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ORIGINAL RESEARCH

Predictive Significance of Sperm DNA Fragmentation Testing in Early Pregnancy Loss in Infertile Couples Undergoing Intracytoplasmic Sperm Injection

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Objective: This study aimed to determine the role of sperm DNA fragmentation as a marker that could predict early pregnancy loss (EPL), either independently or in combination with another marker or markers, after intracytoplasmic sperm injection (ICSI) cycles.

Methods: This prospective descriptive cohort study retrieved data from 162 couples who underwent their first ICSI cycles at the Center for Reproductive Endocrinology and Infertility of Hue University Hospital in Vietnam from May 2018 to December 2019. General characteristics, semen parameters, sperm DNA fragmentation index (DFI), fertilization, embryo development, pregnancy rates, and EPL were assessed. The receiver-operating characteristic (ROC) method was performed to identify the threshold of DFI in EPL. Multivariate analysis was used to demonstrate the relationship between the sperm DNA fragmentation level and EPL.

Results: Of 162 ICSI cycles, 23 (14.2%) involved EPL. There was no significant difference between the sperm DNA fragmentation rate and the overall rate of pregnancy loss, although the negative pregnancy outcome group had a median DFI that was higher than that of the positive pregnancy outcome group (20% vs 17.8%). The ROC analysis indicated that a sperm DNA fragmentation rate of 16.6% was the priority cut-off that could be used to distinguish EPL with a sensitivity of 73.9% (95% confidence interval [CI], 67.15–80.67) and specificity of 47.48% (95% CI, 39.79–55.17). The multivariate analysis confirmed that in female factors such as age, body mass index (BMI), and sperm DNA fragmentation level affected the EPL rate. However, a combination of the sperm DNA fragmentation level and female age or female BMI could not sufficiently predict EPL.

Conclusion: EPL can be affected by multiple factors including sperm DNA fragmentation; however, there is no sufficient evidence indicating that sperm DNA fragmentation, both as a single marker and combined with other markers, is a good predictor of EPL.

Keywords: sperm DNA fragmentation, intracytoplasmic sperm injection, early pregnancy loss, infertility, assisted reproductive technology

Introduction

Infertility affects an estimated 15% of couples; of these couples, male factors account for 20% to 70% of infertility cases, and the percentage of male infertility ranges from 2.5% to 12%.[1] The sperm DNA fragmentation index (DFI) is clinically useful for assessing male fertility potential or as a prognostic test to determine if infertility treatment is necessary.[2,3] In the field of assisted reproductive technology

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(ART) and andrology, sperm DNA fragmentation tests have been recommended to diagnose unexplained infertility, repeated implantation failure, and issues of the varicocele.[4] Male factors, such as sperm DNA damage, can impact not only the fertilization process but also embryo gene expression and development.[5–8] However, the impact of the DFI and embryo quality on pregnancy outcomes is controversial. Several publications have shown an association between high DFI and embryo quality regarding embryo formation[9] and embryo quality scores.[10] A higher DFI is correlated with poor embryo development and lower implantation rate during intracytoplasmic sperm injection (ICSI) cycles for couples with female factor infertility.[11] ICSI is recommended as a better choice than in vitro fertilization (IVF) for improving pregnancy outcomes of patients who have sperm with a high DFI.[12] Conversely, other studies concluded that the DFI was not correlated with blastocyst aneuploidy or morphological grading,[13] total blastocyst number, or the number of blastocysts with good quality.[14]

Recent studies have reported an association between a high DFI and miscarriage after ICSI[11,15,16] and between a high DFI and recurrent natural pregnancy loss.[17] A meta-analysis of 8068 IVF/ICSI cycles reported by 41 articles suggested that sperm DNA damage negatively affected clinical pregnancy following IVF and/or ICSI treatment.[18] However, other studies did not find a significant association between DFI and miscarriage[12] or between DFI and live birth rates[19] of pregnancies associated with both IUI and ICSI cycles.[20]

Furthermore, the DFI value that is a negative factor in pregnancy remains unclear. Different cut-off values for DFI have been presumed for distinguishing between men with normal and abnormal semen characteristics, with predictive values varying from 18%[21] to 26.1%[22] as follows: 19.9% according to a sperm chromatin structure assay (SCSA); 22.08% according to the terminal deoxynucleotidyl transferase UTP nick-end labeling (TUNEL) assay; 24.74% according to the sperm chromatin dispersion (SCD) test; and 48.47% according to the comet assay.[23,24] Benchaib et al reported a four-fold increased risk of miscarriage when the DFI exceeded 15% for pregnancies associated with IVF/ICSI cycles.[25] A DFI threshold between 20% and 30% was used in some studies to evaluate the relationship between DFI and semen parameters, between DFI and embryo quality, as well as outcomes of pregnancies associated with assisted reproductive technology (ART).[26–28] Some studies have shown different DFI cut-off values when using the receiver-operating characteristic (ROC) curve to predict top-quality or grade A embryo formation rates (DFI using the SCD test, 30.7%),[9] to predict the clinical pregnancy rate (DFI using the SDC test, 27.3%),[29] and to predict the miscarriage rate (DFI using the TUNEL test, 13%).[15] As the impact of the DFI on ICSI outcomes as well as EPL after ICSI remains controversial, this study aimed to determine the role of sperm DNA fragmentation alone or in combination with other female factors in the prediction of EPL after ICSI.

Materials and Methods

This prospective descriptive cohort study included data of infertile couples who underwent ICSI from May 2018 to May 2019 at the Center for Reproductive Endocrinology and Infertility of Hue University Hospital in Vietnam. The inclusion criteria were couples with primary infertility undergoing their first ICSI cycle with frozen embryo transfer resulting in pregnancy outcomes and complete follow-up examinations until 12 weeks of gestational age. Exclusion criteria were men who were unable to ejaculate, men who had a general infection or urogenital infection, retrograde ejaculation, sperm from cryopreservation or surgical extraction, extremely low sperm counts (<1 million/mL), and azoospermia. IVF cycles with gamete donors and females with severe endometriosis (grade 3 and grade 4) were also excluded from the study.

The sample size was calculated for the rate estimate investigation as follows: $n \geq Z^2_{\alpha/2} \frac{p(1-p)}{\Delta^2}$

This equation resulted in the following: p = 0.06,[15] Δ = 0.05, α = 0.05, and $Z_{\alpha/2}$ = 1.96 (95% confidence); to estimate prevalence of miscarriage for each IVF cycle, the minimum sample size was estimated to be at least 87 subjects. A total of 162 couples were recruited for the study population.

The study was approved by the ethics committee of Hue University of Medicine and Pharmacy (number H2019/432). Written informed consent was obtained from all patients.

Semen Analysis

Semen samples were collected from men after they masturbated in the laboratory at 3 to 5 days after abstinence. After liquefaction for 30 minutes, semen samples were evaluated for sperm motility, vitality, concentration, and morphology in accordance with the 2010 guidelines of the World Health Organization (WHO).

Sperm DNA Fragmentation

Sperm DNA fragmentation was measured using an SCD test (Halosperm kit®; Halotech, Madrid, Spain), which is a fast method based on controlled DNA denaturation and protein depletion to determine DNA fragmentation in sperm cells. A total of 500 sperms were examined by only one highly trained technician to decrease variability. DFI was expressed as the percentage of sperms with a small halo, the percentage without halo, and the percentage of degraded spermatozoa out of a total of 500 male gametes. The slides were observed by two highly trained technicians who calculated the average percentage of the two results to avoid bias.

Controlled Ovarian Hyperstimulation

Women who underwent IVF cycles were treated with controlled ovarian hyperstimulation using a GnRH antagonist protocol. Recombinant follicle-stimulating hormone (FSH; follitropin alfa) was administered with a starting dose of 225 IU (Gonal F®; Merck KGaA, Darmstadt, Germany) that was adjusted based on the number of developed follicles. Oocyte retrievals were performed 35 to 36 hours after administration of a trigger injection of human chorionic gonadotropin (hCG) 10,000 IU intramuscularly (Pregnyl®; Merck Sharp & Dohme Limited, Hertfordshire, UK) by ultrasound-guided follicle aspiration with a single-lumen needle (Vitrolife, Frölunda, Sweden).

Semen Preparation

Sperm samples were prepared using a two-layer density gradient centrifugation technique using 45% and 90% Silselects plus (Fertipro, Beernem, Belgium). Sperm samples were washed twice with 3 mL Spermrinse (Vitrolife) before being used for ICSI.

Intracytoplasmic Sperm Injection and Embryo Culture

Mature oocytes were determined after the oocyte cumulus complex (OCC) was denuded with HYASE 80 IU (Vitrolife) and pipetted with a 135-µm inner diameter pipette. Insemination via ICSI was performed with a mature oocyte and prepared sperm 3 hours after retrieval. The injected oocyte was cultured in a single drop of 20 µL of G-TL (Vitrolife) covered with 3 mL Ovoil (Vitrolife) under conditions of 6.0% CO_2 and 5.0% O_2. At 16 to 18 hours after the injection, fertilized oocytes were determined by the presence of two pronuclei. Embryos were evaluated according to the Istanbul consensus on day 2. Top-grade embryos were defined as those with all of the following characteristics: four or more cells on day 2, less than 10% fragmentation, and no multinucleated cells.

Embryo Vitrification and Warming

Embryos with fragmentation less than 25% and embryos with more than two blastomeres on day 2 were selected for vitrification. Embryo vitrification was performed with a Cryotop device and commercially available medium (Kitazato, Tokyo, Japan); the protocol was in accordance with the instructions of the manufacturer. After open system vitrification, the embryos were stored in liquid nitrogen.

Artificial endometrial preparation was conducted by using estradiol 8 mg/day (2 mg × 4 tablets; Progynova®; Bayer, Leverkusen, Germany) divided as 4 mg twice daily. Secretory transformation with progesterone (Crinone Gel® 8%; Merck KGaA, Darmstadt, Germany) was administered vaginally at a dose of 90 mg twice daily. Embryos used for transfer were determined when the endometrium had a thickness of 7 mm or more. Embryo warming was performed using a warming solution (Kitazato) according to the manufacturer's instructions. After warming, embryos were cultured in a group, applied with 20 µL of G-TL (Vitrolife), covered with 3 mL of Ovoil (Vitrolife), and stored with overnight under conditions of 6.0% CO_2 and 5.0% O_2.

Embryo Transfer

Embryos were placed in 1 mL of Embryoglue (Vitrolife) for 15 to 30 minutes before being loaded into the catheter (Kitazato); then, they were transferred back to the uterus under transvaginal ultrasound guidance.

Clinical Follow-Up

Serum beta-hCG was measured 14 days after embryo transfer; a result of more than 50 mIU/mL was considered beta-hCG-positive. These women underwent a transvaginal ultrasound scan 2 weeks later. Clinical pregnancy was determined by the presence of a gestational sac and fetal cardiac activity, which were determined at 4 weeks after embryo transfer. The implantation rate was calculated based on the number of gestational sacs per embryo transferred. A biochemical pregnancy was defined as beta-hCG-positive cases without any gestational sac at 4 weeks after embryo transfer. Ongoing pregnancy was defined as fetal development at 12 weeks of gestational age. Moreover, EPL

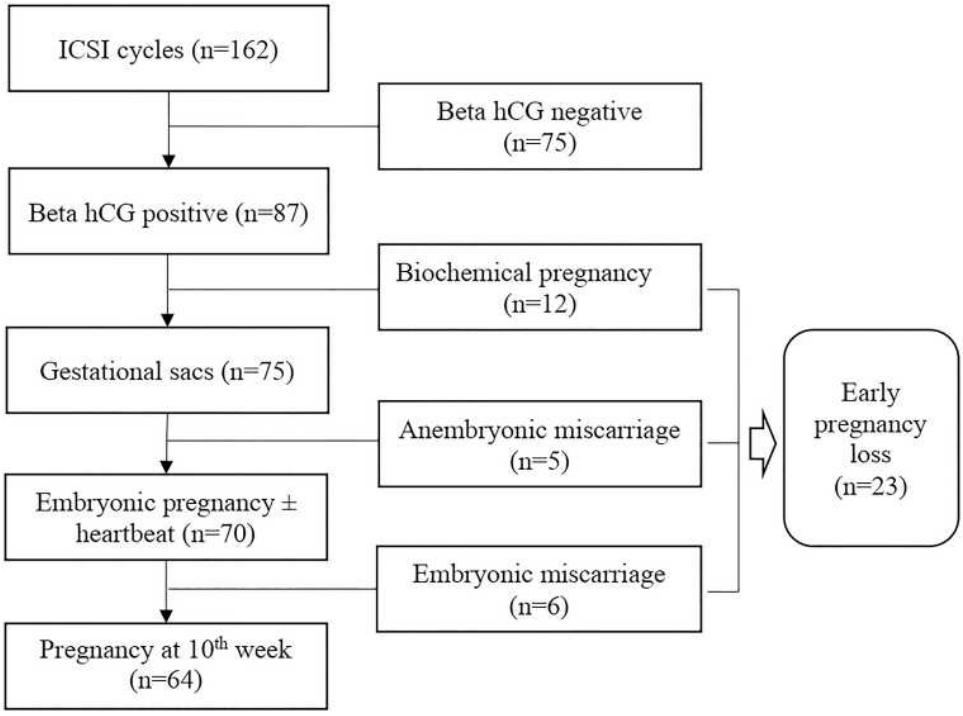

Figure 1 Flowchart of ICSI cycle outcomes.

included biochemical pregnancy, anembryonic miscarriage, and embryonic miscarriage (miscarriage subgroups); this classification was based on the consensus statement of terminology of the European Society of Human Reproduction and Embryology.[30]

Statistical Analysis

Descriptive statistics are demonstrated by the number and percentage for categorical data and by the mean and standard deviation or median with interquartile value (IQR) for continuous data. The chi-square test, Fisher's exact test, Student's *t*-test, and Mann–Whitney test were used to compare the appropriate categorical or continuous variables with ICSI outcomes. The ROC analysis was performed to identify the DFI value to predict ICSI outcomes using the area under curve (AUC) or selecting the optimal DFI threshold to predict EPL. A multivariate analysis adjusted for covariates was performed to demonstrate the association between the sperm DNA fragmentation level and EPL. All tested hypotheses considered p=0.05 as statistically significant.

Results

A total of 162 couples undergoing their first ICSI cycles were recruited. Pregnancy outcomes were followed-up until 12 weeks of gestation. ICSI outcomes are presented in Figure 1. EPL included biochemical pregnancy loss, anembryonic pregnancy, and embryonic miscarriage. The beta-hCG-positive rate of the 162 ICSI cycles was 53.7% (87/162), the ongoing pregnancy rate was 39.5%, and the EPL rate was 14.2%.

Baseline characteristics of couples undergoing ICSI and pregnancy outcomes (positive pregnancy group and negative pregnancy group) are presented in Table 1. There was a significant association among pregnancy, infertility type, and anti-Mullerian hormone (AMH) level. The positive pregnancy rate was 57.6% and 22.2% for those with primary infertility and secondary infertility, respectively (significance, p<0.01). The median AMH level was significantly different between the positive and negative pregnancy outcome groups. Other factors such as infertility duration, male age, and semen parameters were not significantly different between these two groups.

Embryo quality and pregnancy outcomes according to the DFI are shown in Table 2. A statistically significant difference in the DFI was observed only for the clinical miscarriage subgroup (34.5±22.8 vs 22.7±17.2; p=0.03). Although not significantly different, the DFI value was higher for the subgroups with a low fertilization rate (27.4±22.1 vs 22.05 ±15.3), with a low number of usable embryos (29.9±26.5 vs 22.8±16.1), and with EPL (26.3±19.2 vs 23.0±17.2).

Table 1 Baseline Characteristics of Participants and Distribution in Subgroups of Pregnancy Outcomes

Characteristics	Total (n=162)	Pregnancy (+) (n=87)	Pregnancy (-) (n=75)	p-value
Infertile couples				
Infertility duration (years)				
<3	21 (13.0)	10 (47.6)	11 (52.4)	0.55
≥3	141 (87.0)	77 (54.6)	64 (45.4)	
Infertility type				
Primary	144 (88.9)	83 (57.6)	61 (42.4)	0.005
Secondary	18 (11.1)	4 (22.2)	14 (77.8)	
Geography				
Urban	83 (51.2)	44 (53.0)	39 (47.0)	0.86
Rural	79 (48.8)	43(54.4)	36 (45.6)	
Husband				
Age (years)	36 (32–40)	36 (32–39)	37 (32–41)	0.17
Education				
School grade	66 (40.7)	33 (50.0)	33 (50.0)	0.52
College/University	96 (59.3)	54 (56.3)	42 (43.7)	
BMI (kg/m^2)	23.67 (21.63–25.91)	23.03 (21.30–25.53)	24.22 (22.04–26.03)	0.1
Waist (cm)	84 (77–90)	82 (76–89)	85 (80–90)	1.13
WHR	0.88 (0.84–0.91)	0.88 (0.84–0.91)	0.89 (0.85–0.91)	0.21
Semen parameters				
Concentration	32 (22–41)	31 (22–41)	32 (23–42)	0.33
Vitality	79 (72–83)	78 (71–83)	80 (74–84)	0.18
Sperm PR motility (%)	30 (21–39)	28 (15–39)	32 (23–39)	0.08
Morphology	3 (2–5)	3 (2–5)	3 (2–5)	0.23
DFI	18.1 (11.4–31)	18.2 (11–31)	17.8 (11.4–31.6)	0.81
Wife				
Age (years)	33 (30–36)	32(30–35)	33 (30–37)	0.05
BMI (kg/m^2)	20.57 (19.53–22.27)	20.55 (19.53–22.22)	20.70 (19.53–22.60)	0.76
AMH (ng/mL)	2.78 (1.48–4.54)	3.33 (2.1–5.7)	2.16 (0.93–3.55)	0.0003
Miscarriage history				
Yes	37 (22.8)	22 (59.5)	15 (40.5)	0.46
No	125 (77.2)	65 (52.0)	60 (48.0)	

Notes: All continuous variables were presented as mean ± standard deviation (mean ± SD) and were compared between two outcomes groups. The categorical variables were illustrated by the observational number and percentages.

Abbreviations: AMH, anti-Mullerian hormone; BMI, body mass index; DFI, DNA fragmentation index; FSH, follicle-stimulating hormone; PR, progressive; WHR, waist-hip ratio.

The ROC curve analyses showed that varying percentages of sperm DNA fragmentation were used to calculate the optimum sensitivity and specificity of ICSI outcomes (Table 3). The recommended AUC was 0.56 (95% confidence interval [CI], 0.48–0.64) for an EPL rate of 16.6% with sperm DNA fragmentation.

Regarding sperm quality and embryo quality related to the DFI threshold, there were statistically significant differences in the median (IQR) male BMI, waist circumference, and waist-to-hip ratio (WHR) of groups with DFI less than 16.6% and DFI more than 16.6% (p=0.04, p=0.02, and p=0.003, respectively) (Table 4).

Table 2 DNA Fragmentation Index Results in Embryo Quality and Pregnancy Outcomes

Outcomes	DFI					
	n	%	Mean ± SD	Min-Max	Median (IQR)	p
Fertilization rate						
< 75%	43	26.5	27.4±22.1	4.4–81.6	19.4 (11–38.2)	0.42
≥ 75%	119	73.5	22.05±15.3	4.2–88.4	17.8 (11.4–29)	
Usable embryos						
<50%	16	9.9	29.9±26.5	6.2–81.6	18.4 (12.9–41.3)	0.61
≥ 50%	146	90.1	22.8±16.1	4.2 −88.4	18.1 (11–31)	
hCG test						
hCG (+)	87	53.7	23.6±17.1	4.4–81.6	18.2 (11–31)	0.81
hCG (-)	75	46.3	23.3±17.9	4.2–88.4	17.8 (11.4–31.6)	
Biochemical pregnancy						
Yes	12	7.4	23.6±17.1	4.4–81.6	11 (31–97)	0.42
No	150	92.6	23.3±17.9	4.2–88.4	11.4 (31.6–75)	
Clinical pregnancy						
Yes	75	46.3	24.4±17.8	4.4–81.6	18.4 (12–32)	0.51
No	87	53.7	22.7±17.2	4.2–88.4	17.8 (10.4–29.4)	
Miscarriage						
Yes	11	6.8	34.5±22.8	13.4–77	24.8 (17.4–63.8)	0.03
No	151	93.2	22.7±16.8	4.2–88.4	17.8 (11–30.6)	
Pregnancy loss						
Yes	23	14.2	26.3±19.2	5.6–77	20 (13.4–31.8)	0.35
No	139	85.8	23.0±17.2	4.2–88.4	17.8 (11–31)	
Total	162		23.5±17.4	4.2–88.4	18.1 (11.4–31)	

Abbreviations: DFI, DNA fragmentation index; hCG, human chorionic gonadotropin.

Table 3 The Predicted Values of ICSI Outcomes by Sperm DNA Fragmentation

Outcomes	Yes	Threshold	AUC	95% CI of AUC	Se	Sp	PPV	NPV
Beta-hCG positive	87	22.2	0.51	0.42–0.60	43.7	65.3	59.4	50.0
Clinical pregnancy	75	23.6	0.53	0.45–0.61	42.7	71.3	56.1	59.0
Ongoing pregnancy	64	20.8	0.48	0.40–0.56	45.3	59.2	42.0	62.4
Pregnancy loss	23	16.6	0.56	0.48–0.64	73.9	47.5	18.9	91.7

Abbreviations: hCG, human chorionic gonadotropin; AUC, area under curve; Se, sensitivity; Sp, specificity; PPV, positive predictive values; NPV, negative predictive values.

A multivariate analysis of the relationship between the DFI and EPL (Table 5) indicated that EPL was more likely to occur in older women (age, 35 years or older; odds ratio [OR], 5.87; 95% CI, 1.72–20.09; p=0.005), women with an abnormal BMI (OR, 3.20; 95% CI, 1.10–9.28; p=0.032), and women with DFI ≥16 (OR, 3.2; 95% CI, 1.10–9.28; p=0.032).

Table 6 presents the values of the DFI level, female age, and female BMI as predictors (single factor or combined factors) of EPL. The optimal cut-off point for DFI to predict EPL was defined as 16.6%, with a sensitivity of 73.9%, specificity of 47.5%, positive predictive value (PPV) of 18.9%, and negative predictive value (NPV) of 91.7%. Despite a significant association among female age, female BMI, and EPL (Table 5), these were not significant predictors of EPL when the DFI level was adjusted for female age or female BMI. The combination of the DFI and female age can predict EPL with a sensitivity of 43.5%, specificity of

Table 4 Related Factors and Embryo Quality by DNA Fragmentation Index Threshold

Factors	DFI ≥ 16.6	DFI < 16.6	p-value
Husband			
Age (years)	36.5 (32–41)	36 (32–39)	0.91
BMI (kg/m^2)	24.28 (22.2–26.3)	23.03 (21.3–24.9)	0.04
Waist (cm)	85 (79–91)	80.5 (75–88.5)	0.02
WHR	0.897 (0.85–0.9)	0.861 (0.83–0.89)	0.003
Sperm quality			
Concentration (mil/mL)	30 (22–40)	32.5 (22.5–43)	0.09
Vitality (%)	80 (71–83)	79 (73.5–83)	0.74
Progressive motility (%)	30 (20–40)	31 (22.5–38)	0.59
Normal morphology (%)	3 (2–5)	3 (2–5.5)	0.33
Embryo quality			
Blastomeres ≥ 4	79.58 (67.5–100)	80 (66.7 −97.2)	0.99
Fragmentation <10%	90.45 (69.6–100)	85.67 (60–100)	0.36
Grade A embryo	66.67 (50–81.5)	60 (41.4–86.0)	0.49
Usable embryos (%)	100 (90.5–100)	100 (83.3–100)	0.33
Embryos transferred*	2 (2–3)	2 (2–3)	0.51

Notes: *Number of transferred embryos was presented by the median and interquartile range.
Abbreviations: BMI, body mass index; DFI, DNA fragmentation index; hCG, human chorionic gonadotropin; WHR, waist-hip ratio.

Table 5 Predictors of Early Pregnancy Loss in Multivariate Analysis

Predictors	OR Adjusted*	95% CI	p
Husband's age	0.90	0.808–1.01	0.071
Wife's age			
<35	1 (ref)		
≥35	5.87	1.72–20.09	0.005
Wife's BMI			
Normal (18.5-<23)	1 (ref)		
Abnormal (<18.5 or ≥23)	4.18	1.58–11.02	0.004
DFI			
<16.6	1 (ref)		
≥16.6	3.20	1.10–9.28	0.032

Notes: *By adjustment with age of the infertile couples (male and female) in the generalized linear models.
Abbreviations: BMI, body mass index; DFI, DNA fragmentation index; OR, odd ratio.

89.9%, PPV of 36.4%, and a NPV of 89.9%. Similarly, the combination of DFI and female BMI resulted values of 39.1%, 87.8%, 34.6%, and 89.7% respectively.

Discussion

The influence of sperm DNA damage on embryo quality during ICSI cycles is a controversial issue. With the ICSI method, sperm is selected based on motility and morphology, active insemination in the oocyte cytoplasm, and the ability to overcome several barriers of natural selection;[31] however, despite this selection, sperm may have contained damaged DNA.[10] Recent studies have shown indirect effects of sperm DNA damage on fertilization and early embryonic development through the process of genetic material repair that occurred in the oocyte. Logically, embryo development could be delayed; poor embryo quality was found in the group with high DFI.[9,32] The ICSI results of patients with DFI ≥30% indicated a statistically significant decrease in fertilization rate, normal cleavage speed rate, and top-grade embryo rate on day 3 compared to those with DFI <30%.[11] In the present study, a cut-off value of DFI ≥16.6% based on

Table 6 The Values of Exploratory Factors in Prognosis of Early Pregnancy Loss by Independent or in Combined Predictors

Predictors	Pregnancy Loss		Sensitivity (95% CI)	Specificity (95% CI)	PPV	NPV
	Yes	No				
Age						
≥35	14	48	60.9 (53.4–68.4)	65.5 (58.2–72.8)	22.9 (16.1–29.0)	91.0 (86.6–95.4)
<35	9	91				
BMI						
Abnormal	13	34	56.5 (48.5–64.2)	75.5 (68.9–82.2)	27.7 (20.8–34.6)	91.3 (87.0–95.6)
Normal	10	105				
DFI						
≥16.6	17	73	73.9 (67.2–80.7)	47.5 (39.8 −55.2)	18.9 (12.9–24.9)	91.7 (87.4–95.9)
<16.6	6	66				
Age - BMI						
≥ 35 and BMI = abnormal	8	14	34.8 (27.5–42.1)	89.9 (85.3–94.6)	36.4 (29.0–43.8)	89.3 (84.5–94.1)
< 35 | BMI = normal	15	125				
DFI & Age						
≥16.6 and Age ≥35	10	23	43.5 (35.8–51.1)	83.5 (77.7–89.2)	30.3 (23.2–37.4)	89.9 (85.3–94.6)
DFI <16.6 | Age <35	13	116				
DFI & BMI						
DFI≥16.6 and BMI = abnormal	9	17	39.1 (31.6–46.7)	87.8 (82.7–92.8)	34.6 (27.3–41.9)	89.7 (85.0–94.4)
DFI<16.6 | BMI = normal	14	122				

Abbreviations: BMI, body mass index and age of wife; DFI, DNA fragmentation index; PPV, Positive predictive values; NPV, negative predictive values.

the ROC method to predict an increased risk of EPL when analyzing embryogenesis using this threshold, showed no significant differences in the fertilization rate, normal cleavage speed, and top-grade embryo rate on day 2. It should be explained that an increased DFI is insufficient to influence the fertilization process and early embryonic development. In fact, it was reported that the use of spermatozoa with damaged DNA did not affect the results of fertilization and early embryonic development until blastocyst formation.[33,34]

The integrity of sperm DNA has an important role in fertilization and maintaining a normal pregnancy.[35] Especially, sperm DNA damage was supposed to be responsible for increasing early miscarriage risk. It was thought that the lack of proper chromatin structure in sperm due to fragmented DNA may inhibited gene expression and caused EPL.[6,16] An association between sperm fragmentation DNA and IVF/ICSI failure by meta-analyses studies showed that increased DFI raised miscarriage rate.[5,7,37] A previous meta-analysis showed a significant increase in miscarriage for patients with high DFI; this risk increased 2.16-times[36] to 2.48-times[37] for IVF and ICSI. In our study, there were statistically significant differences in the DFI of the miscarriage subgroups; the sperm DFI with a cut-off of 16.6% increased the EPL rate 3.2 times (p=0.032). It is possible that unrepaired damage of sperm DNA may negatively affect the blastocyst development rate and result in a higher risk of EPL.

Although it has been reported that an increased DFI affects the incidence of EPL after ICSI, there is no homogenous DFI cut-off value. Violeta et al reported that DFI >27% was associated with an increased risk of EPL after ICSI (OR, 5.65; 95% CI, 4.32–7.11; p<0.05), including biochemical pregnancies and miscarriages.[38] Lopez et al used the ROC curve to calculate a DFI threshold value of 25.5% to predict successful and unsuccessful IVF/ICSI treatments; therefore, a value more than 25.5% could be associated with a higher probability of failed IVF.[39] The latest study by Borges et al involved the use of an SCD assay indicated a DFI cut-off of 30% resulted in a 2.5-fold miscarriage rate (DFI ≥30%: 42.8% miscarriage rate; DFI <30%: 16.8% miscarriage rate).[11] However, the ROC analysis also revealed a low cut-off value (DFI >13%) for predicting miscarriage.[15]

Several methodologies have been used to assess sperm DNA fragmentation and calculate DFI, such as the

TUNEL assay, comet assay, SCSA, and SCD test. Each method had advantages and disadvantages when used to evaluate sperm DNA fragmentation. The TUNEL assay and SCSA are two standardized sperm DNA fragmentation assessment methods with high repeatability. However, the disadvantages of these two methods is that the test requires expensive equipment and a high concentration of sperm. The comet assay provides visual images of double-strand DNA appearing at the head of the comet and damaged double-strand and single-strand DNA fragments forming the tail part. However, the protocols of the comet assay have not been standardized, and it is difficult to distinguish between endogenous DNA breaks and induced DNA breaks in the alkaline comet assay. The SCD assay is simple, quick, and has high reproducibility; it only requires a microscope, but the results of the SCD assay are greatly dependent on the skills of the person performs the test.[31] Besides, although SCD tests measured fragmentation indirectly that was less sensitive than others, it might reflect later outcomes such as birth delivery and abortion rates.[14,42] Primarily, because of the advantages of the SCD assay, we used it along with the equipment available in our laboratory to assess sperm DNA fragmentation levels during this study. Moreover, previous studies have shown that SCD can help predict the prognosis for male infertility and provide results similar to those of other methods when assessing sperm DNA fragmentation levels.[24]

Female age-related declines in fertility have a greater impact than BMI on the cumulative live birth rate.[40] Jin et al reported that when DFI was >27.3%, the risk of EPL increased significantly for those with reduced and normal ovarian reserves. This suggested that sperm DNA fragmentation testing was particularly useful for couples when the female had a reduced ovarian reserve and high DFI.[29] Similarly, the study by Liang et al revealed that when female age was older than 30 years, women with DFI >30% had lower clinical pregnancy rates and fewer good-quality embryos than women with DFI ≤30%.[41] In our study, the multivariate analysis indicated that female age and female abnormal BMI increased the risk of EPL in IVF cycles by 5.87 times (p=0.005) and 4.18 times (p=0.004), respectively.

Regarding the prediction of EPL, the DFI alone had high sensitivity (73.9%) and high NPV (91.7%) but low specificity (47.5%) and low PPV (18.9%). This means that the DFI value is not a good independent predictor of EPL after embryo transfer. The combination of DFI and other female factors, including age or BMI, did not strongly improve the ability to predict EPL. Increased DFI and female age had higher specificity (83.5%) and NPV (89.9%), but sensitivity and PPV were very low. Similarly, the combination of DFI and female BMI had low sensitivity and low PPV. Lin et al also reported that when the DFI threshold was higher than 27%, the specificity was 85% specificity and the NPV was 96% for predicting miscarriage, but the sensitivity and PPV were very low; in the ICSI group, this DFI threshold for predicting miscarriage had 50% sensitivity, 78% specificity, and 94% NPV, but the PPV was very low.[42]

The strength of the present study confirmed the effects of an increased DFI on the incidence of EPL after ICSI. Moreover, the data of our SCD test recommended a DFI threshold as low as 16.6% to predictive EPL. This study was limited because the choice of test as Halo is less sensitive than other sperm DNA fragmentation tests and this assessment may be a reason for the lack of association. Besides, follow-up to determine the birth rate rather than the ongoing pregnancy rate can provide a better understanding. Moreover, future studies with long-term follow-up may provide further insight into the relationship between DFI and the live birth rate.

In conclusion, the DFI is related to EPL after ICSI. Although it has high sensitivity and high NPV, the DFI should not be used as an independent predictor of EPL because of its low specificity. When DFI is combined with female factors such as age and BMI, its specificity for predicting EPL is improved but its sensitivity is not. This study determined that EPL can be affected by multiple factors, including sperm DNA fragmentation. However, there is insufficient evidence to support sperm DNA fragmentation as a good predictor of EPL.

Abbreviations

AMH, anti-mullerian hormone; AUC, area under curves; ARTs, assisted reproductive techniques; BMI, body mass index; CI, confidence interval; DNA, deoxyribonucleic acid; DFI, DNA fragmentation index; hCG, human chorionic gonadotropin; ICSI, intracytoplasmic sperm injection; SCD, sperm chromatin dispersion; SDF, sperm DNA fragmentation; SCSA, sperm chromatin structure assay; TUNEL, terminal deoxynucleotidyl transferase UTP nick-end labelling; PPV, positive predictive value; NPV, negative predictive value; Median (IQR), median and interquartile range.

Data Sharing Statement

All data are available and would be shown as requested.

Ethics Approval

This study was approved by the Ethics Committee of Hue University of Medicine and Pharmacy, numbered H2019/432. All information and data were encrypted and confidential. This study was conducted in accordance with the Declaration of Helsinki.

Consent for Publication

All authors have been involved in the research work and consent for publication.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

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