ORIGINAL RESEARCH

THE EFFECTS OF SPERM MORPHOLOGY AND MOTILITY ON THE OUTCOME OF INTRACYTOPLASMIC SPERM INJECTION

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ABSTRACT

Objective: The outcome of intracytoplasmic sperm injection (ICSI) treatments was evaluated and compared with sperm morphology and motility classifications in order to determine whether strict criteria or motility could aid in predicting the ICSI outcome.

Patients and Methods: Two-hundred and forty-two of the infertile couples admitted to the clinic, were selected and ICSI treatment was performed. In the study group, female partners were required to have at least 5 oocytes at metaphase II. For male partners only the presence of spermatozoa cells in the semen fluid was necessary. Semen analysis and motility was performed according to the World Health Organisation (WHO) criteria and sperm morphology was assessed according to Kruger's criteria.

Results: There was no significant difference for the ICSI outcome assessment parameters indicating that fertilization and pregnancy rates between the groups were based on the percentages of sperms morphology and motility.

Conclusion: Sperm morphology and motility were accepted as best parameters to evaluate the outcomes of in vitro fertilization (IVF). However, our results showed that ICSI outcomes were independent from these valuable parameters for IVF.

Keywords: Sperm morphology, Sperm motility, ICSI

SPERM MORFOLOJİSİ VE MOTİLİTESİNİN İNTRASİTOPLAZMİK SPERM ENJEKSİYON SONUÇLARI ÜZERİNE ETKİLERİ

ÖZET

Amaç: İntrasitoplazmik sperm enjeksiyonu (ICSI) tedavi sonuçları, sperm morfoloji ve motilite sınıflandırmaları ile karşılaştırılarak değerlendirildi; bu çerçevede Kruger kesin kriterlerinin veya motilitenin, bu sonuçları tahminde yardımcı olup olamıyacağı araştırıldı.

Yöntem: Kliniğe başvuran infertil çiftlerden, 242 si seçilerek ICSI tedavisi uygulandı. Çalışma grubundaki kadın partnerlerde Metafiz II oosit sayısı en az 5 ve üzerinde, erkek partnerlerde ise yanlızca semen sıvısında sperm bulunması gereği öngörüldü. Semen analizi ve motilite değerlendirmesi Dünya Sağlık Örgütü (WHO) kriterlerine göre gerçekleştirildi; sperm morfolojisi ise "Kruger'in kesin kriterleri"ne göre değerlendirildi.

Bulgular: Sperm morfoloji ve motilite yüzdelerine göre oluşturulan gruplar içerisinde ICSI sonuçlarını değerlendirme parametrelerinden, fertilizasyon ve gebelik oranları arasında anlamlı bir fark saptanmadı.

Sonuç: Sperm motilite ve morfolojisi in vitro fertilizasyon (IVF) sonuçlarının değerlendirilmesinde en iyi parametreler olarak kabul edilmektedir. Oysa ki bizim bulgularımız ICSI sonuçlarının IVF için değerli olan bu parametrelerden bağımsız olduğunu göstermektedir.

Anahtar Kelimeler: Sperm morfolojisi, Sperm motilitesi, ICSI

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INTRODUCTION

Since about half of the infertility cases are due to male factors, a detailed sperm analysis became the most important examination to be performed in the approach to the infertile couples. Basically, sperm count, motility and percentage of normal sperms are conventional criteria for semen quality [1]. Among these parameters, sperm morphology and motility are the best criteria for demonstrating the fertilization capacity of a male [2-4]. Sperm motility gives a measure of the integrity of the sperm axoneme and tail structures as well as the metabolic machinery of the mitochondria, and sperm morphology is a surrogate measure of the integrity of DNA packaging and the quality of spermatogenesis [5]. When the effects of sperm morphology were examined from different aspects the following conclusions were drawn: sperm morphology was considered to be the best predictive factor in natural fertilization [6,] intrauterine insemination [7] and ordinary in vitro fertilization [8,9].

The aim of this study was to demonstrate the effects of sperm motility and sperm morphology on fertilization and pregnancy, in couples candidates for ICSI evaluated by means of a detailed sperm examination of the male partner.

PATIENTS AND METHODS

Two-hundred and forty-two patients admitted to our infertility center who had previously undergone ICSI and still wanted to have a child were included in the study. The study group consisted of male patients with sperm in normal semen fluid (sperm parameters were not taken into account) but free of total immotility or any major anomaly (globozoospermia, megalozoospermia) and female patients with metaphase two (MII) oocyte count ≥ 5. Following the assignment of our cases into two groups based on the percentages of sperm with normal morphology (≥5%, < 5%), a third group was formed from patients with a sperm count of <1 million/ml and not subjected to morphological examination.

Initially, a spermiogram test was performed for the male partner. A general evaluation of the samples was performed according to WHO[10] criteria and their morphological evaluation was done by Kruger strict criteria [11]. On the day of ICSI, an appropriate washing method was determined by re-evaluation with respect to the number, motility and morphology. Ovarian stimulation was applied to the female partner to obtain more than one oocyte. During stimulation, urine or recombinant gonadotropins were combined with the gonadotropin releasing hormone (GnRH) agonist/antagonist. Mature oocytes were collected approximately 36-40 hours following urinary human chorionic gonadotropin (hCG) injection. ICSI procedure was performed after preparation of the oocytes. Fertilization was confirmed after 16-18 h by the observation of two distinc pronuclei (2PN) and two polar bodies. The fertilization rate was calculated as the number of fertilized oocytes divided by the total number of mature oocytes for each couple. Embryo transfers were performed by the same clinician on day 2 or 3 according to the number and quality of embryos. Blood hCG levels were examined 14 days after the procedure (B hCG Test. Diagnostics Corp.). Positive test value for pregnancy was > 5. Repeat test was required for patients with a result > 20. Those with a twofold or more increase were considered pregnant. We were not able to follow up pregnancies until labour since most of our patients lived in other cities and were lost to follow up.

Examination Methods:

Semen Analysis Sperm specimens were obtained by masturbation following sexual abstinence for three to five days. The sperm specimens were liquidified in a laminar flow chamber at 37 ºC. After liquidification, 10 µl of semen specimen was placed in a Makler chamber for the determination of spermatozoa count and motility. Also, for morphological evaluation, a smear was prepared by applying 10-20 µl of sperm specimen on a slide.

Preparation of Semen Specimen: The sperm specimens were prepared by "concentration", "gradient" or "swim-up" methods according to sperm count and motility. Sperm specimens with sperm counts less than 0.5 million/ml and motility less than % 10 were prepared by the concentration method. In this procedure, equal amounts of semen specimen and washing medium (G Sperm, Vitrolife) were mixed and transferred to a 15 ml conic tube and centrifuged at 2000 rpm for 10 minutes. The supernatant was removed and the pellet was mixed with 1 ml medium and centrifuged again. Thereafter supernatant was discarded, 0.3 ml culture medium (G Fert, Vitrolife) was added and incubated. The gradient method was used for specimens with sperm counts of less than 5 million/ml and poor motility or having too many scrap cells. For this procedure, 90% and 45% gradient media were prepared (Sperm Grad, Vitrolife). The following were placed in a conic tube in ascending order: 90% sperm grade medium, 45% sperm grade medium and 1 ml semen. The specimen was centrifuged at 1500 rpm for 20 minutes. The pellet on the base was taken by a pipette crossing the gradient and sperm layers, and transferred to a clean tube. It was mixed with 4 ml washing medium and centrifuged at 1500 rpm for 5 minutes. The supernatant was removed again and the pellet was diluted with 0.5-1 ml culture medium depending on the sperm count. Specimens with a good sperm count and motility were prepared with the swim up method. Equal amounts of specimen and washing medium (G Sperm, Vitrolife, Sweden) were placed in a conic tube and centrifuged at 1600 rpm for 10 minutes. After removing the supernatant, 0.5 ml culture medium (G Fert, Vitrolife, Sweden) conditioned with gas in a 5% CO2 incubator was added and placed in the incubator. All specimens were assessed for concentration and motility prior to storage in the incubator until the procedures.

Assessment of Sperm Morphology

Two slides were prepared from each patient for morphological assessment. A specimen of 10µl was placed on a slide and smeared with another slide and the preparation was left to dry. The dried specimens were stained with the Diff Quick method. For this procedure, a Diff-Quick staining set (Allegiance Healthcare Corp., USA) was used. Two hundred sperm were counted for assessment, and examined under immersion oil with a (100X) phase contrast microscope. They were evaluated using Kruger strict criteria [11].

Assessment of Sperm Motility

The motility of the semen specimen was assessed by using liquidified fresh semen.A Makler (Sefi Medikal Instr., Israel) counting chamber was used for counting. Motility assessment was performed by counting at least 200 sperm from 5 different areas according to WHO criteria [10].The sperms were scored for motility evaluation, expressed as grades a to d and the progressive motility rate was calculated as a percentage of (a+b).

Statistical Analysis

Two hundred and forty-two couples were divided into three groups on the basis of sperm percentages with normal morphology: group 1, ≥5% (n=100); group 2, >5% (n=90), and group 3 patients with sperm counts of less than 1 million/ml (n=52). Morphological evaluation was not performed for group 3. In the study groups, mean age, mean number of oocytes, mean MII oocyte number, fertilized oocyte percentage and mean embryo numbers transferred were calculated using the "Univariate Variance Analysis".

All couples were also divided, based only on motility rates ≥50% (n=119) and < 50% (n=123), without considering the morphological evaluation. In two groups, according to motility, the mean age, mean number of oocytes, mean MII oocyte number, fertilized oocyte numbers and mean embryo numbers transferred were calculated using the Student's t-test.

Pregnancy rates of all the groups according to morphology and motility were calculated by "Chi-Square Test". Statistical significance was assessed at $p < 0.05$.

RESULTS

In our study, the fertilization and pregnancy outcomes of ICSI treatment were assessed in 242 couples. Sperm morphology and motility values were classified to determine whether

these parameters were helpful in estimating ICSI outcomes.

There were no difference in the mean age of the female partners, the number of total oocytes, injected metaphase II oocytes and number of transferred embryos in all groups as presented in Table I-II.

The first group included couples with normal sperm morphology ≥5%, the second group included couples with normal sperm morphology < 5% and the third group was formed from patients with a sperm count of <1 million/ml and not subjected to morphological examination. There was no difference for ICSI outcome assessment parameters for fertilization (78.36, 74.26 and 73.09%) and pregnancy rates (59, 54.4, and 57.7) in the groups. The fertilization rate was slightly decreased in the third group; however this did not reach statistical significance (Table I).

When we group the 242 couples of our study based only on motility rates, without considering the morphological evaluation, the mean of fertilized oocytes was 76.66% and 74.78% and the pregnancy rates were 58.80 and 55.30, respectively (Table II). There was no statistically significant difference between the two groups based on motility in terms of fertilization and pregnancy rates.

Table I: Groups based on sperm percentages with normal morphology ≥5% and >5%, and patients with sperm counts less than 1 million/ml for which morphological evaluation was not performed.

	Sperm Morphology≥5 (n=100)	**Sperm Morphology <5 (n=90)**	**Sperm Concentration<1mil/mil (n=52)**	P
Age	31.13±4.62	30.06±4.94	29.25±4.69	0.057
Total oocyte *	13.60±8.03 (12)	13.96±7.64 (13)	12.98±6.32 (12)	0.860
Metaphase II oocyte*	11.12±6.03 (10)	11.59±6.14 (10)	10.56±4.96 (9)	0.738
Number of embryos transferred*	3.67±0.77 (4)	3.53±0.92 (3)	3.48±0.89 (4)	0.417
Fertilization (%)	78.36±17.97	74.26±19.56	73.09 ±20.22	0.182
Pregnancy (%)	59.0	54.4	57.7	0.813

* Mean±SD (Median value)

Table II: Patients also divided into two groups according to progressive motility ≥ 50% or <50%.

	Motility ≥%50 (n=119)	**Motility <50 (n=123)**	**P**
Age	30.59±0.42	30.06±0.44	0.390
Total oocyte*	14.19±8.14 (13)	13.02±6.86 (12)	0.299
Metaphase II oocyte*	11.51±6.02 (10)	10.84±5.68 (10)	0.287
Number of embryos transferred*	3.63±0.76 (4)	3.52±0.94 (4)	0.767
Fertilization (%)	76.66±1.68	74.78±1.79	0.444
Pregnancy (%)	58.80	55.30	0.578

* Mean±SD (Median value)

DISCUSSION

For the semen analysis, traditional manual methods of concentration, motility and morphology measurements are currently the most important parameters for the examination of male infertility [11,12]. The combination of sperm morphology, progressive motility percent and total motile sperm count has been demonstrated to be the best parameter to evaluate the fertility capacity of sperm in IVF [2-4]. Parallel studies were examined and found out that the number of studies and cases that examined ICSI outcomes were less in literature when compared with the number of studies and cases that examined IVF outcomes.

Peter Svalander et al. have classified the semen specimens into three categories according to strict criteria in their study: excellent prognosis (>14%), good prognosis (4-14%) and poor prognosis [13]. It was reported that total immotile sperm injection may have a negative effect on fertilization and pregnancy rates. Total absence of fertilization was demonstrated in outcomes of cases with total immotile sperms or round head sperms [14]. On the other hand, Nagy et al. reported that samples prepared from fresh ejaculate sperm concentration and morphology had no effect on the ICSI outcomes in their study conducted in patients with entirely immotile spermatozoa [15]. We have also found a correlation in fertilization and pregnancy rates with neither morphology nor motility. Our results confirm the findings; however, we have no results for immotile sperm injection outcomes.

Nikolettos et al. have concluded that the chance of a successful pregnancy is low with severe anomalies of the sperm head shape even if fertilization has been achieved in these patients. Moreover, they reported that sperm decondensation defects and DNA anomalies might be the main factors affecting the fertilization capacity of sperm irrespective of its morphology [16].

Evenson and Bianchi mentioned that loose packaged chromatin and damaged DNA were in higher amount in the sperm nuclei of infertile males [17,18]. Also, the potential of having a normal pregnancy from embryos developed from sperms with abnormal head shapes was found to be lower [19]. An inverse proportion was found between the increase of abnormal sperm count and chance of live birth and a longer the time to the first pregnancy was also reported [20].

According to previous reports and our results in ICSI, fertilization may be achieved, even in the presence of a few motile sperm, because natural selection steps are skipped in the presence of abnormal sperm [21,22]. According to results of published reports, four explanations were proposed to clarify for independency of ICSI outcomes from sperm morphology and motility. The first explanation is that normal sperm morphology is required to pass the zona pellucida and barriers in the female reproductive tract. All these barriers are crossed mechanically in the ICSI method [16,23]. The second explanation is reports that sperms with abnormal shaped heads and immotile sperms reduce fertilization, implantation and pregnancy rates [19,23]. However, these sperms are usually not chosen by embryologists in the ICSI procedure [16]. The third explanation is that the sperms chosen from the semen for the procedure may not reflect a clear morphology especially in low magnification. The fourth explanation is that the variations of minor defective sperms at organelle level may affect the ICSI outcomes [7]. These ultrastructural changes can neither be detected by morphologists nor by embryologists in microscopic examinations. To test this last hypothesis, Bartoov et al. developed a method providing a detailed examination of a motile sperm in real-time and named it motile sperm organelle morphology examination (MSOME). The normal morphological structure of the sperm nucleus defined by MSOME has been positively correlated both with fertilization and pregnancy outcomes [24]. The group demonstrated that sperm nucleus morphology is favorable for determining implantation and pregnancy outcomes compared to standard sperm morphology [25].

Studies examining the relationship between ICSI outcomes, sperm morphology and aneuploidy have led us to question the ICSI methods in recent years. Palermo et al. have found an increasing aneuploidy rate in infertile patients undergoing ICSI when they examined the ICSI outcomes and aneuploidy rate in unselected infertile patients. The association between absence of pregnancy and increased aneuploidy was shown in the same study [26].

In the light of these and our studies, we can say that sperm morphology and motility have no effect on the fertilization and pregnancy rates established in the ICSI procedure. However, it is suggested that these two parameters may have a possible effect on the continuing pregnancy and live birth rates. Although selection of spermatozoa with microscopically normal appearance by an embryologist is effective for increasing pregnancy rates, the effect of indiscernible nucleus anomalies on live birth rates will be clarified by future studies.

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