

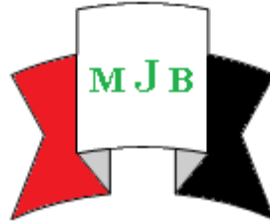
Influence of Sperm Concentration, Morphology, and Motility on The Result of Intracytoplasmic Sperm Injection

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Abstract

Defective Sperm parameters was suggested to play an important role in determining fertility and was proven to be closely related to fertilization and pregnancy rates in the natural fertilization process as well as in ART. To evaluate the influence of defective sperm parameters including concentration, morphology and motility on intracytoplasmic sperm injection outcome represented by fertilization rate, cleavage rate, embryo quality and pregnancy rate.

This a cohort study included 60 infertile couple attended the clinics of infertility treatment center Al-Sadder teaching hospital and underwent intracytoplasmic sperm injection. They are classified into three groups according to their spermogram and ICSI outcome evaluated for each group and compared with each other.

The main results of this study showed no significant difference in fertilization rate, cleavage rate, embryo quality and pregnancy rate among studied groups i.e., no significant difference in ICSI outcome between normozoospermic group and those with defective spermogram. Intracytoplasmic sperm injection outcomes represented by fertilization rate, cleavage rate, embryo quality and pregnancy rate were identical with no significant differences between couples with defective sperm parameters and those with normal spermogram.

Keywords: Intracytoplasmic sperm injection, spermogram, normozoospermia, oligoteratozoospermia, asthenoteratozoospermia, defective sperm parameters.

تأثير التركيز والشكلية الطبيعي والحركة للنطف على نتائج عملية الحقن المجهرية

الخلاصة:

ان وجود اي خلل في المتغيرات الرئيسية للمني يؤدي الى تراجع الخصوبه ومعدلات الحمل سواء في الحمل الطبيعي او في عمليات الانجاب المعان. تقييم تأثير الخلل في المتغيرات الرئيسية للسائل المنوي على نتائج عملية الحقن المجهرية متمثلة بنسب الأخصاب ,نوعية الأجنة ونسب الحمل. شملت هذه الدراسة ستون من الأزواج العقيمين من مراجعي مركز الخصوبه ممن خضعوا لعملية الحقن المجهرية وتم تصنيفهم لمجاميع ثلاثة حسب المتغيرات الرئيسية للسائل المنوي وتمت مقارنة نتائج عملية الحقن المجهرية بين تلك المجاميع لمعرفة مدى تأثير اضطراب هذه المتغيرات على معدلات الاخصاب ونسب الحمل. عدم وجود فرق معنوي ملحوظ في معدلات الاخصاب , نوعية الاجنة ونسب الحمل بين المجاميع الثلاثة. ان اضطراب المتغيرات الرئيسية للسائل المنوي لا يؤثر على نتائج عملية الحقن المجهرية.

Introduction

Infertility is the inability of a sexually active non contraception couple to achieve pregnancy in one year (1). A male factor is solely responsible in about 20% of infertile couples and contributory in another 30- 40% (2).

Routine semen analysis is the basic analysis in the exploration of male infertility. It provides useful data concerning sperm count, sperm motility and viability, sperm morphology, performance of genital glands, and ejaculation.

The key responsibility of ART is the management of infertility including male infertility. Assisted reproductive technology increases the pregnancy probability by a double mechanism; first, it facilitates the interaction between spermatozoa and oocytes, and second, it bypasses seminal abnormalities, such as a reduced number, motility or increased morphological defects of spermatozoa (3).

Impaired sperm parameters was suggested to play an important role in determining fertility and was proven to be closely related to fertilization and pregnancy rates in the natural fertilization process as well as in ART. The paternal genome is transferred to the oocyte in a balanced physical and chemical condition to complement genetic division during embryo development. So the DNA integrity of human spermatozoa contributes significantly to embryonic growth and fetal health (4).

Material and methods

This study included 60 infertile couple complaining from either primary or secondary infertility attended the clinics of infertility treatment center a Al-Sadder teaching hospital and underwent intracytoplasmic sperm injection throughout period from April 2013 to June 2014. All patients were of at least 1 year

duration history of regular unprotected intercourse. All females included in this research were less than 35 years old. The mean age of female partner was 31.31 ± 0.86 years, mean duration of infertility period was 7.53 ± 0.59 years. Those infertile couples were divided into three groups:

1. The male partner has normal semen parameters according to WHO criteria 1999 and female partner has bilateral tubal block, cases of hydrosalpinx and endometriosis were excluded.
2. The male partner has oligastheno-teratozoospermia.
3. The male partner has asthenoteratozoosperm.

The female partners of the last two groups had been evaluated by a gynecologist and they had a normal history, physical examination and gynecological investigations. The male patients were informed that further investigations would done on their samples for academic research purposes and the entire patient's information for this study remained confidential. Semen sample should be produced by masturbation after 2-5 days of abstinence and the time spent noted. The sample were placed in an incubator at 37°C up to 30 minutes to liquefy (5). The liquefied semen is carefully mixed by Pasteur pipette for few seconds, and then examined by macroscopic and microscopic examinations within one hour of collection according to the WHO criteria (1999). Samples then prepared for ICSI by centrifugation-swim up technique as follow:

1. Mix the semen sample well
2. Dilute the semen sample 1:1 with medium (Ferticult) to promote removal of seminal plasma. Transfer the diluted suspension into centrifuge tubes.
3. Centrifuge at 2500 rpm for 5 minutes.

4. Discard the supernatants. Gently, layer 1 mL of medium over the pellet and incubate for 30 min at 37 °C.

5. Gently, remove the uppermost medium which will contain highly motile sperm cells.

A drop (10µL) was taken and put on a slide and covered with a cover slip and examined at a microscope under 40X objective for assessment of sperm concentration, progressive motility and normal morphology. The specimen was used directly for ICSI (6).

The procedure of ICSI was performed using standard protocol:

- Single viable sperm is selected and immobilized, sperm is then aspirated starting from tail into the injection pipette.
- The mature oocyte is held with the polar body at the 6 o'clock position by the mean of holding pipette .The sperm cell is ejected near to the opening of the injector.
- Injection site is at the 3 o'clock position with slight suction of ooplasm to ensure that oolema were penetrated. Then the sperm is introduced into the oocyte with a minimal amount of medium (7).

Oocyte Culture and Evaluation of fertilization:

The normal standardized routine method was used for all patients 16-18 hours after injection oocytes were inspected for fertilization (finding of two pronuclei). Subsequent evaluation of the embryo quality was depending on blastomere number, shape and equality, mononucleation and proportion of fragmentation. Embryos were incubated individually in drops and transferred daily to fresh cleavage medium. Embryos were classified, good quality“ if they were at the four-cell stage at forty eight hr. after injection or at the six- to eight-cell stage, seventy tow hr. after injection with even sized blastomeres and little or no fragmentation .

Results

In this study the influences of sperm parameters (concentration, morphology and motility) on the results of ICSI were evaluated. Table (1) shows the classification of the infertile couples according to their spermiogram and the main SFA parameters in the studied groups.

Table 1: Main spermiogram in studied groups

Parameter	OAT (n=22)	AT (n=17)	N (n=21)	P	WHO (1999) Reference value
Sperm concentration × 10 ⁶ (million/mL)	5.17 ±0.99	33.47 ±3.65	77.19 ±4.69	0.0001*	≥20
Normal sperm morphology (%)	0.11 ±0.01	0.13 ±0.02	0.48 ±0.03	0.288	≥30%
Progressive sperm motility (%)	0.16 ±0.03	0.21 ±0.04	0.60 ±0.02	0.0001*	≥50% grade a+b or ≥25% grade a

Values are expressed as mean ± SEM.

OAT: asthenoteratozoospermia.

AT: asthenoteratozoospermia, N: normozoospermia.

Table 2: Intracytoplasmic sperm injection (ICSI) outcome measured in studied groups.

Parameter	OAT (n=22)	AT (n=17)	N (n=21)	P
Fertilization rate	0.67±0.05	0.69±0.07	0.73±0.05	0.773
Cleavage rate	0.92±0.03	0.95±0.02	0.89±0.05	0.593
Good quality embryo	52 (27.7%)	16(33.3%)	24(55.8%)	0.078
Bad quality embryo	58 (30.6%)	18(37.5%)	11(25.6%)	0.863
Embryonic arrest	79 (41.7%)	14(29.2%)	8(18.3%)	0.573

Values are expressed as mean ± SEM for fertilization and cleavage rate and as a number percent for good quality, bad quality and arrested embryo.

OAT: oligoasthenoteratozoospermia.

AT: astenoteratozoospermia.

N : normozoospermia.

Oligoasthenoteratozoospermic group consist of 22 couple fourteen of them (63.6%) ended with failed ICSI trial i.e. no pregnancy and eight of them (36.4%) had positive pregnancy test two weeks following embryo transfer. Asthenoteratozoospermic group included 17 couple ,the result of pregnancy test is known for fourteen couple and missed for three. Ten out of fourteen (71.4%) had negative pregnancy test and four of them

(28.6%) were pregnant. Finally, normozoospermic group consist of 21 couple the result of pregnancy test is known for nineteen couple and missed for two. Thirteen of them (68.4%) had failed trial and six of them (31.6%) had positive pregnancy test .

No significant difference in positive pregnancy percentage among studied groups (P=0.377) this is illustrated in figure (1).

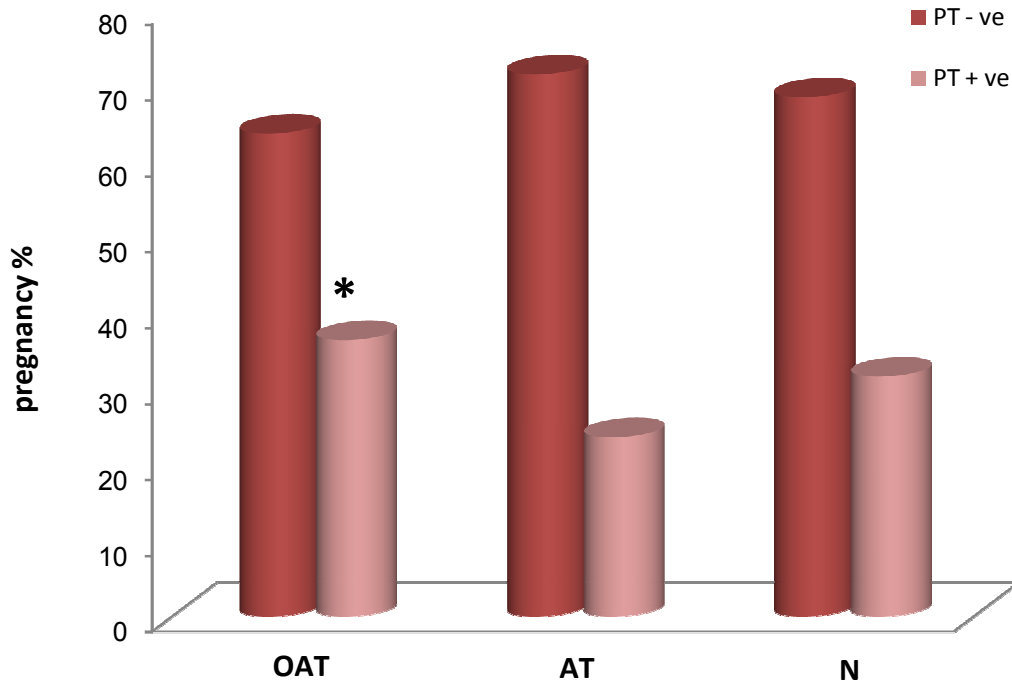


Figure (1): Negative and positive pregnancy percentage in studied groups.

OAT:oligoasthenoteratozoospermia.

AT:asthenoteratozoospermia.

N:normozoospermia.

*No significant difference in pregnancy rate among studied groups P=0.377

Table3: Comparison of the main semen parameters between pregnant and non pregnant patients.

Parameter	Pregnant (n=18)	Non pregnant(n=37)	P
Sperm concentration ×10 ⁶ (million/mL)	31.94±6.91	40.87±6.30	0.385
Normal sperm morphology %	0.25±0.04	0.25±0.03	0.492
Progressive motile sperm %	0.34±0.04	0.31±0.04	0.689

Values are expressed as mean ± SEM.

Discussion

The primary identification of male factor infertility is largely based on the assessment of specific sperm characteristics consequently, standard

semen parameters, as described by the World Health Organization , have been implemented for routine analysis and include sperm concentration , motility and morphology.

The introduction of intracytoplasmic sperm injection (ICSI) in 1992, a technique of in-vitro fertilization using direct insertion of a single sperm into an egg, offered the ability to bypass even some of the most severe etiologies of male sub fertility (6). Accordingly, one of the primary objectives of the present study is to predict the relationship between defective sperm parameters and the result of ICSI procedure.

One of the main results of the present study was that, ICSI outcome was comparable with no significant difference in fertilization rate, cleavage rate, embryo quality ,embryonic development and pregnancy rate among oligoasthenotera to zoospermic group, as the noterato-zoospermic group and normozoospermic group. Other finding of this research that confirm the above mentioned result is that there is no significant difference in sperm count , normal sperm morphology percent and progressive motile sperm between pregnant and non pregnant groups. This finding goes with other researches that found the result of intracytoplasmic sperm injection is not related to the three basic sperm parameters (8,9), and the results of ICSI were shown to be the same with various sperm defects such as severe oligospermia, oligoasthenoteratospermia, and asthenoteratospermia (6). There is a prove that semen parameters, such as concentration and morphology (except for globozoospermia) do not influence the success rates of ICSI and Successful ICSI has also been described for patients with acrosomeless spermatozoa after oocyte activation with Ca-ionophore (10). Also it was found that the use of sperm either with a structural defect or with anomalies of sperm-activating factors, not necessarily associate with clinical consequences. Actually, what happen during ICSI is the selection of sperm for injection at a magnification of $\times 400$ and

the primary factors influencing the choice of sperm for injection are their morphological appearance coupled with their swimming characteristic so that, the establishment of a pregnancy even with compromised ejaculated (dysfunctional) spermatozoa may be attributed to the corrective role of selecting a single spermatozoon for ICSI (11). Therefore, during ICSI a single life sperm with ability to activate oocyte and form pronucleus is necessary but morphology, motility and acrosome status not important (12), consequently, ICSI procedure bypass the problem of reduced sperm count and considered as a treatment of choice for the most severe cases of oligozoospermia.

Motile spermatozoa should be selected for ICSI not because of their movements but with a view to their vitality furthermore progressive motility may be an indicator of adequate metabolic activity of the spermatozoon. This may explain why when viable but immotile spermatozoa were used for ICSI, the resulting embryos were of diminished quality and had poor developmental potential (13).

The importance of sperm morphology has been widely debated, the use of spermatozoa extracted from semen samples with an apparently normal sperm morphology resulted in higher fertilization, implantation and pregnancy rates compared with the injection of an abnormal spermatozoon (3), where as others founded that sperm morphology has no significant prognostic advantage concerning the prediction of fertilization, cleavage and pregnancy outcome in an ICSI (14).

In addition to the corrective role of ICSI that attributed to the selective nature of the procedure sperm preparation which is a prerequisite to all types of ART including ICSI aiming to maximize the chance for fertilization and to obtain

spermatozoa with highest potential for normal fertilization from grossly abnormal semen sample (12) , and this is regarded another explanation to the result of this study.

Conclusion

Intracytoplasmic sperm injection outcomes represented by fertilization rate, cleavage rate embryo quality and pregnancy rate were identical with no significant differences between couples with defective sperm parameters (count, normal sperm morphology and progressive motility percent) and those with normal spermiogram, this is suggest the corrective nature of ICSI procedure and show the unique capability of this technique to treat the most difficult cases of male infertility.

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