Clinical and Experimental Obstetrics & Gynecology

Clin. Exp. Obstet. Gynecol. **2026**; 53(7): 50300
https://doi.org/10.31083/CEOG50300

Original Research

The Impact of Psychological Stress on Treatment Outcomes in Patients Undergoing *In Vitro* Fertilization-Embryo Transfer: A Prospective Cohort Study

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Academic Editors: David H. Barad and Michael H. Dahan
Submitted: 22 January 2026 Revised: 10 March 2026 Accepted: 17 March 2026 Published: 9 July 2026

Abstract

Background: Patients undergoing *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) often experience negative emotions such as anxiety and stress, which may adversely affect treatment outcomes. Given that current emotional assessments rely primarily on subjective scales, identifying objective biomarkers for early intervention is crucial. **Methods:** A total of 236 female patients who underwent IVF/ICSI-ET treatment between March 2024 and March 2025 were enrolled in the study. Saliva samples were collected in a fasting state during the menstrual period and on the day of oocyte retrieval to measure salivary alpha-amylase (SAA) levels. Following sample collection, participants completed the Self-Rating Anxiety Scale (SAS) and the Fertility Quality of Life Questionnaire (FertiQoL). Treatment outcomes were subsequently followed up, and participants were categorized into a clinical pregnancy group and a non-clinical pregnancy group based on clinical pregnancy status. The impact of anxiety on treatment outcomes was then analyzed. **Results:** Menstrual SAA levels were significantly positively associated with SAS scores and significantly negatively correlated with the Social domain of fertility-related quality of life ($p < 0.05$). Menstrual SAS scores were significantly negatively correlated with total fertility-related quality of life ($p < 0.05$). Statistically significant differences were observed between the clinical pregnancy and non-clinical pregnancy groups in terms of menstrual SAA levels, age, cause of infertility, anxiety status, fertilization rate, number of available high-quality embryos, and number of transferred embryos ($p < 0.05$). Menstrual SAA levels, age, cause of infertility, and fertilization rate were independent factors that affected pregnancy outcomes ($p < 0.05$). The area under the curve (AUC) for menstrual SAA in predicting pregnancy failure was 0.719 (95% confidence interval [CI]: 0.655~0.783), with an optimal cutoff value of 58.25 IU/mL (sensitivity 68.8%, specificity 64.3%). **Conclusions:** SAA levels showed a significant positive association with anxiety in IVF/ICSI-ET patients. Elevated SAA levels may increase the risk of treatment failure in IVF/ICSI-ET patients. SAA may serve as a valuable objective biomarker for assessing stress-related fertility outcomes.

Keywords: salivary α-amylase; anxiety; quality of life; *in vitro* fertilization; intracytoplasmic sperm injection; embryo transfer

1. Introduction

Infertility is defined as the inability of a woman of reproductive age to achieve a pregnancy after 12 months of regular, unprotected sexual intercourse [1]. With the accelerating pace of life, worsening environmental pollution, and increasing prevalence of delayed marriage and childbearing, the incidence of infertility has been rising annually [2]. *In vitro* fertilization-embryo transfer (IVF-ET) technology is typically considered a last resort for infertile patients. While it offers hope, the complex and invasive nature of the treatment process, along with its prolonged duration, imposes a significant financial burden on patients. The uncertainty of treatment outcomes, combined with pressure from family and society, often leads to anxiety and other negative emotions during the assisted reproduction process [3].

Currently, there is still some controversy regarding the impact of anxiety on treatment outcomes for IVF-ET patients. While some studies, such as that by Pinarbasi FD et al. [4], report no significant effect, a recent meta-analysis concluded that anxiety significantly reduces the clinical pregnancy rates in IVF-ET cycles [5]. Most negative emotion assessment tools rely on subjective self-reported scales or questionnaires that lack objective indicators and may therefore fail to accurately capture a patient's true emotional status. Salivary alpha-amylase (SAA) is a glycoside hydrolase synthesized and secreted by the salivary glands and it functions in the oral cavity to hydrolyze starch into glucose and maltose. Chronic stress activates the hypothalamic-pituitary-adrenal (HPA) axis and the sympathetic nervous system (SNS), leading to the release of cortisol and SAA. Because SNS activation occurs more rapidly than that of the HPA axis activation, SAA is considered a sensitive marker of the physiological stress response [6]. SAA, as a sensitive indicator of SNS activation, has been identified in recent years as a biomarker for acute stress, chronic stress, and anxiety [7]. Clinical studies have shown that SAA levels are significantly elevated in individuals exposed to stressful environments or experiencing anxiety [8,9]. In addition,

negative emotions such as anxiety in IVF-ET patients can affect family relationships and reduce their fertility-related quality of life. In contrast, a decline in fertility-related quality of life may exacerbate anxiety symptoms [10].

This study integrates SAA measurement with questionnaire-based assessments to examine its correlation with anxiety, fertility-related quality of life, and treatment outcomes among *in vitro* fertilization/intracytoplasmic sperm injection-embryo transfer (IVF/ICSI-ET) patients, thereby providing a theoretical basis for the objective evaluation of anxiety, fertility-related quality of life, and their impact on treatment outcomes in infertile patients.

2. Materials and Methods

2.1 Study Participants

This was a prospective cohort study. Infertile female patients scheduled for IVF/ICSI-ET treatment at the at the Reproductive Medicine Center of the First Hospital of Shanxi Medical University were enrolled between March 2024 and March 2025. Inclusion criteria were as follows: (1) age of 20–38 years; (2) diagnosis of infertility and indication for IVF/ICSI-ET; (3) undergoing controlled ovarian stimulation using an antagonist protocol; and (4) ability to provide informed consent. Exclusion criteria included: (1) organic uterine lesions affecting pregnancy; (2) oral diseases, upper respiratory tract infections, or immune disorders; (3) use of glucocorticoids or psychotropic drugs within the past three months; and (4) exposure to significant life events within the past six months. A total of 249 patients were enrolled in this study, of whom 13 withdrew due to personal reasons or the absence of transferable embryos. Consequently, 236 patients were included in the final analysis. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the First Hospital of Shanxi Medical University (No.: KYYJ-2023-124).

2.2 Research Instruments

General sociodemographic survey: includes information such as age, body mass index (BMI), residence, educational level, monthly family income, cause of infertility, duration of infertility, previous live births, and prior assisted reproduction cycles.

Clinical and embryology laboratory data: basal follicle-stimulating hormone (bFSH), basal luteinizing hormone (bLH), basal estradiol (bE2), duration of gonadotropin-releasing hormone (GnRHa) treatment, dosage of GnRHa, endometrial thickness, number of oocytes retrieved, fertilization rate, number of two-pronuclear (2PN) oocytes, number of utilizable embryos, number of usable high-quality embryos, usable embryo rate, number of embryos transferred.

Self-Rating Anxiety Scale (SAS): developed by William W. K. Zung in 1971 [11], was administered to assess anxiety. This 20-item scale measures patients' subjective anxiety over the prior week using a 4-point rating system. Based on the Chinese norm, the total raw score was multiplied by 1.25 to obtain the standardized index score. A standard score ≥50 indicated the presence of anxiety, with higher scores reflecting greater severity.

Fertility Quality of Life Questionnaire (FertiQoL): this scale was jointly developed by the European Society of Human Reproduction and Embryology (ESHRE) and the American Society for Reproductive Medicine (ASRM) to assess the quality of life in women with infertility [12]. It has been translated and validated in more than 20 languages. The Chinese version was first introduced and applied to women undergoing assisted reproductive technology (ART) treatment by Xu MZ et al. [13] in 2016. In this study, the questionnaire demonstrated high internal consistency, with a Cronbach's α coefficient of 0.907. The FertiQoL consists of two primary modules: a 24-item Core module and a 10-item Treatment module. The Core module evaluates four domains: Emotional, Mind/Body, Relational, and Social. The Treatment module assesses two domains: Treatment Environment and Treatment Tolerability. All items are rated on a 5-point Likert scale (0 to 4). The total score is transformed to a scale ranging from 0 to 100, with higher scores indicating a better fertility-related quality of life [12].

2.3 Research Methods

Participants were instructed to arrive at the hospital at 8:00 AM, fasting, for saliva collection during menstruation (days 2–4) and again on the day of oocyte retrieval. They were required to abstain from vigorous exercise and smoking for at least one hour before collection. To minimize procedural stress, samples were collected before any medical procedures on the day of sampling. Unstimulated saliva was collected using a sterile graduated cylinder. Samples were then centrifuged at 4000 rpm for 10 minutes to separate and remove mucins and cellular debris. The supernatant (2–3 mL) was aliquoted into sterile microcentrifuge tubes and immediately stored at –80 °C to avoid repeated freeze-thaw cycles.

Immediately following saliva collection, participants completed on-site questionnaires, which were verified and collected by research staff. For analysis, thawed saliva samples were measured using a Multiskan Mk3 microplate reader (Thermo Fisher Scientific,Waltham, MA, USA) with onboard software, using a commercially available Enzyme-Linked Immunosorbent Assay (ELISA) kit (Human Alpha-Amylase ELISA Kit, Catalog CSB-E14075h; Wuhan Huamei Biological Engineering Co., Ltd., China) according to the manufacturer's instructions. All samples were assayed in duplicate, and concentrations were calculated using a standard curve. The intra-assay and inter-assay coefficients of variation were both <10%.

Follow-up pregnancy outcomes: absence of pregnancy was defined by a negative serum human chorionic

Table 1. Comparison of SAA level and anxiety scores across different treatment phases.

Characteristics	Menstrual period	Oocyte retrieval day	*Z*/*t*	*p*-value
SAA level (IU/mL)	60.20 (38.90, 131.00)	61.35 (39.25, 154.40)	–1.841	0.066
SAS score	41.96 ± 8.75	42.07 ± 8.90	–0.716	0.475

SAA, salivary α-amylase; SAS, Self-Rating Anxiety Scale.

gonadotropin (hCG) test 14 days after embryo transfer; a positive hCG test at this time point with no subsequent visualization of a gestational sac on transvaginal ultrasound (28–35 days post-transfer) indicated a biochemical pregnancy; confirmation of an intrauterine gestational sac on ultrasound defined a clinical pregnancy. Clinical pregnancies ending before 12 weeks were classified as early miscarriages, whereas those with ongoing fetal heart activity beyond 12 weeks were defined as ongoing pregnancies. In this study, biochemical pregnancies were classified as non-clinical pregnancies. Accordingly, patients were divided into a clinical pregnancy group and a non-clinical pregnancy group for comparative analysis of all indicators.

2.4 Statistical Analysis

Statistical analyses were performed using SPSS 27.0. Normally distributed continuous data are expressed as mean ± standard deviation ($\bar{x} \pm s$) and compared using *t*-tests or one-way analysis of variance (ANOVA). Non-normally distributed continuous data are presented as median with first and third quartiles [Median (Q1, Q3)] and compared using the Mann-Whitney *U* or Kruskal-Wallis *H* test. Categorical variables are reported as n (%), and intergroup comparisons are performed using the chi-square test of independence or Fisher's exact test. Correlations among SAA level, anxiety, and fertility-related quality of life were assessed using Pearson or Spearman correlation analysis, depending on the data distribution. Factors influencing treatment outcomes were analyzed using binary logistic regression. Receiver operating characteristic (ROC) curve analysis was performed using GraphPad Prism 10.4.2 to determine the SAA cutoff for treatment failure. Statistical significance was set at $p < 0.05$.

3. Results

3.1 Comparison of SAA Level and Anxiety Scores Across Different Treatment Phases

Of the 236 patients, 50 (21.2%) met the criteria for anxiety during menstruation, while the remaining 186 (78.8%) did not.Compared with the menstrual phase, both SAA levels and anxiety scores showed no significant changes on the day of oocyte retrieval ($p > 0.05$) (Table 1). Subsequent analyses were based on data from the menstrual cycle.

3.2 Univariate Analysis of Factors Influencing Menstrual SAA Level

Among the 236 patients, the predominant cause of infertility was female factors (65.7%). Most patients were nulliparous (86.4%), and the majority underwent their first assisted reproductive cycle (65.7%). No statistically significant differences in menstrual SAA levels were observed across age, cause of infertility, type of infertility, duration of infertility, number of previous live births, or number of assisted reproductive cycles ($p > 0.05$) (Table 2).

3.3 Correlation Analysis Between Menstrual SAA Level, Anxiety, and Fertility-Related Quality of Life

The total FertiQoL score for the cohort was 77.14 ± 11.73. Domain scores were as follows: Mind/Body, 76.36 ± 17.23; Emotional, 75.46 ± 16.87; Relational, 76.01 ± 12.12; Social, 81.02 ± 14.37; Treatment Environment, 78.20 ± 12.40; and Treatment Tolerance, 75.16 ± 17.38. Correlation analysis revealed that the total FertiQoL score was positively correlated with all six domain scores ($p < 0.01$). Menstrual SAA levels showed a weak but statistically significant positive correlation with menstrual SAS score ($r = 0.155$, $p < 0.05$) and a negative correlation with the Social domain score ($r = –0.138$, $p < 0.05$). Menstrual SAS score were significantly negatively correlated with fertility-related quality of life and all individual domain scores ($p < 0.01$) (Table 3).

3.4 Univariate Analysis of Different Treatment Outcomes

The 236 patients were divided into a clinical pregnancy group ($n = 126$, 53.4%) and a non-clinical pregnancy group ($n = 110$, 46.6%) based on pregnancy outcomes. Intergroup comparisons yielded statistically significant differences in age, cause of infertility, menstrual anxiety status, menstrual SAA levels, fertilization rate, number of usable high-quality embryos, and number of embryos transferred ($p < 0.05$) (Tables 4,5). Among the 126 patients with clinical pregnancy, follow-up through 12 weeks of monitoring revealed that 17 (13.5%) experienced early miscarriage, while 109 (86.5%) progressed to an ongoing pregnancy. Menstrual SAA levels were significantly higher in the early miscarriage group than in the ongoing pregnancy group ($p < 0.01$) (Table 6).

3.5 Multivariate Analysis of Factors Influencing Treatment Outcomes

A multivariate logistic regression model was constructed with clinical pregnancy as the dependent variable, coded as 1 for *not pregnant* and 0 for *pregnant*. Statistically significant variables from the univariate analyses were entered as independent variables. These were coded as follows: age (<32 years = 1, ≥32 years = 2); cause of infer-

Table 2. Univariate analysis of factors influencing menstrual SAA level [M(Q1, Q3)].

Characteristics	*n* (%)	SAA level (IU/mL)	*H/Z*	*p*-value
Age (years)			–1.909	0.056
<32	122 (51.7)	52.45 (35.80, 110.85)		
≥32	114 (48.3)	62.75 (41.10, 166.20)		
Cause of infertility			5.501	0.139
Female	155 (65.7)	61.20 (39.40, 153.50)		
Male	13 (5.5)	44.00 (34.00, 59.65)		
Combined	55 (23.3)	62.00 (39.70, 164.30)		
Unexplained	13 (5.5)	48.80 (24.70, 108.25)		
Type of infertility			–0.837	0.402
Primary	126 (53.4)	61.30 (39.80, 141.23)		
Secondary	110 (46.6)	55.95 (36.70, 126.20)		
Previous pregnancy loss			–0.225	0.822
Yes	92 (39.0)	56.60 (37.33, 146.83)		
No	144 (61.0)	61.25 (38.90, 130.40)		
Duration of infertility (years)			1.804	0.406
<2	72 (30.5)	57.60 (37.23, 183.05)		
2~5	108 (45.8)	59.55 (36.78, 104.40)		
≥5	56 (23.7)	62.70 (42.30, 190.43)		
Previous live births			–0.546	0.585
Yes	32 (13.6)	63.25 (36.50, 110.43)		
No	204 (86.4)	59.60 (39.20, 138.00)		
Assisted reproductive cycle (times)			0.934	0.627
1	155 (65.7)	61.10 (39.40, 153.50)		
2	45 (19.1)	55.90 (35.70, 117.10)		
≥3	36 (15.2)	54.90 (35.30, 190.43)		

Table 3. Correlation analysis between menstrual SAA level, anxiety, and the fertility-related quality of life (*r*).

Characteristics	SAA	SAS	FertiQoL	Mind/Body	Emotional	Relational	Social	Environment	Tolerability
SAA level	1								
SAS score	0.155*	1							
FertiQoL	–0.113	–0.651**	1						
Mind/Body	–0.041	–0.580**	0.831**	1					
Emotional	–0.118	–0.620**	0.838**	0.772**	1				
Relational	–0.087	–0.404**	0.620**	0.324**	0.398**	1			
Social	–0.138*	–0.562**	0.864**	0.674**	0.741**	0.486**	1		
Environment	–0.039	–0.327**	0.598**	0.312**	0.297**	0.410**	0.465**	1	
Tolerability	–0.043	–0.498**	0.795**	0.661**	0.601**	0.406**	0.633**	0.434**	1

** $p < 0.01$; * $p < 0.05$; FertiQoL, Fertility Quality of Life Questionnaire.

tility (unexplained = 1, female = 2, male = 3, combined = 4); and menstrual anxiety status (no = 0, yes = 1). Continuous variables were analyzed on their original form. The analysis identified age, cause of infertility, menstrual SAA level, and fertilization rate as independent predictors of te treatment outcome ($p < 0.05$) (Table 7).

3.6 Predictive Value of Menstrual SAA Level for Pregnancy Failure

ROC curve analysis revealed that the area under the curve (AUC) of menstrual SAA for predicting pregnancy failure was 0.719 (95% confidence interval [CI]: 0.655~0.783). The optimal cutoff value for menstrual SAA was 58.25 IU/mL (sensitivity 68.8%, specificity 64.3%) (Fig. 1).

4. Discussion

Influenced by traditional Chinese cultural concepts, women with infertility experience substantial psychological pressure. Although IVF-ET technology is widely regarded as the most effective infertility treatment, offering hope to patients, it can also trigger negative emotions such as anx-

Table 4. Comparison of questionnaire data on different treatment outcomes.

Characteristics	Clinical pregnancy *n* (%), 126 (53.4)	Non-clinical pregnancy *n* (%), 110 (46.6)	$\chi^2/Z/t$	*p*-value
Age (years)			4.217	0.040
<32	73 (57.9)	49 (44.5)		
≥32	53 (42.1)	61 (55.5)		
BMI (kg/m^2)	23.88 (21.69, 26.46)	23.69 (21.48, 26.39)	–0.286	0.775
Residence			0.003	0.959
Urban	100 (79.4)	87 (79.1)		
Rural	26 (20.6)	23 (20.9)		
Educational level			1.278	0.528
Junior high school and below	27 (21.4)	21 (19.1)		
High school and junior college	47 (37.3)	49 (44.5)		
Bachelor's degree or higher	52 (41.3)	40 (36.4)		
Family monthly income (RMB)			1.127	0.569
<4000	36 (28.6)	36 (32.7)		
4000~7000	66 (52.4)	50 (45.5)		
≥7000	24 (19.0)	24 (21.8)		
Cause of infertility			8.371	0.039
Female	74 (58.7)	81 (73.6)		
Male	6 (4.8)	7 (6.4)		
Combined	36 (28.6)	19 (17.3)		
Unexplained	10 (7.9)	3 (2.7)		
Duration of infertility (years)			4.226	0.121
<2	44 (34.9)	28 (25.5)		
2~5	58 (46.0)	50 (45.5)		
≥5	24 (19.1)	32 (29.0)		
Previous live births			1.235	0.267
Yes	20 (15.9)	12 (10.9)		
No	106 (84.1)	98 (89.1)		
Assisted reproductive cycle (times)			2.583	0.275
1	82 (65.1)	73 (66.4)		
2	28 (22.2)	17 (15.5)		
≥3	16 (12.7)	20 (18.2)		
Menstrual anxiety status			4.571	0.033
Yes	20 (15.9)	30 (27.3)		
No	106 (84.1)	80 (72.7)		
Menstrual SAS score	41.01 ± 7.86	43.03 ± 9.60	–1.779	0.077
FertiQoL total score	77.70 ± 11.54	76.50 ± 11.96	0.782	0.435

BMI, body mass index.

iety. However, whether anxiety levels fluctuate across different stages of IVF-ET treatment remains controversial. Liu YF et al. [14] assessed anxiety in 247 infertile couples at three time points: treatment initiation (T1), trigger day (T2), and day 4 post-embryo transfer (T3), reporting the highest scores at T2. Conversely, Song DH et al. [15] found that anxiety scores were significantly higher at T1 than at T3. Ouyang XP et al. [16] reported that anxiety scores at T3 were significantly higher than at both T1 and T2, with T2 scores slightly exceeding those at T1. In our study, both anxiety scores and SAA levels were modestly elevated on the day of oocyte retrieval (T2) compared with the menstrual period (T1). However, the differences did not reach statistical significance, a trend consistent with the T1-T2 comparison reported by Ouyang XP et al. [16]. The discrepancies among these findings may be attributable to factors such as the subjective nature of assessment tools, heterogeneity of study populations, and differences in sample size.

Currently, the assessment of negative emotions in infertility patients relies predominantly on subjective questionnaires, which are inherently prone to bias and have limited generalizability. In contrast, SAA serves as a viable objective biomarker that can be measured precisely and offers the advantages of cost-effectiveness and noninvasiveness [17]. Its validity as a marker for stress and anxiety has

Table 5. Comparison of clinical characteristics on different treatment outcomes.

Characteristics	Clinical pregnancy *n* (%), 126 (53.4)	Non-clinical pregnancy *n* (%), 110 (46.6)	*Z*	*p*-value
Menstrual SAA level (IU/mL)	43.50 (32.38, 83.75)	85.60 (49.15, 212.33)	–5.923	<0.001
bFSH (mIU/L)	7.05 (5.40, 9.30)	7.20 (5.80, 9.30)	–0.622	0.534
bLH (mIU/L)	3.93 (2.60, 6.00)	4.70 (2.97, 6.20)	–1.021	0.307
bE2 (pg/mL)	32.00 (25.00, 42.71)	35.41 (25.00, 46.00)	–1.114	0.265
Duration of GnRHa	9.00 (8.00, 11.00)	9.50 (8.00, 11.00)	–0.697	0.486
Dosage of GnRHa (IU)	2475 (2100, 2925)	2438 (2025, 3000)	–0.178	0.859
Endometrial thickness (mm)	11.00 (10.00, 13.00)	12.00 (10.00, 13.00)	–1.586	0.113
Number of oocytes retrieved	11.00 (6.00, 17.00)	11.50 (6.00, 17.00)	–0.102	0.918
Fertilization rate (%)	84.50 (67.00, 100.00)	76.00 (60.00, 93.00)	–2.247	0.025
Number of 2PN fertilized oocytes	6.00 (4.00, 10.00)	5.50 (2.00, 10.25)	–1.190	0.234
Number of utilizable embryos	6.00 (4.00, 10.00)	5.50 (2.00, 10.25)	–1.222	0.222
Number of utilizable high-quality embryos	3.00 (1.00, 5.00)	2.00 (0.75, 5.00)	–2.187	0.029
Utilizable embryo rate (%)	45.50 (25.00, 70.00)	33.00 (6.00, 63.25)	–1.676	0.094
Number of embryos transferred			5.096	0.024
1	13 (10.3)	23 (20.9)		
2	113 (89.7)	87 (79.1)		

bFSH, basal follicle-stimulating hormone; bLH, basal luteinizing hormone; bE2, basal estradiol; GnRHa, gonadotropin-releasing hormone; 2PN, two-pronuclear. Endometrial thickness, measured by vaginal ultrasound on trigger day.

Table 6. Comparison of menstrual SAA level and anxiety scores among patients in the clinical pregnancy group.

Characteristics	Early miscarriage *n* (%), 17 (13.5)	Ongoing pregnancy *n* (%), 109 (86.5)	*Z*/*t*	*p*-value
Menstrual SAA level (IU/mL)	140.00 (78.50, 262.00)	40.50 (29.70, 62.75)	–5.092	<0.001
Menstrual SAS score	41.40 ± 7.38	40.95 ± 7.96	0.217	0.829

Table 7. Multivariate logistic regression analysis of factors influencing treatment outcomes.

Characteristics	β	SE	Wald	*p*-value	OR	95% CI
Age	0.606	0.297	4.144	0.042	1.832	(1.023, 3.282)
Cause of infertility (Unexplained)			8.819	0.032		
Female	1.453	0.783	3.443	0.064	4.276	(0.922, 19.842)
Male	1.917	0.967	3.933	0.047	6.801	(1.023, 45.230)
Combined	0.644	0.822	0.614	0.433	1.905	(0.380, 9.542)
Menstrual Anxiety status	0.350	0.370	0.892	0.345	1.418	(0.687, 2.930)
Menstrual SAA level (IU/mL)	0.013	0.003	17.887	<0.001	1.013	(1.007, 1.019)
Fertilization rate (%)	–1.662	0.723	5.283	0.022	0.190	(0.046, 0.783)
Number of usable high-quality embryos	–0.023	0.050	0.211	0.646	0.977	(0.886, 1.078)
Number of embryos transferred	–0.578	0.446	1.682	0.195	0.561	(0.234, 1.344)

SE, standard error; OR, odds ratio; CI, confidence interval.

been well demonstrated [7]. In our study, SAA was significantly associated (though weakly) with continuous SAS scores. Although the impact of anxiety on IVF-ET outcomes remains inconclusive [18], our results indicate that elevated menstrual SAA levels were associated with an increased risk of pregnancy failure. Although menstrual anxiety scores did not differ significantly between the clinical pregnancy and nonpregnancy groups, the anxiety detection rate was significantly higher among patients who experienced pregnancy failure, suggesting a potential adverse effect of anxiety on outcomes. Furthermore, menstrual SAA levels were significantly elevated in the early miscarriage group, suggesting that anxiety may also contribute to early miscarriage. However, after adjustment for confounders, anxiety was not identified as an independent risk factor. This discrepancy may be attributed to individual differences or to patients adopting socially desirable response strategies during questionnaire completion as a psychological defense mechanism, potentially biasing the results and failing to accurately reflect true anxiety levels. Future studies should

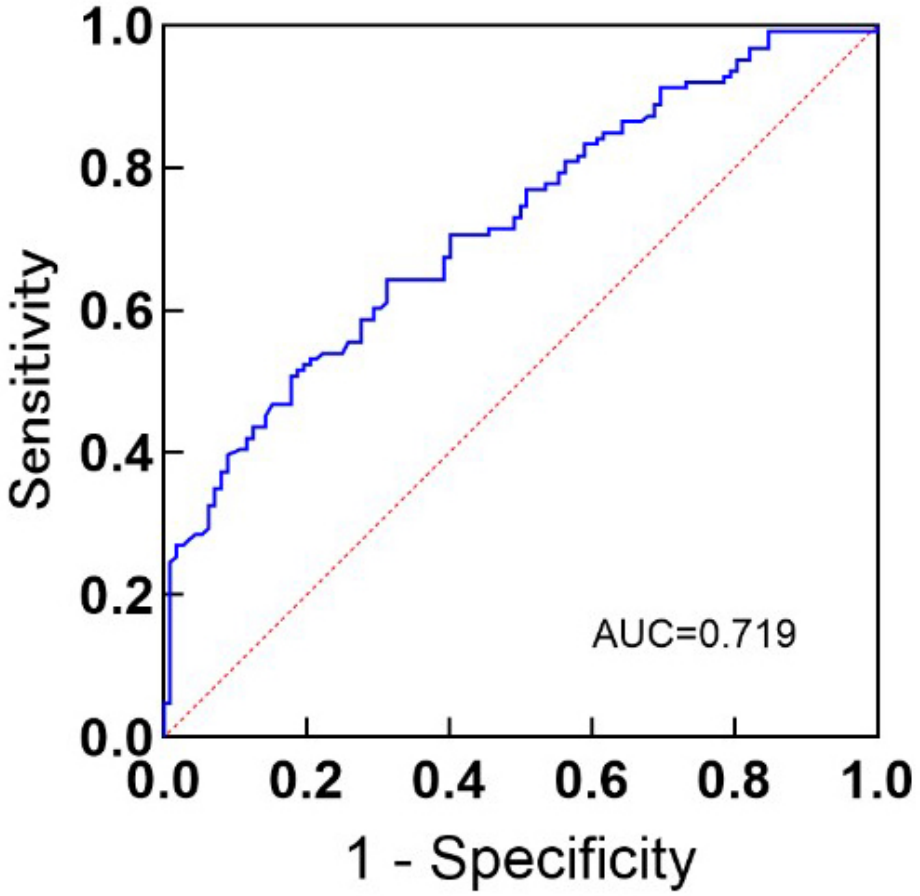

Fig. 1. Predictive value of menstrual SAA for pregnancy failure. AUC, area under the curve. The AUC was 0.719 (95% CI: 0.655~0.783). The optimal cutoff was 58.25 IU/mL (sensitivity 68.8%, specificity 64.3%).

expand sample sizes and ensure questionnaire validity to more accurately investigate this relationship.

In ART clinical practice, patient age, antral follicle count (AFC), oocyte yield, embryo quality, and endometrial receptivity are well-established predictors of pregnancy outcomes [19,20]. Our study identified age, causes of infertility, fertilization rate, and menstrual SAA levels as independent determinants of outcome. Female fertility is known to decline gradually from around age 32 and more rapidly after 37 [21]. Consistent with this, we found patients older than 32 years had a significantly higher risk of treatment failure. Shingshetty L et al. [22] demonstrated that causes of infertility were a predictive factor for IVF outcomes, reporting lower live birth rates in cases of female, male, or combined factor infertility compared with unexplained infertility—a finding corroborated by our study. Higher fertilization rates are associated with increased rates of high-quality embryos, higher utilizable embryo rates, and improved cumulative live birth rates [23]. Consistently, the fertilization rate was significantly higher in the clinical pregnancy group. Additionally, the number of usable high-quality embryos and embryos transferred was significantly higher in the clinical pregnancy group than in the non-clinical pregnancy group. However, after adjustment for confounders, neither variable emerged as an independent factor influencing treatment outcomes. Only five patients with thin endometrium were included in our study, precluding a meaningful analysis of this factor.

To investigate the predictive value of menstrual SAA for pregnancy failure, we performed ROC curve analysis to determine its optimal cutoff value. Our results indicate that elevated SAA levels, once exceeding a certain threshold, may be associated with an increased risk of pregnancy failure. Consistent with the findings of Zhou et al. [24], our study demonstrated that menstrual SAA levels have significant predictive value for IVF pregnancy outcomes. In contrast, Nikolaeva et al. [25] found no such correlation between male SAA and ICSI outcomes, suggesting that the predictive value of SAA may be sex-specific or influenced by differences in sampling timing or study populations. However, the utility of SAA as a biomarker of anxiety or stress may be limited, as ovarian steroid hormones may exert a regulatory effect on SAA secretion [7].

Currently, the FertiQoL, due to its strong reliability and validity, is widely used to assess the correlation between psychological stress and quality of life in infertility

patients [26]. Our analysis revealed that the Treatment Tolerability domain received the lowest scores, suggesting that IVF/ICSI-ET patients have concerns about potential side effects from medications and treatment outcomes. Additionally, the demanding and complex treatment process may interfere with daily work and daily life. In our study, the menstrual SAS scores showed significant negative correlations with both the FertiQoL total score and its individual domains, indicating that higher anxiety levels may be associated with poorer fertility-related quality of life, a finding consistent with prior reports [3]. Meanwhile, a decline in fertility-related quality of life may further trigger or exacerbate anxiety symptoms, potentially creating a vicious cycle [10]. Notably, SAA levels were negatively correlated only with the Social domain of FertiQoL. Currently, the relationship between fertility-related quality of life and treatment outcomes remains unclear. In contrast, Wu MH et al. [27] found no association with pregnancy rates in a specific population, whereas Urata Y et al. [28] reported that higher FertiQoL total scores were correlated with increased clinical pregnancy rates. Our data showed a non-significantly higher total FertiQoL score in the clinical pregnancy group, underscoring the need for further investigation with larger sample sizes or more sensitive objective measures.

Limitations

This study had several limitations. First, the single-center design may limit the generalizability of the findings. Second, anxiety was assessed at only two time points (menstruation and oocyte retrieval day), and anxiety status in infertile patients may change during follow-up due to various factors. Third, the analysis focused exclusively on female stress; male SAA levels were not measured. Fourth, stress-related autonomic activity was assessed using only a single biomarker (SAA). Given these limitations, further well-designed studies, including multicenter prospective cohorts and randomized controlled trials (RCTs), are warranted to validate these findings.

5. Conclusions

In conclusion, infertile patients undergoing IVF/ICSI-ET treatment face pressures from society, family, and the treatment process itself, which can precipitate anxiety, reduce fertility-related quality of life, and potentially compromise pregnancy outcomes. Currently, commonly used psychological assessment questionnaires remain relatively subjective and may not adequately capture individual differences among patients. In contrast, SAA, as a quantifiable objective biomarker, demonstrates promising clinical utility. Our findings indicate that menstrual SAA levels are significantly positively associated with SAS scores, whereas continuous SAS scores are significantly negatively correlated with the fertility-related quality of life. Notably, SAA emerged as an independent risk factor for treatment failure and demonstrated predictive value for this outcome. Therefore, SAA may serve as a valuable objective biomarker for assessing stress-related fertility outcomes, offering a physiological measure to complement subjective questionnaires in identifying patients at risk for treatment failure. Future multicenter, large-cohort RCTs are warranted to further validate the predictive value of combining objective biomarkers for stress assessment on treatment outcomes, while strictly controlling for confounding factors. This will provide a reliable basis for implementing targeted psychological interventions in clinical practice, thereby improving clinical pregnancy and live birth rates of IVF/ICSI-ET treatments.

Availability of Data and Materials

The datasets used and analyzed during the current study are available from the corresponding author upon reasonable request.

Author Contributions

QW: data collection, data analysis, manuscript writing, and data interpretation; XQL: data collection; YJH: data collection, manuscript revision, and final approval. All authors contributed to 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

The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Ethics Committee of the First Hospital of Shanxi Medical University (No.: KYYJ-2023-124). All participants were informed about the study's objective and provided informed consent.

Acknowledgment

Not applicable.

Funding

This research received no external funding.

Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found, in the online version, at https://doi.org/10.31083/CEOG50300.

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