Successful childbirth after intracytoplasmic morphologically selected sperm injection without assisted oocyte activation in a patient with globozoospermia

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ABSTRACT: We here report a successful pregnancy and healthy childbirth obtained in a case of total globozoospermia after intracytoplasmic morphologically selected sperm injection (IMSI) without assisted oocyte activation (AOA). Two semen analyses showed 100% globozoospermia on classic spermogram. Motile sperm organelle morphology examination (MSOME) analysis at ×10,000 magnification confirmed the round-headed aspect for 100% of sperm cells, but 1% of the spermatozoa seemed to present a small bud of acrosome. This particular aspect was confirmed by transmission electron microscopy and anti-CD46 staining analysis. Results from sperm DNA fragmentation and fluorescence in situ hybridization analyses were normal. The karyotype was 46XY, and no mutations or deletions in SPATA16 and DPY19L2 genes were detected. Considering these results, a single IMSI cycle was performed, and spermatozoa were selected for the absence of vacuoles and the presence of a small bud of acrosome. A comparable fertilization rate with or without calcium-ionophore AOA was observed. Two fresh top-quality embryos obtained without AOA were transferred at Day 2 after IMSI, leading to pregnancy and birth of a healthy baby boy. This successful outcome suggests that MSOME may be useful in cases of globozoospermia in order to carefully evaluate sperm morphology and to maximize the benefit of ICSI/IMSI.

Key words: globozoospermia / intracytoplasmic morphologically selected sperm injection (IMSI) / motile sperm organelle morphology examination (MSOME) / sperm morphology / acrosome

Introduction

Globozoospermia, so-called round-headed syndrome, is a sperm defect of low frequency (incidence <0.1% of infertile patients) but is associated with a severe teratozoospermia causing male sterility (Schirren et al., 1971). Globozoospermia is characterized by the absence of acrosome in round-headed spermatozoa, leading to a complete inability to fertilize the oocyte, thus the affected males suffer from infertility. A genetic basis was suggested by the familial distribution of the syndrome (Kilani et al., 2004), and different modes of inheritance have been described (Dam et al., 2007a, 2011). The main morphological defect is characterized by an absent or severely malformed acrosome. The pathogenesis occurs during spermiogenesis and probably originates in acrosomic vesicle fusion impairment and cytoskeleton disorders, although precise mechanisms remain to be elucidated (Dam et al., 2007b). Total (100% round-headed spermatozoa) or partial (<100%) globozoospermia have been described (Holstein et al., 1973; Dam et al., 2011), although it is still unclear whether they are variations of the same syndrome or result from separate disorders. Two globozoospermia types have been reported: complete lack of acrosome with spherical nucleus (type I) or the presence of some residue of acrosome vesicles sometimes associated with other sperm morphology abnormalities (type II).
(Singh, 1992). However, this classification can be misleading and is not used universally.

The introduction of ICSI (Palermo et al., 1992) provided a fertility treatment for patients suffering from globozoospermia (Hamberger et al., 1998). In 1994, the first pregnancy and delivery after micro-injection of globozoospermic spermatozoa was reported by Lundin et al. (1994). However, fertilization rates in ICSI remained severely reduced, mostly because of the inability of the round-headed spermatozoa to trigger oocyte activation. Later reports showed that assisted oocyte activation (AOA) can be achieved by applying calcium-ionophore in such cases (Rybouchkin et al., 1997). However, few live births of healthy children after ICSI for globozoospermia, with or without AOA, have been reported (Table I).

Besides the routine morphological sperm analysis (spermocytogram), Motile Sperm Organelle Morphology Examination (MSOME) offers a more detailed morphological sperm examination, especially of the sperm head, at a high magnification (up to $\times6000$) (Bartoov et al., 2002). Under precise conditions, high magnification can help us to optimize sperm selection before oocyte injection (Intracytoplasmic Morphologically Selected Sperm Injection, IMSI), improving fertilization rate, embryo development and ongoing pregnancy rate (Antinori et al., 2008; Vanderzwalmen et al., 2008).

We performed an IMSI cycle for a patient with fully documented total globozoospermia in order to test whether high magnification selection of spermatozoa with small bud of acrosome would improve IVF outcome. We here report the resulting successful pregnancy and childbirth.

**Case**

A 36-year-old man and his 24-year-old wife (both from Morocco) presented to our assisted reproduction technologies (ART) unit with a primary infertility of 1-year duration. They had no medical previous history, their BMIs were normal and neither tobacco nor toxic exposure was noted. No consanguinity was observed. Two of the patient’s five brothers were also infertile but no data about their infertility was noted regarding the risk of transmitting the pathology to male offspring. Genetic counseling was given according to World Health Organization (WHO) recommendation (WHO, 2010). Both samples showed normal sperm count (52 and $18 \times 10^6$/ml) and motility (30–30% progressive motility) but 100% globozoospermia evaluated on 200 sperm cells by classic spermocytogram with Harris-Shorr coloration, suggesting a total type I globozoospermia (Fig. 1a and b). The MSOME analysis at $\times1000$ magnification (currently used in our laboratory for MSOME and IMSI procedures after high resolution calibration), performed using an inverted microscope equipped with Nomarski differential interference contrast optics, confirmed the round-headed shape for 100% of sperm cells and the lack of acrosomic material. However, 1% of the spermatozoa seemed to present a small bud of acrosome (Fig. 1c and d, Fig. 2a and b). This particular aspect was confirmed by transmission electron microscopy (TEM) observation performed on 150 sperm cells (Fig. 2c) and anti-CD46 staining analysis succeeded in detecting an ionophore-induced acrosome reaction in these few spermatozoa presenting a small bud, validating its acrosomic nature (Fig. 2e).

Complementary analyses were proposed in order to assess the presence of other abnormalities, combining sperm DNA fragmentation analysis by terminal deoxynucleotidyl-transferase-mediated dUTP nick-end-labeling (TUNEL) assay (Weng et al., 2002), sperm fluorescence in situ hybridization (FISH) analysis (Benzacken et al., 2001), somatic karyotyping, and genetic testing of the SPATA16 (Dam et al., 2007a) and DPY19L2 genes (Harbuz et al., 2011). The percentage of DNA fragmentation was within normal values (6%) and the FISH analysis of 500 spermatozoa showed a normal haploid population ($<1\%$ 13,18,21,X,Y aneuploidiies). The patient’s karyotype was 46XY with no sex chromosome mosaicism. No mutation or deletion was detected in the SPATA16 or DPY19L2 genes, respectively (data not shown).

Considering these reassuring results, the multidisciplinary committee suggested sperm donation or adoption but also proposed the possibility of a single IMSI cycle under optimized conditions to evaluate fertilization rate with and without AOA. The couple was fully informed about the diagnosis of globozoospermia, associated with the very low chances of success of an intraconjugal attempt. Genetic counseling was given regarding the risk of transmitting the pathology to male offspring. After written informed consent and approval of the institutional review board at Jean Verdier Hospital, an IMSI cycle was performed. Ovarian stimulation and cumulus–oocyte complex retrieval were carried out by standard procedures, as previously described (Huime et al., 2006). Fifteen oocytes were retrieved and 11 were at metaphase II stage. After semen preparation by density gradient centrifugation, spermatozoa were selected for injection at $\times10000$ magnification for the absence of vacuoles, and only spermatozoa presenting a small bud of acrosome were chosen (Fig. 2a). Sperm selection was particularly tedious and time-consuming. Five oocytes were injected without oocyte activation, and six were injected after oocyte activation with calcium-ionophore A23187 (Tesarik and Testart, 1994), after random allocation. Oocytes were examined for evidence of fertilization 18 h after microinjection. Seven showed normal pronuclear stage, corresponding to a normal fertilization rate of 64%, and the fertilization rates were similar in the two groups, with or without AOA [66% (4/6) and 60% (3/5), respectively]. Embryo quality was evaluated according to criteria previously described (Leniaud et al., 2008). Seven good-quality embryos were obtained and two top-quality embryos (four blastomeres without any fragmentation) obtained without any AOA were transferred at Day 2 after IMSI. Five good quality supernumerary embryos were cryopreserved with a slow freezing protocol in separate straws. Pregnancy was established by a positive beta hCG result 12 days after transfer and confirmed by ultrasonography. The pregnancy went to term without any complication and a healthy baby boy of 3490g was born after 38 weeks of gestation with no congenital abnormality.

**Discussion**

To our knowledge, this is the first report of a pregnancy and birth of a healthy child after IMSI using globozoospermic spermatozoa without AOA.

MSOME, initially developed by Bartoov et al. as a selection tool, may also represent an improvement in the evaluation of semen morphology (Bartoov et al., 2002). Although its applications are still unclear and not validated, MSOME allows the evaluation of live and motile sperm cell morphology at a high magnification (more than $\times6000$). Here, the round-headed shape and the lack of acrosomic material were confirmed during MSOME observation but $\approx1\%$ of
<table>
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<tr>
<th>Technique</th>
<th>Number of couples, births and children</th>
<th>Mean fertilization rate (range)</th>
<th>Sperm TEM</th>
<th>Sperm FISH</th>
<th>Sperm DNA fragmentation</th>
<th>Sperm CD46 staining</th>
<th>MSOME</th>
<th>Somatic karyotyping</th>
<th>Gene testing</th>
</tr>
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<tr>
<td>aICSI</td>
<td>1 couple 11 births 13 children</td>
<td>47.5% (9–89%)</td>
<td>+ / – ^ d</td>
<td>+ / – ^ *</td>
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<tr>
<td>bICSI + AOAc</td>
<td>11 couples 11 births 12 children</td>
<td>72.9% (33–100%)</td>
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<tr>
<td>IMSI</td>
<td>1 couple 1 birth 1 child</td>
<td>63.6%</td>
<td>+</td>
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TEM, transmission electron microscopy; FISH, fluorescence in situ hybridization; MSOME, motile sperm organelle morphology examination; IMSI, intracytoplasmic morphologically selected sperm injection; AOA, assisted oocyte activation.

^aICSI: Lundin et al. (1994), Kilani et al. (1998, 2004), Stone et al. (2000), Nardo et al. (2002), Zeyneloglu et al. (2002), Dirican et al. (2008), Banker et al. (2009), Bechoua et al. (2009) and Sahu et al. (2010).

^bICSI + AOAc: Rybouchkin et al. (1997), Kim et al. (2001), Tesarik et al. (2002), Dirican et al. (2008), Tejera et al. (2008), Egashira et al. (2009) and Kyono et al. (2009).

^cAOA with calcium-ionophore (Rybouchkin et al., 1997; Kim et al., 2001; Tejera et al., 2008; Kyono et al., 2009), mechanical (Tesarik et al., 2002; Dirican et al., 2008) or electrical (Egashira et al., 2009).

^dTEM was performed for eight men (Lundin et al., 1994; Stone et al., 2000, Nardo et al., 2002; Kilani et al., 2004; Dirican et al., 2008; Bechoua et al., 2009; Sahu et al., 2010) and showed total globozoospermia.

^eSperm FISH was performed for two men and showed respectively 2% 13,18,21,X,Y aneuploidies (Zeyneloglu et al., 2002) and normal result (Sahu et al., 2010).

^fSperm DNA fragmentation was performed for one man with a result of 46% in Halosperm technique (Tejera et al., 2008).

^gSperm FISH was performed for one man and showed 0.7% X,Y aneuploidies (Tejera et al., 2008).

^hSomatic karyotyping was performed for one man with a result of 46% in Halosperm technique (Tejera et al., 2008).

^iSomatic karyotyping was performed for five men showing four normal 46,XY karyotype (Dirican et al., 2008; Tejera et al., 2008; Egashira et al., 2009;Kyono et al., 2009) and 1 Down syndrome mosaicism (Kim et al., 2001).
the spermatozoa seemed to present a small bud of acrosome, suggesting that careful selection of spermatozoa for micro-injection, thanks to IMSI, might provide a valid solution for this couple.

It has also been described that morphologically abnormal sperm could be associated with genetic alterations, which could have long-term consequences for the offspring after ART. Considering the total globozoospermia of the patient, we then proposed a comprehensive approach combining an assessment of sperm DNA fragmentation by TUNEL assay, sperm FISH analysis, somatic karyotype and genetic testing of \textit{SPATA16} and \textit{DPY19L2} genes. All results were normal (data not shown).

Globozoospermia originates in a disturbed acrosome biogenesis and, although the underlying causes are still poorly known, the hypothesis of a genetic contribution is supported by several familial cases. Thus, Dam \textit{et al.} described a family with three affected brothers in whom they were able to identify a homozygous mutation in the spermatogenesis-specific gene \textit{SPATA16}, suggesting a crucial role of \textit{SPATA16} protein in acrosome formation (Dam \textit{et al.}, 2007b). Recently, a homozygous deletion of \textit{DPY19L2} gene was also identified in globozoospermia patients, indicating that \textit{DPY19L2} protein is necessary in men for sperm head elongation and acrosome formation (Harbuz \textit{et al.}, 2011). These genetic abnormalities were not detected in our patient but several other genes are probably involved in this pathology.

Several authors reported a low or absent fertilization in ICSI with globozoospermic sperm (Liu \textit{et al.}, 1995; Battaglia \textit{et al.}, 1997; Rybouchkin \textit{et al.}, 1997; Stone \textit{et al.}, 2000; Kilani \textit{et al.}, 2004) and Check \textit{et al.} (2007) also encountered fertilization failure during the only published IMSI cycle. It has been described that fertilization rate can be improved by AOA with calcium-ionophore (Rybouchkin \textit{et al.}, 1997), but its clinical use in human ART is still controversial and limited by insufficient knowledge about the potential cytotoxic, mutagenic and teratogenic effects on oocytes and embryos (Nasr-Esfahani \textit{et al.}, 2010).

The couple was fully informed about all these data and about the very low chance of success of an intra-conjugal attempt. After written informed consent, a single IMSI cycle was performed to provide the best chances to select spermatozoa for microinjection. In order to optimize fertilization rate, AOA was also used for some of the retrieved oocytes. However, we observed a normal and comparable fertilization rate with or without calcium-ionophore. We believe that high magnification may have represented in this case a tool, even a solution, to select round-headed sperms presenting sufficient amount of acrosome and essential proteins needed for fertilization. Nevertheless, it still remains unclear whether IMSI might have applications in other spermatogenesis disorders, as only few data are available (Antinori \textit{et al.}, 2008; Balaban \textit{et al.}, 2011).

Few live births after ICSI for globozoospermia have been reported without AOA, and most of these cases were described as total globozoospermia, using conventional spermocytogram and TEM. We postulate that these pregnancies were perhaps obtained, thanks to a very low percentage of spermatozoa presenting a small bud of acrosome, which was not detected both by conventional spermocytogram, because of insufficient magnification, and by TEM, because of limited number of examined cells. MSOME constitutes an easy tool to evaluate numerous live cells at a high magnification and may be relevant in helping to direct patients into ICSI without or with AOA, depending on whether buds of acrosome are observed or not, respectively.

In conclusion, we here report the first fully documented case of total globozoospermia, for whom strict morphological selection of spermatozoa at a high magnification and transfer of two top quality embryos obtained without AOA resulted in the birth of a healthy child. This successful outcome suggests that MSOME should be useful in the case of globozoospermia in order to carefully evaluate sperm morphology and consider the benefit of ICSI/IMSI.

\textbf{Figure 1} Globozoospermia morphology examination (\textit{a} and \textit{b}) using light microscopy, Harris-Shorr coloration, $\times 1000$ magnification; (\textit{c} and \textit{d}) with MSOME, $\times 10000$ magnification.
Authors’ roles


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References


Figure 2 (a, c and e) Round-headed sperm presenting a small residue of acrosome and selected for IMSI (a) with MSOME examination (c) using TEM (e) with anti-CD46 and DAPI (4(6-diamidino-2-phenylindole) staining; (b, d and f) round-headed sperm with no acrosome and not selected for IMSI, (b) with MSOME examination (d) TEM (f) with anti-CD46 and DAPI staining.
Birth after high magnification ICSI in globozoospermia

2949


