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Research Article

DNA Integrity in Absolute Teratozoospermia Patients and its Impact on Assisted Reproductive Technology Outcome

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Abstract

Numerous studies showed that Sperm DNA Fragmentation (SDF) is inversely correlated to semen parameters and that high SDF is a major contributor to failure of conception. In the present study, the severity of Sperm DNA Fragmentation in absolute teratoozoospermia patients and its impact on ICSI outcome compared to males with normal semen was examined. A prospective study was performed including 36 absolute teratozoospermia and 30 controls. The mean of DNA Fragmentation Index (DFI) using Sperm Chromatin Dispersion (SCD) test in the absolute teratozoospermia was 42.9 ± 13.2, compared to 24.59 ± 6.9 for the controls. The percentage of patients with high DFI (>30) was higher in the absolute teratozoospermia group 78% than in the control group 30%. Moderate DFI (15-30%) was more common in controls 60% than in absolute teratozoospermia 22%. Normal DFI (≤ 15%) was completely absent in absolute teratozoospermia. The pregnancy rate following Intracytoplasmic Sperm Injection (ICSI) in the control group was 66.6% and 50% in the absolute teratozoospermia. The miscarriage rate was 26% in the control group compared to 53% in the absolute teratozoospermia. Live birth was significantly lower in absolute teratozoospermia compared to fertile men; 19% and 52% respectively. High levels of SDF are predominant in absolute teratozoospermia patients consequently affecting the outcome of ICSI.

Keywords: ICSI; Infertility; Sperm DNA fragmentation; Absolute teratozoospermia; Live birth.

Introduction

Male infertility accounts for 50% of infertility cases but since the beginning of Intracytoplasmic Sperm Injection (ICSI), the role of the sperm has been disregarded because ICSI offered a method of fertilization regardless of the severity of male factor

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infertility [1,2]. Research has shown that sperm DNA fragmentation (SDF) during spermatogenesis is inevitable [3]. Researchers have concluded that molecular factors play an important role in male fertility and that the standard semen analysis (count, motility, morphology) cannot be indicative enough of male reproductive competence [4].

Sperm DNA fragmentation (SDF) is a type of DNA damage that comprises the presence of breaks in the DNA backbone either as single or double strand lesions that are present in both fertile and sub fertile men [5]. Such damage was found to impair the normal function of sperm cells to complete successful fertilization or proceed with normal embryonic development. Research has found that high levels of SDF are significantly related to failure of achieving conception naturally or through assisted reproduction [6]. The proposed theories of SDF origin include apoptosis, DNA re-modeling, and reactive oxygen species (ROS), and a variety of factors can predispose patients to these events. These include varicocele, infectious diseases, obesity, diabetes mellitus, lifestyle risk factors and environmental pollutants [7,8]. The broadly agreed upon threshold is a DFI of 30% where samples of a higher DFI are associated with fertility problems [9]. In ICSI, sperm morphology is considered the single most critical feature indicating sperm fertilization potential [10]. Research has found that SDF correlates negatively with conventional semen parameters including sperm morphology [11]. Accordingly, the objective of the following study was to investigate the role of SDF in a specific group of patients; absolute teratozoospermia, which are characterized by complete absence of morphologically normal sperm. Polymorphic teratozoospermia can be caused by gene dysregulation, oxidative stress, failure of DNA repair, xenobiotics, endocrine disruptors, and varicocele [12]. It is widely accepted that the first line of treatment for absolute teratozoospermia is ICSI. However, limited number of studies have focused solely on this group of patients, yielding controversial opinions and to date, information on live birth rates is scarce [13].

Two studies on absolute teratoozoospermia concluded that, there was no difference in outcomes between the absolute teratozoospermia patients and patients who had some morphologically normal sperm [14, 15]. Pereira and colleagues, who described a high live births rate 38.8% for absolute teratozoospermia patients after ICSI, used donor oocytes [15]. By definition, donor oocytes are from women in their 20s or early 30s are likely capable of more extensive DNA repair. In contrast, the study by Braham and colleagues reported a 10% pregnancy rate after ICSI for couples with absolute teratozoospermia and live birth was not documented [17]. However, a study from a different perspective by Kovac demonstrated that 29.2% of men with 100% abnormal sperm structure conceived without assisted reproduction over a period of 2.5 years [18]. The relationship between SDF in absolute teratozoospermia and live births rate was not evaluated in previous studies [15,16]. The following study aims to measure the live births rate after ICSI in absolute teratozoospermia patients in comparison to men with normal semen samples. Additionally, SDF testing was performed for all participants and participants were

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subdivided by SDF level; normal SDF (below 15%), moderate SDF (15-30%), or high SDF (above 30%) [9]. It was hypothesized that absolute teratozoospermia patients would have high levels of SDF and lower ICSI outcomes, including pregnancy rate and live births when compared to men having sperm with normal structure.

Materials and methods

A prospective study was carried out at Dr. Faris Medical center in Cairo, Egypt. Participants were recruited among patients seeking ICSI after experiencing at least one year of infertility. The American University in Cairo's (AUC) Institutional Review Board (IRB) approved all procedures. Written informed consents were obtained from all participants. Data including medical history, lifestyle habits, occupation, age, infertility duration and infertility type (primary or secondary) were obtained from couples on their first visit. Couples were excluded from the study if the male's age was above 45 years if the female's age was over 35 years and in case of a female infertility disorders. The exclusion criteria also included a history of exposure to chemotherapy or radiation, cryptorchidism, globozoospermia, chronic diseases and harmful exposures. Couples seeking ICSI with frozen sperm samples or testicular sperm were not included in the study. The study group (n=42) included couples where the male partner was diagnosed with absolute teratozoospermia (0% normal forms) according to the Kruger strict criteria on two separate visits. The control group (n=30) included couples where the male partners had normal semen parameters, including ≥ 4% normal morphology, according to the 2010 WHO manual.

Conventional semen analysis

Semen samples were collected after three days of abstinence. Semen evaluation was performed in accordance with the WHO 2010 guidelines; it was evaluated for the overall number of sperm cells per mL, sperm motility, percentage of progressive (A+B) motility, and sperm morphology. Samples were considered normal if they had ≥ 39 million spermatozoa per mL, ≥ 40% motile sperm of which ≥ 32% had progressive motility and ≥ 4% normal morphology. Samples were classified as absolute teratozoospermia if 0% of the spermatozoa had normal morphology.

Sperm morphology assessment

Sperm morphology was evaluated in real time using Motile Sperm Organelle Morphology (MSOME) technology. Two hundred sperm cells per sample were examined under MSOME. One mL of the semen sample was placed in a 5mL microdroplet of Earl's Balanced Salt Solution (EBSS) (Thermo Fisher, Massachu-setts, United States). The microdroplet was viewed under inverted microscopy with high-powered Nomarski optics (x6600 magnification) (Leica, DMI 3000B, Germany and Hamilton Thorne, Beverly, Massa-chusetts, USA).

Sperm preparation

Sperm samples were prepared using the GM501 density gradient (Gynemed, Lensahn, Germany) following the manufacturer's instructions. The gradient medium was brought to room temperature, and 2.5 mL of

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the 90% medium was placed under the 45% solution.

The semen sample was dropped onto the top of the gradient medium. Centrifugation was performed at 400g for 15 minutes, and then the supernatant was discarded, and the sperm pellets were re-suspended in sperm preparation media (Origio Sperm Wash, Malov, Denmark).

Sperm DNA fragmentation testing (Halo Test)

Processed sperm were tested with the Halo sperm test kit (INDAS Labs, Madrid, Spain) according to the manufacturer's manual. An amount of 50 µL of the sample was pipetted into an Eppendorf tube (EPT) containing liquefied agarose gel. A drop of 8µL of the mixture was transferred to the center of the well on the provided slides. The slides were stored at 4 °C so that the agarose could solidify, and the denaturant agent was applied. The slide was left for 7 minutes for denaturation to take place. The lysis solution was applied to the slide and left for 20 minutes in order to remove most of the nuclear protein. Ethanol (100%) was applied for two minutes to dehydrate the slide. Staining solution, A was applied and kept for seven minutes, followed by staining solution B. The slides were left to air dry for 15 minutes. The samples were observed under bright field microscopy (x100 objective), and 300 spermatozoa per sample were examined. Degraded sperm, sperm with no halo, and sperm with a small halo were counted as having fragmented DNA, whereas sperm with a large or medium-sized halo were counted as not having DNA fragmentation. The DNA fragmentation index (DFI) was calculated as follows: Total (fragmented+ degraded) sperm cells ÷ Total counted sperm cells × 100 [9].

Ovarian stimulation and ICSI

For ovarian stimulation, all female patients received the short protocol. The standard ICSI procedure was carried out as follows: oocyte pickup (OPU) using vaginal ultrasound guided follicle aspiration and removal of cumulus cells. ICSI was performed after 3-4 hours of OPU. Fertilization was checked 18 hours post ICSI followed by Day 3 embryo grading and continuation of extended culture. Two Blastocysts were transferred in each case on Day 5. Ultrasound-guided embryo transfer was performed using a soft catheter (Labotect, Rosdorf, Germany). Clinical Follow-Up involved Human chorionic gonadotropin (hCG) analysis 12 days following embryo transfer. A transvaginal ultrasound scan was performed five weeks after the transfer, and a clinical pregnancy was identified if at least one gestational sac was present, and a fetal heartbeat was detected. Miscarriages, ongoing pregnancies, and live births were documented.

Statistical methods

Statistical analysis was performed with Statistical Package for the Social Sciences (SPSS) software (SPSS Inc., Chicago, USA), version 20.0. Results were stated as mean (SD) or N (%) when appropriate. The student-t-test was used in assessing the significance of the differences in means in the clinical parametric data between the two groups (patients and controls); however, when more than 2 groups were found, AVOVA test was used. Pearson's chi-squared test was implemented to identify whether there is a

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statistically significant difference between the expected frequencies and the observed nominal data. The Spearman's rank correlation coefficient (r) was measured to describe the linear relationship between each two continuous values. Significance was defined as P <0.05.

Results

Motile sperm organelle morphology examination (MSOME)

Patients (n=42) underwent MSOME testing to confirm the diagnosis of absolute teratozoospermia. MSOME testing confirmed the absolute teratzoospermia diagnosis in 36 patients. The remaining 6 (14.3%) had MSOME results showing 1% normal morphology and were diagnosed with severe teratozoospermia and excluded from the study.

Demographic characteristics of the control group and the absolute teratozoospermia patients

The average age of male participants (control group 33 ± 3.7 and patients 33.55 ± 4.3 years) was not significantly different between the absolute teratozoospermia patients and the control group. The average age of female partners (control group 29.27 ± 2.95 and patients 29 ± 3.74 years) was also not significantly different between the two groups.

Semen characteristics of the control group and absolute teratozoospermia patients

Table 1 compares the semen analysis of the control group to the absolute teratozoospermia patients. A strong significant difference (p=0.0001) was shown between the two groups in the average number of sperms. The average sperm count was 80.97 ± 27.5 (millions/mL) for the control group, compared to 23.06 ± 24 (millions/mL) for the absolute teratozoospermia patients. The difference in sperm motility (p=0.0001) was highly significant between the absolute teratozoospermia and men with normal semen samples. For the control group, it was (56.5%), and for the absolute teratozoospermia patients, it was (25%). The progressive motility (A+B) was higher in the control group (42%) than in the absolute teratozoospermia group (9%), which represents a highly significant difference (p=0.0001). The difference in percentages of fragmented sperm, degraded sperm, and DFI also showed a highly significant difference (p=0.0001) between the two groups. The mean of the cumulative DFI in the absolute teratozoospermia patients was 42.9 ± 13.2, compared to 24.59 ± 6.9 for the control group. Regarding DFI levels, there was a highly significant difference (p=0.0001) apparent between the two groups as illustrated in Figure 1.

The number of patients with high DFI (>30) was higher in the absolute teratozoospermia group (78%) than in the control group (30%). Moderate DFI (15-30%) was more prevalent in the fertile men (60%) than in the absolute teratozoospermia group (22%). None of the patients in the absolute teratozoospermia group had a normal DFI (≤ 15%) compared to (10%) from the control group.

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Parameters		Controls (N=30)	Patients (N=36)	p-value
Count (million/ml)	Mean (SD)	80.967(27.5)	23.056(24)	0.0001
Motility %	Mean (SD)	56.5% (8.8)	25% (13.8)	0.0001
PR (A+B)	Mean (SD)	42% (9.6)	9% (8.5)	0.0001
Abnormal Forms %	Mean (SD)	89.23(4.35)	100% (0)	0.0001
Fragmented %	Mean (SD)	19.45(6)	32.24(10.3)	0.0001
Degraded %	Mean (SD)	5.14(2.33)	10.38(5.4)	0.0001
	Mean (SD)	24.587(6.9)	42.9(13.2)	0.0001
DFI	Normal: ≤15	3(10%)	0	
	Moderate: 15-30	18(60%)	8(22%)	0.0001
	High:>30	9(30%)	28(78%)	0.0001

Table 1: Semen parameters of fertile men and absolute teratozoospermia patients.

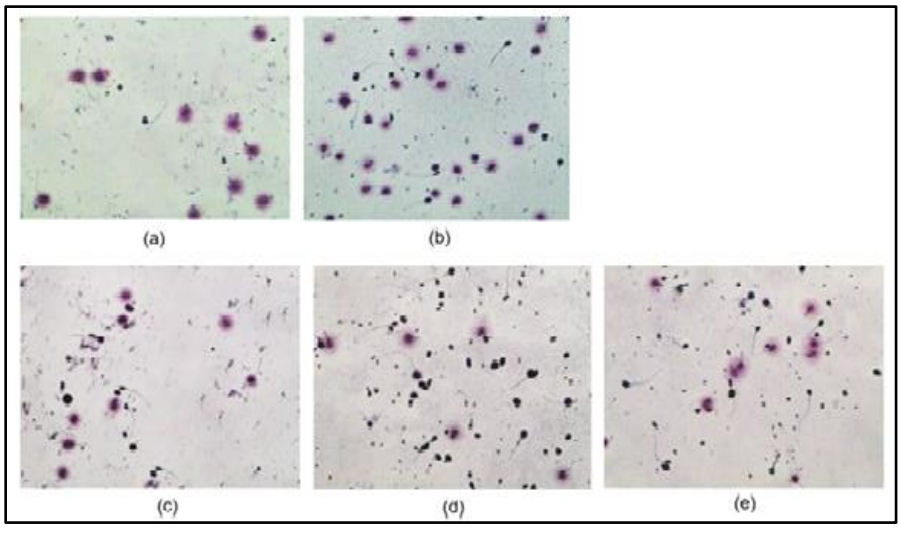

Figure 1: Sperm chromatin dispersion (SCD) test results (a) and (b) of fertile men and (c), (d) and (e) of absolute teratozoospermia patients. A big or medium size halo indicated normal chromatin while a small halo or absent halo showed DNA fragmentation.

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Outcomes		Controls (N=30)	Patients (N=30)	p-value
Fertilization	Mean (SD)	10.13(5.7)	10.53(6.2)	0.795
Cleavage	Mean (SD)	9.73(5.4)	9.6(6)	0.941
Embryo Grade A	Mean (SD)	3.5(2.4)	3.5(3.2)	1
Embryo Grade B	Mean (SD)	3.2(2.2)	2.78(2.4)	0.477
Blastocyst	Mean (SD)	7.7(3.9)	7.25(4.6)	0.677
Pregnancy	Positive	20(66.67%)	15(50%)	0.027*
	Negative	10(33.33%)	15(50%)	
Miscarriage (% from pregnant cases only)	Absent	14(74%)	7(47%)	0.04*
	Present	5(26%)	8(53%)	
Live Birth (of total recruited cases)	Absent	15(48%)	26(81%)	0.014*
	Present	14(52%)	6(19%)	

Table 2: Comparison of ICSI outcome between the controls and Absolute teratozoospermia.

No significance was obtained between the two groups in fertilization, cleavage, embryos grades (A and B) and blastocysts as represented in Table 2. Two couples from the absolute teratozoospermia patients did not receive an embryo transfer: one due to fertilization failure and the other due to cleavage arrest. A statistical significance was shown between the two groups in pregnancy rate (p=0.027). The pregnancy percentage in the fertile group was (66.6%) and (50%) in the absolute teratozoospermia group. Miscarriage was diagnosed either by a decreased serum hCG test performed 48 hours after the initial positive test or by a transvaginal ultrasound showed that there was no embryonic cardiac activity. The results revealed a significant difference (p=0.04) in miscarriage rate between the control group (26%) and the absolute teratozoospermia patients' group (53%). The live birth rate was significantly greater (p=0.014) in the control group (52%) in comparison to the absolute teratozoospermia group (19%). No complications in neonates were reported upon delivery but no further evaluation was performed beyond this stage.

Discussion

The aim of the following study was to assess SDF in absolute teratozoospermia patients in comparison to men with normal semen parameters. Prior assessment of SDF, MSOME was used for diagnosis of absolute teratozoospermia. The results demonstrate that using this technique for diagnosis of sperm morphology could be a promising solution to the problems discussed by Czubaszek and colleagues with regard to choosing the best staining procedure for sperm morphology assessment. The authors indicate that two samples taken from the same ejaculate but stained with different methods and observed under different

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magnification can yield vastly different assessments of the sperm structure. Furthermore, the preparation of slides for microscopic examination of sperm morphology requires great precision, and even minor errors can make the results even more difficult to interpret. They argue that there is no perfect method for sample preparation, and that results vary even regarding the degree to which the results of the various staining methods correlate with each other [20]. MSOME makes it possible to visualize the morphology of living spermatozoa in much more detail than lower levels of magnification of a fixed sample.

In the following study, semen analysis of the absolute teratozoospermia group revealed poorer results in comparison to the control group, with a statistically significant difference in virtually every characteristic measured. The absolute teratozoospermia patients had, on average, a lower number of sperm, a lower rate of motility and progressive motility (A+B) than the men in the control group. The results of this study corroborate previous findings, which show that teratozoospermia tends to occur in tandem with other abnormal semen parameters [21,22]. These results also confirm the findings of Elbashir and colleagues, namely that patients with higher SDF tend to have lower motility, since the same suggested mechanisms that cause sperm chromatin damage, namely abortive apoptosis, and oxidative stress, also impair sperm motility [23]. DNA fragmentation is a feature of spermatozoa that has begun to undergo apoptosis, but the apoptotic pathway is terminated, hence given the name abortive apoptosis [5]. During spermatogenesis, apoptosis maintains equilibrium between the number of germ cells and the number of Sertoli cells by removing defective sperm cells, acting like a “quality control” system [6]. Over production of ROS surpasses the antioxidant capacity of cells eventually leading to oxidative stress which induces damage in sperm structure, function, and DNA integrity [24]. There are two mechanisms by which ROS is produced in a sperm cell: physiological and pathological mechanisms [5]. Normally, sperm cells require small amounts of ROS to regulate cellular functions such as acrosome reaction, capacitation and oocyte fusion thus described as physiological mechanisms of ROS production. According to Henkel and colleagues, sperm cells exhibiting abnormal morphology are also a source of high levels of ROS and subsequently damage their DNA [6]. Furthermore, a state of oxidative stress via pathological mechanisms of ROS production is regarded toxic to the sperm cell due to the high-unsaturated fatty acids forming the plasma membrane that makes sperms highly vulnerable [7]. ROS disrupts the plasma membrane and has a direct impact on sperm motility [10]. ROS plays a key role in DNA damage and previous research has shown that between 20 and 88% of sub fertile men have elevated ROS in their semen [2].

The absolute teratozoospermia patients showed significantly higher levels of DFI showing an average of 42.9% compared to 24.6% in the control group, which has a negative impact on fertilization, division, and implantation. This data is consistent with a recent study on absolute teratozoospermia, which showed an average DFI of 45% [24]. One of the key findings in the study is that the

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majority, 78% of absolute teratozoospermia patients carry DFI higher than 30% and none had normal DFI level. This study also confirmed previous research indicating that high SDF can occur in men with normal semen parameters as measured by conventional analysis [8,26]. This was shown in the control group where 30% of the participants exhibited high DFI while three men only showed normal DFI levels. Based on conventional semen analysis alone, their semen analysis showed normal parameters, and yet they had sought treatment for infertility. This indicates, as Esteves and Agarwal have suggested, that high SDF is the explanation for many cases of unexplained infertility [25].

Although literature on the clinical outcome of absolute teratozoospermia following ICSI is limited, controversial findings were previously reported on the impact of abnormal morphology on ICSI outcome including fertilization, cleavage, embryo quality and blastocyst. Some studies reported results similar to normal sperm outcome, provided that the injected spermatozoon is viable, and others concluded a poor outcome following ICSI with abnormal sperm morphology [26-28]. In the present study, no difference was shown in fertilization, number of cleaved embryos, embryo quality and number of blastocysts between the control group and the absolute teratozoospermia group. Meanwhile, Kim and colleagues noted that DFI is inversely correlated with embryo quality [13]. However, Henkel and colleagues concluded that a sperm cell with DNA damage can fertilize an oocyte and that oocytes can compensate for this damage and are also capable of producing good quality embryos [6]. One of the cases in the absolute teratozoospermia group had total fertilization failure, which is a common finding in absolute teratozoospermia [16]. It has been established that about 5% or less ICSI cycles can result in a complete failure of fertilization, and according to Neri and colleagues, this usually occurs because of an asynchrony in the development of the nucleus and the cytoplasm or because of sperm cytosolic factors [28]. Another patient in the absolute teratozoospermia group who had 65% DFI was able to fertilize 6 out of 10 oocytes, but no embryos progressed to division beyond the zygote stage. Induction of the apoptotic pathway due to late paternal effect, resulting in embryo arrest, is a possible explanation for why men whose DFI is severely high, could experience complete embryo arrest [29,30].

The present study found that some measures of ICSI outcome were worse to a statistically significant degree for the absolute teratozoospermia patients than for men with normal semen. Specifically, the absolute teratozoospermia group had a significantly lower pregnancy rate, greater miscarriage rate, and the number of live births were significantly less. The clinical pregnancy percentage for absolute teratozoospermia patients in this study was 50% while it was 66.7% in the control group. This outcome is in accordance with a study by French and colleagues who have reported a pregnancy rate of 60% in absolute teratozoospermia patients compared to 57% in males with normal semen [27]. In contrast, a study by Braham and colleagues found that the clinical pregnancy rate for absolute teratozoospermia is only 10% [17]. Since the paternal genome

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switches on at the early embryonic stage, researchers have hypothesized that SDF, which tends to be higher in teratozoospermia patients, would lead to low pregnancy rates and high miscarriage rates; they have also expressed concern that selecting spermatozoa with abnormal morphology for ICSI would lead to the formation of chromosomally abnormal embryos [25]. Studying embryo quality, blastocyst formation and even pregnancy rate is not sufficient to show the true effect of SDF on the eventual outcome of the embryo; for purposes of making clinical decisions, it is important to follow up until a live birth is achieved. Patients want to know whether ICSI will result in a healthy live birth, not simply whether it will lead to the transfer of an embryo that has a high possibility of miscarrying.

A statical significance was found between the control group and the absolute teratozoospermia group in the miscarriage rate. For couples in the control group, the miscarriage rate was 26%, while the absolute teratozoospermia couples had 53% rate of miscarriage. The results are in close agreement with a recent study by Haddock and colleagues who reported that SDF testing as a promising new biomarker for miscarriage [30]. Conversely, the results were not consistent with the study by French who reported a miscarriage rate of 4% in absolute teratozoospermia compared to 5% in men with normal semen samples [27]. The causes of miscarriage are notoriously difficult to pin down, making pregnancy loss a challenge in clinical practice of reproductive medicine. Bu and colleagues have identified many possible factors for miscarriage in IVF and ICSI pregnancies, including the age of the female partner, the endometrial measurements during embryo transfer, and whether the procedure is using fresh or frozen embryos [32]. The present study controlled for those factors, with all the women in the study being younger than 35, exclusion of female factor infertility and all embryo transfers performed using two blastocysts. This leaves DNA damage as a likely explanation for many of the miscarriages documented in this study. Likewise, according to Zafar and colleagues, the spermatozoon not only contributes its genome to the zygote, but it also contributes sperm-specific proteins that activate the oocyte [33]. The paternal contribution to oocyte activation could include the modification of free RNAs associated with gene expression, which could ultimately lead to implantation failure [34]. Likewise, according to Sedo and colleagues, sperm DNA damage activates the apoptotic pathway, leading to embryo arrest [30]. Robinson and colleagues describe this phenomenon as a "late paternal effect during the activation of male gene expression" [29].

The second aim of the study was to determine the live births rate in absolute teratozoospermia in comparison to normal sperm. A study by Tasdemir measured pregnancy rate in cases with abnormal sperm head morphology only. They noted an ongoing pregnancy rate of 6% and patients were not followed up to term [16]. The French study measured the live births rate after ICSI in couples where the male partner had absolute teratozoospermia, but SDF was not assessed. The authors reported high rates of live birth (56%) [27]. The study showed that the live birth rate in absolute

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teratozoospermia was 19%, which is significantly lower than the live births rate in the normal semen group, which was 52%. One possible explanation of this is the prevalence of high SDF in the absolute teratozoospermia patients compared to the control group. The results agree with Siva Narayana and colleagues who reported clinical outcome following ICSI in two groups of patients (<30% and ≥ 30%) SDF, who found a highly significant difference in live birth rate between the two groups with lower results obtained from the high SDF group [36]. However, the study was based solely on SDF testing, it did not include absolute teratozoospermia patients and the mean SDF in the study group was 39%. The data suggest that absolute teratozoospermia is associated with high SDF that consequently impairs the outcome of ICSI. Researchers have investigated various ways to reduce the percentage of SDF in a sub fertile semen sample and, in cases where men have high levels of SDF, to select the spermatozoa with the least DNA damage for ART. Ways of reducing SDF include lifestyle changes, antioxidant therapy and shortening the abstinence period before SDF testing, and medical interventions such as hormonal therapy and antibiotics [22]. Perhaps the first and most manageable way to reduce SDF is to decrease exposure to external sources of oxidative stress and endocrine disruptors and this includes smoking cessation, following healthy eating habits (antioxidant rich foods), reducing alcohol consumption, weight management and following protective guidelines in case of exposure to occupational hazards or environmental toxins [25]. The data suggest that absolute teratozoospermia are candidates for SDF treatment prior enrollment in ICSI therapy in order to reduce SDF and achieve better outcomes.

Conclusion

The data obtained from this study underscore the significance of extensive sperm testing as a prerequisite for choosing the most appropriate treatment plan for ART patients. It showed that absolute teratozoospermia is associated with high SDF and that SDF adversely affects live birth rate. Although future large-scale research is needed to confirm this recommendation, this study provides further information on absolute teratozoospermia cases that may be beneficial in-patient counselling and management. Standard semen analysis supplements us with basic information regarding sperm characteristics such as count, motility, and structure but it does not reflect the capability of a sperm to fertilize an egg or in case fertilization occurs, its ability to proceed with developmental processes, and achieve a healthy live birth.

Limitation

A possible limitation of the study was its small sample size. For the present study, time constraints limited the number of couples recruited. Studies of a similar design that include a larger number of patients should be conducted in the near future.

References

1. Schulte RT, Ohl DA, Sigman M, Smith GD. Sperm DNA Damage in Male Infertility: Etiologies, Assays, and Outcomes. J Assist Reprod Genet. 2010;27(3):12. PubMed | CrossRef

Citation: Faris D, Eltaweel N, Fadel M, Faris M, Zada S. DNA Integrity in Absolute Teratozoospermia Patients and its Impact on Assisted Reproductive Technology Outcome. J Gynecol Clin Obstet Reprod Med. 2023;1(2):76-89.
DOI: https://doi.org/10.37191/Mapsci-JGCORM-1(2)-009

2. Cho CL, Agarwal A. Role of Sperm DNA Fragmentation in Male Factor Infertility: A Systematic Review. Arab J Urol. 2018;16(1):21-34. PubMed | CrossRef
3. Arafa M, Elbardisi H, Majzoub A, Agarwal A. Genetics of Male Infertility: A Case-Based Guide for Clinicians. Springer Nature; 2020;14.
4. Selvam MK, Agarwal A. A Systematic Review on Sperm DNA Fragmentation in Male Factor Infertility: Laboratory Assessment. Arab J Urol. 2018;16(1):65-76. PubMed | CrossRef
5. González-Marín C, Gosálvez J, Roy R. Types, Causes, Detection and Repair of DNA Fragmentation in Animal and Human Sperm Cells. Int J Mol Sci. 2012;13(11):14026-52. PubMed | CrossRef
6. Henkel RR, Franken DR. Sperm DNA Fragmentation: Origin and Impact on Human Reproduction. J Reprod Stem Cell Biotech. 2011;2(2):88-108.
7. Leach M, Aitken RJ, Sacks G. Sperm DNA Fragmentation Abnormalities in Men from Couples with a History of Recurrent Miscarriage. Aust N Z J Obstet Gynaecol. 2015;55(4):379-83. PubMed | CrossRef
8. Panner Selvam MK, Agarwal A. A Systematic Review on Sperm DNA Fragmentation in Male Factor Infertility: Laboratory Assessment. Arab J Urol. 2018;16(1):65-76.
9. Yang H, Li G, Jin H, Guo Y, Sun Y. The Effect of Sperm DNA Fragmentation Index on Assisted Reproductive Technology Outcomes and its Relationship with Semen Parameters and Lifestyle. Transl Androl Urol. 2019;8(4):356. PubMed | CrossRef
10. Gosálvez J, Migueles B, López-Fernández C, Sanchéz-Martín F, Sáchez-Martín P. Single Sperm Selection and DNA Fragmentation Analysis: The case of MSOME/IMSI.
11. Mehdi M, Khantouche L, Ajina M, Saad A. Detection of DNA Fragmentation in Human Spermatozoa: Correlation with Semen Parameters. Andrologia. 2009;41(6):383-6. PubMed | CrossRef
12. Oehninger S, Kruger TF. Sperm Morphology and its Disorders in the Context of Infertility. F&S Reviews. 2021;2(1):75-92.
13. Kim GY. What Should be Done for Men with Sperm DNA Fragmentation?. Clin Exp Reprod Med. 2018;45(3):101. PubMed | CrossRef
14. Gómez E, Pérez-Cano I, Amorocho B, Landeras J, Ballesteros A, Pellicer A. Effect of Injected Spermatozoa Morphology on the Outcome of Intracytoplasmic Sperm Injection in Humans. Fertil Steril. 2000;74(4):842-3. PubMed | CrossRef
15. Pereira N, Neri QV, Lekovich JP, Spandorfer SD, Palermo GD, Rosenwaks Z. Outcomes of Intracytoplasmic Sperm Injection Cycles for Complete Teratozoospermia: A Case-Control Study Using Paired Sibling Oocytes. Biomed Res Int. 2015. PubMed | CrossRef
16. Taşdemir I, Taşdemir M, Tavukçuoglu S, Kahraman S, Biberoğlu K. Effect of Abnormal Sperm Head Morphology on the Outcome of Intracytoplasmic Sperm Injection in Humans. Hum Reprod. 1997;12(6):1214-7. PubMed | CrossRef
17. Braham A, Ghedir H, Zidi I, Sallem A, Hajlaoui A, Ajina M, et al. Nuclear Sperm Quality in Total Polymorphic Teratozoospermia and its Impact on Intracytoplasmic Sperm Injection Outcome. Andrologia. 2019;51(5):13252. PubMed | CrossRef
18. Kovac JR, Smith RP, Cajipe M, Lamb DJ, Lipshultz LI. Men with a Complete Absence of Normal Sperm Morphology Exhibit High Rates of Success Without Assisted Reproduction. Asian J Androl. 2017;19(1):39. PubMed | CrossRef
19. Cariati F, D'Argenio V, Tomaiuolo R. The Evolving Role of Genetic Tests in Reproductive Medicine. J Transl Med. 2019;17:1-33. PubMed | CrossRef
20. Czubaszek M, Andraszek K, Banaszewska D, Walczak-Jędrzejowska R. The Effect of the Staining Technique on Morphological and Morphometric Parameters of Boar Sperm. PLoS One. 2019;14(3):0214243. PubMed | CrossRef
21. Iwamoto T, Nozawa S, Yoshiike M. Semen Quality of Asian Men. Reprod Med Biol. 2007;6(4):185-93. PubMed | CrossRef
22. Sabra SM, Al-Harbi MS. An Influential Relationship of Seminal Fluid Microbial Infections and Infertility, Taif Region, KSA. World J Med Sci. 2014;10(1):32-7.
23. Elbashir S, Magdi Y, Rashed A, Ibrahim MA, Edris Y, Abdelaziz AM. Relationship Between Sperm Progressive Motility and DNA Integrity in Fertile and Infertile Men. Middle East Fertil Soc J. 2018;23(3):195-8.

Citation: Faris D, Eltaweel N, Fadel M, Faris M, Zada S. DNA Integrity in Absolute Teratozoospermia Patients and its Impact on Assisted Reproductive Technology Outcome. J Gynecol Clin Obstet Reprod Med. 2023;1(2):76-89.
DOI: https://doi.org/10.37191/Mapsci-JGCORM-1(2)-009

24. Brahem S, Elghezal H, Ghédir H, Landolsi H, Amara A, Ibala S, et al. Cytogenetic and Molecular Aspects of Absolute Teratozoospermia: Comparison Between Polymorphic and Monomorphic Forms. Urology. 2011;78(6):1313-9. PubMed | CrossRef
25. Esteves SC. Clinical Relevance of Routine Semen Analysis and Controversies Surrounding the 2010 World Health Organization Criteria for Semen Examination. Int Off J Braz Soc Urol. 2014;40(4):443–453.
26. Le MT, Nguyen TA, Nguyen HT, Nguyen TT, Nguyen VT, Le DD, et al. Does Sperm DNA Fragmentation Correlate with Semen Parameters? Reprod Med Biol. 2019;18(4):390-6. PubMed | CrossRef
27. French DB, Sabanegh Jr ES, Goldfarb J, Desai N. Does Severe Teratozoospermia Affect Blastocyst Formation, Live Birth Rate, and Other Clinical Outcome Parameters in ICSI Cycles? Fertil Steril. 2010;93(4):1097-103. PubMed | CrossRef
28. Neri QV, Lee B, Rosenwaks Z, Machaca K, Palermo GD. Understanding Fertilization Through Intracytoplasmic Sperm Injection (ICSI). Cell calcium. 2014;55(1):24-37. PubMed | CrossRef
29. Robinson L, Gallos ID, Conner SJ, Rajkhowa M, Miller D, Lewis S, et al. The Effect of Sperm DNA Fragmentation on Miscarriage Rates: A Systematic Review and Meta-Analysis. Hum Reprod. 2012;27(10):2908-17. PubMed | CrossRef
30. Sedó CA, Bilinski M, Lorenzi D, Uriondo H, Noblía F, Longobucco V, et al. Effect of Sperm DNA Fragmentation on Embryo Development: Clinical and Biological Aspects. JBRA Assist Reprod. 2017;21(4):343. PubMed | CrossRef
31. Haddock L, Gordon S, Lewis SE, Larsen P, Shehata A, Shehata H. Sperm DNA Fragmentation is a Novel Biomarker for Early Pregnancy Loss. Reprod Biomed Online. 2021;42(1):175-84. PubMed | CrossRef
32. Bu Z, Hu L, Su Y, Guo Y, Zhai J, Sun YP. Factors Related to Early Spontaneous Miscarriage During IVF/ICSI Treatment: An Analysis of 21,485 Clinical Pregnancies. Reprod Biomed Online. 2020;40(2):201-6. PubMed | CrossRef
33. Ribas-Maynou J, Benet J. Single and Double Strand Sperm DNA Damage: Different Reproductive Effects on Male Fertility. Genes. 2019;10(2):105. PubMed | CrossRef
34. Zafar MI, Lu S, Li H. Sperm-Oocyte Interplay: An Overview of Spermatozoon's Role in Oocyte Activation and Current Perspectives in Diagnosis and Fertility Treatment. Cell Biosci. 2021;11(1):1-5. PubMed | CrossRef
35. Tang SS, Gao H, Zhao Y, Ma S. Aneuploidy and DNA Fragmentation in Morphologically Abnormal Sperm. Int J Androl. 2010;33(1):163-79. PubMed | CrossRef
36. Sivanarayana T, Ravi Krishna C, Jaya Prakash G, Krishna KM, Madan K, Sudhakar G, et al. Sperm DNA Fragmentation Assay by Sperm Chromatin Dispersion (SCD): Correlation Between DNA Fragmentation and Outcome of Intracytoplasmic Sperm Injection. Reprod Med Biol. 2014;13:87-94. PubMed | CrossRef

Citation: Faris D, Eltaweel N, Fadel M, Faris M, Zada S. DNA Integrity in Absolute Teratozoospermia Patients and its Impact on Assisted Reproductive Technology Outcome. J Gynecol Clin Obstet Reprod Med. 2023;1(2):76-89.
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