# Hormones and Sperm Capacitation in Rabbits[1] (35398)

EDWARD D. PLOTKA AND WILLIAM L. WILLIAMS

*Marshfield Clinic Foundation for Medical Research and Education, Marshfield, Wisconsin 54449; and Department of Biochemistry, University of Georgia, Athens, Georgia 30601*

Chang (1) first suggested endocrine involvement in sperm capacitation. In 1958, he showed that sperm can not be capacitated in the uteri of pseudopregnant rabbits with or without gonadotropin or estrogen treatment, or in the uteri of immature, overiectomized, or estrous rabbits treated with progesterone. More recently, Soupart (2) studied sperm capacitiation in the uteri of intact, hypophysectomized, or ovariectomized rabbit does stimulated by human chorionic gonadotropin (HCG), luteinizing hormone (LH), or estrogen. Maximum fertilization was achieved by a single injection of 75 IU of HCG, however, greater doses depressed the number of ova fertilized. Purified LH caused similar effects following hypophysectomy, maximum fertilization being obtained with 200 $\mu$g. Estrogen stimulated capacitation in ovariectomized does producing a diphasic dose–response curve, with maximum stimulation being achieved with 1 $\mu$g of estradiol benzoate/kg of body weight.

Hamner *et al.* (3) found no improvement after estrogen stimulation in ovariectomized rabbits and concluded that estrogen alone cannot maintain optimal conditions for capacitation in rabbits. Later, however, Hamner and Sojka (4) fertilized 86% of ova exposed to rabbit sperm that had been capacitated in the uteri of ovariectomized estrogen-treated does. Bedford (5) used the technique of sperm transfer into the oviducts of recipient females or of ova transfer to the site of capacitation. He concluded that the rabbit oviduct has a greater innate potential for capacitation than does the uterus and that this potential is increased in both oviduct and uterus by estrogen stimulation.

The purpose of our research was to further investigate endocrine involvement in capacitation and to prepare a hormonally controlled animal that is suitable as a continuous capacitor.

*Methods.* Using pentobarbital anesthesia and a flank incision, we removed ovaries unilaterally or bilaterally from New Zealand white rabbits that were sexually mature and nonpregnant. Starting the day after bilateral ovariectomy, does received 1 $\mu$g of estradiol benzoate (in propylene glycol)/kg of body weight/day. Two weeks later, all does were hand-mated to two bucks and injected subcutaneously with either propylene glycol, propylene glycol plus 20-$\alpha$-hydroxyprogesterone, or propylene glycol plus progesterone. Ten hr later, the does were anesthetized, laparotomy was performed via a mid-abdominal incision, and sperm were recovered by flushing each uterus with 2 ml of a Krebs–Ringer phosphate buffer containing 0.25% glucose and 5% heated blood serum. The sperm were concentrated and $5.0 \times 10^4$ were transferred to the oviducts of donor does that had ovulated 2 hr prior to the transfer, *i.e.*, had received 75 IU of HCG 12 hr earlier.

*Results and Discussion.* Low levels of progestin (progesterone or 20-$\alpha$-hydroxyprogesterone) enhance capacitation in the ovariectomized estrogen-treated rabbit and higher levels of progestin depress capacitation (Table I). Because Soupart (2) achieved a maximum average of 54% ova fertilized in his capacitation test, he suggested the possibility of a "partner" in the control of capacitation. In addition, Hilliard *et al.* (6) demonstrated secretion of progestin (progesterone and 20-$\alpha$-hydroxyprogesterone), which is stimu-

[1] This research was supported by Training Grant 5 T01 HD-00140 from the National Institute of Child Health and Human Development, Career Development Award 2 K3 GM4831 from National Institute of General Medical Sciences, and a Ford Foundation Grant.

TABLE I. Effects of Progestin and Estradiol Benzoate (EB) on Capacitation of Spermatozoa in the Uteri of Ovariectomized Rabbit Does.

| Progestin (mg injected with 1 μg of EB; dose/kg of body wt) | Capacitation test system | | |
|---|---|---|---|
| | No. of oviducts inseminated | Ova recovered cleaved/total | % Fertile |
| No progestin | 14 | 32/52 | 62 |
| 20-α-Hydroxyprogesterone | | | |
| 0.5 | 34 | 98/115 | 85 |
| 1.0 | 17 | 47/62 | 76 |
| 1.5 | 14 | 42/64 | 62 |
| 2.0 | 15 | 23/60 | 38 |
| 4.0 | 14 | 10/68 | 15 |
| Progesterone | | | |
| 0.15 | 14 | 48/61 | 79 |
| 0.2 | 16 | 42/72 | 58 |
| 0.4 | 14 | 22/46 | 48 |
| 1.0 | 15 | 30/48 | 44 |
| 2.0 | 12 | 18/51 | 39 |
| 4.0 | 12 | 12/69 | 17 |
| Intact control | 31 | 72/102 | 77 |

lated by gonadotropic hormone during the preovulatory period in the rabbit. From the results of these experiments, it appears that release of progestin is important in helping to provide the optimum environment for sperm capacitation. The enhancing effort of small doses of progestin may be antiestrogenic, because our does were under estrogen domination for 2 weeks prior to experiment. Hamner and Sojka (4) fertilized 86% of ova with rabbit sperm incubated in does treated with 0.5 μg of estradiol cyclopentane-propionate (ECP)/kg for 6 days prior to sperm incubation, and Bedford (5) fertilized all ova using sperm incubated in does that had received 50 μg of ECP for 5 days.

The suppression of capacitation by high doses of progestin may result from endometrial changes in the uterus. Chang (1) achieved a very low level of fertilization (2%) using sperm recovered from pseudopregnant rabbits or from rabbits treated with 25 mg of crystalline progesterone. Also, Soupart (2) achieved a fertilization level of only 5.4% with sperm from pseudopregnant does.

The difference between effective doses of progesterone and 20-α-hydroxyprogesterone might be explained by the relative biologic potencies in the rabbit. Zarrow *et al.* (7) have stated that 20-α-hydroxyprogesterone has 30 to 50% of the activity of progesterone in stimulating rabbit endometrial proliferation.

The level of fertilization (62%) in rabbits treated with 1 μg of estradiol benzoate and no progestin is similar to the level (54%) reported by Soupart (2) but much greater than the level (33%) reported by Chang (1), who injected 1.5 mg of estradiol. However, as Soupart pointed out, doses of estrogen greater than 1 μg/kg of body weight progessively reduce the percentage of fertilized ova in the capacitation test system. Further evidence that the dose used here is optimal is the agreement between the efficiency of fertilization in this study with that achieved in surgically inseminated does (1). Doses of 20-α-hydroxyprogesterone of 2 and 3 mg/kg of body weight given to ovariectomized rabbits receiving estrogen depressed capacitation, but no stimulation was observed at lower doses (8).

In addition to establishing the need for progestin in the capacitation process, our experiments provide an experimental animal valuable for the study of capacitation and decapacitation factor. The ovariectomized rabbit primed with estrogen and progestin can be used several times as a capacitator without delay due to pregnancy or pseudopregnancy. We have used such animals as many as six times (three times a week) without decreased fertility in the capacitation test system (2).

Unilateral ovariectomy of the capacitator did not alter the level of fertilization in the capacitation test system (Table II). It is possible that removal of one ovary may upset the feedback system between the pituitary and gonads and may alter the uterine environment and capacitation. However, the 2 weeks between surgery and experiments may have established a new hormonal balance conducive to capacitation. These experiments demonstrate that an ovary mediates capacitation in both uteri through the general circu-

TABLE II. Effects of Unilateral Ovariectomy on Capacitation of Spermatozoa in the Uteri of Rabbit Does.

| | Capacitation test system | | |
|---|---|---|---|
| Horn of uterus | No. of oviducts inseminated | Ova recovered cleaved/total | % Fertile |
| Adjacent to ovary | 13 | 29/36 | 80 |
| Contralateral to ovary | 18 | 46/60 | 77 |

lation. If it mediated capacitation only in the adjacent horn through direct blood supply, one would expect the rates of fertilization to differ between sperm incubated in the adjacent uterine horn and sperm incubated in the contralateral horn.

*Summary.* Bilaterally ovariectomized does were maintained on 1 μg of estradiol benzoate/kg of body wt/day and given various levels of either progesterone (P) or 20-α-hydroxyprogesterone (20α-OHP) following mating to fertile bucks. Compared with estrogen-treated controls, a single injection of 0.5 and 1.0 mg of 20α-OHP and 0.15 mg of P increased the number of ova fertilized. Higher levels of either progestin reduced the number of fertilized ova recovered in the capacitation test system. A doe thus prepared can be used several times in a short period to capacitate sperm. Unilateral ovariectomy of the capacitator doe had no effect on the number of ova fertilized in the test system.

We thank LeRoy Patrick and Ora Lee Johnson for their assistance in surgery and animal care.

1. Chang, M. C., Endocrinology **63**, 619 (1958).

2. Soupart, P., J. Reprod. Fert. Suppl. **2**, 49 (1967).

3. Hamner, C. E., Jones, J. P., and Sojka, N. J., Fert. Steril. **19**, 137 (1968).

4. Hamner, C. E., and Sojka, N. J., Nature (London) **220**, 1042 (1968).

5. Bedford, J. M., J. Endocrinol. **46**, 191 (1970).

6. Hilliard, J., Archibald, D., and Sawyer, C. H., Endocrinology **72**, 59 (1963).

7. Zarrow, M. X., Yochim, J. M., and McCarthy, J. L., "Experimental Endocrinology, A Sourcebook of Basic Techniques," p. 82. Academic Press, New York (1964).

8. Soupart, P., Biol. Reprod. **3**, 1 (1970).

Received Sept. 2, 1970. P.S.E.B.M., 1971, Vol. 136.