The morphological normalcy of the sperm nucleus and pregnancy rate of intracytoplasmic injection with morphologically selected sperm

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BACKGROUND: Our preceding studies have already demonstrated the advantage of intracytoplasmic morphologically selected sperm injection (IMSI) over the conventional IVF-ICSI procedure in terms of pregnancy rate. This study was undertaken to determine whether the increased pregnancy outcome was attributable to the preferred nuclear morphology of the selected spermatozoa, and not to the special sperm preparation technique modified by IMSI. METHODS: Comparison between two matched IMSI groups, i.e. negative comprising 38 cycles, where no spermatozoa with intact nuclei were available for microinjection; and positive, involving ovum microinjection by spermatozoa with strictly defined morphologically normal nuclei. RESULTS: Implantation and pregnancy rates were significantly higher, and abortion rates significantly lower, in the positive group compared with the negative group (25.0 ± 25.9 versus 5.9 ± 12.9%, F = 15.8, P ≤ 0.01; 52.6 versus 18.4%, χ2 = 9.7, P ≤ 0.01; and 10.0 versus 57.1%, χ2 = 7.1, P ≤ 0.02, respectively). CONCLUSIONS: Implantation and pregnancy by ICSI is associated with morphological nuclear normalcy of sperm. Sperm with a morphologically abnormal nucleus usually have low fertility potential, but some with certain nuclear abnormalities may still be able to produce pregnancy following ICSI.

Key words: intracytoplasmic morphologically selected sperm injection (IMSI)/male fertility/sperm nucleus

Introduction

Sperm morphology has been recognized as the best predictor of outcome for natural fertilization (Bartoov et al., 1993, 1994), intra-uterine insemination (IUI) (Berkovitz et al., 1999) and conventional IVF (Kruger et al., 1988; Liu and Baker, 1992; Mashiach et al., 1992). Recently, increasing evidence has suggested that sperm morphology also plays a significant role in ICSI outcome (Bartoov et al., 2001, 2002, 2003; Tesarik et al., 2002; Chemes and Rawe, 2003; De Vos et al., 2003).

In a recent, prospective, feasibility study we demonstrated that fine morphological integrity of human sperm nuclei is positively associated with fertilization and pregnancy rates following IVF-ICSI. These results were obtained by the application of a new method of unstained, real-time, high magnification motile sperm organellar morphology examination (MSOME) to the leftover sperm fraction selected for microinjection in 100 random couples referred for ICSI (Bartoov et al., 2002). Accordingly, we introduced a modified IVF procedure—intracytoplasmic morphologically selected sperm injection (IMSI)—based on microinjection into retrieved oocytes of selected spermatozoa with strictly defined morphologically normal nuclei. The modified IMSI treatment did, in fact, result in significantly higher pregnancy rates, compared with conventional IVF-ICSI (Bartoov et al., 2001, 2003).

In addition to the strict morphological sperm selection, the IMSI method adapted some minor modifications in sperm preparation which were not conducted in the conventional IVF-ICSI process, i.e. use of a density gradient (Sil Select) in the routine preparation prior to selection, use of polyvinyl pyrrolidine (PVP), low temperature and a glass-bottomed dish during selection, prolonged sperm manipulation post-separation from the seminal fluid and sperm storage prior to microinjection.

Thus, one may claim that the advantage of IMSI is caused by these modified techniques, and is not necessarily related to the nuclear normality of the injected sperm cells. The aim of this study was to determine whether the increased pregnancy outcome was, indeed, attributable to the preferred nuclear morphology of the selected spermatozoa and not to...
the special sperm preparation technique adapted for IMSI. For this purpose, two matched IMSI groups with identical sperm preparation but different selection criteria were compared.

Materials and methods

Patients

Three hundred and nine IVF-IMSI cycles included in our database between the years 2001 and 2003 fit the following criteria: female partner younger than 40 years; three or more retrieved metaphase II ova in the present IVF-IMSI cycle; and IMSI outcome confirmed 3 months after microinjection.

In 38 (12%) of these cycles, we were unable to find any spermatozoa with strictly defined morphologically normal nuclei. Subsequently, all the oocytes within one cycle were injected by ‘second best’ morphologically evaluated sperm cells, selected according to compromised criteria (see MSOME criteria for spermatozoa selected for IMSI). In 126 (41%) of the cycles, the number of retrieved spermatozoa with preferred nuclear morphology was insufficient for all oocytes retrieved within one cycle. Thus, in these 126 cases, the ova were microinjected with spermatozoa with morphologically normal nuclei or with spermatozoa selected under compromised criteria (mixed microinjection). The fertilization rate and the percentage of top embryos at strict microinjection were significantly higher than the parallel variables at compromised microinjection (71.5 ± 23.6 versus 56.6 ± 36.0%, and 30.14 ± 24.2 versus 18.7 ± 26.3%, respectively, t = 4.0, P ≤ 0.01 and t = −2.7, P ≤ 0.02, respectively). The mixed microinjection group was subdivided further into two subgroups: in 98 cycles, embryos derived from both kinds of microinjection were transferred in parallel (mixed transfer), while in the remaining 28 cases only embryos obtained from microinjection into the ova of spermatozoa with strictly defined morphologically normal nuclei were transferred. In 145 cases (47%), all the oocytes were injected with spermatozoa exhibiting the preferred nuclear morphology. Subsequently, all the transferred embryos in these cases were also obtained from microinjection of spermatozoa with strictly defined morphologically normal nuclei.

The pregnancy and abortion rates related to the mixed embryo transfer (n = 98) were 26.5 and 30.7%, respectively. The pregnancy and abortion rates related to embryo transfer following the strict sperm selection (n = 173) were 46.2 and 8.8%, respectively.

It should be noted that the above IMSI outcome data refer to the overall patient group from which the study group was derived and are not analysed further.

Negative group

The 38 cases where the transferred embryos were obtained from microinjection into the oocytes of the ‘second best’ morphologically evaluated sperm cells, selected according to compromised criteria (see MSOME criteria for spermatozoa selected for IMSI), were designated as the negative group. All the participants in this group were informed that only ‘second best’ morphologically evaluated sperm cells were retrievable for microinjection. Nevertheless, these patients wished to continue with the IMSI procedure. A written, informed, consent was obtained from each couple.

Positive group

A comparative study between the IVF-IMSI cycles, involving transfer of embryos derived following compromised versus strict sperm selection was conducted by matching the 38 negative cases with 38 IVF-IMSI cycles, where only embryos derived from microinjection of spermatozoa with strictly defined morphologically normal nuclei were transferred. The matching criteria were female’s age and the number of previous failed ICSI attempts. The time period between the negative and positive matched cases was up to 4 months. The 38 positive matching cases and the remaining 135 cases, where embryos derived from injection of spermatozoa with strictly defined morphologically normal nuclei, were statistically similar in all examined demographic and IMSI outcome parameters.

The examined demographic parameters, i.e. male’s and female’s age, pregnancy expectation (the period between the first attempt to conceive and the IMSI cycle included in this study) and the numbers of previous failed ICSI and IMSI trials were statistically similar in the negative and positive study groups (Table I). Neither of the study groups included any previously reported data.

Routine preparation of sperm fraction

As previously described (Bartoo et al., 2003), the routine selection procedure of motile high density spermatozoa for IMSI was performed on the basis of a two-layer Sil Select density gradient system (FertiPro N.V. Beernem, Belgium). The obtained motile high density sperm fraction was used for further individual sperm retrieval based on MSOME.

Sperm preparation for individual retrieval based on MSOME

About 4000 motile high density sperm cells, obtained from the above fraction, were transferred to a 4-μl observation microdroplet of SPER medium (Medi-Cult, Jyllinge, Denmark) containing PVP solution (ICSITM-100, Vitrolife, Kungsbacka, Sweden).

In order to estimate the morphological state of the sperm nucleus, the technician has to follow the motile sperm cell by moving the microscopic stage in the x, y and z directions for at least 20 s. Therefore, the PVP solution was necessary to slow the sperm speed and, thereby, to prevent the highly motile spermatozoa from disappearing from the monitor screen during measurement. To reduce PVP toxicity, the concentration of the PVP was adjusted to a minimum (range 0–8%), which still enables image analysis. The observation microdroplet was placed in a sterile, glass-bottomed dish (Willco wells BV, Amsterdam, The Netherlands) under sterile paraffin oil (OVOIL-100; Vitrolife, Kungsbacka, Sweden). The sperm cells, suspended in the observation microdroplet, were used for individual retrieval by MSOME. For this purpose, the sterile glass-bottomed dish containing the microdroplet was placed on a microscopic stage over the top of a Uplan Apo ×100 oil/1.35 objective lens previously covered by a droplet of immersion oil. In this way, the motile sperm cells, suspended in the observation droplet, could be examined at high magnification by the inverted microscope (Olympus IX 70, Tokyo, Japan) equipped with Nomarski differential interference contrast optics.

| Table I. Comparisons between the positive and negative study groups in demographic data |
|---------------------------------------------|-----------------|--------------|
| Demographic dataa Study groups              | Positive (n = 38) | Negative (n = 38) |
| Female’s age (years)                        | 32.3 ± 3.3      | 33.3 ± 4.5       |
| Male’s age (years)                          | 35.1 ± 4.5      | 36.1 ± 4.5       |
| Pregnancy expectation (years)               | 4.4 ± 2.9       | 5.4 ± 2.6        |
| No. of previous ICSI cycles                 | 3.0 ± 2.6       | 3.0 ± 2.6        |
| No. of previous IMSI cycles                 | 0.8 ± 1.4       | 1.2 ± 1.9        |

Values are means ± SD.
The success of the MSOME is associated with four image properties. (i) Optical resolution, which depends on microscope optics and on the light source of the microscope: theoretical resolution is light wavelength divided by numerical aperture of objective (in our case ~1.5). This resolution is ~200 nm for blue light and 300–350 nm for red light. When used with the usual white-yellow light, the resolution is ~300 nm. (ii) Image contrast, which is increased by Nomarski optics (special phase contrasting light processing). (iii) Maximal optical resolution ~150, which is defined by the following factors: (a) objective magnification (100×); (b) magnification selector (1.5×); and (c) video coupler magnification 0.99 (UPMTV × 0.3, PE × 3.3). (iv) Magnification of the video system, which could be calculated from the following data: (a) CCD chip diagonal dimension 8 mm; and (b) TV monitor diagonal dimension 356 mm.

Thus, the calculated video magnification (b/a) is ~44.5 (356/8). It is, however, dependent on the size of the display. In our case, the calculated total magnification is ~150 × 44.5 = 6675×. This number is a ratio between the size of the object on display and the real size. The actual digitally enhanced magnification, as determined by a 0.01 mm Olympus objective micrometer, was 6300×.

The digital image processing provides additional image enhancement (special filters) for detail recognition by the operator and tools for morphological measurements. This video enhancement is very important for the MSOME analysis, which requires morphological definition of the size, counter and content of the sperm nucleus.

**MSOME criteria for spermatozoa selected for IMSI**

As described in our previous studies, the strict descriptive criteria for normally shaped nuclei were based on those defined by scanning electron microscopy, i.e. smooth, symmetric and oval configuration (Bartoov et al., 1981, 2002, 2003). For MSOME, the average length and width of this configuration were estimated in 100 spermatozoa with an obviously normal nuclear shape and were found to be 4.75 ± 0.28 and 3.28 ± 0.20 μm, respectively. During sperm selection, a fixed, transparent, celluloid form of a sperm nucleus fitting the normal criteria was superimposed on the examined cell; any sperm cell with a nuclear shape was excluded from selection if it varied in length or width by 2 SDs from the normal mean axes values. According to transmission electron microscopy estimations, the nuclear chromatin content was considered normal if it contained no more than one vacuole, which occupies <4% of the nuclear area (Bartoov et al., 1981). To exclude from selection spermatozoa with abnormal chromatin content, a fixed, transparent, celluloid form of a vacuole with a borderline diameter of 0.78 ± 0.18 μm was superimposed on the examined cell. In the cases with few small vacuoles, the estimation was made ‘by eye’. We must emphasize that spermatozoa with doubtful determination were excluded from selection.

When no spermatozoa with normal nuclei were available for microinjection, the ‘second best’ morphologically evaluated sperm cells with minimally impaired nuclei were retrieved according to the hierarchy presented in Table II and Figure 1. Sperm cells exhibiting an abnormal but oval nuclear shape and a morphologically normal nuclear content were considered for the first choice selection (small or large oval nuclear forms, n = 5 and n = 2, respectively, Figure 1b and c, respectively). Spermatozoa with non-oval, abnormal nuclear shapes, but with normal nuclear content, were retrieved as a second choice (wide or narrow forms, n = 2 and n = 7, respectively, Figure 1d and e, respectively) and sperm cells with regional shape disorder (an extrusion or invagination of the nuclear mass, n = 2, Figure 1f), as a third choice. Sperm cells exhibiting large nuclear vacuoles but normal oval nuclear shape (n = 18, Figure 1g), were considered preferable for selection to those with a non-specific combined nuclear malformation (narrow forms + large nuclear vacuoles, n = 2, Figure 1h). It should be noted that, just as in the conventional ICSI trial, sperm cells with pin, amorphous, tapered, round or multinucleated head shapes were totally excluded from retrieval.

### Sperm retrieval for IVF-IMSI

Morphologically selected spermatozoa were retrieved from the observation droplet and placed into a recipient selection droplet as described earlier (Bartoov et al., 2003). On average, ~20 sperm cells were retrieved per cycle (two spermatozoa per expected retrieved ovum). Two technicians, both of whom underwent training in sperm subcellular morphology (transmission and scanning electron microscopy), were involved in each individual sperm selection procedure. The average duration of this process was ~2.5 h (range 1.5–5.0). During retrieval, the metabolism of the sperm cells was reduced by lowering the temperature to 21°C.

As mentioned above, in the positive group only, spermatozoa with strictly defined morphologically normal nuclei were retrieved and injected into the oocytes. In the negative group, we also tried to inject all the oocytes within one cycle with spermatozoa exhibiting exactly the same nuclear aberration, i.e. all the oocytes of each specific couple were injected with similar sperm. This condition could be accomplished since each semen sample usually exhibited typical nuclear malformations. The distribution of the seven specific aberrations for each patient is demonstrated in Table II.

<table>
<thead>
<tr>
<th>Choice</th>
<th>Specific nuclear malformations</th>
<th>No. of patients</th>
<th>Frequency (%) of malformation within each patient</th>
<th>Pregnancy outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Mean ± SD</td>
<td>Range</td>
</tr>
<tr>
<td>1</td>
<td>Large oval</td>
<td>5</td>
<td>77.9 ± 8.6</td>
<td>68–89</td>
</tr>
<tr>
<td>1</td>
<td>Small oval</td>
<td>2</td>
<td>80.0 ± 28.3</td>
<td>60–100</td>
</tr>
<tr>
<td>2</td>
<td>Wide forms (&gt;3.7 μm width)</td>
<td>2</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Narrow forms (&lt;2.9 μm width)</td>
<td>7</td>
<td>83.5 ± 15.5</td>
<td>60–100</td>
</tr>
<tr>
<td>3</td>
<td>Regional disorder</td>
<td>2</td>
<td>80.0 ± 28.3</td>
<td>60–100</td>
</tr>
<tr>
<td>4</td>
<td>Large vacuoles + normal shape/size</td>
<td>18</td>
<td>86.1 ± 15.4</td>
<td>63–100</td>
</tr>
<tr>
<td>5</td>
<td>Narrow forms + large vacuoles</td>
<td>2</td>
<td>70.9 ± 15.5</td>
<td>60–82</td>
</tr>
</tbody>
</table>

D = delivered; O = ongoing pregnancy; A = abortion.
**Sperm storage prior to microinjection**

Post-selection, the sterile glass-bottomed dish containing the recipient selection droplet was transferred from the Male Fertility Laboratory in Bar-Ilan University to the IVF centre (~30 min away) in an insulated box at ~25°C. The average time between completion of sperm selection and microinjection was ~2 h (range 1–4). About half an hour before microinjection, the dish was incubated at 37°C with an atmosphere of 5% CO₂.

**Preparation for IVF-IMSI**

The preparation for the IVF-IMSI procedure was conducted according to the method described by Van Steirteghem et al. (1993).

**Microinjection**

The transferred, retrieved, cumulus-free ova were placed into drops of SPERM medium prepared in the same glass dish with the recipient droplet. As mentioned above, the latter contained the sperm cells morphologically selected for IMSI. The majority of these cells located in the recipient droplet were easily found by the embryologist at the usual magnification of 200–400x. No shortage of selected spermatozoa was reported in any of the IMSI cycles included in this study. Microinjection of the selected spermatozoa into the oocytes was conducted in SPERM medium. Each microinjected oocyte was immediately transferred to a 4-well dish (Nunc), incubated in 0.5 ml of IVF or ISM 1 medium (Medi-Cult, New York, NY), covered with 0.5 ml of mineral oil (Medi-Cult) at 37°C with an atmosphere of 5% CO₂.

The definitions and calculations of the fertilization rate, percentage of top embryos and implantation rate were described in detail earlier (Bartoov et al., 2003). The pregnancy rate was calculated per transfer. The abortion rate was calculated per clinical pregnancy occurrence.

**Statistical analysis**

All statistical analyses were performed using SPSS for Windows Version 11.0 (SPSS Inc., Chicago, IL). Data were presented as means ± SD for continuous variables, including the numbers of retrieved and injected oocytes, fertilization rate, percentage of top embryos, number of transferred embryos and implantation rate. Comparisons between the positive and negative matched groups in the above variables were performed using univariate analysis of variance (ANOVA). The data for each cycle have been averaged prior to calculating the means. The discrete variables, including IMSI pregnancy and abortion rates, were presented as percentages. Comparison between the matched groups in the above variables was made by χ² tests.

Non-parametric statistical analyses involving Mann–Whitney tests were performed in parallel to the described statistical analyses in order to avoid possible artefacts due to the high standard deviations of some sperm parameters. The non-parametric methods revealed results similar to those reported here.

**Results**

The total values of the IVF-IMSI outcome parameters obtained in the matched positive and negative groups are demonstrated in Table III. Comparison between these two groups, using one-way ANOVA tests, revealed that three of the six continuous variables examined, namely fertilization rate, percentage of top embryos and implantation rates, were significantly higher in the positive group than in the negative group (F = 16.3, $P < 0.01$; F = 4.9, $P < 0.03$; F = 4.0, $P < 0.03$).

<table>
<thead>
<tr>
<th>Study groups</th>
<th>Positive (n = 38) per cycle (total)</th>
<th>Negative (n = 38) per cycle (total)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Continuous variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. of retrieved ova</td>
<td>13.3 ± 5.7 (506)</td>
<td>13.1 ± 6.8 (488)</td>
</tr>
<tr>
<td>No. of injected ova</td>
<td>10.1 ± 4.5 (382)</td>
<td>9.2 ± 4.0 (334)</td>
</tr>
<tr>
<td>Fertilization rate (%)</td>
<td>71.3 ± 20.8 (264)</td>
<td>50.3 ± 24.1 (174)</td>
</tr>
<tr>
<td>Percentage of top embryos (%)</td>
<td>34.9 ± 31.3 (81)</td>
<td>19.4 ± 27.1 (32)</td>
</tr>
<tr>
<td>No. of embryos transferred</td>
<td>3.3 ± 1.2 (125)</td>
<td>3.5 ± 1.4 (113)</td>
</tr>
<tr>
<td>Implantation rate (%)</td>
<td>25.0 ± 25.9 (31)</td>
<td>5.9 ± 12.9 (8)</td>
</tr>
<tr>
<td>Discrete variables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pregnancy rate per transfer (%)</td>
<td>52.6 (20)</td>
<td>18.4 (7)</td>
</tr>
<tr>
<td>Abortion rate (%)</td>
<td>10.0 (2)</td>
<td>57.1 (4)</td>
</tr>
</tbody>
</table>

*Values are means ± SD per cycle.

**Table III. Comparisons between the positive and negative study groups in IVF-IMSI outcome parameters**
and $F = 15.8, P = 0.01$ respectively, Table III). Of the two
discrete variables, pregnancy rate was significantly higher,
and abortion rate significantly lower, in the positive group
compared with the negative group ($\chi^2 = 9.7, P = 0.01$ and
$\chi^2 = 7.1, P = 0.02$ respectively, Table III).

Of the 38 IVF-IMSI cycles with strict sperm selection, 20
(52.6%) pregnancies were achieved. The distribution of sin-
discrete variables, pregnancy rate was significantly higher,
gleton, twin and triplet pregnancies in the positive study
group was 12 (60%), six (30%) and two (10%), respectively.
In 11 couples, delivery has already taken place, and six preg-
nancies are ongoing. In two cases (10.0%), the pregnancies
resulted in first trimester missed abortions and in one couple
an extra-uterine pregnancy occurred.

Of the 38 IVF-IMSI cycles with compromised sperm
selection included in the negative group, seven (18.4%) preg-
nancies were achieved (six singlets and one twin). Three
of these pregnancies resulted in normal deliveries while four
others resulted in a first trimester missed abortion (Table II).
Of the three deliveries, two were achieved following ovum
microinjection of sperm cells with large oval nuclei
(choice 1, Table II) and the third occurred in a case of
narrow formed sperm heads (choice 2). Three missed abort-
cion cases occurred when ovum microinjection was con-
ducted with spermatozoa exhibiting large nuclear vacuoles
(choice 4, Table II). In another abortion case, the sperm cells
exhibited a combined malformation: large vacuoles associ-
ated with narrow formed head shape (choice 5, Table II).

Discussion

In our previous study, we compared the modified IMSI
procedure with classic IVF-ICSI (Bartoov et al., 2003) and
demonstrated the obvious advantage of the former treatment
in pregnancy outcome (Bartoov et al., 2003). In the present
study, using a negative control within the IMSI group, we
confirmed our previous assumption that the remarkable
increase in IVF-IMSI pregnancy rate is associated with the
preferred nuclear normalcy of the sperm cells injected into
the ova.

Indeed, the significantly higher fertilization rate, per-
tage of top embryos, implantation and pregnancy rates, and
the lower abortion rate obtained following strict sperm selec-
tion, compared with the compromised selection, fit our
previous findings. The fact that in the excluded ‘mixed
microinjection’ group (see ‘Patients’), the fertility and top
embryo ratios obtained following injection of strictly selected
spermatozoa were significantly higher than those obtained in
cases of compromised selection has only strengthened these
findings. The present results also confirm the results of
studies by Tesarik et al. (2002) and De Vos et al. (2003)
who reported that sperm morphological quality influences
fertilization, implantation and pregnancy rates following
ICSI.

Our decision to use compromised selection criteria in
cases where spermatozoa with strict morphologically normal
nuclei could not be found was based on the following
assumptions. First, it seems logical to suppose that spermato-
zoa with multiple combined (non-specific) nuclear malfor-
mations reflect extra-chromosomal damage compared with
those with single specific abnormalities. Secondly, we
assumed that within the category of specific morphological
malformations, the existence of large vacuoles in the sperm
nuclei indicates more damage to the nuclear DNA content
and organization than nuclear shape or size impairment. This
assumption is based on preliminary unpublished data,
obtained in our laboratory, which indicate a significant nega-
tive relationship between the size of the nuclear vacuoles and
chromatin stability assessed by the sperm chromatin structure
assay (SCSA). Since, according to Larson et al. (2000), chro-
matin stability appears to influence embryonic development,
we preferred, where possible, to retrieve sperm cells with a
single shape/size nuclear deviation rather than those with a
normal nuclear configuration, but with an impaired content.
Of the specific nuclear defects with normal nuclear content,
we chose to retrieve sperm cells with oval nuclear configur-
ation (either large or small) rather than non-oval sperm cells.
The results of Lee et al. (1996), who demonstrated that no
increase in chromosome aberrations was found in spermato-
zoa with large or small heads, support our approach.

Indeed, all three pregnancies in the negative study group
which resulted in normal deliveries were achieved when
spermatozoa with a single specific nuclear shape malfor-
mation were injected into the ova (choices 1 and 2); two of
them with large oval heads (choice 1). On the other hand, the
four pregnancy cases in the negative study group which
terminated in early missed abortions were achieved by micro-
injection of spermatozoa with large nuclear vacuoles.

The pregnancy rate of 18.4% obtained in the negative
study group raises the question of sensitivity regarding the
strict sperm selection criteria adopted for IMSI. However,
because of the small number of IMSI cycles conducted with
spermatozoa retrieved under compromised criteria and the
low success rate in the negative group, we were still unable
to draw any conclusions concerning the relationship between
specific morphological malformations of the sperm nucleus
and IMSI pregnancy outcome. Further investigation using
compromised sperm selection may prove that spermatozoa
with certain so-called ‘morphological nuclear abnormalities’
are still capable of inducing pregnancy following IVF-IMSI.
If this is the case, then, in order to reduce the false-negative
value of the sperm selection, the preliminary criteria which
we have adopted in our laboratory should be re-evaluated. It
seems, therefore, that at this stage of the research, the elimi-
nation process used in the compromised sperm selection does
not infer that one choice is inherently better than another.

Although the advantage of the modified IMSI treatment
compared with the classic ICSI approach in pregnancy out-
come is quite clear, some important issues have to be con-
sidered prior to introducing this method into a routine IVF
laboratory: (i) a significant increase in the time taken to com-
plete a case; (ii) the need for additional highly trained man
power; and (iii) the additional cost of upgrading for the rou-
tine inverted microscope equipment, including high resol-
ution Nomarsky optics (€15 000) and a micromanipulator
with two memories in space (€12 000). Taking into account
these issues, a busy IVF centre may prefer to obtain sperm
selection services from a separate andrological laboratory. Establishment of special andrological units which will serve several IVF centres seems to us the most practical way to introduce IMSI.

In conclusion, implantation and pregnancy by ICSI is associated with morphological nuclear normalcy of sperm. Sperm with a morphologically abnormal nucleus usually have low fertility potential, but some with certain nuclear abnormalities may still be able to produce pregnancy following ICSI.

The association between specific morphological nuclear abnormalities of the sperm microinjected into the oocyte and IMSI outcome should be investigated further.

References

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