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First successful live birth following the use of MSOME and time lapse for sperm and embryo selections in a patient with severe male factor infertility: A case report

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ABSTRACT

We report a case of healthy live birth from a couple diagnosed with oligoasthenoteratozoospermia (OAT). They failed three ICSI cycles in the past 11 years. In the last cycle, the ovarian stimulation was done with antagonist protocol and six oocytes were aspirated. Semen sample was prepared by one layer gradient. 10 class I spermatozoa were selected by motile-sperm organelle-morphology examination (MSOME) according to Cassuto criteria. The selected spermatozoa were then injected into the MII oocytes. 16–18 h after ICSI, three zygotes was formed and, then, cultured in the time lapse monitoring (TLM) for 3 days. Two best embryos were selected according to the morphokinetic status for transfer. A singleton pregnancy was achieved, resulting in the birth of a healthy baby. This successful outcome shows that use of high technologies of MSOME and TLM procedures are useful for selection of the best spermatozoa and embryos in case of severe male infertility.

Introduction

Intracytoplasmic sperm injection (ICSI) has been a very powerful tool to overcome the infertility in couples with severe male factor infertility [1]. There is still a controversy as to whether sperm morphological defects influence fertilization or subsequent embryo development or not [2]. Since the selection of spermatozoa for ICSI was mainly based on viability, motility and cell morphology [2], there is high interest in developing an optimized sperm selection technique. In this regard, the motile-sperm organelle-morphology examination (MSOME) was introduced, not only for simple observation in terms of a sperm's size and shape, but also for a detailed analysis in real time of the subtle subcellular abnormalities, such as nuclear defects and vacuoles [3]. The application of MSOME for sperm evaluation and selection for ICSI indication, resulted in the development of intra-cytoplasmic morphologically selected sperm injection (IMSI) technique [1]. The IMSI procedure permits selection of spermatozoa free of anatomical defects, thus resulting in the production of more "healthy" blastocysts [4].

Another important factor in assisted reproductive technology (ART) programs is the selection of the best embryo for transfer. Miscarriage to the healthy embryos with the highest implantation potential can result in failed IVF outcomes. It may also cause multiple pregnancies, when numerous embryos are transferred with neonatal and maternal complications [5]. Embryo grading system based on morphology still remains the favorite manner of categorizing embryonic development. However, the assessment of embryo morphokinetic using the time lapse monitoring (TLM) at the cleavage stage has improved the implantation potential [6]. Morphokinetic information also helps the embryologists to classify unclear timing ranges between developmental stages in embryos with the same morphological appearance [7]. Also, TLM has made continuous monitoring of the embryo development during the culture period. It is a secure tool, with no adverse effects on the embryo development, while maintaining stable culture conditions [8]. The study of embryo kinetics through TLM has given rise to new markers for embryo selection, representing an exciting powerful tool for viewing cellular activity and embryogenesis in a coherent, continuous manner [8]. Here, we report the first live birth case using high techniques for both sperm and embryo selection, using MSOME (IMSI) and TLM in a couple diagnosed with severe male factor infertility. They had failed three previous ICSI treatment cycles.

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Case report

A 35-year-old woman and her 41-year-old husband were referred to Reproductive Science Institute, Yazd, Iran. This couple was candidate for ICSI due to oligoastheno teratozoospermia (OAT) with a history of 3 failed ICSI treatment cycles within past 11 years. Briefly, in the first treatment cycle, with 5 mature oocytes, no embryo were formed, in the second cycles, from 4 mature oocytes one embryo with grade C were transferred and finally, in the third cycles, she had 5 oocytes that 2 embryos with grade B, C were formed and transferred, but it did not lead to pregnancy. On examination of the female, the fertility checkup showed no abnormalities and the presence of a normal ovarian reserve: serum anti-Müllerian hormone levels of 3.12 ng/mL and an antral follicle count of 18 on both ovaries, by ultrasound examination of uterus and office diagnostic hysteroscopy. Semen analysis showed a volume of 2 mL, concentration of 8 million/ mL, and 30% motility, of which only 15% had progressive motility. Sperm morphology was reported as 1% normal according to WHO 2010 (Table 1). The patient was diagnosed with severe male infertility and the couple counseled for IMSI and TLM. The study was approved by the Scientific and Ethics Committee of our institution (IR.SSU.MEDICINE.REC.1394.338). A written informed consent was also obtained. The spouse was treated with antagonist protocol using ganirelix (Orgalutran–MSD) and the administration of 150 IU rFSH (Puregon - MSD) daily for 9 days. Oocyte retrieval started 36 h post hCG priming 10,000 IU (Pregnyl - MSD). A total of 6 oocytes were collected, of which 5 were mature and suitable for injection (Table 1). Semen was prepared with the use of one-layer density technique (40%) of Puresperm (Nidacon International) diluted in albuminate Hams F10 medium. After 10 min of centrifugation at 300 g, the pellet was washed twice with Hams F10 and centrifuged for another 5 min at 300 g.

For microinjection technique, the spermatozoa preselecting step was performed at x6600 magnification (MSOME) with the use of a Nomarski-contrast Nikon inverted microscope (TE300; Nikon, Tokyo, Japan). The image's capture and video recording for further analysis was performed with software (OCTAX PolarAIDE; Octax). This optical system required the use of a glass-bottomed dish (Willco GWST 0.17 mm; JCD). An elongated polyvinylpyrrolidone 2-μL drop was placed in this dish, covered with mineral oil (Nidacon International). Motile spermatozoa were selected for injection under high magnification according to the Cassuto classification [9] and transferred into the ICSI dish. Briefly, the primary objective was to select spermatozoa displaying a normal oval head shape, normal base and without vacuoles or with fewer than two vacuoles, representing <4% of the head area (class 1 or 2) as well as absence of both cytoplasmic extrusion and tail defects. However, if not available, the second-best spermatozoa with the least number of vacuoles and/or other abnormalities (grade 3 or 4) were selected. After ICSI, the injected oocytes were placed in culture dishes containing 1 mL G-1 Medium (Vitrolife, Sweden) covered with oil and incubated overnight in incubator at 37 °C and 6% CO_2. 16–18 h post-insemination, the fertilization was evaluated based on the presence of two pronuclei. Three zygotes were then transferred to a PrimoVision Embryo Culture Dish™ (Vitrolife, Sweden) that was pre-equilibrated (37 °C and 6% CO_2) using fresh G-1 medium and mineral oil. The culture dish was placed in TLM in an incubator at 37 °C, 5% O_2 and 6% CO_2. The monitoring system continually took images of the embryos using an inverted microscope every 10 min during culture period.

On day 3, the embryos were evaluated by viewing the TLM images. From the three eight cell embryos, two embryos with ideal morphokinetic parameters (grade A^+ and B^+) were selected for transfer using embryo transfer (ET) catheter (Labotect, Gottingen, Germany) (Table 1, Fig. 1). For embryo selection, t3, t5, s2 and cc2 parameters were used according to hierarchical algorithm [6]. Luteal phase support was done with 100 mg progesterone in oil (Aburaihan Co, Iran), injected daily. Estradiol valerate (Aburaihan Co, Iran) was taken orally at the dose of 6 mg/day from the day of oocytes retrieval, and continued until confirmation of fetal heart by sonography. βhCG was measured 14 days after ET, which was reported positive (>180 IU/L). At 39 weeks of pregnancy, a healthy baby boy was born by cesarean section. The baby weight at the time of birth was 3100 g with the apgar of 9/10 (Table 1).

Discussion

Due to the restrictive laws regarding the number of embryos usable for ET in ARTs, the application of non-invasive methods for gamete and embryo selection is showing a fast grows. ICSI bypasses the natural barriers of reproduction, including the danger of transferring putative negative effects on to the offspring by injecting an abnormal spermatozoon [1]. Therefore, it is reasonable to develop optimized selection techniques, however, most of the enzymatic or genetic tests currently accessible cannot be performed on viable, unfixed spermatozoa. This is the first case report in which the sperm and embryo selection was done with MSOME and TLM techniques. MSOME is the only real-time microscopy not requiring fixation, allowing selecting viable sperm with minimal ultra-structural defects [10]. The detection of large nuclear vacuoles at high magnification using MSOME could be related to DNA fragmentation and denaturation. It is widely accepted that DNA integrity plays an important role in the fertilization process as well as in the embryo development and implantation [1,11]. Recent data available have already demonstrated that the possibility to select spermatozoa for IMSI provided positive results in couples with severe male factor infertility or repeated ICSI failures. The clinical pregnancy rate was prominently improved in the IMSI group, with reduced abortion rates [11,12,2]. This can be explained by the production of more "healthy" blastocysts, without chromosomal abnormalities, thank to selection of defect-free spermatozoa by IMSI.

Despite major developments in ARTs, the selection of the best embryo for ET is still the main challenge. Progresses on this matter will maximize the IVF success rates, also minimizing the risks of multiple pregnancies accompanied by the related maternal and fetal complications [5,8]. Embryo morphology has been the most common method used by embryologists for monitoring the embryo growth and selection of optimal embryo for ET. But, today the frequency of microscopic observations has decreased in order to minimize the potential negative effect of handling the embryos outside the incubator. Additionally, the variation of time-points assessment may contribute to confusing the findings. For instance, day-2 embryos may be at the two-cell stage in the morning of day 2 but, if assessed a few hours later, they can found to be at the four-cell stage. Indeed, standard morphological assessment is vulnerable to the timing of observations and

Table 1
Laboratory and clinical characteristics of the patient.

variables	Sperm count	Progressive motility	Normal morphology	Oocyte number	MII oocyte	2PN	embryo	ET	Live birth
	8×10^6	15%	1%	6	5	3	3	2	1

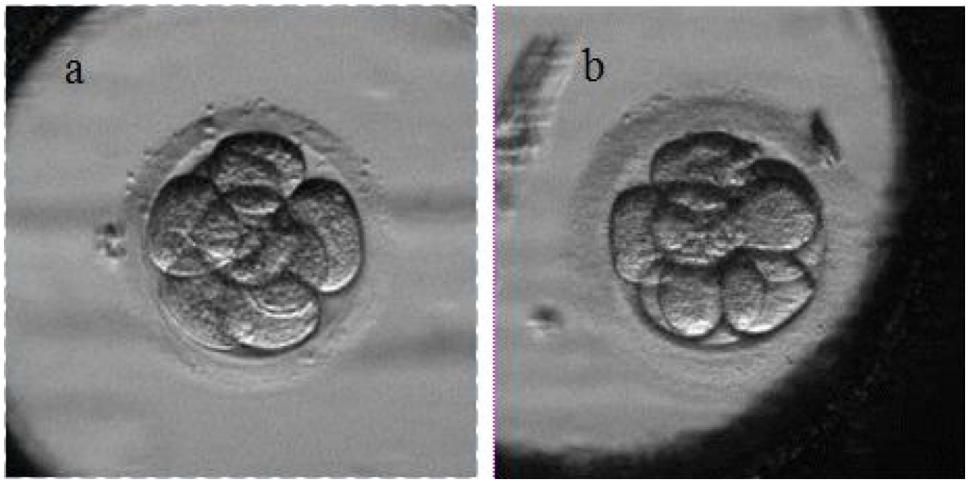

Fig. 1. The two transferred embryos; a: embryo with A^+ grade. B: embryo with B^+ grade according to TLM algorithm [6].

inherent variability in embryo scoring among embryologists. Therefore, the introduction of TLM has offered solutions to overcome some of the difficulties of standard morphological assessment. First, TLM has safely allowed the incubation of embryos in stable culture conditions by minimizing the potential impact of changes in temperature or gas composition. Secondly, it allows for continuous observation of embryo development, thereby increasing our knowledge of embryo kinetics. Lastly, TLM allows for the evaluation of quantitative and qualitative objective parameters, thus reducing inter- and intra-observer variations [13]. Furthermore, without TLM, transient features, such as multinucleation, can easily be missed when embryos are limited to a single daily observation. The detrimental effect of multi-nucleation on embryo implantation, pregnancy, and birth rates has been reported before [14]. Time-lapse imaging and analysis ensure that such phenomena are recorded, allowing embryo deselection, where appropriate [15].

In conclusion, this case report demonstrates for the first time a successful live birth following the use of non-invasive high technology for sperm and embryo selection in a patient with severe male factor infertility following 3 previously failed ICSI treatment cycles. Further studies are needed to prove whether these techniques open a new dimension in infertility treatment program.

Declaration of interest

The authors declare that they have no conflict of interest.

References

[1] Sakkas D. Novel technologies for selecting the best sperm for in vitro fertilization and intracytoplasmic sperm injection. [Review]. Fertil Steril 2013;99(4):1023–9.
[2] Setti AS, Braga DP, Figueira RC, Iaconelli Jr A, Borges E. Intracytoplasmic morphologically selected sperm injection results in improved clinical outcomes in couples with previous ICSI failures or male factor infertility: a meta-analysis [Comparative Study Meta-Analysis Review]. Eur J Obstet Gynecol Reprod Biol 2014;183:96–103.
[3] Taherzadeh S, Khalili MA, Agha-Rahimi A, Anbari F, Ghazali S, Macchiarelli G. Vitrification increased vacuolization of human spematozoa: application of MSOME technology. J Reprod Infertil 2017;18(2):225–30.
[4] Klement AH, Koren-Morag N, Itsykson P, Berkovitz A. Intracytoplasmic morphologically selected sperm injection versus intracytoplasmic sperm injection: a step toward a clinical algorithm. Fertil Steril 2013;99(5):1290–3.
[5] Faramarzi A, Khalili MA, Micara G, Agha-Rahimi A. Revealing the secret life of pre-implantation embryos by time-lapse monitoring: a review. [Review]. Int J Reprod Biomed 2017;15(5):257–64.
[6] Meseguer M, Herrero J, Tejera A, Hilligsoe KM, Ramsing NB, Remohi J. The use of morphokinetics as a predictor of embryo implantation [Research Support, Non-U.S. Gov't]. Hum Reprod 2011;26(10):2658–71.
[7] Yang Z, Zhang J, Salem SA, Liu X, Kuang Y, Salem RD, et al. Selection of competent blastocysts for transfer by combining time-lapse monitoring and array CGH testing for patients undergoing preimplantation genetic screening: a prospective study with sibling oocytes. BMC Med Genomics 2014;7:38.
[8] Mangoli E, Khalili MA, Talebi AR, Ghasemi-Esmailabad S, Hosseini A. Is there any correlation between sperm parameters and chromatin quality with embryo morphokinetics in patients with male infertility? Andrologia 2018.
[9] Cassuto NG, Bouret D, Plouchart JM, Jellad S, Vanderzwalmen P, Balet R, et al. A new real-time morphology classification for human spermatozoa: a link for fertilization and improved embryo quality. [Evaluation Studies]. Fertil Steril 2009;92(5):1616–25.
[10] Boitrelle F, Guthauser B, Alter L, Bailly M, Bergere M, Wainer R, et al. High-magnification selection of spermatozoa prior to oocyte injection: confirmed and potential indications. [Review]. Reprod Biomed Online 2014;28(1):6–13.
[11] Ghazali S, Talebi AR, Khalili MA, Aflatoonian A, Esfandiari N. Large nuclear vacuoles in spermatozoa negatively affect pregnancy rate in IVF cycles. Iran J Reprod Med 2015;13(7):425–32.
[12] Gong Y, Meng XQ, Liu M, Wang J, Xiong F, Zhong Y, et al. [Intracytoplasmic injection of morphologically selected spermatozoa from patients with male factor infertility: clinical and embryo development outcomes]. Nan fang yi ke da xue xue bao = J South Med Univ 2015;35(10):1428–33.
[13] Omidi M, Faramarzi A, Agharahimi A, Khalili MA. Noninvasive imaging systems for gametes and embryo selection in IVF programs: a review. [Review]. J Microsc 2017;267(3):253–64.
[14] Desch L, Bruno C, Luu M, Barberet J, Choux C, Lamotte M, et al. Embryo multinucleation at the two-cell stage is an independent predictor of intracytoplasmic sperm injection outcomes. Fertil Steril 2017;107(1)97–103 e104.
[15] Zaninovic N, Irani M, Meseguer M. Assessment of embryo morphology and developmental dynamics by time-lapse microscopy: is there a relation to implantation and ploidy? [Review]. Fertil Steril 2017;108(5):722–9.