A first prospective study of the individual straight line velocity of the spermatozoon and its influences on the fertilization rate after intracytoplasmic sperm injection

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The straight line velocity of 500 individual spermatozoa was measured immediately before their direct injection into the cytoplasm of 500 metaphase II oocytes. The straight line velocity (VSL) did not have a normal distribution (P < 0.01) and ranged between 0 and 35 µm/s. The fertilization rate (84%) was significantly (P < 0.008) higher in the quartile of the sperm population with the highest VSL compared to the fertilization rate (68%) in the quartile with the lowest VSL. Embryo cleavage and embryo quality were not different in the quartiles of the sperm population used for injection.

Key words: embryo quality/fertilization rate/intracytoplasmic sperm injection/straight line velocity/video microscopy analysis

Introduction

Palermo et al. (1992) reported the first successful use of direct intracytoplasmic sperm injection (ICSI) for treating severe male factor infertility. They fully explored the use of ICSI and clearly demonstrated that the technique is far superior to subzonal insemination (SUZI) and routine IVF for treating severe male factor infertility (Palermo et al., 1993). The fertilization rates of >60% of the injected oocytes published by this team were confirmed by Payne et al. (1994), Svalander et al. (1995) as well as by our own experience (Van den Bergh et al., 1995). The technique has now been applied with similar success using epididymal and testicular spermatozoa (Silber et al., 1995) and fertilization was even achieved by injecting round spermatids (Tesarik et al., 1995). The final outcome of ICSI does not seem to be related to the three basic sperm parameters as reported by Nagy et al. (1995b) and abnormal sperm morphology does not influence the results obtained by ICSI according to Svalander et al. (1996) and Küpker et al. (1995). Because Nagy et al. (1995b) observed differences in the fertilization rates obtained in the five different sperm motility groups and showed a good correlation between motility and fertilization rate, and because Hoshi et al. (1995) obtained better results in ICSI when motile spermatozoa were injected, there might be a possible benefit in selecting the fastest spermatozoon prior to intracytoplasmic injection. ICSI offers a unique situation where we know which particular spermatozoon did fertilize and what kind of embryo was obtained. We therefore measured the straight line velocity (VSL) of each spermatozoon prior to its injection. This is the first kinematic study of the individual velocity of the spermatozoon used in ICSI and the relation with fertilization, embryo cleavage and embryo quality.

Materials and methods

Patients

This study included 46 couples referred to the Fertility Clinic of the Erasmus Hospital of the French-Speaking Free University of Brussels for infertility caused by a severe male factor or because of previous fertilization failure. A total of 47 treatment cycles with ICSI were performed.

Ovarian stimulation and oocyte retrieval

Details of the ovarian stimulation protocol using gonadotrophin-releasing hormone analogue (buserelin acetate, Suprefact spray; Hoechst, Frankfurt, Germany), human menopausal gonadotrophin (Humegon; Organon, Oss, The Netherlands; Pergonal; Serono, Aubonne, Switzerland) and human chorionic gonadotrophin (Pregnyl; Organon; Profasi; Serono), oocyte retrieval through vaginal puncture under ultrasound guidance, embryonic culture in modified Earle’s balanced salt solution (EBSS) and transcervical replacement have been described elsewhere (Englert et al., 1991).

Oocyte preparation

Immediately after oocyte retrieval, cumulus and corona radiata were removed by incubation in HEPES buffered Earle’s balanced salt solution containing 60–80 IU/ml hyaluronidase (Type VIII, Sigma, St Louis, USA) for a time period between 30 s and 1 min. The remaining corona cells were mechanically removed by a hand-drawn glass pipette. The oocytes were then observed under an inverted microscope to assess the presence of a first polar body. They were then further incubated for 3–4 h and ICSI was carried out on all oocytes with a first polar body.

Semen characteristics and preparation

The WHO criteria were used for semen analysis

The initial volume of the semen samples ranged between 0.7 and 14.5 ml with a median of 3 ml. The initial concentration ranged from 1 to 250×10⁶/ml with a median of 10×10⁶/ml. The initial total motility ranged between 6 and 76% with a median of 32%. The initial percentage of progressive motile spermatozoa ranged between 0 and 30% with a median of 0%. None of the samples contained fast forward progressive spermatozoa. In 11 samples the concentration was <2×10⁶/ml and it was impossible to assess the percentage normal morphology on the basis of a minimum of 100 spermatozoa.
The percentage of normal forms ranged from 1 to 40% with a median of 10% for the 36 remaining samples. The glass wool column filtration method was selected, based on our previous positive experience in preparing semen samples for ICSI (Van den Bergh et al., 1996) for the preparation of all the samples. Semen samples were washed first with HEPES-buffered EBSS and then centrifuged for 10 min at 500 g. The pellet underwent a second wash with 10 ml 5 mM Ca\(^{2+}\) T6 medium and was centrifuged again at 500 g for 10 min. The supernatant was removed and the pellet was resuspended in 200 µl T6 medium and was centrifuged again at 500 g. 

Preparation. The washed pellet was then applied to the column and were washed twice with 1 ml of T6 medium just prior to the semen preparation. The washed pellet was then applied to the column and were washed twice with 1 ml of T6 medium just prior to the semen preparation. 

Glass wool filtering. Glass wool columns were prepared by packing 15 mg glass wool microfiber code 112 (Shuller Sales Corporation, Toledo, Ohio, USA) in a 1 ml tuberculin syringe (Becton Dickinson, Erembodegem, Belgium) up to a height of 3–4 mm. The columns were then sterilized for 6 h 30 min at 120°C. The sterilized columns were then used for ICSI. To measure the individual velocity of the spermatozoa we connected to the Olympus IMT inverted microscope with Nomarski optics, normally used for ICSI, a video camera and video tape recorder. 

Results

**Spermatozoon VSL measurement**

To measure the individual velocity of the spermatozoa we connected to the Olympus IMT inverted microscope with Nomarski optics, normally used for ICSI, a video camera and video tape recorder. A data box with timer was installed between the camera and video tape recorder. All measurements were performed at ×300 magnification and on a microscope with heated stage. Temperature was kept constant at 37°C as usually done during the ICSI procedure. The spermatozoa were selected at random. We taped the track of each individual spermatozoa for a minimum of 10 s immediately prior to its injection. Each ICSI procedure was entirely videotaped while the timer was running. By doing so we avoided slowing down the manipulations and overexposing the oocytes. After the ICSI procedure, a playback of the tape enabled us to mark with a pen on the video monitor the start and end-point of the track for each injected spermatozoa and to register the corresponding time period. The distance between these two points was measured and calibrated against a reticule of 10 µm installed under the microscope at the same magnification (×300). This enabled us to express the distance in µm. The obtained distance was then divided by the time period and this was termed VSL (µm/s) according to the manual trajectory analysis described by Mortimer (1994). Motile spermatozoa, with clear continuous oscillations of the flagellum, that did not progress in the 10% PVP and thus covered a distance equal to 0 µm had a VSL of 0 µm/s.

**Assessment of fertilization, embryo cleavage and pregnancy**

The presence of two pronuclei was assessed exactly 18 h after insemination. Oocytes were only considered fertilized if they exhibited two clear pronuclei. Precisely 42 h after injection the cleavage stage was recorded and a score was given to the embryos. The scoring scale is based on presence of anucleated cytoplasmic fragments, regularity and number of the blastomers with a maximum of 6 points per embryo (Puissant et al., 1987). A maximum of three embryos was replaced. Pregnancies were diagnosed by at least two positive human chorionic gonadotrophin (HCG) measurements (>1 mIU/ml) (Beringh Inc., Behring, Germany) 3 days apart, the first not being before day 11 after oocyte retrieval. Clinical pregnancy was defined either by a fetal sac at 5 weeks after oocyte retrieval or by villosity present in miscarriage material.

**Statistical analysis**

Data were analysed with the SPSS software package. Contingency tables were used and if groups were compared two by two, P values were submitted to the Bonferroni correction to avoid type 1 errors.

**Semen glass wool filtrates**

The sperm samples after preparation had a median of 2.4×10^6 cells/ml and a range between 3×10^4 and 45×10^6 cells/ml. The total motility after glass wool filtration had a median of 57% and a range between 10 and 100%. The median of the final percentage of progressive motile spermatozoa was 6% with a range from 0 to 78%.

**Straight line velocity**

Figure 1 illustrates the distribution of the VSL of the 500 spermatozoa used for intracytoplasmic injection. The Kolmogorov–Smirnov test revealed that this distribution was not normal (P < 0.01). The median of the VSL was 6 µm/s and ranged from 0 to 34 µm/s. The quartiles of this distribution were used for further analysis. The lower and upper limits for these four groups were 0–3, 3–5, 5–8 and 8–34 µm/s.

**Fertilization**

Table I gives the activation, the normal two-pronuclear and the polyplody fertilization of the 500 metaphase II oocytes that were injected. Cross-tabulation revealed a significant influence of the VSL on the fertilization (P < 0.05).

**Embryo cleavage and quality**

Table II summarizes the embryo cleavage by giving for each VSL group the number of polyfragmented embryos, the number of uncleaved embryos, the number of embryos with less than four cells and the number of embryos with four or more cells.

![Figure 1. Histogram of the straight line velocity (VSL) of 500 injected spermatozoa (line represents normal distribution).](image)
Table I. Cross-tabulation of the fertilization according to the straight line velocity (VSL)

<table>
<thead>
<tr>
<th>VSL (μm/s)</th>
<th>Fertilization</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unfertilized</td>
</tr>
<tr>
<td></td>
<td>n</td>
</tr>
<tr>
<td>0–3</td>
<td>34</td>
</tr>
<tr>
<td>3–5</td>
<td>20</td>
</tr>
<tr>
<td>5–6</td>
<td>24</td>
</tr>
<tr>
<td>6–34</td>
<td>10</td>
</tr>
<tr>
<td>Total</td>
<td>88</td>
</tr>
</tbody>
</table>

Overall $\chi^2$: $P < 0.05$.

* $P < 0.008$, Bonferroni correction.

Table II. Cross-tabulation of the distribution of the embryo cleavage and the straight line velocity (VSL)

<table>
<thead>
<tr>
<th>VSL (μm/s)</th>
<th>Polyfragmentation</th>
<th>uncleaved</th>
<th>&lt;4-cell stage</th>
<th>≥4-cell stage</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>n</td>
<td>n (%)</td>
<td>n (%)</td>
<td>n</td>
</tr>
<tr>
<td>0–3</td>
<td>12</td>
<td>1</td>
<td>38</td>
<td>37 (42)</td>
<td>88</td>
</tr>
<tr>
<td>3–5</td>
<td>14</td>
<td>6</td>
<td>32</td>
<td>45 (46)</td>
<td>97</td>
</tr>
<tr>
<td>5–6</td>
<td>12</td>
<td>5</td>
<td>28</td>
<td>51 (53)</td>
<td>96</td>
</tr>
<tr>
<td>6–34</td>
<td>7</td>
<td>9</td>
<td>35</td>
<td>57 (53)</td>
<td>108</td>
</tr>
<tr>
<td>Total</td>
<td>45</td>
<td>21</td>
<td>133</td>
<td>190</td>
<td>389</td>
</tr>
</tbody>
</table>

Differences were not significant.

No differences were observed. There was no difference in the proportion of low (score <4) and high quality embryos in the four groups: 49/26, 42/35, 41/38 and 60/32.

Pregnancies

Out of these 47 treatment cycles 46 ended with a transfer. One patient did not have a transfer because all embryos were polyfragmented. Seventeen clinical (37%) and two biochemical (4%) pregnancies were obtained, including four twins. A total of 108 embryos were replaced and 22 yolk sacs were observed corresponding with an implantation rate of 20% per replaced embryo. These 108 embryos were replaced in transfer with a single embryo, 25 transfers with two embryos and 19 transfers with three embryos. The embryos with the highest quality score were always used for transfer. Because the VSL did not influence the quality, embryos with the same quality, but generated by spermatozoa with different VSL, were mixed in the same transfer. It is therefore impossible to withdraw conclusions concerning VSL and embryo implantation rates.

Discussion

Even after the introduction of computer-assisted semen analysis for the assessment of sperm kinematics (David and Katz, 1993), manual trajectory analysis as described above still remains a valid practical method for sperm movement studies (Mortimer, 1994), when sophisticated software is not available.

Several authors have described the correlation between the velocity after semen preparation and the fertilization rate after IVF (Chan et al., 1989; Bongso et al., 1990; Oehninger et al., 1990) but a certain number of studies failed to demonstrate any correlation between fertilization in vitro and objectively assessed motility (Calamera et al., 1989; Barlow et al., 1992; Thanki et al., 1992; Hinney et al., 1993). Papers about sperm motility and ICSI are scarce (Hoshi et al., 1995a,b; Nagy et al., 1995) probably because of the overwhelming success of ICSI in terms of fertilization. All these publications used mean values of motility and/or categories of percentage of motility of the sperm samples and no information is available about the kinetics of each single spermatozoon that was introduced into the oocyte by the ICSI technique. ICSI injection is therefore a unique situation during which we are able to study the characteristics of an individual spermatozoon that eventually will succeed in fertilizing an oocyte and produce an embryo. Our simple videomicrographic study of the VSL shows for the first time a clear relation between the motility of the injected spermatozoon and the fertilization rate. This observation answers the question as to which spermatozoon to select for ICSI.

The goal of fertilization is the union of one and only one sperm nucleus with the female pronucleus within the activated oocyte. For this to occur successfully, several events must occur, including the incorporation of the entire spermatozoon into the oocyte, the completion of meiotic maturation with the extrusion of the second polar body, the metabolic activation
of the previously quiescent oocyte, the decondensation of the spermatozoon nucleus and the maternal chromosomes into the male and female pronuclei respectively and the cytoplasmic migration of the pronuclei, which bring them into apposition (Asch et al., 1995). It is the spermatozoon that introduces into the oocyte the centrosome which nucleates new microtubule assembly to form the sperm aster — an essential step for successful fertilization. (Simerly et al., 1995). It is also the spermatozoon which has to release a sperm-associated oocyte activating factor as described in the model proposed by Dozortsev et al. (1997). Any defects in these mechanisms will result in a fertilization failure. Our study suggests that there is an association between at least one of these defects and a low VSL of the individual spermatozoon.

The major factor controlling sperm motility is the availability of ATP, the energy substrate for the dynein-ATPase, which is produced by the mitochondria and transported to the axoneme through the mediation of the phosphorylcreatine shuttle proposed by Cummins and Yovich (1993). How the reduced motility, generated by any possible defect in this shuttle mechanism, affects fertilization after ICSI is not yet clear, but reduced motility is associated with DNA defects. The association of low motility and DNA abnormalities is mentioned in the literature. Kao et al. (1995) described the highest occurrence of 4977 bp deleted mitochondrial DNA in semen fractions with the lowest motility and a negative correlation between spermatozoon motility and the proportion of 4977 bp fractions with the lowest motility and a negative correlation between spermatozoon motility and the proportion of 4977 bp deleted mitochondrial DNA. A significantly higher incidence between spermatozoon motility and the proportion of 4977 bp fractions with the lowest motility and a negative correlation between spermatozoon motility and the proportion of 4977 bp deleted mitochondrial DNA was found in patients with asthenozoospermia, oligozoosperma and primary infertility compared to normal individuals

Sun et al. (1997) found a negative association between semen analysis parameters and spermatozoa with fragmented DNA. They observed a significant negative association between spermatozoa with DNA fragmentation and the fertilization rate (P < 0.008). They therefore claim that since extremely poor semen samples are the indication for ICSI, there is a high likelihood that spermatozoa with fragmented DNA may be selected by chance and used for oocyte injection, resulting in poor fertilization and/or cleavage as seen from the fertilization obtained in the group with the lowest VSL in our study. The same paper reports a similar association between morphology and DNA fragmentation. However, proper assessment of morphology during the ICSI procedure is impossible.

Lopes et al. (1998) report that fertilization failure in ICSI is associated with DNA fragmentation. The authors suggest that in severe male factor infertility a significant proportion of spermatozoa injected into oocytes may contain fragmented DNA. Injection of oocytes with spermatozoa containing abnormal chromatin will probably result in a lack of decondensation of the spermatozoon and fertilization failure.

Many of the mechanisms of fertilization still have to be fully elucidated; although the present study does not explain the relation between reduced VSL and low fertilization after ICSI, at least it suggests that more motile spermatozoon should be used to obtain a higher fertilization rate.

References


Dozortsev, D., Qian, C., Ermilov, A. et al. (1997) Sperm associated oocyte activating factor is released from the spermatozoon within 30 min after injection as a result of the sperm–oocyte interaction. *Hum. Reprod.*, 12, 2792–2796.


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