Capacitation of Rabbit Spermatozoa: Species Specificity and Organ Specificity.* (31825)

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The necessity for freshly ejaculated spermatozoa to reside in the female reproductive tract of the rabbit and rat a specified length of time in order to gain the capacity to fertilize the ovum was described by Chang (1) and Austin(2). Since these initial observations the requirement for capacitation of sperm has been described in the golden hamster(3) and the ewe(4). However, little progress has been made in elucidating the exact nature of the capacitation process.

One report(5) has indicated that the factors necessary for capacitation of rabbit spermatozoa are present in areas of the body other than the female reproductive tract, *i.e.*, isolated bladder and colon, anterior chamber of the eye and seminal vesicles of the male. The aim of this investigation is to determine if the mechanism of capacitation is similar in several species and to study further the capacitation of rabbit spermatozoa in various hollow organs of the body.

Materials and methods. Semen collected by artificial vagina from 6 New Zealand White rabbits 1 to 2 years old was pooled. The pooled semen was filtered through 2 layers of cheesecloth into a centrifuge tube and diluted 5-fold with saline. The diluted semen was centrifuged at 250 *g* for 7 minutes and the supernatant discarded. The packed sperm were resuspended in saline and injected (0.5 ml; 0.4-2.0 $\times$ 10^8 sperm) into the test organ, *i.e.*, rat uterus, dog uterus, rabbit uterus, oviduct, isolated bladder, isolated colon, and knee joint. When the bladder or colon was used as a test organ, a sac area was isolated from the organ by a purse string suture without interfering with the blood supply. The sac was flushed with saline prior to injection of the washed sperm. Only estrous rats, dogs and rabbits were used. Estrus was determined in these animals by the presence of cornified cells from a vaginal smear(6,7) and the presence of ripe follicles on the ovaries with no corpus luteum present. The washed spermatozoa were incubated 6 to 11 hours in the test organ. The incubated sperm were withdrawn from the test organ with a syringe and checked for motility. One-tenth milliliter of the sperm suspension (1.0-2.0 $\times$ 10^6 sperm) was transferred to the oviducts of egg donors 11.5 to 12.5 hours (approximately 2 hours post ovulation) after intravenous injection of 5 mg P.L.H. (equine pituitary luteinizing hormone—Armour-Baldwin Laboratories) or 100 I.U. of Follutein (human chorionic gonadotropin—E. R. Squibb and Sons). The egg donors were virgin New Zealand White female rabbits 6 to 8 months old, held in quarantine 21 days before use. The eggs were recovered from the donors by flushing the oviducts with 2 ml of saline into a watch glass 20 hours after insemination with the incubated sperm. The eggs were examined under a bright field microscope at 40 and 100 times magnification to determine normal cleavage.

Results. To determine if the mechanism of capacitation was the same in several species, washed rabbit spermatozoa were incubated in the uterus of estrous dogs, rats and rabbits for 6 to 8 hours. It was found that rabbit sperm (Table I) were capacitated in the uterus of both the rat (9 of 33 eggs fertilized) and dog (6 of 27 eggs fertilized) as well as in the rabbit uterus (11 of 38 eggs fertilized). However, sperm incubated *in vitro* in saline with glucose (15 mg/ml) at 37°C failed to fertilize any eggs.

To determine if rabbit spermatozoa could be capacitated in organs other than the female reproductive tract, washed sperm were incubated in the rabbit uterus, oviduct, isolated bladder, isolated colon and knee joint. Table II shows that sperm incubated in the uterus and oviduct fertilize 26 of 53 eggs and 15 of 26 eggs, respectively. However, sperm incubated in the bladder, colon and

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TABLE I. Species Specificity of Capacitation of Rabbit Spermatozoa.

Site of incubation	No. exp	Incubation time (hr)	Ova recovered		
			Total	No. cleaved	% Fertile
Rabbit uterus	4	6–8	38	11	29
Rat "	3	7–8	33	9	27
Dog "	3	6–7	27	6	22
In vitro	1	6	7	0	0

TABLE II. Organ Specificity of Capacitation of Rabbit Spermatozoa.

Site of incubation	No. exp	Incubation time (hr)	Ova recovered		
			Total	No. cleaved	% Fertile
Rabbit Uterus	7	9–11	53	26	49*
Oviduct	4	9–11	26	15	58
Bladder	4	9–10	28	2	7
Colon	3	9–10	26	1	4
Knee joint	1	11	3	0	0
In vitro	2	9–11	11	0	0

* Significantly different from bladder(8), $P < .05$.

TABLE III. Comparison of Fertilizing Ability of Rabbit Sperm Incubated in Uterus and Bladder.

Site of incubation	Incubation time (hr)	Insemination time (hr post P.L.H.)	Ova recovered		
			Total	No. cleaved	% Fertile
Rabbit uterus	9	12	3	2	67
	9	10	5	4	80
" bladder	9	12	6	1	17
	9	10	12	4	33

knee joint fertilized only 2 of 28 eggs, 1 of 26 eggs and 0 of 3 eggs, respectively. Sperm recovered from the bladder, colon and knee joint were similar in concentration ($1.0\text{-}2.0 \times 10^7$ sperm/ml) and motility to those recovered from the uterus or oviduct and gave no hint of their inability to fertilize at a level equivalent to the sperm recovered from the uterus or oviduct. Again, sperm incubated *in vitro* failed to fertilize eggs.

The above results are in conflict with a previous report(5) concerning capacitation of sperm in various organs of the body, where the incubated sperm were placed in the oviducts of the egg donors 9.5 to 10.5 hours after injection of the chorionic gonadotropin. Thus two experiments were designed to duplicate time sequences used in this investigation and the earlier investigation(5). To determine if placing the sperm in the oviduct of the egg donors 2 hours earlier affected the fertilizing ability of the sperm, washed rabbit sperm were incubated in the uterus and isolated bladder for 9 hours before transfer to the oviducts of egg donors 10 and 12 hours after injection of luteinizing hormone. It was found that the fertilizing ability of sperm incubated in the bladder doubled when the sperm were placed in the oviducts of egg donors at 10 hours after P.L.H. as compared with 12 hours after P.L.H. (Table III). However, the fertilizing ability of sperm incubated in the uterus was quite similar at either time of insemination. Thus it is concluded that the rabbit sperm incubated in the bladder gained their ability to fertilize in the reproductive tract of the egg donors.

Discussion. It can be concluded from these results that the factors necessary for capacitation of rabbit sperm are present in the uterus of the estrous dog, rat and rabbit. However, it was found that the environment in the uterus of the estrous dog and rat was not as

entirely compatible with the rabbit sperm as the rabbit uterus. On two occasions it was noted that if the rabbit sperm were incubated in the dog uterus for 8 to 10 hours the sperm became immobilized. In several rats immobilization of the sperm, as well as an occasional overwhelming leukocyte infiltration, was noted when sperm were incubated 8 hours. Because of this experience results are reported only for rabbit sperm incubated in the dog uterus for 6 to 7 hours and the rat uterus for 7 to 8 hours when the sperm remained motile and relatively free of leukocytes. The comparatively low fertilizing rate in the inter-species series of experiments may be due to the short incubation time of 6 to 8 hours and incomplete capacitation of the sperm. In the inter-organ series of experiments where the incubation time was increased to 9 to 11 hours there was a doubling of the fertilizing ability of sperm incubated in the rabbit uterus when compared with the inter-species series of experiments.

Summary. It has been found that washed rabbit sperm may be capacitated in the uterus of an estrous rabbit, rat, and dog, indicating that the capacitation mechanism is similar in these species. It was shown that washed rabbit sperm are capacitated only in the uterus and oviduct of the estrous rabbit and not in the bladder, colon and knee joint, indicating that the factors necessary for capacitation are limited to the female reproductive tract.

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1. Chang, M. C., Nature, 1951, v163, 697.
2. Austin, C. R., Aust. J. Sci. Res. B., 1951, v4, 581.
3. Chang, M. C., Sheaffer, D., J. Heredity, 1957, v48, 107.
4. Mattner, P. E., Nature, 1963, v199, 772.
5. Noyes, R. W., Walton, A., Adams, E. E., ibid., 1958, v181, 1209.
6. Short, D. J., Woodnott, D. P., A.T.A. Manual of Lab. Animal Practice and Techniques, 1963, 285, Charles C Thomas, Springfield, Ill.
7. Hooper, E. E., Hall, A., Dale, H. E., Sm. An. Clin., 1961, v1, 355.
8. Snedecor, G. W., Statistical Methods, 1961, 5th ed., 50, Iowa State Press, Ames.

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Role of Intestinal Microorganisms in Determining Cycasin Toxicity. (31826)

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Cycasin is a compound present in plants of the family *Cycadaceae* which is hepatotoxic and carcinogenic when ingested by small laboratory animals(1). The compound is of special interest because various products from cycad plants are ingested by humans in subtropical and tropical regions of the world(2).

Cycasin is a β-glucoside with the structure methylazoxymethanol-β-*D*-glucoside. The glucose can be removed enzymatically and the purified aglycone has the same hepatotoxic and carcinogenic properties as the glucoside (3). Moreover, although cycasin is not toxic when administered intravenously, subcutaneously or intraperitoneally, the aglycone is toxic by these routes as well as after ingestion.

Previously published experiments have shown that cycasin is nontoxic when ingested by germfree animals, and that it can be recovered quantitatively and unchanged from the urine and feces of these animals(4). In conventional animals, in contrast, as much as 82% of administered cycasin is not recoverable. On the basis of these results, it was proposed that the aglycone is the proximate carcinogen and toxic substance, that