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Comparison of Hyaluronic Acid Binding and Conventional Sperm Selection For ICSI in Patients with Oligozoospermia Cohort

Oligozoospermili Hastalarda ICSI için Hyalüronik Asit Bağlanma ve Konvansiyonel Sperm Seçiminin Karşılaştırılması

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ABSTRACT

Objective: Sperm selection for intracytoplasmic sperm injection (ICSI) is traditionally done by standardized subjective sperm selection (motility/morphology). Physiological selection of spermatozoa for ICSI (PICSI) is a method of sperm binding to hyaluronic acid (HA) that mimics natural sperm selection and selects the most competent sperm. To evaluate the efficacy of physiologic intracytoplasmic sperm injection (PICSI) compared to conventional intracytoplasmic sperm injection (ICSI) in patients with severe or moderate oligozoospermia This is a cohort carried out at Medipol University IVF Center, Istanbul, Turkey. **Materials and Methods:** This is a cohort that includes women < 39 years old with normal ovarian reserves and men with severe or moderate oligozoospermia. Cycles with blastocyst embryo transfer were included. Fertilization; top and good quality (TQ-GQ) blastocyst development, ongoing pregnancy, and miscarriage rates were compared between groups. **Results:** A total of 146 cycles were included: 46 and 100 cycles were carried out with PICSI and conventional ICSI, respectively. There was no significant difference between the groups regarding fertilization, TQ-GQ blastocyst development, ongoing pregnancy, multiple pregnancy, and miscarriage rates. The implantation rate was higher in the PICSI group, but it did not reach significance. The number of retrieved oocytes was the only significant factor affecting blastocyst development and ongoing pregnancy rate. HA-binding rates were correlated with sperm concentration and sperm morphology but not with sperm motility. **Conclusion:** Using HA-binding for sperm selection before ICSI does not seem to have any additional benefit over conventional sperm selection in patients with oligozoospermia.

Keywords: Infertility male; sperm injections, intracytoplasmic; oligozoospermia

ÖZET

Amaç: İntrasitoplazmik sperm enjeksiyonu (ICSI) için sperm seçimi geleneksel olarak standartlaştırılmış subjektif sperm seçimi (hareketlilik/morfoloji) ile yapılır. ICSI için spermatozoanın fizyolojik seçimi (PICSI), doğal sperm seçimini taklit eden ve en yetkin spermi seçen hyaluronik aside (HA) bağlanan bir sperm yöntemidir. Şiddetli veya orta derecede oligozoospermisi olan hastalarda geleneksel intrasitoplazmik sperm enjeksiyonu (ICSI) ile karşılaştırıldığında fizyolojik intrasitoplazmik sperm enjeksiyonunun (PICSI) etkinliğini değerlendirmek. **Gereç ve Yöntemler:** Bu çalışma, normal over rezervine sahip 39 yaş altı kadınları ve şiddetli veya orta derecede oligozoospermisi olan erkekleri içeren bir kohorttur. Blastokist embriyo transferi yapılan sikluslar dahil edilmiştir. Döllenme; en iyi ve iyi kalitede (TQ-GQ) blastosist gelişimi, devam eden gebelik ve düşük oranları gruplar arasında karşılaştırılmıştır. Bu çalışma Medipol Üniversitesi Tüp Bebek Merkezi, İstanbul, Türkiye'de yürütülmüş bir kohorttur. **Bulgular:** Toplam 146 siklus çalışmaya dahil edildi: 46 ve 100 siklus sırasıyla PICSI ve konvansiyonel ICSI ile gerçekleştirilmiştir. Gruplar arasında fertilizasyon, TQ-GQ blastokist gelişimi, devam eden gebelik, çoğul gebelik ve düşük oranları açısından anlamlı bir fark yoktu. İmplantasyon oranı PICSI grubunda daha yüksekti, ancak anlamlılığa ulaşmadı. Alınan oosit sayısı blastosist gelişimini ve devam eden gebelik oranını etkileyen tek anlamlı faktördü. HA bağlanma oranları sperm konsantrasyonu ve sperm morfolojisi ile ilişkiliydi ancak sperm hareketliliği ile ilişkili değildi. **Sonuç:** Oligozoospermi hastalarında ICSI öncesi sperm seçimi için HA-bağlayıcı kullanmanın geleneksel sperm seçimine göre ek bir yararı yok gibi görünmektedir.

Anahtar Kelimeler: İnfertilite, erkek; sperm enjeksiyonu, intrasitoplazmik; sperm enjeksiyonu, intrasitoplazmik

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The ultimate aim of in-vitro fertilization (IVF) is to obtain live birth. Despite the introduction of numerous new techniques, IVF success rates have remained unchanged in the past decade.[1] Embryo development and intracytoplasmic sperm injection (ICSI) outcomes are dependent on both the egg and sperm quality. Although research has focused mainly on female fertility, sperm selection techniques are becoming more popular with the assumption that success rates would improve if competent sperm can be selected for ICSI.

Almost 35-50% of couples attempting pregnancy have male infertility.[2,3] The causes of male infertility include endocrine factors (obesity, diabetes, etc.), previous surgery, sexual disorders, genetic problems, infections, alcohol, tobacco and other drug use, stress, and external factors (temperature, exposure to chemical and environmental contaminants, etc).[2,4] Sperm characteristics play a major role in fertilization and embryogenesis. Male infertility depends on three major sperm parameters: Sperm count, motility, and morphology. These parameters are dynamic and may differ during the life course. In a recent study following the natural course of severe oligozoospermia in infertile males, after more than a six-month follow-up period, approximately 13% of the patients became azoospermic, and in 18% of the patients, the sperm count become low enough that it could only be detected after centrifugation.[5] Thus, sperm selection in these patients during IVF is immensely valuable for both the families and the physicians.

In spermatogenesis, plasma membrane remodeling consists of the formation of zona pellucida and hyaluronic acid (HA) receptors.[6] One of the methods to assess sperm quality is the selection of competent sperm based on its ability to bind to hyaluronic acid. Selecting HA-bound sperm is suggested to be more natural and thus those sperms have been reported to have reduced levels of DNA damage and aneuploidy. This method which mimics natural sperm selection is called physiologic ICSI (PICSI).[7-9]

Although some studies comparing PICSI and conventional ICSI suggest a beneficial effect of HA sperm selection on assisted reproductive technology (ART) outcomes, two recent randomized controlled trials (RCT) showed no statistical difference in embryo quality and pregnancy success.[9-12] These studies did not categorize sperm parameters and usually included males with normal sperm concentration and motility. Only one retrospective study comparing ICSI and PICSI with male factor infertility indicated that cycles that used the PICSI technique had a considerably higher chance of achieving pregnancy. However, the number of patients in this study was inadequate.[2]

OBJECTIVES

In the present study, we aim to evaluate the efficacy of PICSI compared to conventional sperm selection prior to ICSI in patients with severe or moderate oligozoospermia regarding blastocyst development and clinical success rates.

MATERIAL AND METHODS

STUDY CHARACTERISTICS

This is a cohort including cycles carried out at Medipol University IVF Center. In this study, the principles of the Helsinki Declaration were followed and the study was approved by the Medipol University Faculty of Medicine Ethics Committee on April 20, 2020, with the protocol number 10840098-604.01.01 E- 14285

ELIGIBILITY CRITERIA

The study population included women younger than 39 years old with normal ovarian reserve and men with severe or moderate oligozoospermia who were able to give freshly ejaculated sperm for the ICSI procedure. Oligozoospermia was defined as a sperm concentration of less than $15x10^6$ sperm/mL. Severe oligozoospermia was defined as a concentration of less than $5x10^6$ sperm/mL. Cycles in which sperm collection was done other than ejaculation, or cycles including men with vasectomy reversal, or who had gonadotoxic therapy were excluded. Cases with frozen sperm, surgically obtained sperm, or gonadotoxic treatment were excluded from the study. Only cycles with fresh blastocyst embryo transfers (ET) were included.

PRIMARY AND SECONDARY OUTCOMES

The primary outcome of the study was the ongoing pregnancy rate. Secondary outcomes were fertilization, top and good quality (TQ-GQ) blastocyst development rate, and miscarriage rates.

PROCEDURES AND PATIENT FOLLOW-UP

Women included in the study used the standard antagonist protocol. After transvaginal ultrasound, treatment was started with recombinant-follicle-stimulating hormone (r-FSH) (Gonal F®, Merck-Serono Inc., Spain) on the second or third day of the menstrual cycle. The Gonadotrophin dose was adjusted according to the patient's age, body mass index (BMI), antral follicular count, anti-mullerian hormone (AMH), and previous ovarian stimulation responses. Cetrorelix (Cetrotide®, Merck-Serono, Spain) was added when at least one follicle reached a diameter of 12 mm. Recombinant human chorionic gonadotropin (Recombinant-hCG) (Ovitrelle®, Serono, Spain) was given when two or more follicles reached 18-20 mm in diameter.

Oocyte retrieval was performed 36 hours after the trigger injection. After retrieval, oocytes were cultured for 2 hours until the denudation of cumulus-oocyte complexes. Oocyte maturity was evaluated just before ICSI. MII (metaphase phase II) oocytes were identified by the presence of a polar body. ICSI fertilization was checked at 14-18 hours.

Sperm samples for ICSI were collected on the day of egg collection (OPU) after 3-5 days of sexual abstinence. The sperm was kept at room temperature for 30 to 60 minutes to allow liquefaction of the sample. Semen analysis was performed according to World Health Organization criteria. Gradient centrifugation was performed to separate the cellular components of the sperm. PICSI sterile petri box was used for mature sperm selection (PICSI® Sperm Selector, ORIGIO, Denmark PICSI dishes are ready-to-use plastic culture dishes including three 5 ul hydrated HA drops. 2 ul of washed sperm sample was placed closed to HA gel drops and afterward, these two drops were connected by using a micropipette tip under oil (Life Global). Then the dish was kept at 37°C for approximately 5 minutes. Bounded spermatozoa to the HA were collected from the drop by aspirating through their head via an ICSI pipette (ICSI Micropipets; Humagen Fertility Diagnostic-Origio).

After oocyte retrieval, progesterone vaginal gel (Crinone® 8% vaginal progesterone gel, 90 mg – Central Pharma [Contract Packaging] Ltd on behalf of Merck GmbH, Germany) was used twice daily for luteal support. After 5 days, embryo transfers were performed. If pregnancy occurred, progesterone vaginal gel was continued intravaginally twice daily for luteal phase support until 12 weeks of gestation.

Serum beta-hCG was measured 9 days after ET and pelvic ultrasound was performed to confirm intrauterine pregnancy 2 weeks after positive beta-hCG. Clinical pregnancy was defined as the presence of fetal cardiac activity on transvaginal ultrasonography. Biochemical pregnancy was defined as the presence of positive beta-HCG (≥ 15 mIU/ml) but the absence of gestational sac/cardiac activity on ultrasonography.

Fertilization rate (FR) was defined as the percentage of 2 PNs per number of mature oocytes. Implantation rate (IR) was calculated by dividing the number of intrauterine sacs to the number of embryos transferred in each patient. Clinical pregnancy is diagnosed when intrauterine gestational sac fetal cardiac activity is seen.

STATISTICAL ANALYSIS

All data analyses were performed using SPSS For statistical analysis, version 20 (IBM SPSS, Armonk, United States); student's t-test or Mann Whitney U test and chi-square test, and linear logistic regression analysis were used where appropriate. Differences were considered significant when a P value was <0.05.

RESULTS

A total of 146 ICSI cycles were included in this study. Of those cycles, 46 and 100 cycles were carried out with Physiological, hyaluronan-selected intracytoplasmic sperm injection and conventional sperm selection respectively. In 5 cycles no embryo transfers were achieved due to ovarian hyperstimulation syndrome risk, thus they were excluded. Female age (29.2±4.0 versus 29.7±4.1), male age (33.4±4.4 versus 33.2±4.5), and sperm concentration (3.5±3.8 versus 4.4±3.5) were similar between the groups (Tables 1, Table 2)

The number of retrieved oocytes (16.3±9.1 versus 13.1±5.6) were significantly higher and the number of embryos transferred was lower (1.6±0.5 versus 1.9±0.3) in PICSI compared to the control group (Table 3). Mean HA-binding in the PICSI group was 18.3±24.7% (median: 5.5; min: 0, max:80)

There was no significant difference between the groups regarding fertilization (83.6±16.1 versus 83.9±14.1, P = 0.91), TQ-GQ blastocyst development (49.3±22.2 versus 50.0±23.9, P=0.90). The mean implantation rate (IR) was significantly higher in the PICSI group (49.2% versus 31.6%, P=0.09). In accordance with IR, the ongoing pregnancy rate was higher (56.1% versus 43.1%, P=0.16), and the miscarriage rate was lower in the PICSI group (17.9% versus 21.8%, P=0.67), but the difference was not significant (Table 3). Sperm selection with PICSI or conventional method did not affect fertilization, TQ-GQ blastocyst development, ongoing pregnancy, and miscarriage rates. The number of retrieved oocytes was the only significant factor affecting blastocyst development and ongoing pregnancy rate.

The HA-binding ratio was significantly correlated with sperm concentration (r=0.554; P <0.001) but not with sperm motility. When the PICSI group was divided into subcategories according to HA-

TABLE 1: Demographic characteristics of the patients.

	PICSI (n=46)	ICSI (n=100)	p value
Female age	29.2±4.0	29.7±4.1	0.44
Male age	33.4±4.4	33.2±4.5	0.85
Mean Infertility duration (years)	5.10±3.3	5.12±3,1	0.9
Female factor	23.9%	27%	0.16
Previous IVF attempt rate (%)	34.8	36.0	1.0

PICSI=physiologic intracytoplasmic sperm injection; ICSI=intracytoplasmic sperm injection; SD=standard deviation; IVF=in-vitro fertilization.

TABLE 2: Sperm characteristics of the males in the study groups.

	PICSI (n=46)	ICSI (n=100)	p value
Sperm concentration (mean±SD)	3.5±3.8	4.4±3.5	0.18
Total motility (mean±SD)	40.6±12.2	36.3±16.2	0.17
Abnormal sperm morphology (%)	87.8	97.9	0.02

PICSI=physiologic intracytoplasmic sperm injection; ICSI=intracytoplasmic sperm injection; SD=standard deviation.

TABLE 3: Comparison of PICSI and ICSI groups according to cycle characteristics.

	PICSI (n=46)	ICSI(n=100)	p value
Retrieved oocytes (mean±SD)	16.3±9.1	13.1±5.6	0.036
Mean mature oocyte	11.7±7.1	9.8±4.4	0.23
Mean Fertilization rate (%)	83.6±16.1	83.9±14.1	0.91
Blastocyst development rate (%)	49.3±22.2	50.0±23.9	0.90
D5 TQ-GQ embryos rate (%)	49.3±22.2	49.8±23.9	0.9
Transferred embryos (mean±SD)	1.6 0.5	1.9±0.3	0.08
Mean Implantation rate (%)	49.2	31.6	0.09
Multiple pregnancy rate (%)	3.2	1.7	0.66
Miscarriage rate (%)	17.9	21.8	0.67
Ongoing pregnancy rate (%)	56.1	43.0	0.16

PICSI=physiologic intracytoplasmic sperm injection; ICSI=intracytoplasmic sperm injection; SD=standard deviation; TQ-GQ=top and good quality.

binding (<20% versus >20%), there were no significant differences in success rates between <20% or >20% HA-binding groups. In the PICSI group, sperms with normal morphology had significantly higher HA-binding ratios compared to sperms with abnormal morphologies (38.6±31.9 versus. 15.5±22.7; P=0.05).

DISCUSSION

Although the role of the PICSI system for sperm selection in couples with male factor infertility has been previously studied, no report yet analyzed the results in patients with severe or moderate oligozoospermia in detail. Moreover, all previously reported studies reported fertilization rates, but just a few mentioned embryo quality, pregnancy rates, spontaneous abortions, and live birth rates.[13]

Erberelli et al. from Brazil studied male factor infertility and discussed ICSI versus PICSI. They included only 56 ICSI cycles (19 cycles using PICSI and 37 using conventional ICSI). Most of their male patients had teratozoospermia (51.4%). The rates of moderate and severe oligozoospermia in their study group were only 8 patients (56 patients included). They found that clinical pregnancy rates were significantly better in the PICSI group. In our study, we found that the use of hyaluronic acid sperm selection is comparable to ICSI regarding fertilization rate. However, there is a significant improvement in implantation rates. In addition, we found increased ongoing pregnancy and decreased miscarriage rates in the PICSI group, but these differences did not reach significance which might be due to the low number of PICSI cycles.

A recent systematic review determining the efficacy of PICSI in couples with male factor infertility included two prospective studies.[4] Of these studies, Parmegiani et al. did not analyze sperm morphology but considered the total number of spermatozoa and motility.[8] There were no significant differences between PICSI and ICSI in terms of fertilization, embryo quality, implantation, clinical pregnancy, and live birth rates. We found similar results to this review except for the miscarriage and implantation rates.

There are many studies comparing PICSI and ICSI in couples having standard semen quality. These studies also had small sample sizes and reported that PICSI improved embryo quality and live birth rates, and lowered miscarriage rates compared with conventional ICSI in couples having normal semen quality parameters.[8,14-16]

A recent randomized multicentered study from the United Kingdom compared the efficacy of PICSI versus standard ICSI among couples undergoing fertility treatment.[12] In both groups, semen volume, sperm concentration, and forward progressive motility were in normal ranges. Clinical pregnancy, live birth, and premature birth rates did not differ significantly between groups. However, the miscarriage rate was significantly lower in the PICSI group compared to ICSI patients.[12] We also found that the miscarriage rate was lower in PICSI patients compared to the ICSI group in our study with severe or moderate oligozoospermia. In addition to that study, Worrilow KC et al. in a prospective, multicenter, double-blind, randomized clinical trial found a significant decrease in miscarriage rates using hyaluronan in sperm selection compared to conventional ICSI.[17] Worrilow, on the other hand, showed significant clinical improvement in clinical outcomes using PICSI compared to ICSI in his previous studies.[11,15,17,18]

It is suggested that spermatozoa that bind to hyaluronic acid have completed the spermatogenic process of remodeling the plasmatic membrane, cytoplasmic extrusion, and nuclear maturity showing that they have a whole DNA and low frequency of aneuploidies and miscarriages.[4] It is a fact that sperm DNA damage rates are higher in severe or moderate oligozoospermia.[19] Thus, higher abortion rates are not surprising in these patients. We included men with severe or moderate oligozoospermia and their sperm was selected with the PICSI method. The PICSI method chooses the sperms with normal DNA that have higher affinities to bind oocytes for fertilization.[20] Thus, we came to the conclusion that this may affect the reason for lower abortion rates in the PICSI group compared to ICSI patients in our study. Therefore, we believe that this is the reason for the lower abortion rates in the PICSI group compared to ICSI patients, although there were no statistical differences in our study.

CONCLUSION

The present data indicate that using HA-binding for sperm selection before ICSI does not have any additional benefit over conventional sperm selection in patients with oligozoospermia. In cycles with low sperm count, an increased number of oocytes is the most significant predictor of clinical success. As the number of oocytes retrieved increases, TQ-GQ can compensate for low sperm count by increasing blastocyst development and ongoing pregnancy rate.

The available data suggest that in patients with oligozoospermia, there is no additional benefit of using an HA-binder for sperm selection prior to ICSI compared to conventional sperm selection. Due to the lower abortion rates in the PICSI group compared to ICSI patients, we believe prospective studies with larger samples are needed. In cycles with low sperm count, increasing oocyte number may compensate for low sperm count by increasing TQ-GQ blastocyst development and ongoing pregnancy rate.

Source of Finance

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Conflict of Interest

No conflicts of interest between the authors and / or family members of the scientific and medical committee members or members of the potential conflicts of interest, counseling, expertise, working conditions, share holding and similar situations in any firm.

Authorship Contributions

Idea/Concept: *Sevinç Özmen;* ***Design:*** *Sevinç Özmen, Tuba Yelke;* ***Control/Supervision:*** *Sevinç Özmen;* ***Data Collection and/or Processing:*** *Ayşe Karahasanoğlu, Tuba Yelke;* ***Analysis and/or Interpretation:*** *Aylin Pelin Çil;* ***Literature Review:*** *Sevinç Özmen;* ***Writing the Article:*** *Sevinç Özmen, Aylin Pelin Çil;* ***Critical Review:*** *Aylin Pelin Çil;* ***References and Fundings:*** *Ayşe Karahasanoğlu;* ***Materials:*** *Tuba Yelke.*

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