EGYPTIAN ACADEMIC JOURNAL OF

BIOLOGICAL SCIENCES ZOOLOGY

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ISSN
2090-0759

WWW.EAJBS.EG.NET

Vol. 14 No. 2 (2022)

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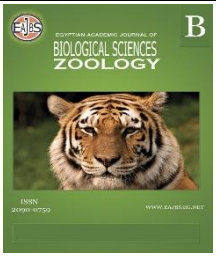
www.eajbs.eg.net

Citation: *Egypt. Acad. J. Biolog. Sci. (B. Zoology) Vol. 14(2) pp: 299-313(2022)*
DOI: 10.21608/EAJBSZ.2022.269377

Egypt. Acad. J. Biolog. Sci., 14(2): 299-313 (2022)

Egyptian Academic Journal of Biological Sciences
B. Zoology
ISSN: 2090 – 0759
http://eajbsz.journals.ekb.eg/



The Influence of Sperm DNA Fragmentation on Blastulationand Implantation Rates in Different Sources of Sperms.

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ARTICLE INFO
Article History
Received:15/8/2022
Accepted:24/10/2022
Available:28/10/2022

Keywords:
Sperm.
Blastulationand
Implantation Rates.

ABSTRACT

Background: It is well understood that a major contributing factor to male infertility is sperm DNA breakdown. Many studies have been carried out to investigate how sperm DNA damage affects the patient reproduction ability. This suggests different outcomes, and the subject remains contentious. We investigated differences in sperm parameters and embryonic developmental steps in our study, and the observations compare the results of ICSI using sperm extracted from testicular on patients with high sperm injury to the results of patients with sperm DNA fragmentation on ejaculate samples. **Materials and Methods:** From March 2017 to December 2019, 418 couples were enrolled in our study at the Algazeera Centre for IVF Giza, Egypt. The parameters of the sperm were evaluated. The value sperm DNA fragmentation index (DFI) was calculated desponded on the sperm chromatin dispersion (SCD) diagnostic test, which was applied to teams in the regulation (sperm DNA fragmentation index < 30%) or abnormal (sperm DNA fragmentation index > 30%) groups. **Results:** decrease in fertilization and blastulation rate in a group of patients suffering high sperm damage on ejaculate samples and our results show no difference between normal ejaculate samples and testicular sperm extrication (SDF) groups. **Conclusion:** no impact was detected on ICSI outcomes (pregnancy rates) between the three groups but a negative effect for sperm DNA fragmentation on fertilization and blastulation rate and blastulation rate depended on the age of women and the number of embryos transferred.

INTRODUCTION

For 44 years of IVF, all studies focus on how can increase the implantation rate. This recently improved it by obtaining blastocysts on day 5. But some factors inhabit embryos to form a blastocyst, the important factor for that male factor, which can investigate by the cornerstone of screening infertility males is semen analysis. Indicators of seminal vesicle and prostate function, respectively, are semen volume and pH level. Sperm concentration, motility, and morphology are mostly determined by testicular function and, to a lesser extent, by post-testicular (epididymal) genital tract function.

Although men who are fertile have greater mean sperm parameters than men who are infertile (concentration, motility, and morphology), these groups overlap significantly (Evenson, DP *et al.,* 1999). Additionally, these factors typically offer poor predictions of reproductive outcomes. To differentiate between productive and unproductive men, identify pregnancy outcomes, and reduce the risk of unfavorable reproductive events, new markers are required. Standard sperm parameters are insufficient. Some data suggest that sperm DNA integrity larger studies are required to determine the clinical significance of evaluating sperm DNA integrity, but markers may be more accurate indicators of male reproductive capacity than standard assessments. (Evenson, D.P. *et al.,* 1999, Spano M, *et al.,* 2000, Zini A, *et al.,* 2001, Guzick DS, *el al.,* 2001). Research on sperm in an era where cutting-edge kinds of assisted reproductive technology are frequently used, DNA damage is particularly important. Direct contact between the sperm and the oocyte, a fusion of the cellular membranes, and the fusion of the male and female gamete genomes are all components of fertilizing. (Primakoff P 2002). The inherent quality of the sperm DNA is important in both the success of this procedure and the following embryogenesis (Ahmadi A, Ng SC 1999). Because sperm DNA integrity is not taken into account in sperm analysis, it has limitations in diagnosing male infertility (De Jonge, 2012). According to research, the sperm analysis results for 15% of evaluated infertile men are within normal parameters (Omran, Bakhiet, & Dashti, 2013; Schulte, Ohl, Sigman, & Smith, 2010). As a result, sperm DNA fragmentation and its connection to pregnancy outcomes have received a lot of interest. incorrect chromatin structure during spermatogenesis DNA damage in sperm is brought on by insufficient apoptosis just before ejaculation or by an excessive amount of reactive oxygen species (ROS). However, the exact mechanism causing this circumstance is yet unclear (Henkel R. *et al.,* 1998, Sanocka-Maciejewska D. *et al.,* 2005 and Schuppe HC, *et al.,* 2008). The sperm chromatin structure assay (SCSA), terminal deoxynucleotidyl transfer as mediated deoxyuridine triphosphates nick end labelling TUNEl, and other tests to assess sperm DNA integrity have all been developed since the original hypothesis—made more than 30 years ago that changes in the chromatin structure of sperm may be related to decreased fertility rates (Evenson, Darzynkiewicz, & Melamed, 1980). (Chohan *et al.,* 2006). The WHO acknowledges the use of several of these assays for "checking the regularity of sperm chromatin and DNA," but not endorsing any of them.

In contrast, with such a diverse range of tests available, it has become increasingly clear that test standardization and consistency in clinical interpretation are essential (Shamsi *et al.,* 2011Until there is standardization in the execution, analysis, and clinical interpretation of DNA fragmentation assays, it is doubtful that DNA fragmentation testing will be officially approved as a part of routine diagnostic testing in andrology laboratories around the world (Fernández *et al.,* 2005).

There are several approaches to evaluating the integrity of sperm DNA, and each has benefits and drawbacks. But the only straightforward, inexpensive, and easily accessible kits are those that are sold commercially and are based on sperm. The chromatin dispersion test really does seem to satisfy these requirements. Other types of DNA fragmentation Assays necessitate specialized equipment, are employment, expensive, and time-consuming (Chohan et al., 2006). As a result, the impact of altered sperm chromatin integrity on post-embryonic development remains unknown (Larson *et al.,* 2000). But its effect on fertilization, blastocyst formation and pregnancy rates is demonstrated in this study. Male infertile with high sperm DFI might think about getting ICSI as a treatment. However, studies have found that ICSI treatment for infertile couples with high sperm DFI results in adverse clinical outcomes, such as high rates of unsuccessful fertilization and clinical pregnancy (Lopes, Sun, Jurisicova, Meriano, & Casper, 1998; Gardner, Schoolcraft, Moffatt, & Sakkas, 2004). The sperm source, on the other hand, may have an

impact on the degree of sperm DNA impairment during sperm preparation operations in an ART cycle, according to the available data. Spermatozoa from the testicles are believed to have more intact DNA signals than spermatozoa from the ejaculate, leading us to believe that using testicular spermatozoa rather than ejaculated spermatozoa would result in a more favourable reproductive outcome in an ICSI protocol. The goal of this study was to make a comparison of the clinical ICSI outcomes of using testicular spermatozoa versus ejaculated spermatozoa in infertile males with high SDF, with a focus on blastulation rates and clinical pregnancy the primary outcome and semen parameters, embryo fertilization and division as the secondary outcome, to help physicians determine the best treatment strategies for infertile males with high sperm DND Fragmentation Level.

MATERIALS AND METHODS

Patients:

The prospective study concerned all the cycles of ICSI performed during a given period in Algazeera Hospital, Giza, Egypt: 418 cycles of ICSI. ART procedures involving ejaculated sperm samples or testicular extracted sperm were included in this study.
The couples were divided into three groups:
A Control Group: within normal sperm parameters of the patient`s (sperm concentration $20X10^6$/ml,motility 50% [types a and b, according to WHO criteria], and morphology 30% normal forms
B Group: patients have high SDF and use ejaculate samples in ICSI
C Group: patients use testicular biopsy sperm extraction because their sperm DNA fragmentation ishigh.

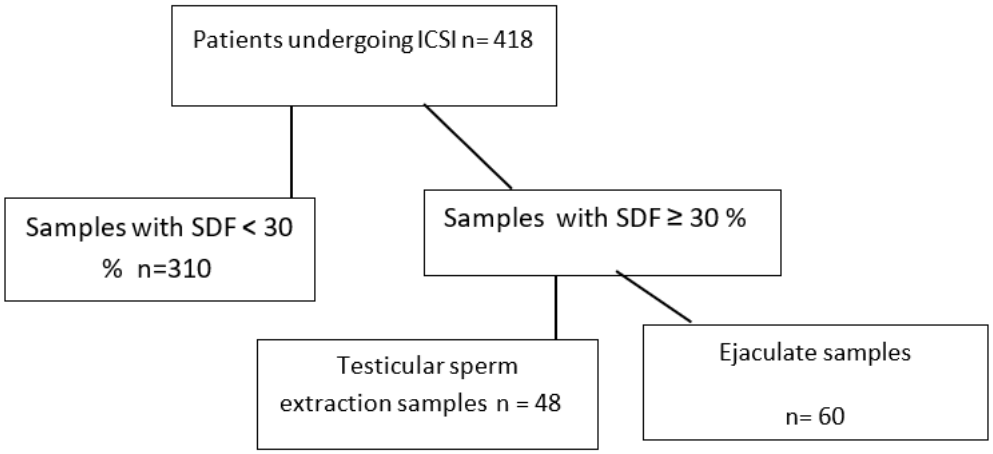

Diagram A for classification of patient`s

Semen Analysis:

After 3-5 days of sexual abstinence, each participant gave a sample of semen, and routine semen analyses were performed by one doctor in accordance with the 5th edition of the WorldHealth Organization (WHO) laboratory manual for the examination and processing of human semen. Sperm parameters such as concentration, progressive motility (PR%), and normal sperm morphology were collected for more study.

Sperm DNA Fragmentation Assessment:

Halosperm® was used to analyze all sperm samples for fragmented DNA. Halosperm® is based on the sperm chromatin dispersion (SCD) technique, It involves a carefully controlled DNA denaturation procedure that makes it easier to remove the spermatozoon proteins later.This is how typical spermatozoa produce halos. by sperm

DNA loops, which are missing in those with damaged DNA, at the sperm's head. Sperm was diluted in culture media with 20. million spermatozoa per milliliter concentration to get the optimum results 0.2 mL of fresh sample semen was diluted in medium sperm concentrations of 5 to 10 million/mL in order to get. The sperm sample was spread out across the sleeve in an agarosemicro gel. After being polymerized, the agarose was chilled and then polymerized.

The slide was exposed to a denaturation solution after the cover had been removed. For 7 min with the answer, The acid denaturation solution (labeled AD tube) was combined with 10 mL of distilled water to create AD (denaturant solution), which was then added to an incubation tray. The material was submerged in the lysis solution for 25 minutes. The slide was then air-dried after being dehydrated in ethanol baths of 70%, 90%, and 100% for a total of three minutes. The sperm were then washed in water and stained with Giemsa. Each slide was air-dried before being viewed at 400x magnification using a Carl Zeiss Primo Starlight microscope to score 500 sperm. Halosperm® creates highly contrasted images of halos that may be carefully assessed utilizing Primo Star microscopy (Carl Zeiss). shows each of the five potential SCD patterns.

Sperm cells with prominent halos (thickness equal to or greater than the length of the core's Sperm cells with medium halos (thickness less than the length of the core's (minor diameter. Sperm cells with small. minor diameter but greater than 1/3 of the core's minor diameter) thickness equal to or less than 1/3 the diameter of the core's minor diameter.

A total of 500 spermatozoa were counted, and those with Degradation of sperm cells fragmented DNA was identified using the manufacturer's instructions below.

SFD (%) = 100 × No. of spermatozoa with fragmented DNA /No. of spermatozoacounted

TESA Procedures:

As previously mentioned, a single doctor performed all TESA operations while under local anesthetic (Esteves, Miyaoka, & Agarwal, 2011) Briefly said, the spermatic cord was injected with about 10 ml of topical anesthetic to induce anesthesia. The spermatic cord, epididymis, and testicle fingers were interconnected during TESA treatments to immobilize the testicle and maintain skin tautness around the scrotum. The process was repeated until we had clear tissue or a spermatozoon in the biopsy needle (Argon Medical Device Inc., Gainesville, USA) that was put into the testicle's inferior pole while controlling the depth of the needle excursion. Under a microscope, one medical professional looked.

Ovarian Stimulation and ICSI Procedure:

Following three weeks of desensitization with GnRH analogues (Decapeptylâ; Ipsen), ovarian stimulation with recombinant FSH (Gonal-Fâ; Serono, or Puregonâ; Organon) was achieved and monitored. Endovaginal echography and plasma estradiol were used. When the follicles reached the desired diameter, 10 000 IU of hCG (Organon) has been administered 36 hours before oocyte retrieval. Under general anesthesia, the oocytes were extracted using a vaginal ultrasonography-guided aspiration.

Procedures for ICSI:

Denudation of oocytes was also collected, as was the on the same day as oocyte denudation, the male partner was asked to produce a semen sample, either by masturbation or TESA, which was then processed for ICSI. Fertilization was also checked, and the presence of two pronuclei and two polar bodies was considered normal fertilization; otherwise, it was considered abnormal. Embryo transfer was completed on the fifth day after the oocyte retrieval. Furthermore, clinical pregnancy was defined as the detection of a quantitative chemical pregnancy test and a fetal heartbeat via ultrasound 6 weeks after embryo transfer.

Assessment of Fertilization and Embryo Quality:

18 hours after fertilization, the resulting fertilized oocytes were placed in global media, they stayed until the fifth day. Embryos cultured after day 3 were transferred to an extended culture medium on day 3. Decide Culture for 3-5 days, depending on development speed and the Morphological quality of the cohort. Selected embryos Transfer to the new droplet on day 3 Selection of embryos for transfer Cleavage/morula stage or immature/Mature blastocyst stage. In blastocyst, in most cases, Maturity (size and distinguishable inner cell mass [ICM] and Trophectoderm) and morphology is the main determinant chosen. Embryo transfer these days Assisted hatching is not performed. this blastocyst classification according to dived Gardner.

Blastocyst classification according to its size from 3:6, ICM classification from A: C and Trophectoderm classification from A: C

Embryo Transfer:

Embryo transfer takes place 5 days after oocyte injection and lasts approximately 115 hours. ETs must be taken carefully after counting blastocysts and transferred at 37 °C usinga dewormed hot plate under a Zeiss stereomicroscope and a sterilized Falcon 1006 Petri dish. For transfer, incubate a 1 ml series filled with 20% Serum Supplement Solution (SSS) for 17 hours and remove air bubbles, connect the filled syringe to the transfer catheter, and after flushing the catheter, aspirate the selected blastocysts into the catheter, via The cervical canal inserts the catheter into the uterine cavity, slowly injects the catheter, and then gradually withdraws the catheter, and ET occurs under ultrasound in the operating room. The advantage of transferring blastocysts on day 5 is bettersynchronization between the embryo and the endometrium. Transferring one or two Blastocysts is the optimal number for transfer to avoid multiple pregnancies but our protocol in ET number of Embryos depends on the woman's age and/or the number of available blastocysts. According to our protocol.

Statistical Analysis:

Data were analyzed using IBM© SPSS© Statistics version 23 (IBM© Corp., Armonk, NY) and MedCalc© version 18.2.1 (MedCalc© Software bvba, Ostend, Belgium Numerical variables were presented as mean and SD and inter-group differences were compared using one-way analysis of variance with an application of the Tukey test for post hoc comparison if needed Categorical variables were presented as numbers and percentages and differences were compared using Fisher's exact test Correlations between numerical variables were tested using Pearson correlation. Multivariable binary logistic regression analysis was used to examine determinants of biochemical pregnancy. To examine determinants of fertilization, division, and blastulation rates, multivariable linear regression was used Two-sided p-values <0.05 were considered statistically significant.

RESULTS

The biochemical pregnancy results in the three groups were the following and no difference between the three groups.

Table 1 the results success rate in the study groups.

Study items		A (number of cases) =310	B (number of cases) =60	C (number of cases) =48	Test statistic	*P value*
Outcomes result	*Negative*	139 (45.2%)	31 (52.9%)	24 (50.0%)	1.893	0.391
	Positive	171 (54.8%)	29 (48.7%)	24 (50.0%)		

Fisher's Exact Test:

In Table 1. Pregnancy tests amongst the three groups did not differ substantially (P > 0.05, p = 0.391). Whatever the circumstances, it was cancelled.

The comparison between three groups showing decrease on fertilization and blastocyst formation was as the following:

Fertilization Rate:

Control group = 83.4 ± 17 %, ICSI with ejaculate samples has SDF group = 75.0 ± 18.5 %, ICSI with TESE group=81.8±17.1 %, where, *p value* was = 0.003 so there is an affect by SDF.

Blastocyst Formation Rate:

Control group = 47.7 ± 18.1 % ICSI with ejaculate samples has SDF group = 38.3 ± 20.8 % ICSI with TESE = 48.1± 20.7 % where *p value* was = 0.002 so there is an affect by SDF.

Therefore, the results had to be confirmed using linear regression, which it has more accurate where:

Independent variable and a corresponding dependent variable. The independent variable is the input, and the corresponding dependent variable is the output.

Table 2. shows the outcomes of a multivariable binary logistic regression analysis for factors that influence biochemical pregnancy. The odds ratio for an increase in the number of embryos transferred was 1.384, and the chance of pregnancy increased with that factor. The odds of a decrease in chemical pregnancy with an increase in the age of the wife factor were less than 1, indicating a negative effect.

Female's age (odds ratio = 0.944, 95% CI = 0.909 to 0.975, P-value = 0.002) and number of E.T (odds ratio = 1.384, 95% CI = 1.119 to 1.722, P-value = 0.004) are independent factors that affect chemical Pregnancy.

Table 2. Effect of age variables and an increase in transplanted embryos on biochemical pregnancy.

Items	Regression coefficient	Stande rError	Wald statistic	P-value	Odds ratio	95% Confidence interval.
†SDF >30% (=1)	-0.317	0.325	0.946	0.337	0.735	0.383 to 1.381
‡ Sperm source FNA (=1)	0.013	0.409	0.003	0.979	1.015	0.463 to 2.223
Female Age	-0.065	0.018	10.062	0.002	0.944	0.909 to 0.975
No of ET	0.329	0.117	8.479	0.004	1.384	1.119 to 1.722
Constant	1.079	0.711	2.302	0.129		

†Descried to DNA fragmentation ≤30% (=0).

‡ Descried to sperm collection by ejaculation (=0).

Table 3 shows the results of multivariable linear regression analysis for determinants of fertilization rate.

Sperm collection by FNA was the only independent determinant of fertilization rate (B = -6.873, SE =3.337, P-value = 0.040).

Table 3. Multivariable linear regression analysis for determinants of fertilization rate

Variable	B	SE	t-value	P-value	$r_{partial}$	$r_{semipartial}$
Constant	79.407					
DNA fragmentation >30% (=1)	-1.330	2.690	-0.494	0.621	-0.024	0.024
Sperm collection by FNA (=1)	-6.873	3.337	-2.060	**0.040**	-0.101	0.100
Age (years)	0.130	0.155	0.836	0.404	0.041	0.040

This table demonstrates the effect of testicular sperm extracted effect in increase fertilization rate more than ejaculated sperm with high DNA sperm.
B = regression coefficient, SE = standard error, $r_{partial}$ = partial correlation coefficient (correlation between independent variable and outcome variable adjusted for the effect of the other variables in themodel), $r_{semipartial}$ = semi partial correlation coefficient (correlation between independent variable and outcome variable adjusted for other independent variables only.
†Descried to DNA fragmentation ≤30% (=0).
‡ Descried to sperm collection by ejaculation (=0).

Table 4. shows the results of multivariable linear regression analysis for determinants of division rate;none of the included variables is a determinant of division rate (all P- values > 0.05) from the motion, not a significant (P ≤ 0.05).

Table 4. Multivariable linear regression analysis for determinants of the division rate:

Variable	B	SE	t-value	P-value	$r_{partial}$	$r_{semipartial}$
Constant	80.937					
DNA fragmentation >30% (=1)†	2.025	2.685	0.754	0.451	0.037	0.037
Sperm collection by FNA (=1) ‡	-2.131	3.331	-0.640	0.523	-0.031	0.031
Age (years)	0.144	0.155	0.932	0.352	0.046	0.046

B = regression coefficient, SE = standard error, $r_{partial}$ = partial correlation coefficient (correlation between independent variable and outcome variable adjusted for the effect of the other variables in themodel), $r_{semipartial}$ = semi-partial correlation coefficient (correlation between independent variable and outcome variable adjusted for other independent variables only.
†Descried to DNA fragmentation ≤30% (=0).
‡ Descried to sperm collection by ejaculation (=0).

Table 5. shows the results of multivariable linear regression analysis for determinants of totalblastulation rate.

Both sperm collection by FNA (B = -9.942, SE = 3.628, P-value = 0.006) and patient's age (B = 0.356,SE = 0.168, P-value = 0.035) are independent determinants of total blastulation rate. Where inject oocytes with testicular sperm extracted increases blastulation rate regard to oocytes injected with sperm with high sperm DNA fragmentation. And age factor was considered and the increase in the wife's age increased the blastulation rate it is according to our data analysis regardless of cancellation cases.

Table 5. Multivariable linear regression analysis for determinants of total blastulation rate.

Variable	B	SE	t-value	P-value	$r_{partial}$	$r_{semipartial}$
Constant	36.857					
DNA fragmentation >30% (=1) †	1.070	2.925	0.366	0.715	0.018	0.018
Sperm collection by FNA (=1) ‡	-9.942	3.628	-2.741	**0.006**	-0.134	0.132
Age (years)	0.356	0.168	2.111	**0.035**	0.103	0.102

B = regression coefficient, SE = standard error, $r_{partial}$ = partial correlation coefficient (correlation between independent variable and outcome variable adjusted for the effect of the other variables in the model), $r_{semipartial}$ = semi-partial correlation coefficient (correlation between independent variable andoutcome variable adjusted for other independent variables only.
†Descried to DNA fragmentation ≤30% (=0).
‡ Descried to sperm collection by ejaculation (=0).

Table 6. shows the results of multivariable linear regression analysis for determinants of late blastulation rate, SDF, and wife age were showed a significant effect in blastocyst formation (*P- value*≤ 0.05). Where increased SDF decrease late blastulation rate, and increase wife age decrease lateblastocyst formation.

Generally, there was no appreciable difference between the groups with an SDF of 30% and those with anSDF of 30% in ICSI in terms of achieving clinical or biochemical pregnancy. However, there are important differences between group B and the control in early and late blastulation, where excessive sperm DNA formation reduces blastocyst development. Additionally, there was no discernible difference between group C and the control group in any of the outcomes.

Table 6. Multivariable linear regression analysis for determinants of late blastulation rate

Variable	B	SE	t-value	P-value	$r_{partial}$	$r_{semipartial}$
Constant	21.674					
DNA fragmentation >30% (=1) †	-3.226	1.684	-1.915	**0.056**	-0.094	0.092
Sperm collection by FNA (=1) ‡	3.304	2.089	1.581	0.115	0.077	0.076
Age (years)	-0.337	0.097	-3.479	**0.001**	-0.169	0.168

B = regression coefficient, SE = standard error, $r_{partial}$ = partial correlation coefficient (correlation between independent variable and outcome variable adjusted for the effect of the other variables in themodel), $r_{semipartial}$ = semi-partial correlation coefficient (correlation between independent variable and outcome variable adjusted for other independent variables only.
Referenced to DNA fragmentation ≤30% (=0). Referenced to sperm collection by ejaculation (=0).

Demographics:

In this part, the figures in the research are explained that the results from the statistical calculations and plenty of illustration to see the results represented in those illustrations.

Figure 1 explains the relationship between the three groups in pregnancy results, as there is no difference in the results resulting from ICSI with the difference in the sperm source used that likely to be the cause of blastocyst trans fear benefits even transfer single blastocyst. While, the figure 2 explains the effect of ICSI by highly sperm DNA fragmentation, where decreased fertilization is less than the control group, which are the normal group and the group TESE group.

Figure 3 explains the effect of sperm DNA fragmentation on division, no effect for high sperm DNA fragmentation on division where the results appear in this figure no different between three groups.

Figure 4 descried the resulting of total blastulation rate where decreased blastulation on day fife by high sperm DNA fragmentation more than other two groups this likely to be the cause effect of paternal genome on day three. Also, The Figure 5 descried the resulting of late blastulation rate where increased late blastulation by high sperm DNA fragmentation more than other two groups this explains that the high sperm DNA fragmentation not arrest blastulation rate and delays it.

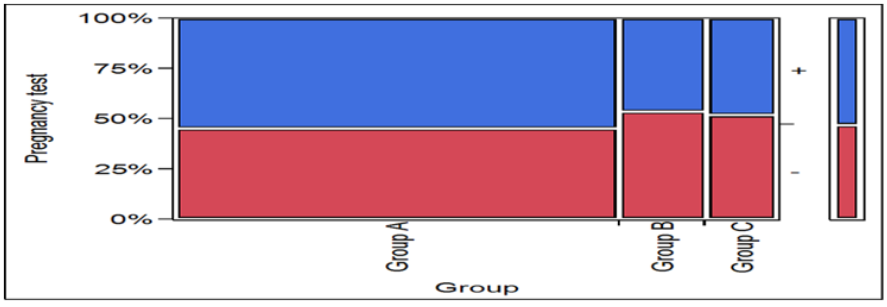

Fig. 1: shows the pregnancy rate in various groups graphically. Visual presentations.

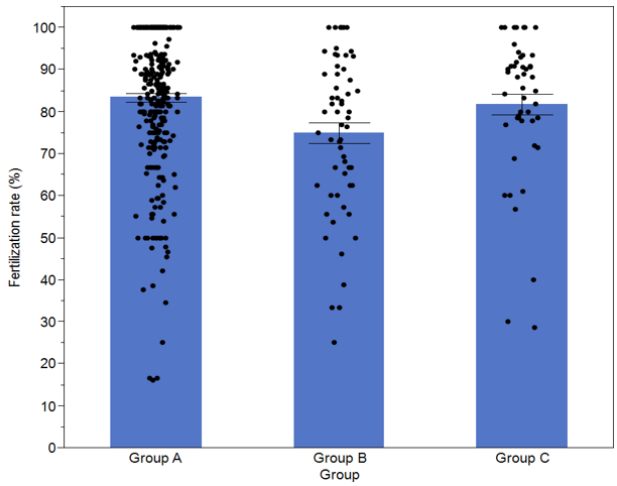

Fig. 2. Graphical presentation of fertilization rates in different groups A, B, and C, increased fertilization in groups A and C (P-value > 0.05). And decrease fertilization in group B (p-value < 0.05).

It is previously mentioned sperm DNA fragmentation showed significance in column group B.

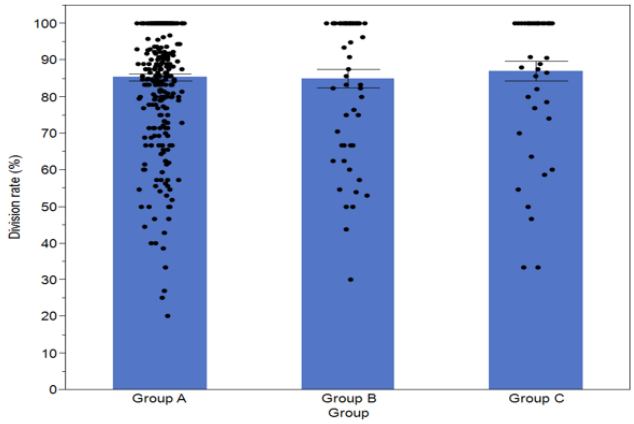

Fig. 3. Mean division rate in the three study groups. Error bars represent the standard error (SE).Dots represent individual observations.

Graphical presentation of division rate in different groups A, B, and C, not significant effect *(p-value* >0.05) between different columns.

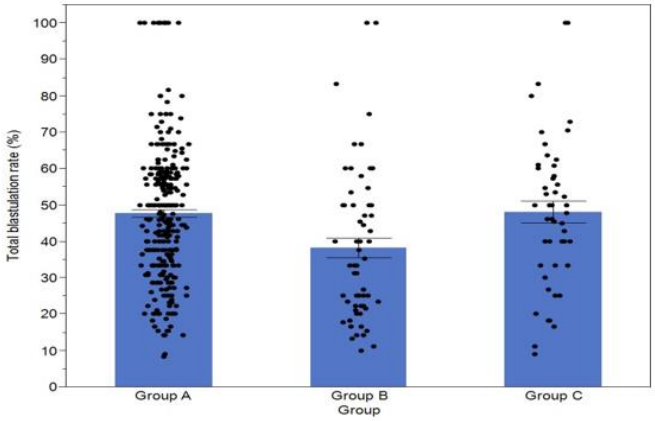

Fig. 4*:* Mean total blastulation rate in the three study groups. Error bars represent the standard error(SE). Dots represent individual observations.

Graphical presentation of fertilization rate in different groups A, B, and C, between blastocyst formations in different three groups. Columns carrying different letters are significantly different atp < 0.05.

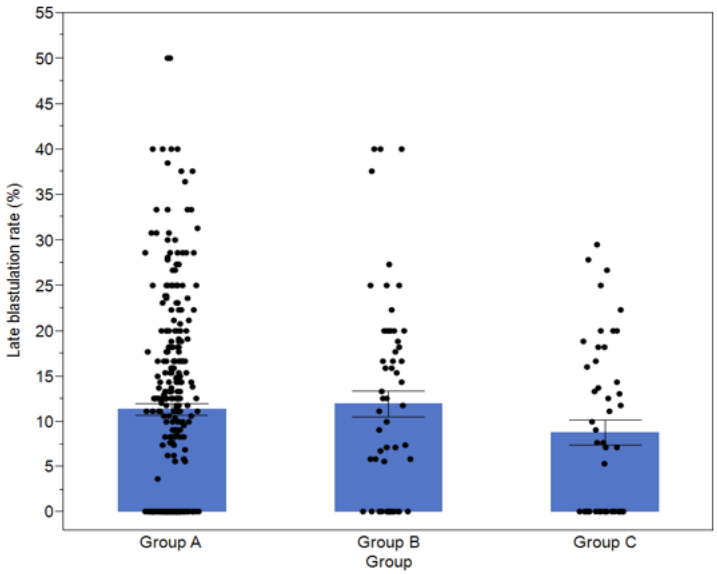

Fig. 5. Mean late blastulation rate in the three study groups. Error bars represent the standard error(SE). Dots represent individual observations.

Graphical presentation of the relationship between late blastulation in different three groups. Columnscarrying different letters are significantly different at p < 0.05.

DISCUSSION

The effects of SDF on semen parameters and the requirement of sperm chromatin analyses in normal laboratory exams have conflicting conclusions. Studies have linked sperm DNA fragmentation to fertility and semen quality standards, In normal laboratory studies of semen, chromatin condensation (SCD) has been proposed as a useful metric to assess male fertility (Hammadeh ME, *et al.*, 2001). There are other ways to identify sperm DNA fragmentation, such as Halosperm, an enhanced SCD test created by Férnandez *et al.*, in 2005, However, some researchers found a negative link in some(Khalili MA, *et al.*, 2006), (Sills *et al.*, 2004) and every parameter were to choose sperm that are normally mobile and do not affect an oocyte's potential to get fertilized (Muratori M., 2008) and (Erenpreiss J, *et al.*,2008) However, our results show host R *et al.*, 2000 found that total and progressive sperm motility increased more in LFG than HFG after semen preparation, showing that lower DNA fragmentation levels benefit more from semen preparation through sperm motility. the head, acrosome, and intermediate portion of sperm, as well as concentration, do not yet correlate with sperm DNA fragmentation, but Cohen- Bacrie *et al.*, 2009 showed a negative relationship between quick motility rate and provides a straightforward method for DNA fragmentation (Cohen-Bacrie P, 2009) That is consistent with our findings, where p =0.049 (previous manuscript khallaf *et al.*, 2022) was found for decreased progression and quick motility. These findings may imply that the embryo's fertilization is influenced by the sperm's DNA fragmentation level but is unaffected by division and developmental potential until day 3 of development (Braude P, *et al.*, 1988) that sperm can be repaired by oocytes and embryo DNA damage, (Ahmadi A, Ng SC *et al.*, 1999), and that the patient'scondition may have contributed to these findings. That agrees with my finding in tables 3 and 4 where the due to and the decrease of fertilization

is likely to be the cause of a large amount of SDF and the ability to repair oocytes with this defect with a small amount of SDF. Sperm from the testicular exhibited less DNA damage than sperm from the ejaculate in infertile males with a high DNA fragmentation rate (Mehta, A., *et al.* (2015), (Moskovtsev, S. I. *et al*., 2010). Which can improve the embryonic potential from oocyte fertilization. (Weissman *et al.,* 2008). discovered that the passage of spermatozoon through the epididymis was associated with loss of sperm integrity and fertilizing ability in a series of only four couples using ICSI cycles using ejaculated spermatozoon (O'Connell, McClure, and Lewis 2002). (Suganuma, Yanagimachi &Meistrich 2005). that reflect my findings, which appeared in table 3. One explanation for this finding is that the mature spermatozoa are crammed with immature spermatozoa that produce ROS as they go through the seminiferous tubule and epididymis, resulting in DNA damage to the mature sperm. (Aitken, R. J. *et al.,* 1997, Ollero, M.*et al.*, 2001). Because spermatozoon DNA is more susceptible to oxidation before going through chromatin condensation in the epididymis, this explanation makes sense. My article's findings imply that oxidative stress experienced during transit through the epididymis may be a greater danger to the success of ICSI than testicular sperm maturation. then use of testicular sperm in these patients to achieve better ICSI outcomes.

A Low and significantly lower cleavage and fertilization rates Note embryo quality and blastocyst formation, especially when Testicular sperm from men with OA and NOA is utilized for ICSI, which is caused by reduced testicular sperm quality, subsequent embryo quality and blastocyst growth are directly impacted. less fruitful than the previous ejaculation Epididymal maturation of sperm Lin M.H., *et al.*,2008. Additionally, research indicates that testes from NOA patients are more likely to have chromosomal aneuploidy than OA patients do. That may, at least in part, account for the reduced embryonic development rates seen in this study's group of patients. This contract with us There was no difference between the control group and the control group A set of testicular sperm extraction as a table (3,4,5) in the study that was presented. DFI has no impact on clinical outcomes following blastocyst transfer. because the pregnancy rate for blastocyst transfer is higher than that of early embryo transfer (day 2 or 3) There have been reports of high levels of in vitro-developed blastocysts (Zorn B *et al.,* 2012, Goker, ENT *et al.,* 2002) In blastocysts table, we saw adverse sperm damage effects. Form Table (5), which resulted in paternal genome effects Flow after 6- 8 cell division, reducing blastocyst formation oppositely related to the number of oocytes extracted. The embryonic cell block is one of the most frequent causes of a decreased blastocyst development rate. Development blockade is made by Apoptosis that begins after the second or third division of the embryo. basic indications include apoptosis and nuclear fragmentation. Abnormalities in the development of microtubule spindles. Balaban B, *et al.*, 2001 Kolibianakis E, *et al.*, 2002. The testicular sperm extraction group increased the blastulation rate of more Ejaculate samples which SDF, Table (5), because Reducing sperm damage is in this group equal to the properties of a typical ejaculatedsample. according to 26 clinical outcomes of ICSI following single blastocyst transfer that have beendocumented Use of TESE similar to standard ICSI Table with normal ejaculation samples (Rossi–Ferragut LM *et al.,* 2003 - Arafa M, *et al.,* 2017, Nagata S, *et al.,* 2003, Nilsson J, *et al.,* 2004).

From table 6 we observed an increased number of late blastocysts compared with another group we believe that high SDF does not make an arrest only but can cause a delay in blastulation rate may be because of the action of oocytes in repairing itself and repairing the DNA damage take time, female aging and oocyte quality plays role .that agree with that who said ought to be taken under consideration is deoxyribonucleic acid fragmentation (Carrell and Liu 2003) report a drop in high-quality embryos and an increase in low-quality embryos on day two when gamete deoxyribonucleic acid fragmentation is substantial. It

has been widely documented that the absence of the epididymal tract in spermatozoa prevents SDF in male reproductive gland gametes (Agarwal, Majzoub, Esteves, Ko, Ramasamy, &Zini, 2016; Agarwal Sharma, Samanta, Durairajanayagam, & Sabanegh, 2016; El Greco Scarselli, & Iacobelli, 2005). We incline to speculate that a higher level of deoxyribonucleic acid fragmentation in ejaculated thawed gametes may confirm a delay in embryo cleavage activation and, consequently, a delay seen in early cellular divisions.

Finally, after ICSI, we found no variations in the pregnancy rates between the 3 groups. highest-scoring embryos for transfer may be the reason why there is no correlation between sperm DNA damage and pregnancy rates. Because the pregnancy rate is affected by age of the female and the number of embryos in the blastocysts stage which is transferred to Table 2. Gerris M.R *et al.,* 2009 where increasedpregnancy chance with the increase in the number of blastocysts in a direct relationship with age and vice versa Table 1 and 2.

Conclusion

The benefit of sperm extraction from the testicles in cases of high sperm DNA. Fragmentation (SDF) gives great results in obtaining the highest fertilization equations and blastocyst formation. Similar to the injection of samples with normal parameters, which are in the results Superior to samples injected with higher SDF.

Acknowledgements

The study's financial support is done by Algazeera IVF Unit.There is no conflict of interest in this article

REFERENCES

Ahmadi A, Ng SC, (1999): Developmental capacity of damaged spermatozoa. *Human Reproduction*, Volume 14, Issue 9, September 1999, Pages 2279–2285, https://doi.org/10.1093/humrep/14.9.2279

Agarwal, A., Sharma, R., Samanta, L., Durairajanayagam, D., & Sabanegh, (2016): Proteomic signatures of infertile men with clinical Varicocoele and their validation studies reveal mitochondrial dysfunction leading to infertility. *Asian journal of andrology*, 18(2): 282–291.Published online 2015 Dec 29. doi: 10.4103/1008-682X.170445

Agarwal, A. Majzoub, A. Esteves, S.C, Ramasamy, E. Ko, R, Zini.A (2016): Clinical utility of sperm DNA fragmentation testing: Practice recommendations based on clinical Scenarios. *Translational Andrology and Urology,* 5(6), 935– 950. https://doi.org/10.21037/tau.

Arafa. M, AlMalki. A, AlBadr. M, (2017): ICSI outcome in patients with high DNA fragmentation: Testicular versus ejaculated spermatozoa. *Andrologia.* 50(1):e12835

Aitken. R. J, Fisher .M.H (1997): Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by the flavoprotein inhibitors diphenylene iodonium and quinacrine. *Molecular reproduction and development,* 47, 468–482, https://doi.org/10.1002/(sici)1098-2795(199708)47:4468::aid-mrd143.0.co;2-s .

Balaban B, Urman B, Isiklar A, (2001): The effect of pronuclear morphology on embryo quality parameters and blastocyst transfer outcome. *Human reproduction;* 16:2357–2361

Braude P, Bolton V, Moore S (1988): Human gene expression first occurs between the four-and eight-cell stages of preimplantation development. *Nature;* 332:459-461

Carrell, D. T., & Liu, L. (2003). Sperm DNA fragmentation is increased in couples with unexplained recurrent pregnancy loss. *Archives of Andrology*, 49, 49– 55 https://doi.org/10.1080/01485010290099390.

Cohen-Bacrie P, Belloc S, Ménézo YJ Clement P,Hamidi J, Benkhalifa M. (2009) :Correlation between DNA damage and sperm parameters: a prospective study of 1,633 patients. *Fertility and Sterility*; 91(5):1801-1805.

Chohan, K. R., J. T. Griffin, M. Lafromboise, C. J. Jonge and D. T. Carrell (2006): "Comparison of chromatin assays for DNA fragmentation evaluation in human sperm." *Andrology and Urology*, 27(1): 53-59

De Jong, I. Schipper, J. S. E. Laven (2012): Serum Anti-Müllerian Hormone Levels in Healthy Females: A Nomogram Ranging from Infancy to Adulthood *the Journal of Clinical Endocrinology & Metabolism*, Volume 97, Issue 12, 1 December 2012, Pages 4650–4655, https://doi.org/10.1210/jc.2012-1440

Erenpreiss. J, Elzanaty. S, Giwercman. A (2008): Sperm DNA damage in men from infertile couples. *Asian Journal of Andrology*; 10 (5): 786-790

Evenson DP, Jost LK, Marshall D, (1999): Utility of the sperm chromatin assay as a diagnostic and prognostic tool in the human fertility clinic. *Human Reproduction*; 14:1039-49

Esteves .SC, Agarwal. A, (2011): Novel concepts in male infertility. Int Braz , Official journal of the Brazilian Society of Urology 37(1):5-15

Evensonz. Darzynkiewiczand M. R. (1980): Relation of Mammalian Sperm Chromatin Heterogeneity to Fertility. *Melamed Science*,Volume 210, Issue 4474 pp. 1131 -1133 DOI: 10.1126/science.744444

Férnandez JL, Muriel L, Goyanes V, Segrelles E,Gosálvez J, Enciso M, (2005). Simple determination of human sperm DNA fragmentation with an improved sperm chromatin dispersion test., *Fertility Sterility journal*, 84: 833- 842

Guzick DS, Overstreet JW, Factor-Litvak P, (2001): Sperm morphology, motility, and concentration in infertile and fertile men. *New England Journal of Medicine,* 345:1388-93.

Goker ENT, Sendag F, Levi R, (2002): Comparison of ICSI outcome of ejaculated sperm with normal, abnormal parameters and testicular sperm, *European Journal of Obstetrics & Gynecology and Reproductive Biology*, 104:129–136.

Gardner DK, Schoolcraft WB, (1999): In vitro culture of human blastocysts. In: Jansen R, Mortimer D, editors. Toward reproductive certainty: *fertility and genetics beyond. London*: *Parthenon* Publishing; 1999. p. 378–88.

Gardner, D.K., Surrey, E., Minjarez, D., Leitz, A., Stevens, J., Schoolcraft, and W.B, (2004): Singl blastocyst transfer: a prospective randomized trial. *Fertility and Sterility*. 81, 551–555

Henkel R, Schill WB (1998): Sperm separation in patients with urogenital infections. *Andrologia*; 30(Supplement 1):91–7.

Host, R, Svend Lindenberg, and Steen Smidt-Jensen, (2000): The role of DNA strand breaks in human spermatozoa used for IVF and ICSI; *Acta Obstet Gynecol Second*; 79: 559–563

Hammadeh ME, Zeginiadov T, Rosenbaum P, GeorgT, Schmidt W, Strehler E. (2001): Predictive value of sperm chromatin condensation (aniline blue staining) in the assessment of male fertility. *Archive of Andrology*. 46(2):99-104.

Jan Gerris M.R. (2009): Single-embryo transfer versus multiple-embryo transfer. *Reproductive Biomedicine journal.* Online Volume 18, Supplement 2, 2009, Pages S63-S70

Kolibianakis E, Bourgain C, Albano C, (2002): Effect of ovarian stimulation with recombinant follicle–stimulating hormone, gonadotropin–releasing hormone–antagonists, and human chorionic gonadotropin, on endometrial maturation on the day of oocyte pick–up. *Fertility and Sterility journal*. 78(5):1025–1059.

Khalili MA, Aghaie-Maybodi F, Anuari M, Talebi AR, (2006): Sperm nuclear DNA in ejaculate of fertile and infertile men: correlation with semen parameters. *Urology and Nephrology Research Center journal;*3(3): 154-159.

Khallaf. M.Y, (2022): Impact of sperm DNA fragmentation on blastocyst formation and pregnancy, *Obstetrics Gynecology international journal. 2022;13(1):32-37.*

Muratori M, Marchiani S, Tamburrino L, Tocci V, FailliP, Forti G (2008): Nuclear staining identifies two populations of human sperm with different DNA fragmentation extent and relationship with semen parameters. *Human Reproduction,* 23(5): 1035-1043.

Mehta, A., Bolyakov, A., Schlegel, P. N. & Paduch, D. A, (2015): Higher pregnancy rates using testicular sperm in men with severe oligospermia. *Fertility and sterility journal 104*, 1382–1387, https://doi.org/10.1016/ 2015.08.008

Moskovtsev, S. I, (2010): Testicular spermatozoa have statistically significantly lower DNA damage compared with ejaculated spermatozoa in patients with unsuccessful oral antioxidant treatment. *Fertility and Sterility journal*, 93, 1142–1146, https://doi.org/10.1016/.2008.11.005.

Nilsson J, Fioretos T, Hoglund M, (2004): Approximate geodesic distances reveal biologically relevant structures in microarray data. *Bioinformatics journal*, 20(6):874–880

Nagata S, Nagase H, Kawane K, (2003): Degradation of chromosomal DNA during apoptosis. *Cell Death & Differentiation journal,* 10:108–116.

Lin MH, Kuo–Kuang .Lee R, Li .SH, (2008): Sperm chromatin structure assay parameters are not related to fertilization rates, embryo quality, and pregnancy rates in vitro fertilization and Intracytoplasmic sperm injection, but might be related to spontaneous abortion rates. *Fertility and Sterility*; 90: 352–359.

Larson KL, De Jonge CJ, Barnes AM, Jost LK, Evenson. D.P, (2000): Sperm chromatin structure assay parameters as predictors of failed pregnancy following assisted reproductive techniques. *Human reproduction,* 15: 1717– 1722.

Lopes S., Sun JG, Jurisicova A., Meriano J. Casper RF, (1998b): Sperm deoxyribonucleic acid fragmentation is increased in poor-quality semen samples and correlates with failed fertilization in intracytoplasmic sperm injection. *Fertility and sterility*, 69: 528– 532.

Omran HM, Bakhiet M, Dashti MG (2013): Evaluation of Age Effects on Semen Parameters of Infertile Males. *Andrology,* 2: 106. doi:10.4172/2167-0250.1000106

O'Connell, M., McClure, N., & Lewis, S. E. (2002) ; Mitochondrial DNA deletions and nuclear DNA fragmentation in testicular and epididymal human sperm. *Human Reproduction*, 17, 1565–1570. https://doi.org/10.1093/humrep/17.6.1565

Ollero, M. *(2001);* Characterization of subsets of human spermatozoa at different stages of maturation: implications in the diagnosis and treatment of male infertility. *Human reproduction (Oxford, England),* 16, 1912–1921

Primakoff P, Myles DG, (2002): Penetration, adhesion, and fusion in mammalian sperm-egg interaction. *Science,* 296:2183-5.

Rossi–Ferragut LM, Iaconelli A, Aoki T, (2003) : Pronuclear and morphological features as a cumulative score to select embryos in ICSI (intracytoplasmic sperm injection) cycles according to sperm origin. *Journal of assisted reproductive genetics*, 20:1–7.

Ryan T. Schulte, Dana A. Ohl, Mark Sigman, and Gary D. Smith , (2010) : Sperm DNA damage in male infertility: etiologies, assays, and outcomes . *Journal of assisted reproductive genetics*, 2010 Jan; 27(1): 3–12. Published online 2009 Dec 12. doi: 10.1007/s10815-009-9359-x.

Spano M, Bonde JP, Hjollund HI, (2000): Sperm chromatin damage impairs human fertility. *Fertility and Sterility*; 73:43-50.

Sakkas, D., E. Seli, D. Bizzaro, N. Tarozzi and G. C. Manicardi, (2004): Abnormal spermatozoa in the ejaculate: abortive apoptosis and faulty nuclear remodeling during spermatogenesis. *Reproductive Biomedicine Online*, 7(4): 428-432.

Shamsi, M B Syed Nazar Imam, and Rima Dada,(2011) :Sperm DNA integrity assays: diagnostic and prognostic challenges and implications in management of infertility . *Journal of assisted reproductive genetics.* 2011 Nov; 28(11): 1073–1085. Published online 2011 Sep 9. doi: 10.1007/s10815-011-9631-8.

Sanocka-Maciejewska D, Ciupińska M, Kurpisz M, (2005): Bacterial infection and semen quality. *Journal of Reproductive Immunology*; 67:51–6.

Schuppe HC, Meinhardt A, Allam JP, Bergmann M, Weidner W, Haidl G, (2008): Chronic orchitis: A neglected cause of male infertility? *Andrologia.*;40:84–91.

Sills ES, Fryman JT, Perloe M, Michels KB, Tucker MJ, (2004): Chromatin fluorescence chracteristics and standard semen analysis parameters: correlations observed in andrology testing among 136 males referred for infertility evaulation. *Obstetrics Gynecology international journal*, 24(1): 74-77.)

Suganuma, R., Yanagimachi, R., & Meistrich, M. L. (2005): Decline in fertility of mouse sperm with abnormal chromatin during epididymal passage as revealed by ICSI.*Human Reproduction*, 20, 3101 – 3108. https: //doi.org/ 10.1093/ humrep/ dei169