Sperm Capacitation Assay in Superovulated Rabbits with Uterine or Oviducal Inseminations[1] (35838)

R. P. WETTEMANN,[2] W. INGALLS, AND H. D. HAFS

Animal Reproduction Laboratory, Department of Dairy Science, Michigan State University, East Lansing, Michigan 48823

Rabbit spermatozoa must reside in the uteri or oviducts for a period of time to develop the capacity to fertilize ova. Fertility of oviducal inseminations performed after ovulation is the usual measure of sperm capacitation (1, 2). A reliable procedure to superovulate rabbits (3) provided a means to increase the number of observations in assays for sperm capacitation. Superovulated ova are fertile when inseminations are performed before the time of ovulation (3); and Maurer *et al.* (4) demonstrated that superovulated ova develop into viable young. However, Varian *et al.* (5) reported that ovulation may occur slightly later after an injection of ovulating hormone into rabbits primed with FSH; and Sietz *et al.* (6) found that the interval between HCG and the first ovulation can be reduced by previous treatment with PMSG.

Since superovulation may alter the intervals from injection of HCG to ovulation and to cleavage of ova, the present experiments were designed principally to determine whether superovulation of test rabbits influences results of sperm capacitation assays. A second objective was to learn whether, in tests of sperm capacitation, the test rabbits could be inseminated in the uterus rather than in the oviduct, because we were concerned that inseminations into the ovarian end of the oviduct may influence the results by displacing that portion of the oviduct.

Materials and Methods. Mature estrous rabbits of mixed breeding and weighing 3 to 5 kg were caged individually for at least 30 days at 18° with a 14-hr photoperiod before assignment to an experiment. In each experiment, semen samples were pooled from six rabbits with proven fertility. Seminal plasma was discarded and the sperm were resuspended to 25 or 500 million/ml of Locke's solution with 100 μg of dihydrostreptomycin sulfate/ml. Portions of these pooled semen samples were used for inseminations requiring freshly ejaculated sperm, and portions (125×10^6 sperm in 0.2 ml) were surgically deposited in each uterine horn of estrous capacitor rabbits which were injected with 100 μg of NIH-LH[3] (7). After 10 hr in capacitator rabbits, the sperm were aspirated from the uteri and concentrated to 25 million/ml.

With uterine inseminations of test rabbits, 2.5 million sperm in 0.1 ml were deposited near each tubo-uterine junction and for oviducal inseminations, 1.25 million sperm in 0.05 ml were deposited about 2 cm into the fimbriated end of each oviduct. Test rabbits were killed by cervical dislocation 34 hr after injection of ovulating hormone (75 IU of HCG[4]). Numbers of ovulation points were determined, ova were flushed from the oviducts, and numbers of blastomeres were determined.

To determine the appropriate time to inseminate superovulated test rabbits in assays for capacitation, 60 rabbits were injected twice daily with 0.4 mg of FSH[5] for 3 days.

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[4] Supplied by E. R. Squibb and Sons, Inc., Cream Ridge, New Jersey.

[5] Armour FSH-P.

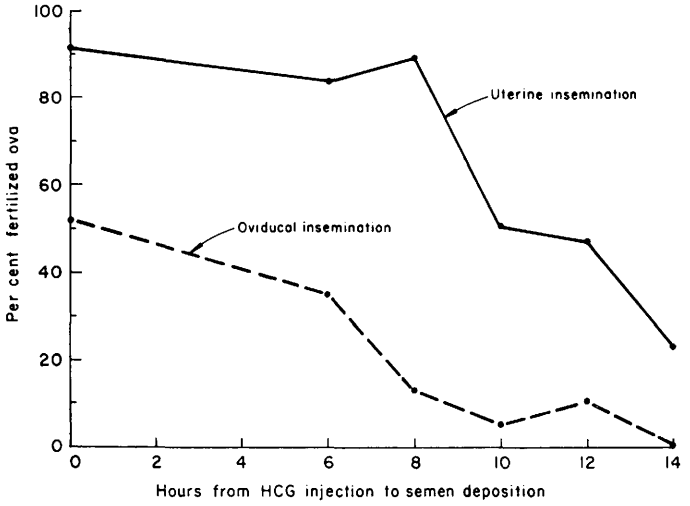

FIG. 1. Fertility of oviducal and uterine inseminations with freshly ejaculated sperm at intervals after injection of ovulating hormone. Numbers of ova represented in each point were from 38 to 93 for oviducal and from 90 to 194 for uterine inseminations. Part of these data for uterine insemination was published previously [Proc. Soc. Exp. Biol. Med. 133, 1002 (1970)].

Five rabbits were inseminated within each uterine horn and 5 rabbits were inseminated within each oviduct at 0, 6, 8, 10, 12, and 14 hr after an ovulating injection of HCG.

In a second experiment, 119 rabbits were injected with 75 IU of HCG, 60 of them following twice daily injection of FSH as in the first experiment. Half of each ovulation group was inseminated in each oviduct and half in each uterine horn adjacent to the tubo-uterine junction. In each of these four treatments, rabbits were inseminated at the time of HCG injection with freshly ejaculated sperm, 13 hr after HCG with freshly ejaculated sperm, or 13 hr after HCG with capacitated sperm.

Results. Normal fertility (84–92%) resulted from uterine inseminations with freshly ejaculated sperm in superovulated rabbits up to 8 hr after HCG, *i.e.*, up to about 2 hr before ovulation (Fig. 1). But at 14 hr after HCG injection, uterine inseminations with freshly ejaculated sperm resulted in only 23% fertilized ova. Maximal fertility (52%) with oviducal inseminations occurred at the time of HCG injection; then fertility decreased with increasing time after HCG and only 5% fertility resulted from oviducal inseminations 10 hr after HCG (near the time of ovulation).

In the second experiment, an average of 22 ova was recovered after superovulation of 60 rabbits and an average of 8 ova was recovered from 59 nonsuperovulated rabbits. Neither sperm capacitation, time of insemination, nor site of insemination had a significant influence on ova recovery (Table I). But a significantly lower portion of ovulated ova were recovered from superovulated rabbits; 83% from controls and 72% from superovulated rabbits.

Oviducal inseminations of control or superovulated rabbits with freshly ejaculated sperm 13 hr after HCG resulted in very low fertility, and similar uterine inseminations resulted in only slightly higher fertility (Table I). Fertility of rabbits inseminated with capacitated sperm at 13 hr after HCG was similar to the fertility of rabbits inseminated with freshly ejaculated sperm at the time of HCG injections. Over all treatments, fertility was significantly higher with uterine than with oviducal inseminations ($p<0.05$).

Neither site of insemination nor superovulation influenced significantly the number of blastomeres (Table II) 34 hr after HCG. But ova recovered from rabbits inseminated with freshly ejaculated sperm at the time of HCG injection had significantly more blastomeres (4.72) than rabbits inseminated with

TABLE I. Fertility and Efficiency of Recovery of Ova from Control or Superovulated Rabbits After Oviducal[a] or Uterine[b] Inseminations.

Sperm	Ovulation	Test rabbits inseminated: Time[c] (hr)	Site	No.	Ova[d] recovered/ovulation points	Ova (%): Recovered	Fertilized
Freshly ejaculated	Control	13	Oviduct	11	64/115	59 ± 9[e]	4 ± 4[f]
		13	Uterus	9	76/82	92 ± 12	20 ± 10
	Superovulated	13	Oviduct	11	276/385	74 ± 8	1 ± 1
		13	Uterus	10	222/328	70 ± 11	5 ± 3
	Control	0	Oviduct	9	62/68	96 ± 14	34 ± 13
		0	Uterus	10	85/100	87 ± 8	71 ± 9
	Superovulated	0	Oviduct	10	194/274	73 ± 7	26 ± 8
		0	Uterus	10	242/325	82 ± 10	80 ± 7
Capacitated[g]	Control	13	Oviduct	10	78/98	84 ± 8	54 ± 11
		13	Uterus	10	76/89	83 ± 7	51 ± 10
	Superovulated	13	Oviduct	9	149/271	58 ± 8	30 ± 6
		13	Uterus	10	211/317	77 ± 8	72 ± 9

[a] Sperm (1.25×10^6 in 0.05 ml) deposited about 2 cm into the fimbriated end of each oviduct.
[b] Sperm (2.5×10^6 in 0.1 ml) deposited near each tubo-uterine junction.
[c] Hours after injection of ovulating hormone.
[d] Ova flushed from oviducts 34 hr after injection of ovulating hormone.
[e] Mean percentage recovery ± standard error among rabbit.
[f] Mean percentage fertility ± standard error among rabbit.
[g] Sperm recovered after uterine incubation for 10 hr in capacitor rabbits injected with 100 μg of NIH-LH at the time of sperm deposition.

capacitated sperm 13 hr after HCG (3.90). Standard errors of the mean number of blastomeres were larger when rabbits were inseminated with freshly ejaculated sperm at the time of HCG injection than when rabbits were inseminated with capacitated sperm.

Discussion. Fertility of uterine inseminations was maximal in superovulated rabbits when inseminations were performed at least 2 hr prior to ovulation. But maximal fertility from oviducal inseminations occurred when inseminations were performed about 10 hr before ovulation. These fertility results are similar to those reported in nonsuperovulated

TABLE II. Average Number of Blastomeres in Fertilized Ova.

Sperm treatment	Ovulation method	Test rabbit inseminated: Time[a] (hr)	Site	No. of blastomeres
Freshly ejaculated	Control	0	Oviduct	4.60 ± 0.60[b]
		0	Uterus	5.25 ± 0.52
	Superovulated	0	Oviduct	4.73 ± 0.27
		0	Uterus	4.31 ± 0.14
Capacitated[c]	Control	13	Oviduct	3.94 ± 0.05
		13	Uterus	3.97 ± 0.08
	Superovulated	13	Oviduct	3.90 ± 0.16
		13	Uterus	3.78 ± 0.19

[a] Hours after injection of ovulating hormone.
[b] Mean number of blastomeres ± standard error among rabbit.
[c] Sperm recovered after uterine incubation for 10 hr in capacitator rabbits injected with 100 μg NIH-LH at the time of sperm deposition.

rabbits (8) inseminated at comparable periods after injection of ovulating hormone. But Ishijima *et al.* (9) found 74% of ova recovered from superovulated Japanese white rabbits were fertile when mating took place 12 hr after HCG injections and 23% fertility occurred at matings 14 hr after HCG. This slightly higher fertility than we found may be related to the PMS and estrogen used to induce superovulation. On the basis of our data, 12 to 14 hr after injection of HCG appears to be an appropriate time to inseminate superovulated test rabbits with sperm to be assayed for capacitation.

Lowest fertility was obtained from oviducal inseminations with freshly ejaculated sperm performed 13 hr after the injection of HCG. In contrast, fertility of inseminations with capacitated sperm performed 13 hr after HCG was comparable to that of inseminations with freshly ejaculated sperm at the time of HCG injection. Overstreet (10) reported that rabbits inseminated with 10,000 capacitated sperm 10 to 13 hr after HCG had higher fertility than those inseminated with untreated sperm at the time of HCG injection. Although we found a comparable difference in fertility among oviducal inseminations in control rabbits, it was not significant and uterine inseminations resulted in the opposite trend. Differences between these results and Overstreet's may be related to numbers of sperm inseminated. These data indicate that few ova will be fertilized when inseminations are performed 13 hr after HCG injection unless the sperm have been capacitated previously.

In agreement with earlier results (3), fertility of ova from control and from superovulated rabbits did not differ appreciably. Higher fertility from uterine than from oviducal inseminations is consistent with previous reports (7, 8), and is probably caused by more rapid sperm capacitation in the uterus than in the oviduct of estrous rabbits.

Varian *et al.* (5) observed that, at 23 to 33 hr after injection of ovulating hormone, fertilized ova from control rabbits were in a later stage of cleavage than ova from superovulated rabbits. We did not find this difference among ova recovered at 34 hr after injection of HCG. Neither was the mean number of blastomeres influenced significantly by site of insemination. Thus, neither of these treatments had significant influence on the time of ovulation. But the means and the standard errors for number of blastomeres were larger when rabbits were inseminated with freshly ejaculated sperm at the time of HCG injection. The more advanced developmental stage of ova recovered from rabbits inseminated with freshly ejaculated sperm at the time of HCG injection may be attributed to fertilization occurring soon after ovulation, while our inseminations with capacitated sperm were performed 2 to 3 hr after ovulation.

We conclude that sperm may be assayed for capacitation with oviducal or with uterine inseminations into superovulated test rabbits. Lower fertility from oviducal than from uterine inseminations may provide a more discriminative assay.

Summary. Rabbits which had been superovulated with FSH and HCG were compared with rabbits ovulated with HCG to assay sperm for capacitation. Inseminations with freshly ejaculated sperm 0 to 13 hr after an ovulating injection of HCG were compared with inseminations with capacitated sperm 13 hr after HCG. Superovulation increased the number of recovered ova from 8 to 22 and depressed percentage of ova recovery, but did not influence fertility significantly. Site of insemination did not influence ova recovery, but uterine inseminations resulted in higher fertility than oviducal inseminations whether they were performed with freshly ejaculated or with capacitated sperm. Inseminations with freshly ejaculated sperm at the time of injection of HCG resulted in more advanced ova development 34 hr after HCG than inseminations with capacitated sperm 13 hr after HCG. The data indicate that sperm may be assayed for capacitation with oviducal or with uterine inseminations into superovulated test rabbits. Lower fertility from oviducal than from uterine inseminations may provide a more discriminative assay.

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