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The movement of human spermatozoa in cervical mucus

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Summary. Movement characteristics of freely swimming spermatozoa were studied with high-speed cinemicrography. At 21°C, flagellar beat frequency was higher in midcycle human cervical mucus than in native semen or Tyrode's solution; the beat shape differed, possessing diminished amplitude and wavelength. Although the spermatozoa swam straighter in the mucus, the progressive swimming speeds did not differ in the three media. Swimming speed and beat frequency were linearly related in semen and in Tyrode, but in mucus the linearity was less certain. In midcycle cervical mucus at 37°C, beat frequencies and swimming speeds were greater than at 21°C, but the trajectories were equally straight, and the distances swum per beat (kinetic efficiencies) did not differ.

Introduction

Residence in cervical mucus is an important phase in the process of sperm transport through the female reproductive tract in many mammals. Quantitative information on the kinetics of sperm entry and residence in the cervix and cervical mucus is available for man (Sobrero & MacLeod, 1962; Tredway *et al.*, 1975), rabbit (Morton & Glover, 1974) and ruminants (Quinlan, Mare & Roux, 1932; Mattner, 1963). It is generally believed that active sperm motility is an essential component of sperm transport through cervical mucus (Overstreet & Katz, 1977), and swimming speed is therefore a useful measure of sperm competency, at least in the initial phase of cervix colonization. However, the physical interaction *in vivo* of ascending spermatozoa with cervical mucus is not necessarily the only process involved; sperm–mucus compatibility may be reflected by additional measures of the physiological status of the gametes. For example, flagellar beat frequency is related to intermediary metabolism (Gibbons & Gibbons, 1972; Lindemann, 1976; McGrady & Nelson, 1976), while beat shape is associated with the resting membrane potential (McGrady & Nelson, 1972, 1973) and the external forces and moments acting upon the spermatozoon (Gibbons, 1975; Brokaw, 1975).

With the exception of the report of Odeblad (1967), there has been relatively little study of the detailed movement characteristics of human or other mammalian spermatozoa in cervical mucus. Visual methods of motility assessment cannot be used to determine sperm movement characteristics with accuracy or precision (van Duijn, Van Voorst & Freund, 1971; Katz & Dott, 1975). The present study was the preliminary phase of a detailed investigation of the biophysics of the interaction between human spermatozoa and cervical mucus.

Materials and Methods

Semen samples (A, B, C, D) were collected by masturbation from 4 healthy adult men after sexual abstinence for at least 72 h, and were utilized within 30 min after reliquefaction. All samples contained at least 50×10^6 spermatozoa/ml and cells with a percentage motility of at least 50% and a grade of progressive motility visually ranked (on a scale of 0–4) at 3 or greater. The 4 samples of mucus (a, b, c, d) were assessed as being from normal women at midcycle, according to the criteria of spinnbarkeit, fern ing, cellularity, and the advice of the collecting physician. The mucous samples were placed in sealed containers and stored at 4°C immediately after collection, and were used within 3 days. The sperm and mucous samples were considered as pairs throughout, i.e. Aa, Bb, Cc and Dd, and only one pair was used in each experiment.

Experiments 1 and 2. These were performed at room temperature (21 ± 1°C). Sperm movement characteristics were first measured in the whole semen. Simultaneously, spermatozoa in an aliquot of the semen were gently washed once, and resuspended in sterile Tyrode's solution (pH 7·4) (Grand Island Biol. Co., Grand Island, New York) at the same concentration as in the semen before assessment of the movement characteristics. Approximately 15 µl of the sperm suspensions (whole semen or washed) were pipetted onto a plane slide and covered by an 18 × 18 mm coverslip (No. 1½) to provide a depth of approximately 45 µm which was adequate for the spermatozoa to swim freely. After assessment of the whole semen, sperm penetration of the mucus was examined as follows. A 22 × 22 mm coverslip (No. 1½) was rimmed with a thin layer of vaseline and a blob of mucus, approximately 10 µl in volume, was placed in the centre with a plunger-capillary micropipette (i.d. 0·08 cm: Drummond Sci. Co., Broomall, Pennsylvania, U.S.A.). A 10 µl drop of semen was pipetted onto the coverslip in close apposition to the mucus and the chamber, 30–40 µm deep, was closed by a microscope slide. A clear interface between the semen and the mucus was obtained, with no spilling of the semen over the mucus. Sperm movement in the mucus was assessed 15 min after the completion of the slide preparation.

Experiments 3 and 4. These were performed at 37 ± 0·5°C. The slide preparation procedure was as described above and the mucus, semen, coverslips and slides were preheated. Immediately after preparation, each slide was placed on a microscope stage maintained at 37°C by a forced hot-air curtain and sperm movement in the mucus was assessed 15 min later.

Sperm movement assessment

Sperm movement characteristics were assessed by high-speed cinemicrography. Films were taken at 100 frames/sec with a Redlake Locam 16-mm camera. A timing light generator, integrated to the camera, provided a precise measure of the framing rate. Dry phase-contrast optics were employed, at a magnification of ×200, and the illumination was stroboscopic. Several fields on each slide were randomly selected and filmed for 6 sec. Processed cine films were analysed frame by frame on a Vanguard Motion Analyser. All motile spermatozoa in each filmed field were analysed. Spermatozoa swimming in cervical mucus occasionally came to an abrupt halt, followed by resumption of the initial trajectory or a marked change in direction. Such changes in sperm movement appeared to be modulated by obstructions within the microstructure of the mucus. The measurements reported here were based upon those portions of the trajectories in which spermatozoa were freely swimming. The following movement characteristics were measured: flagellar beat frequency (obtained from 24 or more consecutive beats); total swimming speed (the total distance per unit time traversed by a point at the junction of the sperm head and midpiece, obtained over the same time sequence as the beat frequency); progressive swimming speed (the distance per unit time measured along the straight line joining the initial and final points of the preceding trajectory); flagellar beat amplitude (in Exp. 1 only), defined as half the maximum transverse displacement of the flagellum with respect to an effective centre line of the beat. The following movement characteristics were also computed on the basis of the preceding measurements: progressiveness ratio, defined as the quotient of progressive swimming speed and total swimming speed, and progressive kinetic efficiency, defined as the quotient (progressive swimming speed)/(beat frequency × sperm body length). In the computation of progressive kinetic efficiency, the length of the human spermatozoon was taken as 50 µm (Gray, 1973).

Within Exps 1 and 2, statistical comparisons between pairs of mean values of beat frequency, amplitude, progressive swimming speed, and total swimming speed were performed by Student's *t* test. The corresponding mean values of progressiveness ratio and progressive kinetic efficiency were compared by the nonparametric Mann–Whitney test. Analysis of variance was used to compare mean values of movement characteristics in cervical mucus at room temperature (Exps 1 and 2) with those at body temperature (Exps 3 and 4). In the comparisons among values of progressiveness ratio and progressive kinetic efficiency, the nonparametric Friedman two-way analysis of variance by ranks was employed (Hollander & Wolfe, 1973). The relationship between progressive swimming speed and beat frequency was studied by linear regression analysis. The data were first analysed independently for each sample in each suspending medium at each temperature. Then the appropriate between-sample variability in the regression lines was examined with pooled regression methods (Snedecor & Cochran,

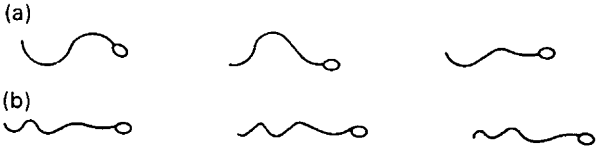
1967) and when permissible the regression lines were recalculated for the pooled data. Unless otherwise indicated, all statistical comparisons were based upon a 5% significance level.

Results

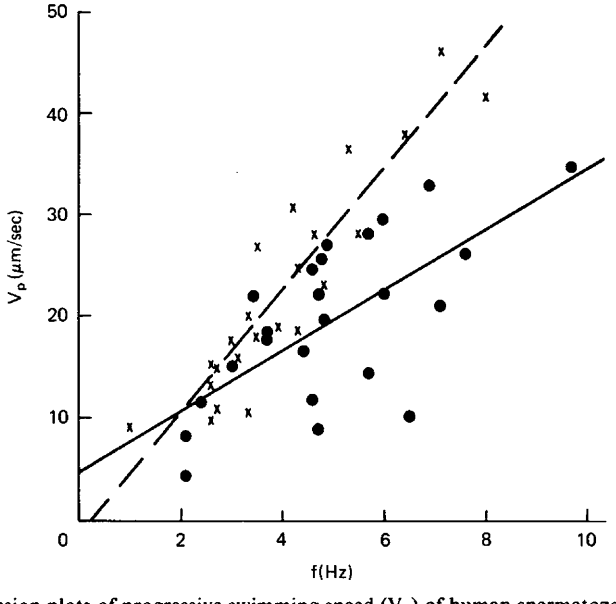
The quantitative results of the study are given in Table 1. At room temperature (Exps 1 and 2) the sperm flagella beat at higher frequencies in the mucus than in semen or in Tyrode's solution, but the swimming speeds in the three media did not differ. The progressive kinetic efficiency of the spermatozoa was lowest in the mucus. The spermatozoa swam straighter and with less variability in mucus than in semen or Tyrode. In Exp. 1 the mean beat amplitudes (± s.e.m.) were 4·38 ± 0·35 μm (semen), 6·73 ± 0·71 μm (Tyrode) and 2·77 ± 0·14 μm (mucus), and were significantly different from each other.

Swimming speed and beat frequency were higher for spermatozoa in cervical mucus at 37°C than at 21°C. However, the progressiveness ratios and progressive kinetic efficiencies did not differ.

The flagellar beat shape in cervical mucus was markedly different from that in the other media. Typical beat shapes, traced directly from cinemicrographs, are shown in Text-figs 1(a) and 1(b). The principal flagellar bending was confined to the distal half of the flagellum, and was characterized by a lower amplitude and wavelength than in semen or Tyrode's solution. There was less pitching of the sperm head in the mucus.



Text-fig. 1. Typical flagellar beat shapes of human spermatozoa in (a) semen or Tyrode's solution, and (b) fresh cervical mucus from a woman at midcycle.

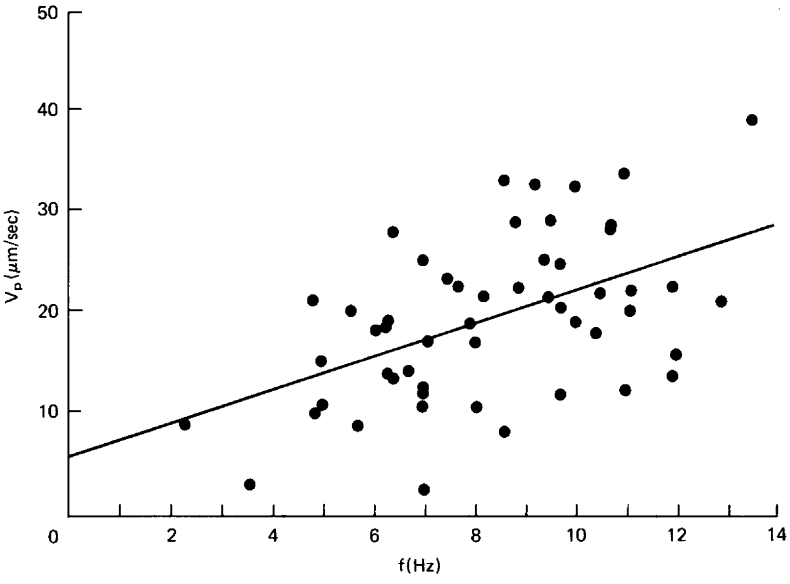


Text-fig. 2. Regression plots of progressive swimming speed (V_p) of human spermatozoa against flagellar beat frequency (f) in semen and in Tyrode's solution at room temperature. In semen the data points are denoted by ● and the regression line by ——, the regression equation being $V_p = 3{\cdot}01f + 4{\cdot}76$ ($n = 24$, $r = 0{\cdot}68$, $P < 0{\cdot}001$). In Tyrode the data points are denoted by × and the regression line by – – – –, the regression equation being $V_p = 6{\cdot}02f + 1{\cdot}62$ ($n = 23$, $r = 0{\cdot}92$, $P < 0{\cdot}001$).

Table 1. Summary of the movement characteristics of human spermatozoa in different media at two temperatures (values are mean ± s.e.m.)

Exp.	Medium	Temperature (°C)	No. of observations	Flagellar beat frequency, f (Hz)	Progressive swimming speed, V_p (μm/sec)	Total swimming speed, V_T (μm/sec)	Progressiveness ratio, V_p/V_T	Progressive kinetic efficiency $(V_p/fL) \times 10^2$
1	Semen	21	10	$5{\cdot}84 \pm 0{\cdot}59^a$	$21{\cdot}39 \pm 2{\cdot}71^a$	$25{\cdot}99 \pm 2{\cdot}57^a$	$0{\cdot}81 \pm 0{\cdot}04^a$	$7{\cdot}40 \pm 0{\cdot}77^a$
	Tyrode		10	$4{\cdot}11 \pm 0{\cdot}43^b$	$21{\cdot}54 \pm 3{\cdot}82^b$	$37{\cdot}23 \pm 4{\cdot}87^b$	$0{\cdot}59 \pm 0{\cdot}07^b$	$10{\cdot}78 \pm 0{\cdot}92^b$
	Cervical mucus		35	$7{\cdot}72 \pm 0{\cdot}37^c$	$18{\cdot}44 \pm 1{\cdot}15^c$	$20{\cdot}46 \pm 1{\cdot}22^c$	$0{\cdot}91 \pm 0{\cdot}02^c$	$4{\cdot}90 \pm 0{\cdot}30^c$
2	Semen	21	14	$4{\cdot}15 \pm 0{\cdot}50^a$	$18{\cdot}58 \pm 2{\cdot}07^a$	$28{\cdot}81 \pm 2{\cdot}67^a$	$0{\cdot}65 \pm 0{\cdot}06^a$	$8{\cdot}54 \pm 0{\cdot}69^a$
	Tyrode		13	$4{\cdot}58 \pm 0{\cdot}50^a$	$22{\cdot}66 \pm 2{\cdot}73^a$	$46{\cdot}64 \pm 4{\cdot}76^b$	$0{\cdot}53 \pm 0{\cdot}07^a$	$9{\cdot}89 \pm 0{\cdot}68^a$
	Cervical mucus		20	$9{\cdot}52 \pm 0{\cdot}54^b$	$22{\cdot}45 \pm 2{\cdot}00^a$	$24{\cdot}65 \pm 2{\cdot}10^a$	$0{\cdot}91 \pm 0{\cdot}01^b$	$4{\cdot}84 \pm 0{\cdot}41^b$
3	Cervical mucus	37	13	$17{\cdot}42 \pm 0{\cdot}50$	$36{\cdot}44 \pm 3{\cdot}78$	$39{\cdot}21 \pm 4{\cdot}35$	$0{\cdot}89 \pm 0{\cdot}02$	$4{\cdot}30 \pm 0{\cdot}50$
4	Cervical mucus	37	23	$15{\cdot}13 \pm 0{\cdot}72$	$34{\cdot}11 \pm 3{\cdot}45$	$35{\cdot}64 \pm 3{\cdot}62$	$0{\cdot}96 \pm 0{\cdot}01$	$4{\cdot}36 \pm 0{\cdot}32$

L denotes total sperm length, taken as 50 μm. Within an experiment means with different superscripts are significantly different, $P < 0{\cdot}05$.

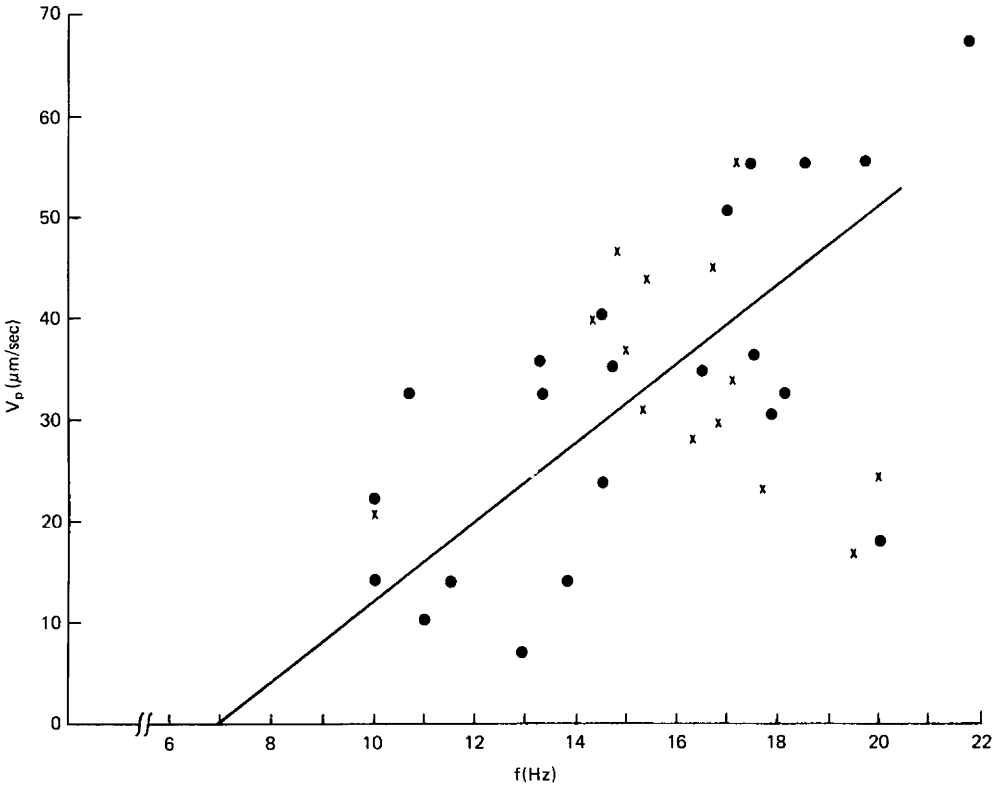


Text-fig. 3. Regression plot of progressive swimming speed (V_p) of human spermatozoa against flagellar beat frequency (f) in fresh, midcycle human cervical mucus at room temperature. The regression equation was $V_p = 1{\cdot}67f + 5{\cdot}90$ ($n = 55$, $r = 0{\cdot}52$, $P < 0{\cdot}001$).

Regression analysis for individual samples in Exps 1 and 2 suggested a predominantly linear relationship between progressive swimming speed and beat frequency in semen and in Tyrode's solution ($P < 0{\cdot}01$ in all cases). An analysis for spermatozoa in semen and in Tyrode revealed no significant differences in the residual variances, slopes or intercepts. The data were therefore pooled to give the regression lines indicated in Text-fig. 2. From the pooled regression analysis it was found that the slope of the regression line in Tyrode's solution was significantly greater than that in semen. There was evidence of a linear relationship between swimming speed and beat frequency in cervical mucus (Text-fig. 3) in Exps 1 and 2 ($P < 0{\cdot}05$ in both cases), and pooling of the data was also permissible. The slope of the regression line in this case was significantly lower than those in semen and in Tyrode. In Exp. 3 there was no evidence of a linear relationship between swimming speed and beat frequency in cervical mucus, but in Exp. 4 the relationship was again predominantly linear (Text-fig. 4).

Discussion

These preliminary results indicate differences in flagellar beat shape and frequency, and in the straightness of the trajectories of human spermatozoa swimming freely in cervical mucus compared to semen or Tyrode's solution. This beat can be distinguished during direct observation by the apparent figure-of-eight through which the distal tip of the flagellum oscillates. We have observed (unpublished) a similar beat shape for human spermatozoa swimming in Tyrode's solution of increased viscosity (addition of 1·5% methylcellulose) or in cervical mucus from an oestrous cow, and for rhesus monkey spermatozoa in cervical mucus from a bonnet monkey. The flagellar contraction mechanism is sensitive to the external forces and bending moments imposed upon the flagellum by its surroundings. In all likelihood, therefore, this beat shape results from the increased external resistances experienced by the flagellum during its undulations within the media. In cervical mucus these resistances must originate from the local hydrodynamic interactions between the flagellum and the microstructure of the mucus.



Text-fig. 4. Regression plot of progressive swimming speed (V_p) of human spermatozoa against flagellar beat frequency (f) in fresh, midcycle human cervical mucus at body temperature. The data points in Exp. 3 are denoted by ×, and a significant linear correlation was not indicated. The data points in Exp. 4 are denoted by ●, and the regression line by ——, the regression equation being $V_p = 4{\cdot}23f - 29{\cdot}31$ ($n = 23$, $r = 0{\cdot}83$, $P < 0{\cdot}001$).

The quantitative linear relationship between progressive sperm swimming speed and beat frequency in semen and in Tyrode's solution is in accordance with first principles of hydrodynamics (Lighthill, 1976), and is consistent with the results of Denehy (1975) for the ram. The slope of the regression line, or the ratio of swimming speed to beat frequency for an individual spermatozoon, reflects the beat shape and the influence of nearby solid objects, such as other spermatozoa. It is less certain from our present results whether such a quantitative linear relationship applies to sperm movement in cervical mucus. Because mucus is an inherently poly-disperse and non-homogeneous material (Gibbons & Sellwood, 1973; Odeblad, 1973), spermatozoa therein encounter variations in the microphysical environment. Visual data on bovine sperm swimming speed in bovine cervical mucus indicate as much variation within individual spermatozoa as between spermatozoa (Tampion, 1966). Odeblad (1967) obtained a non-linear relationship for swimming speed and beat frequency of human spermatozoa at body temperature, a maximum occurring at beat frequencies of about 7 Hz and swimming speeds of 6 μm/sec. Our preliminary data do not exhibit such behaviour.

While the average progressive swimming speed in mucus might appear to have been simply related statistically to the average beat frequency in the different experiments, there was substantially more variability in swimming speed than in beat frequency. Considerable information would therefore be lost by reliance upon measurement of swimming speed alone. Since the relationship between swimming speed and beat frequency in mucus is not yet understood, may not be simple, and may embody undetermined properties of a particular mucous sample, these two movement characteristics must at present be regarded as independent measures of sperm behaviour. Once the rudimentary hydro-

dynamics of the sperm–cervical mucus interaction are understood, precise measurement of sperm movement characteristics in mucus could provide valuable information about the nature of the mucus. For example, the altered beat shape in aged mucus (unpublished) suggests that the mucous macromolecules may have undergone configurational changes.

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References

BROKAW, C.J. (1975) Spermatozoan motility: a biophysical survey. *Biol. J. Linn. Soc.* **7**, *Suppl.* **1**, 423–439.

DENEHY, M.A. (1975) The propulsion of nonrotating ram and oyster spermatozoa. *Biol. Reprod.* **13**, 17–29.

GIBBONS, B.H. & GIBBONS, I.R. (1972) Flagellar movement and adenosine triphosphatase activity in sea urchin sperm extracted with Triton X-100. *J. Cell Biol.* **54**, 75–97.

GIBBONS, I.R. (1975) Mechanisms of flagellar motility. In *The Functional Anatomy of the Spermatozoon*, pp. 127–140. Ed. B. A. Afzelius. Pergamon Press, New York.

GIBBONS, R.A. & SELLWOOD, R. (1973) The macromolecular biochemistry of cervical secretions. In *The Biology of the Cervix*, pp. 251–265. Eds R. J. Blandau & K. Moghissi. University of Chicago Press.

GRAY, H. (1973) *Anatomy of the Human Body*. Ed. C. M. Goss. Lea and Febiger, Philadelphia.

HOLLANDER, M. & WOLFE, D.A. (1973) *Nonparametric Statistical Methods*. John Wiley & Sons, New York.

KATZ, D.F. & DOTT, H.M. (1975) Methods of measuring swimming speed of spermatozoa. *J. Reprod. Fert.* **45**, 263–272.

LIGHTHILL, J. (1976) Flagellar hydrodynamics. *SIAM Rev.* **18**, 161–230.

LINDEMANN, C.B. (1976) Adenosine 3′:5′-cyclic monophosphate (cAMP) stimulation of demembranated bull sperm models. *J. Cell Biol.* **70**, 238a, Abstr.

MATTNER, P.E. (1963) Spermatozoa in the genital tract of the ewe. II. Distribution after coitus. *Aust. J. biol. Sci.* **16**, 688–694.

MCGRADY, A.V. & NELSON, L. (1972) Cationic influence on sperm biopotentials. *Expl Cell Res.* **73**, 192–196.

MCGRADY, A.V. & NELSON, L. (1973) Electrophysiology of bull spermatozoa: correlations with motility. *Expl Cell Res.* **76**, 349–352.

MCGRADY, A.V. & NELSON, L. (1976) Cholinergic effects on bull and chimpanzee sperm motility. *Biol. Reprod.* **15**, 248–253.

MORTON, D.B. & GLOVER, T.D. (1974) Sperm transport in the female rabbit: the role of the cervix. *J. Reprod. Fert.* **38**, 131–138.

ODEBLAD, E. (1967) Motility pattern and propagation rate of spermatozoa in human cervical mucus. *Int. J. Fert.* **12**, 405–409.

ODEBLAD, E. (1973) Biophysical techniques of assessing cervical mucus and microstructure of cervical epithelium. In *Cervical Mucus in Human Reproduction*, pp. 58–74. Eds M. Elstein, K. Moghissi & R. Borth. Scriptor, Copenhagen.

OVERSTREET, J.W. & KATZ, D.F. (1977) Sperm transport and selection in the female genital tract. In *Mammalian Development*, pp. 31–65. Ed. M. H. Johnson. Elsevier/North Holland, Amsterdam.

QUINLAN, J., MARE, G.S. & ROUX, L.L. (1932) The vitality of the spermatozoa in the Merino ewe with special reference to its practical application in breeding. *Rep. vet. Res. Un. S. Afr.* **18**, 381–870.

SNEDECOR, G.W. & COCHRAN, W.G. (1967) *Statistical Methods*. Iowa State University Press, Ames, Iowa.

SOBRERO, A.J. & MACLEOD, J. (1962) The immediate postcoital test. *Fert. Steril.* **13**, 184–189.

TAMPION, D. (1966) Fluctuations in the swimming rate of individual bull spermatozoa. *J. Reprod. Fert.* **11**, 371–377.

TREDWAY, D.R., FORDNEY SETTLAGE, D., NAKAMURA, R.M., MOTOSHIMA, M. UMEZAKI, C.U. & MISHELL, D.R. (1975) Significance of timing for the postcoital evaluation of cervical mucus. *Am. J. Obstet. Gynec.* **121**, 387–393.

VAN DUIJN, C., JR, VAN VOORST, C. & FREUND, M. (1971) Movement characteristics of human spermatozoa analyzed from kinemicrographs. *Eur. J. Obstet. Gynec. Reprod. Biol.* **4**, 121–135.

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