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Which semen analysis correlates with favorable Intracytoplasmic morphologically selected sperm injection (IMSI) outcomes?

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ABSTRACT

Objective: To assess whether a correlation exists between different sperm pathologies and Intracytoplasmic morphologically selected sperm injection (IMSI) outcomes.
Study design: A retrospective cohort study which included couples with recurrent implantation failures (2 or more unsuccessful IVF-ICSI cycles) undergoing their first IVF-IMSI cycle in Hebrew-University Hadassah Medical Center between January 2008 and May 2017.
Results: A total of 170 couples with at least two IVF failures attempting their first IVF-IMSI cycle were included, of them 56 (32.9%) achieved a clinical pregnancy. No correlation was found between clinical pregnancy and a specific abnormal semen parameter. However, a positive correlation with clinical pregnancy was demonstrated when all three semen parameters were abnormal (OR-3.33, p = 0.015).
Conclusions: Our findings suggest that IMSI procedure may be more efficient in severe compound sperm pathologies than in patients with one abnormal sperm parameter. Future prospective trials are required to reinforce these findings and allow formation of clear indications for IMSI.

Introduction

Semen analysis is still the cornerstone in male fertility evaluation, and its importance in clinical practice cannot be underestimated. In cases of male infertility, intracytoplasmic sperm injection (ICSI) is used with motile sperm chosen according to their normal shape morphology under ×200 or ×400 times magnification. However, subtle changes in sperm morphologic characteristics cannot be easily detected under this degree of magnification. Therefore, several techniques were developed to optimize the selection of spermatozoa, including Intracytoplasmic morphologically selected sperm injection (IMSI), which offered selection of motile spermatozoa under ×6000 times magnification and above [1].

Since its first introduction, the benefits of IMSI over ICSI have been a matter of controversy. Some studies demonstrated its advantages regarding fertilization rate [2], implantation, clinical pregnancy and delivery rate [3–6], while others did not show any significant difference in IVF results between the two techniques [7]. This diversity can be explained by variations in the inclusion criteria used in different publications. While some included couples with repeated IVF failures [5,8], others included unselected population [7] or male factor infertility [9].

Infertility attributed to male factor is composed of several pathologies, relating to abnormal concentration, motility or morphology, and therefore should not be regarded as a single disturbance. The benefits of IMSI according to the different alterations in semen parameters have not been assessed previously. Hence, the purpose of our study is to assess IMSI outcomes in regard to different sperm disturbances and by that, defining which subgroup of patients diagnosed with male factor infertility might benefit the most from the IMSI procedure.

Materials and methods

Study design and population

This retrospective cohort study included couples after embryo transfer using fertilization by IMSI in Hebrew-University Hadassah Medical Center between January 2008 and May 2017. Inclusion criteria were couples with recurrent implantation failures (2 or more unsuccessful IVF-ICSI cycles, [5,8]) undergoing their first IVF-IMSI cycle. Data collection included male and female age, gravidity, parity, basal female FSH levels, previous IVF cycles, smoking status,

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diagnosis of varicocele, sperm parameters (volume, concentration, motility and morphology), and variables relating to IVF-IMSI cycle outcomes including fertilization and clinical pregnancy rates.

The primary outcome was clinical pregnancy rate, whereas the primary exposure variables were semen parameters.

In order to standardize sperm parameters, sperm assessment was performed using the World Health organization (WHO) definition and guidelines [10]. These guidelines define normal ejaculate volume of 1.5–7 milliliters (ml), normal concentration of at least 15 million spermatozoa per milliliter, motility of at least 40% and presence of at least 4% normal morphology spermatozoa.

Since we intended to assess the correlation between semen parameters and treatment outcomes we further tested these parameters together with possible confounders including: Male and female age, basal female FSH levels and number of oocytes retrieved.

Additional analysis was held in order to determine whether a correlation exists between patients with one abnormal sperm parameter or Oligoasthenoteratozoospermia (OAT) and IMSI.

Intracytoplasmic morphologically selected sperm injection technique

As was previously described [5], the sperm preparation method is similar to that preformed in the ICSI procedure, though a difference exists in the selection method of the spermatozoa. The selection of spermatozoa for injection included observation of the semen sample under 6600 magnifications by an inverted microscope (equipped with the Nomarski differential interference contrast optics) in order to detect minor morphological defects in the sperm head and its organelles. The motile sperm organelle morphology examination (MSOME) selection criteria regarding the sperm head, involves three parameters: the shape, the presence of vacuoles and the base.

The normal morphology of the nucleus is determined according to its shape and the appearance of the chromatin mass. The nucleus should not demonstrate more than one vacuole involving less than 4% of its area. Motile Spermatozoa with morphologically normal nuclei were retrieved and placed in a dish used for classic ICSI procedure.

Embryo transfer

Women had controlled ovarian stimulation with either antagonist or short agonist protocols. Oocyte retrieval was performed using transvaginal ultrasound guidance. Embryo quality was determined by cell number, symmetry and fragmentation and was graded “A”,”B” or “C” according to the SART grading. Embryo transfer was performed on day 2, 3 or at blastocyst stage according to patient and laboratory preference. Embryo transfer was performed using one of two catheters upon operator preference- the Edwards-Wallace catheter (Classic Embryo Replacement Catheter; Smiths Medical, Hythe, Kent, U.K.) or the SIVF catheter (K-Jets-7019-SIVF; Cook IVF, Eight Miles Plains, Queensland, Australia).

Statistical analysis

Demographic, obstetrical and reproductive characteristics were compared between the successful (defined by evidence of clinical pregnancy) and unsuccessful IVF-IMSI cycles using two-tailed Student’s *t*-test and the χ^2 test for continuous and categorical variables, respectively. For all continuous variables we tested for normality and linearity. An unadjusted logistic regression analysis was then performed in order to assess correlation between male and female factors and treatment outcomes – fertilization rate and clinical pregnancy. In order to neutralize the possibility of confounders such as female and male age, basal FSH levels and number of oocytes retrieved, a further adjusted analysis was done controlling for these variables and each of the semen parameters. Adjusted odds ratios and 95% confidence intervals were calculated for these factors. P value of less than 5% was considered significant. Data were analyzed using the Data Analysis and Statistical Software (STATA)

Results

One-hundred-ninety-nine couples underwent 332 IVF-IMSI cycles in our medical center between January 2008 and May 2017. Of those, 170 couples were included in the analysis with repeated implantation failures (previous 2 or more IVF failures) attempting their first IVF-IMSI cycle. Twenty-nine couples that underwent less than two previous implantation failures (non IMSI cycles) prior to IMSI were excluded. In addition, 133 repeated IMSI cycles were excluded from the study.

Clinical pregnancy was observed in 56 cycles while 114 cycles did not result in pregnancy. Comparison of the basic characteristics of each group showed a slightly older female age (35.73 ± 5.43 vs. 33.79 ± 4.29 years, p = 0.02) and fewer retrieved oocytes and MII oocytes (9.74 vs. 12.95oocytes and 10.22 vs. 7.71, respectively; p < 0.001) in the unsuccessful IVF-IMSI cycles. However, the MII rate was similar (0.81 in the unsuccessful IMSI vs. 0.78 in the successful IMSI).

No difference was found between the groups when comparing other basic characteristics including embryos quality on cleavage stage, blastocyst rates and number of transferred embryos (Table 1).

Similarly, Univariate analysis of the parameters associated with clinical pregnancy, showed significant positive association only with younger female age (OR= 0.93, p = 0.02) and higher number of oocytes retrieved (OR= 1.11, p=>0.001) and not with isolated sperm parameters, male age or basal FSH levels (Table 2). Multivariate analysis, however, showed no significant correlation with any of the abovementioned parameters (Table 3).

Table 1
Comparison of basic characteristics between the successful and unsuccessful clinical pregnancy groups in intracytoplasmic morphologically selected sperm injection cycles.

Variable	Clinical pregnancy	No pregnancy	P value
N	56	114	
Gravidity	1.69±2.10	1.38±1.70	0.31
Parity	0.49±0.74	0.38 ± 0.63	0.29
Smoking	10(17.6%)	18 (18.4%)	0.93
Varicocele	10 (17.9%)	16 (14.0%)	0.52
Basal FSH levels	7.10 ± 4.09	7.32±3.05	0.70
Female age (y)	33.79 ± 4.29	35.73 ± 5.43	0.02
Male age (y)	37.38 ± 5.99	38.44 ± 6.84	0.33
Sperm parameter			
Volume (ml)	2.67±1.48	2.57±1.83	0.73
Concentration (10*6/ml)	26.02 ± 35.24	31.34 ± 41.67	0.42
Motility (%)	27.69±17.79	32.41 ± 23.44	0.19
Normal morphology (%)	6.48 ± 10.89	9.23 ± 12.05	0.16
No. of previous IVF cycles	5.39±3.04	6.16±3.24	0.14
No. of oocytes retrieved	12.95±5.71	9.74 ± 5.49	0.001>
No. of MII oocytes	10.21 ± 5.00	7.71 ± 4.51	0.001>
MII rate	0.78±0.17	0.81 ± 0.18	0.40
% Grade A embryos[a]	71.66±31.65	63.49±35.28	0.10
% Grade B embryos[a]	20.23±20.80	24.38±27.75	0.25
% Grade C embryos[a]	7.20±15.92	10.39 ± 20.75	0.23
Blastocyst rate	18.23%	19.45%	0.89
No. of embryo transferred	2.36 ± 0.82	2.14 ± 0.92	0.08

Data presented as mean ± SD, % or n(%).
IMSI, intracytoplasmic morphologically selected sperm injection; FSH, follicular stimulating hormone.
[a] Embryo quality was determined according to the SART grading.

Table 2
Univariate analysis of the parameters associated with clinical pregnancies in intracytoplasmic morphologically selected sperm injection cycles.

	OR (95% CI)	P value
Female age	0.93 (0.87-0.99)	0.02
Male age	0.98 (0.93-1.03)	0.32
Basal FSH levels	0.98 (0.89-1.08)	0.69
Sperm parameter		
Concentration (10*6/ml)	0.99 (0.99-1.01)	0.42
Motility (%)	0.99 (0.98-1.01)	0.19
Normal morphology (%)	0.98 (0.94-1.01)	0.17
N of oocytes retrieved	1.11 (1.05-1.18)	0.001>

IVF, In vitro fertilization; IMSI, morphologically selected sperm injection; FSH, follicular stimulating hormone.

Table 3
Multivariate analysis of parameters associated with clinical pregnancies in intracytoplasmic morphologically selected sperm injection cycles.

	OR (95% CI)	P value
Female age	0.94 (0.86–1.04)	0.23
Male age	1.02 (0.95–1.10)	0.58
Basal FSH levels	1.05 (0.94–1.17)	0.40
Sperm parameter		
Concentration (10*6/ml)	1.00 (0.99–1.01)	0.53
Motility (%)	0.99 (0.97–1.01)	0.47
Normal morphology (%)	0.98 (0.94–1.02)	0.27
N of oocytes retrieved	.110 (0.99–1.21)	0.053

IMSI, morphologically selected sperm injection; FSH, follicular stimulating hormone.

Additional analysis was drawn to assess whether a correlation exists between different sperm pathologies (one abnormal parameter or Oligoasthenoteratozoospermia - OAT) and clinical pregnancy. This analysis showed a significant correlation between successful IVF-IMSI cycles and OAT (OR = 3.33, p = 0.015). This correlation was also demonstrated after controlling for possible confounders in a multivariate regression analysis. Cases of one abnormal sperm parameter were evaluated separately for each abnormal parameter - concentration, motility and morphology and none of the parameters were correlated with increased clinical pregnancy rates compared with normal sperm cases (Table 4). Regarding fertilization rate, no correlation was obtained with one abnormal parameter in the semen analysis (correlation coefficient=-0.254, p = 0.59) nor with OAT (correlation coefficient=-0.724, p = 0.17).

Comment

A Cochrane review [11] comparing ICSI to IMSI outcomes published in 2013 concluded that there is no evidence IMSI improves live birth and clinical pregnancy rates, however the level of evidence was considered low and heterogeneity was substantial. This was in part attributed to different inclusion criteria used in the absence of clear indications.

In contrast to other publications which compared IMSI to ICSI outcomes [3,7], this study focused on the correlation between semen pathology and IMSI outcomes in order to assess whether there is a subgroup of patients diagnosed with male factor infertility that might benefit the most by preselection of spermatozoa by high magnification approach. Our results demonstrate a correlation between patients with abnormalities in all three semen parameters and clinical pregnancy when IMSI is preformed, whereas no correlation was found when only one semen parameter is abnormal.

Recently there is growing evidence on large variation in semen analysis obtained within and between laboratories [12,13]. This inter-observer variability, which is most profound regarding morphology assessment, should be taken into consideration in clinical judgment of semen analysis. Those differences between observers are in part the result of lack of adherence to the WHO guidelines [14] that were published in order to enhance standardization. We suggest that the probability of a major error in all three semen parameters is lower than in one, and therefore the cases with OAT truly represent impaired semen. In such cases, there might be a benefit from optimal selection of spermatozoa with IMSI by detecting motile and morphologically normal spermatozoa.

Our results correspond with those published by Antirori et al [15] and Setti et al. [2], who demonstrated an advantage in clinical pregnancy rate when IMSI is performed in a population diagnosed with OAT. Interestingly, in our study no correlation was found between abnormal sperm parameters and fertilization rate even in patients with OAT. Similar findings comparing IMSI to ICSI outcomes were previously published [8,16,17] and demonstrated superiority of IMSI regarding clinical outcomes alone, with no effect on preimplantation embryonic development.

High magnification technique enables the detection of sperm cells containing nuclear vacuoles, which were correlated to DNA fragmentation [18]. Sperm nuclear content has an important role in late preimplantation embryo development, a phenomenon referred as the late paternal effect [19–21]. The "late paternal effect" of spermatozoa starts after the embryo genomic activation. Hence, these later stages of embryogenesis may be impaired by abnormalities in DNA content.

Therefore, one might speculate that by optimal selection of spermatozoa without nuclear vacuoles, improvement of later stages of embryogenesis can be achieved. This will be expressed in clinical outcomes and not necessarily in fertilization and early embryo development. However, further prospective studies are required to confirm this theory.

The limitations of this study include its retrospective design, which makes it more prone to bias. Additional limitations were lack of data regarding body mass index (BMI), oocytes retrieval rate and endometrial thickness. This may explain the difference obtained regarding the female age and number of oocyte retrieved. Therefore, we used a multivariate regression analysis in order to control these confounders. Another drawback regarding our cohort lies in the fact we did not exclude couples diagnosed with female factor infertility, although the number of such cases was minor. Nevertheless, this is the first study to characterize which group of patients might benefit from IMSI by using semen parameters. Our cohort of patients is one of the largest published in the field, and by using the recent WHO parameters our definitions are standardize and relevant.

In conclusion, higher prevalence of OAT cases but not just one abnormal parameter was found in successful cycles using IMSI in patients with recurrent IVF failures. Future randomized clinical trials are further required to establish our observation.

Table 4
Comparison of clinical pregnancy rates according to sperm abnormality compared with cases with all three normal sperm parameters group (n = 37).

	N	OR (95% CI)	P value
One abnormal parameter	85 (50.0%)	1.51 (0.61–3.76)	0.38
Three abnormal parameters (OTA)	48 (28.2%)	3.33 (1.27–8.76)	0.015

Comparison was performed using a two-way univariate analysis variance test OTA, oligo-terato-asthenospermia.

Contribution to authorship

Dr Schachter Safrai – analyzed and interpreted the data, drafted the manuscript and revised it critically.

Dr Karavani – analyzed and interpreted the data, drafted the manuscript and revised it critically.

Dr Reuveni-Salzman – collected the data and edited the manuscript.

Dr Gil – collected the data and edited the manuscript.

Dr Ben Meir – contributed substantially to the concept and design, edited the manuscript and approved the final draft.

Ethical approval

This study has been approved in by the Human Investigation Review Board of Hadassah Hebrew University Medical center (IRB approval number: HMO-0479-17).

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Disclosure of interest

None.

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