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Sperm DNA fragmentation index: limited effectiveness on predicting embryo quality in assisted reproduction technology treatments

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Abstract

Purpose Prior sperm DNA fragmentation index (DFI) thresholds for diagnosing male infertility and predicting assisted reproduction technology (ART) outcomes fluctuated between 15 and 30%, with no agreed standard. This study aimed to evaluate the impact of the sperm DFI on early embryonic development during ART treatments and establish appropriate DFI cut-off values.

Methods Retrospectively analyzed 913 couple's ART cycles from 2021 to 2022, encompassing 1,476 IVF and 295 ICSI cycles, following strict criteria. The WHO guidelines directed the semen analysis, while the acridine orange test (AOT) determined the DFI. Male factors (age, BMI, DFI, infertility duration, sperm parameters) and female parameters (age, BMI, AMH, retrieved oocytes) were evaluated. We also assessed embryological parameters like fertilization rate, cleavage rate, and blastocyst quality. Correlations between DFI and embryo quality were examined and DFI cut-off values were established using ROC analysis.

Results The Sperm DFI demonstrated a positive correlation with male age and a negative correlation with sperm motility, concentration, and normal morphology, while showing no relation to BMI. No connection between DFI and embryological parameters in only IVF and ICSI groups was found, but a negative correlation with fertilization rate was seen in all ART cycles. ROC curve analysis revealed a DFI cut-off value of 21.15% having 36.7% sensitivity and 28.9% specificity in predicting high fertilization rate (≥ 80%).

Conclusion Sperm DFI had a negative correlation with fertilization rate, but limited predictive efficacy and no significant link to other embryological parameters. DFI assessments may improve early embryo development prediction during ART treatments, particularly in older males or those exhibiting poor sperm quality.

Keywords Sperm DNA fragmentation index, Assisted reproduction technology, In vitro fertilization, Intracytoplasmic sperm injection, Fertilization rate

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Introduction

Infertility affects 15% of reproductive-aged couples worldwide [1], with male factors responsible for more than 20% of the cases [2]. Despite the increasing maturity of assisted reproduction technology (ART) in recent years, clinicians still face challenges, such as low rates of embryo implantation and clinical pregnancy. The success of ART largely depends on the quality of the embryo, which is strongly influenced by both female and male factors [3]. However, most previous studies mainly focused primarily on the effects of female factors, and it is widely accepted that advanced age, overweight status, and diminished ovarian reserves in women have negative influence on ART outcomes [4, 5]. How sperm-related factors affect embryo quality is rarely studied and the conclusions remain controversial.

Semen analysis is still the golden standard physical examination to assess male fertility so far. However, there are some infertile males in whom no abnormalities can be detected by conventional semen analysis [6]. Sperm DNA fragmentation refers to the accumulation of single- and double-strand sperm DNA breaks. The integrity of DNA is essential for the precise transmission of paternal inheritance. Spermatozoa with defective DNA can theoretically increases sperm aneuploidy and impair male fertility. The sperm DNA fragmentation index (DFI) has therefore emerged as a promising indicator of ART outcomes and is recommended by the 6th edition of the WHO Laboratory Manual for the Examination and Processing of Human Semen to be included as a conventional semen assay [7].

It is currently established that sperm DFI has a negative correlation with semen concentration, sperm motility, and normal sperm morphology [8], yet its influence on male subfertility and particularly embryo quality is still a subject of debate. Some studies proposed that sperm DFI was higher in infertile men than fertile ones and could predict a lower cumulative live birth rate (CLBR) in ART treatments [9, 10]. In contrast, other studies argued that sperm DFI did not provide sufficient information regarding embryonic development or clinical outcomes of ART cycles [11, 12]. In these studies, the varying cut-off values of sperm DFI were selected. Additionally, the absence of strict inclusion criteria for female participants who have a direct impact on embryo quality may be a cause of the disagreement.

In the current study, the sperm DFI was evaluated by an acridine orange test (AOT), conducted under flow cytometry (FCM). The potential impact of the DFI on early embryonic development during in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) cycles was investigated. Furthermore, we sought to establish appropriate DFI cut-off values based on embryo quality in both IVF and ICSI cycles, acknowledging that a uniform cut-off value may not be universally applicable in all cases. To minimize the potential influence of female factors on embryo quality, we stringently controlled for age, weight, and ovarian function of the female participants enrolled in this study.

Materials and methods

Subjects

The data were obtained from IVF/ICSI fresh cycles conducted between January 2021 and December 2022 at the Center of Reproductive Medicine of Zhuhai Maternal and Child Health Care Hospital in Guangdong, China. This study has been performed in accordance with the Declaration of Helsinki and approved by the Ethics Committee of Zhuhai Maternal & Child Health Care. Written informed consent from all enrolled patients was obtained prior to the study.

All participating couples had been diagnosed with infertility, having attempted to achieve pregnancy unsuccessfully for at least one year prior to undergoing ART treatments. The total number of IVF/ICSI cycles during the period from January 2021 to December 2022 was 4,528 cycles from 2,673 couples. All participants should meet the following inclusion criteria: (1) the women must be < 35 years and have a body mass index (BMI) < 28 kg/m^2; (2) the women should have normal period of menstruation and normal levels of anti-müllerian hormone (AMH) > 1.2ng/ml; (3) both partners are nonsmokers and have normal chromosomes. Stringent exclusion criteria were set as follows: (1) sperm obtained by percutaneous epididymal sperm aspiration (PESA) or testicular sperm aspiration (TESA); (2) mixed IVF/ICSI method; (3) the IVF/ICSI treatments for the same couple was greater than 3 cycles; (4) cycles with incomplete data. Finally, 913 couples with their 1,476 IVF and 295 ICSI cycles were enrolled in the study (Fig. 1).

Semen collection and preparation

Fresh semen samples were collected by masturbation on the day of oocyte collection in a dry and sterile cup and placed in a 37℃ incubator for liquefaction. Semen analysis was performed according to the WHO manual [13]. PureSperm® density gradients were used for sperm preparation according to the manufacturer's instructions (Vitrolife, Gothenburg, Sweden). After centrifugation, the upper layer seminal plasma and 45% diluted PureSperm® was aspirated and the remaining 90% layer was collected. After being washed twice with Hams F10 medium and centrifuged at 300 g for 10 min, the pellet was re-suspended in IVF medium and used for oocyte insemination after capacitation.

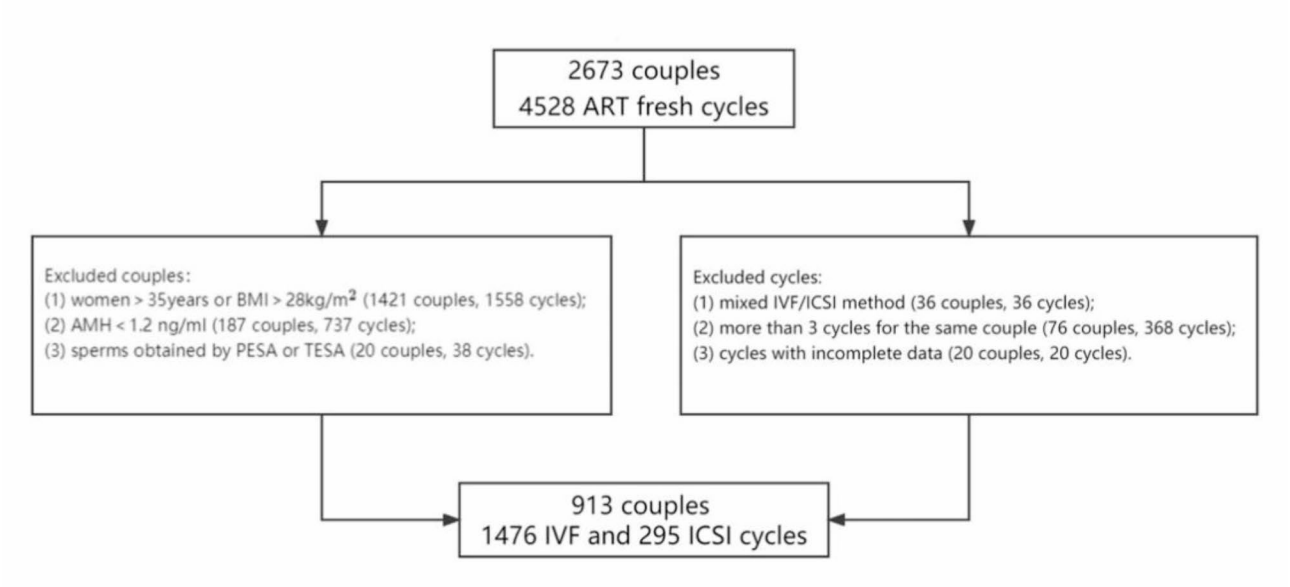

Fig. 1 Flow chart illustrating the details of the enrolled couples/cycles in the study

Evaluation of sperm DFI

The assessment of sperm DNA damage was conducted under FCM, utilizing the AOT in accordance with the manufacturer's instructions (Huakang Biomedicine, Inc., Shenzhen, China). In summary, fresh semen was initially liquefied at 37°C, followed by the incubation of a 10 μL sample with a 90 μL solution of disodium hydrogen phosphate sperm diluent. This solution was then introduced into a FCM test tube and further mixed with 200 μL of pretreatment fluid. After 30 seconds, 600 μL of a mixed solution (comprised of FCM buffer and acridine orange) was incorporated and thoroughly blended for 5 minutes. Specific parameter settings were set as: threshold of 'FSC-H greater than 100000', 'Ungated' storage gate, 560 PerCP channel voltage, 340 FITC channel voltage, and a low sample flow rate. Data were collected from a minimum of 5000 particles, with the sperm DFI computed as the ratio of red to total (red + green) spermatozoa ×100%.

Stimulation protocol

Controlled ovarian stimulation was performed using the gonadotropin releasing hormone agonist (GnRH-a) long protocol or GnRH-antagonist protocol. The women undergoing the long GnRH-a protocol received a dose of 1.25 mg long-acting GnRH-a (Diphereline, Ipsen, France) subcutaneously on day 18–20 of the previous cycle. 14 days later, 150–300 IU recombinant follicle-stimulating hormone (rFSH; Merck Serono, Inc., Darmstadt, Germany) and/or human menopausal gonadotropin (HMG; Lizhu Pharmaceutical, Inc., Guangzhou, China) were provided daily until trigger. GnRH-antagonist protocol was initiated on day 2 or 3 of the cycle with 75–300 IU of rFSH. 0.25 mg of GnRH antagonist (Cetrorelix, Merck serono, Switzerland) was given subcutaneously daily until trigger when the leading follicles reached a mean diameter of 14 mm. For both protocols, if three follicles reached a mean diameter of 17 mm or two follicles reached a mean diameter of 18 mm, 250 μg of recombinant human chorionic gonadotropin (r-HCG; Merck Serono, Inc., Darmstadt, Germany) was injected subcutaneously. Oocyte retrieval was performed 36 h after r-HCG injection via vaginal access guided by transvaginal ultrasound. Oocytes were cultured at 37°C and 6% CO_2 for 3–6 h before IVF/ICSI. The formation of the pronucleus of fertilized eggs was monitored 16–20 h after fertilization. The embryonic development and blastocyst formation were monitored regularly during the continuous cell culture.

Parameters for evaluating study couples and embryo development

The general conditions of the male patients, such as age, BMI, DFI, infertility duration. Sperm quality was assessed using computer-assisted sperm analysis (CASA). Sperm images were captured in the CASA system using a microscope and a corresponding image capture device, software algorithms were then used to identify and count the sperm in the images, calculating the sperm concentration. Subsequently, the system tracked changes in the position of the sperm head to evaluate sperm motility and assessed sperm morphology according to the WHO standards. In female patients, parameters like age, BMI, AMH, and the quantities of retrieved oocytes, and mature eggs (MII, metaphase 2) were assessed.

Time-lapse imaging (Vitrolife A/S, Denmark) was used to evaluate the quality of embryos, with observations

made under time-lapse microscopy until the day of embryo transfer or freezing. Parameters used to assess embryo development in the study included fertilization rate (ratio of fertilized eggs to retrieved eggs), 2PN (2-pronucleate) fertilization rate (ratio of 2PN fertilized eggs to retrieved eggs), cleavage rate (ratio of cleaved embryos to fertilized eggs), quantity of D3 embryos, quantity of high-quality D3 embryos, blastocyst development rate (ratio of blastocysts to all cultured embryos), and the quantity of high-quality blastocysts.

Statistical analysis

Statistical analysis of the data was performed using SPSS version 22.0 (SPSS, Inc., Chicago, IL). The descriptive variables were described as mean ± standard deviation (SD) when normally distributed, and otherwise as median, 25%, and 75%. Variables pertaining to IVF and ICSI groups were compared using the student's t-test if the distribution was normal or the Mann-Whitney test if the distribution was non-normal. Pearson's (normal distribution) or Spearman's rank (non-normal distribution) correlation analyses were used to discern the association among female factors, male factors, notably DFI, and the embryo quality in ART couples. To identify the optimal cut-off value for DFI in predicting embryo development, a receiver-operating characteristic (ROC) analysis was carried out to determine sensitivity and specificity, the area under the ROC curve, and optimal cut-off with 95% confidence intervals (CI). A value of $p < 0.05$ was considered statistically significant.

Results

Comparison of variables between IVF and ICSI cycles

The data presented in Table 1 depicted a total of 1,771 ART cycles, encompassing 1,476 IVF and 295 ICSI cycles. All variables were categorized into three major aspects to assess the quality of the embryos: female factors, male factors, and embryological parameters. As the constraints on the female participants had been scrutinized strictly, female age, BMI, AMH levels, and the quantities of oocytes and MII oocytes appeared to be effectively equalized between IVF and ICSI groups ($p > 0.05$).

In terms of male factors, with the exception of BMI ($p > 0.05$), other parameters like male age and the duration of infertility were notably elevated in the ICSI group in comparison to the IVF group ($p < 0.05$). Conversely, sperm DFI, concentration, progressive motility, and normal morphology were considerably reduced in the ICSI group ($p < 0.05$).

Pertaining to the embryological parameters, the fertilization rate, D3 embryos, blastocyst development rate, and high-quality blastocysts were conspicuously higher in the IVF group when compared with the ICSI group ($p < 0.05$). Contrastingly, 2PN fertilization rate and the

Table 1 Comparison of characteristics between IVF and ICSI cycles

Variables	Total	IVF	ICSI	*P* value
Cycles (n)	1771	1476	295	N/A
Male factors				
Duration of infertility (y)	3.0 (2.0, 4.0)	3.0 (2.0, 4.0)	3.0 (2.0, 5.0)	0.030*
Men age (y)	33.0 ± 3.9	32.8 ± 3.9	33.7 ± 4.0	0.001*
Men BMI (kg/m^2)	24.0 ± 3.4	24.0 ± 3.4	24.2 ± 3.6	0.294
DFI	18.0 ± 10.4	16.7 ± 9.0	24.6 ± 13.9	< 0.001*
Sperm concentration (×10^6/ml)	64.5 ± 34.8	69.1 ± 34.1	41.6 ± 28.5	< 0.001*
Sperm progressive motility (%)	39.3 ± 15.2	41.8 ± 13.6	26.6 ± 16.5	< 0.001*
Sperm normal morphology (%)	4.6 ± 2.1	5.0 ± 1.9	2.4 ± 1.7	< 0.001*
Femal Factos				
Women age (y)	30.7 ± 2.5	30.6 ± 2.6	30.8 ± 2.4	0.174
Women BMI (kg/m^2)	21.6 ± 2.5	21.7 ± 2.5	21.4 ± 2.5	0.052
Women AMH (ng/ml)	4.8 ± 3.0	4.8 ± 3.1	4.6 ± 2.4	0.154
Oocytes (n)	10.0 (6.0, 15.0)	10.0 (6.0, 15.0)	8.0 (5.0, 12.0)	0.755
MII (n)	8.0 (5.0, 12.0)	8.0 (5.0, 12.0)	8.0 (5.0, 12.0)	0.316
Embryological parameters				
Fertilization rate (%)	81.2 ± 19.2	81.8 ± 18.9	78.1 ± 20.8	0.002*
2PN fertilization rate (%)	69.0 ± 20.8	68.2 ± 20.4	73.3 ± 21.9	< 0.001*
Cleavage rate (%)	96.7 ± 12.8	96.9 ± 12.4	95.9 ± 14.8	0.268
D3 embryos (n)	5.0 (3.0, 9.0)	5.0 (3.0, 8.0)	3.0 (6.0, 11.0)	0.016*
High-quality D3 embryos (n)	3.0 (1.0, 5.0)	1.0 (3.0, 5.0)	8.0 (1.0, 4.0)	< 0.001*
Blastocyst development rate (%)	53.9 ± 37.6	55.5 ± 37.3	45.5 ± 38.3	< 0.001*
High-quality blastocysts (n)	1.0 (0, 3.0)	1.0 (0, 3.0)	0 (0, 2.0)	< 0.001*

BMI, body mass index; DFI, DNA fragmentation index; MII, metaphase 2; 2PN, 2-pronucleate; AMH, anti-mullerian hormone

*Significant *P* value ($P < 0.05$)

number of high-quality D3 embryos were notably lower in the IVF group as opposed to the ICSI group ($p < 0.05$), while the cleavage rate between both groups were similar ($p > 0.05$).

Correlations between sperm DFI and male parameters

We analyzed the correlation between sperm DFI and other male impacting factors within the context of the total 1,771 ART cycles (Fig. 2, Related to Table S1). The findings revealed a significant positive correlation between DFI and male age and a strong negative correlation with sperm progressive motility, concentration, and normal morphology ($p < 0.05$). However, no correlation was observed between DFI and male BMI ($p > 0.05$). The same correlation was observed in both 295 ICSI cycles and 1476 IVF cycles (Table S1) .

Correlations between male/female factors and embryological parameters in IVF cycles

Upon analyzing the male influencing factors in these 1,476 IVF cycles (Table 2), we determined that there was no correlation between sperm DFI and any of the considered embryological parameters, including the fertilization rate, 2PN fertilization rate, cleavage rate, the number of D3 embryos and high-quality D3 embryos, the blastocyst development rate, and the count of high-quality blastocysts, all yielding a p-value of > 0.05. There was not a significant correlation between sperm DFI and normal sperm morphology either ($p > 0.05$). Men's age and duration of infertility were significantly and negatively associated with the quantity of D3 embryos and high-quality D3 embryos ($p < 0.05$) but not with any other embryological parameters ($p > 0.05$). Likewise, men's BMI negatively influenced the blastocyst development rate ($p < 0.05$) but did not correlate with other embryological parameters ($p > 0.05$). Sperm concentration was positively correlated with the fertilization rate, D3 embryos, and high-quality D3 embryos ($p < 0.05$) but failed to correlate significantly with other embryological parameters ($p > 0.05$). Lastly, sperm progressive motility was significantly and positively correlated with fertilization and cleavage rates ($p < 0.05$), but no significant correlation was detected with other embryological parameters ($p > 0.05$).

Even after a stringent selection process for female participants, our current data reaffirmed that female factors had a more pronounced influence on embryo quality compared to their male counterparts. Significantly, we observed a positive correlation between AMH and several variables, including fertilization rate, 2PN fertilization rate, and blastocyst development rate, as well as the quantities of D3 embryos and high-quality D3 embryos ($p < 0.05$). Simultaneously, women's age was significantly negatively correlated with blastocyst development rate and the quantities of D3 embryos and high-quality D3 embryos ($p < 0.05$). Aside from the fertilization rate ($p > 0.05$), numbers of retrieved oocytes displayed a noteworthy positive correlation with all other embryological parameters under consideration, including the 2PN fertilization rate, cleavage rate, the quantities of D3 embryos and high-quality D3 embryos, blastocyst development rate, and the quantity of high-quality blastocysts ($p < 0.05$). Meanwhile, MII oocytes showed a positive correlation with all the embryological parameters ($p < 0.05$)

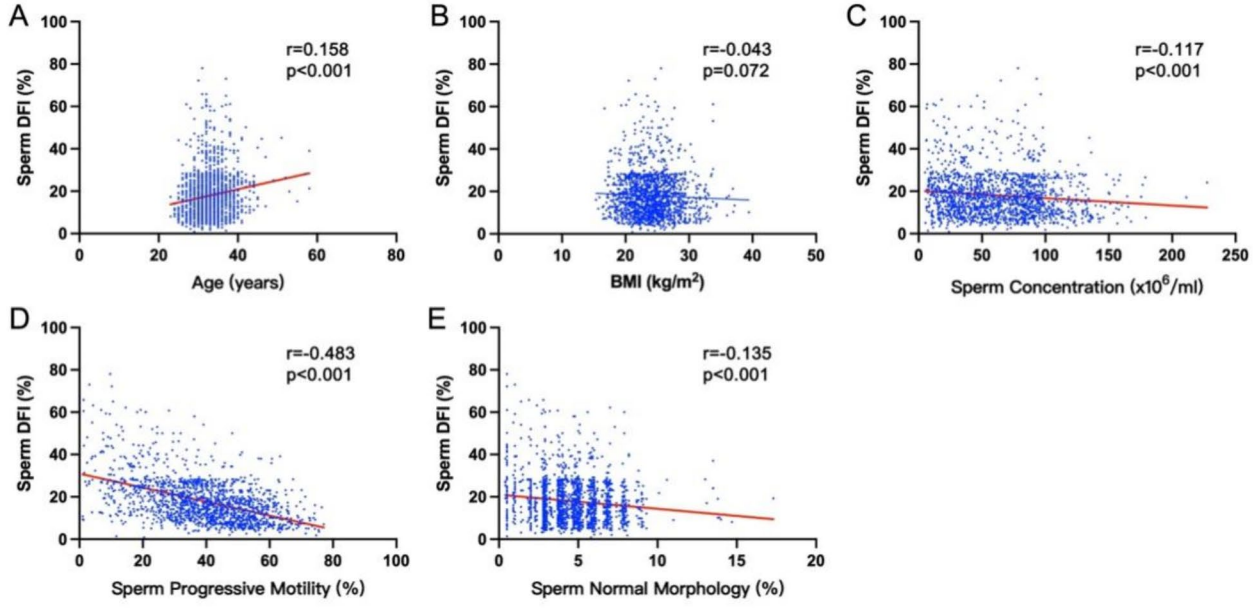

Fig. 2 Association between sperm DFI and men age, BMI, sperm concentration, sperm progressive motility and sperm normal morphology in ART cycles

Table 2 Correlations between male/female factors and embryological parameters in IVF cycles ($n = 1476$)

Variables	Fertilization rate		2PN fertilization rate		Cleavage rate		D3 embryos		High-quality D3 embryos		Blastocyst development rate		High-quality blastocysts	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Femal Factos														
Women age	-0.002	0.940	-0.007	0.801	-0.001	0.871	-0.142	< 0.001*	-0.096	< 0.001*	-0.063	0.016*	-0.015	0.557
Women BMI	-0.013	0.615	-0.021	0.413	-0.031	0.233	-0.035	0.181	-0.010	0.709	-0.034	0.190	-0.001	0.970
Women AMH	0.056	0.031*	0.067	0.010*	0.047	0.070	0.422	< 0.001*	0.405	< 0.001*	0.187	< 0.001*	0.022	0.394
Oocytes	0.046	0.075	0.053	0.043*	0.087	0.001*	0.812	< 0.001*	0.631	< 0.001*	0.340	< 0.001*	0.069	0.008*
MII	0.296	< 0.001*	0.023	0.371	0.119	< 0.001*	0.889	< 0.001*	0.700	< 0.001*	0.380	< 0.001*	0.056	0.032*
Male factors														
Sperm DFI	-0.034	0.197	-0.002	0.930	-0.029	0.274	-0.031	0.241	-0.015	0.560	-0.006	0.809	-0.008	0.771
Duration of infertility	-0.039	0.136	-0.038	0.149	-0.023	0.387	-0.057	0.028*	-0.082	0.002*	-0.049	0.059	-0.008	0.747
Men age	-0.008	0.746	-0.002	0.935	-0.010	0.707	-0.064	0.014*	-0.085	0.001*	-0.051	0.052	-0.025	0.331
Men BMI	-0.008	0.769	-0.001	0.984	-0.009	0.726	-0.046	0.079	-0.038	0.147	-0.057	0.029*	-0.016	0.539
Sperm concentration	0.107	< 0.001*	0.049	0.061	0.042	0.109	0.081	0.002*	0.064	0.014*	0.034	0.192	0.013	0.630
Sperm progressive motility	0.053	0.040*	0.036	0.161	0.055	0.036*	0.036	0.162	0.049	0.059	0.013	0.618	0.005	0.845
Sperm normal morphology	0.012	0.646	0.008	0.771	0.004	0.865	0.044	0.091	0.049	0.059	0.025	0.340	0.009	0.734

*Significant *P* value ($P < 0.05$)

with the exception of the 2PN fertilization rate ($p > 0.05$). Our data demonstrated that women's BMI had no correlation with any of the included embryological parameters ($p > 0.05$).

Correlations between male/female factors and embryological parameters in ICSI cycles

In line with the results of the IVF cycles, the data from the ICSI cycles also demonstrated that sperm DFI and normal morphology did not have a significant impact on embryo quality ($p > 0.05$) (Table 3). According to the correlation analysis between male factors and embryological parameters, the duration of infertility negatively correlated with the quantities of D3 embryos, high-quality D3 embryos, and high-quality blastocysts ($p < 0.05$). Men's age was negatively correlated with the quantities of D3 embryos and high-quality D3 embryos ($p < 0.05$). Men's BMI was only negatively correlated with the blastocyst development rate ($p < 0.05$). Sperm concentration was negatively correlated with the fertilization rate, the quantities of D3 embryos, and high-quality D3 embryos ($p < 0.05$). Sperm progressive motility was negatively correlated with the fertilization rate and the 2PN fertilization rate ($p < 0.05$). In addition, there were no significant correlations observed among other parameters.

In comparison to male factors, female factors demonstrated a more substantial influence on the embryo quality. Specifically, AMH, the quantity of retrieved oocytes, and the number of MII oocytes each exhibited a significant positive correlation with the blastocyst development rate and the quantities of D3 embryos, high-quality D3 embryos, and high-quality blastocysts ($p < 0.05$). Women's age was only negatively correlated with the number of D3 embryos ($p < 0.05$). Furthermore, there was no observed correlation between women's BMI and any of the embryological parameters included in the study ($p > 0.05$).

Correlations between sperm DFI and embryological parameters in total ART cycles

In the study, we tried to merge the IVF and ICSI cycles to boost the sample size and then analyzed the impact of sperm DFI on embryo quality across the total 1,771 ART cycles (Fig. 3, Related to Table S2). The results revealed that sperm DFI had a significantly negative correlation solely with the fertilization rate ($p < 0.05$), whereas it held no correlation with other embryological parameters ($p > 0.05$).

Optimal cut-off value of sperm DFI for the prediction of embryological outcomes

Given the statistical correlation between sperm DFI and the fertilization rate, we used the fertilization rate of ≥ 80% as the threshold and tried to determine the

Table 3 Correlations between male/female factors and embryological parameters in ICSI cycles ($n = 295$)

Variables	Fertilization rate		2PN fertilization rate		Cleavage rate		D3 embryos		High-quality D3 embryos		Blastocyst development rate		High-quality blastocysts	
	r	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value	*r*	*P* value
Femal Factos														
Women age	-0.069	0.240	-0.065	0.266	0.007	0.899	0.127	0.029*	0.084	0.150	0.047	0.424	0.107	0.067
Women BMI	-0.037	0.523	-0.037	0.524	0.033	0.569	0.010	0.859	0.048	0.408	-0.040	0.494	-0.035	0.549
Women AMH	0.010	0.860	-0.018	0.763	0.053	0.363	0.345	< 0.001*	0.331	< 0.001*	0.245	< 0.001*	0.341	< 0.001*
Oocytes	0.015	0.792	0.010	0.868	0.098	0.092	0.639	< 0.001*	0.494	< 0.001*	0.340	< 0.001*	0.540	< 0.001*
MII	0.010	0.858	0.020	0.738	0.105	0.071	0.733	< 0.001*	0.578	< 0.001*	0.391	< 0.001*	0.609	< 0.001*
Male factors														
Sperm DFI	-0.038	0.512	-0.017	0.772	0.045	0.443	0.057	0.332	0.005	0.930	-0.026	0.651	-0.014	0.807
Duration of infertility	-0.062	0.290	-0.045	0.446	-0.016	0.780	-0.129	0.027*	-0.162	0.005*	-0.054	0.359	-0.127	0.030*
Men age	-0.101	0.084	-0.090	0.124	0.088	0.132	-0.058	0.320	-0.118	0.044	-0.063	0.284	-0.157	0.007*
Men BMI	-0.069	0.239	-0.048	0.408	0.061	0.299	-0.023	0.688	-0.008	0.889	-0.097	0.095	-0.010	0.867
Sperm concentration	0.005	0.930	0.037	0.532	0.105	0.070	0.034	0.564	0.014	0.812	0.059	0.314	0.034	0.557
Sperm progressive motility	0.080	0.172	0.033	0.570	0.081	0.168	0.165	0.005*	0.154	0.008*	0.059	0.316	0.166	0.004*
Sperm normal morphology	0.042	0.474	0.006	0.914	0.007	0.911	0.048	0.415	0.016	0.789	0.026	0.651	0.016	0.781

*Significant *P* value ($P < 0.05$)

predictive cut-off value for high fertilization rate with respect to sperm DFI through the ROC curve. Based on the ROC curve analysis, the sperm DFI cut-off point of 21.15% showed 36.7% sensitivity and 28.9% specificity with an area under the curve (AUC) of 0.542 (95% CI: 0.514–0.570, $p < 0.05$) (Fig. 4). However, we also observed that despite the cut-off value of 21.15% showing statistical significance, its predictive power for the fertilization rate was relatively weak.

Discussion

In this study, it was observed that among the male factors incorporated into the IVF cycles, only men's age, duration of infertility, sperm concentration, and progressive motility were shown to have significant correlations with certain embryological parameters. Their influence was particularly pronounced on the quantity of D3 embryos. In both IVF and ICSI cycles, male age and duration of infertility are negatively correlated with the number and quality of D3 embryos, while male BMI is mainly significantly associated with the rate of blastocyst development. Sperm concentration and sperm progressive motility are both related to the fertilization rate. In the case of the ICSI cycles, owing to the selection of competent spermatozoa exhibiting higher fertilization potential, the impact of men's age and sperm concentration on the embryo quality was found to diminish to a certain extent. Nonetheless, a notable correlation was found to persist between men's age and sperm progressive motility with the count of D3 embryos or high-quality blastocysts. Although we have adopted rigorous control measures to account for female factors such as age, weight, and ovarian function in the study in an attempt to minimize their influence on embryo quality, findings still illustrated that female factors nonetheless play a more pivotal role in early embryo development during both IVF and ICSI treatments compared to male factors. Specifically, the levels of AMH in women, the number of oocytes retrieved, and the quantity of MII oocytes play crucial roles in early embryo development, exerting a significant influence across all embryological parameters, including the fertilization rate, 2PN fertilization rate, cleavage rate, the number of D3 embryos and high-quality D3 embryos, the blastocyst development rate, and the number of high-quality blastocysts. This may elucidate the reason most previous studies of ART treatments have primarily centered their attention on the impacts attributable to female factors while contributing less significance to male counterparts.

In conjunction with routine semen analysis, the sperm DFI has now widely become an established standard for the assessment of sperm functionality in ART treatments [14]. However, conclusions regarding the correlation between the DFI and conventional sperm parameters

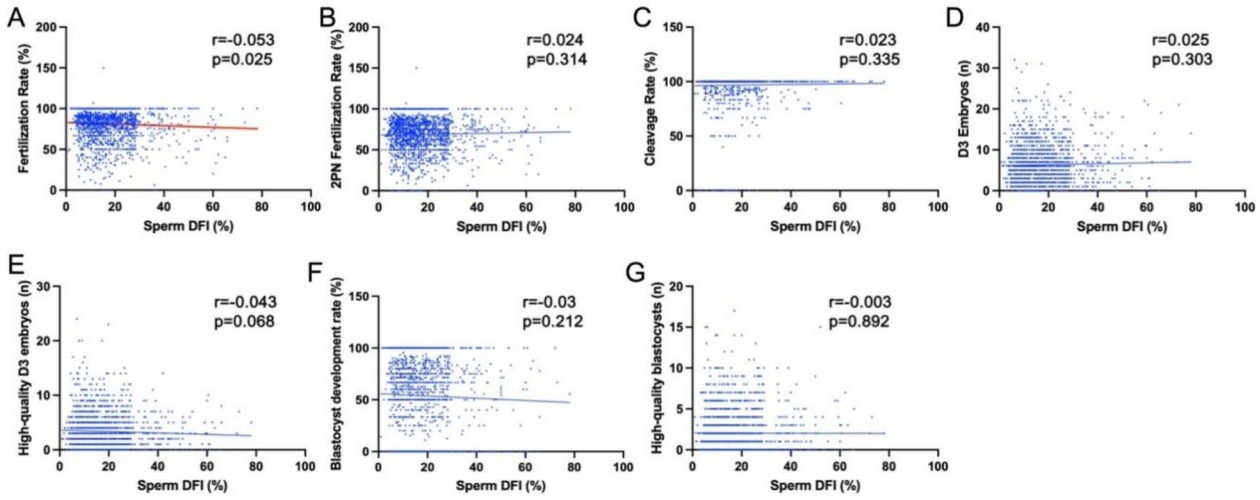

Fig. 3 Correlations between sperm DFI and embryological parameters in ART cycles

remain contentious and disputed. The disagreement likely stems from the inconsistent inclusion criteria for subjects in different studies. For instance, studies focused on generally infertile men had indicated a negative correlation between DFI and other male factors such as men's age, sperm concentration, normal morphology, and/or sperm motility [15, 16]. Consistent with previous findings [17], our study analyzed semen samples from infertile males undergoing ART, IVF and ICSI treatments, revealing that the DFI was positively correlated with male age and negatively correlated with conventional sperm parameters such as sperm motility, concentration, and normal morphology, but bore no relation to male BMI. This may be potentially ascribed to the optimization and selection processes applied to male sperm during ART treatments, which might, to some extent, mask the intrinsic information contained in the semen samples. The present findings underscored that male age significantly impacts early embryo development in both IVF and ICSI cycles. As male age advances, there is a reduction in sperm telomeres' length [18], which has a positive correlation with embryo quality in IVF treatments [19, 20]. Additionally, human spermatozoa are vulnerable to reactive oxygen species (ROS) attacks that may result in sperm DNA fragmentation [21, 22]. ROS can simultaneously exert a severe impact on sperm motility [23]. This relationship was supported by the present data, which showed a significant correlation between DFI and sperm progressive motility. A sperm DFI test is preferentially recommended for infertile men with older age who specifically demonstrate decreased sperm progressive motility.

In this study, it was observed that sperm DFI did not exhibit any considerable correlation with the embryological parameters, regardless of IVF or ICSI cycles. Upon mixing data from both the IVF and ICSI cycles to establish a wider sample size, we discerned that the sperm DFI demonstrated a statistically significant correlation solely with the fertilization rate, leaving all other embryological parameters unaffected. Furthermore, the present data proposed a sperm DFI cut-off value of 21.15%, which offered predictive potential for high fertilization rates (≥ 80%) in ART cycles. This finding was consistent with the majority of previous research suggesting DFI thresholds of around 20% [24, 25]. It is important to note that despite the statistical significance of the 21.15% cut-off value, its predictive specificity and sensitivity were relatively weak. Previous studies suggested that setting a DFI threshold between 15 and 30% could effectively differentiate infertile males [15, 24–26]. However, the emergence of advanced techniques can reduce the need for enhancing the semen parameters [27], as evidenced by the lack of significant differences in sperm DFI among men undergoing ART treatments [28], The present results reinforced this conclusion, emphasizing that the predictive value of DFI for determining the high fertilization rate within ART treatments remained relatively low. Hence, it is proposed that although the sperm DFI carries considerable relevance in evaluating male fertility, it exhibits merely marginal significance in predicting pregnancy outcomes following ART treatments.

The association between maternal factors and pregnancy outcomes of ART treatments has been well investigated. However, the impact of paternal factors on subsequent embryo development following ART treatments has been largely overlooked. Our study highlighted that male sperm parameters seemingly exert their influence during the initial stages of embryo development, from fertilization to the D3 embryo stage. Notably, the influence of sperm DFI on ART embryological parameters was found to be significant solely in the fertilization stage. Meanwhile, female factors appeared to maintain a continual influence throughout the entire process of

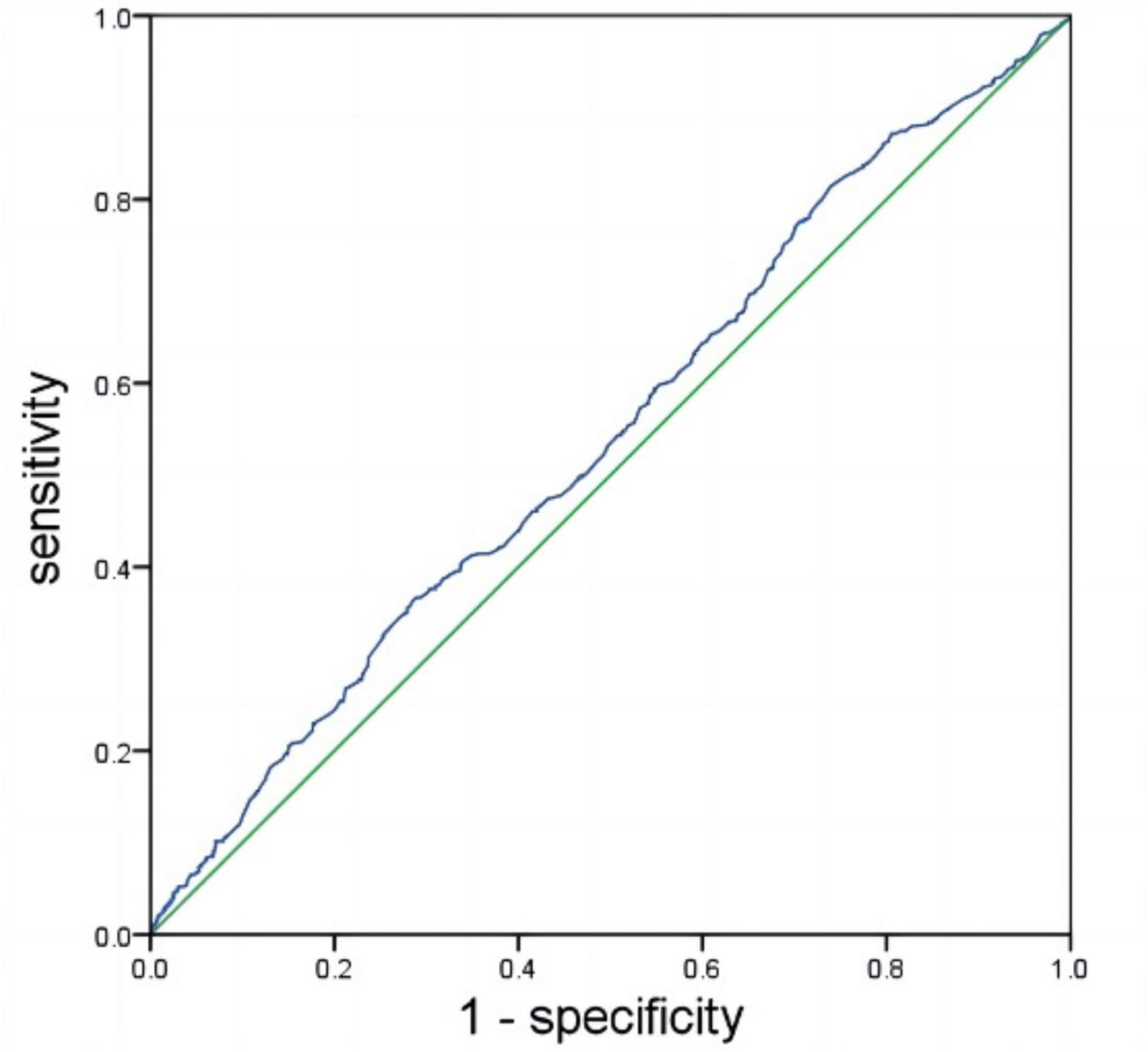

Fig. 4 The receiver operating characteristic (ROC) curve analysis identified an optimal threshold value for sperm DFI to anticipate an embryological outcome typified by a fertilization rate of 80% or higher in ART cycles. The established cut-off value for DFI in predicting a high fertilization rate (≥ 80%) stood at 21.15%. The analysis divulged an area under the curve (AUC) of 0.542 (95% CI: 0.514–0.570, $p < 0.001$)

early embryo development, from the fertilization stage to the formation of the blastocyst. In line with our observations, prior studies have demonstrated that among males exhibiting sperm DFI exceeding 20%, the kinetics of early embryonic progression, spanning from pronuclear formation to the four-blastomere stage, undergo acceleration without compromising pregnancy potential [29]. Similarly, sperm concentration and motility have been shown to modulate the fertilization rate, but without detrimental effects on the production of high-quality blastocysts [30]. Two innovative strategies, one specifically focused on embryos derived from donor oocytes and the other utilizing donor sperm, have been employed to dissect the relative contributions of oocytes and sperm during the initial stages of embryonic cleavage, ultimately confirming the crucial importance of the oocyte [31]. It is suggested that the paternal impact of damaged chromatin on the early embryo development may be mitigated by the active repair mechanisms inherent in oocytes [32]. The competency of the oocyte exerts a more decisive influence on the progression of embryo development [33]. In fact, the number of proteins identified in sperm currently surpasses that in oocyte [34]. However, a comprehensive understanding of the roles that these sperm proteins play in embryo development still needs to be established.

The present study has certain limitations. Firstly, the sperm DFI values can vary depending on the different detection techniques employed. For instance, sperm DNA fragmentation can occur on either one or both DNA strands, with a more profound negative impact of sperm double-strand breaks (DSBs) on reproductive outcomes, while the neutral comet assay is the only technique that can differentiate single-strand breaks (SSBs) from DSBs [35]. To a certain extent, establishing the DFI cut-off value solely on the basis of our single-center data lacks robustness. Therefore, it is imperative to incorporate an additional DFI-related technique and integrate multi-center data in subsequent studies. Secondly, as the enrolled subjects are still within their follow-up period, an analysis of the ultimate pregnancy outcomes is currently unfeasible. This necessitates further exploration into the impact of the sperm DFI on the outcomes of ART treatments, in order to clarify the time-dependent effects of sperm DFI on the entire spectrum of embryonic development processes. Nonetheless, accumulating evidence suggests that contributions from male-derived factors extend well beyond the provision of the male haploid genome to the early embryo, and semen may play a pivotal role in the intricacies of embryogenesis [36].

In conclusion, despite the potential diminishment of the predictive significance of sperm parameters on embryo quality due to the application of semen optimization processes, our data still indicated that sperm DFI could exhibit some predictive power for the fertilization rate during ART cycles. Furthermore, a threshold value of 21.15% has been identified, offering potential predictive utility for high fertilization rates (≥ 80%). Yet, its correlation with other embryological parameters concerning early embryo development appeared to be inconsequential. For older males or those displaying decreased sperm motility, as suggested by routine semen analysis, a combined assay of DFI may heighten the prediction effectiveness for embryo quality throughout ART treatments. Current evidence continues to highlight that female factors predominately exert stronger impact on embryo quality and pregnancy outcome in ART cycles in contrast to male factors. The implications of sperm-contributing factors regarding the pregnancy outcomes of ART and their effects on the subsequent offspring undoubtedly merit further exploration.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12958-025-01345-8.

Supplementary Material 1

Author contributions

J.X. and S.Q. conceived and designed the study. H.J. and S.Q. collected samples, performed experiments and conducted the data analysis. X.X. and H. P. collected samples and conducted the data analysis. Y.L. conducted the data analysis. H.J. wrote the article. S.Q. and J. X. critically revised the article. All authors have been involved in interpreting the data and approved the final version.

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Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate

This study was approved by the Ethics Committee of Zhuhai Maternal & Child Health Care (Approval No: [2018] ICE-22). Written informed consent from all enrolled patients was obtained prior to the study.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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