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Research Article

Reproductive Outcomes in Patients with Severe Oligospermia Undergoing Intracytoplasmic Sperm Injection using Testicular Versus Ejaculated Spermatozoa

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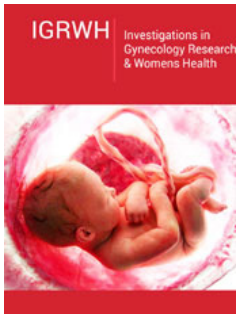

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Abstract

Background: In infertile men with severe oligospermia (sperm concentration <5million/mL) undergoing ICSI the choice between using ejaculated sperm or testicular sperm remains controversial. Testicular sperm have shown lower levels of DNA damage compared with ejaculated spermatozoa from the same individuals.

Objective: To compare intracytoplasmic sperm injection (ICSI) outcome in patients with severe oligospermia using testicular versus ejaculated spermatozoa.

Design: Retrospective cohort study.

Setting: Medina Fertility Center

Materials and Methods: Patients with severe oligospermia who underwent ICSI cycles with either testicular or ejaculated sperm between January 2015 and December 2017 were included in this study. 205 couples met the study criteria after exclusion of cycles with mixed sperm sources. Patients were divided into 2 subgroups according to the source of sperm used in intracytoplasmic sperm injection (ICSI) cycle; (Group A) included 104 patients in which ejaculated sperm were injected while (Group B) included 101 patients where fresh testicular sperm were extracted (TESE) and injected. The medical records of the included couples were reviewed and tabulated regarding demographic data, reproductive history, hormonal profiles and different semen parameters. The reproductive outcomes; fertilization rate, number of high-quality embryos, and pregnancy rate were evaluated and compared in both groups.

Results: There was no significant difference in the fertilization rate between the two subgroups (group A 67.93%, group B 68.01%, p=0.960), the total number of high-quality embryos tended to be higher in the testicular sperm group, but the difference did not reach statistical significance (187 vs 154, P= 0.084). The clinical pregnancy rate was significantly higher in group B where testicular spermatozoa were injected (49.50% vs 35.57%, p=0.044*).

Conclusion: In patients with severe oligospermia, when both sources of spermatozoa are available (i.e. ejaculate or testicular) for the ICSI procedure, TESE should be considered as it offers higher pregnancy rates

Keywords: Infertility; Severe oligospermia; Intracytoplasmic sperm injection; Testicular sperm extraction (TESE)

Introduction

Male factor is an important cause of infertility, isolated male factor accounts for 25% of cases of infertility. The development of the ICSI procedure and surgical sperm retrieval techniques have been of great benefit to achieve pregnancy in men with such conditions [1]. Cases with sufficient number of sperms for injection in the ejaculate (including cases with severe oligospermia, defined most commonly in literature as sperm count below 5 million per ml) [2,3] are injected using ejaculate sperm. Since the testicular sperm may not reach the same maturity as the ejaculated sperm which completes its maturation during its transit in the genital tract, thus with normal semen parameters the fertilization potential and the success rates are higher with ejaculate sperm than with testicular sperm [4]. Evidence shows that infertile men have more sperm DNA damage than fertile men [5]. Apparently, this is more profound in oligospermic men as Moskovtsev et al. [6] analyzed semen from more than

2500 men and found that low sperm counts are associated with higher DNA damage [6]. The American Society of Reproductive Medicine (ASRM) Practice Committee has reported that measuring the DNA fragmentation might be helpful in predicting the outcome of ICSI and Esteves et al suggested that integrity testing should be integrated in the basic semen analysis [7,8] Studies have shown that in men with high DNA fragmentation in their ejaculate sperm, testicular sperm tended to have better quality with lower DNA damage [9]. Researchers have shown that with increased DNA damage, there is reduction in the fertility potential, as well as a drop in most of the outcome parameters of ART, namely the embryo quality, the implantation rate, the clinical pregnancy rate, the ongoing pregnancy rate, and the live birth rate [9,10].

The exact cause for sperm DNA fragmentation remains unclear, but several factors have been accused such as: gonadotoxic exposure to pollutants, varicocele, infections, systemic diseases and advanced age [11-13]. Studies have shown that sperm can be exposed to oxidative stress leading to damage to its DNA while it is being transported in the male genital tract [14,15]. This is supported by the fact that the process of chromatin condensation is still in progress during epididymal transport and that epididymal epithelial cells can produce excessive amounts of free oxygen radicals when exposed to unfavorable conditions [15,16]. Multiple strategies have been proposed to improve the sperm DNA integrity like the use of oral antioxidants for few months before ICSI, varicocelectomy surgery, the use of multiple ejaculates before ICSI, or the use of "intracytoplasmic morphologically selected sperm injection"(IMSI) [17-19]. Unfortunately, none of these strategies has been proven to alleviate the negative effect of DNA damage on ICSI success rates [7]. In infertile men with oligospermia and high ejaculate DNA damage, testicular sperm has been proven to have markedly less DNA fragmentation [20]. This has led to the postulation that in oligospermic men, the use of the testicular sperm with its higher DNA integrity might yield higher success rates.

Materials and Methods

Design: Retrospective cohort study

Setting: Patients with severe oligospermia who underwent ICSI/fresh ET cycles in Medina Fertility Center using either ejaculate or testicular sperm between January 2015 and December 2017 were included in this study. 205 couples met the selection criteria.

Inclusion criteria

i. Sperm count <5 millionml

ii. Normal HSG and normal transvaginal ultrasound

iii. Women age >40 years

iv. Long agonist or antagonist protocols of stimulation

Exclusion criteria

i. Cycles with mixed sperm sources

ii. Obese females with BMI <30kg/m^2

iii. Poor responders

iv. Freeze all cycles

v. Absence of good quality embryos for transfer

The medical records of the included couples were reviewed and tabulated regarding demographic data, reproductive history, hormonal profiles, different semen parameters, cycle performance and outcome.

Analysis of semen parameters

i. Basic sperm parameters (count, motility and morphology) were evaluated in all cases according to the World Health Organization criteria standards (WHO, 2010).

ii. Patients were divided into 2 groups according to the source of sperm used:

A. (Group A): included 104 patients in whom ejaculate sperm was used for injection

B. (Group B): included 101 patients who underwent TESE and fresh testicular sperm were injected.

Outcome analysis

Cases in both groups were compared as regards: Demographic data, hormonal profile on initial work up, different parameters of the semen analysis, number of oocytes retrieved, number of mature Metaphase II oocytes, fertilization rate, number of high quality embryos, chemical and clinical pregnancy rates where chemical pregnancy was defined as a positive pregnancy test with failure to identify a gestational sac two weeks after the pregnancy test, while clinical pregnancy rate was defined as demonstration of a gestational sac with a positive heart beat two weeks after the pregnancy test (30 days after oocyte retrieval). Statistical analysis of the obtained data was carried out using standard SPSS software for Windows version 20. To assess the significance of the differences in the quantitative characteristics, the Student's t-test and F-Fisher criteria were applied, the differences were considered significant at p-value < .05 Qualitative data were described using number and percent and was compared using Chi square test. Abnormally distributed data was expressed using Median (Min.-Max.) and was compared using Mann Whitney test. Normally distributed quantitative data was expressed as Mean±SD and compared using student t-test. While abnormally distributed data was expressed using Median (Min.–Max.) and was compared using Mann Whitney test.

Result

Both groups showed no significant difference as regards the demographic data and the pre-ICSI hormonal profile as well as the semen parameters (Tables 1 and 2). The number of oocytes retrieved per case and the number of mature oocytes were not significantly different between both groups. The fertilization rate was nearly identical in both groups (67.93% vs 68.01, p=0.96) (Table 3). The number of high-quality embryos was higher in the testicular sperm group than in the ejaculate sperm group (187 vs 154), but this difference did not reach statistical significance (p=0.084). The total number of embryos transferred was very

similar between both groups (214 vs 215). Furthermore, there was no significant difference between both groups as regards the numbers of embryos transferred per case (p=0.561) (Table 4). The clinical pregnancy rate was 49.5% in the testicular sperm group which was significantly higher than the 35.57% (p=0.044) observed in the ejaculate sperm group. Both groups had 5 cases of chemical pregnancies. This similarity did not affect the overall pregnancy rate which was significantly higher in the testicular sperm group than the ejaculate sperm group (54.45% vs 40.38%, p=0.0442) (Table 5).

Table 1: Comparison between the two studied groups regarding the demographic data and hormonal profile.

	Group A Ejaculate Sperm (n=104)	Group B Testicular Sperm (n=101)	P
Age(years)			
Male	36.3±6.9	37.7±7.2	0.132
Female	29.4±5.4	30.2±5.4	0.284
Years of infertility Hormonal Profile	4 (0-17)	4(0-22)	0.562
AMH	3(0.1-8.5)	2.1(0-9.5)	0.121
FSH	6(1-26.5)	6.3(2.3-26.2)	0.325
LH	7.8±3	7.9±2.9	0.783
Testosterone	380(2.3-990)	370(116-924)	0.497
Free Testosterone	6.1(0.5-17.3)	5.7(1.3-15.4)	0.298
Prolactin	9.2(2.3-44)	8.3(1.8-39)	0.364

*: Statistically significant at p<0.05

Table 2: Comparison between the two studied groups according to different semen parameters.

	Group A Ejaculate Sperm (n=104)	Group B Testicular Sperm (n=101)	P
Semen Abnormality			
Normal	21(20.2%)	23(22.8%)	0.638
Teratospermia	83(79.8%)	78(77.2%)	0.826
Volume (ml)	3(1-8)	3(0.2-6.6)	0.073
Conc. (×106/ml)	3.8(0.1-4.6)	4.1(0.3-4.9)	0.75
Motile %	5.2(1-45)	3.5(0-46)	0.653

*: Statistically significant at p<0.05

Table 3: Comparison between the two studied groups according to the number of oocytes retrieved, their maturity, and the fertilization rate.

	Group A Ejaculate Sperm (n=104)	Group B Testicular Sperm (n=101)	P
Oocyte number	16(3-25)	14(4-28)	0.492
M2 oocytes	14(2-25)	13(2-26)	0.789
Fertilization rate	67.93%	68.01%	0.96

*: Statistically significant at p<0.05

Table 4: Comparison between the two studied groups according to the number of high-quality embryos, number of embryos transferred (total and per case).

	Group A Ejaculate Sperm (n=104)	Group B Testicular Sperm (n=101)	P
Number of high-quality Embryos	154	187	0. 084
Total number of embryos transferred	214	215	0.982
Embryos transferred per case 1 embryo 2 embryos 3 embryos 4 embryos	 11(10.6%) 78(75.0%) 13(12.5%) 2(1.9%)	 8(7.9%) 73(72.3%) 19(18.8%) 1(1.0%)	0.561

*: Statistically significant at p<0.05

Table 5: Comparison between the two studied groups according to pregnancy outcome.

Outcome	Group A Ejaculate Sperm (n=104)	Group B Testicular Sperm (n=101)	P
Chemical Pregnancy	5(4.80%)	5 (4.95%)	0.96
Clinical Pregnancy	37(35.57%)	50(49.50%)	0.044*
Overall Pregnancy	42(40.38%)	55(54.45%)	0.0442*

*: Statistically significant at $p<0.05$

Discussion

Male factor remains a major cause of infertility. ICSI is considered as a final solution for such cases. In male factor cases of infertility, the success rate largely depends on the quality of the sperm selected for injection. Besides its morphology and motility, sperm DNA integrity is a major determinant of the quality of the injected sperm. Cases with high sperm DNA fragmentation index (DFI) have been shown to have poor ICSI outcomes with lower fertilization rate, pregnancy rate, live birth rate, and higher abortion rate [21,22]. Recent studies have shown that the ejaculated and the epididymal spermatozoa have higher DNA damage than the testicular spermatozoa; this can be explained by the fact that testicular spermatozoa are protected by the Sertoli cells from exposure to damaging factors, and the ability of the genital tract to generate excessive reactive oxygen species (ROS) under the effect of infection, high temperature or exposure to environmental pollutants [12,18,23,24]. The present study was performed to compare the outcome of ICSI in patients with severe oligospermia using testicular versus ejaculated spermatozoa.

This study found no significant difference in the fertilization rate between the two study groups, this was in agreement with the findings reported by Mehta et al. [20], Abhyankar et al. [25], Arafa et al. [26] and Herrero et al. [27] who all showed that even with the use of sperm with high DFI, fertilization can still be achieved, this is because fertilization and early embryo development are mainly controlled by maternal DNA. Paternal factors, on the other hand are involved in later stages of embryo development which can be manifested in the quality of embryos, the pregnancy rate, and the abortion rate. The current study found the high-quality embryos to be higher in the testicular sperm group, the difference though did not reach statistical significance. This finding was in concordance with the results presented by Arafa et al. [26] and Esteves et al. [28]. As regards the clinical pregnancy rate, it was significantly higher in the testicular sperm group than in the ejaculate sperm group (p=0.044). Similar results were reported by Arafa et al. [26], Herrero et al. [27], Esteves et al. [28], Zhang et al. [29] and Mehta et al. [20] performed ICSI with testicular spermatozoa on 24 couples with severe oligospermia who had multiple previous failed cycles with ejaculated sperm; they achieved 50% clinical pregnancy rate in the first cycle using testicular sperm. A limitation of our study was the use of chemical pregnancy rate-instead of abortion rate-where both groups had similar rates as this did not identify the cases which had later miscarriages. In their studies, Arafa et al. [26], Herrero et al. [27], Esteves et al. [28] and Zhang et al. [29] reported a significantly higher abortion rate in the ejaculate sperm group and a significantly higher live birth rate in the testicular sperm group. It is important to note that TESE has its own drawbacks. It is an invasive procedure with added cost and added risk of surgical complications-although of very low incidence-including infection, bleeding, testicular atrophy and decreased testosterone production [30]. Furthermore, Turek et al. [31] reported that testicular sperm are more immature and show a slightly higher incidence of aneuploidy in comparison to ejaculated sperm (12% vs 6%). This finding however remains to be confirmed by larger trials, and because of the fact that ICSI is usually performed to patients who are unlikely to achieve natural conception; a minor elevation in the chance of the children having some health issues is an accepted side effect to having an established higher live birth rate [30].

Conclusion

ICSI using testicular sperm is a promising option for men with severe oligospermia especially those with high sperm DFI. The added cost and minor complications seem to be outweighed by the benefit of the improved reproductive outcome. Furthermore, it could be argued that the more invasive male treatment is opposed by the less invasive female treatment as lower numbers of cycles are needed to achieve live births.

Larger randomized controlled trials are required to validate these findings and to establish whether this treatment strategy can be applied to oligospermic men with high DNA fragmentation alone or to all cases with high DFI regardless of their sperm count. Whether testicular sperm should be used in such cases from the first ICSI trial or be restricted to cases with previous failures should be evaluated in additional studies.

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