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Impact of Seminal Properties, Incubator Type, and Sperm DNA Fragmentation on Pregnancy Outcomes in ICSI and IMSI Techniques: A Retrospective Cohort Study

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Abstract

Background: The selection of the optimal Assisted reproductive technology (ART) technique is challenging in cases with low-quality sperm.

Objective: This research was designed to assess the impact of seminal properties, incubator type, and sperm DNA fragmentation on pregnancy outcomes in intracytoplasmic sperm injection (ICSI) and intracytoplasmic morphologically selected sperm injection (IMSI) techniques.

Materials and Methods: This retrospective cohort research involved 140 couples with males who had high sperm DNA fragmentation, at least one previous failure of implantation or previous miscarriages after ICSI, and women 40 years or younger at least 8 months of marriage without normal pregnancy who were enrolled in the ICSI or IMSI program. Participants were divided into ICSI (n=100) and IMSI (n=40) groups, further categorized by incubator type (humidified vs. dry) and SDF percentage (<20 vs. >20).

Results: There is no significant difference in sperm DNA fragmentation between ICSI and IMSI groups. The ICSI group had higher sperm concentration and motility, while the IMSI group had higher abnormal forms and lower acrosomal index. IMSI group had higher positive clinical pregnancies and cumulative fetal heart development. Cumulative fetal hearts were higher in humidified incubators. Higher sperm DNA fragmentation was associated with lower total motility and was a significant predictor of lower cumulative clinical results in ART at a cutoff value of 4.5%.

Conclusion: IMSI is effective in achieving positive clinical pregnancies and cumulative fetal heart development, particularly in cases with high sperm DNA fragmentation. Humidified incubators further enhanced outcomes. High sperm DNA fragmentation significantly predicted lower ART success.

Keywords: Intracytoplasmic sperm injection, Intracytoplasmic morphologically selected sperm injection, Sperm Quality.

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Introduction

Infertile patients with abnormal sperm parameters display an elevated sperm aneuploidy rate, despite a normal blood cell karyotype. Indications of male infertility, such as elevated follicle-stimulating hormone (FSH) levels, sperm concentration of less than 1 million per ml, and severe teratozoospermia, are significantly associated with higher numbers of aneuploid sperm (1).

Impaired sperm DNA integrity such as sperm DNA fragmentation or denaturation has been related to poor semen quality and may negatively affect embryo development and quality (2). The motile sperm organelle morphology examination (MSOME) allows the selection of the best sperm before oocyte microinjection (3).

There have been some reports on improved implantation and ongoing pregnancy rates using this sperm selection method, which is used for intracytoplasmic morphologically selected sperm injection (IMSI) (4). IMSI enables the selection of motile spermatozoa with fine nuclear morphology and without head vacuoles in real-time at high magnification (> 6000 ×), which are then injected into oocytes (4).

The IMSI method seems to improve the clinical outcome of sperm microinjection of oocytes in terms of embryo quality and development, and, consequently, the embryo implantation and pregnancy outcome (5, 6).

Additionally, the incubator type (e.g., time-lapse dry incubator (TLI) or non-TLI) used for embryo culture and maternal age are associated with oxidative stress. Previous studies have suggested that closed embryo culture systems using TLI improved embryo and clinical outcomes compared with those using non-TLI (1, 7).

There is a paucity of studies that directly assessed the different factors affecting intracytoplasmic sperm injection (ICSI) and IMSI Outcomes including seminal characters, incubator type as well as damaged DNA sperms. Therefore, the purpose of this study was to assess the outcomes of IMSI vs. ICSI and determine the clinical situations in which the use of this assisted reproduction technology is likely to be of greatest value. Also, the study aimed to examine whether humidified or dry benchtop incubators can affect ex vivo and ongoing pregnancy rates of human embryo development. The present study also focused on DFI and embryo development correlations at various stages of development even blastocyst formation and implantation rate.

Materials and Methods

This retrospective cohort research involved a total of 140 cycles of 140 couples with males who had mild or severe male factors with high sperm DNA fragmentation, at least one previous failure of implantation or previous miscarriages after ICSI, and women 40 years or younger, regular menstrual cycles of 25–35 days, two normal ovaries based on transvaginal scan findings, no polycystic ovarian syndrome, no known endometriosis, no gynecological or medical disorders, at least 1 year of marriage without normal pregnancy who were enrolled in the ICSI or IMSI program at Adam IVF Center over two years from 2021 to 2023.

The study was done after approval from the Ethical Committee of the Zoology Department, Faculty of Science, Al-Azher University, and from Adam IVF Center. All participants provided informed written consent.

Couples were excluded if either partner had a previous diagnosis of Human Immunodeficiency Virus (HIV) infection or Hepatitis B or C, patients with endometriosis and polycystic ovaries, and female partners over 42 years old.

In order to investigate the impact of seminal properties on the selection of the optimal ART technique, participants were divided into two groups: the ICSI group (n=100) and the IMSI group (n=40). Then, the cases were further categorized into two groups based on the type of incubator: the humidified group (n=70) and the dry group (n=70). This was done to determine the relationship between the type of incubator and the pregnancy outcome. Following this, the participants were further divided into two groups based on the SDF percentage: SDF<20 group (N=121) and SDF>20 group (N=19) to ascertain the impact of SDF on the ART outcome.

All participants' records were reviewed to obtain the patient's demographic data, laboratory investigations including seminal analysis, sperm DNA fragmentation, and clinical outcomes (fertilization, blastocyst, implantation, and pregnancy rates) of the sperm microinjection procedure, IMSI, or conventional ICSI.

Ovarian stimulation

Controlled ovarian stimulation was performed using GnRH agonist buserelin (Suprefact; Hoechst AG, Frankfurt/Main, Germany) administered from day 22 of the cycle in a daily dosage of 0.6 ml (600pg) s.c. After 14 days, the pituitary desensitization was checked by E2 determination and B-mode ultrasound scan. Once the criteria for desensitization were fulfilled (E2 ≤ 0.05 nmol/l,

follicles ≤ 5 mm in diameter and endometrial thickness ≤ 5 mm), ovarian stimulation with a daily dose of 225 IU highly purified urinary FSH (Gonal-Fâ; Serono, or Puregonâ; Organon) was started. GnRH agonist administration was continued until hCG administration. hCG (Pregnyl; N.V. Organon, Oss, The Netherlands) in a dose of 10,000 IU was administered when 3 or more follicles reached a diameter of 18 mm. Ultrasound-guided oocyte retrieval was performed 34-36 h after hCG administration.

Conventional sperm parameter assessment before an ART attempt

After 2-7 days of abstinence, a complete pure semen sample was collected in a sterile container in a laboratory room by masturbation. Within the initial thirty minutes post sample collection, spermatozoa concentration was obtained by the hemocytometer count chamber techniques, and a wet preparation technique was used to determine sperm motility. Phase contrast optics was used to examine the slides at a magnification of 400x and only morphologically complete sperm were evaluated. Sperm morphology determination was carried through an air-dried, fixed, and Spermic stained (Fertil Pro, Industrie park, Noord, Beernem, Belgium) preparation using brightfield optics. This study's semen analysis parameters were sperm count, morphology, and motility according to the WHO guidelines 2010 (8).

Measurement of DNA Fragmentation Index

The spermatozoa DFI was determined by using the Sperm Chromatin Dispersion test -Halosperm® Kit. Primarily a heated Eppendorf tube with Agarose was put in a bath of water maintained for 5 minutes at 100ºC then it was transferred to another bath of water maintained also for 5 minutes at 37ºC. Then a 50 µl of semen sample was added and mixed. Then 10 µl of the mixed solution was putted on a slide and covered by a covering slip then put on a cold surface for 7 minutes to solidify the Agarose. The covering slips were separated cautiously then slides were promptly immersed horizontally in a plate filled with freshly prepared acidic denaturing solution for 7 minutes at 22°C. Then denaturation was discontinued, and proteins were removed by slides transferal to a dish containing Solution 1 for neutralization and lysis kept at 37°C for 10 minutes, followed by incubation in Solution 2 for neutralization and lysis kept at 37 °C for 5 minutes. The slides were washed in Tris-borate EDTA buffer for 2 minutes, dehydrated by successive ethanol baths of 70%, 90%, and 100% (2 minutes for each), then air dried. Slide staining was done by Eosin for 8 minutes and by Azure B for 8 minutes. The slides were examined by the Oil immersion lens under a simple microscope for the detection of halos that were present around the sperm. Spermatozoa surrounded by halos of large or medium size, typical of dispersed DNA loops were considered to have intact DNA. Spermatozoa surrounded by small, degraded halos or absent halos were considered to have fragmented DNA. Percent of DNA fragmentation was calculated for each semen sample.

Semen preparation

Semen samples were collected by masturbation after 2–5 days of sexual abstinence and were processed for IVF after liquefaction for 15 to 60 min.

IMSI procedure

Preparation and selection of sperm for IMSI have previously been described (8). An aliquot of the sperm preparation was placed in a glass-bottomed dish (WillCo-dish, WillCo Wells BV, Amsterdam, The Netherlands) and examined by the Nomarski interference contrast microscopy with a Leica DFC-280 camera (Leica Microsystems, Nanterre, France) mounted on a Leica DMI 6000 microscope with an immersion objective lens ×100 and camera magnification ×1. Spermatozoa with the smallest relative vacuole area were preferentially selected. If available, spermatozoa without vacuoles or with only small vacuoles were preferentially injected.

Conventional ICSI procedure

Sperm selection for microinjection was performed at a magnification of ×400. Spermatozoa seen to have severe head defects at this magnification were excluded. The procedure of oocyte injection was the same in both the ICSI and the IMSI group and was performed at ×200 magnification using the Hoffman contrast.

Embryo culture

The injected oocytes were transferred to a four-well dish containing 50 µL of culture medium (G1Plus, Vitrolife, Göteborg, Sweden) overlaid with mineral oil (FertiCult Mineral Oil, Fertipro Belgium). The embryos were divided into two groups; alternate sibling embryos were placed in culture in either the humidified incubator or a dry incubator. The fertilization of the oocytes was checked the next day, 16–20 h after microinjection. Embryo quality was assessed on day 2 according to the Giorgetti

classification system (9). Embryos with a score of 3 or 4 have good morphology.

Blastocyst formation

An embryo that has developed into a blastocyst has two different landmarks (two cell components and a fluid cavity). Embryos resulting from ICSI in the IVF lab, which develops naturally in the uterus, usually reach the blastocyst stage on day 5 after insemination. Transfer blastocyst gives high pregnancy outcomes. The Gardner blastocyst grading system assigns three separate qualities to each blastocyst embryo: -Blastocyst development stage – expansion and hatching status Inner cell mass (ICM) scoring, quality Trophectoderm (TE) scoring.

Embryo transfer

ET took place on day 5 after oocytes were injected after scoring using wormed hot plat at 37 C⁰ under Zeiss Stereo Microscope and sterilized falcon dish 1006, for transfer, a 1 ml serial was filled with global total media 20 % with SSS incubated over 17 hours and removed the air bubbles, connect the filled syringe to cook transfer catheter after flushing the catheter the selected blastocysts were aspirated into a catheter, the catheter was passed through the cervical canal into the uterine cavity, the catheter was slowing injected, after which the catheter was withdrawn gradually, ET occurs under Ultrasound in the Operating room.

Assessment of Reproductive Outcome

The reproductive outcome was determined using both biochemical tests and ultrasonography and all women were followed up after their estimated date of delivery (EDD) as is standard practice at Fertility First. A cycle was then classified as successful with a serum Beta human chorionic gonadotropin (β-HCG) of ≥25 IU 18 days after ovulation or oocyte retrieval.

Statistical analysis

Statistical analysis was done by SPSS v27 (IBM©, Chicago, IL, USA). The Shapiro-Wilks test and histograms were used to evaluate the normality of the distribution of data. Quantitative parametric data were presented as mean and standard deviation (SD) and compared between the two groups utilizing an unpaired Student's t-test. Qualitative variables were presented as frequency and percentage (%) and analyzed using the Chi-square or Fisher's exact test when appropriate. Correlation between various variables was done using the Spearman rank correlation equation for non-normal variables/non-linear monotonic relation. The overall diagnostic performance of each test was assessed by ROC curve analysis, The area under the curve (AUC) evaluates the overall test performance (where the area under the curve >50% denotes acceptable performance and area about 100% is the best performance for the test). A two tailed P value < 0.05 was considered statistically significant.

Results

Comparing seminal analysis between ICSI and IMSI techniques

Table 1 shows that there is no significant difference in the age of female or male partners between couples undergoing ICSI or IMSI. The ICSI group had higher sperm concentration and motility, while the IMSI group had higher abnormal forms and lower acrosomal index, suggesting poorer semen quality in males. However, no significant difference in sperm DNA fragmentation was found.

Table 1: Participants' characteristics and seminal analysis of the studied cases

	Group (ICSI) N=100	Group (IMSI) N=40	P-value
Female Age (years)	33.2±5	34.65±5.4	.161
Male Age (years)	38.7±6.4	40.3±5.8	.172
Sperm Concentration (Million /ml)	57.5 (.1_280)	37.5 (.1_100)	.007
Total Motility %	35 (.9_85)	25 (.9_60)	.001
Abnormal Forms %	92.5±5.1	95.8±3.5	<.001
Acrosomal Index %	52.6±13.2	44.6±14.8	.002
Sperm. DNA Fragmentation %	9.9 (1.7_54)	11 (3_35)	.159

Data are presented as mean ± SD or median (max-min) or mean ± SD. IMSI: Intracytoplasmic morphologically selected sperm injection, ICSI: Intracytoplasmic sperm injection.

Clinical outcomes between ICSI and IMSI groups

Table 2 reveals no significant difference in fertility rates between the IMSI and ICSI groups. However, the IMSI group had a higher percentage of positive clinical pregnancies and cumulative fetal heart development, with 47.4% of pregnancies having

two fetal hearts compared to 33.3% in the ICSI group.

Comparing the effect of humidified and dry incubators on clinical outcomes

Table 3 no significant difference in the median number of fertilized oocytes and embryos between dry and humidified incubator groups. Fertilization and blastulation rates were similar between the two groups. The cumulative clinical results showed no significant difference in biochemical pregnancy, missed abortion, positive clinical pregnancy, or negative clinical pregnancy. Cumulative fetal hearts were higher in humidified incubators.

The effect of high SDF (>20) on seminal parameters

Table 4 found that female and male ages were significantly higher in the group with sperm DNA fragmentation (SDF>20) compared to those with SDF<20. Sperm concentration was also higher in the group with SDF>20 with no significant factor between groups. Total Motility % is significantly higher in the group with SDF<20 (35(.9-85)) compared to the group with SDF>20 20(.9-70) (p=.004). This means that as the sperm DNA fragmentation increases, the total motility decreases. This finding suggests that higher sperm DNA fragmentation is associated with lower total motility. Abnormal forms were higher in the group with SDF>20, but the difference was not statistically significant. Acrosomal Index was similar in both groups. (Figure 1)

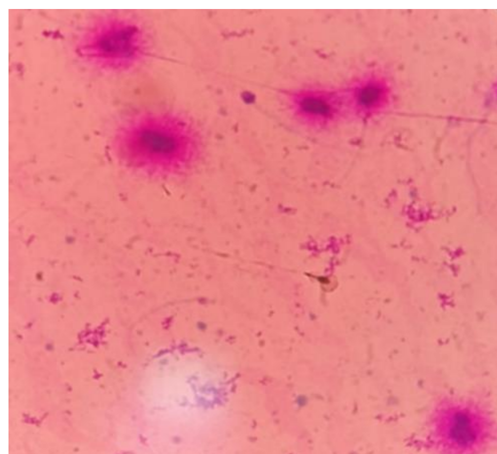

Figure 1. Microscopic sperm morphology with DNA fragmentation.

Table 2: Clinical outcomes between ICSI and IMSI groups

		Group (ICSI) N=100	Group (IMSI) N=40	P- value
No. of fertilized oocyte		5(1-20)	6 (1-35)	.202
No. of embryos		5(0-20)	6 (1-35)	.277
Fertilization Rate %		77.1±20.7	80.35±21.5	.414
Blastulation rate %		52.23±23.4	55.78±24.4	.491
ET Number	0	2(2%)	0	.416
	1	13(13%)	8(20%)	
	2	74(74%)	30(75%)	
	3	11(11%)	2(5%)	
Cumulative Clinical Result	Biochemical pregnancy	1(1%)	0	.359
	Missed abortion	6(6%)	1(2.5%)	
	Positive Clinical Pregnancy	33(33%)	19(47.5%)	
	Negative	60(60%)	20(50%)	
Cumulative Fetal Hearts (Sacs)	One	20(60.6%)	10(52.6%)	.386
	2 sacs	11(33.3%)	9(47.4%)	
	3 sacs	2(6.1%)	0	
Cumulative Transferred embryos		2(1-9)	2(1-6)	.376

Data are presented as median (max-min) or percentage (frequency). IMSI: Intracytoplasmic morphologically selected sperm injection, ICSI: Intracytoplasmic sperm injection. ET: embryo transfer.

Table 3: Effect of different types of incubators on pregnancy outcome

		Group (humidified incubator) N=70	Group (dry incubator) N=70	p-value
No. of fertilized oocyte		6(1-35)	5(1-25)	.262
No. of embryos		6(0-35)	5(1-25)	.316
Fertilization Rate %		77.9±19.9	78.1±22	.958
Blastulation rate %		51.2±25.3	55.2±26.5	.399
ET Number	0	1(1.4%)	1(1.4%)	.772
	1	12(17.1%)	9(12.9%)	
	2	52(74.3%)	52(74.3%)	
	3	5(7.1%)	8(11.4%)	
Cumulative Clinical Result	Biochemical pregnancy	1(1.4%)	0	.736
	Missed abortion	3(4.3%)	4(5.7%)	
	Positive Clinical Pregnancy	25(35.7%)	27(38.6%)	
	Negative	41(58.6%)	39(55.7%)	
Cumulative Fetal Hearts (Sacs)	One	16(64%)	14(51.9%)	.323
	2 sacs	9(36%)	11(40.7%)	
	3 sacs	0	2(7.4%)	
Cumulative Transferred embryos		2(1-6)	2(1-9)	.290

Data are presented as median (max-min) or percentage (frequency). ET: embryo transfer.

The effect of SDF on clinical outcome

Table 5 presented that the median number of embryos, Fertilization rates, and blastulation rates were significantly higher in the group with sperm DNA fragmentation (SDF<20) compared to the group with SDF>20. The ET was significantly higher in the group with SDF<20. Clinical results showed similar frequency of biochemical pregnancy, missed abortion, positive clinical pregnancy, and negative clinical pregnancy. The frequency of fetal hearts (sacs) was similar in both groups, with one sac being more common in the group with SDF<20 group and two sacs being more common in the group with SDF>20 group. However, the frequency of three sacs was higher in the group with SDF<20 group. The cumulative transfer of embryos was also similar in both groups.

Correlation between maneuver group (ICSI or IMSI), incubator type, and Sperm DNA Fragmentation % and outcomes

Table 6 showed that there was no statistically significant correlation between ICSI and IMSI maneuvers, incubator type, and SDF % and outcomes including cumulative clinical results, fetal hearts (sacs), and transferred embryos.

Receiver operating characteristic curve analysis for Sperm DNA Fragmentation % to predict Cumulative Clinical Result.

SDF% is a significant predictor of cumulative clinical result in ART at cutoff value of 4.5%, with a sensitivity of 88% and a specificity of 86%. The AUC was 0.73 (p=0.048). (Figure 1)

Table 4: Participants' characteristics and seminal analysis according to sperm DNA fragmentation

	SDF<20 N=121	SDF>20 N=19	P-value
Female Age (years)	33.2±4.8	36.3±6.3	.014
Male Age (years)	38.2±6.3	41.7±5.7	.056
Sperm Concentration (Million /ml)	50(.1-280)	40(.1-120)	.200
Total Motility %	35(.9-85)	20(.9-70)	.004
Abnormal Forms %	93.1±5	95.5±3.7	.049
Acrosomal Index %	50.9±14.3	46.6±12.5	.219
Sperm DNA Fragmentation %	9.3±4.1	27.4±8	<.001

Data are presented as mean ± SD or median (max-min). SDF: sperm DNA fragmentation.

Table 5: Outcomes between the studied groups according to sperm DNA fragmentation

		SDF<20 N=121	SDF>20 N=19	P-value
No. of embryos		6(1-35)	4(1-12)	.045
Fertilization Rate %		6(0-35)	4(1-12)	.050
Fertilization Rate %		78.8±20.4	72.7±23.9	.241
Blastulation rate %		58(0-200)	42(0-77)	.030
ET Number	0	1(.8%)	1(5.3%)	.017
	1	19(15.7%)	2(10.5%)	
	2	93(76.9%)	11(57.9%)	
	3	8(6.6%)	5(26.3%)	
Cumulative Clinical Result	Biochemical pregnancy	1(.8%)	0	.983
	Missed abortion	6(5%)	1(5.3%)	
	Positive Clinical Pregnancy	45(37.2%)	7(36.8%)	
	Negative	69(57%)	11(57.9%)	
Cumulative Fetal Hearts (Sacs)	One	25(55.6%)	5(71.4%)	.677
	2 sacs	18(40%)	2(28.6%)	
	3 sacs	2(4.4%)	0	
Cumulative Transferred embryos		2.65±1.2	2.5±1.1	.706

Data are presented as median (max-min) or percentage (frequency). ET: embryo transfer.

Table 6: Correlation between maneuver group (ICSI or IMSI), incubator type, and Sperm DNA Fragmentation % and outcomes

	Cumulative Clinical Result	Cumulative Fetal Hearts (Sacs)	Cumulative Transferred embryos
Maneuver group (ICSI or IMSI)	r=-.126	r=.049	r=-.067
	P=.139	P=.729	P=.428
Incubator type	r=-.015	r=.148	r=.0742
	P=.863	P=.295	P=.388
Sperm DNA Fragmentation %	R=.008	R=-.117	R=-.012
	P=.923	P=.409	P=.884

IMSI: Intracytoplasmic morphologically selected sperm injection, ICSI: Intracytoplasmic sperm injection.

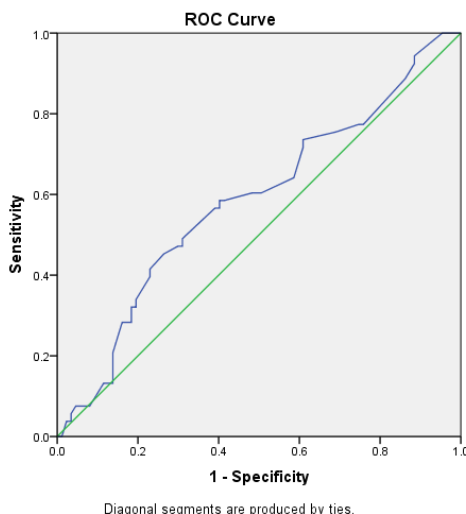

Figure 2. Receiver operating characteristic curve analysis for Sperm DNA Fragmentation % to predict Cumulative Clinical Result.

Discussion

The study aimed to enhance the success rates of IMSI and ICSI procedures to assess the outcomes of these procedures and determine the clinical situations in which the use of this assisted reproduction technology is likely to be of greatest value. Also, the study aimed to determine whether humidified or dry benchtop incubators can affect human embryo development ex vivo and the ongoing pregnancy rates. The present study also focused on DFI and embryo development

correlations at various stages of development even blastocyst formation and implantation rate.

The present study showed that the ICSI group had higher sperm concentration and total motility compared to the IMSI group, but there was no significant difference in sperm DNA fragmentation. The IMSI procedure allows for the selection of sperm with normal morphology and acrosomal status, which may be beneficial for males with poor semen quality (10).

Regarding the relation between the semen parameters and incubator type, the results showed that there are no significant differences in sperm concentration, total motility, abnormal forms, acrosomal index, and sperm DNA fragmentation between the Humidified and dry incubator groups ($p>.154$).

The median (max-min) sperm DNA fragmentation in the humidified incubator group is 9.3 (2-35) %, and in the dry incubator group is 11 (1.7-54) %. The results suggest that there is no preferred type of incubator over another for use according to the seminal characteristic.

Moreover, our study clarified the impact of SDF% on seminal characteristics that can affect ART success. The study found that sperm concentration was not significantly different between the group with low sperm DNA fragmentation (SDF<20) and the group with high sperm DNA fragmentation (SDF>20), suggesting that sperm DNA fragmentation may not be a significant factor in sperm concentration.

However, total motility was significantly higher in the group with low SDF, indicating that higher sperm DNA fragmentation is associated with lower total motility. The percentage of abnormal forms was slightly higher in the group with high SDF, but the difference was not statistically significant.

Several studies have reported a negative correlation between sperm DNA fragmentation and semen parameters, including sperm concentration, motility, and morphology (11, 12).

A recent meta-analysis also found that high sperm DNA fragmentation is associated with lower pregnancy rates and higher miscarriage rates (13). Therefore, assessing sperm DNA fragmentation may be important for male fertility evaluation and treatment.

In the present study there is no significant difference in the number of fertilized oocytes, embryos, fertilization rate, and blastulation rate between the IMSI and ICSI groups. Moreover, the study found that outcomes were better in low sperm DNA fragmentation group (SDF<20) compared to the group with high sperm DNA fragmentation (SDF>20)

Several studies have reported a negative association between sperm DNA fragmentation and embryo development, including blastulation rate (14, 15).

A recent meta-analysis found that sperm DNA fragmentation is negatively correlated with pregnancy rates and positively correlated with miscarriage rates (16). Our study supports these findings, as we found that higher sperm DNA fragmentation was associated with a lower blastulation rate.

According to our results, we found no correlation between sperm concentration, morphology, motility, and blastocyst development. However, Zhou et al. (17) reported that oocytes injected with sperm from an ejaculated sample with high progressive motility had a significantly high chance of reaching the blastocyst stage they also found that no effect of SDF on sperm parameters (except motility and progressive).

The present findings suggested that the use of a humidified incubator does not significantly affect the number of fertilized oocytes or embryos, as well as the fertilization and blastulation rates, compared to a dry incubator. This is consistent with previous studies that have reported no significant differences in embryo development or pregnancy rates between humidified and dry incubators (18).

Our observation showed that the Blastulation rate % is significantly higher in the group with SDF<20 (58(0-200)) compared to the group with SDF>20 (42(0-77)) ($p=.030$). This finding suggests that higher sperm DNA fragmentation is associated with a lower blastulation rate.

Blastocyst formation depends on sperm selection (motility and progressive) that agrees with Zhou et al (17) who demonstrated that a reduced number of motile spermatozoa diminished fertility and embryo quality on day 3 however, if there was a good embryo for transfer, the likelihoods of implantation and pregnancy were similar. Then, it was suggested that the implantation rate was the important parameter to evaluate the ability of an individual embryo to be implanted and it was not associated with sperm quality.

In this study, the implantation and pregnancy outcomes were not significantly different in the group with an SDF >30% and the group with an SDF ≤30% in ICSI cycles, although the blastulation rates were significantly higher in the SDF ≤30% group.
The majority of studies indicate that SDF has negative effects on fertilization and blastocyst formation with no effect on pregnancy outcome after blastocyst transfer (19).
In the present study, the IMSI group had a higher percentage of positive clinical pregnancies and a trend towards higher rates of cumulative fetal heart development compared to the ICSI group, but these differences are not statistically significant.
The study found no statistically significant difference in the number of embryos transferred and clinical outcomes between the humidified and dry incubator groups.
However, the cumulative fetal hearts (sacs) were higher in the humidified incubator group compared to the dry incubator group, although the difference was not statistically significant (p=0.107). These findings suggest that the humidified incubator may have a positive impact on the development of embryos, but further research is needed to confirm these results and establish the clinical significance.
Previous studies have reported conflicting results regarding the use of humidified incubators for embryo culture during IVF procedures. Some studies have suggested that humidified incubators may improve embryo development and increase pregnancy rates, while others have found no significant difference between humidified and dry incubators (18).
Our results reported that there was no statistically significant correlation between ICSI and IMSI maneuvers and outcomes including cumulative clinical results, fetal hearts (sacs), and transferred embryos.
In agreement with our results, Boitrelle et al. (20) reported that IMSI yielded even higher clinical pregnancy and delivery rates per couple (50%, in both cases) in a group of 12 additional, unmatched couples with more than eight ICSI failures.
On the other hand, Fawzy et al. (21) the dry culture environment was associated with lower rates of formed and high-quality blastocysts. This may suggest a correlation between dry culture and the livelihood of the transferred blastocysts.
The decreased rate of compaction on day 3 in the dry culture could have resulted in a lower implantation rate and poorer overall clinical outcomes in this group because of the known correlation between compaction and implantation rates (22).
The current findings showed that there was no significant correlation between sperm DNA fragmentation and cumulative clinical result, cumulative fetal hearts (sacs), and cumulative transferred embryos indicating that sperm DNA fragmentation percentage may not be a significant factor in the outcomes of ART.
Our results were supported by Liu et al. (23) who found that sperm DFI level showed a statistically significant negative correlation with PR% which is consistent with relevant research reports (24, 25).
From the present study SDF% is a significant predictor of cumulative clinical result in ART at cutoff value of 4.5%, with a sensitivity of 88% and a specificity of 86%. The AUC was 0.73 (p=0.048). which is lower than previous studies such as (19, 26, 27)
In this regard, Agarwal et al. (26) demonstrated that DNA fragmentation index (DFI) >30% is consistently associated with negative pregnancy outcomes in natural conception and intrauterine insemination (IUI), the reported cut-off values in predicting ART outcomes are varied (28). Infertility problems may occur when DFI reaches 20–25% and the success of ART decreases as DFI rises (27).
Moreover, Borges et al. (19) found that a 2.5-fold miscarriage rate was observed in cycles with an SDF above the established cutoff (≥30% SDF, 42.8% vs. <30% SDF, 16.8%).
The difference in cutoff values for SDF% in the present study and other studies may be due to differences in study populations, methods used for measuring DNA fragmentation, and outcomes measured. Further research is needed to determine the optimal cutoff value for SDF% for predicting pregnancy outcomes in different populations and methods used for measuring DNA fragmentation.
Hence, SDF testing should be offered to couples with unexplained infertility, and ART failure. A high SDF index would provide a possible explanation for the adverse reproductive outcome, though the exact cut-off values for prediction of ICSI and IMSI success are still debated. Possible interventions to reduce SDF should be implemented.
Our study had certain limitation due to the retrospective design with chance of missed data also, it was a single center study with insufficient sample size, absence of randomization as participants were not assigned a contraceptive technique at random as randomizing patients to a method of contraception was not ethical.

Conclusion

The cumulative fetal cardiac development and the percentage of positive clinical pregnancies were higher with IMSI technique. In addition, the type of

incubator employed in ART may be a critical factor to evaluate, as humidified incubators exhibited higher cumulative embryonic hearts, which implies that they may be more favorable to fetal development. Additionally, higher sperm DNA fragmentation was associated with lower total motility and was a significant predictor of lower cumulative clinical results in ART at a cutoff value of 4.5%.

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