Human sperm volume regulation. Response to physiological changes in osmolality, channel blockers and potential sperm osmolytes

C.H.Yeung, M.Anapolski, M.Depenbusch, M.Zitzmann and T.G.Cooper

Institute of Reproductive Medicine of the University, Domagkstrasse 11, D-48129 Münster, Germany

To whom correspondence should be addressed. E-mail: yeung@uni-muenster.de

BACKGROUND: Volume regulation is an important sperm function because defective sperm cannot negotiate the female tract in an infertile mouse model and swollen human sperm cannot penetrate and migrate through mucus.

METHODS AND RESULTS: The size of sperm from 52 donor ejaculates incubated in medium of female tract fluid osmolality (BWW290) was measured by flow cytometry to be identical to that in homologous semen osmolality (289±351 mosmol/kg), indicating effective volume regulation. Inhibition of anticipated regulatory volume decrease in BWW290 by the channel blocker quinine induced size increases and associated kinematic changes measured by computer-aided sperm analysis. Incubation in L-carnitine, myo-inositol and taurine did not change sperm volume or kinematics, but the presence of glutamate and K+ decreased the efficiency of forward progression indicative of volume increase, suggesting them as potential osmolytes for human sperm. Linear regression suggested correlations of changes in cell volume and in kinematic parameters, and the association of faster forward progressive sperm with smaller cell size. CONCLUSIONS: Sperm volume and its regulation may be crucial to natural fertility. The identification of sperm osmolytes, ion channels and mechanisms involved would contribute to the understanding of male infertility and offer a lead for male contraception.

Key words: organic osmolytes/quinine/regulatory volume decrease/sperm function/sperm motility

Introduction

Mammalian sperm produced in the testis undergo maturation in the epididymis and are stored there until ejaculation (Cooper, 1998). This holds true for man despite claims to the contrary (see Cooper, 1990, 2002; Cooper and Yeung, 2000). In laboratory animals, the epididymis provides sperm with a unique milieu of high osmolality. Upon ejaculation into the female tract, sperm are subjected to a hypo-osmotic challenge that necessitates regulation of cell volume (see Cooper and Yeung, 2003). Defects in sperm volume regulation have recently been identified as the cause of infertility by natural mating in a transgenic mouse model, due to failure in sperm transport into the oviduct (Yeung et al., 2000), despite reproductive success with IVF (Sonnenberg-Riethmacher et al., 1996). Osmotic adjustment upon ejaculation would also be required of human sperm in the female tract, since there are changes in osmolality from ~340 mosmol/kg in the vas deferens (Hinton et al., 1981) to 280–290 mosmol/kg in the female tract environment. Inhibition of cell volume regulation, by in-vitro incubation of ejaculated sperm with the ion channel blocker quinine, leads to failure in the penetration and migration through surrogate mucus (Yeung and Cooper, 2001). Hence, human sperm volume regulation is an important factor in male fertility hitherto not well understood.

Epididymal fluid is characterized by progressive decreases in Na+ and increases in K+ concentrations along the duct. Osmolality is made up by high amounts of small organic molecules including amino acids such as glutamate and taurine, carnitine, glycerophosphocholine (GPC) and myo-inositol (see Cooper and Yeung, 2003). These organic molecules, as well as K+, are commonly used by somatic cells as osmolytes for volume regulation (see Lang et al., 1998; Fürst et al., 2002). The increase in osmolality from testicular to epididymal fluid should induce uptake of osmolytes by epididymal sperm to counteract cell shrinkage. These compounds could then be utilized by sperm upon ejaculation into a relatively hypotonic environment in the female tract, by mechanisms of regulatory volume decrease (RVD) resulting in efflux of osmolytes and cellular water (Cooper and Yeung, 2003). In our preliminary study of murine sperm, it was revealed that the presence of such molecules in the incubation medium, with the exception of GPC, could prevent sperm RVD, thus supporting the hypothesis that these are sperm osmolytes. In the present study, these putative osmolytes were tested on human ejaculated sperm.

When challenged with physiological changes in osmolality, murine epididymal sperm swell considerably upon inhibition of RVD with induction of tail angulation (Yeung et al., 1999,
Materials and methods

**Source of ejaculates and semen analysis**

Fifty-two ejaculates were obtained at the Institute of Reproductive Medicine from healthy men volunteering for a study for hormonal male contraception, which was approved by the Ethics Committee of the University and the State Medical Board, Münster, Germany. The participants gave written informed consent. They were between 18 and 45 years old and did not suffer from chronic illnesses or infections of the urogenital tract and took no medications of any kind. Only prior-to-treatment samples were used for the present study. Semen samples were obtained by masturbation after ≥2 days of abstinence. After liquefaction at 37°C for 30 min, routine semen analysis was carried out according to the protocol of the World Health Organization (1999). Osmolality of the semen sample was measured after liquefaction using a vapour pressure osmometer (Wescor Vapro 5520; Schlag GmbH, Germany) that does not require separation of cells and particles before measurement. Characteristics of the ejaculates are given in Table I.

**Medium**

Each ejaculate was treated with modified Biggers–Whitten–Whittingham (BWW) medium (Biggers et al., 1971) containing bovine serum albumin at 4 mg/ml, with osmolality adjusted to 290 mosmol/kg (that of cervical mucus and uterine fluid; BWW290) or that of homologous semen (BWWsemen). BWWsemen was prepared by mixing appropriate volumes of two BWW solutions with osmolality of 270 and 480 mosmol/kg to achieve the required seminal osmolality. Various BWW290 solutions, each containing a test osmolyte (see Figure 1 for substances and concentrations), were made freshly each day by adding a small volume of the stock solution and omitting the equivalent amount of NaCl, and the final osmolality was checked. Osmolyte stock solutions containing test substances that had an acidic pH (glutamate and taurine) were adjusted to pH 7.0 before use. All BWW solutions contained phenol red as a pH indicator, and were equilibrated in an incubator at 37°C with 5% (v/v) CO₂ in air before use.

**Sperm preparation and incubation**

As indicated in the flow chart of the protocol (Figure 1), 10 μl aliquots of the freshly liquefied ejaculate were dispersed in 250 μl BWWsemen and BWW290 and analysed with the flow cytometer (2 min time-point) after addition of 3 μl propidium iodide (PI, 500 μg/ml phosphate-buffered saline) to stain non-viable cells, as described below. Another dispersed aliquot was incubated at 37°C with 5% v/v CO₂ in air and analysed after 20 min. Aliquots of the ejaculate were also washed (150 μl ejaculate in 1 ml medium, centrifuged at 450 g for 5 min at room temperature) with BWWsemen, BWW290 control medium or BWW290 containing quinine or a test osmolyte listed in Figure 1. The sperm pellet was resuspended in 100 μl fresh solution of the wash medium and incubated. For volume measurement with the flow cytometer at 20 and 40 min of incubation, 15 μl sperm suspension was diluted into 250 μl of the same medium containing 3 μl PI as above.

**Evaluation of sperm volume by flow cytometry**

The laser forward scatter signal of viable sperm as a reflection of sperm cell volume (Yeung et al., 2002a) was analysed by a flow cytometer (Coulter Epics XL, version 3.0; Germany) as described previously (Yeung and Cooper, 2001). The same flow cytometer...
settings were used throughout the study. For each sample, ~10 000 particles were analysed and the mean forward scatter intensity was calculated after gating out the cell debris and aggregates using the forward and side-scatter dot plot and eliminating the non-viable sperm based on PI fluorescence.

It should be noted that the forward scatter signals obtained in terms of channel numbers are relative values that depend on the voltage settings of the photo-multiplier tube, which were chosen to be sensitive enough to detect changes and were kept constant for all experiments in the study. Signals so detected for human sperm are therefore not comparable with those obtained for mouse studies which used different voltage settings (Yeung et al., 2002a,b).

**Simultaneous measurement of sperm volume by flow cytometry and electronic sizing using the Coulter counter**

Aliquots of sperm suspensions diluted from eight ejaculates into BWW medium were measured simultaneously by the flow cytometer as described above and by a Coulter counter (model Z; Beckman Coulter, Germany) as previously described for mouse sperm (Yeung et al., 2002a). Volume was measured in femtolitres by the Coulter counter, and the size distribution of the 10 000–30 000 particles measured in each sample was analysed using the Accucomp software provided by the manufacturer. The mean sperm volume was calculated after gating out debris and cell aggregates. The mean forward scatter signal of all the sperm from an aliquot of the same sample analysed by the flow cytometer was also calculated for comparison. To remove the sperm plasma membrane and cytoplasm in order to reduce sperm size to the minimum, Triton X-100 was added to the sperm suspension at a final concentration of 1% (v/v) for 3 min before reading by both machines.

**Computerized analysis of sperm kinematics (using CASA)**

Washed sperm incubated in various media for 20 min were recorded at 37°C in a 20 μm deep chamber (2X-Cel; Hamilton–Thorne Research, USA) on video tape as described in Yeung and Cooper (2001). For each sample, >200 motile sperm were tracked for 1 s at 50 frame/s, with an average path velocity threshold value of 3 μm/s and a minimum of 26 track points for each motile cell, using a Hamilton–Thorne CASA system (IVOS version 10.8; Hamilton–Thorne Research). To circumvent any non-Gaussian distribution of individual track data, the median values of all sperm tracks in each sample were taken to represent the kinematic parameters of that sample. Data for each treatment group were calculated and presented as mean ± SEM of the n median values from n samples.

**Statistics**

In each experiment, the effects of osmolality and quinine treatment on sperm volume were analysed by comparison with the reference value initially obtained with non-washed sperm in BWWsemen (see Figure 1) and expressed as ratios to overcome basal differences between ejaculates. Data from osmolyte incubation were expressed as ratios of the control incubation (BWW290) in the absence of osmolytes. Analysis of variance was performed using SigmaStat computer software (version 2.03, SPSS Inc., Germany). For each group of osmolyte or quinine treatment, statistical comparisons with controls were done using the Student–Newman–Keuls method. In cases where the normality test failed, one way analysis of variance on ranks was performed followed by Dunn’s comparison.

**Results**

**Comparison of sperm volume measurement by flow cytometry and by the Coulter counter**

The flow cytometric method for sperm volume measurement has been validated for mouse sperm by comparison with the electronic sizing method using the Coulter counter (Yeung et al., 2002a). In the attempt to make a similar comparison of the two methods for human sperm volume measurement, the problem of interference in electronic sizing by debris present in the samples of non-selected, non-washed sperm was encountered as reported by other workers (Brotherton and Barnard, 1974; Brotherton 1975; Spano et al., 1984). Using ejaculates with relatively little debris, the mean volumes of human sperm, ranging from 33 to 37 fl (mean 34.5 fl), were measured by the Coulter counter, with the corresponding forward scatter signals ranging from 550 to 581 (mean 568) channel no. (upper right of Figure 2). After Triton treatment, which should remove plasma membranes and cytoplasm, both methods registered marked decreases in the measurements to mean values of 21 fl and 482 channel no. respectively (lower left in Figure 2).

**Effect of physiological changes in osmolality on sperm volume and kinematics in the presence or absence of channel blocker**

When the ejaculate was diluted in BWWsemen to maintain extracellular osmolality, sperm volume, as reflected by the intensity of laser forward scatter, was maintained over 20 min incubation. Compared with the initial measurement [reference (Ref) indicated in Figure 1], there was no change in the laser forward scatter of visible sperm over 20 min when extracellular osmolality was lowered to that of fluids in the female tract by dilution in BWW290 (n = 32; Figure 3a). Neither was there any difference in volume between the washed sperm incubated in these two different media, although there were slight increases in both washed samples compared with the non-washed samples, indicating some effect of washing. However, when viable sperm were washed and incubated in quinine at 125 or 250 μmol/l in BWW290, there were significant increases in the laser forward scatter compared either with the non-washed
BWWsemen reference (Figure 2) or with the BWW290 washed control (Figures 3a, 4), indicating swelling of intact sperm in the presence of the channel blocker. When the same analysis was performed on dead sperm identified by positive PI fluorescence, there was no response to quinine (Figure 3b). In subsequent experiments, only viable sperm were analysed.

Sperm motility and kinematic parameters were the same whether they were incubated in BWW290 or BWWsemen (Table II). However, in the presence of quinine, there were marked changes in sperm kinematics without alteration to the overall percentage that was motile. These included increases in curvilinear velocity and amplitude of lateral head displacement, with decreases in straight line velocity and in linearity and straightness of the sperm track (Figure 4).

**Effect of incubation in potential osmolytes**

No significant changes were found by flow cytometry in cell volume of sperm incubated up to 40 min in any of the potential osmolytes at the concentrations tested (see Figure 1). Neither L-carnitine, myo-inositol nor taurine alter sperm kinematics (data not shown). On the other hand, the presence of glutamate at both 0.5 and 5 mmol/l, but not 1 mmol/l, resulted in significant decreases in straight line velocity whereas K⁺ at the highest concentration tested led to decreases in straightness and linearity of the sperm track despite having no effect on the laser forward scatter intensity (Table III). Viability and percentage motility were maintained in all incubations (some, but not all, data given in Table III).

**Correlations of kinematic changes with sperm volume**

In order to investigate if the effects on sperm kinematics by quinine, K⁺ and glutamate were related to those on sperm volume as reflected by laser forward scatter, linear regression was performed on these parameters using the ratios of the treatment to individual control values. A larger increase in forward scatter intensity was associated with a larger decrease in straightness of the sperm track (Figure 5), presenting the strongest negative correlation among all regressions ($R = -0.70$, $P < 0.001$). Negative correlations of cell volume changes were also demonstrated with changes in straight line velocity, straightness and linearity (Table IV), and weak, but statistically significant, positive correlations were found between changes in cell volume and in curvilinear velocity and amplitude of lateral head displacement (Table IV, Figure 6).

To examine if sperm kinematic parameters *per se* were influenced by the cell volume, regressions were performed on the absolute values of measurement (Table IV). Among the significant correlations found, higher straight line velocities were associated with smaller forward scatter signals (Figure 7).

**Correlations of semen osmolality with sperm volume and kinematics**

Osmolality of the ejaculate was not correlated with the size (forward scatter) of sperm diluted in BWWsemen. No correlations of seminal osmolality were found with sperm kinematic measurements made in BWWsemen or BWW290. Neither was

---

**Table II.** Kinematic parameters of sperm after washing and incubation for 20 min in BWW medium with osmolality identical to the homologous semen (BWWsemen) or female tract fluids (BWW290)

<table>
<thead>
<tr>
<th></th>
<th>BWWsemen</th>
<th>BWW290</th>
</tr>
</thead>
<tbody>
<tr>
<td>Motility (%)</td>
<td>65 ± 2.5</td>
<td>65 ± 2.5</td>
</tr>
<tr>
<td>Averaged path velocity</td>
<td>43 ± 2.0</td>
<td>43 ± 2.1</td>
</tr>
<tr>
<td>Straight line velocity</td>
<td>28 ± 1.4</td>
<td>30 ± 1.6</td>
</tr>
<tr>
<td>Curvilinear velocity</td>
<td>71 ± 3.5</td>
<td>69 ± 3.6</td>
</tr>
<tr>
<td>Amplitude of head</td>
<td>7.2 ± 0.41</td>
<td>6.5 ± 0.40</td>
</tr>
<tr>
<td>displacement (µm)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Straightness (%)</td>
<td>78 ± 1.2</td>
<td>80 ± 1.3</td>
</tr>
<tr>
<td>Linearity (%)</td>
<td>43 ± 1.4</td>
<td>46 ± 1.7</td>
</tr>
</tbody>
</table>

Values are mean ± SEM ($n = 25$).
the magnitude of the kinematic effects of quinine correlated with individual seminal osmolality.

**Discussion**

The problem with the electronic sizing of human sperm created by the interference of debris in the ejaculates, which is not encountered in measuring epididymal sperm from experimental animals, has been recognized by many earlier workers (see Brotherton, 1975). Elimination of such debris using the detergent Zaponin (Brotherton and Barnard, 1974; Brotherton, 1975; Spano et al., 1984) cannot be applied for the study of functional intact sperm, since the detergent would damage the cell membrane. In the present study, the flow cytometric method already validated for volume measurement of mouse sperm (Yeung et al., 2002a) was employed. Evidence that changes in forward scatter signals detected for human sperm also reflect changes in their volume was provided by comparing data obtained from relatively clean samples by both methods. One crucial advantage in the use of flow cytometry is the possibility to distinguish live and dead cells, so as to eliminate the latter from analysis and improve the quality of the data. This was clearly illustrated in this study by the lack of response of dead sperm to the channel blocker quinine.

Semen osmolality measured in the present study after 30 min of liquefaction varied from 289 to 351 with an average of 318 mosmol/kg, as found by Rossato et al. (2002). In our previous study, a mean ± SD value of 342 ± 21 mosmol/kg from 66 normozoospermic ejaculates was obtained 60–90 min after collection (Yeung and Cooper, 2001). There are reports of similar (Makler et al., 1981) or higher values (Velazquez et al., 1977; Polak and Daunter, 1984; Gopalkrishnan et al., 1989). However, it is well known that semen osmolality increases with time after ejaculation owing to proteolysis and other chemical changes (see Mann and Lutwak-Mann, 1981), e.g. increasing from ~330 mosmol/kg at 50 min to 380 mosmol/kg at 150 min (Abraham-Peskir et al., 2002).

The measured seminal osmolality was lower than that reported in the vas deferens and higher than that of female tract fluids. Therefore during normal copulation sperm experience a stepwise decrease in surrounding osmolality from the cauda epididymidis and vas deferens (342 mosmol/kg; Hinton et al., 1981) via the fresh ejaculate (318 mosmol/kg) to cervical mucus (287 mosmol/kg; Casslén and Nilsson, 1984) and uterine fluid (284 mosmol/kg; Rossato et al., 1996). The

**Figure 4.** Effects of quinine on (a) laser forward scatter, (b) percentage motility and (c) kinematic parameters of washed ejaculated sperm incubated for 20 min. Values are mean ± sem (n = 14–20), expressed as a ratio to the control data from the same experiments. VSL = straight line velocity; VCL = curvilinear velocity; ALH = amplitude of lateral head displacement; BCF = beat frequency; STR = straightness; LIN = linearity. *Significantly different from control value (P < 0.05).

| Table III. Effects of glutamate and K⁺ in the incubation medium for 20 min on sperm size and kinematic parameters |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Osmolyte (n) | % viability | Size (FS) | VSL | VCL | ALH | STR | LIN | % motility |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| Glutamate  |
| 0.5 mmol/l (10) | 0.9863 | 1.000 | 0.876 | 0.989 | 1.112 | 0.959 | 0.954 | 1.050 |
| ± 0.01 | ± 0.004 | ± 0.022* | ± 0.041 | ± 0.057 | ± 0.019 | ± 0.043 | ± 0.066 |
| 1 mmol/l (10) | 1.029 | 1.002 | 0.970 | 1.062 | 1.150 | 0.958 | 0.923 | 1.139 |
| ± 0.014 | ± 0.010 | ± 0.092 | ± 0.064 | ± 0.102 | ± 0.019 | ± 0.036 | ± 0.050 |
| 5 mmol/l (10) | 1.017 | 1.007 | 0.845 | 0.980 | 1.037 | 0.955 | 0.956 | 1.048 |
| ± 0.010 | ± 0.010 | ± 0.080* | ± 0.080 | ± 0.084 | ± 0.036 | ± 0.079 | ± 0.053 |
| K⁺  |
| 10 mmol/l (7) | 1.051 | 0.998 | 1.559 | 1.306 | 1.222 | 1.014 | 1.097 | 1.057 |
| ± 0.008 | ± 0.010 | ± 0.340 | ± 0.249 | ± 0.213 | ± 0.067 | ± 0.192 | ± 0.112 |
| 30 mmol/l (7) | 1.073 | 0.994 | 1.637 | 1.342 | 1.140 | 1.100 | 1.322 | 1.105 |
| ± 0.025 | ± 0.006 | ± 0.277 | ± 0.170 | ± 0.187 | ± 0.090 | ± 0.262 | ± 0.106 |
| 100 mmol/l (8) | 1.008 | 0.998 | 0.936 | 1.100 | 1.220 | 0.926 | 0.862 | 0.973 |
| ± 0.008 | ± 0.003 | ± 0.100 | ± 0.132 | ± 0.158 | ± 0.029* | ± 0.057* | ± 0.040 |

Values are expressed as ratio to the control (BWW alone). Values in each experiment are presented as mean ± SEM from n experiments.

FS = forward scatter signal measured by flow cytometry; VSL = straight line velocity; VCL = curvilinear velocity; ALH = amplitude of lateral head displacement; STR = straightness; LIN = linearity. *Significantly different from control value (P < 0.05).
gradient of change (~60 mosmol/kg) is less than that experienced by murine ejaculated sperm (~80 mosmol/kg). In the present study, dilution of ejaculated sperm into medium with cervical mucus osmolality in the presence of quinine, the wide spectrum blocker of ion channels involved in RVD, also induced both volume increases and kinematic changes. In the absence of quinine, sperm size and kinematics remained unchanged either in BWWsemen or BWW290, indicating that RVD mechanisms were functioning within the physiological range of osmolality.

In the search for potential sperm osmolytes, K+ and small organic molecules found in high concentrations in epididymal fluid of laboratory mammals were tested in the present work. Because of the paucity of data on human (or other primate) epididymal fluid composition, these substances were included in the incubation medium at concentrations found to be effective in inhibiting RVD of murine sperm, presumably by preventing efflux down concentration gradients through opened channels, resulting in the removal of water from the cell (C.H.Yeung, M.Anapolski and T.G.Cooper, unpublished data). However, none was found to be effective in inducing a volume increase in human sperm. When the related, but more sensitive, kinematic parameters were examined, glutamate and K+ were found to have slight effects. In vas deferens fluid (Hinton et al., 1981), K+ is at far higher concentration (111 mmol/l) than myo-inositol (6 mmol/l) and carnitine (6 mmol/l in both vas deferens and cauda epididymidal fluid from one man) (Turner, 1979). This differs from the rat (there are no data from mouse) where all three osmolytes are ~50 mmol/l in caudal fluid (Levine and Marsh, 1971; Hinton and Palladino, 1995). Therefore, K+ could be the major osmolyte utilized by human sperm for RVD.

It is worth noting that uterine (Casslén and Nilsson, 1984) and oviductal fluids are generally rich in K+, with mean values ranging from 7 to 26 mmol/l in women (see Borland et al., 1980) and as high as 30 mmol/l in rabbits oviducts (Burkman et al., 1984). Nevertheless, these levels are well below the intracellular concentrations reported to be 75 mmol/l in human sperm (Patrat et al., 2002) and as high as 120 mmol/l in bovine and murine sperm (Babcock, 1983; Chou et al., 1989), creating large diffusion gradients which would enable efficient efflux of K+ as an osmolyte for volume regulation. Evidence for this is provided by the reversal of the quinine-induced changes in volume and kinematics by the K+ ionophores valinomycin and gramicidin (Yeung and Cooper, 2001). The present finding of an effective concentration of 100 mmol/l but not of 30 mmol/l is in agreement with this hypothesis. Rabbit sperm motility is also inhibited by 50 mmol/l K+ (Burkman et al., 1984), which led the authors to suggest a role of K+ in the regulation of sperm motility in the female tract.

The weak, or lack of, response of human sperm to incubation with potential osmolytes compared with murine sperm was reflected in the difference in their extents of swelling at physiological osmolality induced by quinine. Whereas a 30% increase in laser forward scatter (as indicator of sperm size) is demonstrated in murine sperm (Yeung et al., 2002b), the increase in human sperm is very small, amounting to only a few per cent. One explanation of a lower swelling response to quinine in human sperm is a smaller drop in osmolality from the native fluid of the sperm sample (seminal plasma in man and cauda epididymidal fluid in mouse) to the test medium (an average of 318±290 = 28 mosmol/kg in the human and 415–330 = 85 mosmol/kg in the mouse studies). Another explanation could be the difference in the size of the cytoplasmic droplets which hold most of the sperm cytoplasm. These are relatively large and present in ~80% of mature murine sperm (Cooper and Yeung, 2003). The presence of

![Figure 5. Negative correlation between changes in sperm volume measured as forward scatter signal intensity and changes in straightness (STR) of the swim path of sperm. The regression line is indicated (y = 7.4 – 6.4x).](image)

<table>
<thead>
<tr>
<th>Table IV. Linear regression coefficients (R) of sperm kinematics with sperm volume, and of changes (as ratio to control) in sperm kinematics with changes in sperm volume</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kinematic parameter</strong></td>
</tr>
<tr>
<td>Absolute values</td>
</tr>
<tr>
<td>---------------------</td>
</tr>
<tr>
<td>Straight line velocity</td>
</tr>
<tr>
<td>Averaged path velocity</td>
</tr>
<tr>
<td>Curvilinear velocity</td>
</tr>
<tr>
<td>Linearity</td>
</tr>
<tr>
<td>Straightness</td>
</tr>
<tr>
<td>Amplitude of lateral displacement</td>
</tr>
</tbody>
</table>

*P = 0.012; **P < 0.001. NS = non-significant.
cytoplasmic droplets in dried smears is mostly considered an abnormal morphology of human sperm, but the disruptive forces during smearing and air drying may well disrupt osmotically sensitive organelles. Recently, vesicles around the human sperm mid-piece (MPV) have been reported in wet preparations using differential interference contrast or X-ray microscopy (Abraham-Peskir et al., 2002). The incidence, as well as the size, of these MPV increases with decreasing osmolality of the medium ranging from 450 to 200 mosmol/kg. These authors reported that the association of MPV with reduced motility, in agreement with our previous observation of inhibition of mucus penetration and migration upon inhibition of RVD (Yeung and Cooper, 2001). Therefore visible MPV may be a manifestation of insufficient cell volume regulation.

The present flow cytometric method could offer a rapid analysis of sperm cell volume in semen analysis. It has been reported that in normozoospermic ejaculates, ~30% of sperm, separated by density gradients, have enlarged mitochondrial diameter and low creatine kinase isoform ratios (Gergely et al., 1999) which is an indication of cytoplasmic retention during spermiogenesis (Huszar et al., 1998). The presence of such defective, 'mis-matured' sperm would increase the mean cell volume of the ejaculated sperm. This cause of poor sperm quality may partly contribute to the present correlations of poor kinematics with higher laser scatter signals. Nevertheless, the correlation with the changes in laser scatter signal were stronger than that with the absolute values, reflecting more the functional deficiency in cell volume regulation than the abnormal morphology.

The reversal of both sperm size increase and alterations in kinematics in the presence of quinine by the ionophores valinomycin and gramicidin (Yeung and Cooper, 2001) suggests that the dynamics of flagellation are influenced by the physical dimension/structure of sperm components. In the present study, a linear correlation was established between changes in sperm volume as reflected by laser forward scatter and simultaneous changes in various kinematic parameters, particularly the straightness of the sperm track, which decreases with increases in sperm volume. Although the low values of the correlation coefficients for the other parameters warrant a note of caution, the finding suggests that an increase in cell volume may lead to a decreased efficiency of forward progression (decreases in straight line velocity and linearity) without compromising vigour of flagellar beating (reflected in increases in curvilinear velocity and lateral head displacement).

That there was no correlation between semen osmolality and sperm size indicates that normal sperm can regulate their volume in semen. Nevertheless, weak but significant correlations were demonstrated between sperm size and kinematic parameters, in particular the association of faster forward progression with smaller sperm. Upon cell swelling, the signal transduction leading to channel opening for osmolyte effluxes (if there are concentration gradients) is known to involve diverse pathways including protein kinase A (PKA) and PKC (see Hoffmann, 2000; Fürst et al., 2002), and in some cells increases in intracellular pH and Ca<sup>2+</sup> (MacLeod and Hamilton, 1999). Since such factors could be the direct modulators of motility, it is not clear whether the motility changes found in the present study were effected by any of these factors, besides the direct mechanical effect that volume has on the kinematics. Volume regulation as a component of the complex signal transduction pathways and interplay among various aspects of sperm function warrants further investigation. It also offers a new lead in the development of post-testicular contraceptive.

**Acknowledgements**

We thank Barbara Hellenkemper for technical assistance, and Raphaele Kürtken, Sabine Rehr, Anne Erpenbeck and Daniela Schmidt for routine semen analysis. We also thank Professor Eberherd Nieschlag for support and encouragement. This work was supported by the Deutsche Forschungsgemeinschaft Grant number FOR197/3-1 ‘The male gamete: production, maturation, function’.

**Figure 6.** Positive correlation between changes in sperm volume measured as forward scatter signal intensity and changes in the amplitude of lateral head displacement (ALH) of motile sperm. The regression line is indicated (y = –5.6 + 6.8x).

**Figure 7.** Negative correlation between sperm volume measured as intensity of the laser forward scatter signal and straight line velocity. The regression line is indicated (y = 134 – 0.2x).


Submitted on July 26, 2002; resubmitted on November 26, 2002; accepted on January 15, 2003