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Capacitation in the Rabbit: Stimulation, Inhibition, and Recovery from Inhibition (37938)

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There have been many studies on the influence of hormones on capacitation during the past 15 years (1–8). It has been established that estrogens enhance capacitation in the rabbit uterus while progestagens are inhibitory (1–5). It is not known how soon the uterus recovers from progesterone inhibition or if estrogen speeds recovery.

There is little information (3) available on the effect of estrogen on capacitation in the oviduct, but it appears that estrogen is stimulatory. It is agreed that progesterone apparently does not inhibit capacitation in the oviduct (3–5). The aim of this investigation is to compare the capacitating ability of the oviduct and uterus in the same ovariectomized rabbit with and without estrogen and the ability of the uterus to recover from progesterone inhibition.

Materials and Methods. Semen collected by artificial vagina from 5 New Zealand White rabbits 1–2 years old was pooled. The semen was filtered through 2 layers of cheesecloth into a centrifuge tube and diluted with an equal volume of saline (0.9% NaCl). The diluted semen (0.1 ml; $3–5 \times 10^6$ sperm) was injected through a 23-gauge needle into the uterus via uterine wall puncture or the oviduct via the uterotubal junction of test rabbits weighing 3.6–4.1 kg. The uterotubal junction was always ligated after sperm injections. The rabbits tested for their ability to capacitate sperm included reproductively intact estrous controls, ovariectomized does left untreated for 3–44 days, ovariectomized does treated with subcutaneous injections of 0.5–20.0 μg estradiol cyclopentylpropionate (ECP)/kg in 1.0 ml cottonseed oil (CSO) daily for 6 days, ovariectomized does treated with a sc injection of 1.0 mg progesterone in 1.0 ml CSO daily for 10 days followed by a nontreatment period of 5 and 10 days, and ovariectomized does treated with 1.0 mg progesterone daily for 10 days followed by 0.5 μg ECP/kg/day daily for 0.5 to 10 days. The sperm were incubated 16–18 hr before being aspirated from the test site with a syringe containing saline. The sperm were then checked for numbers and motility. One-tenth milliliter of the incubated sperm suspension (400–3000 sperm) was transferred to the oviduct of egg donors 12.5–13.5 hr (circa 2–3 hr postovulation) after iv injection of 50 IU of human chorionic gonadotropin (HCG) (Follutein, E. R. Squibb and Sons). The egg donors were New Zealand White female rabbits weighing 3.5–4.5 kg. The eggs were recovered from the donors by flushing the oviducts with 2 ml of saline into a watch glass 24–28 hr after insemination. The eggs were examined under a bright field microscope at 50–125 times magnification to determine normal cleavage. For comparison between treatments, the data was arranged in a fourfold contingency table and the significance level ($P \leqslant 0.05$) was determined by the method of Mainland and Murray (9).

Results. Three days after ovariectomy, there was a significant decrease ($P < 0.05$) in the ability of the uterus or oviduct to

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TABLE I. Fertilizing Ability of Sperm After Incubation in the Uterus and Oviduct of the Ovariectomized Rabbit.

	Incubation site					
		Oviduct: eggs examined[a]			Uterus: eggs examined[a]	
Days after ovariectomy	No. of animals	Total	Fertilized (%)	No. of animals	Total	Fertilized (%)
0 (estrous control)	5	43	65	5	44	77
3	6	34	38	4	91	34
6	5	43	30	5	70	41
10	4	23	56	4	56	45
24				3	28	36
44				3	68	41

[a] Eggs are recovered 24 hr after incubated sperm are placed in the oviduct of egg donors.

capacitate sperm when compared to estrous controls. There was no further decrease in capacitating ability after the third day post-ovariectomy (Table I). The ability of the uterus and oviduct to capacitate sperm using our test method was the same. Both organs showed a biphasic response to estrogen (estradiol cyclopentylpropionate ECP, Upjohn Co.). In ovariectomized rabbits, there was a significant ($P < 0.05$) increase in capacitating ability with 0.5 and 1.0 μg ECP/kg/day (Table II).

One milligram of progesterone per day (circa 0.25 mg/kg/day) for 10 days inhibits capacitation in the ovariectomized rabbit uterus (5). Results in Table III indicate that the uterus requires about 10 days to return to its previous nontreated ovariectomized capacitating state after 10 days of progesterone treatment although a significant ($P < 0.05$) improvement is seen in 5 days. If estrogen (0.5 μg ECP/kg/day) is started immediately after 10 days of progesterone treatment, there is a highly significant ($P < 0.01$) increase in uterine capacitating ability by 24 hr (Table IV). This increase is equivalent to the capacitating level reached in 10 days with no estrogen treatment. However, no further increase is seen when estrogen treatment is con-

TABLE II. Fertilizing Ability of Sperm After Incubation in the Uterus and Oviduct of Ovariectomized Rabbits Treated with Estrogen.[a]

	Incubation site					
		Oviduct: eggs examined			Uterus: eggs examined	
Estrogen dose (μg/kg/day)	No. of animals	Total	Fertilized (%)	No. of animals	Total	Fertilized (%)
0	5	43	30	5	70	41
0.5	3	16	56	5	91	91
1.0	5	25	60	5	69	84
2.0	3	12	41	5	71	46
4.0	3	17	35	5	81	62
20.0	3	12	41	5	32	65

[a] Rabbits ovariectomized and injected subcutaneously with estradiol cyclopentylpropionate in 1.0 ml cottonseed oil daily for 6 days before sperm incubation.

TABLE III. Effect of Time on Recovery of Uterine Capacitating Ability After Progesterone Inhibition.[a]

Day of sperm incubation after last progesterone injection	No. of animals	Eggs examined		
		Total	Fertilized	Fertilized (%)
0	6	26	1	4
5	6	34	9	26
10	6	40	15	38

[a] 1.0 mg progesterone in 1.0 ml cottonseed oil injected subcutaneously daily for 10 days after ovariectomy.

tinued for 6 days. The return to optimum capacitating ability did not occur until 10 days after estrogen treatment was started.

Discussion. It was concluded earlier (10) that progestagens both inhibit the capacitating ability of the uterus and depress the physiologic health of sperm by causing immobilization and head and tail separation. Observations made in Table IV show that there is a quick response to estrogen treatment, apparently because of factors specifically dependent on estrogen, but 10 days of treatment is required to return to an optimum environment.

Previously published data demonstrated that the capacitating ability of the oviduct is not suppressed by progesterone as is the uterus (3–5). Perhaps the oviduct's lack of sensitivity to progesterone with regard to capacitation assures the female of a site for completion of this vital process.

The biphasic response of the uterus to estrogen confirms the earlier work of Soupart (2). Estrogens from the adrenals may be enough for maintenance of a constant, but significantly lower, capacitating ability by the reproductive tract after ovariectomy (11).

Summary. The capacitating ability of the oviduct and uterus in the ovariectomized rabbit with and without estrogen treatment was compared. There was a significant decrease in the ability of the uterus and oviduct to capacitate sperm within 3 days after ovariectomy. Both the uterus and oviduct showed a biphasic response to estrogen. Optimum capacitation occurred when ovariectomized rabbits were given 0.5–1.0 μg ECP/kg/day.

The ability of the uterus to recover after inhibition by progesterone was studied. The uterus requires about 10 days to return to its nontreated ovariectomized capacitating state after progesterone treatment is stopped. If estrogen is given when progesterone is stopped, the uterine capacitating ability is

TABLE IV. Effect of Estrogen on Recovery of Uterine Capacitating Ability After Progesterone Inhibition.[a]

Days of estrogen injection	No. of animals	Eggs examined		
		Total	Fertilized	Fertilized (%)
0.5	4	17	2	12
1	3	23	11	48
2	4	28	12	44
4	5	36	20	55
6	5	26	12	48
10	3	16	15	94

[a] Rabbits were treated with 1.0 mg progesterone daily for 10 days before estrogen treatment (0.5 μg ECP/kg/day) was initiated prior to sperm incubation.

restored to the nontreated ovariectomized state in 24 hr. However, only after 10 days of estrogen treatment is optimum capacitating ability regained by the uterus.

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