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Functions of hyperactivated motility of sperm in the oviduct*

Funciones de la motilidad hiperactivada del espermatozoide en el oviducto

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Hyperactivacion of sperm entails increases in the flagellar wave amplitude and the asymmetry of flagellar movement. In simple media on glass slides, hyperactivated sperm are not highly progressive. Nevertheless, we have evidence that hyperactivated sperm within the oviduct possess greater ability to reach the oocyte plasmalemma than normal activated sperm. First, hyperactivated rabbit sperm, flushed from the ampulla at the time of fertilization, have been found to swim in circles of greater radii in shallow chambers than in deep chambers. This behavior would tend to bring sperm away from the shallow chambers of the mucosal folds and into the deep chamber of the center of the oviductal lumen wherein lies the oocyte. Second, a study of mouse sperm, observed through the walls of the oviduct, has revealed that they bind to the mucosal surface, and we have now observed that only hyperactivated flagellar bending movements precede their release. Third, hyperactivated sperm may be more capable of penetrating proteoglycans present in the oviduct. A viscous mucus-like substance that traps sperm has been seen by us on the surface of oviductal mucosa from cows and pigs. In a recent study, we used Ficoll to increase the viscosity of medium to model the viscous environment of the oviductal mucus and cumulus matrix. Hyperactivated hamster sperm demonstrated greater ability to penetrate the viscous medium than activated sperm. Finally, an advanced state of hyperactivation may confer an advantage on sperm penetrating the zona pellucida. In an earlier study, hyperactivated hamster sperm were found to further increase flagellar bending after the acrosome reaction. This could confer an increased potential for generating thrust against the zona. In conclusion, it appears that hyperactivation enhances the ability of sperm to reach the site of fertilization and to penetrate the vestments of the oocyte.

INTRODUCTION

When mammalian sperm recovered from semen or from the caudal epididymis are diluted in medium and placed on a microscope slide, they usually exhibit a progressive form of motility. The flagella beat in a nearly symmetrical pattern, generating low amplitude waves that propel sperm in a straight or slightly curved path. This movement pattern may be termed activated (1, 2). In contrast, sperm recovered from the ampulla of the oviduct near the time of fertilization display a vigorous but relatively nonprogressive movement pattern on microscope slides. This movement, named "hyperactivation" by Yanagimachi (1, 3), is the result of a flagellar wave pattern of increased amplitude and asymmetry (Fig. 1). One approach to understanding whether this apparently nonprogressive pattern of motility enables sperm to reach the oocyte plasmalemma *in vivo* is to test sperm in environments that more closely resemble the oviductal lumen than does culture medium on a glass slide. The results we obtained by using this approach are discussed in this review.

The oviduct and its luminal contents certainly present sperm with a different physical environment than a microscope slide. There are at least four features that may be important considerations in investigating the functions of hyperactivation: 1) the convoluted surface of the oviductal mucosa; 2) the existance of binding sites for sperm on the mucosal epithelium; 3) the presence of highly

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viscous or viscoelastic fluids in the oviduct lumen; and 4) the necessity for penetrating the zona pellucida.

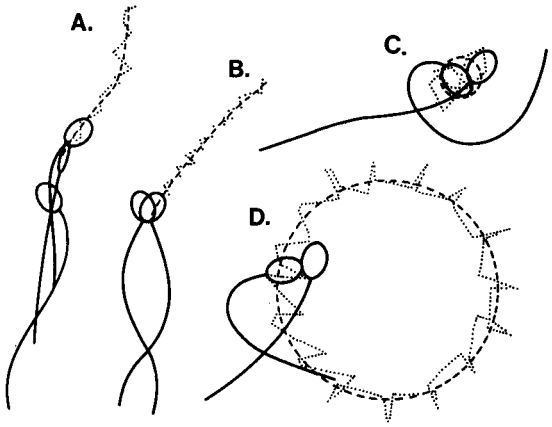

Fig. 1: Movement patterns of rabbit sperm recovered from artificial vaginas (ejaculated sperm) and from oviductal ampullas during the periovulatory period (ampullar sperm). *Dotted lines* represent the curvilinear path of the head/midpiece junction, constructed by joining the points locating the junction on successive video frames (60 frames/sec). *Dashed lines* represent the average path or trajectory of the sperm, obtained by visually judging the path of the center of movement. Sperm heads are 7 μm long. A. Movement pattern of ejaculated sperm that rolled. B. Movement pattern of ejaculated sperm that glided along the surface of the slide. C. Movement pattern of hyperactivated ampullar sperm executing circles of approximately 5 μm radii. D. Movement pattern of hyperactivated ampullar sperm executing circles of approximately 20 μm radii. Reprinted with permission from Biology of Reproduction, reference (23).

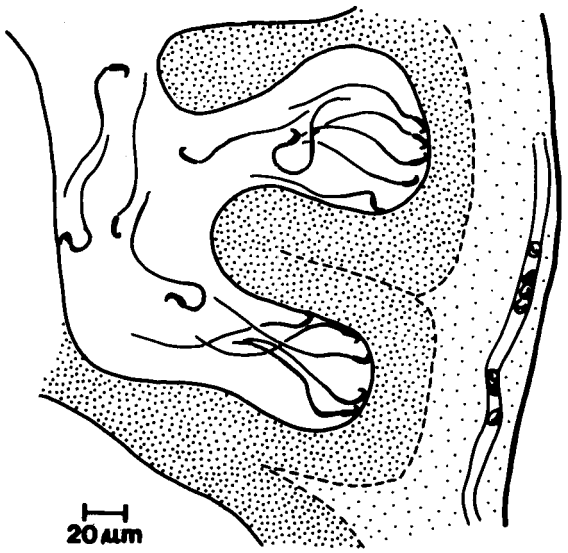

Fig. 2: Tracing from a videotape of mouse sperm moving within the isthmus of an excised oviduct. Mucosal folds are indicated by the densely stippled areas and a capillary is visible in the wall at the right. Most of the sperm are attached to the mucosal surface in two pockets on the right. Two sperm that are headed out of the pockets are executing the acute bends characteristic of hyperactivation.

First, the mucosal surface is curved and convoluted, rather than smooth and flat like a glass or plastic microscope slide. Sperm have been observed trapped in pockets created by mucosal folds (Fig. 2) (4, 7). Thus, mucosal folds may form physical barriers to sperm movement towards the oocyte. The apical surfaces of the mucosal epithelial cells are also complex, being differentiated into microvilli and cilia which may be more difficult for the sperm to move along than a smooth hard surface.

Second, the epithelial surface may inhibit sperm movement by binding sperm. Motile mouse (6), bovine (8, 9), and porcine (10) sperm have been observed to bind tightly to mucosal epithelium *in vitro*. Scanning electron micrographs of porcine oviducts indicate that this may occur *in vivo* as well (7).

Third, sperm may be required to pass through highly viscous and viscoelastic fluids to reach the oocyte. Oviduct fluid contains serum proteins such as albumin (11) that affect its viscosity and shear-thinning properties (2). A mucous substance has been detected in the isthmic lumens of human (12), rabbit (13, 14), bovine (8), and porcine (7) oviducts. *In vitro*, bovine and porcine sperm have been observed to be trapped by a mucous substance produced by mucosal explants (8, 10). After succeeding in passing through viscous oviduct fluids, sperm may also be required to penetrate the viscoelastic cumulus matrix in order to reach the zona pellucida of the oocyte. While it has been proposed that hyaluronidase released from the acrosome dissolves the matrix, some recent work indicates that the acrosome reaction and its accompanying release of hyaluronidase do not occur *in vivo* until the sperm has penetrated through the cumulus to the surface of the zona pellucida (1, 15-17) and that some types of sperm can penetrate cumulus matrix without the aid of hyaluronidase (18).

Fourth, on reaching the oocyte, sperm

must utilize flagellar movement to penetrate the solid glycoprotein network of the zona pellucida. While enzymes apparently play a role in zona penetration, sperm movement is required as well (19-22).

In order to determine whether these features relate to the function of hyperactivation, sperm were analyzed as they moved through the oviductal lumen and artificial systems were used to simulate various physical aspects of the oviductal environment. As a result, we have learned how hyperactivation may be functionally advantageous to sperm for getting out of mucosal folds, breaking free from the epithelium, passing through viscous and viscoelastic fluids, and penetrating the zona pellucida.

RESULTS AND DISCUSSION

Escaping from mucosal folds

Rabbit ejaculated sperm recovered from an artificial vagina, or from the oviductal ampulla of naturally mated does shortly after ovulation, were placed in either 25 μm or 100 μm deep glass chambers (23). The depth of the shallow chambers was less than half of the length of rabbit sperm (60 μm) (24), while the depth of the deep chambers was almost twice the lenght of the sperm. In both types of chambers, the freshly ejaculated sperm continued swimming in linear trajectories. In contrast, more than 93% of the free-swimming ampullar sperm were hyperactivated. They principally swam in circles (Fig. 1); however, sperm in the shallow chambers swam in significantly larger circles than sperm in the deep chambers (23).

Considering these results in relation to the situation faced by sperm in the oviductal lumen, the behavior of hyperactivated sperm could be advantageous for the following reasons. While the circling movement may not be very progressive, it would provide the sperm with a pattern that would increase the likelihood of encountering the oocyte in the oviduct. Sperm swimming in linear trajectories would be less likely to encounter an oocyte, especially if they were unable to turn. They could be more likely to become lost down in the space between mucosal folds. The tendency of the hyperactivated sperm to swim in circles of increased diameter when confined would bring these sperm out of mucosal pockets, which are estimated from the work of El-Banna and Hafez (25) to be 25 μm in width and 200-300 μm deep in the rabbit oviductal isthmus. Once out of the pockets, swimming in small circles would be likely to keep sperm in the center of the oviductal lumen.

The mouse oviduct is transparent enough to permit observation of sperm moving within it. Oviducts were removed from mated mice, placed in a 37°C chamber, and observed and videotaped through modified differential interference contrast optics (6, 26). The movements of the sperm within each oviduct were videotaped for a few minutes, then the recordings were analyzed. The recordings revealed that sperm caught within pockets created by mucosal folds changed direction to head into the central lumen after executing deep flagellar bends characteristic of hyperactivation (26) (Fig. 2). These observations support the hypothesis that hyperactivated movement can serve to move sperm out of mucosal pockets or crypts and into the center of the oviductal lumen.

Release from the oviductal epithelia

Mouse sperm observed within oviducts, and bovine and porcine sperm incubated with oviductal mucosa, have been seen binding to the epithelial surface (6, 8, 10). When washed ejaculated porcine sperm were incubated with explants of porcine oviductal mucosa, neither hyperactivation nor release of sperm from the epithelial surface were observed (10). When previously frozen bull sperm were washed through Percoll and then added to primary cultures of bovine oviductal epithelium, many quickly bound to the epithelium. After al least 12 hr of co-incubation, 30% of the unbound sperm appeared to be hyperactivated (9). In a few instances, sperm were observed to break away from the epithelial surface and commence

swimming in a hyperactivated pattern (Pollard and Suarez, unpublished observations). Further testing is needed to determine whether hyperactivation affects the release of sperm from bovine and porcine oviductal epithelium.

Observations of the behavior of mouse sperm within the oviduct offer stronger support for a role of hyperactivation in release from the oviductal epithelium. Following up on earlier observations *in situ* (6, 26), a series of mice were mated and the oviducts removed, cut away from the mesosalpinx, and uncoiled before they were observed (27). This allowed the paths of individual sperm to be followed for longer periods. Sperm in the oviduct were videotaped using Hoffman modulation contrast optics. Sperm seen on the videotapes were primarily stuck to the oviductal epithelium and only a small percentage of the total number of these sperm were seen to break free (96 out of 778) (27). However, all breakaways were by sperm showing acute flagellar bends indicative of hyperactivation. Most of the sperm that broke free were seen to reattach to the epithelium within 1-2 seconds. Progress appeared to consist of long periods of attachment punctuated by brief periods of free swimming. Hyperactivated flagellar beating seemed to be necessary for sperm to break free, and, thus, to progress along the oviduct.

In order to provide a quantitative evaluation of the motility patterns of free and stuck sperm, the flagellar curvature ratio (the straight-line distance from the head midpiece junction to the first inflection point of the flagellar wave divided by the distance along the tail between these two points (26) was measured for 108 sperm in which the entire principal curvature of the flagellum was in focus. This ratio reflects the degree of curvature in the tail, or amplitude of the wave, and decreases with increasing curvature. It serves as an indicator of hyperactivation since the flagellar curvature increases during hyperactivation (23, 26, 28-30). For the 47 sperm that were not attached to the epithelium, the mean curvature ratio was 0.729 (0.029 SEM). For the 61 sperm that were stuck to the epithelium, the mean curvature ratio was 0.794 (0.019 SEM). Thus, the flagellar curvature of free sperm was significantly sharper than that for sperm stuck to the epithelium (1-factor ANOVA, $p < 0.05$). These values compare well to the results obtained for hyperactivated and activated mouse sperm *in vitro*, 0.758 and 0.840 respectively (26) (and Fig. 3).

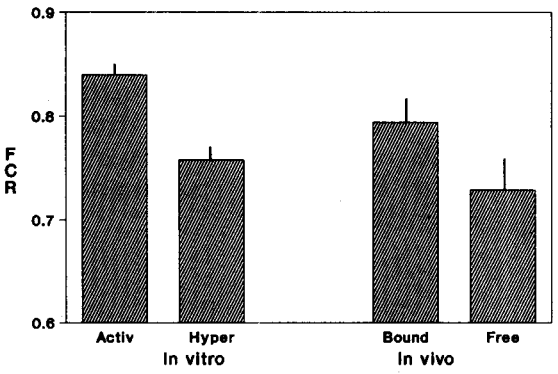

Fig. 3: A comparison of the flagellar curvature ratios (FC) of mouse sperm hyperactivated *in vitro* (data from reference (26)) and *in vivo*. Measurements of sperm incubated *in vitro* were taken from 40 sperm (4 males) 12-15 min after release from the caudal epididymis (*activ*) and from the same number of sperm 90 min after incubation under capacitating conditions (*hyper*). Measurements of sperm hyperactivated *in vivo* were taken from videotapes of sperm in excised oviducts. Sperm that were observed to break free from the mucosal surface during 2 sec of vidotaping were classified as "free" ($n = 47$), while those that remained bound during the videotaping period were classified as "bound" ($n = 61$). The bars indicate the mean of the maximal ratios of all sperm measured in each category. Vertical lines indicate the standard error of the mean. The ratios of sperm hyperactivated *in vitro* were significantly lower ratios than those of activated sperm ($p < 0.05$, 2-Factor ANOVA with male as the second factor), and the free sperm in the oviduct had significantly lower ratios than the bound sperm ($p < 0.05$, 1-Factor ANOVA).

The qualitative observations of sperm with acutely bent tails breaking free from the epithelium and the measurements of flagellar curvature ratio implicate hyperactivation as a mechanism for sperm release from the oviductal epithelium in the mouse. Clearly, in order for a sperm to reach the site of fertilization and make contact with the egg, it must be able to break free of the epithelium. Epithelial binding and hyperactivation may be complementary regulatory mechanisms for

controlling the transport of sperm into the ampulla, acting both to ensure availability of sperm at the time of ovulation and to prevent polyspermy, which may occur if large numbers of sperm enter the ampulla at ovulation (31). Furthermore, the requirement for vigorous hyperactivated movement may serve to filter out abnormal or weak sperm.

Passage through viscous substances

When added to explants of oviductal mucosa from their own species, previously frozen bull sperm (8) and washed ejaculated boar sperm (10) became caught in a mucous substance produced by the explants. In contrast to the ease with which bull sperm penetrate bovine cervical mucus (20, 32), the bull and boar sperm became entrapped in this mucous substance and were not observed to penetrate it deeply or pass through it, even when explants had been taken from estrous females and incubated in estradiol. Hyperactivation might enable sperm to pass through this substance, but this has yet to be observed.

In order to test whether hyperactivation confers a physical advantage upon sperm for penetrating viscous substances, Ficoll was used to create a relatively inert viscous fluid. Hamster sperm were collected from caudal epididymides and capacitated *in vitro* (30). The movements of activated, transitional, and hyperactivated hamster sperm in increasing concentrations of Ficoll were analyzed and compared. At concentrations of Ficoll equivalent to the viscosity of the cumulus matrix (33), it was observed that all of the sperm moved in a sinuous, linear fashion (Fig. 4) (2). Thus, hyperactivated sperm had assumed a progressive pattern of movement. Furthermore, a higher percentage of hyperactivated sperm were able to penetrate the Ficoll solution and did so at a higher velocity than activated sperm (2). The behavior of transitional sperm was intermediate. These observations indicate that hyperactivation confers an advantage on sperm encountering mucus and cumulus matrix in the oviduct.

Penetration of the zona pellucida

Hamster sperm were removed from the caudal epididymis and incubated in a defined medium capable of inducing hyperactivation and the acrosome reaction *in vitro* (34). Hyperactivated sperm were videotaped through phase contrast optics. By stopping the videotapes on individual frames, the state of the acrosome could be ascertained on motile sperm, and flageller bend characteristics could be measured. It was determined that acrosome-reacted, hyperactivated sperm generated flagellar bends of greater asymmetry and amplitude than unreacted, hyperactivated sperm (34). This increase in flagellar bend characteristics can be viewed as an increase in hyperactivation. Increases in flagellar bend amplitude enable sperm to generate greater propulsive thrust (20) and, therefore, provide them with an increased ability to penetrate the zona pellucida.

Conclusion

In the experiments described above, various systems were used to investigate the functions of hyperactivated motility. In most of these studies, the question of function was approached by comparing the behaviors of activated and hyperactivated sperm. Under conditions designed to represent certain characteristics of the oviductal environment, hyperactivated sperm appeared to perform better than activated sperm. In general, it can be concluded that hyperactivation is advantageous in the oviduct. More specifically, the results of these studies indicate that hyperactivation can serve to increase the ability of sperm to move out of oviductal pockets towards the oocyte, to release sperm from the epithelial surface, to enable sperm to penetrate viscous fluids such as oviductal "mucus" and cumulus matrix, and to enhance penetration of the zona pellucida.

REFERENCES

1. YANAGIMACHI, R. (1988) Mammalian fertilization. In: Knobil, E.; Neilll, J.; (eds.) *The Physiology of Reproduction.* New York: Raven Press. pp. 135-185.

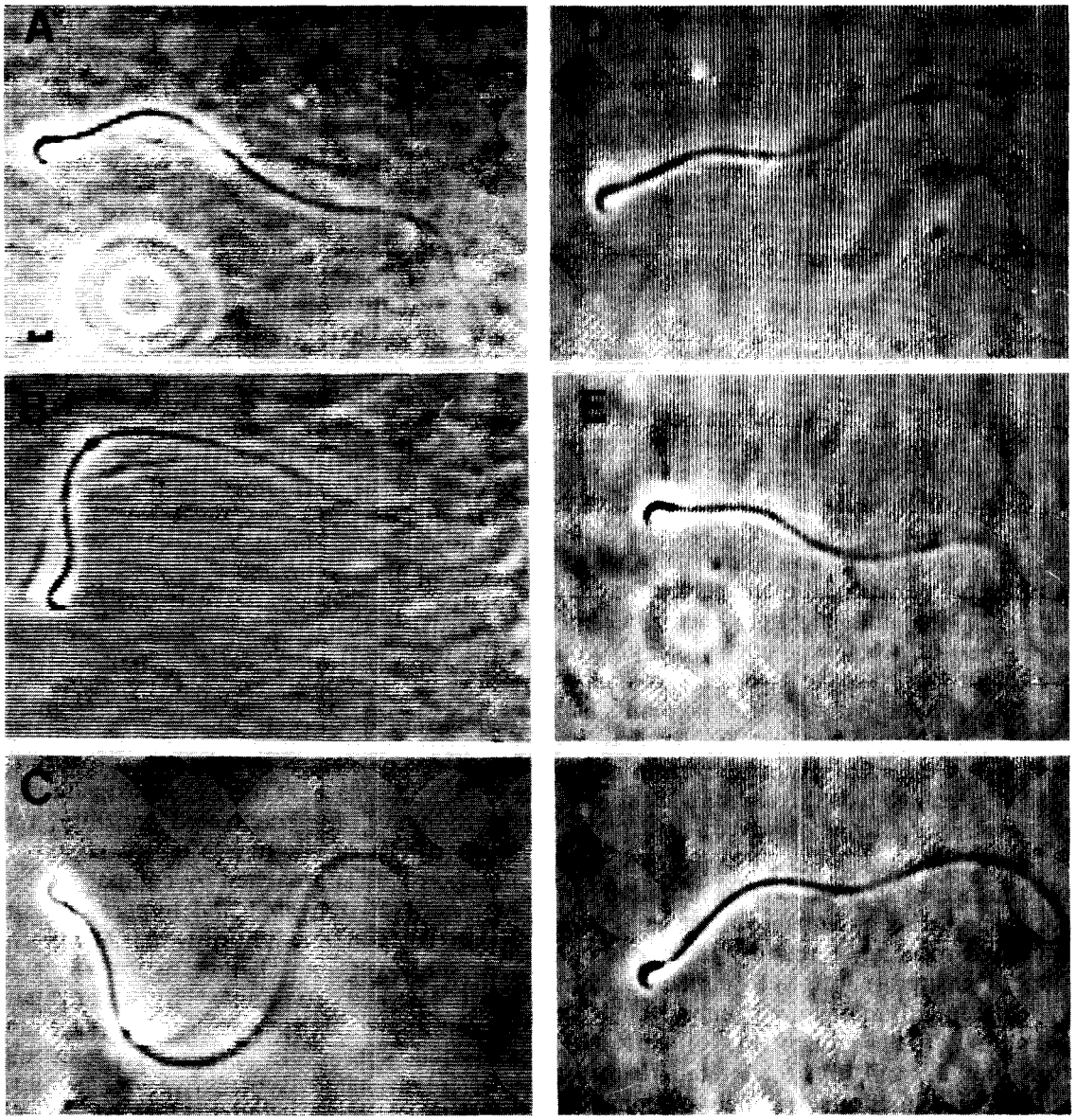

Fig. 4: Movement patterns of hamster sperm in Ficoll solutions. Bar indicates 10 μm. Photographs were taken from videotapes analyzed in reference (2). (A) Activated sperm at 0 hr in medium alone. Flagellar beating was rapid, planar, and of moderate amplitude. (B) Transitional sperm at 3 hr exhibited three-dimensional beats of increased asymmetry and amplitude that produced helical trajectories. (C) Hyperactivated sperm at 4 hr of incubation displayed asymmetrical, two dimensional, high amplitude flagellar beating. (D, E, F) Sperm in 20% Ficoll after 0, 3, and 4 hr, respectively, of pre-incubation in medium.

2. SUAREZ, S.S.; KATZ, D.F.; OWEN, D.H.; ANDREW, J.B.; POWELL, R.L (1991) Evidence for the function of hyperactivated motility in sperm. *Biol. Reprod. 44:* 375-381.
3. YANAGIMACHI, R. (1981) Mechanisms of fertilization in mammals. In: Mastroianni, L.; Biggers, J.D.; (eds.) *Fertilization and Embryo Development in vitro.* New York: Plenum. pp. 81-182.
4. YANAGIMACHI, R.; CHANG, M.C. (1963) Sperm ascent through the oviduct of the hamster and rabbit in relation to the time of ovulation. *J. Reprod. Fert. 6:* 413-420.
5. SMITH, T.T.; KOYANAGI, F.; YANAGIMACHI, R. (1987) Distribution and number of spermatozoa in the oviduct of the golden hamster after mating and artificial insemination. *Biol. Reprod. 37:* 225-234.
6. SUAREZ, S.S. (1987) Sperm transport and motility in the mouse oviduct: observations in situ. *Biol. Reprod. 36:* 203-210.
7. HUNTER, R.H.F.; FLECHON, B.; FLECHON, J.-E. (1987) Pre- and peri-ovulatory distribution of viable spermatozoa in the pig oviduct: a scanning electron microscope study. *Tiss. Cell. 19:* 423-426.
8. SUAREZ, S.S.; DROST, M.; REDFERN, K.;

GOTTLIEB, W. (1990) Sperm motility in the oviduct. In: Bavister, B.D.; Cummins, J.; Roldan, E.R.S. (eds.) *Fertilization in Mammals.* Norwell: Serono Symposia, pp. 111-124.

9. POLLARD, J.W.; PLANTE, C.; KING, W.A.; HANSEN, P.J.; BETTERIDGE, K.J.; SUAREZ, S.S. (1990) Sperm fertilizing capacity is maintained by binding to oviductal epithelial cells. *Biol. Reprod. 44:* 102-107.
10. SUAREZ, S.S.; REDFERN, K.; RAYNOR, P.L. (1990) Interaction of porcine sperm with oviductal explants. *J. Androl 11:* P-23 (Abstract).
11. VIRJI, N.; PHILLIPS, D.M.; DUNBAR, B.S. (1990) Identification of extracellular proteins in the rat cumulus oophorus. *Molec. Reprod. Devel. 25:* 339-344.
12. JANSEN, R.P.S. (1980) Cyclical changes in the human fallopian tube isthmus and their functional importance. *Am. J. Obstet. Gynecol. 136:* 292-308.
13. JANSEN, R.P.S.; BAJPAI, V.K. (1982) Oviduct acid mucus glycoproteins in the estrous rabbit: ultrastructure and histochemistry. *Biol. Reprod. 26:* 155-168.
14. JANSEN, R.P.S. (1978) Fallopian tube isthmic mucus and ovum transport. *Science 201:* 349-351.
15. FLORMAN, H.M.; STOREY, B.T. (1982) Mouse gamete interactions: the zona pellucida is the site of the acrosome reaction leading to fertilization *in vitro. Devel. Biol. 91:* 121-130.
16. STOREY, B.T.; LEE, M.A.; MULLER, C.; WARD, C.R.; WIRTSHAFTER, D.G. (1984) Binding of mouse spermatozoa to the zonae pellucida of mouse eggs in cumulus: evidence that the acrosome remains essentially intact. *Biol. Reprod. 31:* 1119-1128.
17. WASSARMAN, P.M. (1987) Early events in mammalian fertilization. *Annu. Rev. Cell. Biol. 3:* 109-142.
18. TALBOT, P.; DiCARLANTONIO, G.; ZAO, P.; PENKALA, J.; HAIMO, L.T. (1985) Motile cells lacking hyaluronidase can penetrate the hamster oocyte cumulus complex. *Devel. Biol. 108:* 387-398.
19. DROBNIS, E.Z.; YUDIN, A.I.; CHERR, G.N.; KATZ, D.F. (1988) Hamster sperm penetration of the zona pellucida: kinematic analysis and mechanical implications. *Devel. Biol. 130:* 311-323.
20. KATZ, D.F.; OVERSTREET, J.W.; DROBNIS, E.Z. (1989) Factors regulating mammalian sperm migration through the female reproductive tract and oocyte vestments. *Gamete. Res. 22:* 443-469.
21. GREEN, D.P.L.; PURVES, R.D. (1984) Mechanical hypothesis of sperm penetration. *Biochem. J. 45:* 659-662.
22. GREEN, D.P.L. (1987) Mammalian sperm cannot penetrate the zona pellucida solely by force. *Exp. Cell. Res. 169:* 31-38.
23. SUAREZ, S.S.; KATZ, D.F.; OVERSTREET, J.W. (1983) Movement characteristics and acrosomal status of rabbit spermatozoa recovered at the site and time of fertilization. *Biol. Reprod. 29:* 1277-1287.
24. CUMMINS, J.M.; WOODALL, P.F. (1985) On mammalian sperm dimensions. *J. Reprod. Fert. 75:* 153-175.
25. EL-BANNA, A.A.; HAFEZ, E.S.E. (1970) Profile analysis of the oviductal wall in rabbits and cattle. *Anat. Rec. 166:* 469-478.
26. SUAREZ, S.S.; OSMAN, R.A. (1987) Initiation of hyperactivated flagellar bending in mouse sperm within the female reproductive tract. *Biol. Reprod. 36:* 1191-1198.
27. DeMOTT, R.P.; SUAREZ, S.S. (1990) The progress of hyperactivated sperm in the mouse oviduct is intermittent. *J. Cell. Biol. 109:* 2035a. Abstract.
28. YANAGIMACHI, R. (1970) The movement of golden hamster spermatozoa before and after capacitation. *J. Reprod. Fert. 23:* 193-196.
29. FRASER, L.R. (1977) Motility patterns in mouse spermatozoa before and after capacitation. *J. Exp. Zool. 202:* 439-444.
30. SUAREZ, S.S. (1987) Hamster sperm motility transformation during the development of hyperactivation *in vitro* and during epididymal maturation. *Gamete. Res. 19:* 51-65.
31. HUNTER, R.H.F. (1988) Transport of gametes, selection of spermatozoa and gamete lifespans in the female tract. In: Hunter, R.H.F. (ed.) *The Fallopian Tubes. Their Role in Fertility and Infertility.* New York: Springer-Verlag, pp. 53-80.
32. KATZ, D.F.; BLOOM, T.D.; BonDURANT, R.H. (1981) Movement of bull spermatozoa in cervical mucus. *Biol. Reprod. 25:* 931-937.
33. DROBNIS, E.Z.; YUDIN, A.I.; CHERR, G.N.; KATZ, D.F. (1988) Kinematics of hamster sperm during penetration of the cumulus cell matrix. *Gamete. Res. 21:* 367-383.
34. SUAREZ, S.S.; KATZ, D.F.; MEIZEL, S. (1984) Changes in motility that accompany the acrosome reaction in hyperactivated hamster spermatozoa. *Gamete. Res. 10:* 253-265.