The Effect of Sodium Pentobarbital on Capacitation in the Rabbit[1] (35399)

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In 1951, Austin (1) and Chang (2) first demonstrated the need for rabbit spermatozoa to spend a "capacitation" period of 6 to 10 hr in the female reproductive tract before attaining full fertilizing ability. In 1966, Soupart (3) showed that doses of human chorionic gonadotropin (HCG) up to 75 IU enhanced capacitation in the intact rabbit, but that doses of 100 to 400 IU reduced capacitation to very low levels. Later the same worker (4) showed that purified luteinizing hormone (LH) would do the same thing in hypophysectomized does, the optimum dose being 200 μg of purified LH. The level of capacitation, as judged by yield of fertilized ova, achieved by 75 IU of HCG was similar to that achieved by normal mating.

Because the coital stimulus in the rabbit causes release of pituitary LH, in turn inducing ovulation, an additional effect of the released LH might be to augment the capacitation process. In our study we sought to test the hypothesis that coitus enhances capacitation by stimulating pituitary release of gonadotropin.

Materials and Methods. New Zealand white virgin does, 6.5 to 7 months old, were mated three or four times to bucks of proven fertility. These does are designated as capacitator does. Immediately after breeding the capacitator doe was injected intravenously with an anesthetizing dose of sodium pentabarbital (Halatal, Jensen-Salisbury Laboratories, Kansas City, Mo.). The dose of pentobarbital was adjusted to keep each doe anesthetized for 2 hr. For progestin analysis, 2.0-ml blood samples were drawn from the marginal ear vein into heparinized syringes 2, 6, and 10 hr after mating. In the experiments testing the effect of pentobarbital with exogenous HCG, the HCG was administered intravenously while the doe was anesthetized. Spermatozoa were recovered from the uteri of test and control does 10 hr after mating. This was done by injecting and aspirating 3 ml of Krebs–Ringer phosphate solution containing 0.25% glucose and 5% heated rabbit serum (KRP-GS). The spermatozoa were counted, washed, and suspended to a concentration of 10^6 sperm/ml KRP-GS. Then, a volume (0.05 ml) containing 5.0×10^4 spermatozoa was deposited into the infundibular end of the oviduct of each doe that had ovulated 2 hr earlier (ova donors). Twenty-four hr later the does were sacrificed and the oviducts excised. The contents were flushed into a petri dish with saline. Ova were examined for cleavage under 25× magnification.

Progestin was assayed by a modification of Murphy's competitive protein-binding method (5). Blood plasma was extracted with 10 ml of petroleum ether after addition of 2300 cpm of ^{3}H-progesterone. The extract was dried and transferred to 12 × 75-mm plastic tubes (Falcon Plastics) in 1 ml of methanol. One-tenth ml was taken for an estimate of recovery and 0.3 ml was transferred to another tube for assay, leaving 0.6 ml for assay in the first tube. A binding solution was prepared by adding 10,000 cpm of ^{3}H-corticosterone to a 2.5% solution of male dog plasma that had been treated with dextran-coated charcoal. Bound and unbound steroids

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TABLE I. Effect of Sodium Pentobarbital on the Capacitation of Spermatozoa in Naturally Mated Rabbits.

Treatment		Ova recovered		No. of oviducts
Sodium pentobarbital	Mating	cleaved/total	% Fertile	inseminated
+	+	18/44	40.9	12
—	+	23/29	79.3[a]	11
—	—	6/16	38.5	5

[a] $p < 0.05$ for difference between 79.3 and 40.9% and for difference between 79.3 and 38.5%.

were separated using 40 mg of washed Florisil (magnesium silicate).

Results and Discussion. Judged by the percentage of ova fertilized in ova donors, sodium pentobarbital reduced the degree of capacitation to that seen in the estrous does not bred (Table I).

Several workers (6, 7) have shown that barbiturates block ovulation by blocking the endogenous release of LH. Indeed, if LH release from the pituitary stimulates capacitation as Soupart (3) suggests, then blocking the LH release should reduce capacitation to the levels seen in untreated rabbits.

Because mating stimulates release of LH and LH in turn causes synthesis and secretion of progesterone and 20-α-hydroxyprogesterone from the ovary (8), the inhibition of LH release by sodium pentobarbital should prevent that synthesis and secretion. To test this, we collected blood from a limited number of does three times during the 10 hr after mating and assayed total plasma progestin. The results (Table II) further substantiate that sodium pentobarbital reduced capacitation by preventing ovarian release of progestin. In an earlier study (9), we showed that an optimal ratio of estrogen to progestin is necessary to provide an optimum environment for sperm capacitation.

HCG stimulates progestin secretion in unmated estrous does and optimal amounts of exogenous gonadotropin should, therefore, return capacitation to the level obtained in normally mated animals. Indeed 75 IU of HCG restored fertility to the levels achieved in the untreated does mated at the same time (58% vs 60%). This dose of HCG is considered optimal for capacitation (3). It is also noteworthy that the animals given sodium pentobarbital immediately after mating did *not* ovulate within 12 hr of the time when sperm was recoverel from the uterus, but those receiving HCG with pentobarbital did.

Several pharmacologic anesthetics effectively block the release of pituitary gonadotropin (10), and very rapid postcoital injections of anticholinergic agents effectively block copulation-induced ovulation in the rabbit (10). These data lend further credence to the theory that gonadotropins with luteinizing hormone action enhance capacitation, and that this action is mediated through the secretion of ovarian steroids.

Summary. To test the hypothesis that coitus enhances capacitation by stimulating pituitary release of gonadotropin, naturally mated mature rabbit does were treated with sodium pentobarbital. Pentobarbital reduced the number of ova fertilized in the capacita-

TABLE II. Progestin Levels in Does Following Mating or HCG Injection.

Treatment			Progestin levels (ng/ml) (hr following treatment)		
Sodium pentobarbital	Mating	HCG, 75 IU	2	6	10
—	+	—	5.4	40	4.7
+	+	—	3.6	2.8	3.0
—	—	+	8.8	17.7	3.9

tion test system to the level seen in unmated untreated does. Progestin levels measured in peripheral blood of the capacitator does treated with pentobarbital failed to increase following mating. Human chorionic gonadotropin given to pentobarbital-treated does increased the number of ova fertilized in the test system and restored the increase in peripheral progestin levels.

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