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The Effect of Sperm DNA Fragmentation on Invitro Fertilization: From Embryo Development to Clinical Pregnancy Success

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ABSTRACT

The prevalence of infertility affects reproductive-aged couples worldwide, with male characteristics responsible for nearly half of the cases. Traditional semen analysis often fails to diagnose male infertility, as infertile men exhibit normal results. Sperm DNA (Deoxyribonucleic acid) fragmentation (SDF) has gained attention for its diagnostic value in predicting male fertility outcomes, especially in intracytoplasmic sperm injection (ICSI) procedures. The proposed study investigates the effect of SDF on fertilization rates, clinical pregnancy results, and embryo quality. A total of 202 couples were selected from Yana Women Hospital and Fertility Centre, and other hospitals in Kerala. The DNA fragmentation index (DFI) values of the test group (DFI $>$ 30) and the control group (DFI $<$ 30) were used to separate the participants into two groups. The number of retrieved oocytes and MII oocytes showed no significant difference. The number of high-quality embryos was found to be negatively correlated with sperm DFI. The results demonstrated that there was an inverse relationship between clinical pregnancy rate and DFI, which was statistically significant ($p = 0.001$). Additional findings presented that high DFI is significantly related to reduced sperm concentration, inferior morphology, and lower motility. Pearson correlation revealed an inverse relationship between high DFI and clinical pregnancy rate (r = -0.1316, p = 0.001), highlighting the effect of DNA integrity on reproductive success. Therefore, in assisted reproductive technologies (ART) cycles, high DFI levels signify lower fertility rates, clinical pregnancy rates, and embryo quality.

Keywords: *Infertility, Sperm DNA fragmentation, Embryos, Clinical Pregnancy, Intracytoplasmic Sperm Injection.*

1. INTRODUCTION

The incapability to conceive after a year of consistent sexual activity is termed infertility, and it impacts 8% to 15% of couples in the reproductive age range globally [1, 2]. Various methods of treating infertility have been developed and refined over time in an attempt to determine the most effective course of action for each infertile couple. Semen analysis, which examines several standard criteria such as volume of seminal fluid, pH, morphology, motility, and concentration, has been used to identify male infertility based on the study of human semen by WHO's laboratory handbook [3]. The study of male infertility relies heavily on these analyses [4]. Nonetheless, semen analysis results for 15% of infertile men are normal [5, 6], hence it is impossible to diagnose male infertility with certainty in these couples. Thus, SDF and its correlation with reproductive outcomes play a significant role.

According to recent studies, there is a possibility that there are molecular issues even if the sperm seems to be morphologically normal [7, 8]. SDF index (DFI), which is more diagnostic and predictive than routine semen analysis, is used to predict male infertility. DNA fragmentation status for male fertility is classified as "significant lack of (over 30%)," "reasonable (15-30%)," and "high (less than 15% DNA)." [9]. Several additional factors contribute to an increase in DFI, including seminiferous tubule's sperm apoptosis, spermiogenesis remodeling, chromatin packaging's epithelial defects, a rise in reactive oxygen species (ROS), poor diet, obesity, alcoholism, stress, drug use, smoking, and aging. Human spermatozoa typically travel from the testis to the epididymis without motility during sperm maturation. Sperm motility steadily increases when the sperm pass through the epididymis. Therefore, high levels of oxidative stress (OS) brought on as a consequence of imbalanced apoptosis result in reduced sperm motility or an excess of ROS during the sperm maturation procedure [10, 11]. Therefore, exactly how SDF or chromatin abnormalities occur in human spermatozoa is unknown.

In modern times, the SDF test is assessed using a variety of techniques. Sperm Chromatin Dispersion (SCD), comet assay, and Sperm Chromatin Structure Assay (SCSA) are among the assays that are accessible. The halo sperm test is a recently developed, enhanced test that is cost effective and produces more accurate findings than previous tests. In the halosperm test,

sperm with fragmented DNA do not form the same halo of fragmented DNA loops as sperm with non-fragmented DNA. Currently, SCD tests are also provided as commercial kits, which include all the necessary reagents to guarantee the assay's simple application and technical functioning as well as to produce precise and consistent findings in various clinical laboratories.

Numerous studies demonstrate a correlation between low pregnancy rates, poor fertilization rates, low-quality embryos, and ICSI outcomes. Although SDF levels appear to have minimal impact on early embryonic growth before the maternal zygotic change, they are associated with an advanced chance of embryo arrest, lower-quality embryos, and delayed progress to the blastocyst stage after the maternal zygotic transition [12, 13]. Therefore, laboratory measurement of SDF is more crucial than standard semen analysis prior to using sperm for ART. Additionally, this method improves ART outcomes and lessens sperm damage. Examining the effects of SDF on the rate of fertilization, clinical pregnancy outcome, and quality of embryos for infertile couples undergoing ICSI cycles is the aim of this study. The following are the study's primary contributions:

- Examine the SDF effect on embryo quality and fertilization rates in ICSI cycles for infertile couples.
- Evaluate the relationship between clinical pregnancy outcomes and SDF in ICSI cycles.
- Determine the potential benefits of SDF testing in improving ART outcomes for couples undergoing ICSI.

The paper organizes the remaining portion as follows: Section 2 covers existing studies linking DFI to ART success rates. Section 3 details participant selection, sperm analysis protocols, and embryo grading. Section 4 provides results of fertility rates, embryo quality, and clinical pregnancy outcomes between low and high DFI groups. Finally, Section 5 provides an interpretation of the findings, while Section 6 concludes the paper by highlighting the clinical relevance of DFI as a diagnostic tool.

2. LITERATURE REVIEW

Zhang et al. [14] explored the effect of sperm DFI on results in ICSI cycles and invitro fertilization (IVF)-embryo transfer (ET). Using a dataset of 61 cycles from infertile couples undergoing these treatments, the researchers measured DFI with SCD testing, categorizing participants into control ($DFI < 25\%$) and test ($DFI \geq 25\%$) groups, and examined correlations between DFI, ROS, and apoptosis through flow cytometry and immunofluorescence. Results indicated that high DFI was allied with reduced embryo quality, implantation, and live birth rates in IVF-ET cycles, while ICSI outcomes were less affected; however, limitations included a lack of significant findings regarding fertilization rates across both treatment types.

Wang et al. [15] examined the connection between sperm DFI and reproductive outcomes in 176 couples with unexplained infertility. Based on sperm parameters, the participants were split into a control group ($DFI < 25\%$) and an observation group ($DFI \geq 25\%$). Changes in clinical pregnancy, embryo quality, and live birth rate were then analyzed. Results showed that high DFI correlated negatively with the number of high-quality D3 embryos and live birth rates, but not with clinical pregnancy outcomes. However, limitations included a small sample size, reliance on the SCD method for DFI detection, and selection bias from focusing only on initial embryo transfers.

Qi et al. [16] analyzed the relation between semen parameters and sperm DFI, as well as the influence on IVF-ET outcomes, analyzing data from 159 couples undergoing IVF-ET. Participants were separated into two classes based on DFI levels ($< 15\%$ and $\geq 15\%$), with comparisons showing no significant differences in most reproductive outcomes but revealing lower sperm motility, morphology, and embryo quality in the group of high-DFI. Positive correlations between sperm count and DFI and negative correlations with morphology and sperm motility suggested that DFI serve as a predictive tool for early embryonic development, though broader reproductive outcomes remained unaffected. The limitations included a single-center study design and a relatively short observation period.

Li et al. [17] assessed the sperm DFI's impact on birth weight and miscarriage rates in patients undergoing IVF or ICSI, analyzing clinical data from 6,330 Chinese patients. The study employed smooth fitting curve analysis to stratify patients by DFI levels and revealed a negative relation between birth weight and DFI and a positive relation between higher miscarriage rates and higher DFI. Limitations included the study's retrospective design, potential selection bias.

Braga et al. [18] studied the impact of sperm DFI and oocyte dimorphisms on ICSI outcomes, using data from 2,942 fertilized oocytes from 525 patients treated between 2016 and 2019. Researchers compared fertilization, embryo quality, pregnancy rates, and implantation between low ($<30\%$) and high ($\geq 30\%$) DFI groups, found that high DFI combined with oocyte defects, like vacuoles and dark cytoplasm, led to poorer clinical outcomes, including increased miscarriage risk. Limitations included the retrospective design, exclusion of intermediate implantation rates, and variability in selecting sperm for ICSI.

Liu et al. [19] assessed the relationship between sperm DFI and male fertility, specifically its influence on IVF and ICSI outcomes, using data from 1,462 infertile men. The researchers classified participants into three groups based on DFI levels and analyzed correlations with semen parameters, sperm morphology, and antioxidant levels. The major result of the study was that higher DFI was associated with decreased sperm survival, concentration, and pregnancy rates.

The joint impact of conventional sperm DFI and semen parameters on conventional IVF results was investigated by Peng et

al. [20], utilizing a dataset of 1,258 couples from Guangzhou Medical University's Clinical Reproductive Medicine Management System. The researchers applied an unsupervised K-means clustering method to identify coexposure pattern groups, and for statistical analysis, the study employed a multivariate logistic regression model, and found that lower sperm motility, higher sperm DFI values, and reduced semen concentration were related to poor IVF outcomes. The limitations included the reliance on a single cohort.

Farag et al. [21] assessed the effect of SDF, seminal properties, and incubator type on pregnancy outcomes in Intracytoplasmic morphologically selected sperm injection (IMSI) and ICSI techniques, using a dataset of 140 couples with males exhibiting high SDF and a history of failed implantations or miscarriages. The researchers utilized a retrospective cohort design, comparing ICSI and IMSI groups, and found that the IMSI group achieved higher positive clinical pregnancies and cumulative fetal heart development, with humidified incubators further enhancing outcomes. The study's limitations comprised lack of randomization, small sample size, retrospective design, and potential missed data.

Chua et al. [22] assessed the influence of SDF levels on reproductive results in ART cycles, specifically comparing ICSI-only, IVF-only, and IVF–ICSI Split cycles using data from 1148 males across 1600 ART cycles between 2013 and 2022. The researchers employed a retrospective cohort design and found that SDF levels < 15% were related to live birth rates and higher clinical pregnancy, particularly in women under 35 years, with IVF showing the best outcomes in this group. However, the study was limited by mismatched group sizes and the private clinical setting.

In male participants with high SDF, Zhou et al. [23] analysed the clinical and embryological results of ICSI cycles utilizing testicular versus ejaculated sperm. A total of 73 ICSI cycles were analyzed, showing that while testicular sperm had significantly lower SDF levels and a higher blastocyst formation rate, no substantial differences were found in embryo quality, fertilization rates, or clinical pregnancy rates between the two groups.

Although existing studies highlight the relation between high DFI and poorer reproductive outcomes in ART, several research gaps persist. Most studies focus on isolated outcomes such as embryo quality or pregnancy rates, with limited exploration of long-term effects, such as miscarriage rates or birth weight. Furthermore, while methods like SCD testing are commonly used to measure DFI, variability in detection techniques and threshold values may impact findings. There is also a lack of comprehensive investigations that consider the combined influence of DFI and other semen parameters, as well as the effectiveness of different ART protocols in high-DFI cases.

3. MATERIALS AND METHODS

3.1 Study Population

The proposed study selected 202 infertile couples undergoing ICSI cycles. All cycles were selected from Yana Women Hospital and Fertility Centre, Ulloor, Trivandrum, and also recruited from multiple hospitals in Kerala. The participants were chosen based on certain specific selection criteria to ensure the homogeneity of the study. Women were between the ages of 21 and 50, and men were between the ages of 24 and 55. These age groups allowed the study to focus on couples within the reproductive age group. Indications for IVF were also selected as criteria. In order to represent the complicated and multifaceted nature of infertility problems encountered by many couples, these indicators included a range of infertility diagnoses. Indications for IVF were male-factor infertility, tubal factors, unexplained infertility, and endometriosis. The individuals with uterine factors, polycystic ovary syndrome (PCOS), and progressive maternal age were also included in the study. If they were normal responders, ICSI cycles were included, that at least one 2PN zygote was obtained, and 4 to 15 mature oocytes were retrieved. Participants with significant uterine abnormalities or weakened ovarian reserve were not included in the research. The Yana Women Hospital and Fertility Center's ethical committee in Ulloor, Trivandrum, India, provided its approval to this retrospective study.

3.2 Ovarian stimulation and Oocyte retrieval

The ovarian stimulation procedure in this study was carried out using controlled ovarian hyperstimulation (COH), a method intended to encourage the formation of many follicles in order to rise the mature oocytes number offered for fertilization. COH is a common approach in ART that utilizes hormonal medications to induce a robust ovarian response. In this study, with recombinant follicle stimulating hormone (FSH) (NEWMON R, LG Life Science India and Folisurge, Intas Pharmaceuticals Ltd. India) and highly purified human menopausal gonadotrophin (HMG) (IVFM, LG Life Science India), ovarian stimulation was achieved. Transvaginal ultrasonography was used to track the patients' follicular response, which was utilized for adjusting the gonadotropin dosage.

To prevent premature ovulation and allow the eggs to mature adequately, pituitary downregulation protocols were employed to suppress natural hormonal activity in the ovaries in multiple cycles. Two downregulation protocols were used based on individual patient characteristics. During the stimulation phase of the Flexible GnRH Antagonist Protocol, a regular dosage of GnRH antagonist (Ciscure 0.25 mg, Emcure Pharmaceuticals, India) was administered. The antagonist works by quickly suppressing the luteinizing hormone (LH) surge, which prevents premature ovulation and enables controlled maturation of the oocytes. The flexible nature of this protocol allows for adjustments based on the patient's specific response, which can

be beneficial for females at higher risk for ovarian hyperstimulation syndrome (OHSS). In the Midluteal Long GnRH Agonist Protocol, beginning in the mid-luteal phase, patients following this protocol were given a daily dose of a GnRH agonist (Busarlin, 0.3 mg per day, Intas Pharmaceuticals, India). Unlike the antagonist protocol, which provides immediate suppression, the GnRH agonist protocol initially stimulates an increase in gonadotropin release before suppressing it. The midluteal long protocol is typically employed for patients with specific ovarian characteristics, offering a steady suppression of pituitary activity and improved control over the timing of ovulation.

Patients were carefully monitored during the ovarian stimulation procedure in order to evaluate their response and ensure optimal follicular growth. Frequent transvaginal ultrasound imaging was used for monitoring, enabling the assessment of follicular diameter and observing the growing follicles. Additionally, serum estradiol levels were measured periodically, as estradiol levels provide an indicator of follicular activity and ovarian response to stimulation. Adjustments to the gonadotropin dosage were made based on these evaluations, with the goal of achieving controlled ovarian stimulation without overstimulation. As part of the long protocol, 250 μg of recombinant human chorionic gonadotropin (hCG) (Ovidrel, Merck-Serono) was managed subcutaneously when the leading follicle reached a mean diameter of ≥18 mm or two follicles had a mean diameter of ≥17 mm. For the antagonist protocol, a 0.5 mg/d injection of decapeptyl was administered. The final step in the stimulation process involved triggering ovulation and subsequently retrieving mature oocytes. Anesthesia was used to accomplish oocyte retrieval 35–36 hours following hCG injection. In vitro maturation media were used to cultivate immature oocytes if they could be recovered. They were utilized in the ICSI process after being counted as mature oocytes (metaphase II stage-MII).

3.3 Semen analysis

Semen samples were collected using a standardized protocol to ensure consistency and reliability in the results. All male participants were instructed to collect their samples through masturbation after a recommended period , typically between 2 and 7 days of sexual abstinence. This abstinence time is recommended to ensure optimal sperm parameters, as both prolonged abstinence and frequent ejaculation can affect semen quality. The collected samples were deposited into sterile, tightly sealed containers provided by the laboratory. Semen analysis done by microscopic examination assessed the sperm count according to strict WHO 2021 criteria.

This standardized approach provided reliable, clinically relevant insights into various semen parameters, including motility, concentration, volume, and morphology. The primary aspect analyzed was semen volume (mL). Semen volume can be influenced by various factors, including hydration levels, abstinence period, and even certain medical conditions. Sperm concentration, or the density of sperm cells per milliliter of semen, was also measured. Low sperm concentrations (oligospermia) are associated with reduced fertility potential, while extremely low concentrations may indicate underlying issues affecting sperm production. The sperm motility parameters are analysed manually by counting under phase contrast microscopy. Progressive motility and nonprogressive motility are the two classes of sperm motility. Progressive Motility includes sperm with a forward movement, essential for successful fertilization, whereas nonprogressive motility exhibits movement but lacks the linear progression necessary to navigate through the female reproductive tract. Normal sperm morphology is defined as the presence of sperm with the typical head shape, size, acrosomal integrity, and mid-piece and tail structure. As a result, typical sperm have a semen volume of at least 1.5 mL, a morphology of at least 4%, and a motility of at least 40% with a concentration of at least $15 \times 10^6/mL$. Figure 1 illustrates the semen analysis.

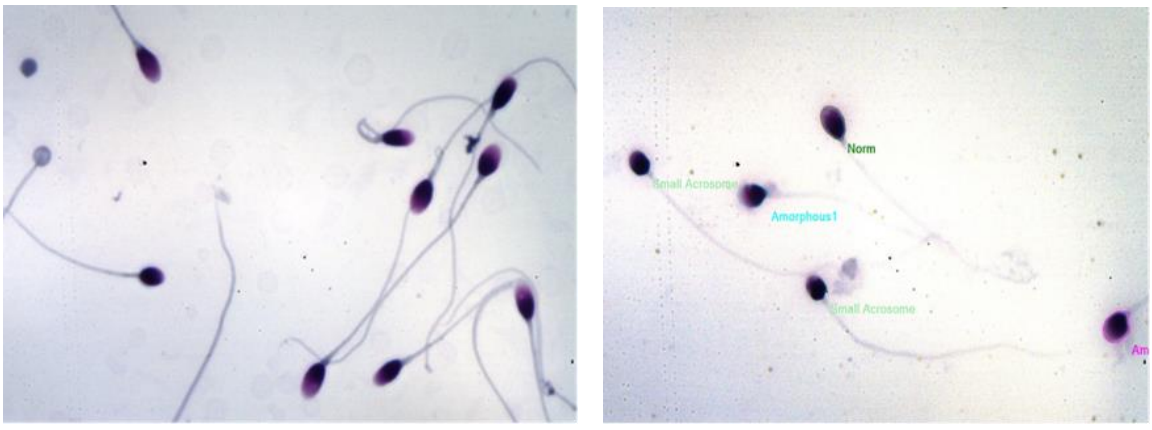

Figure 1. Semen analysis

3.4 SDF

This study evaluated SDF using the Halosperm test, a specialized assay that detects and quantifies DNA damage in sperm cells. SDF shows a crucial role in assessing male fertility, as damaged sperm DNA can negatively affect fertilization,

embryonic development, and pregnancy outcomes. The Halosperm kit was utilized to measure SDF based on the manufacturer's directions. A semen sample, prepared through double gradient washing, was aliquoted for testing. An agarose gel and a tiny Eppendorf tube from the kit were heated at 90 to $100°C$ in a water bath for five minutes, and then they were cooled for five minutes at $37°C$. Following, the mixture of 15 μL was positioned on a pre-coated slide and then enclosed with a coverslip. 25 μL of the semen sample had then been provided to the Eppendorf tube and mixed. To enable the sperm to embed in the microgel, the slides were chilled for five minutes at 4°C. After refrigeration, the coverslips were carefully detached, and for seven minutes, the slides were placed in an acid denaturation solution. The slides were then preserved with a lysis solution for twenty-five minutes. After five minutes of washing in distilled water, they were dehydrated for two minutes each using increasing ethanol concentrations (70, 90, and 100%) before being allowed to air dry. To visualize the sperm, with Diff-Quick solution (solutions I and II), the slides were stained, washed with distilled water, and air-dried at room temperature. At least 300 sperm cells were scored after the stained slides were observed under a light microscope set to 100 × magnification. Sperm cells were classified based on the presence of a halo around the sperm head: large and medium halos specified intact DNA (no fragmentation), while small halos, the absence of halos, or degraded sperm were indicative of DNA fragmentation. Degraded sperm exhibited weak or irregular staining patterns similar to sperm with no halo. The DFI represents the sperm percentage showing DNA fragmentation in the sample as in Equation 1. Figure 2 provides a visual example of the SDF analysis results.

$$DFI\ (\%) = \frac{Number\ of\ sperm\ with\ fragmented\ DNA}{Total\ number\ of\ sperm\ analyzed} \times 100 \quad (1)$$

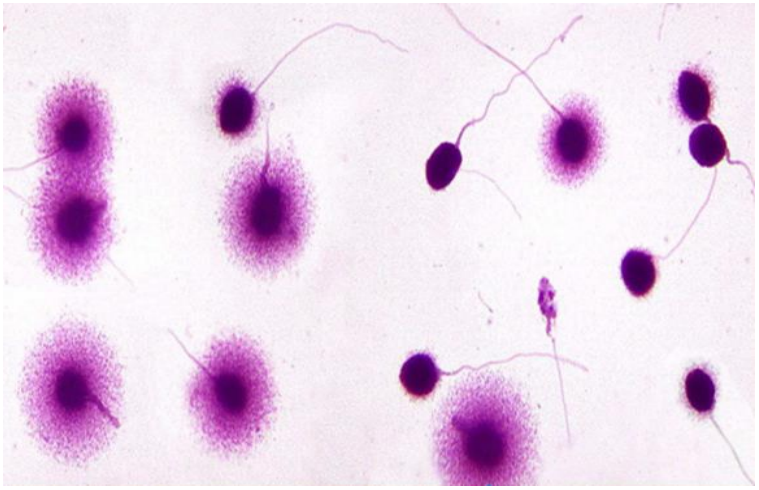

Figure 2. SDF

Samples in the current study were grouped according to DFI levels, using the 30% cutoff threshold. Samples possessing a DFI of $> 30\%$ were thus labeled as high DFI, while samples with a DFI level of $\leq 30\%$ were labeled as low DFI, exhibiting relatively undamaged DNA. This is supported by the findings that high DFI levels are associated with poor reproductive outcomes. These include poor-quality embryos, low fertilization rates, and also reduced pregnancy rates in assisted reproduction technologies.

3.5 ICSI procedure and embryo grading

ICSI is a highly specialized ART technique used to facilitate fertilization by injecting a single sperm cell directly into the cytoplasm of a mature oocyte. This procedure is particularly useful in cases of male infertility, where sperm quality, motility, or count may be insufficient for natural fertilization or conventional IVF. In this study, ICSI was performed to ensure fertilization using carefully selected sperm with high motility and normal morphology, maximizing the chances of successful fertilization. ICSI involves selecting the best sperm with high motility and good morphology, chosen under a microscope by the embryologist to ensure only the healthiest and most viable sperm were used, and is injected into a fertile ovum-forming zygote. Once an ideal sperm cell is identified, a pipette with a sharp end is used for immobilizing the sperm and picks up the immobilized sperm into the pipette.

During the ICSI procedure, the embryologist uses a glass micropipette for holding the mature eggs. The embryologist carefully inserted the injection pipette holding the immobilized sperm through the oocyte's zona pellucida, an outer protective layer, and then into the oocyte's cytoplasm. Aspiration of the ooplasm was performed with a pipette for activating the oocyte for fertilization and injecting the sperm along with the ooplasm into the cytoplasm of the oocyte. After injection, the needle is withdrawn from the oocyte. Next-day oocytes with two pronuclei (2PN) and two polar bodies (PBs) at 14 to 18 hours post-ICSI were defined as fertilized oocytes. The presence of 2PN indicates that the male and female genetic materials have

successfully combined, and the oocyte has completed its fertilization process. Only these fertilized oocytes proceeded to the embryo culture stage.

The embryos were cultured and graded on day 3 of post-fertilization. Then the embryo quality is evaluated by morphological criteria, a widely used method in ART to estimate the developmental potential of embryos and select those most likely to result in successful implantation and pregnancy. In this study, embryos were graded according to size, fragmentation rate, and blastomere symmetry, which are key indicators of cellular health and developmental potential.

Table 1 illustrates the embryo grading. When endometrium reaches the appropriate thickness, typically after one month of oocyte retrieval, progesterone is given to the patient as an injection into a muscle (intramuscular) or as a vaginal gel or pill. The exact day to start with the progesterone is crucial, as it is related to planned thawing and transfer of the frozen embryos to create the best conditions for implantation.

Table 1. Embryo Grading Criteria

Embryo Grade	Characteristics	Expected Development Potential
Grade A	Equal-sized blastomeres, ≤10% fragmentation	High potential for blastocyst development and implantation
Grade B	Equal-sized blastomeres, 10-20% fragmentation	Good potential, viable for transfer with good outcomes
Grade C	Unequal blastomere sizes, 20-50% fragmentation	Moderate potential, used when higher grades are limited
Grade D	Irregular blastomeres, >50% fragmentation	Low potential, generally not recommended for transfer

Clinical pregnancy was confirmed through a two-step verification process. 14 days after the embryo transfer, serum hCG levels were measured. To confirm an intrauterine pregnancy, transvaginal ultrasound was performed on those who had a positive hCG test. The presence of one or more gestational sacs with a fetal heartbeat is clinical pregnancy. Figure 3 displays the cleavage-stage embryos, showing multiple blastomeres.

Figure 3. Embryos showing cell division in early development.

3.6 Statistical Analysis

Qualitative information was stated as frequency and percentage, whereas quantitative variables were presented as mean ±SD. The qualitative variables were compared using the Chi square test. When there were two categories, the t test was used to compare the quantitative variables; when there were more than two categories, the F test was used. A p value of 0.05 was considered statistically significant. To examine the relationships between sperm DFI and various reproductive parameters, including fertilization rate, clinical pregnancy rate, sperm concentration, motility, and morphology, Pearson correlation analysis was conducted. The statistical analyses were achieved using Jamovi version 2.3.28 software, ensuring rigorous and accurate data interpretation.

4. RESULTS AND DISCUSSION

A comparison of semen parameters and sperm DFI was performed between normal (DFI≤30) and abnormal (DFI>30) sperm types, as shown in Table 2. The distribution of these categories was then analyzed across various semen types, including Normal, AT (Asthenoteratozoospermia), OAT (Oligoasthenoteratozoospermia), SOAT (Severe Oligoasthenoteratozoospermia), and others.

Table 2. Sample Type and DNA Fragmentation

Sample type	DFICL				Total	
	Normal (DFI≤30)		High (DFI>30)			
	No	%	No	%	No	%
Normal	86	74.8	10	11.5	96	47.5
AT	11	9.6	22	25.3	33	16.3
OAT	4	3.5	12	13.8	16	7.9
SOAT	6	5.2	27	31.0	33	16.3
Astheno	6	5.2	8	9.2	14	6.9
Terato	2	1.7	5	5.7	7	3.5
Oligo terato	0	0.0	3	3.4	3	1.5
Total	**115**	**100.0**	**87**	**100.0**	**202**	**100.0**

The results, displayed Table 2, highlighted significant differences between normal and abnormal sperm types across different sample categories. Specifically, 74.8% of the samples in the normal DFI category were classified as "normal," while only 11.5% of samples in the high DFI group fell into this category. Conversely, categories such as SOAT showed a higher prevalence in the high DFI (31%) compared to that of the normal (5.2%). These findings underscore the correlation between elevated DFI levels and abnormal sperm types, with a significant Chi-square value of 83.5, $df = 6, p = 0.001$), confirming the statistically significant association between sperm DFI and semen abnormalities. Figure 4 provides the graphical visualization of sample type and DFI.

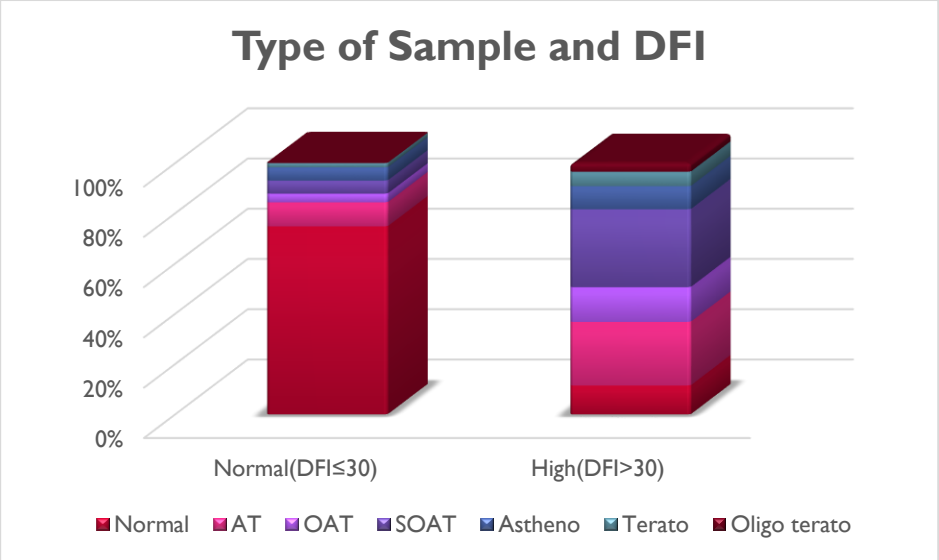

Figure 4. Graphical representation of sample type and DFI

The mean DFI values for different sample types also displayed significant differences as in Table 3. The samples with normal semen parameters had a mean DFI of 25.28 ($\pm$6.36), which was notably lower than the mean DFI of samples categorized

under SOAT, which exhibited a mean DFI of 37.24 ($\pm$8.16). Similarly, oligoteratozoospermia samples recorded the highest mean DFI of 55.00 ($\pm$16.09). This variation in DFI across different semen abnormalities further reinforces the impact of semen type on DNA fragmentation levels. Statistical analysis using the F-test generated a value of 13.6 with a $p-$value of 0.001, representing significant differences in mean DFI values across the groups. These results advise that higher DFI is commonly related to abnormal sperm parameters, and the degree of fragmentation varies significantly with semen abnormality type.

Table 3. DFI and Mean Comparisons

Sample type	N	Mean	Std. Deviation	95% Confidence Interval for Mean		Minimum	Maximum	F	P
				Upper Bound	Lower Bound				
Normal	96	25.28	6.36	26.57	23.99	12.0		13.6	0.001
AT	33	32.70	11.72	36.85	28.54	12.0	49.0		
OAT	16	35.25	12.75	42.04	28.46	12.0	57.0		
SOAT	33	37.24	8.16	40.14	34.35	20.0	63.0		
Astheno	14	32.00	10.63	38.14	25.86	12.0	56.0		
Terato	7	33.14	11.39	43.68	22.61	15.0	45.0		
Oligo terato	3	55.00	16.09	94.98	15.02	40.0	52.0		
Total	202	30.42	10.48	31.87	28.96	12.0	72.0		

The study examined the effect of sperm DFI on embryo quality and clinical pregnancy outcomes in cases where ICSI was employed as in Table 4. The results confirmed that high DFI had a negative influence on fertilization rates, embryo grade, and clinical pregnancy rates. For instance, individuals with a DFI$\leq$30 showed a fertilization rate of 76% (922 out of 1210 eggs), with 69.8% of these fertilized eggs graded as A (high-quality embryos). In contrast, the high DFI group exhibited a lower fertilization rate of 60% (590 out of 971 eggs), with only 47% achieving A-grade status, while 52.8% were classified as lower-quality B and C grades. Thus, the males with elevated DNA fragmentation show poor fertilization rate, poor grade of embryo, and a lower pregnancy rate in the ICSI procedure.

Table 4. Effect of DNA Fragmentation on Clinical Pregnancy and Embryo Quality

Factors	DFI$\leq$30	DFI>30
Fertilization rate % (embryos/ eggs)	922/1210=76%	590/971=60%
A grade Embryos%	644/922=69.8%	278/590=47%
B and C grade Embryos%	280/922=30.3%	312/590=52.8%
Clinical pregnancy rate in%	90/115=78.2%	18/87=20.6%

The Chi-square test exposed a significant association (Chi-square$= 33.8, df = 1, p = 0.001$), underscoring the statistically significant effect of higher DNA fragmentation on embryo quality and clinical pregnancy outcomes. These results clearly indicate that elevated DFI correlates with poor embryo quality, highlighting the critical role of DNA integrity in successful fertilization and embryo growth.

Table 5 illustrates the relationship between embryo quality and DFI. It shows the relationship between DFI and embryo quality. Of the embryos with a DFI $\leq$ 30, 72.2% had an embryo quality rating of $\geq$ 65, whereas only 27.8% had a quality rating of <64. In contrast, for embryos with a DFI > 30, a higher proportion (69%) had a quality rating of <64, and only

31% had a quality rating of ≥ 65. This suggests that embryos with a higher DFI are more likely to have poorer quality. Overall, embryos with lower DFI tend to have better quality, as indicated by the higher percentage of embryos with ratings of ≥ 65. The total number of embryos analyzed was 202, with 115 showing normal DFI and 87 showing high DFI. Figure 5 displays the DFI and embryo quality and demonstrates that a higher sperm DFI is associated with a significantly lower quality of embryos, as a greater proportion of embryos with high DFI show poor quality.

Table 5. DFI and Embryo quality

Quality Embryo Percentage	**DFI**				**Total**	
	Normal (DFI≤30)		**High (DFI>30)**			
	No	%	No	%	No	%
≥65	83	72.2	27	31.0	110	54.5
<64	32	27.8	60	69.0	92	45.5
Total	115	100.0	87	100.0	202	100.0

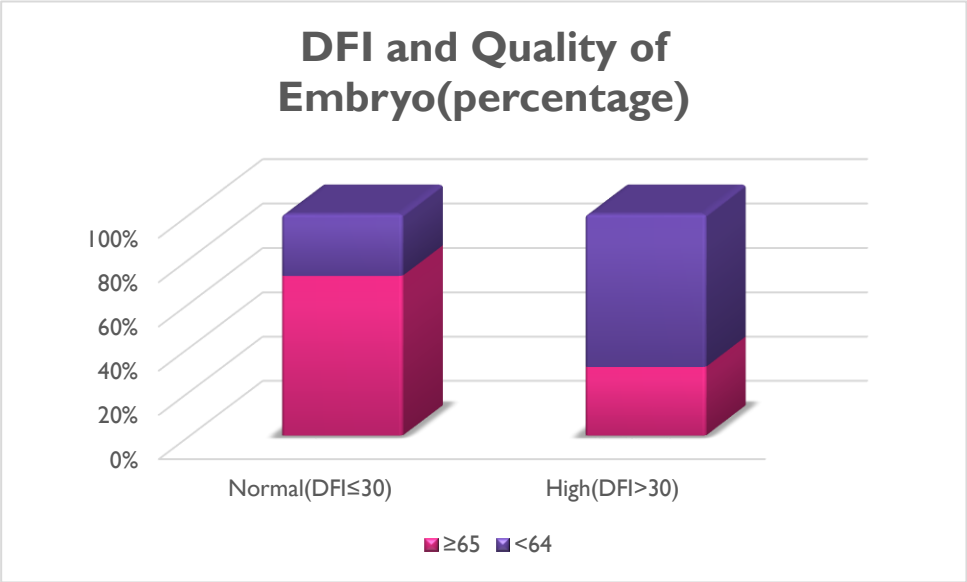

Figure 5. DFI and quality of embryo

Table 6 shows the effect of DFI on clinical pregnancy rates. An analysis of the clinical pregnancy rates further demonstrated the negative effects of high DFI on pregnancy outcomes. Among individuals with a DFI≤30, 79.1% achieved a positive pregnancy outcome, while only 20.7% of those with DFI>30 reached clinical pregnancy. Conversely, the negative pregnancy rate was substantially higher in the high DFI group (79.3%) compared to the normal DFI group (20.9%). This disparity underscores the negative correlation between DFI and pregnancy success rates.

Table 6. DFI and Clinical Pregnancy Rates

Clinical Pregnancy	**DFI**				**Total**	
	Normal (DFI≤30)		**High (DFI>30)**			
	No	%	No	%	No	%
Positive	91	79.1	18	20.7	109	54.0
Negative	24	20.9	69	79.3	93	46.0
Total	115	100.0	87	100.0	202	100.0

The Chi-square test (Chi-square= 68.1, $df = 1, p = 0.001$) confirmed that the difference in clinical pregnancy outcomes between the two groups was statistically significant. These findings underscore the detrimental effect of high SDF on clinical pregnancy rates, signifying that lower DFI levels are associated with improved chances of achieving clinical pregnancy.

Figure 6 provides the graphical representation of DFI and clinical pregnancy rates. This shows that DFI negatively correlates with clinical pregnancy and implantation rate.

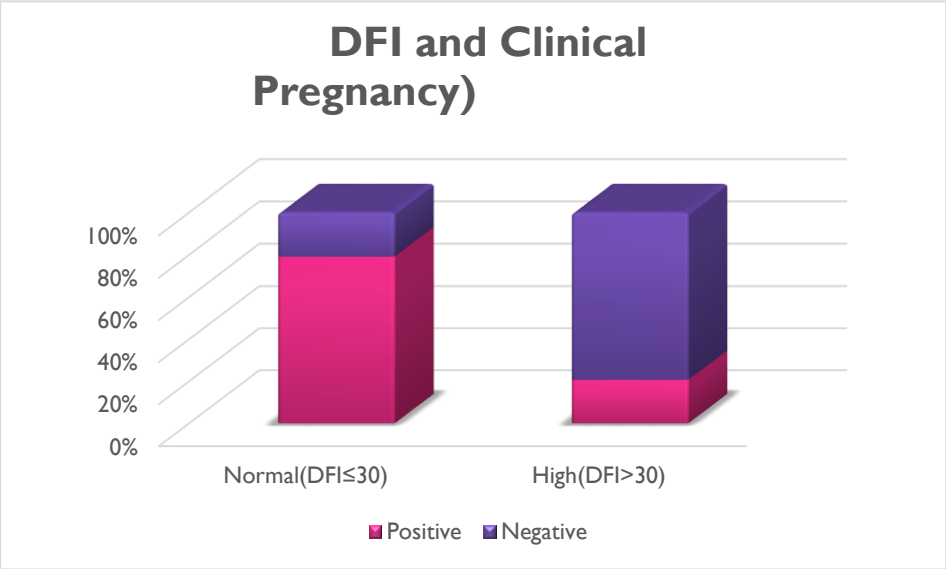

Figure 6. DFI and clinical pregnancy rate

In addition to clinical pregnancy rates and embryo quality, this study examined the relationship between various sperm parameters, including morphology, concentration, and motility, and DFI, as illustrated in Table 7.

Table 7. Comparison of Reproductive Parameters Between Normal (DFI ≤30) and High (DFI >30) Groups

DFI		**N**	**Mean**	**Std. Deviation**	**T**	**p**
Count	Normal (DFI≤30)	115	55.2	33.6	5.90	0.001
	High (DFI>30)	87	28.0	31.5		
Motility	Normal (DFI≤30)	115	40.2	13.3	7.10	0.001
	High (DFI>30)	87	24.3	18.3		
Morphology Normal	Normal (DFI≤30)	115	4.2	1.7	8.50	0.001
	High (DFI>30)	87	2.1	1.8		
MII	Normal (DFI≤30)	115	10.5	5.1	0.902	0.368
	High (DFI>30)	87	11.2	4.8		
No of embryos	Normal (DFI≤30)	115	7.9	4.6	1.90	0.048
	High (DFI>30)	87	6.7	3.6		
High Quality Embryo Percentage	Normal (DFI≤30)	115	72.3	21.4	7.90	0.001

	High (DFI>30)	87	45.3	27.1		

Results indicated that samples with high DFI had significantly lower values in key parameters, including motility, morphology, and high-quality embryo percentage, all of which were statistically significant with p-values of 0.001. Moreover, the fertilization rate, clinical pregnancy rates, and grade A embryo quality were notably lower in the high DFI group, highlighting a significant reduction in reproductive outcomes associated with higher DFI. However, no statistically significant differences were detected for MII oocytes between the two groups (p = 0.368). This proposes that elevated DFI levels are associated with compromised sperm motility, morphology, and embryo quality, all of which are crucial for successful fertilization and clinical pregnancy. Thus, high DFI levels is a useful indicator for predicting lower success rates in assisted reproductive treatments.

Table 8 provides the Pearson correlation results to analyze the outcome of SDF on IVF, embryo quality, and clinical pregnancy. The Pearson correlation analysis between various reproductive parameters and sperm DFI revealed several key findings. There was no significant difference observed in the number of oocytes or MII oocytes retrieved between low and high DFI groups, indicating that DFI did not impact oocyte retrieval. However, a negative correlation was found between high-quality embryos and DFI, suggesting that higher DFI is associated with fewer high-quality embryos. This relationship was statistically significant. Similarly, an inverse relationship between DFI and clinical pregnancy rate was observed, with higher DFI reducing the chances of clinical pregnancy, and this result was also statistically significant.

Table 8. Results of correlation analysis

Reproductive Parameter	**Correlation value**	**Interpretation**
Number of Oocytes Retrieved	0.1	No significant difference observed in the number of oocytes retrieved between low and high DFI groups (p = 0.368).
Number of MII Oocytes Retrieved	0.1	No significant difference observed in MII oocytes retrieved between low and high DFI groups (p = 0.368).
High-Quality Embryos	-0.1277	Negative correlation: Higher DFI is associated with a lower number of A-grade embryos, which is statistically significant (p = 0.001).
Fertilization Rate	-0.1807	Higher DFI is weakly associated with lower fertilization rates, but the correlation is not statistically significant.
Clinical Pregnancy Rate	-0.1316	Inverse relationship: Higher DFI tends to reduce clinical pregnancy rates, the correlation is statistically significant (p = 0.001).
Sperm Concentration	-0.43	Statistically significant reduction in sperm concentration for the high DFI group (p = 0.001).
Sperm Motility	-0.46	Statistically significant reduction in sperm motility for the high DFI group (p = 0.001).
Sperm Morphology	-0.40	Statistically significant reduction in sperm morphology for the high DFI group (p = 0.001).

In terms of sperm quality, significant negative correlations were observed between DFI and sperm parameters, indicating that higher DFI negatively impacts sperm quality.

5. DISCUSSION

SDF is increasingly known as a significant concern in male infertility, characterized by breaks in the DNA strands of spermatozoa [24]. This phenomenon can have profound implications for reproductive results, including fertilization success, pregnancy rates, and embryonic development [25]. It has been established that a notable proportion of infertility cases is due to male factors, often overlooked in traditional assessments that focus predominantly on sperm morphology and motility [26]. Increased SDF has been related to several adverse reproductive outcomes, including unsuccessful pregnancies, miscarriages, and a marked decrease in the efficacy of ART, such as IVF [27]. Understanding the relationship between SDF

and various semen parameters is essential for enhancing fertility assessment and treatment strategies.

Aitken and De Iuliis [25] highlighted the significance of SDF in studies demonstrating that, despite normal sperm morphology, elevated levels of DNA fragmentation can severely compromise reproductive potential. Zini, A., & Sigman, M. [27] indicated that increased SDF may disrupt fertilization processes, impair embryo quality, and contribute to early pregnancy loss. Consequently, there is a growing emphasis on evaluating sperm DNA integrity as a critical factor in the comprehensive assessment of male fertility [24]. Sakkas and Alvarez [28] noted that the identification and measurement of SDF have gained traction in clinical settings, offering a deeper understanding of male reproductive health.

Sperm quality is vital for successful embryo development, fertilization, and clinical pregnancy outcomes, and it also has a significant role in both assisted and natural reproductive cycles. To assess sperm quality, the DFI, which measures the range of DNA damage in sperm, has become a promising means for evaluating sperm health [28]. Studies have shown that increased SDF negatively affects fertilization rates. Furthermore, higher DNA damage is allied with a lower percentage of high-quality embryos and correlates negatively with clinical pregnancy rates. Numerous studies have reported that a higher DFI in sperm negatively impacts fertilization rates [29]. Additionally, embryos from men with high DFI are less likely to be of excellent quality and are more difficult to implant, which increases the risk of pregnancy loss [30, 31, 32]. However, some research has shown no significant differences in oocyte fertilization success, clinical pregnancy rates, early miscarriages, or embryo quality between high and low DFI groups [33, 34, 35]. Nevertheless, research by Zheng et al. has linked elevated sperm DFI to poor embryo quality, along with lower pregnancy rates and reduced fertilization rates in IVF cycles [36].

A study by Niu et al. revealed that while a high sperm DFI does not significantly impact fertilization rates or overall pregnancy outcomes in IVF, it does have a negative effect on embryo quality, particularly on the number of A-grade embryos and the development of blastocysts [36, 37]. Moreover, men with elevated DFI levels are more likely to experience pregnancy loss and early miscarriages compared to those with lower DFI [38]. Additional research by Simon et al. and Osman et al. has confirmed that augmented SDF negatively influences the success of both IVF and ICSI treatments [39, 40]. In a comprehensive analysis by Zhao et al., which pooled data from 14 IVF/ICSI studies with a total of 2,756 couples, it was found that higher SDF levels significantly raised the risk of miscarriage. Their findings suggested that in a fertility clinic performing 1,000 IVF/ICSI cycles annually with a clinical pregnancy rate of 40%, elevated SDF could lead to a reduction of approximately 80 pregnancies, thus decreasing the live birth rate by up to 15% [41]. Additionally, couples undergoing IVF/ICSI with higher SDF were found to have an increased risk of miscarriage compared to those with lower SDF levels. These conclusions were further maintained by a meta-analysis of 23 IVF/ICSI studies involving 6,771 cycles [42], which demonstrated that elevated SDF negatively affected increased miscarriage rates and clinical pregnancy rates, although it did not appear to significantly impact live birth rates (based on 10 studies with 1,785 couples) [43]. When SDF levels, as measured by the Comet assay, exceed certain thresholds, live birth rates are reduced for both IVF and ICSI patients, though the precise extent of SDF's impact on these outcomes is still under investigation [44, 45, 46].

6. CONCLUSION

The study demonstrates that higher sperm DFI levels are associated with significantly poorer reproductive outcomes in ICSI cycles, including reduced fertilization rates, lower embryo quality, early embryo development issues, and reduced clinical pregnancy rates ($p = 0.001$). The reduced live birth rates observed in the high DFI group are likely due to compromised embryo quality, emphasizing the effect of sperm DNA integrity on ART success. Sperm DFI has proven to be a valuable predictor not only of oocyte fertilization rates but also of clinical pregnancy outcomes and high-quality embryo formation, indicating the potential of DNA fragmentation analysis to enhance fertility evaluations. These findings strongly suggest the addition of SDF testing as a standard factor of semen analysis, as it offers insights beyond conventional parameters and allows for a more comprehensive assessment of male fertility. By identifying high DFI levels, clinicians could better develop treatment plans, optimize embryo selection, and ultimately improve ART outcomes for couples facing infertility. This study included a limited range of reproductive parameters without extensive analysis of female factors that could influence outcomes. Further research with larger sample sizes, multiple SDF testing methods, and a broader range of variables is recommended to strengthen the evidence base and improve management strategies in ART.

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