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Article

Study on the Correlation Between Sperm Mitochondrial Membrane Potential, Seminal Plasma Elastase, Seminal Plasma Zinc, and Pregnancy Outcomes of *In Vitro* Fertilization-Embryo Transfer in Male Infertility Patients

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Abstract

Aims/Background: Male infertility accounts for 30%–50% of infertility cases. Sperm mitochondrial membrane potential (MMP) and seminal plasma zinc are crucial for sperm motility and function, while seminal elastase reflects infection and inflammation of the reproductive system. This study aimed to explore the roles of sperm MMP, seminal plasma elastase, and seminal plasma zinc in the outcomes of *in vitro* fertilization-embryo transfer (IVF-ET) among male infertility patients. **Methods:** Clinical data from 179 male infertility patients who visited the hospital between November 2022 and July 2023 were retrospectively analysed. Of these, 85 cases underwent fresh embryo transfer (ET) after *in vitro* fertilization (IVF). According to the pregnancy outcomes, the 85 ET cycles were divided into a pregnancy group (n = 42) and a non-pregnancy group (n = 43). Sperm MMP, seminal plasma elastase, and seminal plasma zinc levels were measured. Spearman correlation analysis was used to assess the correlation between fertilization rate and sperm MMP, seminal plasma elastase, and seminal plasma zinc. Binary logistic regression was performed to identify independent factors influencing pregnancy outcomes. Receiver operating characteristic (ROC) curves were constructed to predict diagnostic values of these factors for pregnancy outcomes. **Results:** The fertilization rate of IVF was positively correlated with sperm MMP (r = 0.362) and seminal plasma zinc (r = 0.415), and negatively correlated with seminal plasma elastase (r = –0.379) (all $p < 0.001$). Among the 85 ET cycles, the clinical pregnancy rate and live birth rate were 49.41% (42/85) and 41.18% (35/85), respectively. Compared to the non-pregnancy group, the pregnancy group had significantly higher sperm MMP and seminal plasma zinc levels ($p < 0.001$ and $p = 0.009$, respectively), and lower seminal plasma elastase levels ($p = 0.002$). Multivariate analysis identified sperm non-progressive motility ($p = 0.013$, odds ratio [OR] = 0.478, 95% confidence interval [CI] = 0.267–0.856), sperm MMP ($p < 0.001$, OR = 1.114, 95% CI = 1.047–1.184), seminal plasma elastase ($p = 0.006$, OR = 0.996, 95% CI = 0.993–0.999), and seminal plasma zinc ($p = 0.024$, OR = 1.188, 95% CI = 1.023–1.381) as independent factors affecting pregnancy outcome. ROC analysis showed that sperm MMP (area under the curve [AUC] = 0.748, 95% CI = 0.644–0.851, $p < 0.001$), seminal plasma elastase (AUC = 0.698, 95% CI = 0.587–0.809, $p < 0.001$), seminal plasma zinc (AUC = 0.664, 95% CI = 0.549–0.779, $p = 0.005$), and the combined indicators (AUC = 0.832, 95% CI = 0.747–0.916, $p < 0.001$) had good predictive values for pregnancy outcomes. **Conclusion:** Sperm MMP, seminal plasma elastase, and seminal plasma zinc are significantly correlated with fertilization rates in IVF. Combined detection of these three indicators provides strong predictive value for pregnancy outcomes in IVF-ET among male infertility patients.

Keywords: male infertility; *in vitro* fertilization; embryo transfer; sperm; mitochondrial membrane potential; elastase; zinc

1. Introduction

Infertility is becoming an increasingly prominent global issue, and the decline in fertility rates has led to numerous social challenges [1]. Statistically, male factors contribute to 30%–50% of infertility cases [2]. In recent years, the incidence of male infertility has shown an upward trend, especially in some industrialised countries and regions [3]. The causes of male infertility are complex, including genetic, environmental, physical, psychological, and lifestyle factors [4]. *In vitro* fertilization (IVF) with embryo transfer (ET) is an important component of assisted reproductive technology and plays a significant role in the treatment of male infertility [5]. Semen parameters, such as concentration, motility, and morphology, are commonly used in clinical practice to evaluate sperm quality. However, these parameters do not accurately reflect male fertility potential, which limits their application in assisted reproductive technologies [6]. This limitation is evident as some infertile men may exhibit normal semen parameters, and semen analysis alone cannot be considered equivalent to an assessment of male fertility [7,8]. Therefore, it is necessary to explore more effective methods for predicting the therapeutic outcomes of IVF-ET in male infertility patients.

Mitochondria serve as the primary energy source for sperm. Through oxidative phosphorylation in the respiratory chain, mitochondria generate Adenosine Triphosphate (ATP), creating a transmembrane proton gradient and establishing the mitochondrial membrane potential (MMP) [9,10]. Sperm MMP reflects the level of energy metabolism and is closely related to sperm motility [9]. Sperm with high MMP have a sufficient energy supply and exhibit higher motility [9]. A previous study explored the correlation between sperm MMP and pregnancy outcomes in IVF, finding that higher sperm MMP was significantly associated with increased fertilization rates, clinical pregnancy rates, or live birth rates; however, no significant differences were found in miscarriage rates [11]. In addition, sperm zinc and seminal elastase are associated with sperm quality [12,13,14]. Zinc ions, which are secreted by the prostate gland, participate in the synthesis and activation of sperm acrosomal enzymes. Moreover, zinc exhibits antioxidant properties and plays a crucial role in clearing free radicals, thereby maintaining the structural and functional integrity of sperm [12]. Seminal elastase, an enzyme with immune functions, is released in large quantities into seminal plasma during infection of the male reproductive system, whose level can be used to evaluate inflammation and infection within the male reproductive system [14]. However, excessive levels of elastase may negatively affect the quality and function of sperm [15]. Previous studies have explored the roles of sperm MMP, seminal plasma zinc, and seminal plasma elastase in assisted reproduction [11,16,17]. However, most of these studies have focused on the application of individual semen indicators in assisted reproductive technology. Given that male fertility is affected by multiple factors, it is necessary to combine several indicators to achieve a more comprehensive evaluation. The integration of multiple parameters could reflect semen quality in multiple dimensions, also contributing to the reliability of prediction in assisted reproduction techniques, as well as to the optimisation of therapeutic regimens. Nevertheless, the combined role of sperm MMPs, seminal plasma zinc, and seminal plasma elastase in IVF-ET remains unclear. This study aims to investigate the role and influence of sperm MMP, seminal plasma elastase, and seminal plasma zinc in the outcomes of IVF-ET. This study may provide a predictive role for male infertile patients in the context of IVF-ET and contribute to the optimisation of fertility assistance programs in assisted reproductive technology.

2. Methods

2.1 Patients

This retrospective study included 179 male infertility patients who visited the Women and Children's Hospital of Ningbo University between November 2022 and July 2023. Clinical data were collected from these patients for retrospective analysis. Inclusion criteria were as follows: (1) patients met the World Health Organization (WHO) diagnostic criteria for male infertility, defined as failure to fertilize the woman after 1 year of regular, unprotected intercourse, without detected female infertility factors [18]; (2) male age $\leq$45 years; (3) female age $\leq$35 years with normal ovarian function; (4) patients undergoing IVF-ET; (5) both partners had normal chromosomal karyotypes; (6) complete clinical data were available. Exclusion criteria included: (1) female patients with ovarian dysfunction, polycystic ovary syndrome, endometriosis, or a history of miscarriages or stillbirth; (2) male patients with azoospermia, severe oligozoospermia, and abnormalities in reproductive system organs such as testicles, epididymis, and vas deferens (including cryptorchidism, varicocele, testicular torsion, etc.); (3) failure to retrieve or obtain oocytes during the IVF cycle; (4) either partner had a history of surgery or medication taken within the previous 3 months; (5) either partner suffered from chronic diseases, cancer, or other disorders; (6) either partner had a history of familial genetic diseases. This study was approved by the Ethics Committee of the Women and Children's Hospital of Ningbo University (Approval No. NBFE-2025-KY-067). All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants. The study design and patient selection process are shown in Fig. 1.

2.2 General Information Collection, Semen Collection, and Semen Parameter Analysis

General demographic and clinical information were collected, including age and body mass index (BMI) for both males and females, duration of abstinence, and history of smoking and alcohol consumption, as well as exposure to passive smoking. Smoking and drinking history were categorized as "Never" and "Quitting". "Never" was defined as no history of smoking or drinking, while "Quitting" was defined as quitting smoking and drinking for more than 3 months. To minimize the impact of tobacco and alcohol on sperm and eggs, both partners were required to quit smoking and drinking for at least 3 months before IVF-ET and to maintain smoking and alcohol cessation during IVF-ET. Passive smoking was defined as the female being passively exposed to tobacco for more than 15 minutes in the workplace or home per day.

After abstinence for 3–7 days, semen samples were collected by masturbation. The samples were incubated at 37 °C for 30 minutes to allow liquefaction, after which semen parameters were assessed. Semen analysis was performed using the CFT-920 semen quality detection system (Jiangsu Ruiqi Life Science and Technology Development Co., Ltd., Xuzhou, China), measuring semen volume, sperm concentration, progressive motility, non-progressive motility, and immotility. Sperm morphology was evaluated using modified Papanicolaou staining (Pasteur staining solution, 20160237, Huakang Biomedical Co., Ltd., Shenzhen, China) to determine the sperm deformity index.

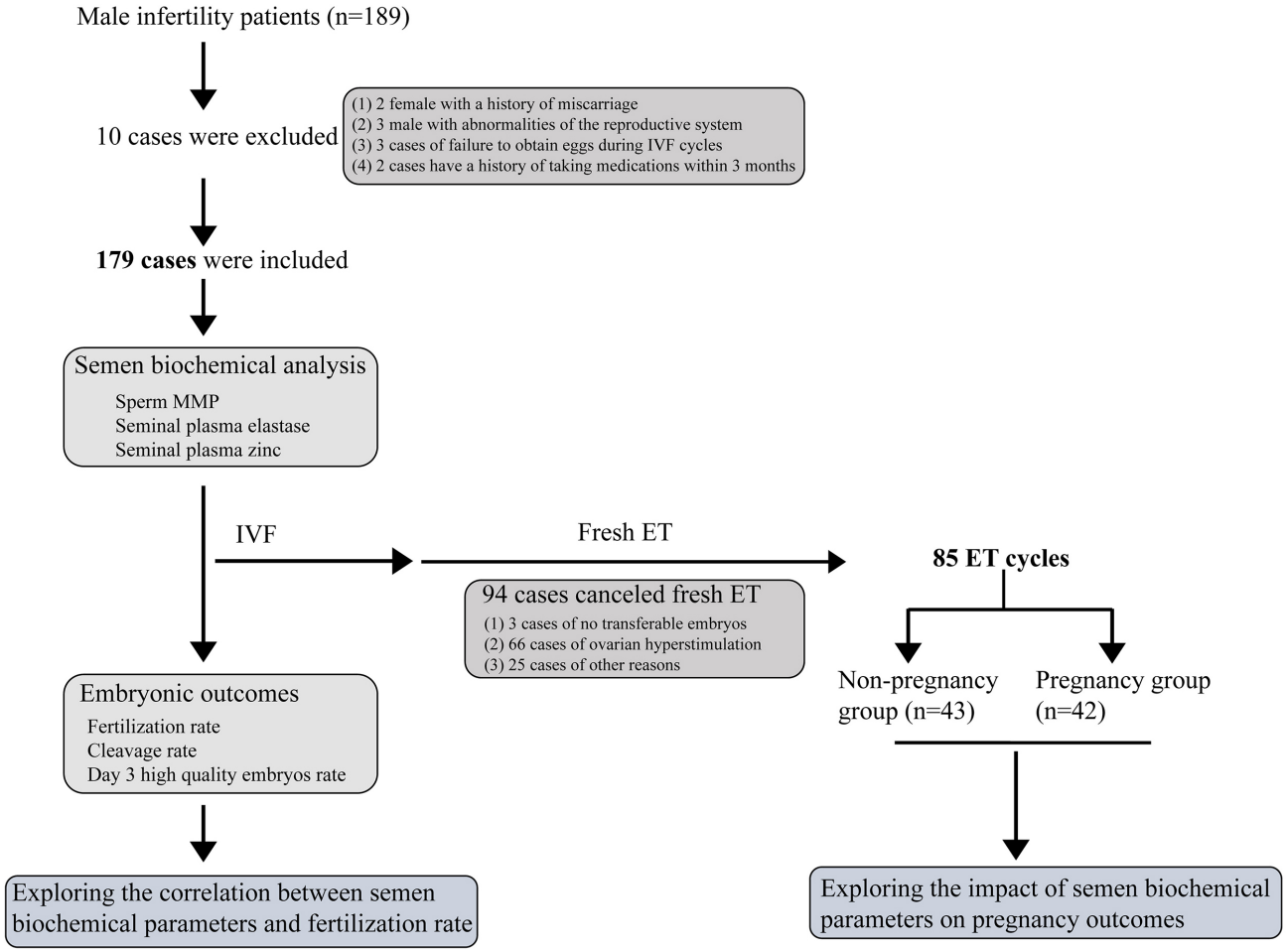

Fig. 1. Flowchart of patient selection and study design. IVF, *in vitro* fertilization; ET, embryo transfer; MMP, mitochondrial membrane potential.

2.3 Detection of Seminal Plasma Elastase and Seminal Plasma Zinc

After centrifugation (3000 ×g, 10 min), levels of elastase and zinc in seminal plasma were measured according to the manufacturer's instructions. Seminal plasma elastase (20152400612) and zinc (20152400613) reagent kits were purchased from Huakang Biomedical Co., Ltd. (Shenzhen, China). Standard samples and quality control samples were included in each assay to ensure accuracy.

2.4 Sperm MMP

After centrifugation, spermatozoa were resuspended in phosphate–buffered saline (PBS) and adjusted to a concentration of 1×10^6/mL. Sperm were stained with 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylimidacarbocyanine iodide (JC-1, FC0711A, 5 mg/mL, Zhejiang Xingbo, Ningbo, China) and incubated at 37 °C in the dark for 10 minutes. Excess dye was removed by washing with PBS. The MMP of sperms were detected using a flow cytometer (BD Calibur, BD Biosciences, Franklin Lakes, NJ, USA), with 10,000 sperm cells assessed per sample. Quality control was performed using high and low target value samples before each detection to ensure the accuracy of the results.

2.5 IVF-ET Cycle

A conventional ovarian stimulation program was adopted to obtain oocytes. Specifically, on day 3 of menstruation, follicle-stimulating hormone (FSH) (H2005 2130, Lizhu Pharmaceutical, Zhuhai, China) and human menopausal gonadotropin (hMG) (H44020673, Lizhu Pharmaceutical, Zhuhai, China) were administered to female participants. Follicular development was monitored via transvaginal ultrasound. When ≥3 follicles reached a diameter of 18 mm, recombinant human chorionic gonadotrophin (HCG) (10000IU, S20210010, Lizhu Pharmaceutical, Zhuhai, China) was administered to trigger ovulation. Next, 36 h after the trigger, transvaginal oocyte retrieval (TVOR) was performed to obtain oocytes.

The obtained ova were cultured in culture plates at 37 °C. After 4 hours, oocytes were inseminated using standard IVF procedures. Zygotes were continuously cultured and monitored regularly under a microscope. Normal fertilization was defined by the presence of two pronuclear (2PN).

All embryos were incubated at 37 °C in an embryo incubator. After 3 days of culture, embryos were evaluated, and high-quality embryos (1–2) were selected for transplantation.

Fresh ET was performed. Subjects received oral progesterone (200 mg/day, H20041902, Xianju Pharmaceutical Co., Ltd., Taizhou, China) starting from the day of oocyte retrieval and continuing until 14 days after embryo transfer. If the subject developed a positive pregnancy, progesterone support was continued until 8 weeks of gestation. After 14 days of embryo transfer, serum HCG levels were measured. After 4 weeks, transvaginal ultrasonography was performed. Clinical pregnancy was determined as serum HCG $\geq$25 IU/L, and observing fetal heartbeat and intrauterine gestational sac.

2.6 Embryonic Outcomes and Pregnancy Outcomes Analysis

A total of 179 couples underwent IVF procedures. Embryonic outcomes were assessed by fertilization rate, cleavage rate, and day 3 high-quality embryo rate. Fertilization rate (%) = number of 2PN/total number of retrieved ova $\times$ 100. Cleavage rate (%) = number of fertilized oocytes divided into embryos/the total number of fertilized oocytes $\times$ 100. Day 3 high-quality embryos rate (%) = number of day 3 high-quality embryos/the total number of embryos $\times$ 100.

After IVF, 85 female patients underwent fresh ET (total 85 transplant cycles). Data from these 85 fresh ET cycles were collected for pregnancy outcomes analysis, including ET rate, clinical pregnancy rate, and live birth rate. ET rate (%) = number of patients receiving fresh ET/total cases $\times$ 100. Clinical pregnancy rate (%) = number of clinical pregnancy cycles/total transplant cycles $\times$ 100. Live birth rate (%) = number of live births/total transplant cycles $\times$ 100. Based on pregnancy outcomes, the 85 cases who received ET were divided into two groups: pregnancy group (n = 42) and non-pregnancy group (n = 43).

2.7 Statistical Analysis

SPSS 22.0 software (IBM, Armonk, NY, USA) was used for statistical analysis. The Shapiro-Wilk test was applied to assess data normality. Normally distributed data are presented as mean $\pm$ standard deviation (SD), while non-normally distributed data are presented as median with interquartile range (IQR). Comparison between two groups was performed using an independent *t*-test for normally distributed data and a Mann-Whitney U test for non-normally distributed data. Categorical variables are presented as n (%), and the chi-square test was used for comparisons between groups. Spearman correlation analysis was used to explore the correlation between fertilization rate with sperm MMP, seminal plasma elastase, and seminal plasma zinc. Independent factors affecting pregnancy outcomes were identified using binary logistic regression analysis. Variance inflation factor (VIF) testing was used to assess multicollinearity. Based on the rule of thumb that at least 10 outcome events per predictor variable (EPV) are required for logistic regression analysis [19], the sample size was calculated. In our study, the pregnancy rate was approximately 49%, and four predictor variables were included in the multiple logistic regression analysis. Sample size = (number of variables $\times$ 10)/pregnancy rate = (4 $\times$ 10)/49% = 82. The sample size in this study (n = 85) was therefore sufficient for binary multivariate logistic regression analysis. Receiver operating characteristic (ROC) curves were plotted to assess the diagnostic value of the influencing factors for pregnancy outcomes. A p-value < 0.05 was considered statistically significant.

3. Results

3.1 The Clinical Information of Patients

A total of 179 cases were included in the study. Table 1 presents the general demographic data, semen parameters, and IVF-ET outcomes of patients. The fertilization rate, cleavage rate, and day 3 high-quality embryo rate were 69.23%, 97.67%, and 65.75%, respectively. Of the 179 cases, 85 underwent fresh ET, resulting in an ET rate of 47.49%. Among these 85 ET cycles, the clinical pregnancy rate and live birth rate was 49.41% (42/85) and 41.18% (35/85), respectively (Table 1).

3.2 The Correlation Between Fertilization Rate and Sperm MMP Seminal Plasma Elastase and Seminal Plasma Zinc

The fertilization rate of IVF was correlated with sperm MMP, seminal plasma elastase, and seminal plasma zinc (all $p < 0.001$), and the correlation analyses (r values) were 0.362, –0.379, and 0.415, respectively (Table 2).

3.3 Comparison of Clinical Information Between the Pregnancy and Non-Pregnancy Groups

Among the 179 couples enrolled, 85 underwent fresh ET after IVF. These 85 transplant cycles were included in the subsequent analysis. The remaining couples did not undergo fresh ET due to the absence of transferable embryos (n = 3), ovarian hyperstimulation (n = 66), or other reasons (n = 25).

Based on pregnancy outcomes, the 85 transplant cycles were divided into two groups: the pregnancy group (n = 42) and the non-pregnancy group (n = 43). There were no significant differences in general demographic characteristics or routine semen parameters between the two groups ($p > 0.05$). Compared with the non-pregnancy group, the pregnancy group exhibited significantly higher sperm MMP and seminal plasma zinc levels ($p < 0.001$, $p = 0.009$, respectively), while seminal plasma elastase levels were significantly lower ($p = 0.002$) (Table 3).

Table 1. General demographic data, semen parameters, and IVF-ET outcomes of patients.

Variables	All patients (n = 179)
General information	
Male age, years	33 (30, 36)
Female age, years	31 (28, 33)
Male BMI, Kg/m^2	24.8 (22.5, 27.4)
Female BMI, Kg/m^2	22.8 (20.0, 25.8)
Abstinence days, days	4 (3, 5)
Male drinking history, n (%)	
Never	72 (40.22%)
Quitting	107 (59.78%)
Male smoking history, n (%)	
Never	91 (50.84%)
Quitting	88 (49.16%)
Female drinking history, n (%)	
Never	106 (59.22%)
Quitting	73 (40.78%)
Female smoking history, n (%)	
Never	127 (70.95%)
Quitting	52 (29.05%)
Passive smokers, n (%)	32 (17.88%)
Semen parameters	
Semen volume, mL	3.4 (2.5, 4.7)
Sperm concentration, 10^6/mL	95.39 (59.69, 152.41)
Sperm progressive motility, %	38 (33, 42)
Sperm non-progressive motility, %	5 (5, 7)
Sperm immotility, %	57 (50, 60)
Sperm deformity index	1.36 (1.20, 1.43)
Sperm MMP, %	51.65 $\pm$ 13.14
Seminal plasma elastase, ng/mL	91.1 (26.6, 421.1)
Seminal plasma zinc, µmol/ejaculation	5.6 (3.6, 8.8)
Embryonic outcomes	
Fertilization rate, %	69.23% (1289/1862)
Cleavage rate, %	97.67% (1259/1289)
Day 3 high-quality embryos rate, %	65.75% (766/1165)
Pregnancy outcomes	
ET rate, %	47.49% (85/179)
Clinical pregnancy rate, %	49.41% (42/85)
Live birth rate, %	41.18% (35/85)

Note: BMI, body mass index; MMP, mitochondrial membrane potential; ET, embryo transfer.

Table 2. Correlation of fertilization rate with sperm MMP, seminal plasma elastase and seminal plasma zinc.

Variables	r	*p*
Sperm MMP	0.362	<0.001
Seminal plasma elastase	–0.379	<0.001
Seminal plasma zinc	0.415	<0.001

Note: MMP, mitochondrial membrane potential.

3.4 Analysis of Influencing Factors for Pregnancy Outcome of IVF-ET

Univariate analysis revealed that several factors significantly affected pregnancy outcomes, including sperm non-progressive motility (p = 0.031, odds ratio (OR) = 0.705, 95% confidence interval (CI) = 0.514–0.969), sperm MMP ($p < 0.001$, OR = 1.097, 95% CI = 1.042–1.154), seminal plasma elastase (p = 0.023, OR = 0.997, 95% CI = 0.995–1.000), and seminal plasma zinc (p = 0.012, OR = 1.149, 95% CI = 1.031–1.281).

The variance inflation factor (VIF) values for all four indicators were lesser than 5, indicating no multicollinear-

Table 3. Comparison of the clinical information of patients between the pregnancy and non-pregnancy groups.

Variables	Pregnancy group (n = 42)	Non-pregnancy group (n = 43)	$t/Z/\chi^2$	p
Male age, years	33 (29, 35)	33 (28, 37)	0.331	0.741
Female age, years	31 (28, 33)	31 (28, 33)	0.190	0.849
Male BMI, Kg/m^2	23.75 ± 3.22	24.46 ± 3.35	0.996	0.322
Female BMI, Kg/m^2	23.00 ± 3.43	22.26 ± 2.88	1.078	0.284
Abstinence days, days	4 (3, 4)	4 (3, 5)	1.885	0.059
Male drinking history, n (%)			0.288	0.591
Never	21 (50.00%)	19 (44.19%)		
Quitting	21 (50.00%)	24 (55.81%)		
Male smoking history, n (%)			0.565	0.452
Never	23 (54.76%)	27 (62.79%)		
Quitting	19 (45.24%)	16 (37.21%)		
Female drinking history, n (%)			1.427	0.232
Never	18 (42.86%)	24 (55.81%)		
Quitting	24 (57.14%)	19 (44.19%)		
Female smoking history, n (%)			0.718	0.397
Never	30 (71.43%)	27 (62.79%)		
Quitting	12 (28.57%)	16 (37.21%)		
Passive smokers, n (%)	5 (11.90%)	6 (13.95%)	0.079	0.778
Semen volume, mL	3.6 (2.5, 4.7)	3.4 (2.5, 4.7)	0.040	0.968
Sperm concentration, 10^6/mL	96.54 (61.72, 157.75)	93.31 (49.21, 178.60)	0.004	0.996
Sperm progressive motility, %	37 (30, 42)	38 (30, 40)	0.070	0.944
Sperm non-progressive motility, %	5 (5, 6)	5 (5, 7)	1.677	0.094
Sperm immotility, %	58 (53, 65)	57 (53, 60)	0.740	0.459
Sperm deformity index	1.38 (1.21, 1.43)	1.36 (1.19, 1.42)	0.603	0.547
Sperm MMP, %	60.04 ± 8.64	49.60 ± 12.83	4.390	<0.001
Seminal plasma elastase, ng/mL	35.7 (16.7, 133.2)	65.6 (35.7, 581.9)	3.147	0.002
Seminal plasma zinc, µmol/ejaculation	7.2 (4.5, 11.9)	4.8 (3.1, 8.1)	2.602	0.009

Note: BMI, body mass index; MMP, mitochondrial membrane potential.

ity among them. Therefore, these four variables were then included in a multivariate logistic regression analysis. The results showed that sperm non-progressive motility (p = 0.013, OR = 0.478, 95% CI = 0.267–0.856), sperm MMP ($p < 0.001$, OR = 1.114, 95% CI = 1.047–1.184), seminal plasma elastase (p = 0.006, OR = 0.996, 95% CI = 0.993–0.999) and seminal plasma zinc (p = 0.024, OR = 1.188, 95% CI = 1.023–1.381) were the independent factors affecting pregnancy outcome (Table 4).

3.5 ROC Analysis

The ROC results showed that sperm MMP (area under the curve [AUC] = 0.748, 95% CI = 0.644–0.851, $p < 0.001$), seminal plasma elastase (AUC = 0.698, 95% CI = 0.587–0.809, $p < 0.001$), seminal plasma zinc (AUC = 0.664, 95% CI = 0.549–0.779, p = 0.005), and combined indicators (AUC = 0.832, 95% CI = 0.747–0.916, $p < 0.001$) have good predictive effects on pregnancy outcomes (Table 5 and Fig. 2).

4. Discussion

Infertility is defined as a couple's failure to achieve a clinical pregnancy after one year of unprotected sexual activity. According to statistics, 10%–15% of couples of childbearing age suffer from infertility, with approximately 30%–50% of cases attributable to male factors [2]. A decline in sperm quality is an important influencing factor for male infertility. However, routine semen parameters are not effective indicators of male fertility, as functional defects in sperm can also impact male fertility. An increasing number of infertile patients are choosing to use assisted reproductive technology to achieve their pregnancy and fertility desires. Therefore, a rational and effective evaluation of semen is an important strategy to minimize adverse outcomes in IVF-ET. In this study, we found correlations between sperm MMP, seminal plasma elastase, seminal plasma zinc, and the fertilization rate in IVF. Furthermore, sperm non-progressive motility, sperm MMP, seminal plasma elastase, and seminal plasma zinc were identified as independent factors affecting the pregnancy outcome of IVF-ET. Combined detection of sperm MMP, seminal plasma elastase, and seminal plasma zinc demonstrated good predictive value for pregnancy outcomes in IVF-ET. In addition, there was no significant difference in routine semen parameters between the pregnant and non-pregnant groups. A previous study has also reported no significant differences in rou-

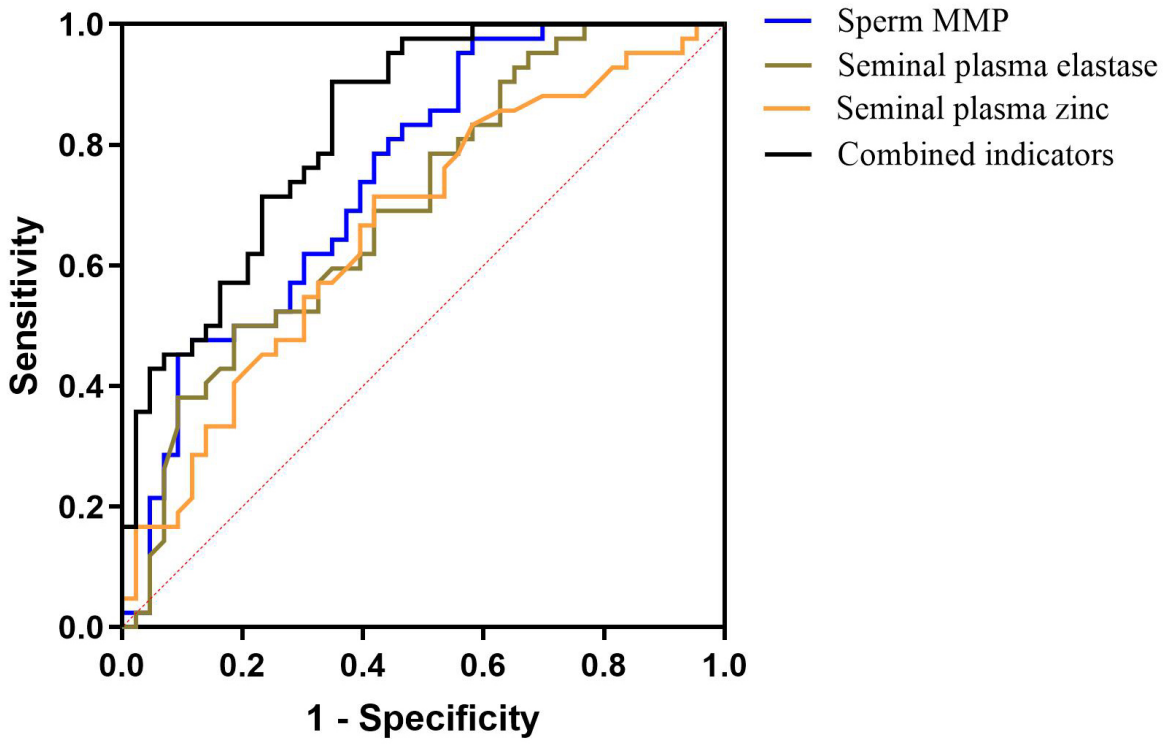

Fig. 2. Receiver operating characteristic (ROC) curves of sperm MMP, seminal plasma elastase, seminal plasma zinc, and combined indicators in predicting pregnancy outcomes. MMP, mitochondrial membrane potential.

tine semen parameters between pregnant and non-pregnant groups in intrauterine insemination [20], which is similar to our findings. This also indicated that routine semen parameters have limitations in predicting pregnancy outcomes.

Mitochondria are the energy factories of cells, producing adenosine triphosphate (ATP) through the electron transport chain and oxidative phosphorylation. During this process, a transmembrane potential difference is generated due to the different proton concentrations across the mitochondrial membrane, which is referred to as MMP. The production of ATP provides energy for effective movement of sperm and supports the acrosome reaction necessary for fertilization [21]. Previous research has reported a correlation between sperm MMP levels and sperm motility [9]. In this study, sperm MMP was positively correlated with the fertilization rate in IVF ($r = 0.362$). Increased sperm MMP contributed to improved pregnancy outcomes in IVF-ET, and sperm MMP demonstrated good predictive value for pregnancy outcomes. Sperm with high MMP exhibited greater motility and enhanced ability to undergo the acrosome reaction. Moreover, mitochondrial function is required during capacitation to penetrate the egg coats [22]. A previous study also reported similar conclusions: researchers collected samples from 85 infertile men and found that low levels of sperm MMP negatively impacted natural fertility outcomes [23].

Zinc is a trace element essential for maintaining male reproductive function. Zinc inhibits oxidative damage caused by hydrogen peroxide, improves sperm motility, and protects sperm membrane integrity [24]. Research has shown that dietary supplementation with zinc sulfate contributes to improved sperm viability and survival indices [25]. Zinc deficiency is associated with delayed testicular development, impaired spermatogenesis, sex hormone deficiency, oxidative stress, and inflammation [26]. A study showed that seminal plasma zinc levels in men with low sperm number were higher than those in men with azoospermia, and that zinc levels affected sperm count, motility, and morphology [27]. In this study, seminal plasma zinc was positively correlated with the fertilization rate in IVF-ET ($r = 0.415$). Seminal plasma zinc was also identified as an independent factor affecting the pregnancy outcome of IVF-ET, and demonstrated good predictive value for pregnancy. The possible mechanism is that zinc ions activate G protein-coupled receptor 39 (GPR39), which catalyzes the production of cyclic adenosine monophosphate (cAMP). Subsequently, cAMP activates the protein kinase A (PKA)-Src-epidermal growth factor receptor (EGFR) cascade and phospholipase C, thereby increasing intracellular calcium ion concentration, promoting sperm capacitation and the acrosome reaction [28]. A prospective study reported a positive correlation between seminal plasma zinc levels and positive pregnancy progression in assisted reproductive therapy [17], which is consistent with our findings. however, other studies have reported different conclusions. For example, Chen et al. [29] reported that zinc exhibited dose-

Table 4. Binary logistic regression analysis for the influencing factors of pregnancy outcome.

	Univariate analysis					Multivariate analysis				
	β	SE	Wald	OR (95% CI)	p	β	SE	Wald	OR (95% CI)	p
Male age	0.013	0.051	0.062	1.013 (0.916–1.120)	0.803					
Female age	–0.001	0.068	0.000	0.999 (0.874–1.142)	0.987					
Male BMI	–0.067	0.068	0.979	0.935 (0.819–1.068)	0.322					
Female BMI	0.076	0.070	1.156	1.078 (0.940–1.238)	0.282					
Abstinence days	–0.290	0.166	3.058	0.748 (0.541–1.036)	0.080					
Male drinking history (Quitting)	–0.234	0.435	0.288	0.792 (0.337–1.858)	0.592					
Male smoking history (Quitting)	0.332	0.442	0.564	1.394 (0.586–3.317)	0.453					
Female drinking history (Quitting)	0.521	0.438	1.419	1.684 (0.714–3.971)	0.234					
Female smoking history (Quitting)	–0.393	0.465	0.715	0.675 (0.271–1.679)	0.398					
Passive smokers	–0.182	0.649	0.079	0.833 (0.234–2.971)	0.779					
Semen volume	–0.033	0.089	0.142	0.967 (0.813–1.151)	0.706					
Sperm concentration	0.000	0.003	0.011	1.000 (0.995–1.005)	0.915					
Sperm progressive motility	0.008	0.020	0.157	1.008 (0.969–1.048)	0.692					
Sperm non-progressive motility	–0.349	0.162	4.651	0.705 (0.514–0.969)	0.031	–0.737	0.297	6.175	0.478 (0.267–0.856)	0.013
Sperm immotility	0.005	0.019	0.074	1.005 (0.969–1.043)	0.785					
Sperm deformity index	0.629	1.690	0.139	1.877 (0.068–51.502)	0.710					
Sperm MMP	0.092	0.026	12.719	1.097 (1.042–1.154)	<0.001	0.108	0.031	11.795	1.114 (1.047–1.184)	<0.001
Seminal plasma elastase	–0.003	0.001	5.202	0.997 (0.995–1.000)	0.023	–0.004	0.001	7.683	0.996 (0.993–0.999)	0.006
Seminal plasma zinc	0.139	0.055	6.282	1.149 (1.031–1.281)	0.012	0.173	0.077	5.076	1.188 (1.023–1.381)	0.024

Note: BMI, body mass index; MMP, mitochondrial membrane potential; OR, odds ratio; CI, confidence interval.

Table 5. ROC analysis for the sperm MMP, seminal plasma elastase, seminal plasma zinc, and combined indicators in predicting pregnancy outcomes.

	AUC	*p*	Sensitivity	Specificity	95% CI	Cut-off value
Sperm MMP	0.748	<0.001	0.976	0.415	0.644–0.851	47.56
Seminal plasma elastase	0.698	<0.001	0.500	0.814	0.587–0.809	35.55
Seminal plasma zinc	0.664	0.005	0.714	0.581	0.549–0.779	5.15
Combined indicators	0.832	<0.001	0.905	0.651	0.747–0.916	/

Note: MMP, mitochondrial membrane potential; AUC, area under the curve; CI, confidence interval; ROC, receiver operating characteristic.

dependent toxicity to spermatocytes at subthreshold levels, with an inverted "U"-shaped trend observed between seminal plasma zinc levels and both sperm progressive motility and total motility, indicating that both high and low zinc levels could affect semen quality. In our study, we did not observe a negative impact of high levels of seminal zinc on fertilization rates or pregnancy outcomes in IVF-ET, which may be related to the small sample size. Larger cohort studies are needed to further explore whether high seminal zinc levels can impair sperm function and interfere with fertilization and pregnancy outcomes. Seminal plasma elastase is an important indicator of male reproductive tract infections and may have a negative impact on male fertility. Elastase is distributed in various cells, such as neutrophils and macrophages [16]. During infection, neutrophil counts in semen increase, and these cells remove antigens through phagocytosis and cytokine secretion. However, this immune response can also damage semen, disrupting the function and maturation of the semen [16]. In our study, we found a negative correlation between seminal plasma elastase and the fertilization rate in IVF (r = –0.379). The level of seminal plasma elastase in the pregnant group was significantly lower than that in the non-pregnant group. Logistic regression analysis showed that seminal plasma elastase was an independent factor affecting pregnancy, and it demonstrated a good predictive effect on pregnancy outcomes. Previous studies have reported conflicting results regarding the association between elastase and semen quality. Elevated elastase can increase the production of reactive oxygen species (ROS), leading to lipid peroxidation and increased permeability of sperm membranes, thereby disrupting membrane integrity. In addition, elevated elastase reflects a higher level of inflammation, which may disrupt the function of the epididymis, indirectly affecting sperm development [15]. A study reported that seminal plasma elastase levels were higher in patients with acute epididymitis compared to uninfected individuals [30]. Liu et al. [13] reported that after ureaplasma urealyticum infection, increased seminal polymorphonuclear elastase affected liquefaction time and viscosity, resulting in decreased sperm motility. Another study reported that compared with normal males, patients with oligospermia and teratozoospermia had elevated levels of seminal plasma leukocyte elastase, but no significant correlation was observed between leukocyte elastase and inflammatory factors [31]. Another study reported that there was no significant correlation between leukocyte elastase and semen parameters [15]. These inconsistent findings regarding elastase and semen quality may be related to differences in study subjects.

In the ROC analysis conducted in this study, sperm MMP, seminal plasma zinc, and the combined indicators demonstrated higher sensitivity but lower specificity, whereas seminal plasma elastase exhibited lower sensitivity and higher specificity. A possible reason is that sperm MMP and seminal plasma zinc are closely related to the vitality and function of sperm, resulting in high sensitivity for predicting pregnancy outcomes. However, non-pregnant individuals may also present with elevated levels of sperm MMP and seminal plasma zinc, which results in lower specificity of these two indicators for predicting pregnancy outcomes. In contrast, seminal plasma elastase is associated with pathological conditions such as inflammation and infection, thereby conferring higher specificity but lower sensitivity in predicting pregnancy outcomes.

This study has several limitations. First, the sample size was relatively small, and the study design was retrospective, which may introduce bias into the results. Therefore, the conclusions drawn should be validated in larger, prospective cohort studies. Additionally, this study was conducted at a single center. Conducting corresponding research in multiple regions/centers is needed to confirm the applicability of these findings to a broader and more diverse population. Furthermore, due to the short period of data collection and the fact that some patients did not undergo fresh ET due to various factors, cumulative pregnancy rates (including frozen ET) were not analysed. It remains unclear whether sperm MMP, seminal plasma zinc, and seminal plasma elastase affect cumulative pregnancy rates. Future studies with extended sample collection periods are warranted to investigate the effects of these seminal plasma biochemical parameters on cumulative pregnancy rates.

5. Conclusion

Overall, the fertilization rate in IVF is significantly correlated with sperm MMP, seminal plasma elastase, and seminal plasma zinc. Sperm non-progressive motility,

sperm MMP, seminal plasma elastase, and seminal plasma zinc are identified as independent factors affecting the pregnancy outcomes of IVF-ET. The combined assessment of sperm MMP, seminal plasma elastase, and seminal plasma zinc demonstrates good predictive value for pregnancy outcomes in IVF-ET.

Key Points

• A total of 179 infertile males were included in the study, and 85 embryo transfer (ET) cycles were collected and analysed.

• Among 85 ET cycles, the clinical pregnancy rate and live birth rate were 49.41% (42/85) and 41.18% (35/85), respectively.

• The fertilization rate in IVF was correlated with sperm mitochondrial membrane potential (MMP) ($r = 0.362$), seminal plasma elastase ($r = –0.379$), and seminal plasma zinc ($r = 0.415$).

• Sperm non-progressive motility, sperm MMP, seminal plasma elastase, and seminal plasma zinc were independent factors affecting pregnancy outcomes.

• The combination of sperm MMP, seminal plasma elastase, and seminal plasma zinc provided better predictive value on pregnancy outcomes in IVF-ET than any individual indicator.

Availability of Data and Materials

All data included in this study are available from the corresponding authors upon reasonable request.

Author Contributions

ZC designed the research. JC and YZ performed the study and collected the data. JC analysed the data. ZC drafted the first draft. All authors contributed to the important editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

This study was approved by the Ethics Committee of the Women and Children's Hospital of Ningbo University (Approval No. NBFE-2025-KY-067). All procedures were conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all participants.

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Conflicts of Interest

The authors declare no conflicts of interest.

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