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Research Article

Use of Testicular Sperm Increases Fertility Outcome in Oligo-asthenoteratozoospermia Patients with Failed IVF/ICSI Cycle

Arzu Yurci*

Memorial Kayseri Hospital IVF Center, Gynecology Obstetrics & Reproductive Medicine, Kayseri, Turkey

***Corresponding Author:** Arzu Yurci, Memorial Kayseri Hospital IVF Center, Gynecology Obstetrics & Reproductive Medicine, Kayseri, Turkey.

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Abstract

Aim: To assess the impact of testicular sperm on fertility outcome in patient with oligo-asthenoteratozoospermia (OAT) who had previously failed ICSI cycles performed using ejaculated sperm.

Methods: 107 patients with a diagnosis of OAT and having at least two previous unsuccessful ICSI with ejaculated sperm were included in the study. Contrary to previous ICSI trials, it was decided to use testicular sperm in all cases this time. The primary outcome measures were fertilization rate (FR), ß hCG positivity, clinical pregnancy rate (CPR) and live birth rate (LBR).

Results: While fertilization was achieved in 17.4 out of of 107 couples using ejaculated sperm, 11 cases were positive for beta hCG and 5 cases were found to have heartbeats. Live birth did not occur in any of the patients who underwent ICSI with ejaculated sperm. In the testicular sperm group fertilization was detected in 44.6 of the embryos, beta-hCG positivity in 51 cases and CPR in 41 cases. Each parameter was found significantly higher than the ejaculated sperm group. Live birth was achieved in 36 patients using testicular sperm.

Conclusion: The use of surgically obtained testicular sperm significantly increased both CPR and LBR in OAT patients in whom pregnancy could not be achieved with ejaculated sperm.

Keywords: *Intracytoplasmic Sperm Injection; Ejaculated Sperm; Microdissection Testicular Sperm Extraction; Apoptosis Score; Live Birth Rate*

Introduction

OAT patients continue to be one of the most difficult disease groups in our infertility practice. ART should be the first treatment option [1] for OAT patients whose female partner is over 35 years old. In cases where the female partner is under the age of 35, the following treatment options can be tried before ART; (i) Cessation of smoking, (ii) weight reduction, (iii) reduced alcohol intake, (iv) continuing physical activity, (vi) scrotal cooling, changes in clothing and working conditions [1]. However, the evidence available for the use of these treatment options is not very strong. Our possibilities to treat infertile men with OAT with medical methods are also very limited. Data regarding the beneficial use of recFSH, aromatase inhibitors, antioxidant supplementation and androgen use in these patients is limited [2-4]. In contrast to these treatments, there are studies showing that treatment with the antiestrogens tamoxifen or clomiphene has a significant improvement in pregnancy rates in OAT patients as well as there are publications showing that there is no benefit [5,6]. The

evidence that life style change increases fertility in OAT cases is not sufficient [7]. Currently, our most powerful weapon for OAT couples to achieve pregnancy is ART. However, in OAT patients we refer to ART, use of ejaculated sperm for ICSI do not always allow us to achieve pregnancy [8].

Because of the missing evidence from randomized studies, routine ICSI application with testicular sperm is not recommended in OAT patients. On the other hand it is an acceptable view to perform ICSI with surgically obtained testicular sperm in OAT couples where pregnancy cannot be achieved in two or more ICSI cycles with ejaculate sperm [1]. However, it is a decision that should be taken jointly by talking to these couples. The scientific rationale for using testicular sperm for ICSI in OAT patients is not clear. However, in ICSI cases with ejaculated sperm, an increase in sperm DNA fragmentation was found in oligospermic men who could not achieve pregnancy [9]. Similarly, the percentage of DNA fragmentation was found to be less in testicular sperm compared to ejaculated sperm [10,11]. All these data are evidence for using testicular sperm if pregnancy could not be achieved in one or more ICSI performed with ejaculated sperm. Unfortunately, very few studies have investigated the effect of ejaculated versus testicular sperm on fertility outcome within the same cohort of OAT patient group. When the literature is reviewed, there are no comprehensive studies showing the effects of ejaculated versus testicular sperm on clinical pregnancy and live birth rates in men with OAT [12]. The present study was, therefore, planned to investigate the effect of testicular sperm on fertilization rates (FR), clinical pregnancy rates (CPR), and live birth rates (LBR) in OAT patients who had previously failed at least two ICSI attempts with ejaculated sperm, and who had accepted to undergo microsurgical testicular sperm extraction (mTESE).

Materials and Methods

107 patients with a diagnosis of OAT and having at least two previous unsuccessful ICSI with ejaculated sperm were included in the study. Contrary to previous ICSI trials, it was decided to use testicular sperm in all couples this attempt. OAT patients participating in the study were selected from among the couples who applied to the Memorial IVF-center between 2018 - 2020. Verbal and written consents were obtained from all couples participating in the study. Information about the mTESE method to be used to obtain testicular sperm was explained to all couples. Patients were provided with sufficient knowledge about the possible advantages and complications of the mTESE method. Couples with female factor infertility in addition to OAT were excluded from the study. What we mean by unsuccessful cycle is failure to obtain live birth in ICSI trials using ejaculate sperm. Demographics and reproductive history of the men with OAT and their female partners were recorded.

OAT patients with a history of previous mTESE, undescended testis, azoospermia, cryptozoospermia, varicoceles requiring treatament, testicular trauma affecting sperm parameters, unilateral orchiectomy, chemo-radio therapy, were not included the study. The diagnosis of OAT for each patient was made according to the definition in the "WHO laboratory manual for the examination and processing of human semen" (fifth edition, 2010). The combination of the following three criteria was accepted as OAT: (i) the total number of spermatozoa in the ejaculate must be below the lower reference limit, (ii) the percentage of progressive and motile spermatozoa must be below the lower reference limit, and (iii) the percentage of sperm with normal morphology must also be below the lower reference limit.

Semen samples were analysed at least twice. Patients who were found to have problems during the sperm collection and transport process, abstinence duration longer or shorter than recommended, as well as high fever, and use of any medication that affects sperm production or motility in the last 3 months were excluded from the study (1). Sperm DNA fragmentation of 47 patient with OAT was evaluated with the in situ Cell Death Detection Kit with fluorescein isothiocyanate (FITC). DNA fragmentation was examined in both ejaculted and testicular sperm samples of the same patient. In the remaining 60 cases, FITC test could not be performed due to severe low sperm counts and technical reasons. Apoptosis results were reported as normal (apoptosis rate 0 - 13%), high risk (apoptosis rate 13 - 35%), and poor prognosis (apoptosis 35 - 100%).

All men with OAT underwent a local and systemic physical examination to evaluate signs of undescended testes, orchiectomy, and hypogonadism. In order to assess the volume and consistency of testes and epididymis scrotal physical examination was performed. Testicular volume was also assessed by ultrasonography. Doppler ultrasonography was also used for confirmation of physical findings. OAT patients were also evaluated for the total or partial absence of deferent ducts [1]. In patients with palpable varicocele with OAT, treatment decision was made by talking to couples. Surgical treatment was recommended for varicocoele in males with abnormal semen parameters. Endocrine evaluation in all men with OAT was performed. In addition to karyotype analysis microdeletions in the long arm of chromosome Y (Yq), called azoospermia factor (AZF) were also assessed.

Standard antagonist protocol was applied for controlled ovarian stimulation. Treatment was started on the 2nd or 3rd day of the cycle with recombinant FSH, which was dosed individually. Gonadotrophin-releasing hormone antagonist was started on the 5th or 6th day of stimulation. Recombinant hCG (Ovitrelle, Merck-Serono, 250 mg, Modugno, BA, Italy) treatment was initiated when at least three follicles with a diameter of 16-17 mm were detected in ultrasonographic evaluation. 36 hours after hCG application oocyte pick-up was performed with 17-gauge needle (Cook Medical, Bloomington, IN, USA) guided by trans-vaginal ultrasonography. Conventional mTESE procedure was applied to all OAT patients. Detailed information about mTESE application can be found elsewhere. Fresh testicular sperm obtained after mTESE were used for ICSI.

The primary outcome measures were fertilization rate (FR), ß human chorionic gonadotrophin (hCG) positivity, clinical pregnancy rate (CPR) and live birth rate (LBR) both in ejaculated and testicular sperm groups. Both groups were compared with each other in terms of FR, CPR and LBR. Since the patients had more than one ICSI attempt with ejaculate sperm before, the results of the last ICSI attempt with ejaculated sperm and live birth could not be achieved and the results of the first ICSI trial with testicular sperm were compared. Fertilization rate was accepted as percentage transformation of micro injected oocytes into two pronuclei. Serum ß hCG values were measured 14 days after embryo transfer. After the following 2 weeks clinical pregnancy was confirmed by presence of gestational sac and fetal cardiac activity on ultrasonographic examination.

Statistical analysis

Statistical analyses were performed on SPSS v21 (SPSS Inc., Chicago, IL, USA). The Kolmogorov-Smirnov test was used to determine whether variables were normally distributed. Data are given as mean ± standard deviation or median (minimum - maximum) for continuous variables according to normality of distribution, and as frequency (percentage) for categorical variables. Comparisons between techniques were performed with the Wilcoxon Signed Ranks test for quantitative data and with the McNemar test for qualitative data. Two-tailed p-values of less than 0.05 were considered statistically significant.

Results

Demographics characteristics scuh as maternal and paternal age, maternal gravity and parity, number of ICSI attempts were shown in table 1. Fertilization rate, beta hCG positivity, CPR and LBR were shown in table 2. The mean age of men was 37.48 ± 10.02 and the mean age of women was 32.47 ± 6.37.

Female age	32.47 ± 6.37
Male age	37.48 ± 8.82
Abnormal male chromosome	4 (3.9%)
Y micro-deletion	1 (1.0%)
Gravidity	0 (0 - 5)
0	76 (74.5%)
1	19 (18.6%)
≥ 2	7 (6.9%)
Parity	0 (0 - 2)
0	91 (89.2%)
1	10 (9.8%)
2	1 (1%)
Abortion	0 (0 - 4)

0	81 (79.4%)
1	14 (13.7%)
≥ 2	7 (6.9%)
IVF attempt	2 (1 - 8)
1	26 (25.5%)
2	25 (24.5%)
3	24 (23.5%)
4	13 (12.7%)
≥ 5	14 (13.8%)
Numbers of TESE	1 (1 - 6)
1	90 (88.2%)
≥ 2	12 (11.8%)
Varicocele	17 (16.7%)
Varicocelectomy	14 (13.7%)
Undescended testicle history	5 (4.9%)

Table 1: *Summary of demographics characteristics.*
Data are given as mean ± standard deviation for continuous variables according to normality of distribution and as frequency (percentage) for categorical variables.

	Method		
	Ejaculation (n = 107)	**microTESE (n = 107)**	**P**
Fertilization ratio	17.4 (0-80)	44.6 (0 - 100)	< 0.001
Beta-hCG	11 (10.3%)	51 (47.7%)	< 0.001
Clinical pregnancy	5 (4.7%)	41 (38.3%)	< 0.001
Live birth rate	0	36 (33.6%)	< 0.002

Table 2: *Comparison of two groups in terms of fertility outcome.*

The abnormal male chromosomes detected in 4 cases were as follows: 46,X,der(Y);47,XXY; 45,X,t(8;15)(q22;15)|29|/46,X,i(Y)(q10),t(8;15)(q22;q15)|21|; and 46,X,Yinv9p11q12. Y chromosome micro-deletion (AZFc) was detected in only one case. While fertilization was achieved in 17.4 out of of 107 couples who underwent ICSI using ejaculated sperm 11 cases were positive for beta hCG and 5 cases were found to have heartbeats. Live birth did not occur in any of the patients who underwent ICSI with ejaculated sperm. Since live birth could not be achieved using ejaculate sperm in 107 cases, ICSI was performed using testicular sperm in all 107 patients. In the testicular sperm group, fertilization was detected in 44.6 of the embryos. This ratio was found to be significantly higher than the group using ejaculated sperm (17.4% vs 44.6%, $p < .001$). Beta-hCG positivity was detected in 51 cases in the testicular sperm group, and this ratio was significantly higher than the ejaculated sperm group (47.7% vs 10.3%, $p < .001$). Similarly, clinical pregnancy was achieved in 41 cases who underwent ICSI with testicular sperm and this ratio was approximately 7.5 times higher than the ejaculated sperm group (38.3% vs. 4.7%, $p < .001$). Live birth was achieved in 36 patients who underwent ICSI with testicular sperm. The difference between two groups in terms of LBR was statistically significant (0% vs. 33.6%, $p < .002$).

In the ejaculated sperm group, the number of cases with normal apopitosis score was 8 (17%), while this number was 43 (91.4%) in the teseticular sperm group, and the difference was significant ($p < .021$). While the number of patients with high risk apopitosis score was 28 (59.5%) in the ejaculated sperm group, this number was found to be 4 (8.5%) in the testicular sperm group, and the difference was significant ($p < .001$). In the ejaculated sperm group, 11 patients with apopitosis score with poor prognosis were detected, while no patients with poor prognosis were found in the testicular sperm group (Table 3).

	FITC Apoptosis Score		
	Normal (0 - 13%)	**High risk (13 - 35%)**	**Poor prognosis (35 - 100%)**
Ejaculated sperm (n = 47)	8 (17.0%)	28 (59.5%)	11 (23.4%)
Testicular sperm (n = 47)	43 (91.4%)	4 (8.5%)	-
p value	0.021	0.001	

Table 3: *FITC apopitosisscore in ejaculatedandtesticular sperm of 47 men with OAT.*

Discussion and Conclusion

The absolute and only indication for using testicular sperm for ICSI is azoospermia patients who do not accept donation. Another indication for testicular sperm use is infertile cases with high sperm DNA damage. Of course, the indication in the latter is not as clear as in azoospermic patients. In addition to the above, use of testicular sperm has a positive contribution to pregnancy rates in cases of severe male infertility such as cryptozoospermia, oligospermia, oligoasthenoteratozoospermia and necrozoospermia. However, since mTESE is an invasive procedure, the use of this method in infertile male cases other than azoospermia should be decided by considering the profit/loss ratio [13-15]. Our study is the most comprehensive study investigating the effects of testicular sperm versus ejaculated sperm on fertility outcome in OAT cases. In the current study we clearly showed that using testicular sperm significantly increases reproductive outcome in OAT patients who cannot conceive with ejaculated sperm. We found a 2.5-fold increase in fertilization rates, a five-fold increase in beta hCG positivity, and an 8-fold increase in clinical pregnancy rates in our patient group, where pregnancy could not be achieved in at least two previous ICSI trials with ejaculated sperm. More importantly, while LBR was 0 in previous ICSI cycles with ejaculated sperm, LBR was found as 33.6% in ICSI with testicular sperm. In summary, live births occurred in 36 OAT patients after ICSI using testicular sperm. A study conducted by Mehta., *et al*. [9] demonstrated that making ICSI with surgically retrieved sperm in men with severe oligospermia was related with a 50% clinical pregnancy and live-birth rate for couples who had previously failed one or more ICSI attempt with ejaculated sperm. These results correspond exactly to our results. We do not clearly know the exact reason for the improvement in reproductive outcome in OAT cycles using testicular sperm, because of the limited literature data. Studies investigating the effects of use of fresh testicular versus fresh ejaculated sperm on fertility outcome within the same cohort of OAT patients are insufficient. Moreover, the patients participating in these studies are more severe cases of oligospermia or cryptozoospermia than the diagnosis of OAT [13-15]. Only a study by Weissman., *et al*. [13], the impacts of using ejaculated versus testicular sperm on implantation and pregnancy rates in four couples with severe OAT were compared. In most of these studies, increased rates of both implantation and clinical pregnancy were reported with the use of testicular sperm in cases of cryptozoospermia, oligospermia, oligoasthenoteratozoospermia, necrozoospermia and virtual azoospermia [14-16].

One of the possible reasons for the improvement in reproductive outcome in testicular sperm cycles may be that the DNA of surgically obtained sperm is less damaged than ejaculated sperm. Studies demonstrated that DNA damage in ejaculated sperm is about 3 to 5 times higher when compared to DNA damage in testicular sperm [9,17]. In our study, the abnormal FITC apoptosis score in ejaculate sperm was found to be approximately ten times higher than the FITC scores of the testicular sperm group. On the other hand, the normal apoptosis test score was 5 times higher in the testicular sperm group than in the ejaculated sperm group. Therefore, in addition to the deterioration

in ejaculate sperm parameters of OAT patients, the increase in DNA damage index may be the main reason for the decrease in pregnancy rates. In agreement with our findings, Guzick., *et al*. [18] reported that the combination of the three parameters including total number of spermatozoa, pecentage of progressively motile spermatozoa, and percentage of morphologically normal spermatozoa lower than the defined thresholds significantly decreases the ICSI outcome in infertile men. Adding a high DNA fragmentation index to these parameters further reduces the reproductive outcome in OAT cases. Oligozoospermic men with high DNA damage the possibility of having a live baby was significantly higher with the use of surgically retrieved sperm compared to ejaculated sperm. This data and our findings together supports the role of sperm DNA damage in failed ICSI cycles in OAT patients [9].

Spermatozoa of men with OAT might expose sheer stress and nuclear DNA damage during migration within the male genital tractus that may cause to deterioration in sperm parameters and ICSI results [19,20]. Then we can prevent both oxidative stress and sperm DNA damage by using testicular sperm. Consistent with this, Esteves., *et al*. showed better ICSI outcomes with testicular sperm in the oligozoospermic men compared to men who used ejaculated sperm. Similar results were also shown by Akanksha Mehta., *et al*. [9] on oligozoospermic male patients. In the meta-analyzes conducted on this subject, it was pointed out that the use of testicular sperm significantly increased reproductive outcome in patients with abnormal sperm parameters and without azoospermia [12,21]. However, the results obtained from both meta-analyzes were not sufficient to make a definite judgment both in terms of patient selection criteria and in terms of using frozen sperm in some studies and fresh sperm in some studies.

The second possible reason for the increase in clinical pregnancy and live birth rates in OAT patients using testicular sperm is decreased oxidative stress. Indeed, when immature spermatozoa migrate from the seminiferous tubule to the epididymis during ejaculation, they produce reactive oxygen species, which may adversely affect sperm DNA quality. Sperm DNA damage that occurs as a result of defects in eliminating ROS in seminal fluid negatively affects fertility outcome by preventing sperm motility and fertilizing capacity as well as the formation of quality embryos. In conclusion, using testicular sperm in these cases may increase the rates of pregnancy and live births by decreasing both ROS production and sperm DAN damage due to ROS [22-25]. However, sperm migration in the male reproductive system is actually a kind of maturation process. During this migration, the sperms that can withstand the oxidative stress complete their maturation and reach the female reproductive system. For these reasons, less DNA fragmentation in testicular sperm does not mean that everything is normal in terms of reproductive biology. Testicular sperm may contain less DNA damage, but it is an immature sperm and its aneuploidy rates are higher [17,19,26]. Therefore, we can say that the underlying reason for the increase in clinical pregnancy and live birth rates with the use of testicular sperm in our OAT patients is not only decreased sperm DNA damage. Decreased DNA fragmentation and ROS production in testicular sperms may contribute to the improvement in reproductive outcome in OAT patients, but testicular sperm may have other healing properties that we do not know.

Lack of detailed data on previous failed ICSI trial cycles is one of the main limitations of our study. Although the American Society of Reproductive Medicine does not recommend the routine use of sperm DNA fragmentation assays in infertility work-up, another handicap is that we cannot apply apoptosis test to all OAT patients [27]. The severely low sperm count has limited our assessment of DNA damage in many patients. Despite this limitations we can list the most important factors that distinguish our study from the studies mentioned above as follows; (i) all of the cases were diagnosed with OAT based on semen analysis performed at least twice, (ii) the number of patients participating in the study was 107 provides sufficient power in interpreting the results, (iii) failure to obtain results from at least two ICSI trials with ejaculated sperm strengthens our hand in terms of ethics in order to use testicular sperm, (vi) and finally, the fact that the patients given fresh ejaculated or fresh testicular sperm are in the same cohort allows us to compare the effects of two different sperm types on reproductive outcome more clearly.

The most suitable group for using testicular sperm after azoospermia is oligozoospermia cases with high sperm DNA damage. However, the indication in these cases is not as clear as in azoospermia. In our study, FITC apoptosis test could only be performed on 47 cases. While the DNA damage values obtained from the ejaculated samples of the OAT patients who underwent the apoptosis test were found to

abnormal in 83% of the cases and normal in only 17% of the cases. On the other hand, in the testicular sperm group, apoptosis test was normal in 91% of the cases, while it was abnormal in only 8.5% of the cases. As a result, doing mTESE reduced sperm DNA damage in OAT patients. In the light of our findings and data from other studies, collecting sperm and performing ICSI by performing mTESE in OAT patients with high sperm DNA damage in ejaculate samples can significantly increase fertility outcome. However, it would not be correct to make a clear comment on this subject, as FITC test cannot be performed on all OAT patients. Although our study was not randomized, it is important in terms of patient selection criteria and the number of cases, as it shows that performing ICSI with testicular sperm in OAT cases leads to a significant increase in clinical pregnancy and live birth rates. Nevertheless, randomized studies comparing the fertility outcomes using ejaculated spermatozoa versus testicular spermatozoa in men with OAT are still needed.

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Conflict of Interest

The authors declare that they have no conflict of interest.

Bibliography

1. Colpi GM., *et al*. "European Academy of Andrology guideline Management of oligo-astheno-teratozoospermia". *Andrology* 6 (2018): 513-524.
2. Jung JH and Seo JT. "Empirical medical therapy in idiopathic male infertility: Promise or panacea?" *Clinical and Experimental Reproductive Medicine* 41 (2014): 108-114.
3. Foresta C., *et al*. "Recombinant FSH in the treatment of oligozoospermia". *Expert Opinion on Biological Therapy* 9 (2009): 659-666.
4. Showell MG., *et al*. "Antioxidants for male subfertility". *The Cochrane Database of Systematic Reviews* (2014): CD007411.
5. Chua ME., *et al*. "Revisiting oestrogen antagonists (clomiphene or tamoxifen) as medical empiric therapy for idiopathic male infertility: a meta-analysis". *Andrology* 1 (2013): 749-757.
6. Jungwirth A., *et al*. "European Association of Urology guidelines on Male Infertility: the 2012 update". *European Urology* 62 (2012): 324-332.
7. Fuentes A., *et al*. "Recent cigarette smoking and assisted reproductive technologies outcome". *Fertility and Sterility* 93 (2010): 89-95.
8. Al-Inany HG., *et al*. "Gonadotrophin-releasing hormone antagonists for assisted reproductive technology". *The Cochrane Database of Systematic Reviews* 4 (2016): CD001750.
9. Mehta A., *et al*. "Higher pregnancy rates using testicular sperm in men with severe oligospermia". *Fertility and Sterility* 104 (2015): 1382-1387.
10. Greco E., *et al*. "Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa". *Human Reproduction* 20 (2005): 226-230.
11. Arafa M., *et al*. "ICSI outcome in patients with high DNA fragmentation: Testicular versus ejaculated spermatozoa". *Andrologia* 50 (2017): e12835.

12. Esteves SC., *et al*. "Reproductive outcomes of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with high levels of DNA fragmentation in semen: systematic review and meta-analysis". *Fertility and Sterility* 108 (2017): 456-467.

13. Weissman A., *et al*. "Pregnancies and live births following ICSI with testicular spermatozoa after repeated implantation failure using ejaculated spermatozoa". *Reproductive BioMedicine Online* 17 (2008): 605-609.

14. Hauser R., *et al*. "Virtual azoospermia and cryptozoospermia—fresh/frozen testicular or ejaculate sperm for better IVF outcome?" *Journal of Andrology* 32 (2011): 484-490.

15. Ben-Ami I., *et al*. "Intracytoplasmic sperm injection outcome of ejaculated versus extracted testicular spermatozoa in cryptozoospermic men". *Fertility and Sterility* (2013): 991867-991871.

16. Amirjannati N., *et al*. "Comparison of intracytoplasmic sperm injection outcomes between spermatozoa retrieved from testicular biopsy and from ejaculation in cryptozoospermic men". *Andrologia* 44.1 (2012): 704-709.

17. Moskovtsev SI., *et al*. "A comparison of ejaculated and testicular spermatozoa aneuploidy rates in patients with high sperm DNA damage". *Systems Biology in Reproductive Medicine* 58 (2012): 142-148.

18. Guzick DS., *et al*. "National Cooperative Reproductive Medicine Network. Sperm morphology, motility, and concentration in fertile and infertile men". *The New England Journal of Medicine* 8.345 (2001): 1388-1393.

19. Greco E., *et al*. "Efficient treatment of infertility due to sperm DNA damage by ICSI with testicular spermatozoa". *Human Reproduction* 20 (2005): 226-230.

20. Popal W and Nagy ZP. "Laboratory processing and intracytoplasmic sperm injection using epididymal and testicular spermatozoa: what can be done to improve outcomes?" *Clinics* 68 (2013): 125-130.

21. Abhyankar N., *et al*. "Use of testicular versus ejaculated sperm for intracytoplasmic sperm injection among men with cryptozoospermia: a meta-analysis". *Fertility and Sterility* 105 (2016): 1469-1475.

22. Zini A., *et al*. "Sperm DNA damage is associated with an increased risk of pregnancy loss after IVF and ICSI: systematic review and meta-analysis". *Human Reproduction* 23 (2008): 2663-2668.

23. Suganuma R., *et al*. "Decline in fertility of Mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI". *Human Reproduction* 20 (2005): 3101-3108.

24. Matas C., *et al*. "Effects of centrifugation through three different discontinuous Percoll gradients on boar sperm function". *Animal Reproduction Science* 127 (2011): 62-72.

25. Swanton A., *et al*. "Azoospermia: is sample centrifugation indicated? A national survey of practice and the Oxford experience". *Fertility and Sterility* 88 (2007): 374-378.

26. Moskovtsev SI., *et al*. "Testicular spermatozoa have statistically significantly lower DNA damage compared with ejaculated spermatozoa in patients with unsuccessful oral antioxidant treatment". *Fertility and Sterility* 93 (2010): 1142-1146.

27. Practice Committee of the American Society for Reproductive Medicine. "The clinical utility of sperm DNA integrity testing: a guideline". *Fertility and Sterility* 99 (2013): 673-677.

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