Influence of sperm movement parameters on human sperm–oolemma fusion

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Flagellar dyskinesia is characterized by abnormal sperm movement parameters and a negative sperm mucus penetration test. It is associated with structural pathologies of the axonemal complex (lack of outer dynein arms), of the periaxonemal complex (sliding spermatozoa and periaxonemal dyskinesia), or of both structures (short flagella). Even during *in vitro* fertilization, dyskinesia prevents the spermatozoon from getting through the egg vestment. However, in some cases, fertilization has been achieved using subzonal insemination. Flagellar dyskinesia is therefore an interesting model for investigating the role of sperm movement in the fusion process between the spermatozoon and the oolemma. Thirty-one patients requiring assisted fertilization were included in the study. Fifteen had spermatozoa in which the flagellum lacked outer dynein arms, 11 had anomalies of the periaxonemal complex (five with sliding spermatozoa and six with periaxonemal dyskinesia) and five had spermatozoa with short flagella. Seven men who produced spermatozoa with normal movement were selected as controls. Movement was evaluated using a computer-assisted analyser, and penetration was assessed using zona-free hamster eggs. At 37°C, in semen, the dyskinetic spermatozoa had reduced straight line and curvilinear velocity and lateral head displacement compared with controls ($P < 0.01$). In the Percoll-selected sperm suspension, the only difference was that spermatozoa with periaxonemal anomalies maintained a narrow lateral head displacement compared with the controls ($P < 0.001$). After 3 h of incubation at 37°C, the lateral head displacement of dyskinetic spermatozoa had not changed, while that of the controls showed a significant increase (4.5 to 5.6 μm ; $P < 0.05$). The results from the sperm penetration assay for the spermatozoa lacking outer dynein arms were lower than those of the controls (47% versus 77%; $P < 0.05$) and the results for sliding spermatozoa and spermatozoa with periaxonemal dyskinesia were even lower (25% and 34%, respectively; $P < 0.01$). The fertilization rates after subzonal insemination were 46.5% for spermatozoa lacking outer dynein arms, 36.1% for spermatozoa with short flagella, 24.8% for sliding spermatozoa and 17.3% for spermatozoa with periaxonemal dyskinesia. There was a significant correlation between the curvilinear velocity of the Percoll-selected sperm suspensions and their fertilization rates after subzonal insemination ($r = 0.5$; $P < 0.05$) and their sperm penetration assays ($r = 0.7$; $P < 0.001$). The data provide evidence that sperm velocity is correlated with the ability to fuse with the oolemma.

Introduction

In addition to the percentage of motile forms, sperm movement parameters are critical to the fertilizing ability of spermatozoa. Indeed, adequate lateral head displacement of the spermatozoon is required for it to penetrate the cervix (Aitken *et al.*, 1986) and abnormal movement prevents spermatozoa from getting through the cervical mucus (Aitken *et al.*, 1985, 1986;

Mortimer *et al.*, 1986; Katz *et al.*, 1989). In cases of flagellar dyskinesia, standard *in vitro* fertilization (IVF) techniques resulted in low or even nil fertilization rates (Wolf *et al.*, 1993). Different aetiologies have been described for flagellar dyskinesia (Jouannet *et al.*, 1983; Escalier *et al.*, 1984, 1990; Feneux *et al.*, 1985). Diagnosis of flagellar dyskinesia is based on three criteria: (1) on the demonstration of abnormal sperm movement by computer-assisted semen analysis; (2) on tests of functional sperm movement inefficiency, such as negative cervical mucus penetration tests (David *et al.*, 1993); and (3) by electron

microscope examination of the flagella, to identify structural abnormalities. Dyskinesia results from either a modification of the axonemal complex, such as the lack of outer dynein arms (Jouannet *et al.*, 1983; Escalier *et al.*, 1984) or of the periaxonemal structures such as sliding spermatozoa (Feneux *et al.*, 1985; Serres *et al.*, 1986), or other specific periaxonemal dyskinesia. When both the axonemal and the periaxonemal structures are truncated, the result is the syndrome of the short flagella. However, sperm movement parameters are affected differently by the various types of flagellar dyskinesia and dyskinesia does not prevent the spermatozoon from fusing with the oolemma. It has been shown that sperm penetration performed with spermatozoa lacking outer dynein arms is half of that of controls (Courtot *et al.*, 1985). Several cases of pregnancy have been reported that resulted from subzonal insemination with spermatozoa lacking outer dynein arms where previous IVF attempts had failed (Wolf *et al.*, 1993). In addition, 52 of 90 human oocytes were fertilized when subzonal insemination was performed compared with none of 23 oocytes when standard IVF was used (Wolf *et al.*, 1993). Subzonal insemination is, therefore, a useful model for studying the influence of sperm movement parameters on the sperm-oolemma fusion process. In the present work the movement parameters of spermatozoa were studied in semen, in Percoll-selected sperm suspension and after incubation for 3 h in a capacitation medium. The correlation between these data and the fertilizing ability of the spermatozoa was evaluated using sperm penetration assays and their fertilization rates after subzonal insemination.

Materials and Methods

Patients

Thirty-one men with infertility characterized by extremely low fertilization rates during IVF or, more often, complete fertilization failure and a negative cervical mucus penetration test were included in the study. Spermatozoa from these patients underwent a movement analysis using a computer assisted sperm analysis system. When abnormal sperm movement parameters were found (Jouannet *et al.*, 1983; Feneux *et al.*, 1985), spermatozoa were further analysed by electron microscopy. Spermatozoa from fifteen patients lacked outer dynein arms; on each section of the flagella at least six outer dynein arms were lacking. Eleven of these patients presented this anomaly in 100% of their spermatozoa, and the remaining four had at least 60% of their spermatozoa with this abnormality (Fig. 1). Eleven patients presented periaxonemal anomalies. The periaxonemal structures of these patients were modified without specific associated axonemal anomalies. Among these anomalies, modifications in the order of termination of the dense fibres, or of their number, and malformation of the longitudinal columns were found: in this group with periaxonemal anomalies, five patients had sliding spermatozoa with an abnormal order of termination of the dense fibres along the principal piece and often an abnormal number or position of their longitudinal columns. Movement of sliding spermatozoa is characterized by a very small amplitude of the flagellar

Fig. 1. Electron micrograph of the principal piece of human sperm flagella. The outer dynein arms are absent. Scale bar represents 1 µm.

bend and a discordance between the high beat frequency and the low cell rotation frequency. The remaining six patients in this group presented other types of periaxonemal dyskinesia (Fig. 2) with unilateral thickening of the fibrous sheet instead of individual column and anomalies of the dense fibres. These anomalies are associated with a slow development and propagation of the flagellar wave. The five other patients in the study had short flagella syndrome characterized by a very short principal piece and disorganization of the axonemal and periaxonemal structures. For all these patients, the relevant anomaly was found in at least 60% of their spermatozoa. Sixty-five attempts using subzonal insemination were carried out involving 551 oocytes.

Seven men were selected for the control group. They belonged to a group of patients with unexplained IVF failure who were included in the subzonal insemination programme. Their spermatozoa had normal movement parameters, presented a positive cervical mucus penetration test and a zona-free hamster egg penetration test result of over 60%.

Processing of spermatozoa

Ejaculates were collected by masturbation at the laboratory after 3 days of sexual abstinence. An aliquot was kept for determination of semen characteristics and sperm movement analysis using computer-aided sperm analysis. The spermatozoa were layered over a two-step (47.5% and 95%) Percoll gradient (0.5 ml per layer) and centrifuged for 20 min at 300 *g*. The pellet containing the highly motile sperm fraction was removed and washed twice by centrifugation for 10 min at 600 *g* with 5 ml B2 medium (Api System, Marcy l'Etoile). The final sperm pellets were resuspended in 0.2–0.5 ml B2 medium. The sperm movement parameters were then determined again

Fig. 2. Electron micrographs of human sperm flagella showing periaxonemal anomalies. (a) Normal position of the two longitudinal columns; (b) and (c) abnormal position of the longitudinal column; (d) and (e) only one column is present, in normal (d) and abnormal (e) position. (f) Thickening of the fibrous sheath considered as a third column. Scale bars represent 0.1 μm .

in the Percoll selected sperm suspensions and after incubation for 3 h at 37°C in 5% CO_2 in air.

Analysis of spermatozoa

The percentage of motile spermatozoa was estimated under a microscope ($\times 200$) using a Bausch and Lomb CAT 312 995 $\times 2$ objective lens and a CCD camera. For morphological analysis, 100 spermatozoa were examined ($\times 1000$) on a Shorr-stained smear (Shorr Staining; Merck, Darmstadt) and classified according to the criteria of David *et al.* (1975). The motility of the aliquots of Percoll-selected sperm suspensions was analysed at 37°C using a computer-assisted semen analyser (HTM 2030 version 7.2; Hamilton-Thorn Research, Danvers, MA). The following settings were used: frame at frame rate, 20 at 25 s^{-1} ; minimum contrast, 8; minimum size, 10; low and high size gates, 0.6 and 1.8, respectively; low and high intensity gates, 0.6 and 1.7, respectively; non-motile head size, 7; non-motile intensity, 110; medium average path velocity value, 25 $\mu\text{m s}^{-1}$; low average path velocity value, 10 $\mu\text{m s}^{-1}$; slow cell motile, yes; percentage progressive cells equated with a straightness of $> 80\%$, regardless of average path velocity. An aliquot of the sperm suspension was diluted to 10^6 cells ml^{-1} with B2 medium and loaded into a 200 μm deep flat glass microcapillary tube (Vitro Dynamics, Rockaway, NJ) that was transferred to the analyser at 37°C. At least 150 motile spermatozoa were scored to obtain mean values for the following movement parameters: curvilinear velocity, straight line velocity, amplitude of lateral head displacement, linearity (straight line velocity/curvilinear velocity), and beat cross frequency.

Electron microscope study of sperm flagella

Semen samples were fixed for 1 h in 2.5% (v/v) glutaraldehyde in 0.1 mol Sørensen's buffer l^{-1} , washed in buffer supplemented with 4% (w/v) sucrose before being embedded in 2% agar. After dehydration in a graded series of ethanol, small pieces of agar were embedded in Araldite. Sections (0.05 μm) were stained with uranyl acetate (4% (w/v) in 70% (v/v) ethanol, for 20 min) and 10% (w/v) lead citrate (10 min) and examined in a Siemens Elmiskop CT 150 transmission electron microscope.

The ultrastructural assessment was made on transverse sections through the sperm tails which provide quantitative observations on the various axonemal and periaxonemal structures, and on longitudinal sections, which provide non-quantitative observations, such as the extent of the anomaly along the tail and possible associations. Dynein arms were considered to be absent from a section if at least six of the nine elements were missing.

Zona-free hamster egg penetration assay

Sexually mature, female golden hamsters were super-ovulated by an injection of 40 iu pregnant mares' serum gonadotrophin (Folligon; Intervet, Angers) and ovulation was induced with 40 iu hCG (Chorulon; Intervet, Angers) 48 h later, beginning on day 1 of the oestrous cycle. The oocytes were collected in BWW medium supplemented with 3.0 mg HSA ml^{-1} fraction 5 (Sigma, St Louis, MO). Cumulus cells were removed by brief exposure to 0.1% (w/v) hyaluronidase type III (Sigma). Zonae pellucidae were removed using 0.1% bovine trypsin (0.1% w/v) (Sigma).

Table 1. Human sperm movement parameters analysed using computer-assisted sperm analysis in semen, after Percoll selection and after 3 h of incubation in the capacitation medium

Type of spermatozoa	n	In semen				Sperm movement parameters Percoll-selected spermatozoa				Percoll selected spermatozoa after 3 h incubation			
		VSL ($\mu\text{m s}^{-1}$)	VAP ($\mu\text{m s}^{-1}$)	VCL ($\mu\text{m s}^{-1}$)	ALH (μm)	VSL ($\mu\text{m s}^{-1}$)	VAP ($\mu\text{m s}^{-1}$)	VCL ($\mu\text{m s}^{-1}$)	ALH (μm)	VSL ($\mu\text{m s}^{-1}$)	VAP ($\mu\text{m s}^{-1}$)	VCL ($\mu\text{m s}^{-1}$)	ALH (μm)
LODA	15	17.8 ± 5.1 ^a	25.7 ± 5.2 ^a	34.4 ± 7.3 ^a	2.3 ± 0.9 ^a	29.8 ± 6.3 ^a	42.5 ± 11.0 ^a	60.5 ± 14.3 ^a	3.8 ± 1.5	34.2 ± 10.0 ^a	49.1 ± 10.1 ^a	66.4 ± 9.0 ^a	4.3 ± 1.7
PA	11	19.2 ± 5.2 ^a	23.1 ± 5.6 ^a	29.1 ± 8.7 ^a	1.9 ± 0.7 ^a	38.1 ± 16.5 ^c	45.7 ± 16.1 ^c	53.7 ± 17.8 ^a	2.9 ± 0.9 ^a	43.9 ± 17.3 ^c	50.6 ± 15.7 ^c	59.0 ± 16.1 ^a	3.0 ± 0.9 ^a
SF	5	21.7 ± 6.8 ^a	27.7 ± 4.9 ^a	33.3 ± 3.8 ^a	2.0 ± 0.1 ^a	46.0 ± 8.9 ^c	51.3 ± 7.5 ^c	60.0 ± 7.8 ^c	3.6 ± 1.9	49.0 ± 9.6	53.0 ± 6.2	62.7 ± 3.2	3.6 ± 1.9
NS	7	39.8 ± 8.9	48.0 ± 10.1	62.7 ± 13.0	4.0 ± 0.9	80.7 ± 22.8	88.3 ± 21.7	99.6 ± 19.3	4.5 ± 0.4	83.6 ± 17.2	94.3 ± 15.9	113.4 ± 12.9	5.6 ± 1.2 ^b

Results are given as mean ± SD. LODA: lack of outer dynein arms; PA: periaxonemal dyskinesia; SF: short flagella syndrome; NS: normal spermatozoa (control group). VSL: straight line velocity. VAP: average path velocity. VCL: curvilinear velocity; ALH: lateral head displacement.

^aSignificantly different from controls ($P < 0.001$). ^bSignificantly different from ALH in the Percoll-selected sperm suspension ($P < 0.05$). ^cSignificantly different from controls ($P < 0.01$).

Semen was collected on the day before the test and was allowed to liquefy for 30 min at 37°C. After determination of the sperm characteristics, it was layered over a two-step Percoll gradient (95% and 47.5%) and centrifuged for 20 min at 300 g. BWW medium, with an osmolality of 410 mosmol kg⁻¹, was used for washing and recovery of the pellet. Volume, number, motility and morphology of spermatozoa were assessed in the Percoll-selected sperm suspension. The suspension then underwent capacitation for 20 h at 20°C in air before use. Five oocytes were incubated for 3 h per 30 µl drop containing 2.5×10^6 motile spermatozoa ml⁻¹ under mineral oil. Incubation was performed in air with 5% CO₂ and thirty oocytes were used per test. At the end of the 3 h period, the oocytes were washed, transferred to a clean microscope slide and compressed to a depth of approximately 30 µm with a 22 mm × 22 mm coverslip mounted on four paraffin wax supports. Control incubations were carried out using a pool of frozen donor spermatozoa and usually resulted in more than 60% of the oocytes containing decondensed sperm heads.

Oocyte preparation

Superovulations were performed in the Gynaecological Departments of the Hôpital A. Beclere (R. Frydman, Clamart), of the Hôpital International de la Cité Universitaire (Paris) (Ph. Granet and G. Sarrot, Paris), of the Hôpital Saint Vincent de Paul (Paris) (S. Epelboin), and of the Hôpital Jean Verdier (Bondy) (J. N. Hugues). Follicular growth was stimulated by human menopausal gonadotrophins (hMG) associated with a GnRH agonist. Ovulation was induced by administration of 10 000 iu hCG and the oocytes were collected 36 h later using a transvaginal ultrasound procedure. The cumuli were washed and placed individually in a 30 µl drop of B2 medium under equilibrated oil. Cumulus cells were removed by exposure of the oocytes to 0.1% (w/v) hyaluronidase (Type III, Sigma) in B2 medium, and the nuclear status of the oocytes was assessed.

Subzonal insemination

Subzonal insemination was performed as described by Wolf *et al.* (1993) on oocytes at metaphase II, 6–8 h after collection. Two Narishige micromanipulators and one inverted IMT-2 microscope (Olympus) were suitable for carrying out the procedure. The number of spermatozoa that had to be micro-injected to obtain diploid fertilization was not known; therefore, between one and 16 spermatozoa were chosen randomly from among those with forward progressive movement and normal morphology and microinjected into the perivitelline space.

Analysis criteria

Oocytes were checked 16–18 h after subzonal insemination for evidence of fertilization. Oocytes exhibiting two pronuclei were considered as normally fertilized and the zygotes were kept in culture for a further 24 h. Only regularly cleaved embryos were transferred into the uterus using a Frydman catheter (CCD, Paris).

Statistical analysis

The relationship between the IVF success rates, the results of the sperm penetration assay and the sperm characteristics were examined by Spearman's rank correlation coefficients using the Statworks package. The results of the sperm penetration assay and comparisons between the movement parameter values for the different types of dyskinesia were performed using the Mann–Whitney non-parametric test.

Results

The sperm concentration from patients with flagellar dyskinesia did not differ from that of the controls. All the dyskinetic sperm samples had a reduced percentage of motile forms of normal forms (except those exhibiting periaxonemal dyskinesia) compared with the controls.

Effect of Percoll selection on sperm parameters

The velocity parameters of normal spermatozoa increased after Percoll selection in the capacitation medium when compared with their values in semen (Table 1). The straight line velocity and average path velocity doubled while the curvilinear velocity showed a 60% increase, from 62.7 to 99.6 µm s⁻¹. However, the lateral head displacement value did not change significantly (4.0 and 4.5 µm, respectively). During capacitation in the inseminating medium, the velocity parameters of normal spermatozoa showed a moderate increase, while the lateral head displacement showed a significant change from 4.5 to 5.6 µm ($P < 0.05$). All types of dyskinetic spermatozoa had lower velocities than did the controls ($P < 0.001$) in semen. They also had significantly smaller lateral head displacements ($P < 0.05$). The velocity parameters of all the dyskinetic sperm increased, but remained significantly different from the control values ($P < 0.01$), after Percoll selection. After Percoll selection, lateral head displacement values for spermatozoa lacking outer dynein arms and spermatozoa with short flagella increased such that they were no longer significantly different from the value for normal spermatozoa. Spermatozoa with periaxonemal anomalies showed little variation in their lateral head displacement which remained significantly smaller than that of the controls (2.9 µm versus 4.5 µm; $P < 0.001$).

Effect of incubation period on Percoll-selected spermatozoa

After 3 h incubation, the velocity of the spermatozoa with short flagella increased slightly while the velocity parameters for spermatozoa lacking outer dynein arms and with periaxonemal dyskinesia increased between 10 and 15%, but remained significantly different from control values ($P < 0.01$). Lateral head displacement values of spermatozoa with periaxonemal anomaly and with short flagella did not change but that for spermatozoa lacking outer dynein arms showed a 13% increase.

Sperm penetration assays

The results of the sperm penetration tests using zona-free hamster oocytes are presented (Table 2). Spermatozoa from

Table 2. Sperm penetration assay of dyskinetic and control human spermatozoa

Type of spermatozoa	n	Mean $\pm$ SD	Range
Short flagella	1	22	
Sliding spermatozoa	4	25.3 $\pm$ 9.3	12–33*
Periaxonemal dyskinesia	6	34.3 $\pm$ 13.2	16–56*
Lacking outer dynein arms	13	47.0 $\pm$ 26.5	8–93**
Controls	7	77.7 $\pm$ 20.2	44–100

Significantly different from controls (* $P < 0.01$; ** $P < 0.05$).

patients with short flagella syndrome do not migrate well through the Percoll gradient; therefore, only one patient of this group had the sperm penetration test. Sliding spermatozoa and spermatozoa with periaxonemal dyskinesia had the most impaired scores compared with controls ($P < 0.01$). The difference between the results of the sperm penetration tests for spermatozoa lacking outer dynein arms and the controls was less but still significant ($P < 0.05$). There was a correlation between the fertilization rate after subzonal insemination and the results of sperm penetration test for dyskinetic sperm (62 cycles, $r = 0.3$; $P < 0.05$). The correlation for spermatozoa with periaxonemal anomalies was higher (9 cycles, $r = 0.7$; $P < 0.05$). Analysis of the correlation between the sperm movement parameters from dyskinetic and control spermatozoa and their sperm penetration results showed that the curvilinear velocity of Percoll-selected suspensions was the most significant variable ($r = 0.7$; $P < 0.001$).

Fertilization rates

The overall fertilization rate of the 62 attempts using subzonal insemination was 41.8%. The rate varied from 31.3% for spermatozoa with periaxonemal anomalies to 43.7% for spermatozoa lacking outer dynein arms. The diploid fertilization rate was almost half the fertilization rate and followed the same pattern. Ten pregnancies resulted from 49 embryo transfers, of which four ended in early termination and six resulted in healthy babies. The correlation between the fertilization rate after subzonal insemination for all types of flagellar dyskinesia and all sperm movement parameters when evaluated in the Percoll-selected spermatozoa were significant for curvilinear velocity ($r = 0.5$; $P < 0.05$) (Fig. 3) and lateral head displacement ($r = 0.4$; $P < 0.05$). When evaluated after 3 h of incubation the correlations were curvilinear velocity ($r = 0.5$; $P < 0.002$), average path velocity ($r = 0.3$; $P < 0.05$) and lateral head displacement ($r = 0.3$; $P < 0.05$).

Discussion

This study provides an analysis of the movement parameters of dyskinetic spermatozoa in semen, after Percoll selection and after 3 h of incubation in the capacitation medium used for insemination. The results indicate a correlation between fertilization rates after subzonal insemination and movement parameters of dyskinetic spermatozoa.

Several characteristics are common to all types of dyskinesia. In semen, movement parameters are reduced. A significant reduction was observed in sperm velocity (straight line, curvilinear and average path velocities) as well as lateral head displacement. All the values of their movement parameters increased when the spermatozoa were in the capacitation medium after Percoll selection, although they still remained lower than control values. It is significant that the movement parameters of dyskinetic spermatozoa did not improve during the first 3 h of incubation contrary to the situation for normal spermatozoa for which the value of lateral head displacement increased from a mean of 4.5 μm to 5.6 μm . All the patients in the study were infertile and had failed to achieve pregnancy with standard IVF, justifying the use of subzonal insemination. It has been shown that for patients with autoimmune sperm antibody, increases in values of lateral head displacement during incubation are linked to the ability of spermatozoa to pass through the egg vestment (Zouari *et al.*, 1993). With dyskinetic spermatozoa the absence of an increase in lateral head displacement is also associated with poor IVF results.

Despite low movement parameters, gamete fusion can occur when spermatozoa are delivered close to the oolemma. Gamete fusion has already been reported with spermatozoa lacking dynein arms using subzonal insemination (Wolf *et al.*, 1993). In the present report evidence is provided that the ability of spermatozoa to fuse with the oocyte is significantly correlated with the curvilinear velocity of the Percoll-selected spermatozoa. Interestingly, lateral head displacement, which has been shown to be the most important criterion for sperm fertilizing ability during standard insemination (Zouari *et al.*, 1993) has less significance for the sperm–oolemma interaction. These results confirm, in a human–human system, the relation between sperm movement parameters and fusiogenic ability reported by Aitken *et al.* (1994) in the human–hamster system. The movement characteristic identified in their study as being of most prognostic value for the sperm–oocyte fusion was the average path velocity and not the curvilinear velocity as in the study reported here. The difference between both these movement parameters was not significant in the study by Aitken *et al.* (1994); this discrepancy is probably due to the use of microcapillary tubes in our analysis of the sperm movement parameters instead of Microcell and Makler chambers. The depth of these devices is 200 μm , 20 μm and 10 μm , respectively.

The mechanism underlying this association between curvilinear velocity and fertilization rate after subzonal insemination is unknown. The association may indicate that sperm movement is necessary for the sperm–oolemma fusion process. In mice, sperm adhesion treatment in which spermatozoa were held against the ooplasmic membrane after subzonal insemination resulted in a significantly increased fertilization rate (Kobayashi *et al.*, 1992). However, applying the sperm adhesion treatment to immotile spermatozoa did not result in fertilization. This suggests, at least in mice, that sperm motility may be involved in fertilization even after adhesion of the sperm head with the oocyte membrane. This is consistent with two other studies also performed in mice. In the first study, Barg *et al.* (1986) showed that immobilized spermatozoa do not fertilize the oocyte. In the second study, Klemm and Engel (1991) showed that the microinjection of immotile spermatozoa resulted in phagocytosis rather than in sperm–oocyte fusion.

Fig. 3. Correlation between fertilization rates after subzonal insemination and curvilinear velocity. The curvilinear velocity is (a) measured in the Percoll-selected sperm suspension (VCL₀) and (b) measured again after incubation for 3 h in the capacitating medium (VCL₃). The lines represent the regression lines. Subzonal inseminations performed with less than five oocytes were not included. Fertilization rate: percentage of microinjected oocytes.

However, fertilization of human eggs with immotile spermatozoa has been reported (Bongso *et al.*, 1989), and immotile spermatozoa from men with Kartagener's syndrome penetrate zona-free hamster oocytes (Aitken *et al.*, 1983). Preliminary investigations performed in our laboratory using subzonal insemination with spermatozoa with flagellar dyskinesia linked to metabolic disorders (results not shown) indicated that they almost never fertilized despite high curvilinear velocities. All these data suggest that the relationship between curvilinear velocity and ability of spermatozoa to fuse with the oocyte is not linked to frequency of collisions with the oocyte. We speculate that curvilinear velocity is indicative of the ability of the spermatozoon to fuse. Curvilinear velocity is the result of the forward progression and of the lateral head displacement. Since lateral head displacement represents the propulsive

strength of the spermatozoa (David *et al.*, 1981), the ability of a spermatozoon to increase its lateral head displacement in the capacitation medium may reflect the adaptation of its metabolic pathway to the fertilizing conditions, and this adaptation is not possible for dyskinetic spermatozoa.

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