

# Hyperactivated Motility in Sperm

# Minireview

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Hyperactivated motility was first described by Yanagimachi (1969, 1970), who observed that hamster sperm incubated in follicular fluid or serum exhibited a vigorous movement characterized by high-amplitude flagellar bends. He also saw this movement in hamster sperm within the oviductal ampulla at about the time of fertilization (Yanagimachi, 1970). He called this movement pattern "activation," but later changed the term to "hyperactivation" to distinguish it from the initiation of motility in epididymal or vas deferens sperm when diluted in seminal plasma or medium (Yanagimachi, 1984). I will use the term activated in this review to refer to the swimming pattern exhibited by most sperm in fresh semen (Fig. 1).

Since Yanagimachi's initial reports, hyperactivation has been observed in sperm from a number of mammalian species, either during capacitation *in vitro*, flushed from the oviduct near the time of fertilization, or observed through the translucent walls of rodent oviducts (reviewed by Yanagimachi, 1994). Human sperm exhibit hyperactivated motility when incubated under capacitating conditions *in vitro* (Burkman, 1984; Mortimer and Mortimer, 1990).

When placed in low-viscosity medium, hyperactivated sperm appear quite vigorous, but generally nonprogressive. They often swim in circles, which is the result of highly asymmetrical flagellar beating (Fig. 1). However, the physical environment of the oviduct is quite different from that of microscope slide chambers, and this form of flagellar bending may aid the sperm in reaching the plasma membrane of the oocyte. Hyperactivated sperm penetrate viscous (Suarez et al, 1991) and viscoelastic substances (Suarez and Dai, 1992) more effectively than activated sperm, which may enable them to penetrate mucoid oviductal secretions and the extracellular matrix of the cumulus oophorus. When swimming in viscous or viscoelastic substances, hyperactivated sperm swim more

progressively and their movement patterns appear like those of activated sperm in medium (Suarez et al, 1991; Suarez and Dai, 1992). Hyperactivated sperm also penetrate the zona pellucida far more effectively than activated sperm, even when both have undergone the acrosome reaction (Stauss et al, 1995). It has also been proposed, based on observations of the behavior of rodent sperm within oviducts, that hyperactivation aids sperm in escaping pockets and grooves in the oviductal mucosa and increases their chance of encountering oocytes, due to their frequent directional changes (Suarez and Osman, 1987). Finally, hyperactivation may enable sperm to detach from oviductal mucosa, to which many become bound when they enter the oviduct. The binding creates a reservoir of sperm in the oviduct. In hamsters (DeMott et al, 1995), horses (Lefebvre et al, 1995), and cattle (Lefebvre and Suarez, unpublished observations), binding has been demonstrated to involve carbohydrate recognition, much like lectin/ligand interactions and sperm/zona binding. Changes in the surface of the sperm head that occur during capacitation *in vitro* prevent binding from occurring (Smith and Yanagimachi, 1991; Lefebvre and Suarez, 1996), but detachment of sperm already bound may require the increased pulling force provided by hyperactivation. Only sperm exhibiting hyperactivated flagellar bending were observed to detach from the mucosa of oviducts in mice (DeMott and Suarez, 1992). Thus, there are several ways in which hyperactivation can improve the ability of sperm to reach the oocyte plasma membrane *in vivo*.

Definitions and descriptions of hyperactivation vary in the literature. This is most likely because the swimming patterns formed by hyperactivated sperm depend upon the length and thickness of the flagellum (reviewed by Yanagimachi, 1994). There is considerable interspecies variation in the thickness of the outer dense fibers of the flagellum, which affects the bending pattern that results from the sliding of the microtubules in the axoneme (Baltz et al, 1990). The main distinguishing characteristic of hyperactivation is an increase in flagellar bending amplitude, which can be detected in computer-automated semen analysis systems as an increase in average lateral head movement (ALH; Mortimer and Mortimer, 1990). In many species, this increase occurs in only the principal or reverse bend of the flagellum, resulting in a highly asymmetrical beat pattern and helical or circling swimming trajectories. Helical patterns result when the flagellar beating is three-dimensional, while circling results

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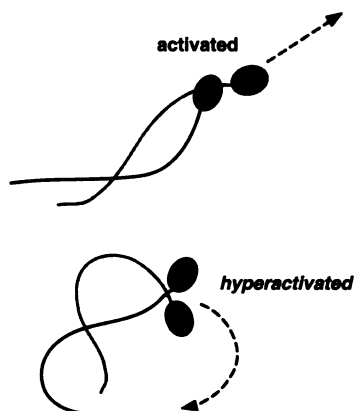


FIG. 1. Diagram of basic patterns of activated and hyperactivated sperm movement for sperm in medium in microscope slide chambers.

when flagellar beating is confined to a plane. Various terms have been devised to describe the resultant swimming trajectories, such as figure-eight, whiplash, dancing (reviewed by Yanagimachi, 1994), star-spin, thrashing, and circling (reviewed by Burkman, 1990). These descriptors aid viewers in identifying hyperactivation in a particular species of sperm.

A transitional phase between activation and hyperactivation has been reported for some species, including human sperm (Mortimer and Mortimer, 1990). It is particularly apparent in hamster sperm incubated under capacitating conditions *in vitro*. In deep slide chambers, hamster sperm assume a helical movement pattern some time before they exhibit a planar, circular pattern. The maximal flagellar bending amplitude is less than that of fully hyperactivated sperm and the beating is three-dimensional, rather than two-dimensional (Suarez, 1988).

Hyperactivation has also been described as biphasic, because some sperm are observed to switch back and forth from high-amplitude, asymmetrical bending to low-amplitude, symmetrical bending. This behavior is particularly common among human sperm *in vitro* and might contribute to underestimating the percentage of hyperactivated sperm obtained by computer-aided sperm analysis (CASA) analysis of samples collected for less than 1 second. Because hyperactivation is reversible, as discussed below, it may be more accurate to say that sperm are only hyperactivated when they are actually exhibiting high-amplitude bending. For those instances when sperm resume activated or transitional movement, they undoubtedly retain the ability to hyperactivate. When more is known of the mechanisms regulating hyperactivation, a universal agreement may be reached on a definition.

Actually, very little is known of the biochemical pathways regulating expression of hyperactivated motility. It has been demonstrated that extracellular  $\text{Ca}^{2+}$  is required to maintain hyperactivation *in vitro* (Yanagimachi, 1982; Fraser, 1987) and we have shown that the concentration

of intracellular free calcium ( $\text{Ca}^{2+}_{in}$ ) is higher in hyperactivated than activated sperm (Suarez et al, 1993).  $\text{Ca}^{2+}_{in}$  is greater in both the head and tail of hyperactivated hamster sperm when compared with activated sperm at the same time point in the same sample (Suarez and Dai, 1995). The increase in the tail is relatively greater than in the head (Suarez and Dai, 1995); perhaps this is because the chief or only site of calcium influx for hyperactivation is in the tail. Hamster sperm that have undergone acrosome reactions have even higher  $\text{Ca}^{2+}_{in}$  in both the head and tail, although the relative increase is greater in the head (Suarez and Dai, 1995). The flagellar bending of the tail is even more acute in acrosome-reacted/hyperactivated sperm than in acrosome-intact/hyperactivated sperm (Suarez et al, 1984; Suarez and Dai, 1995). This implies that the influx of  $\text{Ca}^{2+}$  that occurs during induction of the acrosome reaction also affects the flagellar axoneme. In demembrated rat sperm, the flagellar bend amplitude correlates directly with the amount of calcium in the medium (Lindemann and Goltz, 1988). All of this information indicates that  $\text{Ca}^{2+}$  is involved in the switch from activated to hyperactivated movement. The mechanism for raising intracellular calcium in sperm may involve opening plasma membrane calcium channels because hyperactivation ceases when these channels are blocked by antagonists (Stauss et al, 1995). However, little is known of the biochemical pathway that opens calcium channels. It is also possible that the inorganic calcium channel blockers  $\text{Ni}^{2+}$  and  $\text{Ca}^{2+}$  inhibit hyperactivation by entering the sperm and acting directly upon the flagellar axoneme (Kanous et al, 1993).

It is important to recognize that hyperactivation is a reversible event and requires a sustained elevation of intracellular calcium in order to be expressed. This is in contrast to the acrosome reaction, which, by its very nature, cannot be reversed. The biphasic behavior of so-called hyperactivated sperm could be the result of changing intracellular calcium levels.

Flagellar bending asymmetry, which is characteristic of hyperactivation in many species, can occur in all flagella and is characteristic of most ciliary beating. Flagellar beating results when dynein ATPase anchored to one member of a fused pair of microtubules moves along a neighboring microtubule. Due to physical constraints, the sliding is converted to bending. Nine fused pairs of microtubules, called doublets, are arranged in a circle around a central pair of microtubules. There is evidence that doublets numbered 1–4 are responsible for bending in one direction, whereas doublets 5–9 bend the flagellum in the opposite direction. The sets of doublets and their associated proteins are not exactly alike: for example, they differ in sensitivity to  $\text{Ni}^{2+}$  (Kanous et al, 1993). Therefore, flagellar beat asymmetry can be controlled by differential stimulation of the two groups of doublets.

There are some candidates for inducers of hyperactivation *in vivo*. These might be considered signals that switch on hyperactivation, presumably by a signal transduction pathway that elevates intracellular calcium. Hyperactivation occurs spontaneously in the majority of mouse and hamster sperm incubated under capacitating conditions in medium that contains bicarbonate (Neill and Olds-Clarke, 1987; Boatman and Robbins, 1991); however, this does not necessarily mean that fertilization *in vivo* is normally the result of spontaneous hyperactivation. Mouse and hamster sperm also spontaneously acrosome react; however, the zona pellucida and progesterone have been demonstrated to induce acrosomal exocytosis specifically (reviewed by Yanagimachi, 1994). Both of these spontaneous events may be the result of a gradual increase in permeability of sperm membranes to calcium, associated with cell aging or, more specifically, oxidative damage (Alvarez et al, 1987). It can be argued that there should be one or more specific inducers for hyperactivation, because it is important to regulate the location and timing of hyperactivation *in vivo*. Sperm that hyperactivate before reaching the oviduct are unlikely to fertilize, because hyperactivated sperm may be unable to pass from the uterus into the oviduct (Shalgi et al, 1992). The number of hyperactivated sperm in the oviduct appears to rise near the time of ovulation (Cooper et al, 1979), indicating that there is a specific signal in the oviduct associated with hyperactivation. Keeping hyperactivated motility or all motility suppressed (Cooper et al, 1979) until the time of ovulation would ensure that there would be a supply of sperm from the oviductal reservoir for fertilization when ovulation does occur.

Because hyperactivation increases in the oviduct near the time of ovulation, inducers could be secreted by oviductal epithelium into the lumen at this time or could enter the lumen with the oocyte. In order to be considered a candidate for the signal for hyperactivation, a factor must produce a response within several minutes, rather than hours. This criterion has been used to distinguish physiological or true acrosome reactions from spontaneous or false reactions (Yanagimachi, 1994) and is also appropriate for investigation of hyperactivation. In order to respond to acrosome reaction-inducing signals, sperm must have undergone capacitation (reviewed by Yanagimachi, 1994). It is not known if there is any prerequisite for sperm to achieve responsiveness to hyperactivation-inducing signals. However, activated sperm may be physically capable of hyperactivating, because the hyperactivated form of flagellar bending was observed in hamster sperm recovered from the proximal caudal epididymis (Suarez, 1988). There are no reports of a systematic search for physiological inducers, especially searches that apply these criteria. However, there are some studies that point to candidates. Follicular fluid and its component

progesterone have been demonstrated to induce hyperactivation in human sperm (Mbizvo et al, 1990), although sperm were assayed at least 1 hour after treatment in most reports. Progesterone raises intracellular  $Ca^{2+}$  in human sperm and can induce acrosome reactions in capacitated human sperm (Thomas and Meizel, 1989; Meizel and Turner, 1991). Progesterone is also present in oviductal fluid, although the concentration does not appear to rise at ovulation (Libersky and Boatman, 1995). There are conflicting reports on the effect of oviductal epithelium and its secretions on hyperactivation, but a rapid response has not been reported.

#### *Hyperactivation and Capacitation*

Capacitation is a poorly defined and poorly understood sequence of events that prepares sperm for fertilization; more specifically, to undergo the acrosome reaction. Many authors define hyperactivation as a subset of capacitation, because it often occurs *in vitro* at some point during the capacitation process. However, the two events are not inexorably linked. In mouse sperm carrying the t haplotype, hyperactivation occurs prematurely *in vitro*, but capacitation occurs on schedule (Olds-Clarke, 1989). By lowering the bicarbonate concentration in medium, hamster sperm may be fully capacitated without becoming fully hyperactivated (Boatman and Robbins, 1991; Stauss et al, 1995). In other media formulations, completion of hyperactivation will precede completion of capacitation by an hour or more (DeMott et al, 1995). These observations support the argument that the pathway to hyperactivation diverges from that of capacitation, if indeed the two are ever united.

#### *Hyperactivation and Chemotaxis*

Some human spermatozoa within a semen sample respond chemotactically to some samples of human follicular fluid *in vitro* (Ralt et al, 1994). Hyperactivation provides a mechanism for chemotaxis and was observed to occur in these experiments. Chemotaxis was assayed *in vitro* using various glass slide chambers and capillary tubes. It is important to consider the differences between the physical environment for sperm *in vitro* and *in vivo*. The complex and changing conformation of the oviductal lumen and the stirring of fluid by cilia and muscular contraction could disturb gradients of signals, but hyperactivation could still serve to bring sperm to oocytes. As discussed above, in the physical environment of the oviduct, hyperactivation may enable sperm to escape mucosal pockets and perhaps trace out a search pattern for oocytes by virtue of the fact that hyperactivated sperm frequently change direction. Hyperactivation may also allow sperm to progress toward the oocyte by enabling them to detach from the mucosal epithelium. If a signal for hyperactivation emanates from the cumulus mass, the closer sperm

are to the oocyte, the more frequently they would detach from epithelium and escape from mucosal pockets. This type of effect does not require an undisturbed gradient. So, it is difficult to determine whether classical chemotaxis occurs *in vivo* or hyperactivation brings sperm to eggs via a more complex interaction of events.

### Clinical Applications

Some studies have linked development of hyperactivation in human sperm with *in vitro* fertilization success (Wang et al, 1993) and success after artificial insemination (Johnston et al, 1994). CASA systems can be used to determine objectively the percentage of hyperactivated human sperm in a sample (Mortimer, 1995). Because premature hyperactivation could hinder sperm from reaching the site of fertilization *in vitro*, fertility of a semen sample would probably be linked to low incidence of spontaneous hyperactivation (de Lamirande and Gagnon, 1993) and high incidence of induced hyperactivation. As more information becomes available on physiological signals for hyperactivation, testing for hyperactivation may become a useful aspect of semen analysis.

### Summary

The functions of hyperactivation may fit together to create the following scenario. Sperm that enter the oviduct bind to the mucosal epithelium. Near the time of ovulation, hyperactivation helps them to detach from the epithelium, escape mucosal pockets, and move through oviductal mucus. As sperm reach the ampulla, frequent changes in direction may enable them to encounter the cumulus mass. Then, hyperactivation assists them in penetrating the cumulus matrix and, after acrosome reacting, the zona pellucida. A factor or factors in the periovalutary oviduct or follicular fluid may induce hyperactivation by raising intracellular calcium levels in sperm. Knowledge of mechanisms regulating hyperactivation may be used to develop new clinical tests for fertility of semen samples.

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