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Impact of Sperm DNA Fragmentation on Blastocyst Formation and Ongoing Pregnancy Rates.

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Abstract

Background *Sperm DNA fragmentation is a well-known cause of male infertility. Many studies have been conducted on the effect of sperm DNA damage on reproductive potential, with varying results and the subject remains controversial. In this study, we looked at differences in sperm parameters and Intracytoplasmic sperm injection (ICSI) outcomes based on patients' sperm DNA fragmentation levels (DFLs).*

Methods *We are using Kit depending on sperm chromatin dispersion (SCD) test, was used to determine the DFLs. Patients were divided into two groups: those with low DNA fragmentation (LFG 30 percent) and those with high fragmentation (HFG >30 percent).*

Results *Our analysis showed that the poor quality of embryos on day three and low blastocyst formation on day 5 with high sperm DNA fragmentation and improvement by testicular biopsy sperm extraction.*

Conclusion *There was no difference in ICSI outcomes but has a negative effect on fertilization and blastocyst formation when making a comparison between the three groups, there was a decrease in blastocyst formation on ejaculation samples from the group with high sperm DNA fragmentation.*

Keywords *Sperm.; DNA fragmentation; Infertility; Male factor; sperm. ICSI; Blastocyst formation.*

Introduction

From 1978, when the first In vitro fertilization (IVF) baby was born, Assisted Reproduction Technology (ART) has come a long way with tremendous improvement in IVF results. Many challenges embryologists and clinicians have been facing in order to achieve higher pregnancy and healthy fetuses **[**1**]**

One of the major developments was improvement of embryo transfer on day fife or transfer blastocyst which has man advantage over early stage embryo transfer. In vivo, the cleavage or early stages of an embryo are naturally in the fallopian tube and the blastocyst stage is the most biologically correct stage for embryos to be in the uterus **[**2**]**, thus there is a physiological synchronization of the blastocyst stage embryo with the endometrium **[**3**]**.

Blastocyst stage embryo has higher implantation potential when compared with cleavage stage embryos. Since only those embryos with activated genome reach the blastocyst stage. This is reported to benefit IVF in; improving pregnancy rates and reducing high incidence of multiple gestation, the most advantage for blastocyst transfer is few embryos to be transferred. **[**4**]** reports suggest transfer blastocyst improvement successes rate. But some factors inhabit embryos to reach for blastocyst stage. The biggest of these factors is appeared when it related to male factor which cases delayed in embryo developmental when all female factors is suitable for get baby **[**5**]**.

Male factor is the single most common cause of infertility, with sperm defects representing 30% to 50% of clinical infertility cases. Male infertility is indeed becoming an epidemic problem affecting almost 5% of the male population **[**6**]**.

In order to assess male factor infertility a semen analysis is performed to determine the number, shape and movement of the spermatozoa in a sample. According to World Health Organization(WHO) 2010 criteria for a normal semen analysis. But Conventional semen parameters for differentiation between fertile and infertile male are accepted to have limited diagnostic value for male infertility and not accurate for ART success.

Sperm DNA (DeoxyriboNucleic Acid) fragmentation testing answer many questions IVF pioneers spend many years thinking about it **[**7,8,9,10**]** and has been suggested by some to be more robust than conventional semen parameters as a predictor of outcome **[**9,11.12**]**.

In contrast, sperm DNA fragmentation has been shown to be an important biomarker of male infertility **[**13**]** Recent studies talks about Sperm DNA integrity are vital for successful fertilization, embryo development **[**14**]** and transmission of genetic material to the offspring. DNA fragmentation is the most frequent DNA anomaly present in the male gamete that has been associated to poor semen quality, low fertilization rates, impaired embryo quality, and preimplantation development and reduced clinical outcomes in assisted reproduction procedures.

In another word abnormal embryo and blastocyst development have been linked to poor sperm quality. specifically, sperm DNA fragmentation index (DFI) which decreased improvement of success rate for the results. Pilot studies showed that high levels of DNA fragmentation decrease fertility in patients using ART even in man with completely normal standard semen parameters. In this study we have analyzed sperm DNA fragmentation in candidates for ICSI in order to establish the relationships between the proportion of sperm with fragmented DNA and various factors, embryo development, blastolysion rate and the rate of ongoing pregnancies , and if testicular biopsy sperm extraction with patient has sperm DNA fragmentation is solution to improve the mention or not.

Materials and methods

Patient selection

A total of 418 couples undergoing ICSI for the first time between the years 2017 and 2019 were included in this study. This cohort represents the total number of couples who underwent ICSI in our unit, with no additional exclusion criteria. The couples were classified into three groups according to sperm source use in ICSI as follows:

- Group 1: men with normal semen parameters (sperm concentration ≥ 20 × 106/mL, motility ≥ 50% **[**types a and b, according to the WHO criteria**]** and morphology ≥ 30% normal forms), which served as the control group
- Group 2: men with high sperm DNA fragmentation use ejaculate sample use in ICSI.
- Group 3: men with high sperm DNA fragmentation use testicular biopsy sperm extraction.

Sperm collection and DNA fragmentation analysis

Semen analysis and SDF were among the investigations conducted on the male partner. Masturbation was used to collect sperm samples after 3–5 days of no intercourse. After allowing the sample to liquefy, the semen samples were analyzed following WHO protocols from 2010. (2010, 5th Edition). Then prepare the sample by DCG, SDF was determined using technic Test Kit This kit assesses the degree of DNA damage in a human spermatozoon via a process known as sperm chromatin dispersion (SCD), which is the root cause of male infertility. This method "involves denaturation and controlled lysis of the sample in an appropriate medium and can be used with both fresh and frozen samples." Spermatozoa with intact DNA produce a dispersion halo as a result of chromatin released by proteins, which can be easily observed using fluorescence or bright field microscopy. Spermatozoa with fragmented DNA, on the other hand, will not produce this halo. The procedure is as simple as a routine leucocyte count" **[**15**]** Using the Fernandez protocol, the SDF level cut-off was set at 30%. **[**16**]**

Assisted reproduction techniques

Controlled ovarian stimulation, oocyte recovery, and ICSI were all carried out exactly as described **[**14**]** On days 1, 2, and 5 following ICSI described scoring systems.**[** 17 **]** From two to on the day of the transfer, the 3 embryos with best-scoring were implanted in the patient's uterine cavity on Day 5 following ICSI.

Procedure TESA

The area around the cord was injected with a local anesthetic. In the middle and the upper and lower poles of the testes, a 23-G needle was used to aspire. When the needle came to the center and the aspiration was carried out at different angles at each puncture location, constant negative pressure was applied on the syringe. Sperm intake testicular was unilaterally performed in all patients. Since there was no major difference in the physical examination between the two tests, the proper test was selected for TESA according to the routine preference of the surgeon. The successful TESA procedure was accepted when sufficient numbers of sperm with normal morphology were obtained and discontinued. Pathologic examinations were not routinely performed. All TESA procedures were performed by the same senior urologist.

Embryo classification or blastocyst scoring

An embryo that has developed to blastocyst having two different landmarks (two cell components and fluid cavity).The Gardner blastocyst grading system allocates 3 distinct qualities to each blastocyst embryo, table 1, 2, and 3.

Blastocyst development stage – expansion and hatching status

Inner cell mass (ICM) scoring, or quality - Trophectoderm (TE) scoring, or quality

Table 1 blastocyst scoring according to development stage:

Expansion grade	Blastocyst development and stage status
1	Blastocoel cavity less than half the volume of the embryo
2	Blastocoel cavity more than half the volume of the embryo
3	Full blastocyst, cavity filling the embryo
4	Expanded blastocyst larger than the embryo, with thinning of the shell
5	Hatching out of the shell
6	Hatched out of the shell

Table 1 describes blastocyst development and stage status

Table (2) ICM grading

I cm grade	Inner cell quality
A	Many cells. Tightly packed
B	Several cells. Loosing grouped
C	Very few cells

Table 2 describe inner cell quality

Table (3) Trophectoderm grading

TE grade	Trophectoderm quality
A	Many cells, forming a cohesive layer
B	Few cells, forming a loose epithelium
C	Very few large cells

Table 3 describe Trophectoderm quality

Results

TESA vs. ejaculated spermatozoa ICSI outcomes, A comparison of the fertilization outcomes of two sequential ICSI attempts, the first with ejaculated spermatozoa and the second with testicular spermatozoa, revealed no significant difference in fertilization, cleavage rates, clinical pregnancy, or the proportion of embryos with good morphological appearance. The ovarian stimulation protocol was the same in both attempts, and no discernible differences in oocyte quality or quantity were observed. Using ejaculated spermatozoa, however, only one blastocyst formation was reduced as a result of sperm DNA fragmentation. This was in contrast to the results of the attempts using testicular spermatozoa.

1-The relation between sperm count, motility and morphology with sperm DNA fragmentation:

Fresh semen analysis and DNA fragmentation index (n = 370) table 4

Sperm parameters	Total Mean ±SD	DFI ≥30% (n=60)	DFI<30% (n=310)	*P* value
PH	7.03±0.27	7.05±0.27	7.13±0.29	0.384
Volume (ml)	1.55±0.89	1.51±0.77	1.68±0.89	0.253
Concentration (mil/ml)	34.11±13.58	32.12±13.15	34.35±14.13	0.122
Progressive motility (%)	22.77±13.25	15.11±13.59	32.29±13.6	0.049
Viability (%)	79.02±9.30	78.62±12.29	78.89±8.45	0.422
Abnormal morphology (%)	95.71±2.57	96.10±2.78	95.59±2.49	0.167

Table 4 The dependence of parameter variables (PH, volume, concentration, motility, morphology, and vitality) on the DFI variable is shown in table 3 .there was no correlation between DFI and PH, volume, concentration, and abnormal morphology but with motility. Where $p \geq 0.05$.

Cases items	Ejaculate sperm	Testicular sperm	*P* -value
Sperm DNA fragmentation	40.65±10.35	8.34±3.89	0.01

The p value = 0.01, the SFD deceased by testicular sperm extraction

Table 5 the difference between ejaculated sperm and testicular sperm extraction with high sperm DNA fragmentation.

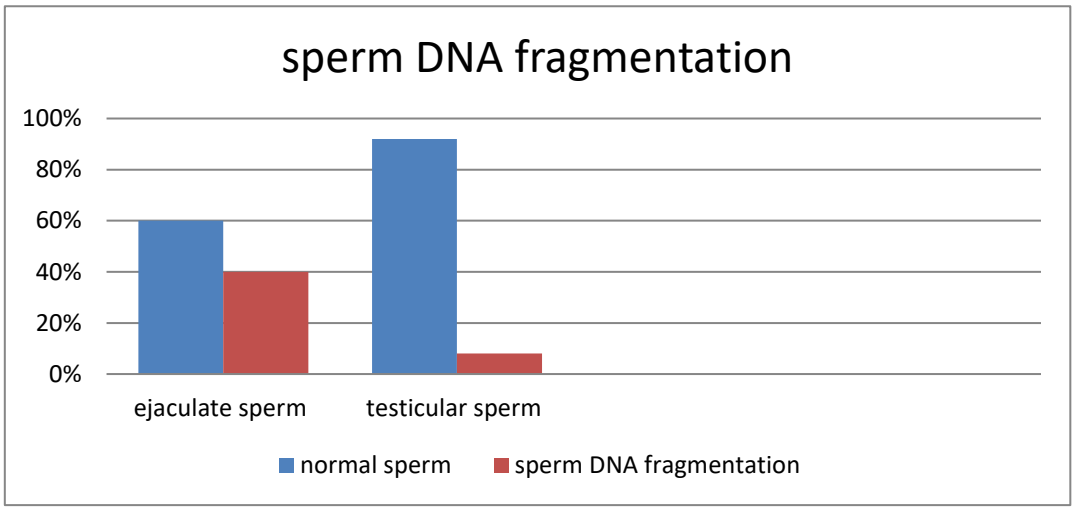

Figure 1 sperm DNA fragmentation percentage on a different source of sperm .The sperm DNA fragmentation decreased in testicular sperm extraction that appear with our results for the same cases have high sperm DNA fragmentation in ejaculated samples (figure 1)

	Ejaculated sample with high SDF	Testicular biopsy
Number of values	10	10
Minimum	30.00	8.780
25% Percentile	30.25	8.650
Median	40.00	8.000
75% Percentile	50.73	10.67
Maximum	78.00	18.00
Mean	40.00	8.067
Std. Deviation	10.00	1.155
Std. Error of Mean	5.774	0.6667
Lower 95% CI of mean	15.16	5.798
Upper 95% CI of mean	64.84	11.54
Sum	300.0	126.00

Table 6 for statistical analysis between Ejaculated sample with high SDF and Testicular sperm extraction. This test was done for 10 cases with high sperm DNA fragmentation by making the comparison between ejaculate sperm with high sperm DNA fragmentation and testicular sperm extracted for the same patients. our observation of the testicular sperm extraction decreased sperm DNA fragmentation. There is a significant correlation between sperm DNA fragmentation and testicular sperm where p =0.01.

Variable	Group A (n=310)	Group B (n=60)	Group C (n=48)	F(2,415)	*P-value
Age (years)	**30.6±5.3**	**29.0±6.0**	**28.6±6.1**	**4.193**	**0.016**
Sperm DNA fragmentation (%)	**12±7.3**	**50.3 ±14.6**	**54.2 ±13.3**	**2.072**	**0.153**

The relationship between wife`s age with testicular sperm extracted group age rate may be an effect on increase implantation chance group C data are significantly different at $p < 0.05$

Table 7. The relationship between wife`s age and value of sperm DNA fragmentation for different groups of sperm source

Variable	Group A (n=310)	Group B (n=60)	Group C (n= 48)	F (2,415)	P-value*
Number of injected oocytes	15.6 ± 8.7	13.4 ± 6.8	15.1 ± 7.8	1.71	0.181
Number of fertilized oocytes	12.6 ± 7.1	10.3 ± 6.3	12.3 ± 6.8	2.739	0.066
Number of divided oocytes	10.5 ± 6.0	8.7 ± 5.9	10.7 ± 6.1	2.471	0.086

Table 8. Effect of sperm DNA fragmentation on fertilized and divided oocytes in the three study groups

The rate of fertilization was not significantly different between groups A, B, and C ($p < 0.05$) numerically. The cleavage rate was also not different among the groups. No statistically significant difference was observed in embryo quality score between group a, group B, and C. Rates of embryo development on day 3 were similar in the three groups; as shown in Table 3, the degree of sperm DNA fragmentation and the fertilization rate, cleavage rate, embryo quality score, and embryo developmental rate were not correlated in the three groups.

Variable	Group A (n=310)	Group B (n=60)	Group C (n=48)	F(2,415)	P-value*
Total number of blastocysts	5.9 ± 4.1	3.3 ± 2.1	6.0 ± 4.4	11.043	<0.001
Number of late blastocysts	1.7 ± 1.7	1.3 ± 1.1	1.4 ± 1.6	1.851	0.158
Number of transferred embryos	3.0 ± 0.9	2.6 ± 1.0	2.7 ± 1.0	6.635	0.001

Table 9. Effect of sperm DNA fragmentation on blastocyst formation

Blastocyst formation depended on the quality of embryos which was seen on day 2 from OPU day, and the quality of embryos increased with integrity sperm was injected so from the previous table increase blastulation with low sperm DNA fragmentation or testicular sperm extraction in group A & C and decrease blastocyst formation in group B, even late blastulation rate in group B near to late blastulation in group A & C .because the embryo developmental considered delayed. From this table we are sure the sperm DNA fragmentation positive correlation effect in blastocyst formation on day 5 .it is attributed to the low number of embryos transferred due to the decreasing number of blastocyst formations.

Variable	Group A (n=310)	Group B (n=60)	Group C (n=48)	F(2,415)	P-value*
Fertilization rate (%)	83.4 ± 17.0	75.0 ± 18.5	81.8 ± 17.1	5.988	0.003
Division rate (%)	85.3 ± 16.4	85.0 ± 19.1	87.1 ± 19.1	0.249	0.780
Total blastolysion rate (%)	47.7 ± 18.1	38.3 ± 20.8	48.1 ± 20.7	6.522	0.002
Late blastolysion rate (%)	11.4 ± 11.1	12.0 ± 11.3	8.8 ± 9.3	1.324	0.267

Table 10. Comparison between the groups according of fertilization division blastocyst formation and late blastulation rate with percent data

In this table fertilization and total blastocyst formation effect in group B where increase sperm DNA fragmentation decreases the fertilization and blastulation with percentage, data were p-value with fertilization rate <0.05 and blastulation rate p-value = 0.02 less than 0.05.

Variable		Group A (n=310)	Group B (n=60)	Group C (n=48)	Test statistic	P-value*
Pregnancy test	*Negative*	140 (45.2%)	32 (53.3%)	23 (47.9%)	1.893	0.388
	Positive	170 (54.8%)	28 (46.7%)	25 (52.1%)		

Data are number (%) *. Fisher's exact test

Table 11. The biochemical pregnancy rate in the three study groups

The pregnancy test was not significantly different between the three groups were P > 0.05 (p = 1.893) regardless of which cases it was canceled.

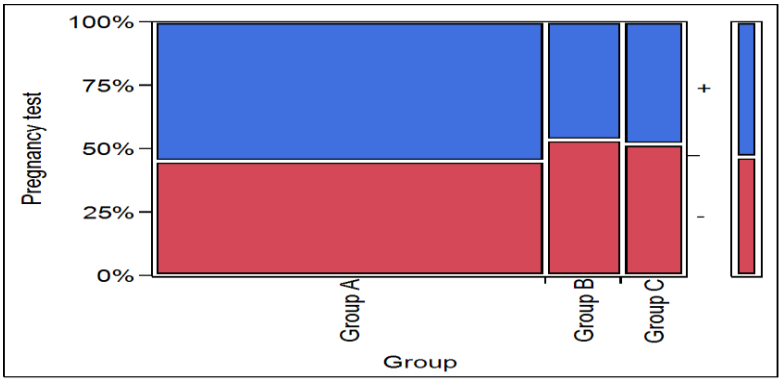

Figure (2) explains the relationship of pregnancy in the three groups subject to the laboratory study and the percentage of each group and shows the number of cases positive and negative of pregnancy examination

Graphical presentation of pregnancy rate in different groups Graphical presentations. No difference between the three groups where $p > 0.05$ so the result show not significant in pregnancy rate (figure2)

Variable	B	SE	Wald	P-value	Odds ratio	95% CI
DNA fragmentation >30% (=1)†	**-0.315**	**0.325**	**0.941**	**0.332**	**0.730**	**0.386 to 1.379**
Sperm collection by FNA (=1)‡	**0.010**	**0.402**	**0.001**	**0.980**	**1.010**	**0.460 to 2.220**
Age (years)	**-0.060**	**0.019**	**10.064**	**0.002**	**0.942**	**0.907 to 0.977**
Number of transferred embryos	**0.323**	**0.111**	**8.485**	**0.004**	**1.381**	**1.111 to 1.716**
Constant	**1.079**	**0.711**	**2.302**	**0.129**		

Table 12. effect of increase number of embryos which transferred and age factors with biochemical pregnancy.

B = regression coefficient, SE = standard error, Wald = Wald statistic, 95% CI = 95% confidence interval.

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

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

Table 12 shows the results of multivariable binary logistic regression analysis for determinants of biochemical pregnancy, Increase pregnancy chance with an increased number of embryos which transferred were p-value < 0.05 and odds ratio 1.381 and chemical pregnancy decrease with increased age wife factor were p-values < 0.05 but odds ratio less than 1 so it`s a negative effect. Patient's age

(odds ratio = 0.942, 95% CI = 0.907 to 0.977, P-value = 0.002) and number of transferred embryos (odds ratio = 1.381, 95% CI = 1.111 to 1.716, P-value = 0.004) are independent determinants of biochemical pregnancy. Overall, There was no significant change in obtaining clinical pregnancy, biochemical pregnancy in the group with an SDF <30% compared with the group with an SDF ≥of 30% in ICSI. But the significant difference between group B and control in blastulation and late blastulation where high sperm DNA formation decreases blastocyst formation. In addition in group C, no significant difference in all results compared with a control group.

Discussion

The correlation between ICSI clinical outcomes and sperm sources has long been a topic of contention and disagreement. Recent investigations have indicated that when ICSI is performed with Ejaculation, the clinical outcomes are better than when ICSI is performed with TESE, **[**18**]** . Many recent studies have found that the clinical outcomes of ICSI with TESE are equivalent to ICSI with Ejaculate regardless of blastocyst aneuploidy percentage. **[**19**]** Furthermore, these studies demonstrate that TESE's ability to promote embryonic development to the blastocyst stage is comparable to that of the Ejaculation sample, and that blastocyst transfer clinical results in cycles using TESE are equivalent to being in cycles using Ejaculate sample because the sperm DNA fragmentation decreased with testicular sperm extraction. That improvement our study the blastulation rate increased with testicular sperm extraction than ejaculate sample with high sperm damage table (9). but we observed low blastocyst formed with ejaculation sample with high sperm DNA fragmentation even with Select normal sperm which decreased or minimize sperm DNA fragmentation, **[**20**]**. Supposed that in ICSI a trial of choosing a motile and a morphologically normal sperm, which has a possibility to have an intact DNA. But a normal sperm could have a damaged DNA .As an intensified DNA fragmentation is linked to abnormal sperm characters and abnormal motility , as concluded by the studies by **[**21-22**]** Table 4 .

This study observed has a negative impact on fertilization rate between the two groups, table 9.10 which disagreement with the findings of **[**24**]** as table 8 who all showed that fertilization can be achieved even with sperm with high DFI. This is since maternal DNA is primarily responsible for fertilization and early embryo development. Paternal factors, on the other hand, are engaged in later stages of embryo development, which may be seen in embryo quality, and pregnancy rate, and no difference in pregnancy outcome due to the ability of the oocyte to repairing sperm damage that increased with maternal age where when maternal age was younger the ability increased and amount

of sperm damage play role with variability in the case in its sperm damage take chance with some oocytes which retrieved that agreement with our results numerically as table 8 only, but disagreement with results percentage (Table 10)that more accurate correlation, and maybe that depends on the amount of sperm damage and the number of oocytes which retrieval during ovum pick up table 7, 11 and 12. **[**14- 25**]** .Oocyte DNA repair mechanisms may theoretically explain this. The ability of oocytes to repair damage to sperm DNA is tightly correlated to female age and oocytes can only repair a limited amount of sperm DNA damage, since, among the group, there's depend on female age and number of blastocysts which transfer table 7 and 12. That agreement with **[**14, 23**]** previous studies, that the oocytes can repair the damaged sperm DNA.

In most studies, When DNA is slightly fragmented, sperm might be self-repaired then resumes the capability to fertilize the oocyte continue development, the oocyte is also capable of repairing damaged sperm DNA, on the other hand when the oocyte equipment is not adequate for repairing DNA damage , the embryo could not develop nor implant in the uterus. **[**14 -26- 27**]** proposed that the paternal genome is inactive during early embryonic growth stages (pre-fertilization, fertilization) and becomes actively tangled at an advanced stage (D3 cleavage-stage onwards) of embryonic growth, a singularity that was named as 'late paternal effect'. **[**27**]**, The late paternal effect on embryo development is positively associated with sperm DNA fragmentation .**[** 27, 28**]** , Sperm DNA fragmentation has also been positively connected with advanced paternal age in infertile couples **[**29**]**, Asthenozoospermia **[** 30 **]** as well as low testicular volume **[**31**]**. sperm DNA fragmentation is not routinely measured in practice. A cleavage rates, low fertilization , and markedly diminished embryo quality and blastocyst formation were noted , especially when testicular sperm from OA and NOA males were utilized for ICSI , This is due to testicular spermatozoa are less mature and subsequently less capable for fertilizing than the ejaculated ones as the final steps of sperm maturation occur in epididymis **[**32**]** Moreover, researches revealed a higher possibility of chromosomal aneuploidies in testicular spermatozoa from NOA patients as compared to OA patients.

This may explain, at least partly, the lower embryo development rates observed in this group of patients, in the present study. That agreement with our presented study where no difference between the control group and a group of testicular sperm extraction as a table (8, 9, 10 and 11). After blastocyst transfer, DFI did not affect clinical outcomes as Blastocyst transfers give a better a pregnancy rate than embryos transferred at an earlier stage (day 2 or 3), where human blastocysts developed in vitro have been reported to achieve high implantation rates **[**33,34**]** We observed a negative impact for sperm damage on blastocyst formation table (4) that resulted in the effect of paternal genome expiration after division 6-8 cells which decreased blastulation inversely proportional with the number of retrieved

oocytes. One of the most reasons which decrease blastocyst formation rate is the embryo cell block. The developmental block was triggered by apoptosis, which began after the second or third embryo cleavage. Basic symptoms, such as nuclear fragmentation, of apoptosis and aberrations in the spindle formation of microtubules. **[**35, 36**]**. blastulation rate increased with testicular sperm extraction more than ejaculate sample with high DNA damage, table 9 because of decreased sperm damage table 5,6 which increased in epididymis by computation of sperms, and testicular sperm extraction similar to normal ejaculate sample parameters, that agreement with **[**36**]** reported that clinical outcomes of single blastocyst transfer after ICSI using epididymal or Testicular sperm extraction were similar to those of standard ICSI using normal Ejaculate sample table 8,9,10, 11 and 12. **[**37**]**

Conclusion

The capacity of TESE to assist embryonic development to the blastocyst stage was compared to that of Ejaculate in patients with significant sperm DNA fragmentation in this study. The clinical outcomes of blastocyst transfer in ICSI cycles utilizing TESE were comparable to those in ICSI cycles utilizing a normal Ejaculate sample with less than 30% sperm damage. As a result, the current research is significant. When the appropriate number of oocytes are obtained and motile sperm are found in ICSI cycles utilizing TESE, the current study suggests that blastocyst transfer may be explored.

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