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Biochemical Luteolysis: *in Vitro* DNA Metabolism by Luteized Ovaries of Immature Pseudopregnant Rats[1] (37907)

DELPHINE BARTOSIK, DONALD H. SZAROWSKI, AND MARTIN L. STONE
(Introduced by S. Shulman)
The Department of Obstetrics and Gynecology, New York Medical College, 1249 Fifth Avenue, New York, New York 10029

Physiological luteolysis is a phrase which we have proposed to characterize, as an event, the abrupt, dramatic, and irreversible decrease in progesterone secretion by the luteal ovary (1–3). This event normally occurs at the end of the luteal (progravid) phase of the mammalian reproductive cycle. In rats, when Day 1 is counted as the day on which fresh ova can be found in the Fallopian tubes, then physiological luteolysis is found to occur by the morning of Day 11 (1–3).

We presume that there is an event during the life span of the corpus luteum cell the inevitable result of which is physiological luteolysis. We propose to call this event "Biochemical Luteolysis." The nature of this primary event and the time when this event takes place are questions of interest. In the present report we have investigated the possibility that there is a decreased number of luteal cells and/or a decreased rate of cellular proliferation which is temporally synchronized with physiological luteolysis. The DNA content of luteal ovaries and the rate of synthesis of ovarian DNA on each of Days 1 through 14 of the pseudopregnancy induced in immature rats (3) were quantitated.

Materials and Methods. Nineteen-day-old female rats were obtained from the Holtzman Laboratory (Madison, Wisconsin) and housed in animal quarters with lights on between 0500 and 1900. At 26 days of age (0900), each rat was given 50 IU of pregnant mares serum (PMS), followed in 56 hr by an injection of 25 IU of human chorionic gonadotrophin (HCG). Day 0 is the day of HCG injection, and Day 1 is the day of estrus, the day in which fresh ova can be found in the Fallopian tube, or the first day in which there are corpora lutea in the ovary.

Groups of 10 animals were decapitated between 0900 and 1000 on each of Days 1 through 14 of pseudopregnancy. 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 begun by adding thymidine-^{14}C and continued at 37° in a Dubnoff Metabolic shaking incubator for exactly 120 min, with 100% oxygen as the gaseous phase. Thymidine-*methyl*-^{14}C 1.0 μCi/0.1 ml of 0.154 *M* NaCl (sp. act. = 54.6 mCi/mmole), was the isotope studied. At the end of the incubation the buffer was poured off and the tissues rinsed in ice-chilled 0.154 *M* NaCl. After adding 1.0 ml of 10% trichloroacetic acid (TCA), the flasks were kept frozen until processed.

After thawing, the pieces of ovaries were transferred to a glass homogenizer with a motor-driven glass pestle and homogenized in ice-chilled 10% TCA. The homogenates were then quantitatively transferred, and after thorough mixing, the precipitate was

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

washed 4 times with 2 ml of ice-chilled 10% TCA. Extraction of the lipids was carried out using successive 2-ml washes, first with sodium acetate saturated ethanol (4), then ethanol:chloroform (3:1) and diethyl ether. The dry-powder residue which contains the acid-insoluble lipid-free extract of the ovarian tissue was then solubilized by incubating in the presence of 1.0 *M* NaOH at 37° for 18 hr. Aliquots of the acid-insoluble lipid-free extract of the incubated ovaries were then taken to quantitate the radioactivity and the DNA content. Radioactivity was measured using a Nuclear Chicago Mark II Liquid Scintillation Spectrophotometer, after suspending the solubilized tissue sample in Bray's solution (5). DNA was quantified by the Diphenylamine reaction as described by Volkin and Cohn (6).

Results and Discussion. The daily changes in the DNA content and in the *in vitro* thymidine-^{14}C incorporation by highly luteinized ovaries of immature pseudopregnant rats are presented in Fig. 1. The data presented are the mean ± SE of ten animals for each day studied. The major changes, either in the DNA content or in the DNA synthesis, have taken place by Day 4 of pseudopregnancy. The DNA content of luteal ovaries was increased to maximal values by Day 3 and remained essentially unchanged through Day 13, suggesting a constant population of luteal cells. The incorporation of thymidine-^{14}C into the DNA of luteal ovaries was highest on Day 1, decreased rapidly through Day 4 and remained essentially unchanged during the subsequent days of observation.

Horikoshi and Wiest (7) have studied the temporal variations in ovarian steroid

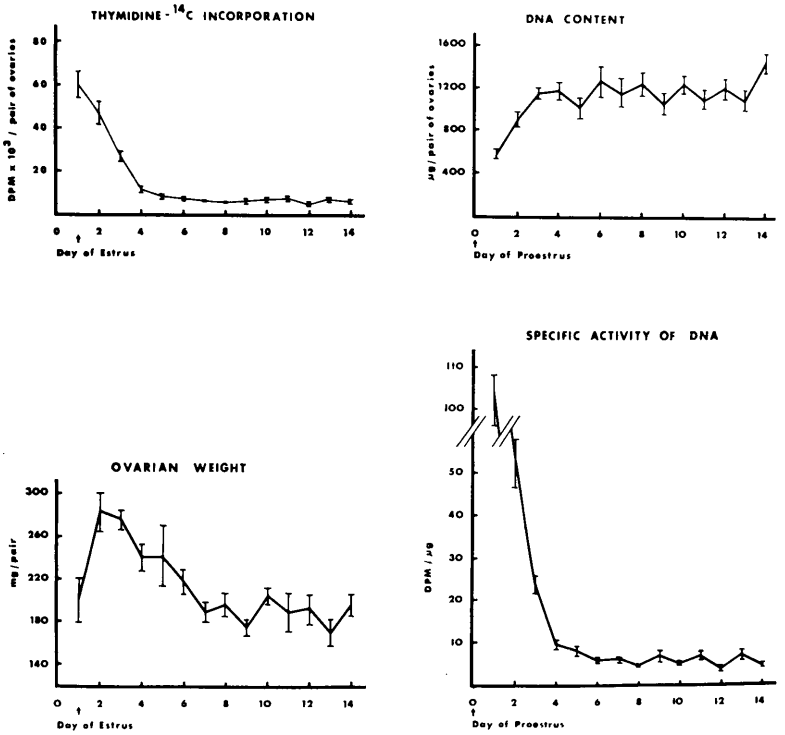

FIG. 1. Daily changes in the *in vitro* DNA metabolism of highly luteinized ovaries of immature pseudopregnant rats. The data presented are the mean ± SE. The animals were studied on each day.

secretion by immature pseudopregnant rats. The changing pattern of progesterone secretion was similar to that observed in the adult pseudopregnant rat (7, 8). Physiological luteolysis has occurred by the morning of Day 11 (1, 3). During pseudopregnancy, the changing pattern of progesterone secretion is not temporally synchronized with the changing pattern of DNA content or DNA synthesis which we have observed in the luteal ovaries (Fig. 1).

Bassett (9) studied mitotic activity in the corpus luteum of pregnant rats. Mitotic activity was highest on the first day of observation (Day 2), decreased steadily through Day 6 and remained essentially unchanged through Day 12 of pregnancy. Thus, the day-to-day changes in DNA metabolism which we observed studying the whole ovary of immature rats were qualitatively similar to the changes reported for the corpus luteum of pregnancy.

Summary. Physiological luteolysis is the abrupt, dramatic, and irreversible decrease in progesterone secretion by the corpus luteum-bearing ovary. Neither an acute decrease in the number of luteal cells nor an acutely decreased rate of cellular proliferation could be demonstrated to occur in temporal synchrony with physiological luteolysis.

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