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Original article

The role of testicular sperm extraction in repeated intracytoplasmic sperm injection failures using ejaculate sperm

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Abstract

Objective: To assess the role of testicular sperm in improving the success rates among patients who have repeated failed intracytoplasmic sperm injection cycles using ejaculated sperm. **Design**: A retrospective review of records. **Materials and Methods**: The study included 110 partners with repeated failed ICSI using ejaculate sperm were allocated into two groups retrospectively, one of them underwent testicular ICSI and the other underwent ejaculate ICSI. The two groups were well matched regarding their age of wife, age of husband, duration of marriage, history of previous marriage and previous children. **Results**: Showed that there was no statistically significant difference between patients with second ejaculate ICSI and patients with second testicular ICSI regarding the number of embryos and pregnancy rate (P- value>0.05). Patients underwent second testicular ICSI had a fertilization rate insignificantly higher than patients underwent second ejaculate ICSI.

Conclusions: ICSI using testicular extracted sperm may be considered an alternative to usingsecond ejaculated sperm in the treatment plan of infertile couples especially if increased SDF. We infer that increased SDF may be the main cause of repeated failures of ICSI using ejaculated sperm which remains to be proved by various studies. Higher pregnancy and live birth rates are associated more with higher testicular biopsy motility per drop, testicular biopsy concentration, Number of embryosand the fertilization rate. This study recommends that men with repeated failed ejaculate ICSI with high DNA fragmentation can gain benefit from testicular ICSI in the form of increased fertilization rate, number of embryos and live birth rate.

1. Introduction:

Infertility is defined as the inability to conceive after 1yr of well-timed unprotected intercourse with a prevalence of about 13-14% of reproductive-aged couples [1]. Since the establishment of *in vitro* fertilization, especially ICSI which allowed the consistent ability of a viable spermatozoon to penetrate an oocyte, about half of the couples still fail to achieve successful fertilization, a condition referred to as unexplained infertility [2].

Palermo et al accomplished the first successful attempt of ICSI using sperm from oligoteratoasthenospermic males in 1992 [3].

By the mid-nineties, the fertilizing capability of human testicular sperm had been understood [4,5]. From that point forward, the use of testicular spermatozoon became the standard approach to circumvent infertility in patients with obstructive azoospermia. Later this strategy also was used to yield sperms in non-obstructive azoospermic patients [6]. Despite the fact that sperm retrieval and the outcome of ICSI are highly successful in OA patients, it remains a struggle in NOA patients, both clinically and in the laboratory [2].

Various surgical methods are used, each with a distinct success rate and a different outcome. The use of TESE or TESA (FNA) almost always results in sperm recovery in approximately 100 percent of OA patients, according to most studies. TSE or micro-TESE [7] are the therapies of choice for NOA patients, with sperm recovery rates reaching up to 40% to 50% in some cases, and micro-TESE being 1.5 times more successful at retrieval of sperm than TESE [8].

In the current state of knowledge, there is no conclusive evidence that testicular or

ejaculated sperm yields superior results in successful ICSI, as measured by fertilisation rate, quality of the embryo, rate of implantation, and pregnancy rate stay [9].

Recently, the use of testicular sperm rather than ejaculated sperm for in vitro fertilisation (ICSI) has attracted increased attention as evidence of improved pregnancy outcomes has emerged in cases of male factor infertility utilising the traditional method. As established in a recent meta-analysis that included 5 trials and 143 patients who served as their own controls, testicular sperm have reduced DNA fragmentation rates when compared to ejaculated sperm in males with significant sperm DNA fragmentation in the ejaculate. Testicular sperm taken from the same men was compared to ejaculated sperm to determine the SDF [10].

SDF is assumed to occur in the epididymis. Hence in certain cases, testicular sperm is relied on rather than ejaculated sperm to yield sperm with lower SDF levels before transiting through the epididymis [11]. Additionally, it's been demonstrated that ICSI using extracted testicular sperm provides higher live birth rates in comparison with laboratory methods like Physiological intracytoplasmic sperm injection and IMSI in couples with high SDF in semen [11].

SDF levels in sperm are higher than in testis for a variety of reasons, one of which is the sperm's vulnerability to oxidative damage, particularly during the epididymis transit period [12]. When sperm transit through the male reproductive canal is hampered by oxidative stress, apoptosis can occur. This event has been documented in both animal and human research, and it may be responsible for the high SDF in ejaculated sperm in infertile men [13]. Age, obesity, smoking, and environmental exposure to toxicants are all potential sources of oxidative stress, as are specific clinical conditions such as varicocele and subclinical genital infection, as well as general factors such as smoking and obesity. Providing that the epididymis is bypassed in chosen ICSI candidates, this oxidative-induced damage to sperm chromatin may be avoided in such individuals [14].

Bradley et al. conducted a study in which they evaluated 448 cycles in which sperm injections were performed using ejaculate and testicular sperm. To choose sperm with greater DNA integrity for ICSI, PICSI and IMSI had been used in the previous procedure. According to their findings, LBRs with Testi-ICSI (49.8 percent) were significantly greater than with IMSI (28.7 percent) and PICSI (28.7 percent) (38.3 percent). It was possible to attain the lowest LBR (24.2 percent) when no procedure was used to select sperm for ICSI.[11]. According to a recent survey research including infertility experts from 19 countries, the practise of testicular sperm extraction for ICSI in couples with unexplained infertility is gaining popularity. Approximately 67 percent of those who responded admitted that an abnormal SDF test result would influence their decision to use testicular sperm rather

than ejaculated sperm for ICSI in the future [15].

2. Aim Of The Work:

This study aims to assess the role of testicular sperm in improving success rates among patients who have a history of failed ICSI cycles utilizing ejaculated sperm.

3. Materials And Methods:

Study Design: A retrospective descriptive study was conducted to assess the role of using testicular sperm in improving the fertility potential in patients who had failed ICSI using ejaculated sperm.

Study Site: The study was carried out in Cairo and Beni-Suef by using metadata (secondary data analysis) collected at Bedaya Fertility and IVF Hospitals (Cairo/Beni-Suef) as well as El-Nada Fertility Center.

Study Time Frame: The study was carried out over six months divided into the following stages:

Preparatory phase (2 months): during which the following activities were done: Designing the study. Reviewing the latest literature on ICSI and methods to enhance related fertilization rates. Preparing the study tools. Submission for research ethical committee approval; faculty of Medicine, Beni-Suef University. Data collection phase (1 month). Data management phase (1 month). Discussion phase: (1 month). Thesis editing phase (1 month).

Sample size: A sample of 110 couples with a history of repeated failed ICSI performed with ejaculated sperm. These were patients attending Bedaya and El-Nada fertility centers combined throughout 2018. A sample size of 110 patients gave power of study equal 95% at an alpha error equals 0.05. By using G power program version 3.1 for windows the sample size was calculated at an alpha error 0.05, power 95% and an infertility rate anticipated to be 50%, the total sample size was 55 couple in each group.

Inclusion criteria: Male factor infertility couple or unexplained primary or secondary infertility couple who had a history of previous failed ICSI using ejaculated sperm.

Exclusion criteria: Associated female factor

Data collection tool:

The records included: Age of both partners, Number of previously failed ICSI cycles, Semen parameters. Medical and surgical history, Testicular examination, Testicular biopsy data, Fertilization rate, Pregnancy rate, Live birth rate & Take-Home-Baby rate.

Data Analysis: The data was coded to fit the program of statistical analysis SPSS version 25 under windows10.A random sample of 10% of cases was selected and reviewed to ensure an adequate quality of data.

Statistical tests:

Description of qualitative variables was by frequency and percentage. Description of quantitative variables was in the form of mean and standard deviation (mean ± SD). Chi-square (χ^2) test was used for comparison of qualitative variables with each other. Comparison between quantitative variables was

carried by using: Student t-test of two independent samples. One-way ANOVA test (analysis of variance) was used instead of t- test. For more statistical analysis; suitable statistical tests of significance were used. P-values < 0.05 is considered as statistically significant.

Ethical Consideration: Administrative approvals were sought from the head of selected facilities. Ethical approval was sought from Beni-Suef University Ethical Committee. They assured that data was confidential and anonymous. Sociodemographic data was recorded for identifying the characteristics, not identity. Data was secured, anonymous, and was obtained from the center without patient identity.

4. Results:

Table (1): Baseline characteristics of the studied couples:

Characteristics	Second testicular ICSI no=55(%)	Second ejaculate ICSI no=55(%)	P-value
Age of husband(years):			
Mean±SD	39.4±8	39.5±8.2	
Range (min-max)	22-60	27-60	0.916
Median	39	39	
Age of wife:			
Mean±SD	32.2±5.6	31.6±5.7	
Range (min-max)	22-44	22-44	0.638
Median	32.00	30	
Duration of marriage:			
Mean±SD	8.1±4.3	7.9±4	
Range (min-max)	2-24	2-18	0.715
Median	8	7	
History of previous marriage	1(1.8%)	1(1.8%)	1.000
History of previouschildren	10(18.2%)	9(16.4%)	0.999

Table (1) showed that there was no statistically significant difference between patients with second ejaculate ICSI and patients with second testicular ICSI regarding their characteristics (P-value>0.05)

Table (2): Comparison between both groups regarding the seminal profile of the semen analysis and after testicular sperm extraction of the studied husbands:

Seminal parameters	Second testicular ICSI no=55(%)	Second ejaculateICSI no=55(%)	P-value
Volume			
Mean±SD	2.7±0.9	**3.3±1.17**	
Range (min-max)	1-4.5	**1-5**	**0.004***
Median	3	**3**	
Conc			
Mean±SD	17.8±14	20.9±11.7	
Range (min-max)	1.2-48	8-45	0.211
Median	12	20	
Motility			
Mean±SD	13.3±12.3	**24±13.9**	
Range (min-max)	0-40	**9-60**	**<0.001****
Median	10	**20**	
DNA Fragmentation			
Mean±SD	47.6±20.5	**60.6±20.9**	
Range (min-max)	15-90	**20-90**	**0.002***
Median	46	65	

Table (2) showed that there was no statistically significant difference between patients with second ejaculate ICSI and patients with second testicular ICSI regarding sperm concentration in ejaculate (P-value>0.05). Patients underwent second ejaculate ICSI had a higher ejaculate volume, motility, and DNA fragmentation than patients underwent second testicular ICSI.

Table (3): Testicular biopsy data

Test biopsy motility/drop	
Mean±SD	2.9±2
Range (min-max)	0-8
Median	3
Test biopsy conc.	
Mean±SD	2.6±2
Range (min-max)	0.2-7.0
Median	2

Table (4): Comparison between outcomes of using second testicular sperm extraction and outcomes of using second ejaculate sperm among the studied husband:

Outcomes	Second testicular ICSI no=55(%)	Second ejaculate ICSI no=55(%)	P-value
Fertilization rate			
Mean±SD	**55±37.4**	41.7±25.3	
Range (min-max)	**0-100**	15-100	**0.031***
Median	**73**	35	
Number of embryos			
Mean±SD	2.8±2	2.7±2.5	
Range (min-max)	0-12	0-9	0.765
Median	2	2	
Pregnancy rate			
Get pregnancy	24(43.6%)	20(36.4%)	0.436
Failed to get pregnancy	31(56.4%)	35(63.6%)	

Table (4) showed that there was no statistically significant difference between patients with second ejaculate ICSI and patients with second testicular ICSI regarding the number of embryos and pregnancy rate (P- value>0.05). Patients underwent second testicular ICSI had a fertilization rate insignificantly higher than patients underwent second ejaculate ICSI.

Table (5): Univariate analysis of the factors affecting getting pregnancy among the patients underwent testicular ICSI:

Characteristics	Got pregnancy **n**=24(43.6%)	Failed to getpregnancy **n**=31(56.4%)	P-value
Age of husband (years): Mean±SD Range (min-max) Median	 38.8±7.6 22-56 39	 39.8±8.5 27-60 39	 0.865
Age of wife: Mean±SD Range (min-max) Median	 31.9±5.4 22-41 32	 32.4±5.9 22-44 30	 0.777
Duration of marriage: Mean±SD Range (min-max) Median	 8±3.6 3-17 7.5	 8.3±4.8 2-24 8	 0.834
History of previous marriage	1(1.8%)	0(0%)	0.634
History of previous schildren	3(12.5%)	7(22.6%)	0.336
Volume Mean±SD Range (min-max) Median	 2.4±0.7 1-3 2.5	 **2.9±0.97** **1-4.5** **3**	 0.022*
Conc Mean±SD Range (min-max) Median	 13±9.7 1.2-31 12.5	 **21.6±15.8** **2-48** **12**	 0.020*
DNA Fragmentation Mean±SD Range (min-max) Median	 51.5±21.7 15-90 48	 45.4±18.6 15-90 43	 0.309
Motility Mean±SD Range (min-max) Median	 11.1±11 1-35 10	 14.8±13 0-40 10	 0.301
Test biopsy motility/drop Mean±SD Range (min-max) Median	 **4.4±1.9** **1-8** **5**	 1.7±1.5 0-5 1	 **<0.001****
Test biopsy conc: Mean±SD Range (min-max) Median	 **3.8±2.4** **0.5-7** **3**	 1.7±1.5 0.2-5 2	 **<0.001****
Number of embryos: Mean±SD Range (min-max) Median	 **4.3±3.3** **1-12** **3.5**	 1.7±2.1 0-5 1	 **<0.001****
Fertilization rate: Mean±SD Range (min-max) Median	 **76.6±25.4** **40-100** **86.5**	 38.3±36 0-86 50	 **0.001****

Table (5) showed that there was unexpectedly statistically significant increase of the seminal volume and concentration among the couples who failed to get pregnancy. The statistically significant increase of the Test biopsy motility/drop, Test biopsy conc, Number of embryos and the fertilization rate were associated more with getting pregnancy.

Table (6): Univariate analysis of the factors affecting getting pregnancy among the patients underwent second ejaculate ICSI:

Characteristics	Got pregnancy n=20(36.4%)	Failed to get pregnancy n=35(63.6%)	P-value
Age of husband(years):			
Mean±SD	39±8.2	39.8±8.4	0.733
Range (min-max)	28-60	27-60	
Median	39.5	39	
Age of wife:			
Mean±SD	30.9±5.8	32.1±5.7	0.465
Range (min-max)	22-44	22-44	
Median	29	31	
Duration of marriage:			
Mean±SD	7.3±3.9	8.3±4.1	0.377
Range (min-max)	2-17	2-18	
Median	6	7	
History of previousmarriage	0(0%)	1(2.9%)	0.446
History of previouschildren	5(25%)	5(14.3%)	0.322
Volume			
Mean±SD	3.5±1.4	3.1±0.7	0.187
Range (min-max)	1-5	2-5	
Median	4	3	
Conc			
Mean±SD	**31.8±9.8**	14.8±7.4	**<0.001****
Range (min-max)	**20-45**	8-38	
Median	**33**	12.5	
DNA Fragmentation			
Mean±SD	44.7±24.5	69.7±11.3	**<0.001****
Range (min-max)	20-90	45-90	

Median	37.5	74	
Motility Mean±SD Range (min-max) Median	 **36.4±16.8** **10-60** **40**	 16.9±3.3 9-22 16	 <0.001**
Number of embryos:			<0.001**
Mean±SD Range (min-max) Median	**5.2±2.4** **2-9** **4.50**	1.2±1.1 0-3 1	
Fertilization rate: Mean±SD Range (min-max) Median	 **70.8±16.7** **45-100** **70**	 25.1±9.4 15-45 20	 <0.001**

Table (6) showed that among patients underwent second ejaculate ICSI, there was a statistically significant increase of motility, concentration, number of embryos and fertilization rate among the couples who get pregnancy than patients with failure ICSI. Also, he lower DNA fragmentation was significantly associated with getting pregnancy.

5. Discussion:

About 13-14% of reproductive-aged couples are unable to achieve a successful pregnancy after 1 yr or more of frequent unprotected sexual intercourse, so infertility is considered as a major health care problem of different communities [1]. The adoption of ARTs notably ICSI has been able to overcome male factor infertility by allowing the consistent ability of a viable spermatozoon to activate an oocyte [2]. Palermo et al accomplished the first successful attempt of ICSI using sperm from OAT males in 1992 [3]. Ever since, researchers have strived to introduce novel approaches to obtain best quality sperm to improve ICSI results. Of these approaches, was the suggestion of surgical sperm retrieval in cases of recurrent ICSI failures using seminal sperm [16]. In recent years, claims of improved pregnancy outcomes with testicular sperm for patients of infertility, particularly those with high SDF in the ejaculated sperm, have fueled interest in using testicular sperm in preference to ejaculated sperm for in vitro fertilisation (ICSI) [10].

So, this study was conducted to assess the role of testicular sperm versus the ejaculate sperm in repeated failed ICSI. The study included 110 partners with repeated failed

ICSI were allocated into two groups retrospectively, one of them underwent testicular ICSI and the other underwent ejaculate ICSI. The two groups were well matched regarding their age of wife, age of husband, duration of marriage, history of previous marriage and previous children.

This study showed that was no statistically significant difference between patients with second ejaculate ICSI and patients with second testicular ICSI regarding sperm concentration in ejaculate (P-value>0.05). Patients underwent second ejaculate ICSI had a higher ejaculate volume, motility, and DNA fragmentation than patients underwent second testicular ICSI. Despite the non-significant difference between patients with second ejaculate ICSI and patients with second testicular ICSI regarding the number of embryos and pregnancy rate, there were still higher number of embryos and pregnancy rate among testicular ICSI group. In addition, patients underwent second testicular ICSI had a higher fertilization rate than patients underwent second ejaculate ICSI.

This finding is consistent with the findings of a study conducted by Westlander and colleagues, who indicated that testicular sperm can produce more dependable fertilisation rates than other sperm types [17]. Also, our study was supported by previous study done by Esteves et al. [10] observed that the clinical characteristics of couples who had ICSI utilising testicular sperm versus ejaculated sperm were not statistically different, but that the birth rate in the Testi-ICSI group was considerably greater than in the Ejaculate-ICSI group.

High levels of reactive oxygen species (ROS) in the seminal fluid are associated with poor embryo development in humans, which may explain the current findings [18]. When it comes to the accumulation of oxidative damage as sperm proceed through the male reproductive canal, there is very little information available. They hypothesised that the presence of immature spermatozoa, which are recognised sources of endogenous ROS, could cause harm to mature sperm during transit through the epididymis, according to Ollero et al. [19]. As a vasovasostomy site begins to stenose, it is possible that the local environment within the epididymis will become even more strained. If there is an increase in pressure on the testicular side of the anastomosis, increasing epididymal dysfunction may occur, particularly in a system that has already experienced iatrogenic blockage [20]. Utilization of testicular sperm, as demonstrated in our work, may circumvent this potentially harmful local environment, resulting in enhanced IVF-ICSI outcomes in patients with similar characteristics. The lower rate of fertilisation in the ejaculate ICSI group may also be explained by increased DNA fragmentation, as previously observed in patients with elevated DNA fragmentation and recurrent IVF-ICSI failure using ejaculated sperm; additionally, they discovered that testis-derived samples had significantly less DNA

damage. Although the rates of fertilisation and embryo development were not significantly different between testis and ejaculated samples [21].

Another reason for testicular ICSI's advantage over ejaculate ICSI Apoptosis induced by testicular circumstances and oxidative stress during sperm transit via the male reproductive canal may account for the increased positive for Spermatic DNA fragmentation of ejaculated sperm from infertile individuals. This oxidative-induced sperm chromatin damage can be prevented in chosen ICSI candidates by bypassing the epididymis and using testicular sperms [13,22].

In contrast to our findings, Lu et al. discovered a small advantage for ejaculated sperm in terms of fertilisation rates and embryo quality across a vast number of centres [23]. Similarly, Gnoth and colleagues reviewed 337 IVF-ICSI cycles and discovered no difference in clinical outcomes between ejaculated and testicular sperm for males with azoospermia, but both of those studies were retrospective and hence susceptible to bias [24].

Furthermore, when testicular sperm was compared to ejaculated sperm, a meta-analysis discovered no statistically significant improvement in ICSI pregnancy rates in azoospermic men [25].

6. Conclusion:

In conclusion, ICSI using testicular extracted sperm may be considered an alternative to using second ejaculated sperm in the treatment plan of infertile couples especially if increased SDF. We infer that increased SDF may be the main cause of repeated failures of ICSI using ejaculated sperm which remains to be proved by various studies. No statistically significant difference between patients with second ejaculate ICSI and patients with second testicular ICSI regarding the number of embryos and pregnancy rate Higher pregnancy and live birth rates are associated more with higher testicular biopsy motility per drop, testicular biopsy concentration, Number of embryos and the fertilization rate.

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