PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY AND MEDICINE 151, 726-729 (1976)

Peripheral Plasma Progesterone during Egg Transport in the Rabbit (39294)

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Hilliard and her colleagues (1-4) have studied extensively the changes in ovarian steroid secretion which occur between mating and ovulation in the rabbit. Recently, these studies were extended to include the period from ovulation to implantation (5). These investigators reported that steroid production remained low during egg transport, but the developing corpora lutea were secreting some progesterone. Unfortunately, it is impossible to determine the temporal changes in progesterone production from their data since only one animal was used at each of only three time periods during egg transport.

The influence of ovarian steroids on the physiological control of egg transport is not clear. Boling (6) suggested that preovulatory progestins "trigger" an increase in oviductal contractility which causes a more rapid transport of eggs; he also suggested that normal tubal transport is related to the acute withdrawal of estradiol prior to ovulation. However, these experiments only relate to egg transport through the ampulla. Other investigators (5) have suggested that a gradual increase in estradiol secretion during the final 24 hr that eggs are in the oviduct aids the passage of eggs through the uterotubal junction into the uterus. The work of Hilliard and Eaton (5) has been interpreted by some investigators to mean that tubal egg transport occurs during a period when blood levels of ovarian steroids are undetectable (7, 8).

The following experiments were designed to determine the postovulatory progesterone and estradiol secretion patterns because it seemed likely that egg transport may be regulated to a large extent by changes in the secretion of these ovarian steroids.

Materials and methods. Fifteen New Zealand rabbits which had been individually caged for at least 3 weeks were used. Sequential 3- to 4-ml blood samples were collected by heart puncture and placed in ice-cold tubes containing heparin (10 IU/ml). The plasma was harvested, frozen, and stored at −20° for subsequent assay. Samples were collected every 6 hr beginning 12 hr before and continuing to 96 hr after mating (seven animals), human chorionic gonadotropin (hCG) injection (five animals), or saline injection (three animals). Animals were injected in the marginal ear vein with 100 IU hCG (0.5 ml, Nutritional Biochemicals Corp., Lot No. 1882) or an equivalent volume of 0.9% NaCl vehicle. Recent ovulation points and/or large follicles were counted when the animals were sacrificed at the end of the blood sampling period.

Plasma progesterone and estradiol were measured using the specific antisera previously described (9, 10). The validity of these extraction and assay procedures for rabbit plasma was confirmed by determining the absence of an interfering plasma blank using ovariectomized rabbit plasma, the demonstration of quantitative recovery of steroid added to ovariectomized rabbit plasma, and the demonstration of parallelism.

A repeated measures analysis of variance procedure was used on the progesterone data from the mated and hCG-injected animals. This test was used to determine if there was a significant increase in plasma progesterone with time and if there were significant differences between the mated and hCG-injected group.

Results. Only follicles were observed on the ovaries of three saline-injected rabbits at the time of sacrifice. Three animals which had been mated, but did not ovulate, also had only follicles on the ovaries. Four mated animals did ovulate and had 9.3 ± 0.6 (mean ± SE) recent ovulation points; this was not significantly ($P > 0.05$) different from the number of ovulation points in the five hCG-injected animals (8.6 ± 1.5).

The plasma progesterone changes for the mated-ovulated, hCG-injected, and saline-

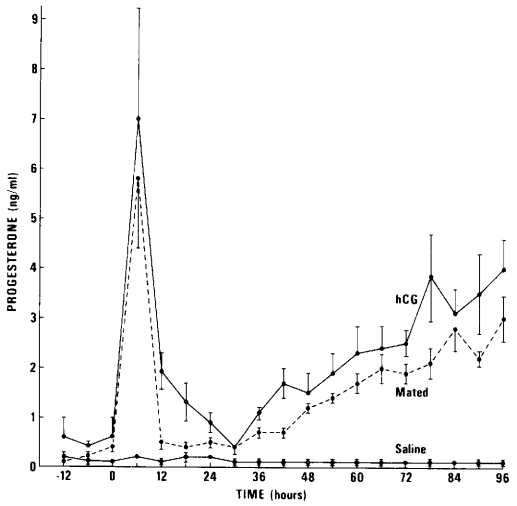

FIG. 1. Peripheral plasma progesterone concentrations (ng/ml) in mated, hCG-injected, and saline-injected New Zealand rabbits. Time 0 is the time of mating or injection. The increase with time beginning at 30 hr is significant ($P < 0.001$) in the mated and hCG-injected groups; there are no significant ($P > 0.05$) differences between these two groups at any of the times after 30 hr.

injected animals are shown in Fig. 1. Data for the mated rabbits that did not ovulate are not shown, as these progesterone values did not differ significantly from the saline injected animals. Peripheral plasma progesterone remained at basal levels of 0.1–0.2 ng/ml in the saline-injected animals throughout the sampling period. Progesterone was at its greatest concentration at 6 hr after mating or hCG injection, and then fell to basal levels at the time of ovulation. This decline was not as abrupt in hCG-injected animals as it was in mated animals. In both groups, plasma progesterone concentrations began to increase at about 30–36 hr and continued to increase for the remainder of the blood collection period. This gradual, progressive increase with time was significant ($P < 0.001$) in both groups. There were no significant ($P > 0.05$) differences between the plasma progesterone concentrations of these two groups at any time after ovulation.

Peripheral plasma estradiol was variable throughout the sampling period. The mean concentrations for all groups fluctuated between nondetectable levels and 12 pg/ml, without any definite change before or after the time of ovulation.

Discussion. The results reported here for the preovulatory increase in progesterone secretion following an ovulation-inducing stimulus are in agreement with other investigators (1–5) who measured ovarian vein concentrations of progestins. For the most part the rapid increase in progestins has been attributed to the release of 20α-hydroxypregn-4-en-3-one by the ovarian interstitial tissue. It was stated (5) that little change could be detected in the ovarian vein concentration of progesterone. Our results, and those of others (11), indicate that there is also a significant release of progesterone prior to ovulation.

At 12 hr, when ovulation was occurring (12, 13), peripheral plasma progesterone had fallen to basal levels in the mated animals and to 27% of the 6-hr peak value in hCG-injected animals. The more gradual decline in plasma progesterone between 12 and 30 hr in the hCG-injected animals may be due to a prolonged stimulation imposed by the relatively longer half-life of hCG as compared to endogenous LH in the mated animals.

In both mated and hCG-injected rabbits, peripheral plasma progesterone was at basal levels at 30 hr, and began to increase between 30 and 36 hr. Thereafter there was a progressive increase in plasma progesterone concentration, reaching a concentration of 2.0–2.4 ng/ml at 72 hr when eggs are normally passing into the uterus. This is the first report of a significant increase in plasma progesterone with time during egg transport. Other investigators (5) used too few animals to determine the progesterone secretion pattern, or reported (14) that peripheral plasma progesterone was approximately 2.6 ng/ml on all of the first 3 days after mating.

Eggs are transported through the ampulla very rapidly after ovulation, and then remain at the ampullary-isthmic junction for several hours before continuing their passage into the isthmus (15). That eggs do move progressively through the isthmus has been reported by several laboratories. Polidoro *et al.* (7) expressed the mean location of eggs as a percentage of the total length of

the oviduct: At 24 hr after hCG the eggs had moved 54% of the length of the oviduct to the area of the ampullary-isthmic junction; at 36, 48, 60, 72, and 84 hr after hCG the eggs had moved 62, 69, 71, 79, and 96%, respectively, of the oviduct length. Pauerstein *et al.* (16) determined the temporal distribution of eggs in several sections of the oviduct after hCG: (a) At 18 and 24 hr after hCG 100% of the eggs were located in the ampulla or ampullary-isthmic junction; (b) at 36 hr after hCG only 18% of the eggs were in the ampullary-isthmic junction while 82% of the eggs were in the distal third of the isthmus; (c) at 48 hr after hCG the eggs were evenly distributed in the distal and middle thirds of the isthmus; (d) by 60 hr after hCG only 31% were in the distal third while 66% were in the middle third of the isthmus; and (e) at 63 hr after hCG only 10% remained in the distal third, and 90% of the eggs were located in the middle third of the isthmus. These reports demonstrate that the eggs, after residing in the proximal ampulla and ampullary-isthmic junction, begin to move into the isthmus between 24 and 36 hr after hCG. Furthermore, once movement into the isthmus begins, the eggs gradually and progressively pass through the isthmus.

The initiation of movement of eggs from the ampullary-isthmic junction into the isthmus (7, 16) is temporally related to the increase in peripheral plasma progesterone concentrations shown in this report. In addition, the progressive movement of eggs through the isthmus occurs as plasma progesterone gradually increases between 30 and 72 hr after the induction of ovulation. These temporal relationships between egg transport and plasma progesterone support the suggestion that the postovulatory secretion of progesterone facilitates the progressive movement of eggs through the oviduct into the uterus (17). Since a postovulatory increase in estradiol secretion was not observed and an earlier report presented information from only one animal $3^1/_2$ days after coitus (5), the importance of estradiol secretion changes in promoting passage of eggs through the uterotubal junction (5) remains equivocal.

Summary. Peripheral plasma progesterone concentrations were measured in New Zealand rabbits every 6 hr beginning 12 hr before and continuing until 96 hr after either natural mating, hCG injection, or saline injection. The number of ovulation points in naturally mated animals (9.3 ± 0.6, mean ± SE) was not significantly different from that in hCG-injected animals (8.6 ± 1.5). There was a surge in progesterone secretion following both mating and hCG injection. Plasma progesterone concentrations reached a peak prior to ovulation and then fell to basal levels at the time of ovulation. Beginning at approximately 30 hr after the ovulation-inducing stimulus, there was a progressive, significant ($P < 0.001$) increase in plasma progesterone concentration, which continued for the duration of the sampling period. The initiation of the postovulatory increase in progesterone secretion corresponds temporally with the movement of eggs from the ampullary-isthmic junction into the isthmus. The progressive increase in plasma progesterone between 30 and 72 hr after the induction of ovulation corresponds with the gradual movement of eggs through the isthmus into the uterus. The data suggest that movement of eggs through the oviductal isthmus is influenced by the postovulatory secretion of progesterone.

We appreciate the statistical assistance of Dr. K. R. Lamborn and the technical assistance of Mrs. K. K. Forbes and Mrs. J. M. Noteboom.

1. Hilliard, J., Archibald, D., and Sawyer, C. H., Endocrinology **72,** 59 (1963).
2. Hilliard, J., Endroczi, E., and Sawyer, C. H., Proc. Soc. Exp. Biol. Med. **108,** 154 (1961).
3. Hilliard, J., Hayward, J. N., and Sawyer, C. H., Endocrinology **75,** 957 (1964).
4. Hilliard, J., Spies, H. G., and Sawyer, C. H., Endocrinology **82,** 157 (1968).
5. Hilliard, J., and Eaton, L. W., Jr., Endocrinology **89,** 522 (1971).
6. Boling, J. L., in "The Mammalian Oviduct" (E. S. E. Hafez and R. J. Blandau, eds.), p. 163, Univ. of Chicago Press (1969).
7. Polidoro, J. P., Howe, G. R., and Black, D. L., J. Reprod. Fertil. **35,** 331 (1973).
8. Croxatto, H. B., and Ortiz, M.-E. S., Gynecol. Invest. **6,** 215 (1975).
9. deVilla, G. O., Jr., Roberts, K., Wiest, W. G.,

Mikhail, G., and Flickinger, G., J. Clin. Endocrinol. Metab. **35,** 458 (1972).
10. England, B. G., Niswender, G. D., and Midgley, A. R., Jr., J. Clin. Endocrinol. Metab. **38,** 42 (1974).
11. Grinwich, D. L., Kennedy, T. G., and Armstrong, D. T., Prostaglandins **1,** 89 (1972).
12. Harper, M. J. K., J. Endocrinol. **22,** 147 (1961).
13. Harper, M. J. K., J. Endocrinol. **26,** 307 (1963).
14. Fuchs, A.-R., and Beling, C., Endocrinology **95,** 1054 (1974).
15. Blandau, R. J., in "The Mammalian Oviduct" (E. S. E. Hafez and R. J. Blandau, eds.), p. 129, Univ. of Chicago Press (1969).
16. Pauerstein, C. J., Anderson, V., Chatkoff, M. L., and Hodgson, B. J., Am. J. Obstet. Gynecol. **120,** 299 (1974).
17. Spilman, C. H., and Harper, M. J. K., Gynecol. Invest. **6,** 186 (1975).

Received October 29, 1975. P.S.E.B.M., 1976, Vol. 151.