

Semen Characteristics and Embryo Outcomes in IVF

Mohadese Dashtkoohi; M.D.¹, Mohammad Haddadi; M.D.¹, Mostafa Saeedinia; M.D.²,
Fedyeh Haghighollahi; M.Sc.¹, Masoumeh Masoumi; Ph.D.¹, Zohreh Heidary; Ph.D.¹

1 Vali-E-Asr Reproductive Health Research Center, Family Health Research Institute, Imam Khomeini Hospital Complex, Tehran University of Medical Sciences, Tehran, Iran

2 Shahid Beheshti University of Medical Sciences, Tehran, Iran

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Abstract

Objective: Infertility is a global health challenge, affecting many couples worldwide. Male infertility contributes to 20–50% of cases. Although semen analysis parameters are widely regarded as key indicators of male fertility, their association with in vitro fertilization (IVF) success remains debated. This study evaluated the relationship between specific semen parameters and grade A embryo formation in IVF among infertile men.

Materials and methods: This retrospective cross-sectional study was conducted at a referral infertility center from March 2019 to March 2021, involving 104 men diagnosed with male-factor infertility. Semen parameters, including sperm count, motility, morphology, and volume, were analyzed. The primary outcome was the formation of at least one grade A embryo, defined as a successful IVF outcome. Statistical analyses included chi-square tests and logistic regression.

Results: The median age differed significantly between the successful and unsuccessful IVF groups (36 vs. 38 years, $p=0.050$). No significant differences were observed in semen volume, sperm count, motility, or morphology between groups. Logistic regression revealed that younger age was associated with a higher likelihood of grade A embryo formation ($OR=0.935$, $p=0.012$), whereas semen parameters showed no significant association with embryo quality.

Conclusion: This study found no significant association between semen parameters and grade A embryo formation in IVF, suggesting that traditional semen analysis has limited predictive value for embryo quality. Although younger age was associated with a higher likelihood of success, the effect size was small ($OR=0.935$, $p=0.012$), and its clinical impact may be limited. These findings highlight the potential for successful embryo development despite suboptimal semen parameters and underscore the need for a broader approach to assessing male fertility beyond standard semen analysis.

Keywords: Fertilization in Vitro; Semen Analysis; Infertility

Introduction

Infertility, a global health challenge affecting at least

180 million couples, presents a significant burden on individuals, families, and healthcare systems. Iran, with a prevalence ranging from 10.3% to 24.9%, is not exempt from this issue (1, 2). Recognizing the intricate interplay between male and female factors in infertility, a comprehensive evaluation and treatment

Correspondence:

Dr. Zohreh Heidary

Email: zheidary2016@gmail.com



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approach is essential (3). Male infertility, contributing to approximately 20-50% of cases, demands particular attention (4).

Nowadays, assisted reproductive technologies (ARTs), such as in vitro fertilization (IVF), offer hope to many infertile couples (5). While semen parameters are considered crucial indicators of male fertility, the strength of the association between these parameters and IVF success remains controversial. Contradictory findings in the literature, coupled with the recent implementation of the "Rejuvenation of the Population and Protection of the Family" law in Iran, underscore the need for further investigation (6).

This study aims to evaluate the relationship between semen parameters and the formation of grade A embryos during IVF in infertile men seeking treatment at a referral academic center. By identifying key factors associated with high-quality embryo development, we seek to contribute to optimizing treatment strategies and improving patient outcomes.

Materials and methods

Study design and Participants: This retrospective cross-sectional study was held between March 2019 and March 2021 at a referral infertility center at Imam Khomeini Hospital, affiliated with Tehran University of Medical Sciences. The study protocol received approval from the institutional review board and ethics committee of Tehran University of Medical Sciences under reference code IR.TUMS.IKHC.REC.1402.003.

The inclusion criteria required that participants be men aged 20 years or older who had been diagnosed with male factor infertility, supported by semen analysis data based on the latest World Health Organization (WHO) guidelines for semen analysis published in 2021 (7). Couples had to be undergoing IVF as part of their fertility treatment plan, and their semen analysis data, including characteristics such as sperm count, motility, morphology, and volume, had to be available. Additionally, both partners had to provide written informed consent to participate in the study.

Exclusion criteria encompassed cases where infertility was solely attributed to female factors or where male-factor infertility was absent. Additionally, men diagnosed with azoospermia were excluded, as our focus was on individuals with measurable semen parameters. Participants with incomplete semen analysis or IVF outcome data were not included. To ensure homogeneity in treatment

protocols, we also excluded couples with multiple prior IVF cycles involving varying protocols or differing sperm analysis parameters. Finally, individuals unable or unwilling to comply with study protocols or provide necessary data were not considered for inclusion.

Sample Size Calculation: The sample size was determined based on data from the Santi et al. study (8), which analyzed the level (alpha) of 0.05 and a statistical power of 0.8, the required sample total number of embryos among couples who achieved pregnancy and those who did not during ART. A significance size was calculated to be 104 participants.

Variables and definitions: Data collected from clinical records included age, occupation, marital duration, current alcohol consumption, opium addiction, current smoking, history of varicocele, and detailed sperm analysis (motility, volume, count, and morphology).

Semen samples were collected at the Vali-E-Asr Infertility Clinic following 1-7 days of sexual abstinence (9). All samples were processed within one hour of collection, maintained at 37 °C, and analyzed using a computer-assisted semen analyzer to assess volume, total sperm count, motility, and morphology (10). Only the most recent semen analysis performed prior to the IVF procedure was included in the study. All parameters were evaluated according to the latest World Health Organization (WHO) guidelines for semen analysis (7). Normal sperm parameters were defined as total motility $\geq 42\%$, semen volume ≥ 1.4 mL, total sperm number ≥ 39 million/ejaculate, and normal forms $\geq 4\%$. A successful IVF outcome was defined as achieving at least one embryo of grade A quality, while the absence of any grade A embryo was considered a failed IVF attempt. Embryo grading was performed on day 3 of development by two independent embryologists based on criteria including the number of cells, degree of fragmentation, and similarity indices of the embryo (11, 12). Embryos were cultured using commercially available IVF media and incubated at 37 °C in an environment with 5% CO₂ and 20% O₂. Embryo evaluation was conducted according to the criteria outlined by Tesarik et al. (13).

Statistical Analysis: Normality of all variables was assessed using the Kolmogorov-Smirnov test. Since the data did not follow a normal distribution, quantitative variables were described using the median (interquartile range), and qualitative variables were reported as number (%). The Chi-square test

was employed to compare qualitative variables between the successful and unsuccessful IVF groups. Additionally, univariate and multivariate logistic regression analyses were conducted to test the effect of independent variables. Data were analyzed using the Statistical Package for Social Sciences (SPSS), version 26. A p-value of less than 0.05 was considered statistically significant.

Results

The baseline characteristics of study participants in the successful and failed IVF groups are presented in Table 1. The median age in the successful group was 36 years (IQR 31-39), which was significantly younger than the median age in the failed IVF group at 38 years (IQR 32-45), with a p-value of 0.050. Employment status showed no statistically significant difference between the two groups ($p = 0.107$), with similar distributions across governmental, non-governmental, and unemployed categories.

The median duration of marriage was similar between the two groups, with a median of 5 years (IQR 4-8) in the successful IVF group and 5 years (IQR 2.25-8) in the failed IVF group ($p = 0.124$). Lifestyle factors such as alcohol consumption, opium addiction, and current smoking did not show significant differences between the groups, with p-values of 0.412, 0.390, and 0.898, respectively.

Regarding medical history, the history of varicocele was comparable between the successful (26.7%) and failed (22.7%) IVF groups ($p = 0.647$). Sperm parameters, including semen volume, total sperm number, total motility, and normal morphology, did not show statistically significant differences between the two groups, with p-values of 0.243, 0.162, 0.143, and 0.229, respectively. However, the total number of embryos was significantly higher in the successful IVF group with a median of 5 (IQR 4-9) compared to 2 (IQR 1-3) in the failed IVF group ($p = 0.001$).

Logistic regression analysis was conducted to explore the relationships between various factors and successful IVF outcomes among infertile males (Table 2). In the univariate analysis, age showed a significant association with IVF success, with an odds ratio (OR) of 0.959 (95% CI: 0.929-0.990, $p = 0.010$), indicating that younger age was related to higher chances of successful IVF. This relationship remained significant in the multivariate model, with an OR of 0.935 (95% CI: 0.887-0.985, $p = 0.012$).

Other variables, such as the duration of marriage, current smoking status, alcohol consumption, sperm volume, total sperm number, sperm motility, and normal sperm morphology, were not significantly associated with successful IVF in either the univariate or multivariate analyses.

Table 1. The baseline characteristic and semen parameters of infertile men among successful and failed IVF groups

Characteristics	Total n= 104	Failed IVF n= 44	Successful IVF n= 60	P-value*
Age (year)	37 (32-41)	38 (32-45)	36 (31-39)	0.050*
Job				0.107
Governmental	31 (30.7%)	18 (41.9%)	13 (22.4%)	
Non-governmental	24 (23.8%)	8 (18.6%)	16 (27.6%)	
unemployed	46 (45.5%)	17 (39.5%)	29 (50.0%)	
Marriage duration (year)	5 (3-8)	5 (2.25-8)	5 (4-8)	0.124
Alcohol consumption	5 (4.8%)	3 (6.8%)	2 (3.3%)	0.412
Opium addiction	1 (1.0%)	0	1 (1.7%)	0.390
Current smoking	23 (22.1%)	10 (22.7%)	13 (21.7%)	0.898
Varicocele	26 (25.0%)	10 (22.7%)	16 (26.7%)	0.647
Sperm parameters				
Semen volume ≥ 1.4 ml	73 (70.2%)	33 (75.0%)	40 (66.7%)	0.243
Total sperm number ≥ 39 million/ ejaculation	20 (19.2%)	6 (13.6%)	14 (23.3%)	0.162
Total motility ≥ 42 percent	22 (21.2%)	12 (27.3%)	10 (16.7%)	0.143
Normal Morphology ≥ 4 Percent	62 (59.6%)	31 (70.5%)	31 (51.7%)	0.229
Total number of embryos	3 (1-6)	2 (1-3)	5 (4-9)	0.001*

The data is presented by n (%) and median (interquartile range). *P-value less than 0.05 is considered significant. Chi-square and Mann-Whitney U tests were used. A successful IVF outcome was defined as achieving at least one embryo of grade A quality, while the absence of any grade A embryo was considered a failed IVF attempt

Table 2. Binary Logistic Regression Analysis of Factors Associated with Successful IVF in Infertile Males

Variables	Raw results			Multivariable analysis		
	OR	95%CI	P-value	OR	95%CI	P-value
Age	0.959	0.929-0.990	0.010	0.935	0.887-0.985	0.012
duration of marriage	1.016	0.970-1.067	0.502			
Current smoking	0.918	0.506-1.666	0.778	-	-	-
Alcohol consumption	1.042	0.274-3.959	0.952	-	-	-
Sperm volume>1.5 ml	0.991	0.514-1.910	0.979	0.619	0.230-1.671	0.344
Sperm number> 15 million/ml	0.983	0.621-1.554	0.941	2.912	0.897-9.460	0.075
Sperm motility> 4 percent	0.846	0.534-1.339	0.476	0.421	0.143-1.237	0.115
Normal sperm> 4 percent	0.448	0.197-1.020	0.056	0.441	0.180-1.083	0.741

The data is presented by n (%) and median (interquartile range). *P-value less than 0.05 is considered significant. Chi-square and Mann-Whitney U tests were used. A successful IVF outcome was defined as achieving at least one embryo of grade A quality, while the absence of any grade A embryo was considered a failed IVF attempt

Discussion

The predictive value of various parameters in standard semen analysis for male fertility remains contentious (14). This study found that sperm parameters, including volume, motility, morphology, and count, are not associated with IVF successful results. Our finding aligns with the study by Danis et al., which also reported that sperm morphology is not a reliable predictor of IVF success (15).

While our study focused on grade A embryo formation as an indicator of IVF success due to its relevance to early embryo quality and data availability in our cohort, we acknowledge that clinical outcomes such as pregnancy and live birth rates are critical for assessing overall IVF success (16).

Similarly, Kohn et al. concluded that sperm morphology should no longer be a primary factor when selecting appropriate ART techniques (17). In contrast, Donnelly et al. found through a clinical trial that sperm motility and morphology significantly influence pregnancy outcomes and IVF success (18). However, a meta-analysis conducted in 2022 revealed no significant association between semen parameters and ART pregnancy rates. This suggests that ART techniques can effectively address concerns related to semen quality (19). Additionally, Villani et al.'s research indicated that sperm morphology could significantly predict pregnancy outcomes through ARTs, which contradicts our findings (20). Historically, a high number of abnormal sperm was believed to decrease pregnancy chances.

However, inconsistent evaluation methods across different laboratories have led to varying results regarding sperm morphology assessment (19).

Recent meta-analyses further suggest that abnormal sperm morphology is not a reliable

predictor of reduced pregnancy rates in ART settings (21). The importance of sperm morphology in ART outcomes remains a topic of debate within the field (22). Given the decreased reliability of sperm morphology testing, clinicians should not rely solely on percentage thresholds when making ART decisions. Instead, a comprehensive evaluation of multiple reproductive factors is recommended (17).

The variability among study groups, differences in staining methods, laboratory practices, scoring classifications, and the choice between manual and computer-assisted semen analyzer (CASA) scoring are significant factors contributing to the inconsistent predictive power of sperm analysis (22). Understanding the significance of sperm morphology in ART outcomes requires the analysis of large-scale case series.

Moreover, Liu et al. suggested that combining sperm parameters with sperm function tests could serve as a prognostic tool for selecting between IVF or ICSI for infertile men (23). Sperm function tests, such as those measuring DNA fragmentation, may predict miscarriage risk and reduce successful pregnancy rates through ARTs (24). Incorporating molecular assessments of sperm function in the evaluation of infertile men could lead to more appropriate treatment choices and better predictions of success rates (25).

Additionally, our results indicate that increasing age is significantly associated with a reduced likelihood of successful IVF, based on both univariate and multivariate analyses. However, this association loses statistical significance after adjusting for sperm parameters, and the effect size remains small, raising questions about its clinical relevance. The limited statistical power of our study, due to the small effect size and a sample size of only

104 participants, may have constrained our ability to detect stronger associations between age or other factors and IVF outcomes. Nevertheless, other studies have demonstrated that aging negatively impacts sperm quality. For example, Lu et al. reported that paternal age over 40 is associated with reduced IVF success rates, independent of sperm parameters (26).

Limitations: This study was conducted at an academic referral center for infertility and has several limitations. Its retrospective design led to missing data, and some important variables, such as male hormone levels, were not documented in the records. This limited our ability to perform more comprehensive statistical adjustments. A major limitation is the absence of detailed information on female partners, including ovarian reserve markers (e.g., AMH levels), stimulation protocols, and embryo transfer details—all of which are known to impact embryo quality and IVF outcomes. These data were inconsistently recorded in our retrospective dataset and, therefore, could not be included in the analysis. Additionally, we defined IVF success as the formation of at least one grade A embryo, while clinical pregnancy rate and live birth rate are more commonly used outcomes in reproductive research. Future studies should incorporate these measures to provide a more clinically relevant assessment. Furthermore, only the most recent semen analysis was included, while repeated analyses over time may offer a more accurate assessment of male fertility, and the accuracy of these laboratory tests was not rigorously validated. Finally, our sample size calculation was based on previous literature, but the observed effect size was smaller than anticipated, leading to lower statistical power.

Conclusion

Our findings challenge the traditional view that standard semen parameters are strong predictors of IVF success. While we found no significant association between semen volume, count, motility, or morphology and the formation of grade A embryos, younger paternal age was associated with a higher likelihood of embryo formation. However, the effect size was small ($OR = 0.935$, $p = 0.012$), and the clinical relevance of this finding remains uncertain. Given the limitations of semen analysis alone in predicting embryo quality and IVF outcomes, a more comprehensive assessment incorporating female partner factors, embryo development over time, and pregnancy outcomes is

needed. These results suggest that successful embryo development may still be possible despite suboptimal semen parameters, providing reassurance to couples undergoing fertility treatment.

Conflict of Interests

Authors declare no conflict of interests.

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