Sperm Capacitation after Injection of LH or HCG in Rabbits[1] (34615)

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Sperm capacitation (1, 2) in the rabbit uterus occurs more rapidly when estrogen is the predominant gonadal hormone (3). Soupart (4, 5) reported that sperm capacitation proceeds more rapidly after mating than after surgical insemination into the uterine lumen, and that an injection of 75 IU of HCG enhances sperm capacitation similarly to mating in rabbits. These observations prompted us to compare the influence of HCG with that of LH on sperm capacitation.

Materials and Methods. Mature estrous rabbits of mixed breeding and weighing 3 to 5 kg were used for capacitator (6) and test rabbits. Each rabbit was caged individually for at least 1 month at 18° with a 14-hr photoperiod before assignment to an experiment.

In any experiment, semen samples were pooled from 2 to 4 rabbits with proven fertility. Seminal plasma was discarded and the sperm were resuspended to 500 million/ml of Locke's solution with 100 μg of dihydrostreptomycin sulfate /ml. Under ether anesthesia, the uterine horns of capacitator rabbits were exposed via a midventral incision and 90 to 200 million sperm (at least 50% motile) were injected within each uterine horn near the tubouterine junction with a 26-gauge needle. HCG[3] (0, 50, 75, 100, or 300 IU) or NIH-LH-B_5[4](0, 100, 250, 500, 1000, or 2000 μg-equivalents of NIH-LH-S_1) in 0.85% sodium chloride was injected intravenously into capacitator rabbits immediately after sperm deposition within the uterus. Ten hr later, capacitator rabbits were anesthetized and sperm were aspirated from their uteri. When we injected 1.0 ml of Locke's solution into each uterine horn before aspiration, we recovered an average of 4.3% (SD = 5.0%) of the sperm.

The fertility of sperm inseminated into superovulated (7) test rabbits was our measure of sperm capacitation. Test rabbits were surgically inseminated, at 12.5 hr after an ovulating injection of 75 IU of HCG, with 0.3 to 2.5 million sperm deposited near the tubouterine junction in each uterine horn or about 1.5 cm in the infundibular end of the oviduct. Control test rabbits were inseminated with freshly ejaculated sperm at the time of, or 12.5 hr after, the ovulating injection. Approximately 36 hr after ovulating injections, test rabbits were killed by cervical dislocation. Ova were flushed from the oviducts and observed for cleavage with bright field (35×) and with phase contrast (250×) illumination. The data were analyzed with analysis of variance and orthogonal contrasts.

Results. To determine the appropriate time to inseminate superovulated test rabbits with sperm to be assayed for capacitation, 2.5 million freshly ejaculated sperm, suspended in Locke's solution, were deposited surgically within each uterine horn at 0, 6, 8, 10, 12, or 14 hr after an ovulating injection of HCG. Fertility from inseminations 10 to 14 hr after injection of HCG was reduced greatly below that resulting from inseminations earlier than 10 hr (Fig. 1). This reduced fertility is similar to that reported in nonsuperovulated rabbits (8) inseminated at comparable peri-

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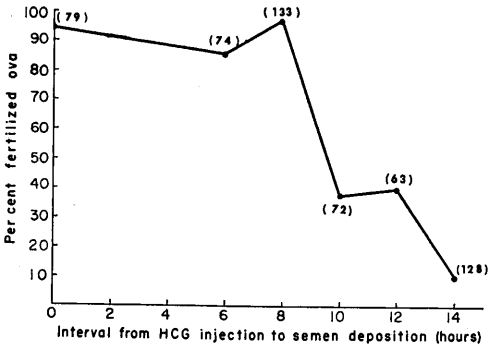

FIG. 1. Fertility of uterine inseminations performed with freshly ejaculated sperm at varying intervals after injection of HCG. (Number of ova in parentheses).

ods after injection of ovulating hormone. On the basis of the data in Fig. 1, 12.5 hr after injection of HCG was selected as an appropriate time to inseminate superovulated test rabbits with sperm to be assayed for capacitation. Control test rabbits inseminated in capacitation experiments described below confirm the above results; of 274 ova recovered from 8 test rabbits inseminated within the uterus with freshly ejaculated sperm 12.5 hr after an ovulating injection of HCG, only 18 ova were fertilized. Sperm inseminated into the uterus at the time of HCG injection were fertile as 94 of the 103 ova recovered from 7 test rabbits were fertilized (Tables I and II).

When freshly ejaculated sperm were deposited into the oviducts 12.5 hr after the injection of HCG, only 1 of 161 ova recovered from 10 test rabbits was cleaved (Tables I and II). Seventy of 168 ova recovered from 7 test rabbits inseminated into the oviducts at the time of HCG injection were fertilized.

Injections of 300 IU of HCG into capacitator rabbits significantly ($p<0.05$) inhibited fertility relative to all other levels of HCG (Table I). But, fertility was not altered, relative to saline injected rabbits, when sperm were incubated in capacitator rabbits injected with 75 IU of HCG. The reduced fertility with 300 IU HCG and the failure to alter fertility with 75 IU HCG occurred with ov-

TABLE I. Fertility of Spermatozoa after 10-hr Uterine Incubation in Rabbits Injected with HCG.

Sperm treatment	Test rabbits inseminated			Ova[c]	
	Time[b] (hr)	Site	No.	Recovered (no.)	Fertilized (%)
Freshly ejaculated	12.5	Oviduct	6	99	0 ± 0
	12.5	Uterus	4	151	5 ± 2
	0	Oviduct	4	100	50 ± 15
	0	Uterus	3	36	94 ± 3
Capacitated,[a] 0 IU HCG	12.5	Oviduct	4	26	62 ± 22
	12.5	Uterus	4	87	85 ± 10
50 IU HCG	12.5	Oviduct	3	72	57 ± 25
	12.5	Uterus	4	97	93 ± 4
75 IU HCG	12.5	Oviduct	5	47	70 ± 18
	12.5	Uterus	4	99	88 ± 12
100 IU HCG	12.5	Oviduct	3	56	41 ± 21
	12.5	Uterus	4	62	100 ± 0
300 IU HCG	12.5	Oviduct	4	90	22 ± 11
	12.5	Uterus	4	89	49 ± 21

[a] Sperm recovered after 10-hr uterine incubation in capacitator rabbits (four at each level of HCG).

[b] Hours after injection of ovulating hormone.

[c] Ova flushed from oviducts 36 ± 2 hr after injection of ovulating hormone. Mean percentage fertility ± among test rabbits standard error.

TABLE II. Fertility of Spermatozoa after 10-hr Uterine Incubation in Rabbits Injected with LH.

Sperm treatment	Test rabbits inseminated			Ova[c]	
	Time[b] (hr)	Site	No.	Recovered (no.)	Fertilized (%)
Freshly ejaculated	12.5	Oviduct	4	62	2 ± 1
	12.5	Uterus	4	123	11 ± 8
	0	Oviduct	3	68	29 ± 18
	0	Uterus	4	67	90 ± 17
Capacitated,[a] 0 μg LH	12.5	Oviduct	4	32	44 ± 17
	12.5	Uterus	4	84	44 ± 20
100 μg LH	12.5	Oviduct	4	35	34 ± 22
	12.5	Uterus	5	107	92 ± 5
250 μg LH	12.5	Oviduct	3	21	24 ± 18
	12.5	Uterus	4	81	86 ± 11
500 μg LH	12.5	Oviduct	3	101	56 ± 19
	12.5	Uterus	4	145	87 ± 9
1000 μg LH	12.5	Oviduct	4	76	50 ± 3
	12.5	Uterus	5	137	87 ± 11
2000 μg LH	12.5	Oviduct	4	100	67 ± 17
	12.5	Uterus	3	82	90 ± 11

[a] Sperm recovered after 10-hr uterine incubation in capacitator rabbits (four at each level of LH).

[b] Hours after injection of ovulating hormone.

[c] Ova flushed from oviducts 36 ± 2 hr after injection of ovulating hormone. Mean percentage fertility ± among test rabbits standard error.

iducal and with uterine inseminations. Average fertility from uterine insemination was significantly greater than that from oviducal insemination ($p<0.01$). The interaction between level of HCG and site of insemination did not approach significance ($p\cong0.50$).

Although fertility of sperm recovered from capacitator rabbits injected with LH generally was enhanced (Table II), none of the differences among levels of LH was significant ($p>0.50$). There was no indication of diminished fertility with high levels of LH. Similar to the results in the HCG experiment, rabbits inseminated into uteri had significantly ($p<0.01$) higher fertility than those inseminated into oviducts.

Discussion. In agreement with our observation that fertility was depressed ($p<0.05$) when capacitator rabbits were injected with 300 IU of HCG, Soupart (5) also observed inhibition of sperm capacitation in rabbits injected with high levels of HCG (200 or 400 IU). But he reported significantly enhanced sperm capacitation in rabbits injected with 75 IU of HCG. We found no significant increase in fertility, relative to saline-injected rabbits, after rabbits were injected with 50, 75, or 100 IU of HCG. The major difference between Soupart's results and ours is the relatively low fertility (15.4%) he observed for sperm incubated in control estrous rabbits. Variable rates of sperm capacitation in control rabbits (see standard errors in Tables I and II) may be related to the observation (9) that less than half of isolated nonpregnant rabbits are receptive to males and permit copulation. We speculate that the variable mating behavior and the variable rates of capacitation in isolated ("estrous") rabbits may be associated with variable ovarian steroid hormone status.

In contrast to the influence of high levels of HCG, high levels of LH did not inhibit fertility of sperm incubated in the uterus. In

general, the fertility of sperm incubated in control rabbits (Table II) was less than that of sperm incubated in capacitator rabbits injected with LH. But this difference was insignificant ($p>0.50$) because of large variation of fertility among test rabbits. Soupart (10) observed enhanced fertility of sperm incubated in hypophysectomized rabbits injected with 200 μg of LH, but markedly reduced fertility when 400 μg of LH was injected. Thus, the influence of injected LH on sperm capacitation in the uterus may depend upon the pituitary.

The difference in influence of high levels of LH and HCG on sperm capacitation (Tables I and II) is reminiscent of a similar difference in progestin synthetic response of bovine luteal tissue to high levels of LH and HCG *in vitro* (11). The cause, in either case, is obscure. The reduced fertility we observed after injection of 300 IU of HCG is not due to the LH property of HCG, because injection of 2000 μg of LH did not inhibit sperm capacitation. Based upon ovarian ascorbic acid depletion, 1 IU of HCG is equivalent to 1 μg of NIH-LH-S_1 (12).

Higher fertility with uterine than with oviducal inseminations was also reported by Adams and Chang (8) and probably reflects more rapid sperm capacitation in the uterus than in the oviduct of estrous rabbits. That we found no interaction between site of insemination and level of gonadotropin indicates that either site of insemination may be used to assess the influence of these gonadotropins on sperm capacitation.

Summary. Sperm incubated in uteri of rabbits given intravenous injections of LH or HCG were assayed for capacitation in superovulated test rabbits inseminated either within the uterus or within the oviduct 12.5 hr after an injection of ovulating hormone. Injection of LH at levels up to 2000 μg had no significant influence on sperm capacitation, but 300 IU of HCG reduced fertility. Test rabbits inseminated within the uterus had significantly higher fertility than those inseminated within the oviduct. The results indicated that sperm may be inseminated at either location in experiments to assess the influence of these gonadotropins on capacitation.

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