Review Article

Factors Regulating Mammalian Sperm Migration Through the Female Reproductive Tract and Oocyte Vestments

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Mechanisms of mammalian sperm migration through the female reproductive tract and ovum vestments are described. The perspective is biophysical as well as biochemical and morphological, and the focus is upon the role of sperm motility in these processes. Sperm forward progression is characterized as an interactive process between the cell and its environment, and the mediation of flagellar bend propagation by the physical properties of its surroundings is described. These properties, together with flagellar beat kinematics, sperm morphology, and surface properties, determine the magnitude of the forces generated by sperm and their consequent rate of progression. Sperm interactions with the cervical mucus, the cumulus oophorus, and the zona pellucida are described. The poorly understood affinity of the sperm surface for the macromolecules of the mucus, cumulus, and zona is stressed, as is the viscoelastic structural mechanical resistance of these biopolymers to sperm motion. The kinematics and consequences of hyperactivated sperm motion are presented, with emphasis on objective characterization of such motion (as a biomarker), along with analysis of the mechanical advantage that such motion may confer on spermatozoa during egg-vestment interaction.

Key words: sperm, transport, mucus, cumulus, zona pellucida, fertilization

INTRODUCTION

Flagellar movement is a fundamental expression of the vitality of a sperm cell, and is essential for its reproductive function. The active mechanism of flagellar motion produces a sequence of principal and reverse bends [Gibbons and Gibbons, 1974; Woolley, 1977]. These can have varying degrees of curvature and can be initiated and propagated along the flagellum at different rates. Flagellar bending results in a distribution of local forces and torques against the surrounding fluid. In accordance with Newton's Third Law, these are resisted by equal and opposite local forces and torques. In order to satisfy Newton's Second Law, the summation of the local local external forces and torques over the sperm head and tail must be zero. As a consequence, there

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must be a net translation of the sperm cell (unless it is abutted against an immovable
object), that is, swimming velocity.

In a general biophysical sense, mammalian sperm swim optimally; the principles
of hydrodynamics and optimal control theory demonstrate that the smooth flagellar
traveling waves, commonly seen in low-viscosity media, tend to maximize the sperm’s
propulsive velocity with respect to the hydrodynamic power output (Pironneau and
Katz, 1974). Mammalian sperm must use their swimming ability to reach the
oocyte–cumulus complex, and the forces due to flagellar motion assist them in pene-
trating the egg vestments to reach the oolemma.

Our studies of these processes are hampered by their complexity and by appar-
ent variation among species. Some of this biological variation is real; for example, the
site of semen deposition may be the vagina, cervix, or uterus. These environments dif-
fer not only with respect to their distance from the site of fertilization but also in their
chemical and physical characteristics. Some variation is only virtual; for example, long
sperm swim faster than short sperm, and introduction of appropriate hydrodynamic
scaling concepts can condense and unify our description of certain aspects of sperm
motion. From a large body of research on the biology of sperm motility and transport,
a number of concepts and themes emerge:

1. Vigorous motility is necessary in certain sites and at certain times to insure success-
ful sperm migration to the site of fertilization and transit through the granulosa cell
investments and zona pellucida of the oocyte.
2. However necessary, motility alone is not sufficient as an active sperm-transporting
mechanism. The physical and chemical activity of the female reproductive tract
are also required.
3. The kinematics of flagellar bending are not constant during the natural history of
the sperm cells. The epithelial surfaces and secretions of the female tract, as well as
the egg investments, appear to modulate sperm motility and may coordinate the
transport and union of the gametes. Inhibition of flagellar activity may be associ-
ated with the temporary retention of sperm in anatomical reservoirs of the female
tract, while resumption of vigorous motion is coincident with the continuation of
sperm migration.

In this article, we will discuss a number of new results and concepts that bear on
our understanding of how sperm migrate through the female reproductive tract and
reach the oolemma. There are many review articles that summarize comprehensively
our basic knowledge of mammalian sperm transport [e.g., Mortimer, 1978; Over-
street, 1983; Hunter, 1987]. Rather than reiterating the classical studies and present-
ing a chronology of sperm transport, we will emphasize recent observations relative to
the cell biology of sperm interaction with the female tract. We will discuss the bi-
physical factors that may modulate the rate of sperm migration. Since sperm must
move through polymeric secretions during passage to the egg(s), the properties of
these secretions (including cervical mucus and the cumulus oophorus) play an impor-
tant role in the modulation of sperm migration. The structural–mechanical resistance
to sperm motion by the macromolecules of these secretions is important in this interac-
tion, as is the affinity between the sperm surface and these molecules. We will also
discuss our understanding of the kinematic details of flagellar movement in relation to
sperm progression in vivo, particularly the significance and complexity of hyperactivated motion.

THE PHYSICAL PROPERTIES OF THEIR ENVIRONMENTS AFFECT SPERMATOZOA

Viscoelastic Properties of Secretions Through Which Sperm Migrate

Within the female tract spermatozoa must traverse polymeric fluid secretions with complex rheological or flow behavior. These include cervical mucus, the cumulus oophorus, and perhaps an oviductal mucus in the lower isthmus. Macromolecular networks or complexes within these secretions provide structural resistances to flagellar forces. As a consequence, the effective viscosities and elasticities of these secretions are significantly greater than those of the culture media in which sperm are commonly observed in vitro. Knowledge of sperm responsiveness to variations in viscosity and elasticity (i.e., viscoelasticity) is therefore essential for understanding sperm movement in vivo.

The viscosity of the suspending fluid can influence sperm flagellar motion [Brokaw, 1975; Rikmenspoel, 1984; Ishijima et al., 1986]. In a simple fluid, the local force produced by motions of the sperm body is proportional to the product of fluid viscosity and the local velocity of the body. If viscosity increases, the flagellum must push harder to achieve the same local velocity, with a resulting increase in power output. The control mechanism for flagellar bending responds to changes in viscosity by altering the pattern and rate of flagellar motion. Initially, the three-dimensional flagellar beat becomes virtually planar, and the rolling frequency of the sperm head is greatly reduced; that is, the sperm is less likely to rotate about the axis of propulsion. For human and bull spermatozoa, as viscosity continues to increase, the bending in the proximal flagellum is diminished (with lower effective amplitude and wavelength); in the distal flagellum an increase in bending is observed, often as one or more complete waves with very small wavelength [Rikmenspoel, 1984; Ishijima et al., 1986]. These movements, which are conspicuous in human sperm swimming in unstretched human cervical mucus [Katz and Berger, 1980], are often referred to as figure-of-eight motions, since the distal tip of the flagellum can be seen to trace such a pattern.

The specific response of the flagellar bending mechanism to elastic properties of the sperm's environment is poorly understood. When a sperm pushes against a purely elastic material, the resistive force is proportional to the extent of displacement of the material, not the rate of displacement, as is the case with a purely viscous material. This response would tend to constrain the amplitude of flagellar beats more than the frequency of beating. As we shall discuss below, sperm movement in the cumulus may be strongly influenced by elasticity.

The Cervical Mucus

Immediately following coitus in primates and ruminants, spermatozoa encounter the cervical mucus, a fluid with complex physical properties. The physical properties of the mucus contribute to several important cervical functions in these species, including exclusion of seminal plasma; the selective exclusion of certain sperm cells with morphological and, possibly, functionally abnormality; and the retention and conservation of the sperm for later migration to the upper tract. The physical proper-
ties of mucus derive from its mucin macromolecular component or microstructure, consisting largely of a glycoprotein of large molecular weight. Mucus granules, <1 μm in diameter, are exocytosed by goblet cells in the cervical epithelium. Immediately after secretion, the granules undergo a swelling and transformation process, with intramolecular bonding replaced by intermolecular bonding during formation of the whole mucus gel [Wergin, 1979; Meyer and Silberberg, 1980; Tam and Verdugo, 1981; Verdugo et al., 1987].

At the microscopic level, the whole mucus appears to contain a network of fibrous, interconnected macromolecules with interstices that contain low-viscosity fluid (the mucus plasma). These interstices are small compared to the size of the sperm body [Chretien et al., 1975; Katz and Berger, 1980; Tam et al., 1982; Poon and McCoshen, 1985; Yudin et al., 1989]. Consequently, the sperm must push or burrow through the mucus, distorting if not rupturing its macromolecular network [Yudin et al., 1989]. As spermatozoa directly contact the mucus macromolecules, their surfaces are subjected to high shearing rates that can relocate or remove molecules attached to the plasma membrane. Decapacitation factors [reviewed in Yanagimachi, 1981; Bedford, 1983; Clegg, 1983; Fraser and Ahuja, 1988] may be included in the surface components removed during sperm migration through mucus. Sperm–mucus interaction may therefore contribute to the initiation of capacitation in some species. The migration of human sperm through a column of human cervical mucus in vitro appears to be as effective as centrifugation in washing sperm for capacitation [Overstreet et al., 1980; Gould et al., 1984]. Human spermatozoa recovered from mucus after insemination appear to have achieved capacitation in vivo [Gould et al., 1984, 1985; Lambert et al., 1985].

The mechanical resistance of mucus to sperm depends on the structural–mechanical properties of the microstructure, the viscosity of the mucus plasma, and the size and shape of the sperm head and flagellum. There is variation among species in these biological factors, as evidenced by comparison of the human and bovine. Ovulatory human mucus is less hydrated than bovine mucus, and, as a consequence, the intermolecular interstices or pores within human mucus are smaller, and its viscosity is higher [Wolf et al., 1977; Katz and Berger, 1980; Tam et al., 1980, 1982]. The bovine sperm flagellum (length 60 μm) therefore encounters less resistance per unit length from its native mucus than does the human sperm flagellum (length 50 μm). Bull sperm in bovine mucus do not display the figure-of-eight bending of the distal flagellum, which is characteristic of human sperm in their mucus and which is the hallmark of elevated external viscosity. However, the bull sperm head is larger (10 μm × 5 μm × 1.5 μm vs. 5 μm × 3 μm × 1.5 μm) for the human, and this size difference in sperm head dimensions is greater than the species difference in mucus pore size. Thus, the hydrodynamic drag on the bull sperm head in bovine mucus may be proportionally greater than that encountered by the human sperm in human mucus. This combination of factors may explain the fact that bull sperm slow their swimming velocity when passing from semen to mucus in vitro (typically going from 90 μm/sec to 60 μm/sec), whereas normal human sperm swim at approximately the same rates in mucus (typically 25–45 μm/sec) and in the lower viscosity semen [Katz and Dott, 1975; Katz et al., 1978a, 1981, 1982a].

Both human and bovine sperm flagella beat at surprisingly high rates in mucus (typically 20–25 Hz) given the striking effects their native mucus secretions have on beat shape (human) and propulsive velocity (bovine). Such behavior does not occur...
when the sperm are subjected to increased viscosity in model fluids such as methycel­
lulose or Ficoll [Rikmenspoel, 1984; Ishijima et al., 1986; Katz, unpublished observa­
tions]. The elastic properties of the mucus, which derive from the inherent flexibility
of the mucus macromolecules and their interconnections, may account for this dis­
crepancy. Exactly how the interplay between mucus viscosity and elasticity could act
to increase the mechanical efficiency of sperm motion is not yet understood. Model
hydrodynamic analyses in our laboratory demonstrate its feasibility [Powell et al.,
1988].

The topology of the cervical mucus microstructure is not homogeneous and
appears to be a mosaic, with microdomains of differing resistance to sperm [Odeblad,
1968; Odeblad and Rudolfsson, 1973; Katz and Overstreet, 1982; Yudin et al., 1989].
Three types of mucus heterogeneity may be important:

1. The exterior borders of the mucus have a more compact microstructure than the
interior, resulting in increased resistance to sperm.
2. The body of mucus within the cervical canal may be composed of different regions,
with varying resistances to sperm.
3. The mucus at the epithelial surfaces, near the sites of secretion, may be different
from mucus near the center of the cervical canal, in part because its structure is in
the process of transformation and, in part, because peripheral regions are less sub­
ject to external shearing by the intraluminal pressure changes.

The compacted mucus microstructure at the mucus borders, for example, at the
semen–mucus interface, has greater structural integrity than that in the mucus inte­
rior and a greater resistance to sperm motion [Katz et al., 1987; Yudin et al., 1989].
As a consequence, the kinetics of penetration by sperm at the semen–mucus interface
are different from those of sustained sperm migration through the mucus interior
[Cummings et al., 1984]. Three factors appear to contribute, interactively, to sperm
penetration at the semen–mucus interface: 1) The sperm themselves, that is, their ade­
quate motility and numbers; 2) seminal enzymes; and 3) external forces due to visceral
contractility. Human seminal proteases have been shown to have mucolytic activity
[Moghissi and Syner, 1970]. Dilution of human semen with artificial medium, as
compared with its own plasma, reduces the relative numbers of sperm that enter
mucus in vitro [Overstreet et al., 1980b]. However, sperm in media devoid of enzymes
are capable of penetrating mucus, although intrinsic sperm enzymes themselves are
not believed to facilitate mucus penetration [Beyler and Zaneveld, 1979]. External
shearing of the interface in vitro [Katz et al., 1987], designed to model the effects of
pressure gradients in vivo [Fox et al., 1970], also increases mucus permeability to
sperm.

In recent studies of sperm–mucus interaction in vitro, a multivariate statistical
approach has been applied to data from objective measures of both semen quality and
mucus penetration [Aitken et al., 1985, 1986; Mortimer et al., 1986]. The numbers of
sperm entering mucus have been shown to depend on the numbers of progressively
motile sperm in the semen, as well as on the progressive or straight-line swimming
velocity and the amplitude of lateral head displacement. That both increased velocities
and lateral head motions facilitate mucus entry suggests that increased sperm shear­
ning of the interface may reduce its resistance to sperm penetration [Aitken et al., 1985;
Feneux et al., 1985; Mortimer et al., 1986].
Our laboratory examined the simultaneous effects of sperm numbers and swimming characteristics on mucus penetration [Cummings et al., 1984]. We found that many fresh peri-ovulatory human mucus specimens, classified as normal by clinical criteria, are not readily penetrated if the sperm concentration in semen is less than $10 \times 10^6$ sperm/ml. This sperm concentration is below the normal clinical range but is frequently observed in semen of fertile men. Some sort of sperm cooperation at the interface appears necessary for penetrating such mucus specimens. However, there are other mucus specimens, indistinguishable by clinical criteria, for which there is no apparent sperm concentration threshold. Collectively, these studies demonstrate that there is no unique set of conditions that enable sperm to enter mucus; these studies illustrate the inadequacy of indirect methods for assessment of mucus penetrability.

Sperm interaction with the semen–mucus interface may be the first step in filtration by mucus of weak and/or otherwise abnormal sperm (see below). However, successful penetration across the semen–mucus interface does not ensure successful sperm migration through the mucus. Sperm movement beyond the interface is influenced by the heterogeneity of the mucus interior. Slide preparations display spatial variation in the optical density of the mucus, as well as differential sperm penetration into different regions. Electron-microscopic studies of the mucus ultrastructure reveal spatial differences in the topology of the macromolecular network [Chretien et al., 1975; Yudin et al., 1989]. Since the forces of mucus collection are likely to have an homogenizing effect upon mucus, the heterogeneity observed in vitro is likely to underestimate the degree of variation within the mucus in vivo.

The origin of this heterogeneity derives in part from the mucus secretory process itself. Odeblad and co-worker [Odeblad, 1968; Odeblad and Rudolfsson, 1973] stressed that different cervical mucosal glands could secrete mucus with different biophysical properties. The sperm themselves could also contribute to heterogeneity via their physico-chemical effects on the mucus. In vitro studies in bovines [Katz et al., 1981] and humans [Katz et al., 1982a] demonstrated that vanguard sperm penetrating the interior of a column of mucus swim faster than following sperm, which arrive at the same location at a later time. Since the flagellar beat frequencies and shapes were not different between the vanguard and followers, it appears that the vanguard sperm had altered the mucus.

The theory that the cervix could serve as a reservoir for sperm in some species, and that sperm may be stored in the crypts of the cervical epithelium, has been advanced for many years [Quinlan et al., 1932; Mattner, 1963; Tredway et al., 1975; reviewed in Overstreet, 1983]. This idea was derived from observations of the parallel swimming of sperm in mucus stretched in vitro [Tampion and Gibbons, 1962; Mattner, 1966]. It was inferred that the mucus microstructure had been oriented along the direction of external shearing, thereby constraining sperm to swim along this direction. There is now direct ultrastructural confirmation of this phenomenon [Chretien et al., 1975; Yudin et al., 1989]. Such sperm behavior led Mattner and colleagues to postulate, over 20 years ago, that a comparable phenomenon occurs in vivo [Mattner, 1966; Gibbons and Mattner, 1971]. The notion is that visceral contractility and the forces of mucus outflow tend to align the microstructure longitudinally within the cervical canal. Entering spermatozoa would therefore be guided toward the endocervix and/or sites of mucus secretion, for storage. Hoglund and Odeblad [1977] presented a complementary hypothesis, in which low-viscosity strings of mucus were proposed to extend out from particular cervical glands into the lumen of the canal.
These models, although attractive, have not been verified experimentally [Overstreet, 1983]. No direct observations have been made of mucus organization in vivo. We lack insight about the possible mechanism(s) for sperm retention and release from the cervical mucus or mucosal surfaces. It is possible that physical or chemical properties of the microenvironment at the mucosal surfaces suppress sperm motion [hypothesized by Hoglund and Odeblad, 1977]. Certainly, mucus near the mucosal surfaces is less subject to external shearing, so that sperm in this region would be harbored, to some extent. It is also possible that sperm retention in the cervix could involve direct adherence to the epithelial surfaces (see below).

The Cumulus Oophorus

Like cervical mucus, the cumulus oophorus is composed of a microstructure with interstices that are small compared to the size of the sperm head [Yudin et al., 1988]. Penetrating sperm must push their way through this network, often coming into direct contact with the macromolecules. In so doing, the sperm displace and possibly rupture the macromolecules [Yudin et al., 1988]. There have been some direct observations of sperm motility within the cumulus [Cummins and Yanagimachi, 1982; Suarez et al., 1983, 1984; Corselli and Talbot, 1986, 1987; Cherr et al., 1986; Katz et al., 1986; Drobnis et al., 1988c]. These studies have primarily involved hamster gametes. In this species, the sperm flagellar beat within the cumulus is different from the figure-of-eight movement displayed by sperm in media of elevated viscosity. The sequence of principal and reverse bends in the cumulus results in two basic patterns of motion [Drobnis et al., 1988c]: 1) low-amplitude sinusoidal-like waves, which result in forward progression of the sperm; and 2) non-propagated high-curvature flexions of the midpiece, which produce a hatchet-like movement of the sperm head, and which do not result in forward progression. The sinusoidal flagellar motions sometimes produce a snakelike progression of the sperm body, in which the entire flagellum follows exactly in the path of the sperm head. Such motion appears to resemble the crawling motility described by Phillips [1972] for mouse sperm collected from the oviduct. Capacitating human sperm can penetrate the hamster cumulus [Drobnis et al., 1987; Katz et al., 1988b], and the same two patterns of flagellar movement are also observed in this heterologous system. Thus, human sperm move in a qualitatively different manner in cervical mucus and in the hamster cumulus. These observations imply that the viscoelastic properties of the cumulus, which are experienced by sperm, are different from those of the cervical mucus. Initial hydrodynamic arguments suggest that the serpentine motion in the cumulus may result from a strong elastic response to flagellar bending [Katz et al., unpublished].

The role of sperm hyaluronidase in modifying the cumulus resistance to sperm is controversial [reviewed by Talbot, 1985]. Our electron-micrographs of hamster sperm within the cumulus reveal deformation but no large-scale degradation of the microstructure adjacent to the sperm body [Yudin et al., 1988]. By direct observations of cumulus penetration we have gained the impression of a wake of disaggregated microstructure along the sperm path, but such structural modifications are difficult to confirm in thin section. It is also possible that sperm enzymes may act to reduce the stiffness of the cumulus microstructure, thereby facilitating sperm penetrability. Where sperm are bound to the zona pellucida, the surrounding regions of cumulus appear to be devoid of microstructure. Whether these open regions are the result of enzymatic activity and/or sustained mechanical shearing by the flagellum is not yet known.
The Zona Pellucida

The physical properties of the zona pellucida present the final barrier to sperm union with the oolemma. The resistance of the zona to the sperm has not been characterized, and the physical and chemical interactions between the sperm and zona are currently subjects of intense study and debate [see Yanagimachi, 1981; Moore and Bedford, 1983; Green and Purves, 1984; Green, 1988; Baltz et al., 1988; Drobnis et al., 1988d]. The thickness of the zona is of the same order of magnitude as the length of the sperm head; thus its principal resistive effect is against the head, not the flagellum. In most mammals the oocytes are fully invested at the time of fertilization, and the sperm flagellum is constrained to move within the inner cumulus and/or the corona radiata. Due to their altered viscous and elastic properties, the mechanics of force generation in these microenvironments are significantly different from those in a simple culture medium. Recent refinements in the measurement and hydrodynamic analysis of sperm flagellar motions during zona penetration have increased substantially our appreciation of the magnitude of the stresses exerted by the sperm head against the zona material [Drobnis et al., 1988d; Katz et al., 1988a]. The relative importance of sperm enzymes in reducing the zona resistance, as compared with active forces generated by the flagellum, has not been ascertained [see Green, 1988].

Recent studies of the mechanical properties of the zona have utilized exclusively the concepts of elasticity [Green, 1987; Cheng et al., 1987; Drobnis et al., 1988a]. Direct solutions of the equations of elasticity [Cheng et al., 1987] for data from capillary suction experiments [Drobnis et al., 1988a] have provided the first information on the mechanical properties of zonae from different species. We determined the modulus of elasticity E, which increases in magnitude with the stiffness of a material. We found that the pig zona (E = 200–250 kdyn/cm²) is stiffer than the mouse zona (E = 100–200 kdyn/cm²) which, in turn, is stiffer than the hamster zona (E = 50–80 kdyn/cm²). These differences are similar to those predicted by species differences in solubility [Dunbar, 1983]. However, none of the above studies characterized zona properties on the scales of time, space, and stress (force per unit area) experienced by individual sperm heads during zona interaction. Our capillary suction experiments did detect a 56% reduction in zona deformability in early two-cell hamster embryos compared with pre-fertilization oocytes. This is the first mechanical confirmation of the zona hardening that follows fertilization in this species, in which no hardening phenomenon is detectable by other methods [Chang and Hunt, 1956; Inoue and Wolf, 1975]. In the mouse, where zona hardening is easily detected by solubility methods [reviewed by Wolf, 1981], we were also able to demonstrate a significant reduction in deformability. Thus, zona hardening may contribute, albeit as an ancillary factor, in the post-fertilization block to polyspermy.

**EFFECTS OF SOLID SURFACES ON SPERM MOTION**

The spaces through which sperm migrate in vivo may have transverse dimensions of the same magnitude, or they may be smaller than the length of the sperm body. In some locations, such as the uterotubal junction (UTJ) and the lower oviductal isthmus, the spaces are small enough to restrict sperm passage physically. The UTJ appears to function as a mechanical valve in many species, including those with intrauterine semen deposition such as pigs, horses, and dogs, and also in some rodents [see
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Gaddum-Rosse, 1981; Overstreet, 1983]. Even if the spaces enclosing sperm are large enough to permit passage, the presence of a nearby surface will have a profound effect upon sperm movement. When a sperm is swimming near a solid surface at a distance less than its own body length, the fluid motions induced by the flagellum are constrained by the surface. This phenomenon causes an increase in the dissipation of hydrodynamic energy, and, consequently, the flagellum must push harder to achieve a local velocity equal to that attained in the absence of a nearby boundary [Katz et al., 1975]. Thus, the flagellum develops more force per unit velocity, that is, it can push harder given the available energy. Because this wall effect also increases the hydrodynamic drag on the sperm head, the potential for increased active flagellar thrust is countered by increased passive resistance to the head.

As in the case of increased viscosity, it is reasonable to expect that the flagellar bend propagation mechanism will respond to the wall effect, and there is some experimental evidence in vitro demonstrating such a response. For example, rabbit and rhesus monkey sperm in diluted semen near a surface swim with less curvature of the proximal flagellum and with a more two-dimensional beat than when in the midplane of a deep-slide preparation [Suarez et al., 1983; Katz and Phillips, 1986]. The seminal spermatozoa of other mammals appear to behave similarly, including those of the bull, ram, and stallion. Hyperactivated rabbit sperm flagella beat with greater midpiece curvature when they are in shallow slide preparations than when in the deep ones [Suarez et al., 1983]. When sperm become constrained to beat two-dimensionally, their flagellar waves become less symmetrical, resulting in circular trajectories [e.g., Rikmenspoel, 1962; Phillips, 1972; David et al., 1981]. Typically, these circles have radii whose magnitude is comparable to the length of the flagellum. This wall effect becomes more pronounced as the depth of the preparation decreases. The relatively short human spermatozoa, whose heads are less flat, retain their three-dimensional flagellar beat (as assessed by the rolling of the sperm head) until constrained to swim in a relatively shallow space.

When observing spermatozoa with a microscope, it is common to focus within a lamina 10 \( \mu \text{m} \) or less beneath the coverglass, because more sperm are seen in this region. This familiar observation results from a hydrodynamic capturing phenomenon by the surface of the coverglass, which was first reported by Rothschild [1963] over 20 years ago. Later experimental studies also stressed this tendency [Phillips, 1972; Winet et al., 1984]. We can now interpret the phenomenon as resulting from the amplification of hydrodynamic forces on the sperm body. The flagellar bending mechanism responds by propagating two-dimensional waves, and the overall force balance on the sperm body inhibits motions away from the vicinity of the surface.

It is likely that these wall effects, so conspicuous in vitro, play a role in sperm migration in vivo. During much of their journey along the female reproductive tract, spermatozoa move in close proximity to epithelial surfaces. The two-dimensional flagellar wave propagation, with planar orientation of the sperm body, is well suited to migration along such surfaces, as initially implied by the description of sperm crawling along surfaces [Phillips, 1972]. The hydrodynamic capturing phenomenon would act to retain many sperm close to surfaces, especially those cells exhibiting relatively symmetrical, continuous flagellar beats. These sperm would progress along surfaces but could be trapped and eliminated from transport if led into cul-de-sacs of the epithelium. The presence of fluid currents due to ciliary activity would act to reorient such sperm; this mechanism, along with visceral contractility, may contribute to sperm
transport through the oviduct. Even hyperactivated motion, which commonly produces non-progressive swimming in slide preparations in vitro (see below), could act to prevent sperm trapping within the enfoldings of the tubal epithelium. Katz and Yanagimachi [1980] reported gliding motion of hamster spermatozoa along the epithelial surfaces when observed through the transparent wall of the oviductal ampulla. They emphasized that the continuously reorienting thrust produced by the hyperactivated beat of such sperm appeared constrained by the surfaces, resulting in progressive swimming.

**THE PROPERTIES OF THE SPERM SURFACE CAN MODULATE ITS TRANSPORT**

**Interaction With Macromolecules**

The foregoing discussion has focused upon structural-mechanical features of the secretions of the female tract and egg investments that resist sperm motion. For example, sperm must push aside and/or break through the microstructure of the cervical mucus and cumulus in order to penetrate these gels. The electrochemical affinity of molecules on the sperm surface for the molecules of these secretions provides an additional source of resistance to sperm penetration. There is increasing evidence that such surface affinities, as well as adherence of spermatozoa to epithelial surfaces, can modulate sperm passage through the female tract and vestments of the oocytes.

The properties of the human sperm surface are known to influence penetration of cervical mucus. When antisperm antibodies are present on the sperm surface, sperm with vigorous motility may be unable to swim for more than a few body lengths into cervical mucus [Fjallbrant et al., 1968, 1969; Jager et al., 1981; Bronson and Cooper, 1987]. Either by physical entanglement or by chemical linkages, antibodies interact with sperm in such a way as to resist forward progression. Similarly, antibodies secreted into the mucus can link to penetrating sperm, causing an analogous impediment to motion. The interaction of human seminal sperm with cervical mucus, both in vivo and in vitro, is characterized by significant reductions in the number of morphologically abnormal cell types that penetrate [Perry et al., 1977; Fredricsson and Bjork, 1977; Hanson and Overstreet, 1981]. Morphologically abnormal human sperm, as a group, have inferior motility compared with normal sperm in the same ejaculate [Overstreet et al., 1981; Katz et al., 1982a, b]. However, this differential swimming ability is not large enough to account for the exclusion of such a large proportion of abnormal sperm from mucus penetration. Basic hydrodynamic reasoning dictates that the variations in the dimensions of the sperm heads alone will not generate sufficient drag to prevent mucus penetration by these sperm. The origin of the increased resistance to these abnormal sperm may lie in their surface interactions with the mucus macromolecules. Studies with capacitated sperm have indicated that a more generalized cellular dysfunction may be associated with morphological abnormalities [Morales et al., 1988a]. Surface changes in the sperm cell are closely related to the functional alterations of capacitation (see below). It is possible, therefore, that the dysfunctional state of the sperm cell is revealed in its earliest interaction with the cervical mucus.

Sperm capacitation involves alterations in the plasma membrane, which may be initiated to remove inhibitory molecules from the sperm surface, and it may ultimately result in the exposure of receptors for binding to the zona pellucida and induction of
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the acrosome reaction [Yanagimachi, 1981; Bedford, 1983; Clegg, 1983; Fraser and Ahuja, 1988]. These membrane changes may also be directly related to changes in flagellar motion, for example, in altering membrane ion conductance. Little attention has previously been directed to additional effects of these surface changes, for example as they may relate to sperm transport. A number of recent studies provide evidence of such effects. There are reports that acrosome-reacted, hyperactivated hamster spermatozoa stick to the outer surface of the cumulus in vitro [Suarez et al., 1984; Corselli and Talbot, 1986]. Hyperactivated motility per se (see below) may not be necessary for cumulus penetration, but freshly collected, uncapacitated epididymal hamster sperm, however vigorous, cannot penetrate the cumulus [Cummins and Yanagimachi, 1986; Corselli and Talbot, 1987]. Thus, changes occurring on the hamster sperm surface during in vitro capacitation appear necessary to prevent sperm adherence to the cumulus matrix and cells, but following the acrosome reaction sperm may again become adherent to these structures. Both of these observations have biological relevance. Prematurely acrosome-reacted sperm outside the cumulus may not be capable of successful zona binding and penetration; vigorous but non-capacitated sperm that prematurely reach the cumulus are also unsuited for successful zona interaction. In addition, hamster sperm within the cumulus, exhibiting similar beat kinematics, can have varying forward velocities [Drobnis et al., 1988c]. Such variation may reflect differences in the surface properties of these spermatozoa, as well as in the local mechanical resistance of the cumulus to sperm motion.

Recently, we have been conducting comparative studies of human sperm interaction with fresh ovulatory human cervical mucus and post-ovulatory hamster cumuli [Drobnis et al., 1987, Katz et al., 1988b]. The latter has been used as a potential surrogate for the human material, since it can be collected from the oviducts with minimal handling before application of sperm. The human spermatozoa are subjected to a standard swimup and washing procedure and are then incubated in high HSA capacitation medium. These sperm retain the ability to penetrate mucus during the first 2-4 hours of incubation. Thereafter, despite maintenance of vigorous flagellar motility, they lose the ability for sustained mucus penetration, becoming encumbered within a few body lengths of the mucus interface. During the same interval that sperm are able to penetrate mucus, they lack the ability to penetrate the hamster cumulus. However, they develop this capacity at approximately the same time at which they cease to penetrate mucus. The implication is very clear that sperm surface changes, rather than motility, are responsible for these distinctions. It is unclear whether these in vitro phenomena have counterparts in vivo.

**Adherence to Epithelial Surfaces**

The electrochemical properties of the sperm plasma membrane also create a potential for affinity to epithelial tissue. Capacitation-induced changes in surface properties could therefore interact with changes in flagellar activity to modulate sperm transport. In the cervix and lower oviduct, sperm adherence to epithelial surfaces has been observed and is thought to play a role in regulation of sperm transport [see Overstreet, 1983; Suarez, 1987]. As mentioned above, in species with intravaginal insemination and which have a mucus-laden cervix, the cervical mucosa have been implicated as a site of sperm storage [Insler et al., 1980]. In ruminants and in the rabbit, large numbers of cervical sperm are sequestered at the mucosal surfaces and can only be recovered by flushing with detergent [Mattner, 1966, 1968; Edey et al.,
1975; Overstreet et al., 1978]. These observations could be explained by sperm adherence to the mucosal surfaces. It is not known whether such sperm are irreversibly trapped, or whether capacitation-mediated changes in sperm surface properties may result in their subsequent release, as suggested by the in vitro studies.

More recently, the oviductal isthmus has received considerable attention as a site of sperm retention and possible storage in several mammals [reviewed in Hunter, 1975; Overstreet, 1983; Hawk, 1987]. In the rabbit, sperm retention in the lower isthmus is associated with reversible cessation of flagellar activity [Overstreet and Cooper, 1975; Cooper et al., 1979; Johnson et al., 1981, Burkman, 1984], a tenacious mucuslike secretion [Jansen, 1978; Jansen and Bajpai, 1982], and sperm adherence to the epithelial cilia [Cooper et al., 1979]. In the hamster, sequestered sperm in the caudal isthmus are generally found within the folds of the epithelium, in close association with the mucosal surfaces [Smith et al., 1987]. Recently, Suarez [1987] has directly observed mouse spermatozoa within the UTJ junction, tubal isthmus, and ampulla of the excised female tract. She described motile and non-motile sperm adhering by their heads to the epithelial surfaces of both the UTJ and isthmus. Sperm accumulated within the lower isthmus but did not advance beyond it until 1–2 hours prior to ovulation. The lower isthmic reservoir contained sperm within the lumen as well as those adhering to epithelial surfaces. At times the adherent sperm were seen to break free of their attachments and assume vigorous motion, resembling hyperactivation, within the lumen. Both vigorous and weakly motile sperm were seen in the lumen, often in adjacent regions. Many questions remain to be answered about the possible role of the isthmus as a sperm reservoir, instead of a trap. It will be necessary to determine which subpopulation of isthmic sperm is released in vivo, and whether some uterine sperm can directly traverse the lower oviduct and contribute to the population that actually engages in fertilization.

It is noteworthy that neither Suarez nor Katz and Yanagimachi [1980] observed sperm adhering to the ampullar epithelium in situ. This could have been due to capacitation-induced changes in the surfaces of sperm reaching that location. However, as indicated by Suarez [1987], differences in the properties of the ampullar epithelial surfaces also could have been responsible.

HYPERACTIVATED MOTILITY IS MORE UBIQUITOUS THAN PREVIOUSLY APPRECIATED AND MORE KINEMATICALLY COMPLEX

History and Terminology

Attention to the hyperactivated motion of mammalian spermatozoa is now almost 20 years old. During the late 1960s, Yanagimachi [1969; 1970] and Gwatkin [Gwatkin and Anderson, 1969] referred to recognizable changes in motion associated with capacitation of epididymal hamster sperm in vitro. These were first illustrated in the following year by Yanagimachi [1970]. Two years later, Phillips [1972] illustrated and stressed the asymmetric flagellar beat patterns and crawling trajectories of mouse sperm collected from the uterus and oviduct (which may or may not have been capacitated). There followed the identification of motility hyperactivation in the spermatozoa of most mammals studied, whether they were undergoing capacitation in vitro after collection from the male or in vivo following recovery from the female [see e.g., Fraser, 1977; Katz and Overstreet, 1980; Yanagimachi, 1981; Cooper, 1984]. The motivation for studying hyperactivation has been twofold: 1) it is a useful biologi-
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cal marker as related to capacitation; 2) it may mechanically promote a number of sperm functions, including transport through the oviduct, and penetration of the cumulus and zona pellucida. However, despite the increasing attention to this visually striking phenomenon, it still lacks objective kinematic definition; the site, mechanisms, and kinetics of its onset are unclear; and its role in sperm function is not fully understood.

Subjective visual assessments have led to a remarkable set of metaphors used to characterize the swimming trajectories of hyperactivated sperm. Such motions have been referred to as “bobbing” [Gwatkin and Anderson, 1969], “high amplitude” [Yanagimachi, 1970b], “serpentine” [Yanagimachi, 1972], “whiplash” [Cooper et al., 1979], “figure-of-eight” [Fraser, 1977], and “darting” [Corselli and Talbot, 1986]. Clearly, terms such as these do not permit standardization or rigor in the identification of hyperactivated sperm. Definition and interpretation of hyperactivation should derive from analysis of the sperm flagellar beat, which is the biophysical cause of the motion. In the mathematical delineation of flagellar beat kinematics, it is convenient to distinguish between the pattern and vigor of motion. There is also biological motivation for this distinction, since the cellular and molecular mechanisms that regulate these two facets of motion may not be identical [Brokaw and Gibbons, 1975; Hoskins et al., 1979; Tash and Means, 1983]. When the flagellar beat is relatively symmetrical and continuous (as for most sperm in semen or cervical mucus), simple geometric parameters can be used to describe its sinusoidal character, for example, beat frequency and effective amplitude and wavelength. However, when the beat is less symmetrical and more intermittent and/or irregular (as for hyperactivation), these simple parameters become inadequate. Here it is necessary to focus on the curvature of the principal and reverse bends as they are developed and propagated along the flagellum. For example, the maximum bending in the midpiece and proximal principal piece has been quantitated in terms of a radius of curvature [Johnson et al., 1981]—a dimensionless “curvature ratio” that measures its departure from a straight line [Suarez et al., 1983, 1984]—and the angle subtended between the tangent lines to the flagellum through the head–midpiece junction and the first inflection point distal to that junction [David et al., 1981; Cooper, 1984]. We will elaborate below on the need for more detailed measures of flagellar beat kinematics.

Since the velocity and trajectory spanned by the sperm head are the hydrodynamic consequence of the flagellar beat, it is possible to use their measurement alone in drawing some inferences about that beat. This approach is potentially useful, since it is more directly amenable to computer-assisted sperm analysis (CASA); however, it does contain less kinematic and mechanical information. Analyses of sperm head velocity and the pattern of head motion have been used in the study of hyperactivation [see, e.g., Suarez et al., 1983, 1984; Burkman, 1984; Suarez and Osman, 1987; Olds-Clarke, 1986; Katz et al., 1986]. The local speed along the curvilinear path traced by the sperm head, that is, the “curvilinear velocity” (VCL) is commonly used for the former. For the latter, two basic strategies are applied. The straightness of the trajectory can be quantitated in terms of three dimensionless ratios incorporating the lengths (per unit time) of the curvilinear path (VCL), a spatially averaged path that eliminates the wobble of the sperm head while preserving the basic curvature of the path (VAP), and the straight line between the initial and final positions on the trajectory (VSL) [see Katz et al., 1978a; Suarez et al., 1983; Olds-Clarke, 1986].

These ratios are referred to as: The “linearity,” LIN = VSL/VCL (formerly
termed the “progressiveness ratio”); the “wobble,” $WOB = \frac{VAP}{VCL}$ (formerly termed the “curvilinear progressiveness ratio”); and the “straightness,” $STR = \frac{VAP}{VSL}$ (formerly termed the “linear index”). Thus, $LIN$ is intended to measure the straightness of the trajectory in some overall geometric sense; $WOB$ measures the wobble of the sperm head about the average path; and $STR$ measures the straightness of the average path. More recently the wobble or “yaw” of the sperm head has been measured in terms of an “amplitude of lateral head displacement,” $ALH$ [David et al., 1981; Aitken et al., 1982a,b,c 1986; Mortimer et al., 1986]. This is defined in analogy to the amplitude of a sine wave. That is, it is the maximum, or average perpendicular distance between the instantaneous position of the sperm head and the corresponding position of its spatially averaged path.

It should be emphasized that the values of all these measures of sperm head motion are sensitive to a number of operational features of the experimental technique, including 1) the accuracy in spatial measurement, which relates to the magnification and spatial resolution of the measurement system and to the size of the sperm head; 2) the time resolution of the system, namely, the framing rate in relation to the speed of the sperm head; 3) the mathematical algorithms used to calculate the average sperm path and the amplitude of lateral head displacement. These issues are discussed, for example, in a number of reports [Katz et al., 1985; Katz and Phillips, 1986; Katz and Davis, 1987; Suarez and Osman, 1987; Davis and Katz, 1988; Mortimer et al., 1983]. It is also possible to measure the instantaneous turning angle of the sperm head about its direction of translation as a parameter akin to $ALH$, which may be more geometrically robust [Katz and Davis, 1987]. That is, its values are independent of the algorithms for determining an average path, and therefore it may more consistently measure sperm head wobble for paths of greatly differing shape.

Most important, there remains a need to establish a biological definition of the hyperactivated state, as a precedent to its kinematic delineation. This could relate to the state of capacitation and/or time of collection from the female, as well as to the fluid environment about the sperm. Once a population or subpopulation of sperm have been biologically identified as hyperactivated, then a set of geometric parameters could be established to characterize the motion. Historically, however, the opposite or inverse approach has been taken; a subpopulation of sperm within a suspension has been subjectively classified as hyperactivated, only after which their movement has been analyzed.

The Origin and Prevalence of Hyperactivation

It is biologically reasonable to propose an operational definition of hyperactivation as related to the completion of capacitation. Sperm motions that may geometrically resemble hyperactivation have been reported for fresh epididymal sperm [Acott et al., 1983; Suarez, 1988] and for other clearly non-capacitated cells [Katz et al., 1986]. Despite this kinematic similarity in such biologically disimilar sperm populations, we do not yet understand whether the cellular mechanisms underlying these motions are identical. Therefore, we shall focus here on hyperactivation as an expression of the final phases if not the completion of capacitation. The success of mammalian in vitro fertilization systems has led to a continuing increase in the number of species for which hyperactivation has been identified. These systems permit precise relation of hyperactivation to the timing and success of fertilization. The original in vitro studies were in the golden hamster [Yanagimachi, 1969, 1970; Gwatkin and
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Anderson, 1969; these were soon followed by the guinea pig [Yanagimachi and Usui, 1974] and mouse [Fraser, 1977]. In the 1980s primates were added to the list, including the rhesus monkey [Boatman and Bavister, 1984; Hoskins and Wolf, personal communication] and the human [Mortimer et al., 1983; Burkman, 1984].

In all of these species, a subpopulation of hyperactivated sperm has been visually identified prior to and/or at the time of in vitro fertilization. Often, the proportion of such cells increases during the capacitation process. It should be appreciated that the details of the kinematics and onset of hyperactivation may vary not only among these species but may also depend upon the biochemical and biophysical conditions of sperm culture and observation. For example, as noted above, the wall effect causes hyperactivated rabbit spermatozoa to beat with greater midpiece curvature than when they are in a deep preparation [Suarez et al., 1983]. In the hamster, the onset and maintenance of hyperactivation in vitro is promoted by a "motility-stimulating factor" [Bavister and Yanagimachi, 1977], later shown to be taurine [Mrsny et al., 1979; see also Meizel et al., 1980].

In addition to the many in vitro studies, a large body of work exists on the development of hyperactivation within the female in vivo. There have been a few direct observations of hyperactivated rodent spermatozoa within the excised, transmurally illuminated female tract. Katz and Yanagimachi [1980] analyzed golden hamster spermatozoa in the oviductal ampulla at the expected time of fertilization and also 2 hours later. Virtually all the sperm identified were classified as motile and hyperactivated. Suarez and Osman [1987] analyzed mouse spermatozoa in the uterus, UTJ, and oviduct at different times post mating [see also Suarez, 1987]. They found evidence of hyperactivation in sperm within the cranial uterus by 1–2 hours post-coitus (pc), as well as in the isthmus and ampulla of the oviduct. Lower isthmic sperm were initially subdued (see below) but began to display hyperactivation at 1 hour prior to fertilization. Some ampullar sperm appeared to be hyperactivated at the latter time.

Hyperactivated motility has also been reported for sperm collected by flushing from the female tracts of the hamster [Cummins and Yanagimachi, 1982], mouse [Phillips, 1972; Olds-Clarke, 1986; Suarez, 1987; Suarez and Osman, 1987], rabbit [Cooper et al., 1979; Suarez et al., 1983], sheep [Cummins, 1982], and rhesus and cynomolgus monkeys [Behboodi et al., 1987]. Rabbit sperm in whole ampullar fluid exhibit hyperactivation 6 hours pc [Suarez et al., 1983], and there was some evidence, in these same experiments, of hyperactivation of a small subpopulation of uterine sperm at the same time. Analogously, a small proportion of rhesus and cynomolgus monkey sperm, aspirated directly from the uterus 6 hours after mating, appear to be hyperactivated [Behboodi et al., 1987].

These studies of hyperactivation in vivo are instructive in establishing when and where such movement may occur; such information is essential in establishing its biological function (see below). However, most of the previous studies did not address the kinetics of the onset of hyperactivation, the details of which are essential if sperm movement is to be monitored as a marker for capacitation. Some information on the evolution of hyperactivated sperm movement is available for rabbits and golden hamsters. In the rabbit, a transition to hyperactivation has been observed in sperm preparations collected from the lower oviductal isthmus [Cooper et al., 1979; Overstreet and others, 1980c; Johnson et al., 1981; Burkman, 1984]. As noted above, the lower isthmus is thought to be a sperm reservoir for this species, in which immobilization of spermatozoa may contribute to their retention. Thus, the onset of hyperactivation in the rabbit
in vivo has been viewed as a reinvigoration process. In some culture media and in ampullar fluid, lower isthmic rabbit sperm initially exhibit low-amplitude flagellar beats, resulting in extremely slow forward progression if the sperm are free to swim. This flagellar movement becomes punctuated by episodes of high-curvature whiplash motions (the classic form of hyperactivation). The biphasic motion continues, but the subdued phase is replaced by relatively symmetrical, moderate-amplitude, high-frequency beats that propel the sperm rapidly forward. This transition to vigorous motion involves a 20-fold increase in sperm hydrodynamic power output [Burkman, 1984]. With other flushing media (including isotonic sucrose), hyperactivation is induced immediately upon dilution of the isthmic contents, without any obvious transitional state [Burkman, 1984]. The significance of this variable response to in vitro conditions is not understood. Rabbit spermatozoa, collected from the ampulla of the oviduct during the time of fertilization, continue to display biphasic motion, although the durations of each phase appear to vary within and among sperm [Cooper et al., 1979; Suarez et al., 1983]. There is little knowledge of rabbit sperm hyperactivation during capacitation in vitro, although recent work suggests that it does occur [R. Young, personal communication].

Sperm hyperactivation has been studied the most thoroughly in the golden hamster. The great majority of observations have been of epididymal sperm cultured in vitro [e.g., Yanagimachi, 1970; Katz et al., 1978b; Suarez et al., 1984; Ishijima et al., 1986; Corselli and Talbot, 1986, 1987; Cherr et al., 1986; Cummins and Yanagimachi, 1986; Katz et al., 1986; Suarez, 1987]. Under these conditions, hyperactivation does not immediately follow a subdued state of motion [as in the rabbit oviduct in vivo, Cooper et al., 1979; Overstreet et al., 1980; Johnson et al., 1981] but, rather, emerges in sperm that have already been swimming vigorously. Consequently, the onset of golden hamster sperm hyperactivation in vitro is not a reinvigoration process and is not accompanied by an increase in sperm power expenditure [Katz et al., 1978b].

In general, the flagella of hyperactivated spermatozoa generate beats of decreased symmetry, involving bends of increased curvature in the midpiece and proximal principal piece. Many studies have indicated that the movements of at least some hyperactivated sperm are intermittent (as in the rabbit), with phases of relatively lesser or greater progressiveness. The less progressive phase is the classic high-curvature motion and is most commonly described subjectively as whiplash and as figure-of-eight motion (the latter referring to the pattern traced by the sperm head, a different use of this metaphor than for sperm in mucus). The more progressive movements are sometimes called darting motion [Corselli and Talbot, 1986] because of the jerky but, on the average, progressive swimming trajectories observed. Recently, Suarez [1987] has analyzed the onset of golden hamster sperm hyperactivation in vitro, emphasizing a discrete intermediate state immediately preceding final whiplash motion. This state, termed helical motion, consists of a more three-dimensional but relatively progressive swimming trajectory through which the sperm head traces a helix. Bending in the proximal flagellum is greater than in the non-hyperactivated state but less than in whiplash motion.

**The Kinematics and Functional Significance of Hyperactivation**

The original report of hyperactivation for golden hamster sperm described the movement as “high amplitude” and contained drawings of trajectories that were rela-
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tively progressive [Yanagimachi, 1970]. Later studies referred to less progressive, more highly curved whiplash or figure-of-eight motions and began to measure flagellar beat parameters [Katz et al., 1978b; Suarez et al., 1984]. Still later, the maximal flagellar bending was related to the orientation of the hook of the sperm head [Ishijima et al., 1986; Katz et al., 1986; Drobnis et al., 1988b,c,d]. We have begun to appreciate that the population of hyperactivated sperm exhibits a diversity of movement patterns, both within and among cells. In our current studies of hamster sperm hyperactivation, we have cultured uterine sperm from mated females and also epididymal sperm [Cherr et al., 1986; Katz et al., 1986; Drobnis et al., 1988b,c]. We have focused on the continuous sequence of principal and reverse bends for individual spermatozoa, in relation to the hook of the sperm head, over a number of flagellar beat cycles. The hooked head in these rodent species provides an orientation reference for relating external flagellar curvature to specific axonemal elements [Wooley, 1977; Mohri and Yano, 1980; Ishijima et al., 1986]. For sperm observed in culture medium, we have identified two distinct types of high-curvature flagellar bending (Fig. 1), both of which result in asymmetric motions.

1. Extreme principal bends are restricted to the proximal midpiece, bringing it close to the convex edge of the head. Such bends are not propagated, and the head moves in a rapid, hatchet-like fashion.

![Fig. 1. Definition of principal (p) and reverse (r) bends for a hamster spermatozoon. A,B: Low curvature bends, which produce a sinusoidal-like motion. C,D: Two distinct types of higher curvature bending, exhibited by many sperm during hyperactivated motion (see text). C shows an extreme principal bend in the proximal midpiece, producing a "hatchet-like" motion. D shows an extreme reverse bend, which can produce a "figure-of-eight"-shaped trajectory.](image-url)
2. Extreme reverse bends occur more distally in the midpiece, bringing the tip of the hook of the sperm head close to the flagellum. These bends are propagated along the flagellum, followed by principal bends of lesser curvature. The resulting trajectory of the head may be helical [Suarez, 1988]. If the bends are quite extreme, and not followed by principal bends of significant curvature, the trajectory resembles a figure-of-eight.

The sequence of motions for individual sperm can be quite complex and includes lower curvature bends that result in more progressive motion.

The preceding kinematic descriptions are useful in the interpretation of recent studies on sperm motility during penetration of the cumulus and zona pellucida [Drobnis et al., 1988b,c,d]. Our work suggests that classical hyperactivation may not be an absolute requirement for cumulus penetration [Drobnis et al., 1988c]. Vigorous epididymal hamster sperm, incubated in capacitation medium for an intermediate period short of that expected for the completion of capacitation, will penetrate the cumulus but do not undergo the acrosome reaction on the zona pellucida. This is consistent with earlier work [Cummins and Yanagimachi, 1986; Corselli and Talbot, 1987]. During initial penetration of the cumulus surface by hyperactivated sperm, they appear to burrow their way into the matrix by means of symmetrical, low-amplitude, high-frequency beats [Drobnis et al., 1988c]. They resume high-amplitude, asymmetric beats after penetration of the surface. In our experiments, hyperactivated spermatozoa observed in the cumulus displayed patterns of movement qualitatively similar but quantitatively different from those observed in the low-viscosity fluid surrounding the cumulus (Fig. 2). Sperm flagellar motion exhibited two basic patterns: 1) continuous propagation distally along the flagellum of sinusoidal waves, sometimes including extreme reverse bends and often resulting in forward progression; 2) hatchet-like motions of the sperm head produced by extreme principal bending of the midpiece and proximal flagellum, always without forward progression. The distinctions in hyperactivated movement within versus outside the cumulus appeared to be due in large part to the sperm's response to the viscoelastic properties of the matrix material.

The movement of hyperactivated sperm on the zona pellucida is affected by the presence of the cumulus, the integrity of the acrosome, and the tenacity of adherence. Katz and Yanagimachi [1981] described the flagellar movements of hyperactivated hamster sperm on the cumulus-free zona as being of high amplitude and as more symmetrical than those of free-swimming hyperactivated sperm. Cherr et al. [1986], Katz et al. [1986], and Drobnis et al. [1988d], using Nomarski interference contrast optics, were able to study sperm attached to cumulus-invested zonae. Initial attachment, analogous to that on cumulus-free zonae [Yanagimachi, 1981; Katz and Yanagimachi, 1981; Cummins and Yanagimachi, 1982; Yanagimachi and Phillips, 1982; Suarez et al., 1984; Katz et al., 1986], was often but not always non-rigid, and was accompanied by fairly symmetrical, large-amplitude beats. There was a close temporal association between the completion of the acrosome reaction and the onset of tight binding of the sperm head; that is, its appearance of being rigidly anchored to the surface of the zona material. Tightly bound sperm displayed intermittent episodes of higher-frequency low-amplitude beating. This apparent change in flagellar motion could have been related to the occurrence of the acrosome reaction and/or tight binding of the sperm head to the zona.
In our recent studies we have analyzed the flagellar movements of hamster sperm throughout the process of zona penetration [Drobnis et al., 1988d]. During zona penetration the sperm display two intermittent types of motion (Fig. 3): 1) symmetrical, sinusoidal beats, having low amplitude and high frequency (mentioned above); 2) repeated asymmetric beats, resulting from extreme principal bends, which appear to be the counterpart of the hatchet motions in the cumulus. With the sperm head anchored on or in the zona, these latter motions resemble the beats of a cilium. During such strokes the tip of the sperm head is seen to rock back and forth in the zona, giving the impression of a cutting motion. Hydrodynamic analysis indicates that, during these high-curvature strokes, the net mechanical effect of the flagellum is a lever action as opposed to a propeller action, in forcing the head against the zona material. The peak forces generated by this lever action can reach a few thousand microdynes, two orders of magnitude greater than the propulsive thrust developed by more symmetrical, sinusoidal beats [Drobnis et al., 1988d].

Importantly, no extreme reverse bends have been seen in sperm penetrating the zona material. This underscores the importance of reporting both the direction of flagellar bend curvature and the magnitude of that curvature in characterizing hyperactivation. For example, free-swimming hyperactivated sperm are known to exhibit increased midpiece curvature following the acrosome reaction [Suarez et al., 1984]. However, the increased curvature seen in sperm during in vitro capacitation in low-
viscosity media is of reverse bends. We have never seen such flagellar bends in sperm on the zona pellucida, even before the completion of the acrosome reaction.

Since hyperactivation was first described by Yanagimachi (1969, 1970) and Gwatkin and Anderson (1969), a number of biological functions have been proposed for this striking motion [see Yanagimachi, 1970; Katz et al., 1978b; Suarez et al., 1983, 1984; Yanagimachi, 1981; Cummins, 1982]. These include:

1. Development of the forces necessary to detach the sperm head from epithelial adherence.
2. Increased stirring of the fluid in the local microenvironment about a sperm, thereby promoting exchange of metabolites and/or other stimulatory factors.
3. Facilitation of migration within the confined spaces of epithelial enfoldings, preventing entrapment.
4. Expansion of the area traversed within the ampullar lumen, thereby increasing the probability of contact with the cumulus and/or zona.

5. Generation of increased forces applied to the sperm head, promoting penetration of the cumulus and zona pellucida.

There is ongoing attention to all these proposed functions. However, much of the evidence in support of them is circumstantial. It is difficult at best and impossible at worst to separate changes in hyperactivated sperm motion from changes in other facets of cell function, that is, metabolic activity and enzyme release. Elucidation of the functional role(s) of hyperactivation will require careful integration of biophysical, biochemical, and ultrastructural perspectives.

There is also ongoing attention to the identification of hyperactivation as a biological marker related to capacitation. Such interest has now been extended to the human, where identification of hyperactivation in sperm subjected to the conditions of in vitro capacitation offers promise as a diagnostic aid in assessing sperm competence. If hyperactivation is to be objectively monitored as a biomarker for capacitation, then the relationship between its biological presence and kinematic expression must be established more thoroughly. Recent studies in rodents are an important step in this direction. Suarez and Osman [1987] compared changes in hamster sperm kinematics within the female reproductive tract with those during in vitro capacitation. Neill and Olds-Clarke [1987] defined mouse sperm hyper activation in relation to the composition of the incubation medium and to capacitation in vitro. As such work continues, it must be remembered that the kinematics of the onset and maintenance of hyperactivation may not be identical for all spermatozoa. Thus, methods of measurement and analysis that focus on subpopulations of sperm within a suspension will be necessary.

The methods of experimental measurement and analysis of the kinematics of hyperactivation also need to be optimized and standardized. Measures of flagellar beat are generally more laborious to obtain than those of head motion, but they may be necessary in some contexts, especially regarding the functional significance of hyperactivation. Simple identification of hyperactivation per se may be less demanding but will clearly benefit from multivariate approaches in which several measures of sperm motion are jointly assessed.

CONCLUSIONS

We have attempted to provide a comprehensive overview of the current state of knowledge of biophysical aspects of sperm migration and egg vestment penetration. We have described some of the factors that push sperm forward as well as those that resist sperm motion, and we have commented on techniques and requirements for incisive sperm motion analysis. These concepts are fundamental to our understanding of the biological mechanisms of sperm transport. However, we recognize that the perspective developed in this article emphasizes only one of a number of phenomena responsible for sperm transport to the oolemma. We have focused here largely on the autonomous migration of spermatozoa, that is, their active role in the sperm transport process.

The union of sperm and oocyte also depends on actions of the female reproductive tract, in which the spermatozoa may be passive participants. These are biophysical phenomena and include the muscular contractions of the female viscera and the
activity of the cilia lining many of the lumina within the tract. The role of these passive transport processes may not be limited to pumping actions, fluid movements, and maintenance of luminal patency. The active and passive transport mechanisms are interactive; muscular contractions move spermatozoa, and sperm cells may stimulate or inhibit contractility. Coordination and promotion of gamete union could be considered a primary biological strategy, and it appears to be a process with much redundancy, to guard against reproductive failure. The complexity of sperm transport and fertilization in vivo is appreciated but not understood. The technology is now available to significantly advance our understanding in this area. A comprehensive knowledge of the molecular biology of gamete union now seems attainable. Comparable understanding of the sperm transport mechanisms that precede and permit fertilization will be more difficult to obtain and should present an important challenge to reproductive biologists in the next decade.

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