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Rotational and translational motions of human spermatozoa: angle dependence of dynamic laser light scattering

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Abstract. We have studied how the dynamic components of laser light scattered from human spermatozoa depend on the scattering angle. This was done by investigating the halfwidth of the intensity autocorrelation function. A model of the spermatozoa as freely rotating and translating linear objects was adequate to describe the scattered light. Rotational motions determined the halfwidth of the intensity autocorrelation function at very small scattering angles and contribution from translational motions was dominant at scattering angles larger than 20 degrees. The contribution from translational motions increased with increasing scattering angle. We found a nearly linear relationship between the translation speed and the rotation frequency. However, the ratio between the two properties varied more than expected from the methodological error. Therefore we introduced a propelling efficacy as a concept to describe the swimming efficiency. This property might contain important information about the swim characteristics.

Key words: Laser light – Human spermatozoa – Scattering angles – Rotational and translational motions

Introduction

Laser light has been used for many years to study the motions of spermatozoa. At the beginning the characteristic speed was evaluated from the frequency component of the intensity of the scattered light (Dubois et al. 1974; Jouannet et al. 1977). Later, analysis with intensity autocorrelation functions (ACF) was preferred. The evaluation of important motility parameters from the ACF requires a theory describing how the intensity varies as the spermatozoa change their position in the laser beam. Point particles moving linearly for periods of time longer than the characteristic times of the ACF have a mean translation speed that scales with the inverse of the scattering wave vector (Nossal 1971).

In some aspects of light scattering it is sufficient to model spermatozoa as point particles (Finsy et al. 1979; Frost and Cummins 1981). The dependence of the ACF on the scattering angle was studied on spermatozoa from abalone and pig. It showed that the product between the scattering wave vector and the half width of the ACF is constant at 45° and 90° scattering angle, thus supporting the point scattering model (Shimizu and Matsumoto 1977).

Bull spermatozoa, on the other hand, show orientation effects in the scattered light and may therefore not be modelled as point scatterers (Harvey and Woolford 1980). A model with Rayleigh-Gans-Debye approximated ellipsoids moving in helical trajectories predicts the features seen in the experimental scaling curves (Craig and Hallett 1982). However, there is a discrepancy in the absolute value between the calculated and measured halfwidths of the ACF for normally swimming bull spermatozoa. The halfwidth of the ACF is determined primarily by rotational motions for these disk-shaped ellipsoids.

When the spermatozoon is regarded as a chain of anisotropic light *scatterers*, each influenced by a constant torque, the rotation frequency may be obtained from depolarized light at scattering angles almost equal to 0° (Shimizu and Matsumoto 1980). This model has a halfwidth of the ACF that is independent of the scattering angle and is similar to that of freely rotating linear molecules (Berne and Pecora 1976).

It is important to establish the relation between the translation speed and the rotation frequency, and its influence on the light scattering data. The present study investigated human spermatozoa and how the dynamic properties in scattered light depended on the scattering angle. These properties have never been investigated in detail before for human spermatozoa. Special interest was focused on the influence of coupling between the translation speed and the rotation frequency.

Abbreviations: ACF, Autocorrelation function; $\tau_{1/2}$, halfwidth; RGD, Rayleigh-Gans-Debye; SD, Standard deviation

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The relation between translation speed and rotation frequency in human spermatozoa was investigated by introducing a propelling efficacy as a ratio between these two properties. Our data show that this parameter has a much larger variation than can be explained from the errors and supports previous observations that the propelling efficacy of human spermatozoa depends on experimental conditions.

Previous examinations of the speed distribution of human spermatozoa have come to different conclusions. One study proposed a "Saclay" distribution (Jouannet et al. 1977), which is a skewed speed distribution, while other studies proposed rather symmetrical distributions (Shimizu and Matsumoto 1977; Makler et al. 1981). We have chosen a Maxwell function for both the translation speed and the rotation frequency. It is well known from kinetic gas theory and provides a closed solution of the ACF. Like the "Saclay" distribution the Maxwell distribution is also skewed, although less so. Furthermore, Maxwell distributed speeds and rotation frequencies were assumed in the previous study that showed a good correlation between light scattering and video micrography of the corresponding properties (Gottlieb et al. 1991).

Experimental

Material

Fresh samples from healthy donors were used to investigate the halfwidth of the intensity autocorrelation function (ACF) and its dependence on the scattering angle. The concentration was about 100 million spermatozoa per millilitre. The measurements were carried out at 20 °C. Sixty-four other samples were used to investigate the methodological and biological variance of the mean translation speed, rotation frequency and propelling efficacy. They had 6 to 250 million spermatozoa per millilitre and a high rate of motile spermatozoa as estimated by means of microscopic inspection. The samples were obtained from healthy donors and patients at the Department of Andrology, Karolinska Hospital, Stockholm.

Methods

We have studied the scaling properties of the scattered light by means of the half-width ($\tau_{1/2}$) of the intensity autocorrelation function (ACF) and its dependence on the scattering angle. The spermatozoa were modelled as rotating and translating linear objects. Two cases were investigated: one where the translation speed and the rotation frequency were coupled and one where these two properties were uncoupled. The $\tau_{1/2}$ of the models were calculated with reasonable values of the parameters (Table 1) and compared with experimental data at different scattering angles.

In order to estimate the degree of coupling between the translation speed and the rotation frequency we determined the correlation between these two properties from measurements. The ratio between the translation speed and the rotation frequency was called propelling efficacy. In particular, the variance of this property was investigated. An expectation value of the standard deviation was determined from the methodological errors in the mean translation speed and the mean rotation frequency. The expectation value was compared with the actual standard deviation of the propelling efficacy.

Table 1. Parameter values in the calculation of the halfwidth of the autocorrelation function

Parameter	Value
Mean rotation frequency	4.5 Hz
Rotation diffusion constant	2.0 Hz
Fraction of swimming spermatozoa	0.7
Mean translation speed	60 μm/s
Wavelength of laser light	514.5 nm

The halfwidth of the intensity autocorrelation function. The halfwidth was defined as the time shift $\tau_{1/2}$ for which the intensity ACF decreased to half of its amplitude

$$G^{(2)}(\tau_{1/2}) = 0.5\,(G^{(2)}(0) + G^{(2)}(\infty)) \quad (1)$$

Relation between the field and the intensity autocorrelation function. The Gaussian approximation (Berne and Pecora 1976) can most likely be applied in the analysis of our data because it applies for ligth scattering by sea chestnut spermatozoa with a system similar to ours (Shimizu and Matsumoto 1976). This implies that the relation between the intensity and the field ACF is

$$G^{(2)}(\tau) = A\,[G^{(1)}(\tau)]^2 + B \quad (2)$$

where B corresponds to the mean square of the scattered intensity and A/B is a property that decreases with increasing number of detected coherence areas (Berne and Pecora 1976).

Translational motions. We calculated the mean translation speed by assuming the spermatozoa to scatter the light like point particles. The field autocorrelation function (ACF) for point particles moving in straight lines over distances much longer than the reciprocal scattering wave vector $\boldsymbol{q}$ (Nossal 1971) is

$$G_T^{(1)}(\tau) = \alpha \int p(v)\,\frac{\sin(q v \tau)}{q v \tau}\,dv + (1-\alpha)\,e^{-q^2 D_T \tau} \quad (3)$$

where v is the translation speed, q the scattering wave vector, α the fraction of actively moving spermatozoa and D_T the diffusion constant. The scattering wave vector, $\boldsymbol{q} = 4\pi n \sin(\theta/2)/\lambda$, depends on the scattering angle θ, the wavelength of the light λ and the refractive index n.

The second term appears from the fraction of particles moving by translational diffusion only. We approximated this term with a constant $1-\alpha$ because diffusing spermatozoa have a much longer mean decay than actively moving spermatozoa. The mean decay of the intensity ACF $1/(2 D_T q^2)$ may be estimated for purely diffusing particles using the Stokes relation $D_T = k T/(6\pi\eta R_h)$. It is 0.5 seconds for spheres with a hydrodynamic radius $R_h = 2$ μm

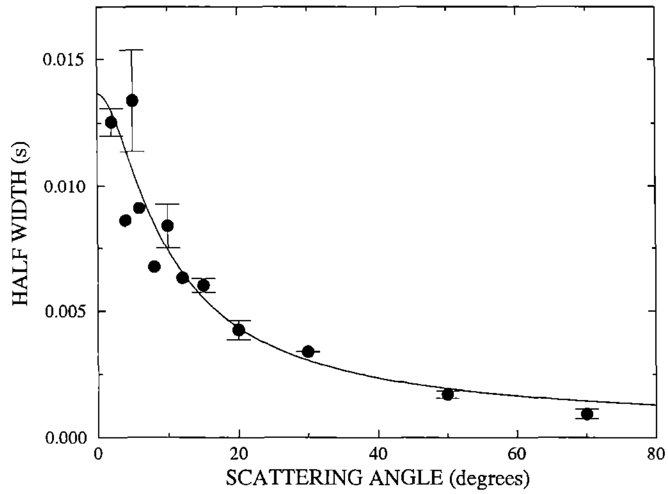

Fig. 1. Halfwidth of intensity autocorrelation function of dynamic laser light scattering from human spermatozoa as measured (*dots*) and calculated from a model of freely rotating and translating linear objects (*solid line*). The standard deviation is indicated with errror bars at some scattering angles

in a solution with a viscosity $\eta = 4\,\text{cP}$ at room temperature, a scattering angle $\theta = 22°$ and a wavelength $\lambda = 633$ nm of the light. The corresponding mean decay in experiments was normally about 50 times faster.

Contributions from all particles with various speeds may be integrated by introducing a speed distribution function, $p(v)$. We have used a Maxwell function

$$p(v) = 4\pi \left(\frac{3}{2\pi \langle v^2 \rangle}\right)^{3/2} v^2\, e^{-3v^2/2\langle v^2 \rangle}, \tag{4}$$

for the fraction of spermatozoa with active motion. The Maxwell speed distribution function is characterised by $\langle v^2 \rangle$, which relates directly to the square of the mean translation speed, $\langle v \rangle^2 = 8\langle v^2 \rangle/(3\pi)$.

The point scattering model (3) with a Maxwell speed distribution (4) yields a Gaussian shaped field ACF (Hallett et al. 1978; Thyberg and Rigler 1986)

$$G_T^{(1)}(\tau) = \alpha\, e^{-q^2 \langle v^2 \rangle \tau^2/6} + (1-\alpha). \tag{5}$$

An expression for the halfwidth of the intensity ACF can be derived

$$\tau_{1/2} = \sqrt{-6 \ln\left(1 - \frac{1 - \sqrt{\frac{1+[1-\alpha]^2}{2}}}{\alpha}\right) \Big/ (q^2 \langle v^2 \rangle)} \tag{6}$$

from the definition of the halfwidth of intensity ACF (1) and the relation between the intensity and the field ACF (2).

Rotational motions. The rotation frequency was assessed from the *depolarized* component of the scattered light by modelling the spermatozoon as a chain of rotatable rigid ellipsoids of revolution (Shimizu and Matsumoto 1980). The field ACF of such a model

$$G_R^{(1)}(\tau) = e^{-6D_R\tau}\,[\alpha \textstyle\int p(\omega)\, P_2(\cos\omega\tau)\, d\omega + (1-\alpha)], \tag{7}$$

includes an angle frequency ω (which is related to the rotation frequency, $f = \omega/2\pi$) of the rotational motion and its distribution function $p(\omega)$. P_2 is the second order Legendre polynomial. The fraction, $1-\alpha$, of spermatozoa with no active motions are diffusing as well as that fraction, α, with active motions. The rotational diffusion constant D_R corresponds to rotational diffusion of the flagella. The same expression describes the correlation function of freely rotating linear molecules (Berne and Pecora 1976). This model and an assumed Maxwell distributed angular frequency (4) express the field ACF analytically (Rigler and Thyberg 1984)

$$G_R^{(1)}(\tau) = e^{-6D_R\tau}\{\alpha[(\tfrac{3}{4} - \langle \omega^2 \rangle \tau^2)\, e^{-2\langle \omega^2 \rangle \tau^2/3} - \tfrac{3}{4}] + 1\}. \tag{8}$$

An expression that allows a numeric determination of the halfwidth of the intensity ACF can be derived

$$e^{-12D_R\tau}\{\alpha[(\tfrac{3}{4} - \langle \omega^2 \rangle \tau^2)\, e^{-2\langle \omega^2 \rangle \tau^2/3} - \tfrac{3}{4}] + 1\}^2 = \tfrac{1}{2}, \tag{9}$$

from the definition of the halfwidth of intensity ACF (1) and the relation between the intensity and the field ACF (2).

Uncoupled translational and rotational motions. A model with linear objects with statistically independent translational and rotational motions allows for separate treatment of each part (Berne and Pecora 1976). Such an assumption leads to a field autocorrelation function (ACF) that is a product between the two parts.

$$G_{\text{UTR}}^{(1)}(\tau) = e^{-6D_R\tau} \cdot \left[\alpha \int p(v) \frac{\sin(qv\tau)}{qv\tau}\, dv \int p(\omega)\, P_2(\cos\omega\tau)\, d\omega + (1-\alpha)\right]. \tag{10}$$

Both integrals may be solved independently.

$$G_{\text{UTR}}^{(1)} = e^{-6D_R\tau}\{\alpha[(\tfrac{3}{4} - \langle \omega^2 \rangle \tau^2)\, e^{-2\langle \omega^2 \rangle \tau^2/3} + \tfrac{1}{4}] \cdot e^{-q^2\langle v^2 \rangle \tau^2/6} + (1-\alpha)\}. \tag{11}$$

The halfwidth of the intensity ACF was calculated by numerical methods.

Coupled translational and rotational motions. A model for coupled translational and rotational motions means that the translation speed is proportional to the rotation frequency, $v = k\omega$. The field ACF

$$G_{\text{CTR}}^{(1)}(\tau) = e^{-6D_R\tau}\left[\alpha \int p(\omega) \frac{\sin(kq\omega\tau)}{kq\omega\tau}\, P_2(\cos\omega\tau)\, d\omega + (1-\alpha)\right]. \tag{12}$$

was calculated by numerical integration with Simpson's method. The halfwidth of the intensity ACF was then obtained from the definition of the halfwidth (1) and the relation between the intensity and the field ACF (2).

Evaluation of the dynamic parameters. The parameters of (5) and (8) have been evaluated by means of least-squares non-linear fitting (Marquardt 1963).

Correlation coefficient. $$r = \frac{\sum_i (x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\left(\sum_i [x_i - \bar{x}]^2\right)\left(\sum_i [y_i - \bar{y}]^2\right)}} \tag{13}$$

The methodological error in $\langle v \rangle$ *and* $\langle f \rangle$. Three measurements or more were taken on each sample to determine the mean translation speed $\langle v \rangle$ (5) and the mean rotation frequency $\langle f \rangle$ (8). Only those samples with a well-defined value of $\langle v \rangle$ and $\langle f \rangle$ were selected. The variance of each property was smaller than the mean value in at least 2 measurements. The methodological errors of $\langle v \rangle$ and $\langle f \rangle$ were estimated by the standard deviation of each property for each sample.

The propelling efficacy and its error estimate. The propelling efficacy ε was defined as the ratio between $\langle v \rangle$ and $\langle f \rangle$ and the error estimate by

$$\delta\varepsilon = \sqrt{\left(\frac{\delta \langle v \rangle^2}{\langle f \rangle}\right)^2 + \left(\frac{\langle v \rangle\, \delta \langle f \rangle}{\langle f \rangle^2}\right)^2} \qquad (14)$$

where $\delta \langle v \rangle$ and $\delta \langle f \rangle$ were the methodological errors in $\langle v \rangle$ and $\langle f \rangle$.

Apparatus. The measurements at various scattering angles were performed at a temperature of 22 °C with an argon-ion laser (wavelength $\lambda = 514.5$ nm) and a digital correlator (Langley-Ford model 1096). The system has previously been described elsewhere (Rigler and Thyberg 1984). The series of measurements to estimate the methodological errors of speed, rotation frequency and propelling efficacy were performed at 37 °C with an He-Ne laser ($\lambda = 632.8$ nm), a PC-based digital correlator and a photomultiplier detector (Hamamatsu R 928).

Both the systems had vertically polarized incident light and two pinholes defining a detection area smaller than or approximately equal to one coherence area. The rotation frequency was assessed from the horizontally polarized light component (depolarized light detection) at a scattering angle of 0°.

Results

The halfwidth ($\tau_{1/2}$) of the experimental intensity autocorrelation function (ACF) decreased from about 13 ms at 2° to 0.9 ms at 70° (Fig. 1). The same dependence on the scattering angle was found for the model with uncoupled translation speed and rotation frequency (11). The $\tau_{1/2}$ calculated with this model deviated no more than the natural variances of the experiments at scattering angles up to 50°. A coupled translation speed and rotation frequency (12) had an almost identical $\tau_{1/2}$ (Fig. 2) to that of uncoupled motions (10). At a scattering angle of 0° these models, as well as the model dealing with rotational motions only (9), had an identical $\tau_{1/2}$. The point scattering model (6) on the other hand, describing only translational motions deviated much from all the other models at small scattering angles. At scattering angles larger than 20°, however, the point scattering model deviated very little from the models comprising both translational and rotational motions.

The product between the scattering wave vector and the halfwidth ($q\tau_{1/2}$) increased systematically with increasing scattering angle at angles smaller than 15° (Fig. 3) and remained constant between 15° and 50°.

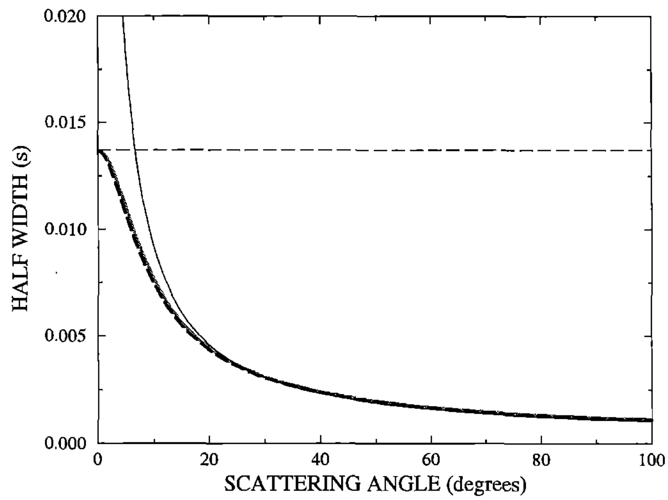

Fig. 2. Halfwidth of intensity autocorrelation function of dynamic laser light scattering calculated from different models. Human spermatozoa were modelled as translating point particles (*solid line*), freely rotating linear objects (*thin dashed line*), linear objects with a translation speed coupled to the rotation frequency (*solid grey line*) and linear objects with a translation speed uncoupled to the rotation frequency (*thick dashed line*)

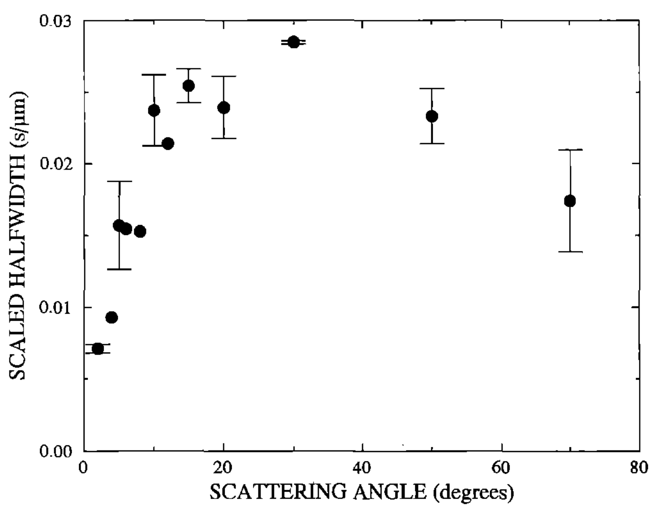

Fig. 3. The product between the scattering wave vector and the half width of the intensity autocorrelation function of light scattered from human spermatozoa at various scattering angles

We found that the mean translation speed $\langle v \rangle$ increased with increasing mean rotation frequency $\langle f \rangle$ (Fig. 4). A linear regression between $\log(\langle v \rangle)$ and $\log(\langle f \rangle)$ was carried out in order to find the best fitting polynomial of the type $\langle v \rangle = \varrho \langle f \rangle^n$ between the properties. We got $n = 1.05$, which suggested a linear relationship between $\langle v \rangle$ and $\langle f \rangle$. The correlation coefficient (13) was $r = 0.74$. A linear regression between $\langle v \rangle$ and $\langle f \rangle$ had a similar value, $r = 0.75$.

The propelling efficacy $\varepsilon = \langle v \rangle / \langle f \rangle$ among different samples had an average value of 13.6 μm and a relative standard deviation of 43%. The distribution (Fig. 5 c) was wider than expected from the methodological errors in $\langle v \rangle$ and $\langle f \rangle$. The expected relative error $\delta\varepsilon$ (14) was on average 27%. Corresponding distributions of $\langle v \rangle$

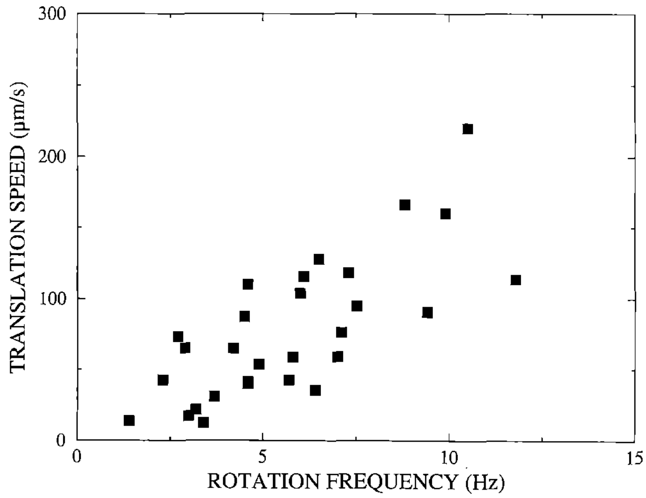

Fig. 4. The relation between the mean translation speed $\langle v \rangle$ and the mean rotation frequency $\langle f \rangle$ in human spermatozoa

(Fig. 5a) and $\langle f \rangle$ (Fig. 5b) were also wider than the methodological errors. The methodological errors were on average 7% for $\langle v \rangle$ and 24% for $\langle f \rangle$.

Discussion

The relation between the dynamic components of the scattered light and the scattering angle depends on the structure and motility of the scattering objects. A previous study concluded that spermatozoa from pig and abalone scatter light like point particles (Shimizu and Matsumoto 1977). However, the conclusion was based on measurements at two scattering angles only: 45° and 90°. Another study proposed that bull spermatozoa act as Rayleigh-Gans-Debye (RGD) approximated rotating and translating ellipsoids (Craig and Hallett 1982). The halfwidth ($\tau_{1/2}$) of the intensity autocorrelation function (ACF) of the model predicts the features seen in the experimental scaling curves, however not the absolute values. The conclusion in the latter study was based on measurements at many scattering angles, all above 15°.

We have studied the dynamic components of scattered laser light from human spermatozoa at a range of scattering angles from 2° to 70°. Particularly the results for scattering angles smaller than 15° revealed new and interesting features. The product between the scattering wave vector $\boldsymbol{q}$ and $\tau_{1/2}$ increased sharply with increasing scattering angle (Fig. 3). None of the previous light scattering models can explain this. The point scattering model has a constant $q\tau_{1/2}$ and RGD approximated rotating and translating ellipsoids does not seem to have that steep increase in $q\tau_{1/2}$ with increasing scattering angle either (Craig et al. 1982). Neither could it have been caused by Brownian particles. Such particles have a decreasing $q\tau_{1/2}$ with increasing scattering angle since the $\tau_{1/2}$ for diffusing particles is proportional to q^{-2} (Cummins et al. 1969).

Our model (11) explained the features in the experiments very well. The ACF as a product of two decaying parts had a $\tau_{1/2}$ that was determined primarily by the one

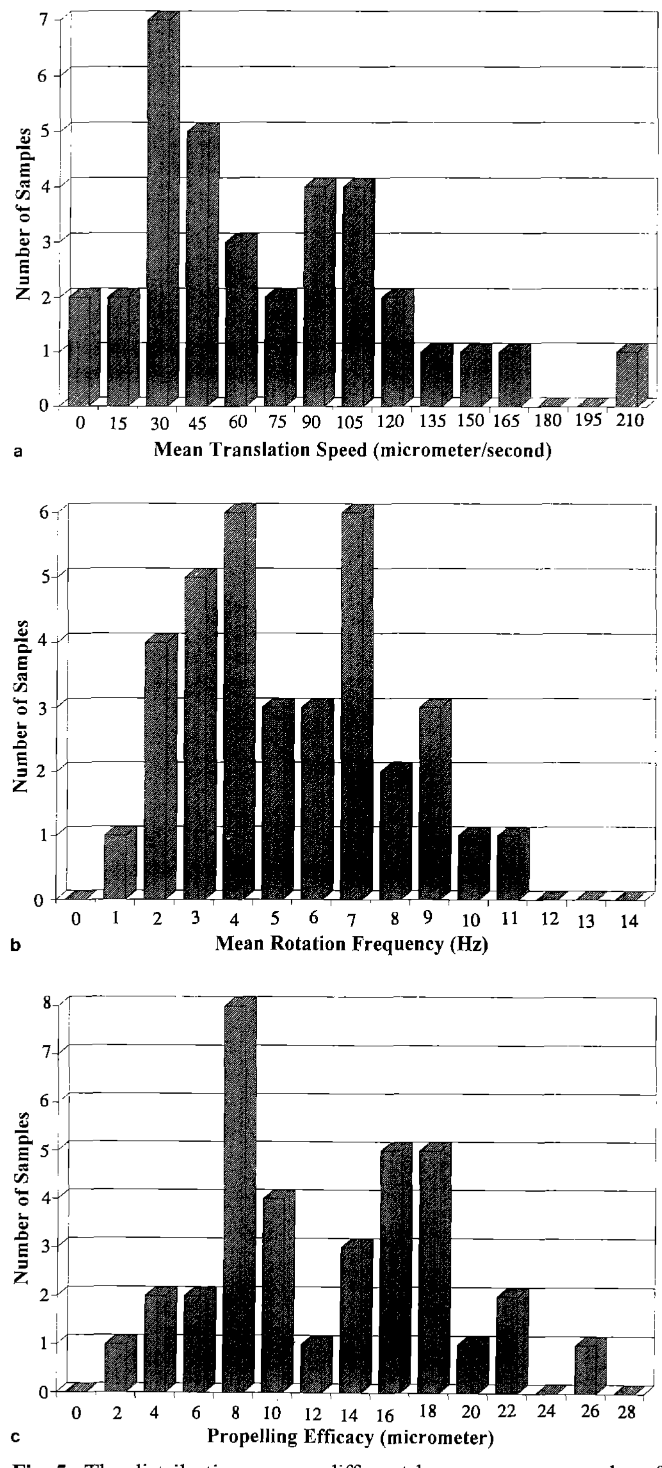

Fig. 5. The distribution among different human sperm samples of **a** the mean translation speed, **b** the mean rotation frequency and **c** the propelling efficacy

with the fastest decay. The mean decay was constant for the part with rotational motions while it decreased with increasing scattering angle for the part concerning translational motions. At a scattering angle of 0° the scattering wave vector is zero and consequently the translational part decayed infinitely slowly. Rotational motions therefore dominated the ACF at very small scattering angles. That was also the reason why the model describing rotational motions only (7) had an identical $\tau_{1/2}$ at zero angle (Fig. 2).

The contribution from translational motions increased with increasing scattering angle and was dominating at angles larger than 20°. At these angles the ACF could as well be approximated with the point scattering model (3), which leads to a constant $q\tau_{1/2}$ at large scattering angles. It should be pointed out that at these angles the size of the sperm head, which is the main scatterer, is of the same order as the inverse scattering wave vector $\boldsymbol{q}$. Exceeding this limit by either increased size or decreased q may introduce other contributions to $\tau_{1/2}$ than those from translational motions.

The ACF of the proposed model with uncoupled motions (10) was derived with the assumption that the translational and rotational motions were statistically independent. This assumption had no influence on the results, because the $\tau_{1/2}$ of the models with uncoupled and coupled motions (12) were approximately equal (Fig. 2). They matched the experimental $\tau_{1/2}$ very well over a large range of scattering angles (Fig. 1). The two parts of the model, one of which describes rotational motions and the other translational motions, have properties that correlate very well with those obtained with another method, video micrography (Gottlieb et al. 1991). Thus the present model has at least two excellent features. Not only does it describe the scattering angle dependence correctly, its dynamic properties also correlate very well with those obtained from direct analysis of the motional trajectories (video analysis).

Bull spermatozoa have a translation speed that is proportional to the rotation frequency (Rikmenspoel et al. 1960). We found a high correlation between these properties for human spermatozoa as well (Fig. 4). Within the accuracy of the method, however, one would expect an even better correlation. An error estimate of the correlation was provided by the propelling efficacy since it is the ratio between the translation speed and the rotation frequency. The measured propelling efficacy had a relative standard deviation (SD) of 43% as compared to the expected one of 27%. We interpret this as an indication of biological variances within the samples. We have previously observed populations of human spermatozoa with different propelling efficacy. The population selected by swim-up methods had higher propelling efficacy than those in fresh semen (Gottlieb et al. 1991). The propelling efficacy might therefore contain important information about swimming characteristics.

It could be argued that special types of motions, such as helical motions, may influence the relation between $\langle v \rangle$ and $\langle f \rangle$ at different q values. From our analysis it was evident that $\tau_{1/2}$ can be described by a unique set of $\langle v \rangle$ and $\langle f \rangle$ over the whole range of q investigated (Fig. 1). This means that we do not see such influence, if it exists, within the significance of our data.

A Maxwell function has a SD of approximately 0.42 of the mean, which means that the speed of a randomly chosen spermatozoon deviates on average 42% from the mean. The SD decreases with the number of spermatozoa as $1/\sqrt{n}$. Each measurement contained information from about 10^2 to 10^4 spermatozoa depending on the sperm concentration, the fraction of motile cells, the mean translation speed and the time span of a measurement. With these numbers and a Maxwell probability function for the speed the SD should be about 1% for both variables, not 7% for $\langle v \rangle$ or 24% for $\langle f \rangle$ that we got. We think that the accuracy did not reach the theoretical value because fresh semen samples contain light scattering particles other than spermatozoa. At least the procedure to evaluate $\langle v \rangle$ (5) by model fittings did not influence the accuracy of that property. The relative SD for $\langle v \rangle$ and the halfwidth of the ACF were approximately equal. Furthermore, sperms that are selected by means of swim-up procedures normally have a better accuracy in $\langle v \rangle$ and $\langle f \rangle$.

The model for rotational motions (7), in contrast to translational motions, had correlations between the properties, which decreased the accuracy. The property $\langle f \rangle$ had a bigger relative SD than the relative halfwidth of the ACF. One reason contributing to this is that the model has one more variable than the model for translational motion. We have, for example, found that the model with uncoupled translational and rotational motions (10) had worse accuracy. This model has even more variables. It is therefore better to evaluate $\langle v \rangle$ and $\langle f \rangle$ separately at appropriate scattering angles than to try to assess both properties simultaneously.

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