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Effect of Calcium on Motility and Fertilization by Rat Spermatozoa *in Vitro* (39989)

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It is well established that Ca^{2+} ions effect both motility and fertilizing capacity in mammalian spermatozoa (1–3). However, it is apparently not known if these two aspects of sperm cell function have similar Ca^{2+} concentration requirements. Such a correspondence might be anticipated if maximum sperm motility was necessary for fertilization. Regarding this possibility, Yanagimachi (4) has described the motility of *in vitro* capacitated epididymal hamster spermatozoa as "extraordinarily active." On the other hand, Overstreet and Cooper (5) report that there is a marked reduction in motility among rabbit sperm in the isthmus of the oviduct.

Data are presented in this report concerning the effect of Ca^{2+} ions on motility and fertilizing ability of cauda epididymal rat spermatozoa in a chemically defined medium. While sperm motility is ordinarily appraised by subjective means, in the present study it was preferable to use a quantitative index of motility. For this purpose, a previously described (6) measure of sperm motility has been employed that relies on the turbulence generated in a suspension of highly motile spermatozoa.

Materials and methods. Sprague–Dawley rats from Charles River Breeding Laboratory were used in these experiments. Sperm cells and eggs were obtained and incubated in a manner similar to that described previously (6, 7). The incubation medium, modified Krebs–Ringer bicarbonate, was prepared to contain between 0 and 6.8 m*M* Ca^{2+}. The incubations were conducted in a humidified atmosphere of 5% CO_2 and 95% air at 37°.

An index of high sperm motility during incubation was provided by the number of turbulence swirls forming at the edge of a 0.1-ml suspension, which was covered with paraffin oil and contained 3×10^6 spermatozoa (6). The sperm cells had been obtained by sectioning a tubule in the cauda epididymis of a mature rat (body weight, 500 g). Sperm fertilizing ability was estimated from the number of fertilized eggs obtained after incubation for 13 to 14 hr with cauda epididymal rat spermatozoa. The eggs were removed from the medium with a fine glass pipet, stained with 0.25% lacmoid, and examined under a phase-contrast microscope (×250). Eggs were judged to be fertilized when they showed pronucleus formation and a fertilizing sperm tail.

Results. Figure 1 indicates that cauda epididymal spermatozoa failed to generate turbulence swirls when incubated in Krebs–Ringer bicarbonate medium lacking Ca^{2+}. This reveals that the cation is required for vigorous motility by rat sperm cells. An adverse effect on motility was also observed at Ca^{2+} concentrations higher than 1.7 m*M*. In medium with 3.4 m*M* Ca^{2+} there was vigorous motility for about 2 hr, however, turbulence declined rapidly and was not apparent after 4.5 hr. Both the maximal intensity and duration of turbulence in sperm suspensions with 5.1 m*M* Ca^{2+} were markedly reduced. Medium having 1.7 m*M* Ca^{2+} maintained sufficiently high sperm motility to permit some turbulence in the suspension for at least 15 hr. It should be noted that sperm motility could be observed in these suspensions for several hours after they had ceased to show turbulence.

Sperm fertilizing capacity was sensitive to Ca^{2+} levels in the medium (Fig. 2). At Ca^{2+} concentrations of 2.6 and 3.4 m*M* the respective fertilization rates were 83% (44/53) and 91% (59/65). In medium containing 1.7 and 6.8 m*M* Ca^{2+} the rates of fertilization were 31% (17/55) and 13% (3/23), respectively. At 6.8 m*M* Ca^{2+} the medium had a slightly cloudy appearance, indicating that saturation had been reached.

Discussion. These results show that motility and fertilization by epididymal rat spermatozoa differ in their dependence on Ca^{2+} ions. Evidently the maximization of sperm

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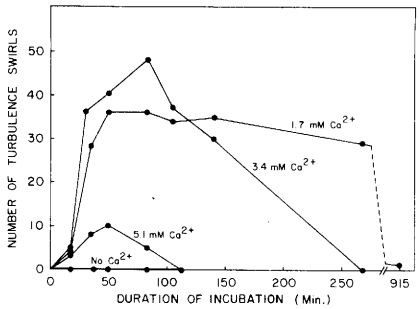

FIG. 1. High motility in suspensions of cauda epididymal rat spermatozoa at different Ca^{2+} concentrations. Sperm motility is related to the number of turbulence swirls that form at the edge of a 0.1-ml suspension, containing 3×10^6 spermatozoa, during incubation.

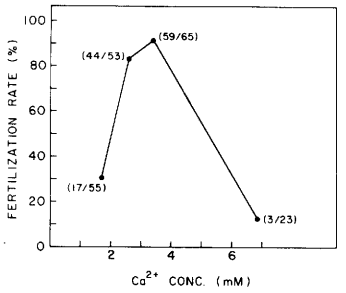

FIG. 2. Effect of Ca^{2+} ions on fertilization *in vitro* of rat eggs. The eggs were incubated with epididymal spermatozoa (0.5×10^6 sperm/ml) in a modified Krebs–Ringer bicarbonate medium containing 10 mg of albumin/ml. The number of fertilized eggs and the total number are given in parentheses.

motility is not necessary for fertilization in this species. It should be noted, however, that this conclusion is based on estimates of motility in the whole sperm population, and it is conceivable that sperm cells responsible for fertilization actually had higher motility than the data indicate.

Results have been reported by Barros and his associates (8) which suggest that alterations in the pattern and vigor of sperm motility are not essential for fertilization. They have shown that noncapacitated guinea pig spermatozoa can penetrate zona pellucida-free hamster eggs *in vitro* when the acrosome reaction is induced by placing these sperm between a coverslip and glass slide for a period of minutes. Guinea pig spermatozoa usually require 12 to 18 hr of incubation under the conditions used in this study before they undergo the acrosome reaction and express fertilizing ability (8, 9). Fusion between the plasmalemma and outer acrosomal membrane, which is the basis of the mammalian acrosome reaction (10), is evidently the only impediment to fertilization by noncapacitated sperm cells. Other changes that might be associated with prolonged incubation, such as changes in respiration and motility, apparently have only secondary significance.

There is good agreement between the fertilization rate data in Fig. 2 and the results presented by Miyamoto and Ishibashi (3). However, the comparison is limited since media with Ca^{2+} concentrations between 1.71 and 5.13 m*M* were not examined in the latter investigation. From the incidence of the acrosome reaction among guinea pig sperm after 4 hr of incubation (11), medium with 3 m*M* Ca^{2+} is twice as effective as that with only 1 m*M* Ca^{2+} for promoting this process. The occurrence of a similar dependence on Ca^{2+} ions in rat spermatozoa would apparently account for the present results, which show that rat sperm cells display high fertilizing capacity at 2.6 and 3.4 m*M* Ca^{2+}. On the other hand, the reported differences in fertilization rate could also reflect Ca^{2+} effects on rat ova. The present observations serve to indicate, however, that the optimization of sperm motility may not be mandatory for fertilization.

Summary. Motility and the expression of fertilizing ability by cauda epididymal rat spermatozoa, incubated in a chemically defined medium, were demonstrated to differ in their dependence on Ca^{2+} ions. Motility was optimal at 1.7 m*M* Ca^{2+}, whereas fertilization rates were significantly higher at 2.6 and 3.4 m*M* Ca^{2+}. These results are interpreted as indicating that sperm motility may not have to be maximized for fertilization to occur.

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1. Davis, B. K., Hunt, D. M., and Chang, M. C., Proc. Soc. Exp. Biol. Med. **147,** 479 (1974).
2. Yanagimachi, R., and Usui, N., Exp. Cell Res. **89,** 161 (1974).

3. Miyamoto, H., and Ishibashi, T., J. Reprod. Fert. **45,** 523 (1975).
4. Yanagimachi, R., J. Reprod. Fert. **23,** 193 (1970).
5. Overstreet, J. W., and Cooper, G. W., Nature (London) **258,** 718 (1975).
6. Davis, B. K., Proc. Soc. Exp. Biol. Med. **151,** 240 (1976).
7. Davis, B. K., and Niwa, K., Proc. Soc. Exp. Biol. Med. **146,** 11 (1974).
8. Barros, C., Berrios, M., and Herrera, E., J. Reprod. Fert. **34,** 547 (1973).
9. Yanagimachi, R., J. Reprod. Fert. **28,** 477 (1972).
10. Barros, C., Bedford, J. M., Franklin, L. E., and Austin, C. R., J. Cell Biol. **34,** Cl (1967).
11. Rogers, B. J., and Yanagimachi, R., Biol. Reprod. **15,** 614 (1976).

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