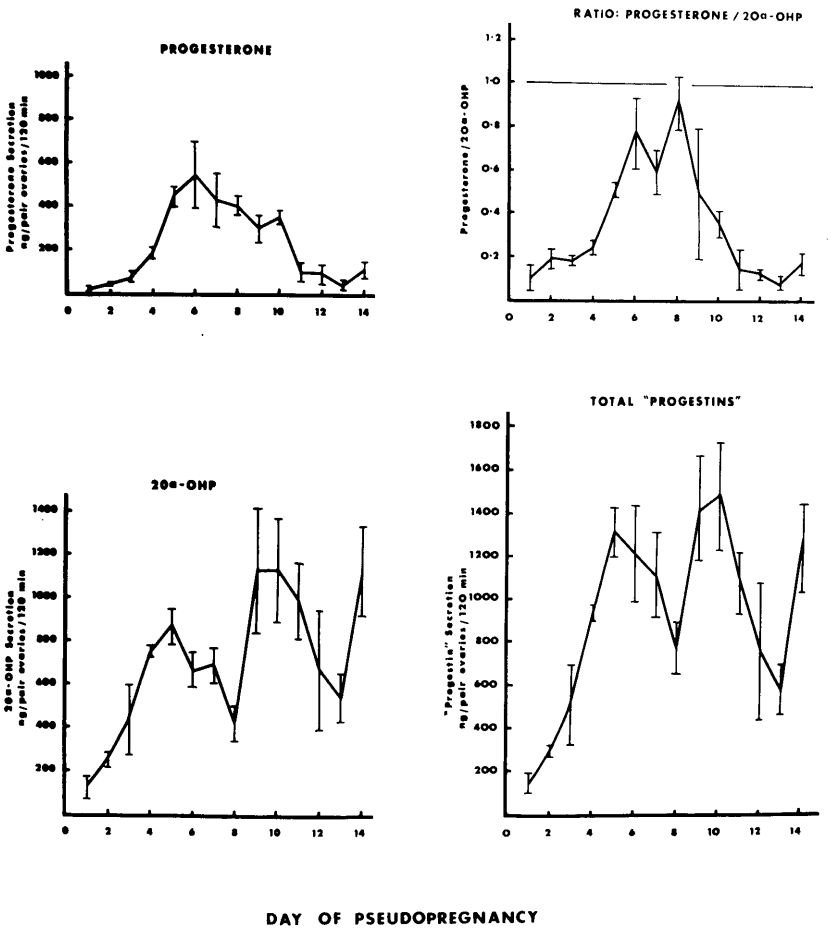

FIG. 1. Daily changes in the *in vitro* progestin secretion by highly luteinized ovaries of immature pseudopregnant rats. The data presented are the mean ± SE. Four animals were studied on each day.

crease dramatically to a maximum value on Day 6; to decrease gradually until Day 10; to decrease abruptly between Days 10 and 11; and to remain low for the subsequent several days of observation.

The *in vitro* 20α-OHP secretion rate was observed to increase rapidly between Days 1 and 5 of pseudopregnancy; to decrease until Day 8; to increase abruptly between Day 8 and Day 9; to remain high through Day 11; to decrease until Day 13; and to increase again by Day 14. The weights of the highly luteinized ovaries, observed on each day of pseudopregnancy, are presented in Table I (mean ± SE). The ovarian weights remain high through Day 5, drop considerably by Day 6, and remain essentially unchanged during the subsequent days of observation. No new ovulations were observed during the latter days of pseudopregnancy. Clearly, the decreased progesterone secretion between Day 10 and Day 11 is not related to a concomittantly decreased ovarian weight.

The day-to-day changes of the *in vitro* progestin secretion described in this report are qualitatively similar to the *in vivo* changes for similarly treated immature rats (10). The pattern of *in vitro* progestin secretion also extends studies of the temporal pattern of 20α-hydroxysteroid dehydrogenase activities in similarly treated rat ovaries (15). Moreover, with respect to the changing pattern of progestin secretion,

TABLE I. Weights of Highly Luteinized Ovaries on Days 1–14 of Pseudopregnancy[a]

Day of pseudopregnancy	Ovarian weight (mg)
1	228 ± 15[b]
2	237 ± 11
3	231 ± 35
4	254 ± 16
5	225 ± 7
6	181 ± 10
7	162 ± 2
8	182 ± 6
9	175 ± 14
10	172 ± 9
11	170 ± 10
12	193 ± 15
13	177 ± 12
14	197 ± 10

[a] The pair of ovaries from each of four animals was studied.

[b] ±SE.

the PMS-HCG-treated pseudopregnant rat is qualitatively similar to the adult pseudopregnant rat (10, 13, 14).

Physiological luteolysis (9, 11) is a phrase which we have proposed to describe, as an event, the acute, dramatic, and irreversible decrease in progesterone secretion by the luteal ovary. Physiological luteolysis is to be distinguished from histological luteolysis, or the morphological regression of the corpus luteum. Histological and physiological luteolysis have been called, respectively, structural luteolysis and functional luteolysis by Malven (12).

Summary. Pseudopregnancy was induced in immature rats by the administration of PMS-HCG. On each of Days 1–15, five pair of highly luteinized ovaries were quartered and incubated *in vitro* for 120 min. The concentration of progesterone and 20α-hydroxypregn-4-en-3-one (20α-OHP) in the incubation medium was then determined.

The temporal pattern of *in vitro* progestin secretion was similar to the temporal pattern of *in vivo* progestin secretion rates for similarly treated rats. Physiological luteolysis occurs by the morning of Day 11.

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