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Comparative analysis of chromosomal abnormalities in embryos from TESE-derived sperm versus naturally ejaculated sperm in assisted reproductive technologies

Abstract

A major obstacle to achieving pregnancy in assisted reproductive technology is high sperm DNA fragmentation (SDF) which results in embryos with chromosomal abnormalities. Although sperm retrieved by testicular sperm extraction (TESE) has low SDF as compared to the ejaculated sperm, there is little data comparing the prevalence of chromosomal abnormalities in the resulting embryos. The objective of this study was to compare rates of chromosomal abnormalities in embryos generated either by TESE or ejaculated sperm with increased SDF. A prospective cohort study on 400 embryos was carried out. The preimplantation genetic testing for aneuploidy (PGT-A) was achieved by next-generation sequencing (NGS). The sperm DNA fragmentation was measured by Halosperm G2 assay. Embryos were cultured to the blastocyst stage. The trophectoderm biopsies were performed on day-5. The rates of euploid, aneuploid and mosaic embryos were compared by multivariate logistic regression and mixed-effects models that accounted for female age and ovarian reserve.

The TESE-derived embryos showed significantly higher percentage of euploid embryos (67.8%) as compared to those derived from ejaculated sperm with high SDF (48.2%, p = 0.003). The multivariate logistic regression indicated that the sperm source (TESE / ejaculate) was an independent predictor of euploid embryos [OR = 1.85; 95% CI = 1.05 - 3.26; p = 0.034]. The probability of having euploid embryos decreased by 6% for every 1% increase in the SDF. The increased age of female was a major negative predictor [OR = 0.92 per year; 95% CI = 0.88 - 96; p = 0.001]. The results of this study showed that extraction of testicular sperm presented statistically significant benefit in the creation of euploid embryos over ejaculated sperm with high SDF. These results warrant the use of TESE as a clinical treatment in high SDF cases to increase the success of IVF and decrease embryo wastage.

Keywords: TESE, DNA fragmentation, preimplantation genetic testing, aneuploidy, embryo quality, male infertility, assisted reproduction, ejaculated sperm

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Introduction

Male infertility is one of the serious worldwide reproductive health problems, as this type of infertility is observed in about 50 percent of all infertile spouses, and about 7 percent among all men.[1,2] Male infertility etiology is multifactorial that involves genetic issues, hormonal imbalances, anatomical barriers, lifestyle, and environmental factors.[3] Diagnosis should be based on history, physical examination, semen analysis, hormonal analysis, imaging and genetic testing.[2] The sperm DNA fragmentation (SDF) is one of the molecular pathways of male factor infertility that can be used as a critical determinant of assisted reproductive technology (ART) outcomes. Higher levels of SDF are linked with lower fertilization, a disrupted embryo growth, high levels of miscarriages, and poor live births, despite the regularity of conventional semen parameters.[4,5] The negative association with the number of chromosomally normal embryos highlights the clinical importance of SDF especially when SDF is greater than 30%.[5]

The sperm DNA re-organizes radically during spermiogenesis, with a density increase of about sixfold relative to somatic nuclei due to substitution of histones with protamines.[6] This restructuring results in very compact, toroidal DNA structures that are needed to accommodate the mammalian genome into the small sperm nucleus and allows it to be safeguarded against mechanical and chemical bombardments.[7] DNA is bound by the protamine 1 (P1) and protamine 2 (P2) proteins via arginine-rich domains in the shape of doughnuts of about 50 kilobases each.[7] In addition to structural organization, sperm cells possess intricate epigenetic reminiscences such as DNA methylation signals, histone retention signals and non-coding RNA that jointly control the early embryonic gene expression.[8] Disturbances in these epigenetic characteristics related to the high paternal age, environmental exposure, and oxidative stress were found to be associated with developmental changes in the embryo and poor offspring phenotype.[8] Sperm mitochondria are another important element of reproductive potential that generate adenosine triphosphate (ATP) molecules needed to provide motility, capacitance and acrosomal reaction.[9] Mitochondrial dysplasia is linked with low motility of sperm, excessive DNA damage and poor embryo development, which directly leads to infertility in males.[9,10]

Sperm DNA fragmentation is caused by several mechanisms, such as oxidative stress, apoptosis, age-related alteration and environmental exposure.[11] SDF is specific to ejaculated sperm since it is transferred through the epididymis and male reproductive tract and undergoes oxidative stress, apoptotic signals, and centrifugal processes of forces that cluster DNA damage.[12,13] The effects of high SDF are not only limited to impairment of fertilization. There is a direct correlation

between high SDF and more chromosomal abnormalities in resultant embryos, especially the aneuploidies of a paternally origin.[14] This is because defective sperm DNA, due to improper repair through oocyte repair mechanisms, will cause mitotic errors in the initial embryonic divisions and eventually leads to the production of aneuploid embryos with low implantation potential and high susceptibility to miscarriage.[14,15] The testicular sperm extraction (TESE) and its forms (percutaneous TESA, standard TESE, microsurgical micro-TESE) are surgical methods to extract sperm directly out of testicular tissue without involving the epididymis and ejaculatory ducts.[16] The method is especially effective in men with azoospermia and with extreme SDF, because the testicular sperm normally undergoes much less DNA fragmentation than ejaculated sperm of the same male.[13,17] The Figure 1 shows male reproductive system components and different ways the sperm can be obtained.

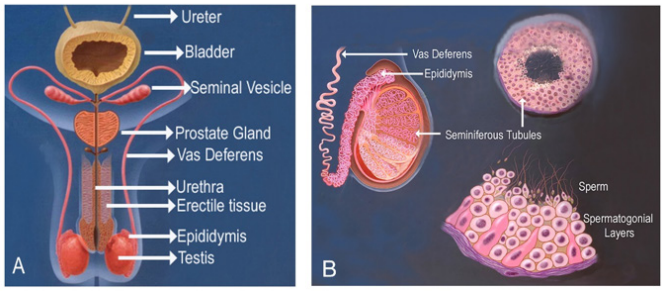

Figure 1 (A) Male reproductive system components. The sperm can be obtained by ejaculation, aspiration from testis/epididymis, or extraction from the testes. (B) Microscopic representation of testicular tubules, epididymis, vas deference and spermatogenesis.

The lesser fragmentation of TESE-derived sperm is indicative of the biological fact that testicular sperm are not subjected to the oxidative insults, apoptotic cues and mechanical damage that ejaculated sperm undergo during epididymal transit and ejaculation.[12] Direct comparative studies of paired samples showed that the amount of DNA damage in testicular sperm is significantly lower than that of ejaculated sperm, especially in men with a high SDF.[17] The result of TESE-ICSI cycles demonstrates good outcomes with fertilization rate of 62 - 68% and live birth rates per embryo transfer of 25 - 29% which is comparable to results using other sources of sperm.[18] The correlation between lowered testicular sperm DNA fragmentation and the consequent embryonic chromosome integrity is, however, not wholly characterized in controlled comparative studies. Even though substantial literature has been written about SDF effects on fertility as well as TESE done to retrieve sperm, there is a large research gap in the direct comparative study of embryonic genetic abnormalities of TESE-derived and ejaculated sperm. Although TESE-derived sperm exhibits less DNA fragmentation by definition, direct empirical studies that have directly attributed this biological capability to a lower rate of embryonic aneuploidy are scarce.[5,19] The available literature on TESE-derived sperm is biased towards retrieval success rates, fertilization rates, and overall pregnancy/live birth outcomes in non-obstructive azoospermia cases,[19,20] and has little embryo-level chromosomal profiling information. On the other hand, numerous studies have been done on naturally ejaculated sperm showing that high SDF, advanced age of a male, low semen quality, and high embryo aneuploidy rates are associated with high SDF[5,21] although not directly compared to TESE sperm in similar study populations.[5] This research aimed at filling this research gap by offering a systematic, controlled comparative analysis of embryonic chromosomal integrity between these two sperm sources; TESE and ejaculated with varying levels of sperm DNA fragmentation. The specific objectives of this study were: to determine differences in the euploidy rates of embryos derived from TESE and ejaculated sperm with SDF>30%; to predict development of euploid embryos after controlling for female age, oocyte quality, and ovarian reserve; to compare embryo morphology between TESE and ejaculated sperm derived embryos; and to determine implantation, pregnancy and live birth rates after single euploid embryo transfer.

Materials and methods

This was a multicenter prospective cohort study at 2 branches (Riyadh and Makkah) of Thuriah Medical Center. We studied 400 ICSI embryos: 200 from TESE and 200 ejaculated sperm derived. The inclusion criteria for TESE derived embryos were male with obstructive azoospermia; partner age between 18 and 45 years; normal ovarian reserve; and first or second ICSI cycle. The inclusion criteria for ejaculated sperm group were SDF > 30%; partners age between 18 - 45 years; normal ovarian reserve; and first or second ICSI cycle. In both groups, patients with history of recurrent miscarriages, and female factor infertility were excluded.

The TESE was performed on site under local anesthesia. The extracted tissues were subjected to mechanical and enzyme treatments to isolate viable sperm. To obtain a clean sperm population, the tissues and suspension were processed by density gradient technique. The ejaculated sperm cells were obtained by masturbation after 3 to 5 days of sexual abstinence. The density gradient technique was used to obtain motile sperm. The SDF was determined by Halosperm G2 assay. Each sample was evaluated in triplicate, and the average value was used for statistical analysis.

The Ovarian stimulation, oocyte collection, denudation, ICSI, and embryo culture, and embryo biopsy were performed per standard protocols in both groups. The blastocysts were graded per the Gardner scoring system and biopsies were performed on day-5. The biopsied cells were lysed and put into polymerase chain reaction (PCR) tubes, which were then taken to the genetics laboratory for PGT-A by NGS. The results were classified as: Euploid, normal chromosomal count (46, XX or 46, XY); aneuploid (monosomy, trisomy, polysomy); and Mosaic.

The demographic, clinical, and laboratory variables were entered into a standardized electronic database controlled by differing data of double-entry validation. Statistics were done using SPSS version 26.0 (IBM, Armonk, NY). Comparisons of baseline traits between groups were done using independent t-tests (continuous variables) and chi-square tests (categorical variables). Chi-square test was used to compare groups in terms of euploid rates. With the help of exact binomial techniques, ninety-five percent confidence intervals were obtained. The mixed-effects logistic regression was developed to assess the independent impact of sperm source on probability of producing euploid embryos, conditions on female age, and measures of ovarian reserve. Likewise, the study center had a random effect to explain the center level variation. The Pearson correlation coefficients were estimated to assess the inter-relationships between important variables (SDF, aneuploidy rate, female age, oocyte parameters). The statistical significance was set at $p < 0.05$ (two tailed).

This research study was approved by the Thuriah Medical Centre's institutional review board. Each of the participants gave written informed consent following extensive information on study goals, procedures, risks, and benefits. The information was de-identified and stored in safe password-coded databases. The results of genetic testing were relayed to the participant using the laid down counseling procedures.

Results

Table 1 shows the baseline demographic and clinical characteristics of groups. The TESE and the ejaculated sperm groups consisted of 100 couples and 200 embryos each. Non-significant differences were observed between groups regarding baseline demographic characteristics, indicating adequate group matching. Table 2 shows sperm DNA fragmentation percentages. The ejaculated sperm group had significantly higher DNA fragmentation (39.7 ± 8.3 %) as compared to the TESE sperm group (18.4 ± 6.2 %), p<0.001).

Table 1 Demographic and clinical characteristics of the study groups

Characteristic	TESE group (n=100)	Control group (n=100)	P-value*
Maternal age (years)	32.4 ± 4.2	33.1 ± 4.8	0.28
Paternal age (years)	38.7 ± 5.1	39.2 ± 5.6	0.42
BMI maternal (kg/m²)	24.6 ± 3.2	24.2 ± 3.5	0.48
BMI paternal (kg/m²)	26.8 ± 3.9	27.1 ± 4.2	0.62
Duration of infertility (years)	4.2 ± 2.1	4.5 ± 2.3	0.35
Cycle number	1.3 ± 0.5	1.4 ± 0.6	0.41
Basal FSH (mIU/mL)	6.8 ± 1.9	6.5 ± 1.7	0.31
Anti-Müllerian hormone (ng/mL)	3.2 ± 1.5	3.4 ± 1.6	0.42
Antral follicle count	12.4 ± 3.1	12.7 ± 3.3	0.51

*Significant at p<0.05

Table 2 DNA Fragmentation Index (DFI) comparison between groups

Parameter	TESE group	Ejaculated sperm group	P-value*
DFI mean (%)	18.4 ± 6.2	39.7 ± 8.3	<0.001
DFI range (%)	8–28	30–62	—
DFI SD	6.2	8.3	—

*Significant at p<0.05

Table 3 shows ICSI cycle outcomes and embryo development in both groups. The number of mature oocytes, fertilization rates, and number of mosaic embryos were similar in both groups. However, day-3 cleavage rate, blastocyst rate, and number of euploid embryos were higher in TESE group, whereas the number of aneuploid embryos were higher in ejaculated sperm group. The most common abnormality in embryos of both groups was chromosome 16 aneuploidy. The second and third most common abnormalities were found for chromosomes 21 and 22, respectively (Table 4).

Table 3 Cycle outcomes and embryo development in study groups

Parameter	TESE sperm	Ejaculated sperm	P-value*
Mature oocytes (MII) retrieved	11.2 ± 3.4	10.8 ± 3.2	0.38
Fertilization rate (%)	64.2 ± 12.1	61.8 ± 13.5	0.28
Day 3 cleavage rate (%)	78.6 ± 8.9	75.3 ± 10.2	0.09
Blastocyst formation rate (%)	58.4 ± 14.3	47.2 ± 15.8	0.001
Embryos reaching blastocyst stage	6.5 ± 2.1	5.1 ± 2.3	0.003
Euploid embryos	135 (67.8%)	96 (48.2%)	0.003
Aneuploid embryos	52 (26.0%)	89 (44.5%)	0.001
Mosaic embryos	13 (6.5%)	15 (7.5%)	0.63

*Significant at p<0.05

Table 4 Distribution of chromosomal abnormalities in aneuploid embryos

Chromosome	TESE sperm (n=52)	Ejaculated sperm (n=89)	Frequency
Chromosome 16	8 (15.4%)	18 (20.2%)	Most common
Chromosome 21	6 (11.5%)	14 (15.7%)	2nd most
Chromosome 22	5 (9.6%)	11 (12.4%)	3rd most
Chromosomes 13, 18	4 (7.7%)	9 (10.1%)	—
Sex chromosomes (X,Y)	7 (13.5%)	12 (13.5%)	—
Other autosomes	12 (23.1%)	15 (16.9%)	—
Polyploidy	4 (7.7%)	10 (11.2%)	—

Embryo genetic stability index (EGSI) is an indicator of the embryo's genetics stability. We suggest the new Embryo Genetics Stability Index (EGSI) a hierarchical classification of embryos to four levels of genomic stability based on NGS-PGT-A. This index is a combination of euploidy, severity of ploidy, mosaic threshold and chaotic arrangement, which is uniquely coupled with paternal SDF in our groups.

EGSI-1 includes euploid embryos which have high stability, have **n**ormal 46 XX / XY, and are transferable. The **EGSI-2 consists of aneuploid embryos which has low genetic stability and includes** one or several abnormalities like monosomy, trisomy, or polysomy. Such embryos are non-viable. The EGSI-3 includes mosaic embryos which have moderate Stability, have less than 50% abnormal cells, and are transferable based on the chromosomes involved. The EGSI-4 consists of chaotic embryos which are not stable, have >50% abnormal cells, or have polyploidy. Such embryos are not transferable. The distribution of embryos based on this classification is given in Table 5. The highest number of euploid embryos was observed from TESE-sperm.

Table 5 Distribution of embryos according to the embryo genetic stability index

EGSI category	Description	TESE-sperm (n=200)	Ejaculated sperm (n=200)	P-value * (Chi-square)
Euploid (1)	Normal karyotype	135 (67.8%)	96 (48.2%)	0.003
Aneuploid (2)	1-3 chromosomal anomalies	42 (21.0%)	70 (35.0%)	0.001
Mosaic (3)	<50% abnormal cells	13 (6.5%)	15 (7.5%)	0.63
Chaotic (4)	>3 anomalies/ polyploidy/chaos	10 (5.0%)	19 (9.5%)	0.04

*Significant at p<0.05

Multivariate logistic regression analysis

The euploid embryo production was predicted against a mixed-effects logistic regression model to examine the independent predictors.

a) Sperm Source (TESE vs. Ejaculate): OR = 1.85 (95% CI: 1.05 - 3.26), p = 0.034)

b) DNA Fragmentation Index (per 1% change): OR = 0.94 (95% CI: 0.91- 0.98), p = 0.003).

c) Female Age (per year): OR = 0.92 (95% CI: 0.88 – 0.96), p = 0.001)

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d) Number of Oocytes Retrieved: OR = 1.08 (95% CI: 0.95 - 1.23), p = 0.29.

Ortmann | Ortmann: OR = 1.12 (95%-CI: 0.891.41), p = 0.34

Significant at $p < 0.05$.

The source of sperm was a predictive variable independent of the female age and ovarian reserve parameters in predicting the production of euploid embryos. A unit change of DFI led to odds decrease of 6 percent of getting euploid embryos. The maternal age was found to be a strong negative predictor whereby one extra year was found to reduce the probability of the euploid by 8%. Blastocyst morphology quality and chromosomal normality were found to have strong relationships. Gardner scored embryos in category A exhibited 63.6 percent euploidy rates, and in category C, 42.9 percent.

Discussion

This study provides strong indication that the source of sperm plays a significant role in determining the embryonic chromosomal integrity. The TESE-obtained sperm had higher number of euploid embryos (67.8%) than 48.2% in the ejaculated sperm group with high sperm DNA fragmentation (p=0.003). Sperm source was an independent predictor of production of euploid embryos after adjusting it in relation to the female age and ovarian reserve [OR=1.85; 95% CI=1.05 - 3.26; p=0.034].

The TESE group had better EGSI profiles, with OR=2.1 (95% CI: 1.2 - 3.7, p=0.01) of EGSI-1 (Euploid) to ejaculated sperm group in adjusted-regression, and it was independent of maternal age. The DFI. EGSI-4 (Chaotic) had been reduced by 50% in TESE (5.0 vs. 9.5) and low testicular SDF was directly connected to testicular genomic chaos. This new index helps in the interpretation of PGT-A, which predicts implantation in cases of high SDF. These results build on earlier results of differences in the SDF by directly associating them with the chromosomally competent embryos. This is a clinically significant improvement in learning about mechanisms of male factor infertility and optimizing ICSI treatment.

The chromosomes normality in TESE embryos is probably due to the lower level of genetic damage in testicular sperm. The testicular sperm avoids the epididymis and ejaculatory ducts and therefore the testicular sperm are less affected by oxidative stress, apoptotic cues, and mechanical damage resulting in damage to DNA of ejaculated sperm.[12,13,17] Upon the introduction of fragmented paternal DNA into the oocyte, endogenous mechanisms of repairing oocytes might fail to restore DNA damage completely and instead the damage is carried into early embryonic divisions. This induces chromosomal instability and aneuploidy by a variety of possible pathways, including a defective recovery of meiosis division, centrosome dysfunction and an abnormal formation of mitotic spindle.[14,22] These results provide a factual basis to reason the use of TESE sperm as a treatment option in the instances of infertility due to male factors where the indicators of azoospermia are not regarded as the typical cases of azoospermia. Men who have failed to correct high sperm DNA fragments following the improved lifestyle and medical optimization can consider using TESE - sperm to achieve better embryonic chromosomal competence, and consequently better ICSI success. The multivariate analysis shows a decline of 6% of euploid probability with 1% of increase of DFI measures the clinical value of DNA fragmentation on embryo quality. This justifies the need to identify and treat men with high SDF.

These findings agree with the existing literature that suggests the benefits of TESE in the context of reducing sperm DNA fragmentation.[13,17] Further, it offers direct relationship of SDF with the chromosomal integrity of resulting embryos. The difference between the rates of euploidy (67.8% vs. 48.2%) is a clinically significant difference that may be translated to significant improvement in cumulative live birth rate. These results provide evidence-based information on relationship between sperm DNA fragmentation and the chromosomal integrity of resulting embryos.

This study has some limitations. Each group included only 200 embryos and was conducted in hot climate of Saudi Arabia. Further studies are needed in larger groups of embryos and other environments to validate these results. The trophectoderm biopsies utilize only few cells from each embryo. Due to the presence of mosaicism in embryos, the euploidy rates need to be interpreted with caution.

Conclusion

The results of this study indicate that sperm retrieved from testicular aspiration produce embryos of significantly higher chromosomal integrity than those produced from ejaculated sperms with high DNA fragmentation. This results in improved IVF success rate and efficiency of embryo utilization. The sperm source is an independent predictor of production of euploid embryos after adjusting it in relation to the female age and ovarian reserve. The probability of having euploid embryos decreased by 6% for every 1% increase in the SDF. These results also provide evidence-based information for patient counselling in cases of high sperm DNA fragmentation in ejaculated sperm. The maternal age was found to be a strong negative predictor whereby one extra year was found to reduce the probability of the euploid by 8%.

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Conflicts of interest

Author has no conflicts of interest to declare.

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