BIOLOGY OF REPRODUCTION 44, 375–381 (1991)

# Evidence for the Function of Hyperactivated Motility in Sperm[1]

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## ABSTRACT

**After insemination, mammalian sperm undergo a striking change in flagellar beat pattern, termed hyperactivation. In low-viscosity culture medium, nonhyperactivated sperm flagella generate relatively symmetrical, low-amplitude waves, while hyperactivated sperm flagella generate an asymetrical beating pattern that results in nonprogressive movement. Since sperm encounter highly viscous and viscoelastic fluids in the female reproductive tract, the progress of hyperactivated sperm was compared with that of nonhyperactivated and transitional sperm in media of increasing viscosity. Hamster sperm obtained from the caudal epididymis were incubated in a medium that promotes capacitation. After 0, 3, and 4 h of incubation, the majority of the sperm exhibited, respectively, activated, transitional, and hyperactivated motility. At each of these time points, aliquots of sperm were removed from incubation and added to solutions of 0, 5%, 10%, 20%, and 30% Ficoll in medium. Samples containing mostly hyperactivated sperm (4 h) maintained higher swimming and flagellar velocities and were able to generate greater forces in response to increased viscous loading than activated sperm (0 h). Transitional sperm (3 h) showed an intermediate response. The paths of hyperactivated sperm through solutions of 20% and 30% Ficoll were considerably straighter than those made through medium alone. This is the first demonstration that hyperactivation can confer a mechanical advantage upon sperm in the oviduct where they may encounter viscous oviductal fluid and a viscoelastic cumulus matrix.**

## INTRODUCTION

After insemination, mammalian sperm must pass through the cervix (in some species), uterus, uterotubal junction, and oviductal isthmus to reach the oocyte in the oviductal ampulla. These female organs impose different physicochemical environments upon the sperm that can affect their flagellar activity and consequently their progress toward the oocyte. Chemical properties of luminal fluids, which comprise secretions and serum transudates, influence sperm cell physiology and the capacitation process [1, 2]. Physical properties of luminal fluids and epithelial surfaces may also affect fertilization by influencing sperm movement [3–5], but it is not yet known how these factors affect sperm transport. In the female tract, sperm undergo a striking alteration in flagellar motion, termed hyperactivation [5, 6]. In low-viscosity fluid in vitro, hyperactivated sperm exhibit asymmetrical flagellar beats of large amplitude and curvature, producing trajectories that are irregular and/or highly curved. In contrast, caudal epididymal or freshly ejaculated sperm tend to swim with lower amplitude, more symmetric flagellar beats along trajectories that are nearly straight [3, 5, 7]. Since hyperactivated sperm have been recovered predominantly from the oviduct [3, 8–10], it has been proposed that hyperactivation assists the sperm in reaching the oocyte or penetrating its vestments in vivo [3, 5]. Nevertheless, the means by which this vigorous, but nonprogressive movement assists the sperm in reaching the oocyte plasma membrane have not been fully elucidated.

In studying the role of hyperactivation in sperm transport and fertilization, it is important to account for the effects of physical properties of the sperm's environment. For example, when hyperactivated hamster sperm enter the cumulus extracellular matrix, a highly viscoelastic gel, there are striking changes in their movement characteristics [11]. Hyperactivation may begin in the oviductal isthmus [10], an environment whose secretions include a mucus-like material [12–15]. Thus, biologically relevant environments in which to study hyperactivation must include media of elevated viscosity and elasticity. Measurement of the characteristics of sperm motion in such environments is required to assess the role of flagellar activity in transport and fertilization [5]. Such characteristics can also be applied to computations of forces generated by sperm, which provide further insight into the mechanisms whereby flagellar motion enables sperm to reach the oolemma [5]. In the present study, such computations have been performed. We have investigated whether elevated viscosity alters the force-generating ability of hamster sperm. Such sperm were studied immediately after collection from the epididymis (the "activated" state), during transition to hyperactivated motion (the "transitional" state), and after assumption of hyperactivated motion (the "hyperactivated" state). Our hypothesis

Accepted October 3, 1990.
Received June 28, 1990.
[1]This research was supported in part by NIH grant HD-19584 (S.S.S.) and NSF grant 86-16134 (D.F.K.). College of Veterinary Medicine, Univ. of Florida Journal Series No. 262.
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was that the hyperactivated state enables sperm to generate increased forces in response to elevated viscous resistance, thereby confering a mechanical advantage on such sperm. We used Ficoll to elevate the viscosity of the medium, since it is a highly soluble, relatively inert polymer that rapidly forms homogeneous solutions [16]. Viscous properties of these solutions were directly measured over a range of shear rates relevant to sperm motion.

## MATERIALS AND METHODS

### *Materials*

Retired breeder male golden hamsters (*Mesocricetus auratus*) were purchased from Simonsen Laboratories, Inc. (Gilroy, CA) and maintained under a 14L:10D diurnal cycle. All inorganic chemicals were purchased from Mallinckrodt, Inc. (Paris, KY) and organics from Sigma Chemical Co. (St. Louis, MO), except for HEPES buffer and Fraction V BSA, which were from Calbiochem, Behring Diagnostics (La Jolla, CA).

A hamster sperm capacitation medium modified from Mrsny and Meizel [17], which included 25 mM HEPES buffer, 12 mg/ml BSA, and 0.5 mM hypotaurine [18], was used for all experiments. The pH was adjusted to 7.6 with NaOH, then the medium was filtered through a 0.22-μm Millex-GV sterile filter (Millipore, Bedford MA) and equilibrated with 5% $CO_2$ in air. At equilibration, the pH was approximately 7.45. Ficoll solutions were made from 400 kDa Ficoll (Sigma, F-9378) dissolved directly in medium. Methyl cellulose (Sigma, M-0512) was also dissolved directly in medium.

Slide chambers used for videotaping sperm were prepared as follows. A solution of 0.4% agar (G. Cornwall, pers. comm.) was prepared in filtered water (Milli-Q system, Millipore) by heating to nearly boiling. The temperature was lowered to 60°C and then glass slides were briefly submerged, the solution was wiped off of one side, and the slide was dried in a vertical position. Number 1 glass coverslips (22 × 30 mm) were also submerged and then dried vertically. Glass beads measuring 100 μm in diameter were mixed into silicon grease (E.Z. Drobnis, pers. comm.) and four posts of the mixture were applied to the slides in the area where the four corners of the coverslip would make contact. Forty microliters of sperm suspension was added onto the center of the slide, then a coated coverslip was pressed down onto the four posts until stopped by the glass beads, creating a chamber approximately 100 μm deep.

### *Sperm Preparation and Analysis*

Sperm were removed from the caudal epididymides by puncturing the ductus epididymis, drawing out the contents with fine tweezers, then immediately immersing them in 37°C medium. Sperm numbers were adjusted to 2 × $10^6$/ml and the sperm were incubated in 1 ml of medium in 1.5-ml Eppendorf tubes open to 5% $CO_2$ in air. After 0, 3, and 4 h of incubation, 50-μl aliquots of sperm were removed and mixed with Ficoll dissolved in medium to achieve final Ficoll concentrations of 0, 5%, 10%, 20%, and 30%. The sperm suspensions in Ficoll were transferred to prewarmed slide chambers that were then placed onto a microscope stage warmed to 37°C by an Arenberg Sage Air Curtain (Jamaica Plain, NY). Videotaping was performed immediately after mixing sperm with each concentration of Ficoll, such that each treatment spent an equal amount of time in the Ficoll.

In addition to videotaping for assessment of motility, assessment of capacitation of sperm was made by directly determining the percentage of motile, acrosome-reacted sperm after 5 h incubation. Samples of sperm were placed on uncoated slides and the acrosomal status was determined by examining the heads of 100 motile sperm via phase-contrast optics at 400× magnification.

The videotaping was performed with a Tritronics shuttered videocamera (Burbank, CA) set to 1/500-sec exposures and recorded on a 3/4-inch JVC U-Matic video cassette recorder (model CR06600U, Victor Co. of Japan) at the rate of 60 fields/sec. The passage of time in 1/100 sec was simultaneously recorded with the image of the sperm, using a ForA video time/date generator (model VTG 33, Los Angeles, CA). A 25× Zeiss phase-contrast objective was used. At least ten microscope fields were recorded for each sample.

For motility evaluation, measurements were taken of the first sperm to enter each of ten microscope fields for each sample. Measurements were taken directly from individual video fields on a Panasonic CCTV monitor by using a Grafbar GP7 Sonic Digitizer (Science Accessories Corp., Stratford, CT) coupled to a MacIntosh 512K computer (Apple Computer, Inc., Cupertino, CA). The output of the digitizer was converted to point-to-point measurements by a program written in Microsoft Basic (Microsoft Corp., Redmond, WA) by W. Gottlieb. The measurements consisted of the amplitude and half wavelength of the greatest principle flagellar bend (Fig. 1), the flagellar beat frequency, and the progressiveness. The progressiveness was measured as straight line velocity divided by average path velocity (VSL/VAP) [5], each velocity measurement being made over a period of 1 sec. The values obtained were not normally distributed, so the nonparametric Randomization Test was used to compare treatment effects [19] and medians are reported rather than means. Differences with a probability of chance occurrence below 0.05 were considered to be significant.

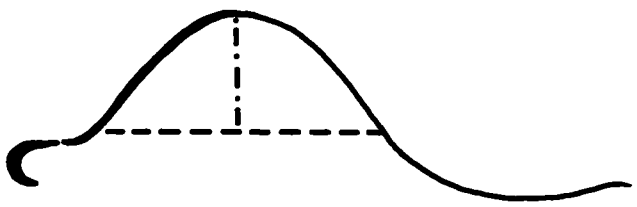

FIG. 1. Illustration of measurement of flagellar bend amplitude (–•–) and half wavelength (– – –).

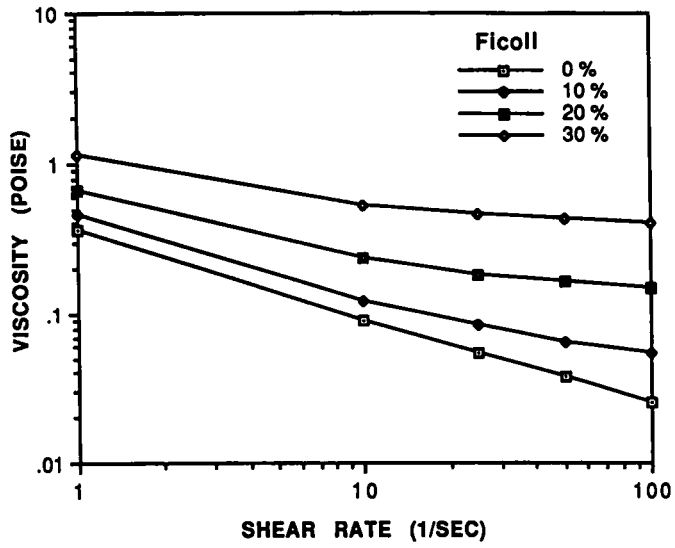


FIG. 2. Shear-rate dependence of viscosity of solutions of hamster sperm capacitation medium with different concentrations of Ficoll. Measurements were made on a Weissenberg Rheogoneometer.

The viscoelastic properties of the Ficoll solutions were measured using a Weissenberg Rheogoneometer [20]. The procedure involved applying a range of steady shear rates to each solution. This experimental range was designed to span the estimated range of shear forces encountered by sperm in vivo [11].

## RESULTS

The hamster sperm culture medium was determined to display shear-thinning behavior, in which viscosity decreases with increased applied shear rate (Fig. 2 and APPENDIX). The degree of shear thinning decreased with increasing Ficoll concentration, and was found to be due to the presence of serum albumin in the medium rather than Ficoll.

Incubation of sperm in culture medium lacking Ficoll produced the changes in movement pattern associated with hyperactivation and capacitation of hamster sperm in vitro [18, 21]. Three incubation time points were chosen for examination because the majority of sperm exhibited distinct

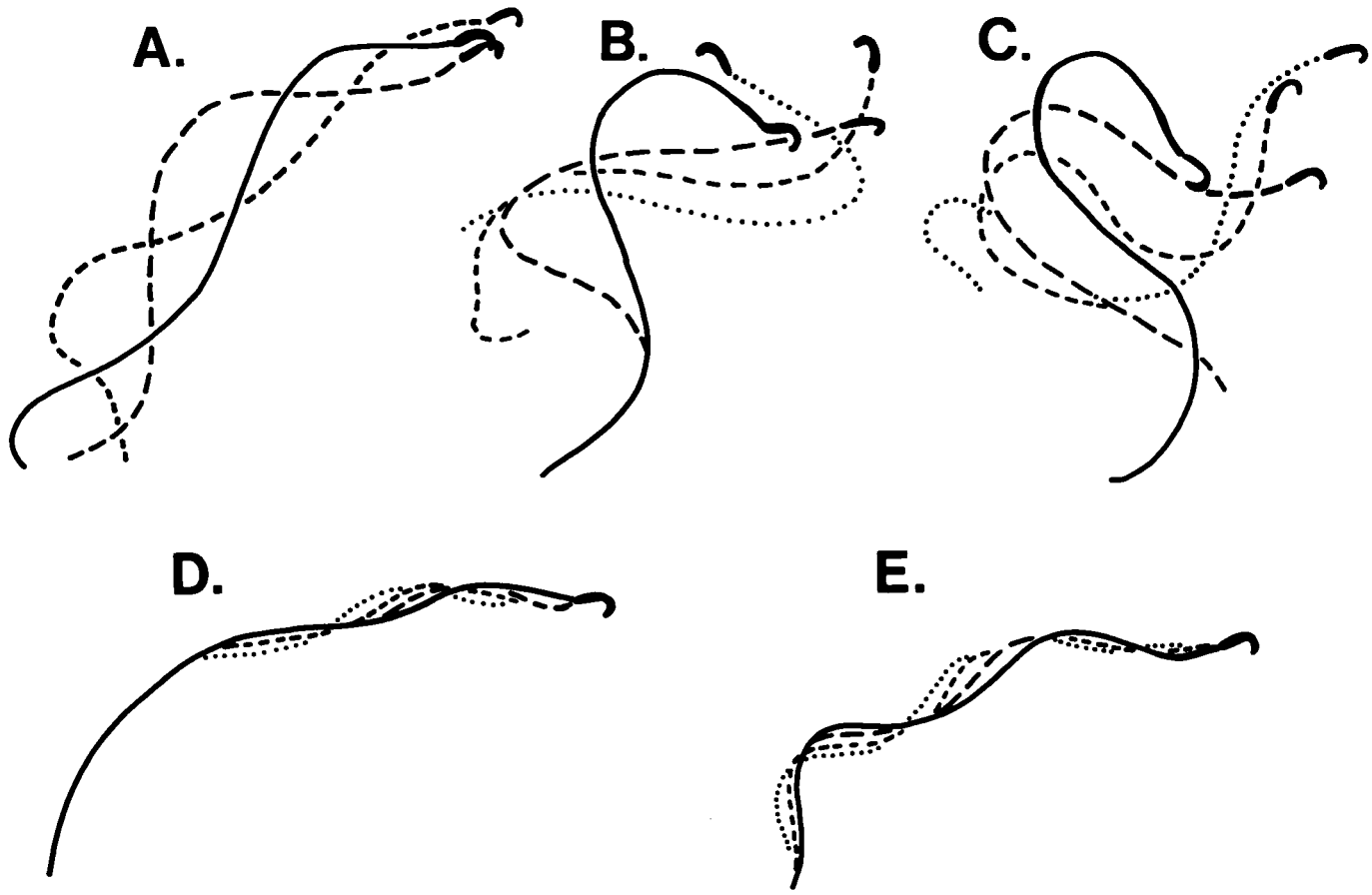


FIG. 3. Movement patterns of hamster sperm during capacitation in vitro, traced from individual frames of videotape. The tracing of the first video frame is a solid line and subsequent frames are indicated by line fragments of decreasing length. (A) *Activated* sperm at 0 h in medium alone. Flagellar beating was rapid, planar, and of moderate amplitude. (B) *Transitional* sperm at 3 h exhibited three-dimensional beats of increased asymmetry and amplitude that produced helical trajectories. (C) *Hyperactivated* sperm at 4 h of incubation displayed asymmetrical, two-dimensional, high-amplitude flagellar beating. (D) Sperm in 35% Ficoll after 0 h of preincubation. (E) Typical movement pattern of sperm in 20–30% Ficoll after 0, 3, and 4 h of preincubation in medium.

swimming patterns at each point: 0 h for *activated* [6] motility, distinguished by moderate amplitude, quasisymmetrical flagellar beats (Fig. 3A); 3 h for *transitional* motility, distinguished by larger amplitude, more three-dimensional beats, and helical trajectories (Fig. 3B) [18]; and 4 h, for *hyperactivated* motility, distinguished by high curvature, asymmetric beats (Fig. 3C) [18]. After 5 h of incubation, the mean percentage of motile sperm lacking acrosomes was 38.0 (range 31 to 52; n = 5), indicating that capacitation was occurring under these conditions [18].

Sperm movement displayed viscosity/Ficoll concentration dependence at each of the three incubation time points. The effect was visually striking at the highest Ficoll concentration; here sperm flagella at all time points exhibited a pattern of two-dimensional, low-amplitude and low-wavelength beats resembling ripples of a surface wave on water (Fig. 3E). This pattern occurred even after 3 h of incubation, when most control sperm were exhibiting conspicuous three-dimensional beats and trajectories. The dampening effect of the Ficoll could be reversed by diluting with medium, indicating that the effect was mechanical rather than physiological. At the highest viscosities (20% and 30% Ficoll), there were no significant differences across time points for flagellar amplitude or wavelength (Tables 1A and 1B). The common swimming patterns at increased viscosity signified an increased directionality of swimming for transitional and hyperactivated sperm (Table 1C). In medium with >10% Ficoll, the trajectories of transitional and hyperactivated sperm were as straight as those for activated sperm. Moreover, the percentages of free-swimming, space-gaining motile cells were higher for the 3-h and 4-h incubation times. Thus, in 20–30% Ficoll there were higher percentages of forward-moving sperm during the onset and development of hyperactivation than at the time of original sperm preparation (Table 1D).

In contrast to similarities in the patterns of sperm motion at elevated viscosity, there were incubation-time-dependent differences in the ability of sperm to penetrate the Ficoll solutions. Although swimming velocities were suppressed by increasing viscosity, sperm incubated for 4 h swam faster than those at 3 h, which swam faster than those at 0 h (Table 1E). The product of flagellar amplitude (μm) and beat frequency (Hz, which estimates maximum flagellar velocity) was significantly elevated for the 4-h sperm suspensions (median = 55, range 31–71, for 30% Ficoll; median 32, range 18–37 for 0% Ficoll).

Since the maximum flagellar velocity was only detected to be significantly greater among 4-h sperm than among 0-h sperm in 30% Ficoll, 25% and 35% Ficoll were tested (the latter being the maximum concentration that could be achieved repeatedly). In this case, for each sample, 20 sperm were measured in 20 different microscope fields, and sperm were only tested at 0 h and when a peak of hyperactivation was observed, which was shortly after 3 h of incubation. In this experiment, 77% of the motile sperm were activated at the first time point and 72% of the motile sperm were hyperactivated at the second time point. As seen on Table 2, the flagellar velocity remained significantly higher among hyperactivated sperm samples at both concentrations of Ficoll, attributable to the maintenance of higher bend amplitudes. In 35% Ficoll, the flagellar bend amplitude of 0 h sperm could frequently be seen to diminish as the wave moved down the flagellum (Fig. 3D). Also, only 7% of the

TABLE 1. Movement parameters of sperm suspended in viscous Ficoll solutions.*

| % Ficoll | 0 h | 3 h | 4 h |
|---|---|---|---|
| | A. Flagellar beat amplitude (μm)[a] | | |
| 0 | 23 (19– 26) | <34 (22– 52) | <37 (26– 49) |
| 5 | 18 (16– 29) | <33 (19– 39) | 30 (27– 38) |
| 10 | 17 (14– 20) | 20 (14– 22) | <23 (16– 33) |
| 20 | 10 (6– 15) | 11 (9– 12) | 12 (9– 13) |
| 30 | 5 (4– 10) | 6 (6– 9) | 9 (4– 11) |
| | B. Half-wavelengths of flagellar beats (μm)[b] | | |
| 0 | 81 (69– 93) | 72 (56– 96) | 68 (56– 89) |
| 5 | 72 (63– 97) | 79 (56– 86) | 68 (52– 85) |
| 10 | 74 (70– 77) | 74 (56– 84) | 70 (56– 83) |
| 20 | 61 (50– 72) | 61 (55– 64) | 61 (51– 67) |
| 30 | 51 (47– 57) | 47 (42– 56) | 53 (45– 58) |
| | C. Progressiveness (×100)[c] | | |
| 0 | 80 (76– 94) | >49 (40– 60) | >34 (30– 44) |
| 5 | 91 (84– 93) | >62 (52– 87) | 63 (57– 74) |
| 10 | 95 (93– 95) | >86 (76– 89) | 80 (75– 88) |
| 20 | 96 (86– 97) | 90 (84– 93) | 91 (86– 94) |
| 30 | 75 (38– 84) | 85 (68– 95) | 83 (68– 86) |
| | D. Percentage of free-swimming sperm[d] | | |
| 0 | 70 (65– 80) | 80 (51– 86) | 80 (60– 94) |
| 5 | 60 (39– 71) | <79 (59– 91) | 81 (53– 91) |
| 10 | 66 (62– 89) | 83 (53– 86) | 82 (57– 88) |
| 20 | 54 (41– 70) | <81 (46– 88) | 76 (30– 85) |
| 30 | 14 (0– 31) | <34 (31– 56) | 43 (34– 69) |
| | E. Average path velocities (μm/sec)[e] | | |
| 0 | 161 (85–185) | <278 (152–281) | <287 (211–358) |
| 5 | 117 (55–155) | <201 (107–244) | 185 (147–214) |
| 10 | 72 (43– 82) | <80 (59– 90) | <110 (77–144) |
| 20 | 31 (17– 46) | <33 (31– 43) | <47 (33– 50) |
| 30 | 9 (4– 21) | <15 (9– 23) | <27 (15– 30) |

*Median values from 5 replicates (50 sperm) are given; ranges are in parentheses. Significant differences ($p < 0.05$) between entries in the columns are indicated by "<" symbols. For more detailed description of measurements, see Katz et al. [5].

[a]Flagellar beat amplitudes, taken from the principle flagellar bend when it reached the point of maximal development.

[b]Half wavelengths of the principle flagellar bend at the point of maximal development.

[c]Progressiveness (STR), measured as straight line velocity (net distance divided by time) divided by average path velocity (distance of path tracing averaged to remove lateral movements of the sperm head, divided by time in sec).

[d]Percentage of free-swimming sperm, i.e., sperm undergoing space-gaining movement. For this measurement, all sperm present in the initial video frame of each microscope field were categorized.

[e]Average path velocities of sperm (VAP)

TABLE 2. Movement parameters of sperm suspended in 25% and 35% Ficoll solutions.*

| | Activated | | Hyperactivated | |
|---|---|---|---|---|
| A. Beat frequency (Hz) | | | | |
| Control | 11.1 | (9.7– 13.7) | 6.8 | (5.3– 10.3) |
| 25% Ficoll | 5.2 | (3.4– 4.2) | 4.9 | (3.9– 7.0) |
| 35% Ficoll | 4.5 | (3.3– 7.0) | 3.7 | (2.5– 4.9) |
| B. Bend amplitude (μm) | | | | |
| Control | 20.1 | (12.1– 24.8) | 39.7 | (26.8– 49.0) |
| 25% Ficoll | 9.3 | (4.3– 15.0) | 14.9 | (6.7– 9.1) |
| 35% Ficoll | 7.0 | (2.4– 11.8) | 11.3 | (5.6– 20.0) |
| C. A × B* | | | | |
| Control | 213.2 | (151.0–297.6) | 279.4 | (207.6–387.9) |
| 25% Ficoll | 55.1 | (14.8– 90.5) | 70.9 | (37.3–121.0) |
| 35% Ficoll | 29.8 | (10.8– 73.2) | 42.4 | (19.9– 74.0) |

*Each Entry represents the median (range) of 20 sperm.
*In section C, the beat frequencies have been multiplied by the bend amplitude to provide an estimate of maximum flagellar velocity; in 0, 25%, and 35% Ficoll, the samples taken from hyperactivated sperm populations achieved higher values than those from activated populations ($p < 0.05$).

motile sperm from the activated sample were progressive (i.e. space-gaining), but 97% of the motile sperm from the hyperactivated sample were progressive.

Hydrodynamic computations of flagellar thrust were performed for the first experiment, taking into account the shear-thinning viscosity (see APPENDIX). Beat-averaged values of viscosity were used for the flagellum, and values for the sperm head were based upon shear rates for the average path velocity. Average values of flagellar thrust for 4 h, 3 h, and 0 h, respectively, were: 155 μdyne, 114 μdyne, and 77 μdyne at 30% Ficoll; and 82 μdyne, 72 μdyne, and 67 μdyne at 20% Ficoll. Thus, the transitional and hyperactivated sperm actually developed greater thrust at increased viscous loading.

## DISCUSSION

These results probably underestimate the superiority of hyperactivated sperm for penetrating viscous media, because sperm to be measured were selected randomly. Activated sperm could not be excluded from the 3- and 4-h measurements, nor could transitional sperm be excluded from the 0- and 4-h measurements, because the swimming patterns of these sperm in 20–30% Ficoll could not be distinguished from each other.

Rikmenspoel [22] observed that activated free-swimming bovine sperm, exposed to increased viscosity (by addition of methyl cellulose to medium) responded with flagellar beats of decreased amplitude and frequency. Human sperm, held by their heads on a suction pipette, behaved analogously, and showed more two-dimensional flagellar motion at increased viscosity [4]. However, in these two species, the flagellar bending becomes confined to the distal portion at elevated viscosity. In contrast, bending is maintained throughout the principal piece of hamster sperm. The reasons for this species distinction are not known. It is noteworthy that the hamster sperm flagellum is about three times the length of human and bull sperm [23], the outer dense fibers surrounding its axoneme are larger [24–26], and it possesses greater tensile strength [27]. Thus, mechanical parameters that influence the flagellar bending mechanism are different for the hamster and this distinction may have contributed to the qualitatively different response to elevated viscosity in this species.

Mammalian sperm evidently remain in the activated state characteristic of ejaculated sperm until they traverse the uterotubal junction [9, 28]. They become hyperactivated at some point in the oviduct [3, 8–10]. In some species, a viscous mucus-like secretion has been observed in the lumen of the caudal isthmus [13, 15]. In addition, sperm are usually confronted by a viscoelastic cumulus extracellular matrix in the ampulla [11]. Activated hamster sperm reportedly do not penetrate the cumulus efficiently in vitro [11, 29]. The present results indicate that such activated sperm may not generate sufficient flagellar forces for cumulus penetration or to overcome possible adherence to granulosa cells contained in the cumulus matrix.

The movement of sperm in viscous oviductal fluid has not been observed in controlled experiments. There is circumstantial evidence, however, that flagellar beating may be depressed by oviductal fluid. The flagellar beating of rabbit sperm removed from the oviductal isthmus was dampened unless the isthmic fluid was diluted with medium [30]. The flagellar curvature of mouse sperm, measured in situ, was determined to be minimal in the caudal isthmus during the preovulatory period [10]. Elevated viscosity may have contributed to such diminished motion.

We have not yet performed the nonlinear analysis of sperm power output in shear-thinning fluids. Nevertheless, the increasing values of (amplitude) × (frequency) for hyperactivated sperm at high viscous loading, when compared with those of activated sperm, suggest that these cells may be responding with increased energy expenditure as well as force generation.

The movements of hamster sperm penetrating the hamster cumulus in vitro were analyzed by Drobnis et al. [11]. Forward progression was associated with sinusoidal-like beats similar to those we observed in Ficoll, although the hyperactivated sperm were also reported to produce more asymmetric "hatchet beats" that did not propel them forward through the cumulus matrix. The effective viscosity of the hamster cumulus matrix was estimated to be on the order of 1 poise [11], which is comparable to the values for the 30% Ficoll medium in the present study. Thus our analysis provides at least a first approximation of the biomechanics of sperm/cumulus interaction. The flagellar beat frequencies and amplitudes of hyperactivated sperm in 30% Ficoll were comparable to those reported as occurring in the cumulus [11], although in the cumulus the beat amplitudes tended to dampen as the waves progressed distally. Wavelengths were approximately 30% greater in 30% Ficoll

than in the cumulus. The differences in swimming patterns observed in Ficoll and cumulus matrix may have been due to differences in the macromolecular structures of the two materials. The principal macromolecule of the cumulus matrix is hyaluronic acid, a linear molecule averaging 4 000 kDa in mass, while the Ficoll used in this study is a globular molecule averaging 400 kDa. The long, unbranched chains of hyaluronic acid are likely to produce greater elasticity than Ficoll, but might also result in lower local microviscosity [16]. We conducted additional experiments substituting 1.5% methylcellulose (a long-chain polymer) and observed similar effects as those for 30% Ficoll reported here (data not shown); therefore, we would predict similar results in vivo for sperm behavior in viscous material comprised of long-chain molecules.

It is noteworthy that the presence of serum albumin in the media created a rheological "shear-thinning" effect; here, the effective viscosity is reduced as the applied shear rate increases. In the context of a motile sperm, this means that the resistance per unit velocity of the suspending fluid actually diminishes as the local velocity of the body becomes higher. As seen in Figure 2, this phenomenon occurred at all Ficoll concentrations, and will need to be taken into account in all studies involving the mechanics of sperm motion in albumin-containing media. Shear-thinning, like the more general phenomenon of viscoelasticity, alters the efficiency at which a sperm swims, and is currently the subject of further study by us. Serum albumin is abundant in the luminal fluid of the mammalian oviduct [2], and so should be included in any artificial environment design to study sperm movement.

In summary, hamster sperm in all three motility states responded to increasing concentrations of Ficoll with decreased flagellar amplitude, wavelength, beat frequency, and swimming velocity. Hyperactivated sperm, however, maintained progressive motility to a greater extent than activated sperm. Hyperactivation actually caused the sperm flagellum to generate greater forces in response to increased viscous loading, while the activated sperm flagellum continued to generate approximately equal forces at all viscosities. This is the first demonstration of a direct mechanical advantage conferred upon sperm by hyperactivation, which could facilitate passage through the oviduct and ovum vestments to the oolemma.

## ACKNOWLEDGMENTS

The authors wish to thank Dr. Xiao-bing Dai for technical assistance and Dr. John Donald and Mr. Robert P. DeMott for editing the manuscript. Mr. Wayne Gottlieb wrote the Basic program used for converting signals from the sonic digitizer into useful data.

## REFERENCES

1. Hunter RHF, Fallopian tube fluid. In: Hunter RHF (ed.), The Fallopian Tubes. Their Role in Fertility and Infertility. New York: Springer-Verlag; 1988: 30–51.
2. Leese HJ. The formation and function of oviduct fluid. J Reprod Fertil 1988; 82:843–856.
3. Suarez SS, Katz DF, Overstreet JW, 1983. Movement characteristics and acrosomal status of rabbit spermatozoa recovered at the site and time of fertilization. Biol Reprod 1983; 29:1277–1287.
4. Ishijima S, Oshio S, Mohri H. Flagellar movement of human sperm. Gamete Res 1986; 13:185–197.
5. Katz DF, Overstreet JW, Drobnis EZ. Factors regulating mammalian sperm migration through the female reproductive tract and oocyte vestments. Gamete Res 1989; 22:443–469.
6. Yanagimachi R. Mechanisms of fertilization in mammals. In: Mastroianni L, Biggers JD (eds.), Fertilization and Embryo Development In Vitro. New York: Plenum Publishing Company; 1981: 81–182.
7. Yanagimachi R. The movement of golden hamster spermatozoa before and after capacitation. J Reprod Fertil 1970; 23:193–196.
8. Cummins JM. Hyperactivated motility patterns of ram spermatozoa recovered from oviducts of mated ewes. Gamete Res 1982; 6:53–64.
9. Olds-Clarke P. Motility characteristics of sperm from the uterus and oviducts of female mice after mating to congenic males differing in sperm transport and fertility. Biol Reprod 1986; 34:453–467.
10. Suarez SS, Osman RA. Initiation of hyperactivated flagellar bending in mouse sperm within the female reproductive tract. Biol Reprod 1987; 36:1191–1198.
11. Drobnis EZ, Yudin AI, Cherr GN, Katz DF. Kinematics of hamster sperm during penetration of the cumulus cell matrix. Gamete Res 1988; 21:367–383.
12. McDaniel JW, Scalzi H, Black DL. Influence of ovarian hormones on histology and histochemistry of the bovine oviduct. J Dairy Sci 1968; 51:754–761.
13. Jansen RPS. Fallopian tube isthmic mucus and ovum transport. Science 1978; 201:349–351.
14. Jansen RPS, Bajpai VK. Oviduct acid mucus glycoproteins in the estrous rabbit: ultrastructure and histochemistry. Biol Reprod 1982; 26:155–168.
15. Suarez SS, Drost M, Redfern K, Gottlieb W. Sperm motility in the oviduct. In: Bavister BD, Cummins JM, Roldan ERS (eds.), Fertilization in Mammals. Norwell: Serono Symposia; 1990: 111–124.
16. Berg HC, Turner L. Movement of microorganisms in viscous environments. Nature 1979; 278:349–351.
17. Mrsny RJ, Meizel S. Potassium ion influx and $Na^+$, $K^+$-ATPase activity are required for hamster sperm acrosome reaction. J Cell Biol 1981; 91:77–82.
18. Suarez SS. Hamster sperm motility transformation during the development of hyperactivation in vitro and during epididymal maturation. Gamete Res 1988; 19:51–65.
19. Overstreet JW, Cooper GW, Katz DF. Sperm transport in the reproductive tract of the female rabbit. II. The sustained phase of transport. Biol Reprod 1978; 19:115–132.
20. Walters K. Rheometry. London: Chapman & Hall; 1975.
21. Katz DF, Cherr GN, Lambert H. The evolution of hamster sperm motility during capacitation and interaction with the ovum vestments in vitro. Gamete Res 1986; 14:333–346.
22. Rikmenspoel R. Movements and active moments of bull sperm flagella as a function of temperature and viscosity. J Exp Biol 1984; 108:205–230.
23. Cummins JM, Woodall PF. On mammalian sperm dimensions. J Reprod Fertil 1985; 75:153–175.
24. Pedersen H. Ultrastructure of the ejaculated human sperm. In: Bacetti B (ed.), Comparative Spermatology. New York: Academic Press; 1970: 133–142.
25. Flaherty SP, Winfrey VP, Olson GE. Localization of actin in human, bull, rabbit, and hamster sperm by immunoelectron microscopy. Anat Rec 1988; 221:599–610.
26. Brito M, Figueroa J, Vera JC, Cortes P, Hott R, Burzio LO. Phosphoproteins are structural components of bull sperm outer dense fiber. Gamete Res 1986; 15:327–336.
27. Baltz JM, Williams PO, Cone RA. Dense fibers protect mammalian sperm against damage. Biol Reprod 1990; 43:485–491.
28. Gaddum-Rosse P. Some observations on sperm transport through the uterotubal junction. Am J Anat 1981; 160:333–341.
29. Cummins JM, Yanagimachi R. Development of the ability to penetrate the cumulus oophorus by hamster spermatozoa capacitated in vitro, in relation to the timing of the acrosome reaction. Gamete Res 1986; 15:187–212.
30. Overstreet JW, Katz DF, Johnson LL. Motility of rabbit spermatozoa in the secretions of the oviduct. Biol Reprod 1980; 22:1083–1088.
31. Hancock GJ. The self-propulsion of microscopic organisms through liquids. Proc R Soc Lond 1953; 217:96–121.
32. Lighthill J. Mathematical biofluiddynamics. Philadelphia: Society of Industrial and Applied Mathematics; 1975.
33. Higdon JJL. A hydrodynamic analysis of flagellar propulsion. J Fluid Mech 1979; 90:685–711.
34. Gray J, Hancock GJ. The propulsion of sea urchin spermatozoa. J Exp Biol 1955; 32:802–814.

35. Dresdner RD, Katz DF, Berger SA. The propulsion by large amplitude waves of uniflagellar microorganisms of finite length. J Fluid Mech 1980; 97:591–621.
36. Johnson RE, Brokaw CJ. Flagellar hydrodynamics: a comparison between resistive force theory and slender body theory. Biophys J 1979; 25:113–127.
37. Katz DF, Yanagimachi R, Dresdner RD. Movement characteristics and power output of guinea-pig and hamster spermatozoa in relation to activation. J Reprod Fertil 1978; 52:167–172.
38. Lighthill J. Flagellar hydrodynamics. SIAM Rev 1976; 18:161–230.

## APPENDIX

In hydrodynamic analysis of sperm motion, there is an instantaneous balance between the active thrust and torque developed by the undulations of the flagellum, and the passive drag and resistive moment of the overall sperm body (flagellum plus head). A common application is the computation of sperm propulsive velocity from data on the kinematics of the flagellar beat [31–33]. Comparison of such computed velocities with their experimentally measured counterparts provides a test of the accuracy of a particular method of hydrodynamic analysis. Sperm hydrodynamic analysis has focussed almost exclusively upon Newtonian fluids to date. In such fluids, the resistance to motion is entirely viscous (e.g. there is no elastic component). The local fluid stress is proportional to the local fluid rate of strain, and the proportionality factor, i.e. the viscosity, is constant. Extensive mathematical analysis of flagellar hydrodynamics in Newtonian fluids has shown that the technique known as "resistive force theory" is a relatively accurate and mathematically tractable method, and this approach has been widely used in biological and biophysical studies [e.g. 11, 32, 34–36].

In shear-thinning fluids, such as the Ficoll-albumin solutions used in the present study, the viscosity is not constant, but depends upon the rate of strain, or shear rate (cf. Fig. 1). Consequently, classical resistive force theory does not strictly apply. However, it is possible to adapt and test this method for generating an approximate flagellar hydrodynamic analysis for shear-thinning fluids. The approach focusses not upon the instantaneous force and moment balances upon the sperm body, but on their beat cycle-averaged values. The fundamental assumption is that although the shear-rate-dependent viscosity varies locally along the sperm body throughout a flagellar beat cycle, it can be replaced by its cycle-average values in expressions for cycle-averaged force and moment balances. In so doing, separate values apply to the active, undulatory motions of the flagellum and the passive, rigid-body movements of the entire sperm (flagellum plus head). This amounts, mathematically, to a linearization of the time-dependent force and moment balances. The experimental data of the present study permit testing of the accuracy of this approach, since both flagellar beat kinematics and sperm propulsive velocity were measured. Our biological objective is to compute the flagellar thrust T. This is equal and opposite (in direction) to the overall drag on the sperm body, which is the sum, $D_f + D_h$, of the drags on the flagellum and head, respectively. That is,

$$T = D_f + D_b \tag{1}$$

For periodic, and relatively symmetric flagellar beats, the following expressions can be used to compute T, $D_f$ and $D_h$:

$$T = \lambda f L (C_N - C_L)\left[\frac{2\pi^2 b^2/\lambda^2}{1 + 2\pi^2 b^2/\lambda^2}\right] \tag{2}$$

$$D_f = \frac{VL}{1 + 2\pi^2 b^2/\lambda^2}\left[C_L + C_N(2\pi^2 b^2/\lambda^2)\right] \tag{3}$$

$$D_b = 6\pi\nu A k V \tag{4}$$

Here, $\lambda$, b, and f are flagellar wavelength, amplitude, and beat frequency, respectively; L is flagellar length; $C_N$ and $C_L$ are the normal and longitudinal resistive force coefficients; V is average path velocity; ($\nu$) is viscosity; A is the effective radius of the sperm head; and k is a geometric factor taking into account the asymmetry of the head [37].

The expressions for $C_N$ and $C_L$ are [38]:

$$C_n = \frac{-4\pi\nu}{\ln\left(\frac{2\pi r}{\lambda}\right) - 0.616} \tag{5}$$

$$C_L = \frac{-2\pi\nu}{\ln\left(\frac{2\pi r}{\lambda}\right) - 0.116} \tag{6}$$

where r is the flagellar radius.

In our application of these expressions to a shear-thinning fluid, we utilize different values of $\nu$ for the flagellum (equations 5 and 6) and for the head (equation 4). In each case, we determine an effective average shear rate, and use Figure 2 to find the corresponding, cycle-average viscosity for the particular concentration of Ficoll. For the sperm head, the effective shear rate is V/A. For the undulatory component of flagellar motion, i.e. the active beat, the effective shear rate for thrust is a beat cycle-average velocity divided by an effective length scale. Since the flagellar beat is approximately sinusoidal (especially for high concentrations of Ficoll), a simple calculation shows that the cycle-average velocity is approximately $4bf\lambda$. An effective hydrodynamic length scale for periodic movements of a flagellum was shown by Lighthill [32] to be approximately 0.1 $\lambda$. It follows that the effective shear rate for the flagellum is $40\ bf/\lambda$. For the hamster sperm we take L = 182 μm, A = 4.5 μm, and k = 0.89 [37].

We have applied these expressions to the data in Table 1 and Figure 2. The following table illustrates the results for the 30% Ficoll media. Values are based upon average over the five replicate experiments. We see that the difference between averages for T and for $D_f + D_h$ is 12% for activated sperm, 16% for transitional sperm, and 10% for hyperactivated sperm. Comparable agreement was obtained for lower concentrations of Ficoll. This degree of agreement lends credence to our method for computing flagellar thrust for shear-thinning Ficoll/albumin media.

| Sperm State | Activated | Transitional | Hyperactivated |
|---|---|---|---|
| T | 77* | 114 | 155 |
| $D_f$ | 62 | 89 | 160 |
| $D_h$ | 6 | 8 | 12 |

*Values of sperm forces components ($10^{-6}$ dynes) in media containing 30% Ficoll.

An alternative approach would be to weight separately the values of viscosity for the normal and tangential force coefficients by measures of the effective normal and tangential velocities. Calculations based on the mean square velocities (mean values are 0) yield slightly increased values of thrust, which do not alter the conclusions drawn here.