Does Kruger's strict criteria have prognostic value in predicting ICSI clinical results?

B. Sariibrahim[1], E. Cogendez[2], S. Kayatas[2], M.R. Asoglu[1], I. Koleli[3], L. Bakir[4]

[1]*Siverek State Hospital, Department of Obstetrics and Gynecology, Sanliurfa*
[2]*Zeynep Kamil Teaching and Researching Hospital, Department of Reproductive Endocrinology and Infertility, Istanbul*
[3]*Malatya State Hospital, Department of Obstetrics and Gynecology, Malatya*
[4]*Bakırköy Teaching and Researching Hospital, Department of Obstetrics and Gynecology, Istanbul (Turkey)*

Summary

Objective: The purpose of this study was to compare clinical results of ICSI for different sperm morphology subgroups divided according to Kruger's classification system. *Materials and Methods:* This retrospectively study was conducted at Zeynep Kamil Training and Researching Hospital in Istanbul (Turkey). The study included 332 intracytoplasmic sperm injection (ICSI) cycles. The patients were under 37 years of age with primary infertility who were admitted to the Department of Reproductive Endocrinology and Infertility, from January 2005 to June 2009. The patients were divided in three groups based on Kruger's strict criteria. Normal sperm morphology was less than 4% in group 1, between 4-14% in group 2, and greater than 14% in group 3. All patients underwent ICSI and embryo transfer (ET) following controlled ovarian hyperstimulation (COH). The groups were compared to the rates of fertilization, implantation, clinical pregnancy, abortion, and live birth. *Results:* Pregnancy occurred in 132 (39.7%) of all ICSI cycles. There was no statistically significant difference between regarding groups regarding the rates of fertilization, implantation, clinical pregnancy, biochemical pregnancy, abortion, and live birth. *Conclusion:* The authors concluded that the normal sperm morphology defined by Kruger's strict criteria and sperm motility will not be able to predict prognosis of ICSI cycles.

Key words: Kruger's criteria; ICSI; Normal sperm morphology.

Introduction

Infertility is a problem that affects approximately 10-15% of all reproductive-aged couples. During the recent years, significant developments have occurred in the treatment of infertility. The success rates have increased in the treatment of infertility through improvements of assisted reproductive techniques, although these are expensive, time-consuming, and stressful treatments for infertile patients. The selection of appropriate treatment techniques is essential for the success of assisted reproductive techniques. Therefore, ovarian reserve and semen quality should be determined before commencing treatment [1].

The importance of the male factor has been known for a long time in infertility etiology. Currently, intracytoplasmic sperm injection (ICSI) has been introduced worldwide in the treatment of male-factor infertility. ICSI is a viable option for men who have serious impairment of spermatogenesis, even of a single living sperm [2].

Semen analysis is the classic and routine test for evaluating male-factor infertility. However, standard semen analysis cannot show the full fertilization potential of sperm. According to Kruger, normal sperm morphology is classified according to three categories: "less than 4%", "4-14%", and "greater than 14%". In the case of less than 4% normal morphology, the fertilization rate per each oocyte is 7.6% with in vitro fertilization (IVF). The fertilization rate however increases up to 63.9% in samples having more than a 4% normal morphology [3]. As a result of these findings, the World Health Organization (WHO) reduced the normal limits of morphology up to 30%.

The aim of present study was to compare the clinical results of ICSI for different sperm morphology subgroups divided according to Kruger's classification system.

Materials and Methods

Patients

This retrospectively study was conducted at Zeynep Kamil Training and Researching Hospital in Istanbul (Turkey). The study included 332 ICSI cycles. The patients were under 37 years of age with primary infertility who were admitted to the Department of Reproductive Endocrinology and Infertility, from January 2005 to June 2009.

Patients who had sperm obtained by invasive method (TESE), laparoscopically-diagnosed severe endometriosis, polycystic ovarian syndrome, clinically significant systemic or endocrine disease, history of severe ovarian hyperstimulation syndrome in previous IVF practice, history of more than three failed assisted reproductive technology (ART) application, history of uterine surgery, presence of endometrial pathology (submucous myoma, synechia, polyps, uterine septum), cases using frozen embryos, and cases with inadequate sperm for morphologic evaluation in semen analysis < $1x10^6$/ml), were excluded from the study.

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Sperm morphology assessment

Sperm specimens were evaluated for total sperm count, motility, progression, and morphology in preparation for ICSI. Sperm specimens were stained using Diff-Quik (Dade Behring, Newark, Delaware) and evaluated on the basis of Kruger's strict criteria. The rate of normal sperm forms was determined in each specimen after scoring 100 sperm. Uniformity of grading in the laboratory was routinely monitored for inter-technician variation. Density gradient centrifugation over a 90% layer of isolate was used to prepare sperm. Sperm cells were classified as morphologically normal or abnormal under inverted microscope used for micromanipulation (x400 magnification, Hoffman modulation). A sperm cell was considered as morphologically normal if the head was normal (normal shape, normal size, having an acrosome, and lacking midpiece or tail defects). In addition, the rate of abnormal motility was accepted as less than 50%. The patients were divided into subgroups based on normal sperm morphology. Three subpopulations were created. Normal sperm morphology was determined as < 4% in group 1, 4-14% in group 2, and > 14% in group 3.

IVF procedure

Controlled ovarian hyperstimulation (COH) was performed with long protocol using gonadotropin-releasing hormone (GnRH) agonist (a) in 227 patients and antagonist (ant) protocol in 105 patients. In the long protocol, GnRHa was initiated on day 21 of the menstrual cycle. Recombinant follicle-stimulating hormone FSH (r-FSH) or human menopausal gonadotropin (hMG) was begun on day 2 of menstruation. In the antagonist protocol, the gonadotropin treatment was administered on day 3 of menstruation with r-FSH or hMG using 150-450 IU / day doses. The antagonist treatment was begun on day 5-7 of treatment or until follicle diameter was 13-14mm. 10,000 IU hCG were injected intramuscularly when the largest follicle diameter was 18 mm. Ultrasound guidance was used to transvaginally aspirate and collect oocytes after 36 hours from human chorionic gonadotropin (hCG) administration. Oocytes were cultured and mature oocytes were fertilized by ICSI procedure. Fertilization was controlled 18-20 hours after the ICSI procedure. Selected embryos were transferred by using a catheter. Pregnancy testing was performed 15 days after the embryo transfer (ET). Clinical pregnancy was determined by ultrasonographic screening of the fetal sac at seven weeks pregnancy

Statistical evaluation

Data were given as mean ± standard deviation (mean ± sd) or percentage (%). Analysis of data was assessed by using NCSS 2007 software package program. One-way ANOVA and Chi-square tests were used in comparisons of data. A $p < 0.05$ was considered statistically significant.

Table 1. — *Relationship of sperm morphology subgroups.*

	Group 1 NSM < 4% (n = 247) mean ± sd	Group 2 NSM = 4-14% (n = 57) mean ± sd	Group 3 NSM > 14% (n = 28) mean ± sd	*p*
[a]Age (years)	29.4 ± 3.9	29.9 ± 4.4	30.2 ± 3.7	> 0.05
[a]Duration of infertility (years)	7.6 ± 6.1	7.3 ± 3.8	7.7 ± 3.9	> 0.05
[a]Basal FSH (IU)	7.3 ± 2.2	7.9 ± 2.7	8.0 ± 2.5	> 0.05
[a]Total dose of gonadotropin	2492 ± 1057	2637 ± 1048	2815 ± 1012	> 0.05
[a]Endometrial thickness during ovulation	10.4 ± 2.1	10.4 ± 2.3	9.6 ± 2.1	> 0.05
[a]Number of oocytes collected	11.3 ± 6.5	11.4 ± 6.4	11.1 ± 7.8	> 0.05
[a]Embryo quality number of grade 1 on day 2	3.5 ± 3	3.3 ± 2.3	3.1 ± 2.7	> 0.05
number of grade 1 on day 3	4.6 ± 2.9	4.7 ± 2.9	4.8 ± 3.0	> 0.05
[a]Number of transferred embryos	2.5 ± 0.7	2.4 ± 0.8	2.6 ± 0.7	> 0.05
	%	%	%	
[b]Fertilization rate	58.2	60.2	61.2	> 0.05
[b]Positive pregnancy	39.7	42.1	35.7	> 0.05

NMS: Normal sperm morphology according to Kruger's strict criteria
[a] One-way ANOVA test was used. [b]Chi-square test was used.
$p < 0.05$ was considered statistically significant.

Table 2. — *The causes of infertility in the three groups.*

	Group 1 NSM < 4% (n = 247) % (n)	Group 2 NSM = 4-14% (n = 57) % (n)	Group 3 NSM > 14% (n = 28) % (n)	*p*
Only male factor	53.4 (132)	31.5 (18)	32.1 (9)	< 0.05
Only tubal factor	13.8 (34)	33.4 (19)	42.9 (12)	< 0.05
Unexplained	32.0 (79)	35.1 (20)	25.0 (7)	< 0.05
Both male and tubal factor	0.8 (2)	0 (0)	0 (0)	

NMS: Normal sperm morphology according to Kruger's strict criteria
Chi-square test was used. $p < 0.05$ was considered statistically significant.

Results

A total of 166 pregnancies occurred in 132 of all patients. Twenty-eight pregnancies were aborted clinically, 13 patients were biochemically pregnant, and the remaining 91 patients had live births. Seventy out of 91 patients delivered later than 37 weeks and the remaining 22 patients delivered before 37 weeks. Eighteen patients had twins and eight patients had triplets.

Table 1 shows the relationship of sperm morphology subgroups based on Kruger's strict criteria. The average maternal age, duration of infertility, basal FSH, total dose of gonadotropin, endometrial thickness during ovulation, numbers of oocytes collected, number of grade 1 embryos on days 2 and 3, numbers of ET were similar in sperm morphology subgroups (Table 1, One-way ANOVA test, $p > 0.05$). There was no statistically significant difference between the groups in terms of fertilization rates and positive pregnancy (Table 1, Chi-square test, $p > 0.05$). There was no statistically significant difference between groups 1 and 2, when the causes of infertility were compared. However, tubal factor was lower in group 3 than groups 1 and 2, while male factor was higher in group 3 than in groups 1 and 2 (Table 2, Chi-square test, $p < 0.05$). The rates of implantation, clinical pregnancy, biochemical

Table 3. — *ICSI outcomes in the three groups*.

	Group 1 NSM < 4% (n = 247) %	Group 2 NSM = 4-14% (n = 57) %	Group 3 NSM > 14% (n = 28) %	*p*
Implantation rate	29.2	29.4	29.7	> 0.05
Clinical pregnancy rate	35.2	38.5	35.7	> 0.05
Abortion rate	8.1	8.7	10.7	> 0.05
Live birth rate	27.1	29.8	25.0	> 0.05

NMS: Normal sperm morphology according to Kruger's strict criteria.
Chi-square test was used. $p < 0.05$ was considered as statistically significant.

pregnancy, abortion, and live birth are shown in Table 3. There was no statistically significant difference for these rates (Table 3, Chi-square test, $p > 0.05$). The rates of sperm motility less than 50% were 26% in group 1, 23% in group 2, and 16% in group 3. A significant difference was not found between the groups (Figure 1, Chi-square test, $p > 0.05$).

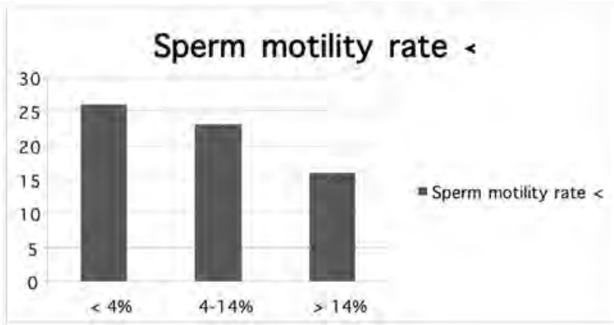

Figure 1. — The rate of sperm motility less than 50%.

Discussion

It is known that sperm morphology may affect fertilization rates in intrauterine insemination and IVF [3,4]. It is controversial whether the fertilization rate is associated with sperm morphology for ICSI. In contrast, it was shown that sperm morphology is an excellent indicator determining fertilization capacity for conventional IVF in the literature [5, 6].

French and *et al*. [7] examined 1,074 ICSI cycles. They stratified patients into groups by using Kruger's strict criteria and eight subgroups were created: 0%, 1%, 2%, 3%, 4%, 5%-7%, and > 7% according to normal sperm morphology. They found that Kruger's strict morphology was not correlated to fertilization rates, clinical pregnancy rates, live birth rates, blastulation, or blastocyst quality, and selection of sperm with normal morphology under microscopic examination was a sufficient ideal for ICSI outcomes. At the same time, they showed that the rates of motile sperm were reduced with an increase of Kruger' strict criteria abnormality, and the rates of fertilization, clinical pregnancy, and live births were not negatively affected. In the present study, the patients were divided into three subgroups based on Kruger's strict criteria as < 4%, 4-14%, > 14% normal sperm morphology. Also, the authors did not find adverse effects in the rates of fertilization, clinical pregnancy, and live birth between the groups. In addition, the authors did not see a significant difference between the groups in terms of the mean number of grade 1 embryos on days 2 and 3. The rate of motile sperm was reduced with decreasing of normal sperm morphology according to Kruger's strict criteria in the study population. As a result, ICSI outcomes were not affected from the rates of normal sperm morphology and motility in the present study.

Slavender *et al*. [8] evaluated 354 ICSI cycles. Semen samples were separated according to Kruger's strict criteria (lower 4%, 4-14%, and over 14%). They found that the rates of fertilization were 61.9%, 66.8%, and 61.6%, respectively and the rates of pregnancy were 18.9%, 24.9%, and 28.3%, respectively. These differences were not statistically significant between the groups. In the present study, the rates of fertilization and pregnancy were 58.2% and 39.7% for group 1, 60.2% and 42.1% for group 2, 61.2% and 35.7% for group 3. There was no statistically significant difference between the groups. Furthermore, the mean number of oocytes collected and ET were similar in all groups as parallel to the literature [7-9].

Many researchers revealed that ICSI cycles with sperm morphology based on Kruger's strict criteria did not affect fertilization and pregnancy rates [8, 10, 11]. This can be explained by the selection of the most normal looking sperm under microscope from even the most impaired specimens by embryologist during ICSI.

In conclusion, according to the results, in this study the authors consider that normal sperm morphology based on Kruger's strict criteria had no prognostic value in predicting ICSI clinical outcomes. In ICSI procedure, selection of the most normal looking sperm cell under microscope may be provided for excellent results for infertile couples. Also, sperm motility does not seem to be sufficiently assisted in ICSI procedure. This subject should be elucidated by randomized prospective studies.

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Address reprint requests to:
B. SARIİBRAHIM, M.D.
Adress: Yavuz Sultan Selim Mahallesi
Mevlana Caddesi
Körfezkent Sitesi, B-7 Blok
D:34, 41780 Körfez/Kocaeli (Turkey)
e-mail: baharsariibrahim@hotmail.com