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Influence of Calcium Ions on Fertilization in the Rabbit (38369)

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Calcium ions have been shown to promote *in vitro* fertilization of eggs from mice (1) and guinea pigs (2). As the acrosome reaction (3–5) failed to occur when the spermatozoa were placed in Ca^{2+} deficient media, these ions appear to play an essential role in the process of capacitation. Holmdahl and Mastroianni (6) provided indirect evidence that the cation is significant for fertilization *in vivo* by revealing that Ca^{2+} levels in rabbit oviduct fluid increase shortly following ovulation. The present experiments were undertaken to find out if the Ca^{2+} concentration in suspensions of uterine capacitated rabbit spermatozoa could significantly influence fertilization following oviductal insemination and, if so, to establish whether Ca^{2+} ions can diminish the action of a seminal plasma fraction (7–9) that is known to inhibit the fertilizing capacity of sperm cells.

Materials and Methods. Mature female rabbits, New Zealand strain, were obtained from a local breeder for these experiments. The animals were kept under constant temperature (22 ± 1°) and light (7.00–19.00 hr) and provided *ad lib* Purina Chow and water. They were used within three weeks of their arrival in the colony. Capacitated spermatozoa were obtained by flushing the uterus of a doe, bred three times 11–12 hr before autopsy, with 4 ml Hanks solution (Difco). After sedimenting the sperm suspension at 700*g* for 20 min, the cell pellet was resuspended in 0.2 ml Hanks solution, modified to contain 20, 50, 70 and 150 μg Ca^{2+}/ml.

Some sperm suspensions were treated with whole or fractionated seminal plasma. Seminal plasma was obtained after sedimentation of semen at 1000*g* for 30 min. The semen used was collected with an artificial vagina from fertile bucks of mixed strain. Fractionation of seminal plasma was performed by ultracentrifugation on a discontinuous sucrose density gradient containing 20, 40 and 60% sucrose zones (7). The fraction (Fr I) isolated at the 40/60% sucrose interface was used for decapacitation of sperm cells recovered from the uterus of a mated doe. To establish if Ca^{2+} could prevent decapacitation, 0.1–0.2 mg protein of seminal plasma Fr I, that had been dialyzed against Hanks solution, was added directly to sperm suspensions (vol. 0.2 ml) with 50 or 150 μg Ca^{2+}/ml.

About 25 μl sperm suspension was inseminated into each oviduct of an anesthetized doe with a fire glazed pipet, 2–3 hr after ovulation. Does were anesthetized with 30 mg/kg body weight of sodium pentobarbital (Diamond) and four animals were inseminated in each experiment. Ovulation was induced by iv injection of 90 IU human chorionic gonadotrophin (Squibb),

TABLE I. Effect of Ca^{2+} Ions on the Fertilizing Capacity of Rabbit Spermatozoa.

Ca^{2+} conc. (μg/ml)	No. of does (oviducts)	No. of sperm inseminated	Eggs		Fertilization rate (%)
			Total	Fertile	
20	8	$0.5–2.5 \times 10^5$	39	9	23
50	8	$0.5–2.5 \times 10^5$	36	16	45
70	8	$0.5–1.0 \times 10^5$	43	33	77
150	8	$1.0–4.2 \times 10^5$	33	32	97

TABLE II. Decapacitation of Rabbit Spermatozoa by Seminal Plasma FR I at Different Ca^{2+} Concentrations.

Ca^{2+} conc.	No. of sperm inseminated	Eggs					
		Control oviduct			Treated oviduct[a]		
		Total	No.	(%) fertile	Total	No.	(%) fertile
50	1.25–2.5 × 10^5	20	9	(45)	20	0	(0)
150	1.0–4.2 × 10^5	33	32	(97)	25	1	(4)

[a]Treated sperm suspension contained 0.5–1.0 mg protein/mg of Fr I.

which was administered 12–13 hr before insemination. The does were autopsied on the following day. Excised oviducts were flushed with 1–2 ml isotonic saline into a Petri dish and the recovered eggs were found with the aid of a dissecting microscope. Fertilized eggs were usually four to eight cells at the time of their recovery. All the eggs were stained with 1% lacmoid and mounted on a glass slide to permit microscopic examination for evidence of fertilization.

Results. The fertilization rate achieved with rabbit spermatozoa was clearly enhanced by elevated levels of Ca^{2+} (Table I). Over the concentration range employed (20–150 μg Ca^{2+}/ml), the probability of fertilization increased with the amount of Ca^{2+}. It can be seen that over this range of Ca^{2+} concentrations there was a fourfold difference in the rate of fertilization.

It is apparent from the results given in Table II that Ca^{2+} ions did not prevent decapacitation within the concentration range used in these experiments. When uterine capacitated spermatozoa were exposed to 0.5–1.0 mg protein/ml Fr I from rabbit seminal plasma in the presence of 50 or 150 μg Ca^{2+}/ml, almost complete suppression of fertilization resulted. Fertilization by untreated spermatozoa varied with the Ca^{2+} concentration, as already noted (Table I).

Discussion. The present results establish that *in vivo* fertilization of rabbit eggs is strongly influenced by the Ca^{2+} level present in a suspension of oviductally inseminated spermatozoa. This finding is in agreement with observations showing Ca^{2+} ions enhanced *in vitro* fertilization in mice and guinea pigs (1, 2). Although hamster spermatozoa have been reported to become immotile in media with low Ca^{2+} (10), suspensions of rabbit sperm cells used in the present experiments did not appear to be influenced in this way. As sperm cells in Ca^{2+} deficient media in the *in vitro* fertilization experiments (1, 2) did not undergo the acrosome reaction, these ions could increase fertilization by promoting sperm capacitation. It has been known for some time that Ca^{2+} ions can induce the acrosome reaction in invertebrate sperm (11). The acrosome reaction is characterized by fusion of the plasmalemma and outer acrosomal membrane (5) and Ca^{2+} binds to membrane phospholipids and, moreover, is considered to be a significant factor in the fusion of membranes (12). Fusion between the plasmalemma and outer acrosomal membranes, therefore, is a plausible site of action for Ca^{2+} ions. Since rabbit spermatozoa were decapacitated even in the presence of a relatively high Ca^{2+} concentration (Table II), membrane vesicles in seminal plasma attributed with inhibiting sperm fertilizing capacity (7) do not apparently compete for Ca^{2+} in exerting this effect. The mechanism of action of these membrane vesicles on mammalian spermatozoa is currently under investigation.

Summary. Fertilization of rabbit eggs following oviductal insemination of uterine capacitated spermatozoa was shown to depend on Ca^{2+} levels in the sperm suspension. Decapacitation of rabbit spermatozoa by seminal plasma Fr I occurred even in the presence of 150 μg Ca^{2+}/ml. The results indicate Ca^{2+} ions were important for expression of fertilizing capacity by rabbit spermatozoa, but they were not implicated in decapacitation.

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