Human Fertility, 2015; 18(2): 81–86

ISSN 1464-7273 print/ISSN 1742-8149 online
DOI: 10.3109/14647273.2014.956812

informa
healthcare

ORIGINAL ARTICLE

Sperm morphological normality under high magnification predicts laboratory and clinical outcomes in couples undergoing ICSI

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Abstract

The objective of this study was to evaluate whether 'motile sperm organelle morphology examination' (MSOME) is correlated with the outcome of ICSI. A total of 14400 spermatozoa from 72 couples undergoing ICSI were analysed by MSOME (x6600) and graded into four groups: grade I, normal form and no vacuoles; grade II, normal form and lesser than or equal to 2 small vacuoles; grade III, normal form greater than 2 small vacuoles or at least one large vacuole and grade IV, large vacuole and abnormal head shapes or other abnormalities. The correlations between the proportion of morphologically normal spermatozoa (grade I + II) and ICSI outcomes were assessed. The proportion of grade I+ II spermatozoa was lower in patients with oligoasthenoteratozoospermia (OAT) compared to patients with other types of semen alterations (10.6% vs. 17.0%, $p = 0.001$). The proportion of grade I+ II spermatozoa was positively correlated with blastocyst formation ($S = 8.31$, R^2:13.5%, $p = 0.014$) and implantation rates ($S = 8.32$, R^2: 7.9%, $p = 0.030$). The proportion of grade I + II spermatozoa was higher in patients with ongoing pregnancy in comparison with those who had a miscarriage (23.2% vs. 10.8%, $p = 0.007$). Sperm morphological normality was lower in oligoasthenoteratozoospermia patients but correlated with blastocyst formation, implantation and miscarriage rates in couples undergoing ICSI. MSOME may be valuable in predicting ICSI outcomes.

Keywords: *MSOME, ICSI, semen analysis, sperm, vacuole*

Clinical trial ID: *NCT01731639*

Introduction

Sperm morphology has been described as one of the major determinants of male *in vitro* fertility potential (Kruger et al., 1986, 1987, 1988; Lundin et al., 1997; De Vos et al., 2003). However, the standard morphological evaluation of random stained cells from the ejaculate is of limited value during ICSI. A new method for detailed morphological evaluation of motile spermatozoa in real time, called 'motile sperm organelle morphology examination' (MSOME) relies on the assessment of spermatozoa morphology under a magnification of at least 6000x (Bartoov et al., 2001).

The MSOME assesses mainly the nucleus, which is the most important sperm organelle in influencing ICSI outcomes, for both shape and the presence of vacuoles. Moreover, the factors that cause these sperm alterations seem to affect the molecular mechanisms regarding DNA integrity, chromosome status, and chromosome segregation (Franco et al., 2008; Garolla et al., 2008; Vanderzwalmen et al., 2008).

Relationships between morphological sperm normality through MSOME and the fertilisation rate, percentage of high-quality embryos, implantation rate, pregnancy rate, and miscarriage rate have previously been published. However, it has still to be elucidated whether the proportion of abnormal sperm in a sample correlates with the outcome of ICSI cycles. The goal of this study was to evaluate whether MSOME can be used as a prognostic tool to predict ICSI success.

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(Received 22 January 2014; revised 9 May 2014; accepted 11 June 2014)

Materials and methods

Experimental design, patients and inclusion criteria

This prospective non-randomised study analysed semen samples of 72 couples undergoing their first ICSI treatment as a result of male factor infertility. The inclusion criteria were as follows: women aged lesser than or equal to 38 years, undergoing ICSI, with regular menstrual cycles of 25–35 days, normal basal FSH and LH levels, body mass index (BMI) less than 30 kg/m^2, presence of both ovaries and an intact uterus, absence of polycystic ovaries, endometriosis, or gynaecological/medical disorders and a negative result in screening for sexually transmitted diseases. No patient had received hormone therapy for at least 60 days preceding the study.

Written informed consent was obtained, in which the patients agreed to share the outcomes of their own cycles for research purposes, and the study was approved by the local institutional review board (protocol 411/2012).

Controlled ovarian stimulation

On day 3 of the cycle, ovarian stimulation was commenced with 225 IU of recombinant FSH (rFSH, Gonal-F®, Serono, Geneve, Switzerland) on a daily basis (day 1 of ovarian stimulation = S1). On S4, the recombinant FSH dose was reduced to 150 IU until the visualization of at least one follicle greater than or equal to 14 mm, at which time, administration of cetrorelix acetate began (Cetrotide; Serono, Geneve, Switzerland) 0.25 mg subcutaneously (SC). When ≥ 3 follicles attained a mean diameter of ≥ 17 mm, hCG injection (Ovidrel®; Serono, Geneve, Switzerland) 250 μg was administered SC. Oocyte retrieval was performed 35 h later, through transvaginal ultrasonography.

Luteal phase support was supplemented with vaginal administration of 600 mg of micronized progesterone (Utrogestan®; Farmoquímica, Rio de Janeiro, Brazil) starting one day after oocyte retrieval and continuing until 12 weeks of gestation in the presence of a positive hCG test.

Oocyte preparation

Retrieved oocytes were maintained in culture media (Global® for fertilization, LifeGlobal, Connecticut, USA) supplemented with 10% protein supplement (LGPS, LifeGlobal, Connecticut, USA) and covered with paraffin oil (Paraffin oil P.G., LifeGlobal, Connecticut, USA) for 2–3 h before cumulus cell removal. Surrounding cumulus cells were removed after exposure to a HEPES buffered medium containing hyaluronidase (80IU/ml, LifeGlobal, Connecticut, USA). The remaining cumulus cells were mechanically removed by gently pipetting with a hand-drawn Pasteur pipette (Humagen Fertility Diagnostics, Charlottesville, USA).

Oocyte morphology was assessed using an inverted Nikon Diaphot microscope (Eclipse TE 300; Nikon®, Tokyo, Japan) with a Hoffmann modulation contrast system under 400X magnification, just before sperm injection (3–4 h after retrieval). Oocytes observed to have released the first polar body were considered mature and used for ICSI.

Semen sample collection and preparation

All semen samples were collected by masturbation after ejaculatory abstinence. After liquefaction for 30 minutes at room temperature, the semen samples were evaluated for ejaculate volume, colour, pH, sperm count, sperm motility and sperm morphology (Bartoov et al., 2002, 2003; Berkovitz et al., 1999, 2005; Berkovitz et al., 2006a, 2006b; Hazout et al., 2006; Gonzalez-Ortega et al., 2010; Knez et al., 2011, 2012; Wilding et al., 2011; El Khattabi et al., 2013; Klement et al., 2013).

A two-layered density gradient centrifugation technique, performed according to the manufacturer's instructions, was used for the sperm preparation (90% Isolate and 50% Isolate, Irvine Scientific, Santa Ana, CA, USA). Sperm count and motility were analysed in the recovered fractions.

ICSI

Oocytes that were observed to have released the first polar body were considered mature and were used for ICSI. ICSI was performed according to Palermo et al. (1992), in micro-injection dish, prepared with 4 μL droplets of buffered medium (Global® w/HEPES, LifeGlobal, Connecticut, USA), and covered with paraffin oil on a heated stage at 37.0 ± 0.5°C of an inverted microscope. The spermatozoa were selected for ICSI under x400 magnification.

MSOME

Immediately after ICSI, a total of 200 spermatozoa from each sample were analysed at high magnification using an inverted Nikon Diaphot microscope equipped with high-power differential interference contrast optics (DIC/Nomarski). The total calculated magnification was x6,600. An aliquot of the sperm cell suspension was transferred to a microdroplet of modified human tubal fluid medium (Global® w/HEPES, LifeGlobal, Connecticut, USA) containing 8% polyvinyl pyrrolidone (PVP; Irvine Scientific, Santa Ana, CA) in a sterile glass dish (FluoroDish; World Precision Instrument, Sarasota, FL). The dish was placed on a microscope stage above an Uplan Apo x 100 oil/1.35 objective lens previously covered with a droplet of immersion oil.

The sperm cells were graded into four groups according to the presence or size of the vacuoles: grade I, normal form and no vacuoles; grade II, normal form and lesser than or equal to 2 small vacuoles; grade III, normal form greater than 2 small vacuoles or at least one large vacuole and grade IV, large vacuole and abnormal head shapes or other abnormalities (Vanderzwalmen et al., 2008).

Fertilisation, embryo quality and embryo transfer

Approximately 16 h after ICSI, fertilisation was confirmed by the presence of two pronuclei and the extrusion of the second polar body. Embryos were maintained in a 50 μL drop of culture medium (Global®, LifeGlobal, Connecticut, USA) supplemented with 10% protein supplement (LGPS, LifeGlobal, Connecticut, USA) covered with paraffin oil in a humidified atmosphere under 6% CO_2 at 37°C for 5 days.

High-quality embryos were defined as those showing 8–10 cells on the third day of development, less than 15% fragmentation, symmetrical blastomeres, an absence of multinucleation and an absence of zona pellucida dysmorphisms. The evaluation of blastocyst morphology has been described elsewhere (Gardner and Schoolcraft, 1999).

Embryo transfer was performed on day 5 of development using a soft catheter with transabdominal ultrasound guidance. One to three embryos were transferred per patient depending on embryo morphology and patients' age.

Clinical follow-up

A pregnancy test was performed 12 days after embryo transfer. All women with a positive test had a transvaginal ultrasound scan 2 weeks after the positive test. A clinical pregnancy was diagnosed as foetal heartbeat.

Data analysis and statistics

The proportion of grade I+II spermatozoa was compared between the groups of patients with oligoasthenoteratozoospermia (OAT) and other types of semen alterations and between the groups who experienced pregnancy and miscarriage. The correlations between the percentage of grade I+II spermatozoa and ICSI outcomes, including (i) fertilization rate, (ii) high-quality embryos on day 3, (iii) blastocyst formation rate and (iv) implantation rate were assessed. Regression analyses, controlled for maternal and paternal ages, and the number of mature oocytes, were used to investigate the influence of grade I+II spermatozoa on (i) blastocyst formation rate, (ii) implantation rate, (iii) pregnancy rate and (iv) miscarriage rate.

High-quality embryo rate on day 3 was calculated as a proportion of zygotes obtained, and blastocyst formation rate was calculated as a proportion of day 3 embryos. Implantation rate was calculated per embryos transferred. Pregnancy rates were calculated per transfer. Miscarriage was defined as pregnancy loss before 20 weeks.

Data are expressed as mean ± standard deviation for continuous variables, while percentages were used for categorical variables. Mean values were compared by Student's *t* parametric test or Mann–Whitney non-parametric test. Percentages were compared by the Chi-squared or Fisher exact test, only when expected frequency was five or fewer. Spearman's rank correlation results are presented as correlation coefficients (r) and *p* values. Data from the linear regressions are presented as the regression coefficient (RC), R^2, slope and *p* value. The results of the logistic regression are presented as the odds ratio (OR), *p* value, and 95% confidence interval (CI). Data analysis was conducted using MINITAB 16 Software.

Results

A total of 40 patients with OAT and 32 patients with other types of semen alterations were included in this study (17 teratozoospermic, 10 asthenoteratozoospermic and 5 astenozoospermic patients). The mean male age was 40.2 ± 8.8 years. In the native semen sample total sperm count was 37.9 ± 33.0 million, percentage of progressive spermatozoa was 45.1 ± 13.6%, non-progressive spermatozoa was 21.3 ± 6.5% and percentage of normal forms was 2.6 ± 1.6%. In the prepared semen sample total sperm count was 5.0 ± 4.4 million, percentage of progressive spermatozoa was 87.5 ± 13.4% and non-progressive spermatozoa was 15.8 ± 4.9%.

Of 14,400 spermatozoa analysed under high-magnification, 260 were classified as grade I (1.8%), 1,843 as grade II (12.8%), 1,166 as grade III (8.1%) and 11,131 as grade IV (77.3%).

The mean female age was 36.8 ± 5.7 years. The stimulation outcomes were mean total dose of FSH administered, 1908.3 ± 628 IU; number of follicles, 14.0 ± 8.6; number of oocytes retrieved, 8.9 ± 5.9 and number of mature oocytes, 7.7 ± 5.2. The ICSI outcomes were fertilisation rate, 72.5%; high-quality embryos rate on day 3 of development, 69.8%; blastocyst formation rate, 32.5%; transferred embryos, 1.6 ± 0.5; proportion of cycles with embryo transfer, 83.3% (60/72); implantation rate, 23.9% (23/96); pregnancy rate, 33.3% (20/60) and miscarriage rate, 20.0% (4/20).

Descriptive statistics of OAT and non-OAT subgroups are provided in Table I. The percentage of grade I+II spermatozoa was significantly lower in patients with OAT compared to the other semen alterations (10.6% vs. 17.0%, $p = 0.001$).

The correlations results are shown in Table II. There was no correlation between the percentage of grade I+II sperm and paternal age. Although weak, there were positive correlations between the percentage of grade I+II sperm and total sperm count and percentage of progressive sperm in the native and prepared semen sample.

There were no correlations between the percentage of grade I+II sperm and fertilization rate and high-quality embryos rate on day 3. However, the percentage of grade I+II sperm was positively correlated with blastocyst formation and implantation rates (Table II).

Regression analyses results demonstrated that the percentage of grade I+II sperm positively influenced blastocyst formation (slope: 8.31, R^2: 13.5, $p = 0.014$) and implantation rates (slope: 8.32, R^2: 7.9, $p = 0.030$).

Table I. Semen parameters and stimulation and ICSI outcomes for OAT and non-OAT subgroups.

Variable	OAT group ($n = 40$)	Non-OAT group ($n = 32$)	p value
Male age (year-old)	39.8 ± 9.4	40.6 ± 7.6	0.691
Native sperm sample			
Total sperm count (million)	22.1 ± 14.1	53.7 ± 32.4	< 0.001
Sperm motility (%)	39.0 ± 5.8	51.2 ± 10.3	< 0.001
Sperm morphology (%)	2.5 ± 1.1	2.7 ± 2.1	0.632
Prepared sperm sample			
Total sperm count (million)	2.0 ± 1.7	7.2 ± 4.7	< 0.001
Sperm motility (%)	71.3 ± 15.2	98.5 ± 0.9	< 0.001
Female age (year-old)	36.0 ± 5.0	36.8 ± 4.9	0.500
Total FSH administered (IU)	1837.5 ± 655.4	1996.9 ± 590.2	0.284
Number of follicles	14.9 ± 6.9	13.0 ± 8.6	0.398
Number of oocytes retrieved	9.7 ± 5.0	8.1 ± 5.3	0.191
Number of mature oocytes	8.5 ± 4.8	6.9 ± 5.1	0.169
Fertilization rate (%)	71.4	73.5	0.763
High-quality embryos rate on D3 (%)	68.5	71.0	0.547
Blastocyst formation rate (%)	27.0	38.0	0.042
Implantation rate (%)	20.4	26.8	0.502
Pregnancy rate (%)	8/27 (29.6)	12/33 (36.4)	0.583
Miscarriage rate (%)	3/8 (37.5)	1/12 (8.3)	0.138

There were no significant differences in the percentage of grade I+ II spermatozoa between the patients in which pregnancy was achieved or not (14.5% vs. 17.0%, $p = 0.376$); however, the percentage of grade I+ II spermatozoa was significantly higher in patients with ongoing pregnancy as compared to those who had miscarriages (23.2% vs. 10.8%, $p = 0.007$).

Despite no influence having been seen in the odds of pregnancy (OR: 1.21, CI: 0.91–1.40, $p = 0.243$), the percentage of grade I+ II sperm was a determinant of the decreased odds of miscarriage (OR: 1.53, CI: 1.23–2.49, $p = 0.007$).

Discussion

The impact of sperm morphology on ICSI success has been a matter of debate. With the advent of MSOME, it is now possible to examine sperm morphological normality at the sub-cellular level (Bartoov et al., 2001). Furthermore several reports have recently supported an association between sperm abnormal morphology through MSOME and DNA damage (Garolla et al., 2008, de Almeida Ferreira Braga et al., 2011, Wilding et al., 2011, Cassuto et al., 2012, Franco et al., 2012, Hammoud et al., 2013).

Table II. Correlation results between the percentage of grade I+ II sperm and (i) semen parameters and (ii) ICSI outcomes.

	Percentage of grade I+ II sperm	
Variable	R	p value
Paternal age	− 0.160	0.181
Native sperm sample		
Total sperm count	0.325	0.005
% of progressive sperm	0.248	0.036
Prepared sperm sample		
Total sperm count	0.236	0.036
% of progressive sperm	0.242	0.041
Fertilization rate	− 0.194	0.112
High-quality embryos on D3	− 0.122	0.337
Blastocyst formation rate	0.367	0.014
Implantation rates	0.280	0.030

The aim of this study was to evaluate whether the MSOME can be used as a prognostic tool in the prediction of ICSI success. Our results demonstrate that the proportion of normal spermatozoa is significantly lower in patients with OAT compared to other types of semen alterations, consistent with previous observations that normal spermatozoa derived from OAT semen samples have alterations in their physiologic status related to DNA integrity, chromosome status and chromosome segregation (Burrello et al., 2004). Nevertheless, it is important to emphasise that a high proportion of abnormal sperm have been reported even in normozoospermic patients (Perdrix et al., 2012).

In addition, our results showed that the proportion of normal spermatozoa analysed under high magnification positively correlated with blastocyst formation and implantation rates, and was a determinant of the decreased odds of miscarriage in couples undergoing ICSI. These findings are consistent with the literature arguing that irreparable irregularities of the paternal genome affects blastocyst development (Vanderzwalmen et al., 2008). It is well known that the effects of the injection of an abnormal spermatozoon is not usually detected before the 4–8-cell stage, when major expression of paternal genes is initiated and activation of the embryonic genome occurs (Vanderzwalmen et al., 2008). Hence, it is plausible that sperm could affect embryo development only after the activation of the embryonic genome. This effect is known as the 'late paternal effect' and could lessen implantation potential and give rise to an increased risk of miscarriage (Tesarik et al., 2004). In addition, the findings of a recent meta-analysis demonstrated a significant relationship between the level of sperm DNA fragmentation and spontaneous pregnancy loss (Robinson et al., 2012).

The main drawback of MSOME is that it is time-consuming. Another disadvantage is the lack of precise values, leading to a subjective high magnification analysis (Perdrix et al., 2012). On the other hand, MSOME presents two major advantages: small intervariation between two consecutive analyses (Oliveira et al., 2010) and the fact that it is an unstained, real-time assessment of spermatozoa that can be performed prior to ICSI, in order to select the best spermatozoon for oocyte injection.

It is important to emphasize that the majority of patients will have a higher percentage of grade 3–4 spermatozoa than grade 1–2. However, MSOME provides a stricter evaluation criterion for sperm morphology and enables the identification of abnormalities more accurately than the analysis of stained samples under 1,000x (Oliveira et al., 2009). Conventional semen analysis has been used to aid in the selection of the most appropriate treatment for infertile couples by artificial insemination, conventional IVF or ICSI. Our results suggest that the evaluation of sperm morphology under high magnification can be used as a tool to select couples who would benefit from conventional ICSI or IMSI. For that to occur would require the establishment of a threshold for the proportion of normal sperm.

Declaration of interest: The authors report no declarations of interest. The authors alone are responsible for the content and writing of the paper.

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