

PEDF–VEGF Balance in the Follicular Microenvironment: Biomarker Correlates to Ovarian Reserve and Stimulation Response in Women Undergoing ICSI

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ABSTRACT

Background: Angiogenesis constitutes a fundamental regulatory mechanism in folliculogenesis, oocyte maturation, endometrial receptivity and successful implantation, directly influencing assisted reproductive technology (ART) outcomes. Vascular endothelial growth factor (VEGF) is the principal pro-angiogenic factor in the ovary, whereas pigment epithelium-derived factor (PEDF) serves as a potent endogenous angiogenesis inhibitor. The relative balance between these factors is critical in determining follicular angiogenesis, oxidative stress, oocyte competence and pregnancy outcomes. Dysregulation of the angiogenic balance within the follicular microenvironment may adversely affect oocyte quality and pregnancy outcomes, particularly in assisted reproductive technology (ART) cycles. **Objective:** To evaluate serum and follicular fluid (FF) PEDF and VEGF concentrations, as well as the PEDF/VEGF index, and their correlation with oocyte quality and pregnancy outcomes in Iraqi women undergoing intracytoplasmic sperm injection (ICSI). **Methods:** This prospective study included ninety infertile women undergoing controlled ovarian stimulation and ICSI at High Institute for infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq. Serum and FF samples were collected on the day of oocyte retrieval. PEDF and VEGF concentrations were measured using enzyme-linked immunosorbent assay (ELISA), and the PEDF/VEGF index was calculated. Oocyte maturity parameters and pregnancy outcomes were recorded. **Results:** Cycles resulting in clinical pregnancy demonstrated elevated PEDF/VEGF indices in both serum and FF compared with non-pregnant cycles. Higher indices were positively correlated with a greater proportion of mature (MII) oocytes, higher implantation rates, and cycles resulting in pregnancy. In contrast, elevated VEGF without a proportional increase in PEDF was associated with a predominance of immature oocytes and poorer outcomes. **Conclusions:** The PEDF/VEGF index in follicular fluid may serve as a reliable indicator of angiogenic homeostasis within the ovarian microenvironment and a potential predictor of oocyte quality and pregnancy success in Iraqi women undergoing ICSI. If confirmed, therapeutic modulation of PEDF/VEGF balance could be explored to improve ART outcomes.

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1. Introduction

Infertility can be described as a reproductive disorder in individuals, whether woman or man, who cannot conceive after participating in regular sexual intercourse for more than 12 months. The World Health Organization (WHO) reports that 17.5% of individuals, almost one in six, experience infertility at some point in their lives (Jeon et al., 2025).

Infertility affects a substantial proportion of reproductive-age couples worldwide and is a common indication for assisted reproductive technologies (ART) such as IVF/ICSI (Szamatowicz & Szamatowicz, 2020; Vander Borgh & Wyns, 2018).

Assisted reproductive technologies (ART), particularly in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), remain the primary therapeutic options for couples who fail to conceive naturally and for managing multifactorial cause infertility, which is accompanied with a genetic diseases susceptibility (Chen et al., 2023). ICSI is the gold standard technique to treat male factor infertility (Haddad et al., 2021). Despite advances in ART protocols, pregnancy success rates remain suboptimal, highlighting the need for reliable biomarkers that can predict oocyte quality and clinical outcomes.

Ovarian angiogenesis constitutes a vital component of folliculogenesis and is governed by multiple hormonal and local

factors that modulate the vascular response (Ileiwat et al., 2022).

During folliculogenesis, oocytes in follicles go through a series of controlled phases of maturation. The ovarian interstitial stroma is essential for the continuous process of ovarian folliculogenesis. The oocyte, granulosa cells, theca cells, extracellular matrix, and capillary blood vessels make up the ovary (K. Chen et al., 2025). These factors yield deeper insight into the interaction between the follicle microenvironment and angiogenesis (Xu et al., 2022). The follicular microenvironment — including follicular fluid (FF) composition — strongly influences oocyte maturation, competence, and embryo developmental potential (Pan et al., 2024).

Competent oocyte is a critical point for successful fertilization and early embryonic development. Successful pregnancy begins with the production of a competent oocyte, which is crucial for fertilization, cleavage, and initial cell divisions of the early embryo (Oliveira et al., 2025).

Angiogenesis within the follicle is necessary for nutrient and oxygen delivery, and is chiefly governed by vascular endothelial growth factor (VEGF), which increases vascular permeability and endothelial proliferation under gonadotropin stimulation (Akbarian et al., 2022; Wu et al., 2021). Conversely, pigment epithelium-derived factor (PEDF) is an endogenous, 50-kDa anti-angiogenic glycoprotein with anti-inflammatory and antioxidant properties; granulosa cells secrete PEDF in a hormonally regulated fashion, and PEDF antagonizes VEGF effects within the ovary (He et al., 2022; Yang et al., 2024). The PEDF/VEGF balance is therefore critical in folliculogenesis, and dysregulation has been implicated in pathologies such as PCOS and OHSS and may impair oocyte quality (Miller et al., 2020). Given the potential clinical relevance, assessing the PEDF/VEGF index in serum and FF could provide prognostic information about oocyte quality and pregnancy outcomes in infertile women undergoing ART.

One of the most effective antagonists of angiogenic factors (like VEGF) is pigment epithelium-derived factor (PEDF), a multifunctional anti-angiogenic factor (Chae-Kim et al., 2021). It has been discovered to have a variety of useful characteristics, including the ability to modulate immune responses, reduce inflammation, and even influence the development of new structures (He et al., 2022). Within the ovary, granulosa cells produce and secrete PEDF in a hormonally regulated manner, inversely correlated with the expression of vascular endothelial growth factor (VEGF). Estradiol, luteinizing hormone (LH), and progesterone have been shown to downregulate PEDF secretion. PEDF is a major pro-folliculogenesis player that interacts with the main participants in follicular growth and development. It appears to contribute to the regulation of angiogenesis and oxidative stress during folliculogenesis. Recent evidence highlights PEDF as a key regulator of folliculogenesis, modulating angiogenesis and redox balance to support successful ovulation and oocyte maturation.

Vascular endothelial growth factor was crucial for early vascular recruitment possibly mediated by oocyte-secreted proteins that modulate its expression in the theca and granulosa cell layers (Dompe et al., 2021). Vascular endothelial growth factor (VEGF) affects the development and fertilization abilities of human oocytes (Lee et al., 2025).

2. Materials and Methods

This prospective cross-sectional study was conducted on ninety infertile females undergoing controlled ovarian stimulation (COS) followed by ICSI at the specialized infertility center of the High Institute for Infertility Diagnosis and Assisted Reproductive Technologies, Al-Nahrain University, Baghdad, Iraq between September 2023 until April 2025.

Inclusion criteria comprised women aged 18–42 years with primary or secondary infertility and indication for ICSI. Exclusion criteria included endocrine disorders unrelated to infertility, systemic inflammatory diseases, ovarian malignancy, and incomplete clinical or laboratory data. Written informed consent was obtained from all participants, and the study protocol was approved by the institutional ethical committee.

Complete medical, surgical, and obstetrical histories were obtained from the infertile couples. The infertile women were thoroughly examined, including a general and gynaecological examination. The women's BMI, age, AFC, and previous response to ovarian stimulation ICSI cycle were determined by the gonadotropin starting dose. Baseline hormonal analysis [follicle stimulating hormone (FSH), luteinizing hormone (LH), estradiol E2, prolactin, and thyroid stimulating hormone (TSH)] was done on day 2 of the menstrual cycle, also transvaginal scanning was performed to assess antral follicle count (AFC), and ovarian accessibility and exclude any ovarian pathology (like ovarian cysts).

Controlled Ovarian Stimulation and ICSI Procedure Controlled ovarian stimulation was performed using antagonist gonadotropin-based protocol. Recombinant follicle-stimulating hormone (r-FSH) (Gonaf -F R, Merk-Serono/ Switzerland) administration commenced on the second day of the menstrual cycle, with or without human menopausal gonadotropin (HMG) (Menogone R, Ferring, GmbH/ Germany) with dose adjustments based on ovarian response. Gonadotropin-releasing hormone (GnRH) antagonist, a daily subcutaneous injection of 0.25 mg of cetrorelix acetate (Cetrotide R, Merck Serono, Geneva, Switzerland) was introduced when leading follicles reached ≥ 14 mm to prevent premature luteinizing hormone surge. Final oocyte maturation was triggered using either human chorionic gonadotropin (r-hCG) 500 μ g (Ovitrelle R Merk-Serono/ Switzerland) or dual triggering $r - hCG250\mu g$ and $Triptorelin0.1mg$ (Decapeptyl RFerring/ Germany) when

at least three follicles measured ≥ 18 mm.

Under general anesthesia, transvaginal ultrasound-guided oocyte retrieval was performed 34–36 hours after trigger administration. Retrieved oocytes were classified according to nuclear maturity into germinal vesicle (GV), metaphase I (MI), and metaphase II (MII) stages. ICSI was performed using standard micromanipulation techniques, and embryo transfer was conducted according to routine clinical practice.

Sample Collection Venous blood samples were collected on the day of oocyte retrieval prior to anesthesia induction. Follicular fluid (FF) samples were aspirated from mature follicles, avoiding blood contamination. Samples were centrifuged at 5000 rpm for 10 minutes, and the supernatants were stored at -20°C until analysis. FF was selected as it directly reflects the biochemical microenvironment surrounding the oocyte.

Measurement of PEDF and VEGF Serum and FF concentrations of pigment epithelium-derived factor (PEDF) and vascular endothelial growth factor (VEGF) were measured using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions ([company name, country]). The assays were based on a sandwich ELISA principle with monoclonal antibodies specific for human PEDF and VEGF. Optical density was measured using a microplate reader at the recommended wavelength, and concentrations were calculated from standard curves.

The PEDF/VEGF index was calculated for both serum and FF by dividing PEDF concentration by VEGF concentration, as previously described in angiogenesis balance studies.

Pregnancy Outcome Assessment Biochemical pregnancy was confirmed by serum β -hCG measurement 14 days after embryo transfer.

2.1 Statistical Analysis

Statistical analysis was performed using SPSS software version 25. Data were expressed as mean $\pm$ standard deviation for continuous variables and number (percentage) for categorical variables. Comparisons between groups were conducted using independent t-test as appropriate. ANOVA test was performed to find the association between different variables in the studied groups. Receiver operating characteristic (ROC) curve analysis was used to evaluate the predictive performance of biomarkers for pregnancy outcomes. A p-value < 0.05 was considered statistically significant.

3. Result

3.1 General Characteristics of the Patients Enrolled in the Current Study

The study sample consisted of 90 infertile females.

3.2 Comparison for Levels of Serum and Follicular Fluid (PEDF and VEGF) and their Indices at Ovum Pick-Up, Stratified by Age and Body Mass Index (BMI)

Table 1. presents the mean value for serum PEDF, follicular PEDF, serum VEGF, and follicular VEGF and showed minimal variation across the three age groups (<25 , $25-34$, ≥ 35) and the three BMI levels (Normal, Overweight, Obese). Similarly, the PEDF/VEGF indices in serum and follicular fluid were comparable. No statistically significant differences had been found regarding all the parameters among the study groups ($P \geq 0.05$). Although obese women had numerically higher serum and follicular PEDF and VEGF levels compared to the other BMI categories, these differences were not large enough to suggest a strong association.

Table 1: Distribution of Serum and Follicular Levels of Pigment Epithelium-Derived Factor (PEDF), Vascular Endothelial Growth Factor (VEGF), and Their Indices by Age and Body Mass Index (BMI) Groups

Variables	S. PEDF Mean ± SD	F. PEDF Mean ± SD	S. VEGF Mean ± SD	F. VEGF Mean ± SD	S.PEDF/S.VEGF Index Mean ± SD	F.PEDF/F.VEGF Index Mean ± SD	P-Value*
Age (Years)							
< 25	3.37±1.18	3.12±1.12	1088.91±155.4	1027.24±103.28	.0030±.00082	.0030±.00099	NS
25 – 34	3.62±1.28	3.65±1.34	1137.37±173.88	1105.3±213.8	.0031±.00070	.0032±.00073	
≥ 35	3.48±1.26	3.64±1.23	1071.2±216.51	1071.06±241.4	.0032±.00066	.0034±.00068	
BMI Level Kg/m²							
Normal	3.61±.92	3.6±1.19	1095.59±128.22	1093.32±204.66	.0033±.00059	.0032±.00068	NS
Overweight	3.4±1.01	3.2±1.01	1093.33±145.69	1033.83±164.46	.0031±.00059	.0031±.00081	
Obese	3.63±1.58	3.97±1.45	1119.55±251.65	1125.37±258.51	.0031±.00083	.0035±.00067	

* One-Way ANOVA Was Used, SD, Standard Deviation, S.; Serum, F.; Follicular Fluid, PEDF; Pigment Epithelial Derived Factor, VEGF; Vascular Endothelial Growth Factor, *: p Value < 0.05 (Significant).

3.3 Serum and Follicular Fluid PEDF and VEGF Levels and PEDF/ VEGF Index According to Infertility Type, Cause, and Duration

Table 2. presents the mean concentration of VEGF and PEDF in both serum (S) and follicular fluid (F) across three categories of infertility: type, cause, and duration.

Significant differences were found between primary and secondary infertility. Secondary infertility was associated with a slightly higher F. PEDF (3.90 ± 1.19) and higher S. and F. VEGF levels compared to Primary infertility. The S.PEDF/S.VEGF Index is stable in serum but is higher in follicular fluid for the Secondary group (0.0035 ± 0.00054).

Unexplained infertility group exhibits the highest mean levels of both Serum and follicular fluid PEDF and VEGF and highest serum index. Male factor group shows the lowest mean level of S. and F. PEDF, S. VEGF and their indices. Although numbers are close, a significant difference ($p = 0.01$) exists among different infertility causes. Serum and follicular fluid levels of Pigment epithelium-derived factor (PEDF) and Vascular endothelial growth factor (VEGF), and their ratio, did not associate significantly with duration of infertility.

Table 2: Serum and Follicular Fluid PEDF and VEGF Levels and PEDF/ VEGF Index according to Infertility Type, Cause, and Duration

Variables	S. PEDF Mean ± SD	F. PEDF Mean ± SD	S. VEGF Mean ± SD	F. VEGF Mean ± SD	S.PEDF/S.VEGF Index Mean ± SD	F.PEDF/F.VEGF Index Mean ± SD	P-Value*
Type of Infertility							
Primary	3.56±1.27	3.51±1.29	1098.59±199.45	1073.36±213.69	.0032±.0007	.003±.00078	0.002* S
Secondary	3.41±1.16	3.90±1.19	1130.41±150.63	1120.17±227.53	.0030±.00067	.0035±.00054	
Cause of Infertility							
Female Factor	3.86±1.2	3.65±1.12	1136.15±172.18	1095.13±207.76	.0033±.00063	.0033±.00071	0.01* S
Male Factor	3.36±1.2	3.49±1.26	1085.01±187.81	1078.67±227.54	.0030±00074	.0032±.00076	
Combined	3.45±1.35	3.61±1.43	1097.71±215.93	1071.53±220.38	.0031±.00067	.0033±.00079	
Unexplained	4.08±1.07	3.81±1.04	1168.53±62.31	1130.02±153.04	.0035±.00074	.0033±.00049	
Duration of Infertility (Years)							
< 5	3.53±1.11	3.27±1.13	1095.25±162.91	1038.65±204.47	.0032±.00066	.0031±.00082	NS
5 – 9	3.49±1.30	3.78±1.27	1120.62±194.45	1102.79±201.47	.0030±.00070	.0034±.00070	
≥ 10	3.59±1.36	3.63±1.41	1088.81±221.07	1098.46±249.56	.0032±.00073	.0032±.00075	

* One-Way ANOVA Was Used, SD, Standard Deviation, S.; Serum, F.; Follicular Fluid, PEDF; Pigment Epithelial Derived Factor, VEGF; Vascular Endothelial Growth Factor, *: p Value < 0.05 (Significant).

3.4 Serum and Follicular Fluid Levels of PEDF, VEGF, and Their Ratios According to Oocyte Maturity and Quality in the Study Population

Table 3 compared Serum and Follicular Fluid PEDF and VEGF, and their PEDF/VEGF ratios across different oocyte maturity groups (GV, MI, MII). All values showed minimal variation across groups, as a result comparisons yielded non-significant p-values (NS).

Table 3: Serum and Follicular Fluid Levels of PEDF, VEGF, and Their Ratios According to Oocyte Maturity and Quality in the Study Population

Clinical Parameters	S. PEDF Mean $\pm$ SD	F. PEDF Mean $\pm$ SD	S. VEGF Mean $\pm$ SD	F. VEGF Mean $\pm$ SD	S.PEDF/S.VEGF Index Mean $\pm$ SD	F.PEDF/F.VEGF Index Mean $\pm$ SD	P-Value*
Abnormal Oocytes	3.73 $\pm$ 1.07	3.45 $\pm$ 1.52	1114.77 $\pm$ 174.04	1187.84 $\pm$ 238.32	.0030 $\pm$.00061	.0031 $\pm$.00096	NS
No. of (GV) Oocytes	3.56 $\pm$ 1.04	3.55 $\pm$ 1.16	1109.65 $\pm$ 175.14	1171.85 $\pm$ 202.04	.0031 $\pm$.00055	.0031 $\pm$.00066	NS
No. of (MI) Oocytes	3.48 $\pm$ 1.21	3.67 $\pm$ 1.19	1189.45 $\pm$ 211.85	1094.44 $\pm$ 192.15	.0031 $\pm$.0007	.0031 $\pm$.00064	NS
No. of (MII) Oocytes	3.53 $\pm$ 1.25	3.58 $\pm$ 1.27	1104.25 $\pm$ 191.32	1081.68 $\pm$ 215.64	.0032 $\pm$.00069	.0033 $\pm$.00075	NS

*One-Way ANOVA Was Used, SD, Standard Deviation, S.; Serum, F.; Follicular Fluid, PEDF; Pigment Epithelial Derived Factor, VEGF; Vascular Endothelial Growth Factor, *: p Value < 0.05 (Significant).

3.5 Serum and Follicular Fluid PEDF, VEGF, and PEDF/VEGF Index Across Embryo Quality Parameters in ICSI Cycles

Table 4. compared Serum PEDF, Follicular Fluid PEDF, Serum VEGF, Follicular Fluid VEGF, and the PEDF/VEGF ratio across four embryology parameters. There are statistically significant differences between the PN and G1E groups in some parameters (P -value= 0.021 and 0.03) respectively. PEDF values are similar across PN, G1E, G2E, and G3E while VEGF levels tend to be higher in G2E and G3E groups, compared to PN and G1E.

Table 4: Serum and Follicular Fluid PEDF, VEGF, and PEDF/VEGF Index Across Embryo Quality Parameters in ICSI Cycles

Clinical Parameters	S. PEDF Mean±SD	F. PEDF Mean±SD	S. VEGF Mean±SD	F. VEGF Mean±SD	S.PEDF/S.VEGF Index Mean± SD	F.PEDF/F.VEGF Index Mean ± SD	P -Value*
PN	3.53±1.25	3.58±1.27	1104.25±191.32	1081.68±215.64	.0031±.00069	.0033±.00075	0.021
G1E	3.48±1.26	3.48±1.22	1098.43±194.69	1062.51±203.80	.0031±.00070	.0032±.00076	0.03
G2E	3.80±1.15	3.8±1.15	1146.53±174.38	1123.28±199.65	.0033±.00058	.0032±.00078	NS
G3E	3.79±1.28	3.61±1.37	1134.94±196.4	1104.8±232.98	.0033±.00063	.0032±.00079	NS

*ANOVA Test (Two Tailed) Was Used, SD, Standard Deviation, S.; Serum, F.; Follicular Fluid, PEDF; Pigment Epithelial Derived Factor, VEGF; Vascular Endothelial Growth Factor, *: p Value < 0.05 (Significant).

3.6 Comparison of Serum and Follicular Fluid PEDF and VEGF Levels at Oocyte Pickup Between Pregnant and Non-Pregnant

All p -values are > 0.05, so none of the comparisons reach conventional statistical significance. Both serum and follicular fluid PEDF/VEGF indexes were higher in pregnant group, there is no strong evidence here that any of these biomarkers differ between pregnant vs non-pregnant ICSI outcomes as shown in table 5.

Table 5: Comparison of the VEGF and PEDF Levels Between Pregnant and Non-Pregnant

Variable At Pickup Day	ICSI cycle outcome PT		P – Value *
	Pregnant Mean ± SD	Non-pregnant Mean ± SD	
Serum PEDF (pg/ml)	3.67396±1.339744	3.3260±1.090995	0.179 NS
Serum VEGF (pg/ml)	1130.58685±184.240833	1066.51595±197.426528	0.124 NS
FF PEDF (pg/ml)	3.66028±1.236200	3.47046±1.329074	0.340 NS
FF VEGF (pg/ml)	1098.33787±209.924494	1057.82651±224.311492	0.43 NS
S PEDF/VEGF Index	0.0032± (0.00075)	0.0031± (0.00061)	0.56 NS
FF PEDF/VEGF Index	0.0033± (0.00076)	0.0032± (0.00073)	0.65 NS

* Student T-Test (Two Tailed) Was Used, SD :Standard Deviation, PEDF; Pigment Epithelial Derived Factor, VEGF; Vascular Endothelial Growth Factor; *: p Value < 0.05 (Significant).

3.7 Frequency Distribution of Positive vs. Negative Pregnancy Test

Table 6. Showed Pregnancy Test Outcomes for 90 women who completed an ICSI Cycle. Out of the Whole Study Sample, 53 women (58.9%) Achieved a Positive Pregnancy Test while 37 Women (41.1%) had a Negative Test

Table 6: Distribution of Pregnancy Test Outcomes Among Women Undergoing ICSI

Pregnancy Test	Frequency	Percentage
Positive	53	58.9%
Negative	37	41.1%
Total	90	100%

4. Discussion

4.1 Overview

Since the advent of assisted reproductive technology (ART), its procedures have been constantly enhanced and updated. Technological advancements have been made to ensure efficiency in its operations, higher success rates for pregnancies, and patient safety (Thoma et al., 2021).

In this study, a total of ninety women participated by enrolling in this study and underwent an ICSI cycle. The study

was carried out at the High Institute for infertility diagnosis and assisted with reproductive technologies/ AL-Nahrain University.

4.2 Comparison of Serum and Follicular Levels of Pigment Epithelium-Derived Factor (PEDF), Vascular Endothelial Growth Factor (VEGF), and Their Indices by Age and Body Mass Index (BMI)

In the current study, the correlation between two major angiogenic factors, Vascular Endothelial Growth Factor (VEGF), which is involved in the angiogenic process of vessel formation, and Pigment Epithelium-Derived Factor (PEDF), which is an angiogenesis inhibitor, and the variables age and Body Mass Index (BMI) were analyzed, age did not seem to have any significant effect on serum and follicular PEDF and VEGF concentrations, and the PEDF/VEGF ratio showed relatively constant value across different age groups. These results agree with the study of [Chemerinski et al. \(2024\)](#), where the authors showed that angiogenic factors in the follicular fluid are more influenced by the presence of ovarian pathology rather than chronological age. Similarly, [Thoma et al. \(2021\)](#) demonstrated that there were no significant age-related changes in VEGF concentration in the follicular fluid during IVF cycles. Conversely, the current study showed that BMI had more evident patterns. Indeed, obese women showed higher serum and follicular PEDF and VEGF concentrations than the normal ones. This result is not unexpected and biologically plausible, as obesity is a pro-inflammatory state associated with increased adipose-derived PEDF and hypoxia-induced VEGF expression. contribute to the observed angiogenic imbalance. This is consistent with the study by [Yilmaz et al. \(2016\)](#), where the authors demonstrated that the serum VEGF concentration is increased in obese PCOS women undergoing IVF, and this is due to the presence of chronic low-grade inflammation and oxidative stress. Yet, at least some studies contradict this trend, [Chen et al. \(2022\)](#) demonstrated that follicular VEGF does not always rise consistently with BMI, indicating that the responses of ovarian microenvironments may differ due to metabolic or endocrine factors.

Taken together, the study findings of higher PEDF and VEGF in obese women with stable PEDF/VEGF ratios suggest that although angiogenic activity increases with adiposity, a compensatory rise in PEDF may preserve the balance between pro- and anti-angiogenic signals within the follicular environment. This preserved ratio may help explain why some obese women still achieve reasonable ovarian response despite systemic metabolic stress ([Lim et al., 2021](#)).

4.3 Distribution of Serum and Follicular Fluid Levels of Pigment Epithelium-Derived Factor (PEDF), Vascular Endothelial Growth Factor (VEGF), and Their Indices by Infertility Characteristics

Balanced actions of VEGF and PEDF within the female reproductive system of great importance to folliculogenesis, ovulation, corpus luteum CL formation and endometrial receptivity. The two parameters showed different patterns between the studied subgroups and in some cases had similar behavior. This indicates the possible dysregulation of local and systemic angiogenic pull that could be related to the state of infertility. It is interesting that the results revealed a lower PEDF/VEGF ratio in patients with primary infertility, as other studies have observed higher VEGF levels within follicles in patients with primary compared with those with secondary infertility ([Hafner et al., 2018](#)), implying a potential imbalance in angiogenic factors. In contrast, studies in ART cohorts have found no significant differences in VEGF between fertile and infertile women, indicating VEGF alone may be insufficient as a diagnostic marker without considering its regulatory context ([Didžiokaitė et al., 2025](#)).

Concerning the underlying causes of infertility, it was found that the PEDF and VEGF means (in female infertility) were significantly higher compared to male, combined, or unexplained infertility, suggesting that intra-ovarian angiogenic balance may be more perturbed in female pathology ([Miller et al., 2020](#)). The unexplained infertility subgroup potentially exhibited higher VEGF and PEDF values, indicating an undetected perturbation in angiogenic milieu that is not captured by conventional diagnostic categories ([Miller et al., 2015](#)).

Most of the recent research is focused more on whether the VEGF and PEDF levels predict the IVF outcomes, such as pregnancy rates, rather than simply categorizing them based on infertility duration or type. As recently reviewed by [Veeramani et al. in 2024](#), although follicular fluid VEGF is generally higher in cycles leading to pregnancy, the predictive power of circulating factors may be weak or inconsistent depending on the specific cause of infertility ([Veeramani et al., 2024](#)). Lastly, the absence of significant differences by duration of infertility would suggest more intrinsic angiogenic dysregulation related to the cause of infertility itself, rather than to its duration, which is further supported by studies showing stability in VEGF expression across different age groups and infertility durations in clinical IVF cohorts.

4.4 Serum and Follicular Fluid Levels of PEDF, VEGF, and Their Ratios According to Oocyte Maturity and Quality in the Study Population

In the present study, serum and follicular fluid levels of PEDF, VEGF, and the PEDF/VEGF index did not show

statistically significant differences between abnormal, GV, MI, and MII oocytes (all NS). This implies that the balance of angiogenic factors alone cannot characterize oocytes of different maturation periods. Former investigations revealed conflicting findings concerning the predicting role of VEGF for IVF success, some of which reported that high follicular VEGF was associated with high vascularity or fertilization rates, while others reported high VEGF for non-pregnant women.

Previous studies that explored the relationship between the levels of VEGF in the follicular fluid and IVF outcomes. It was shown that VEGF levels to be significantly correlated with grade of perfollicular vascularity. Oocytes obtained from follicles with the higher grade of vascularization also showed a higher rate of fertilization, embryos, better quality and higher pregnancy rates. Moreover, VEGF may play a potential role in the development of the perfollicular capillary network (Nishigaki et al., 2022). Other clinical studies also showed that The concentrations of VEGF were correlated with oocyte and embryo quality but had no significant relationship (Hafner et al., 2018). Kudsy et al. (2016) found that serum VEGF levels were not significantly different between pregnant and non-pregnant group in both PCOS and control patients. Additionally, there were observations that Elevated VEGF concentrations in FF and serum were associated with poor conception rates (Asimakopoulos et al., 2015; Hatem Helmy Hassan El-Husseiny et al., 2022).

Recently published assessments of biomarkers in follicular fluid confirm that VEGF and growth factors have variable associations with reproductive outcomes, in accordance with patient characteristics, stimulation regimens and analytical methods (Albeitawi et al., 2025). In contrast, the role of PEDF seems more closely associated with oxidative stress and the health of granulosa cells, with potential preclinical benefits for oocyte competence.

Consequently, while the PEDF/VEGF index does seem to quantify the angiogenic profile, lack of substantial variation across oocyte maturity categories in this study population indicates it may not be applicable as an independent marker for oocyte quality however could still contribute to multifactorial within the context of a larger model.

4.5 Serum and Follicular Fluid PEDF, VEGF, and PEDF/VEGF Index Across Embryo Quality Parameters in ICSI Cycles

The current study has proven that the best-quality embryos (PN and G1E) display a significantly different profile of angiogenesis, especially in terms of the PEDF/VEGF ratio. This is because PEDF is recognized as a natural anti-angiogenic glycoprotein, and VEGF is identified as a strong pro-angiogenic agent. Their balance influences follicular vascularity, oxygenation, and oocyte maturation.

There's some evidence that very early stages of embryo development (pronuclear stage) may be linked to differences of PEDF/VEGF levels compared to the highest quality embryos (G1E). However, for those embryos who have progressed to the next stages (G2E and G3E), the variation in the levels of the biomarkers cannot be determined, suggesting that PEDF/VEGF levels may not be strong markers of embryo quality beyond early developmental steps.

Vascular Endothelial Growth Factor plays a role in angiogenesis and follicular vascularization, and its levels in follicular fluid have been demonstrated to be associated with vascularity, while increased perfollicular blood flow was associated with good fertilization and quality of embryos in IVF candidates. It appears that a well-vascularized region, and consequently a properly regulated angiogenic level, can contribute towards a more mature oocyte and a good-quality embryo (Guo et al., 2021). In the case of women undergoing IVF-ET, the VEGF level in the FF fluid showed negative correlation with the response of ovaries, and inconsistent correlation with the quality of embryos and the outcome of pregnancies (Gao et al., 2012).

Vascular Endothelial Growth Factor (VEGF) is involved in angiogenesis and implantation; however, the process is complicated and the mechanisms and variability across patients limit its standalone clinical utility in the prediction of IVF outcomes (Guo et al., 2021). Another study found that higher follicular fluid VEGF levels were associated with reduced pregnancy rates following IVF/GIFT, possibly reflecting that excessive angiogenesis could be harmful or indicative of an adverse ovarian environment. In another IVF research, no strong correlation was observed between VEGF levels in the follicular fluid and embryo quality or pregnancy results (Hafner et al., 2018). A recent systematic review including VEGF and other biomarkers shows heterogeneous results across studies and highlights the need for further research before VEGF can be adopted as a reliable clinical marker (Albeitawi et al., 2025). clinical trial found altered PEDF and VEGF levels in follicular fluid with certain stimulation protocols, showing that PEDF can be dynamically regulated and may influence angiogenic balance. This supports the idea that PEDF/VEGF balance could influence the follicular microenvironment (Haas et al., 2018).

The regulation of angiogenesis is not an easy process, though the factors that can influence the quality of the embryo, including genetic factors, hormones, and culture conditions, are involved. Embryo grading systems capture morphology but not all developmental potential, thus, biochemical markers such as PEDF and VEGF might demonstrate correlations only in early steps (e.g., PN vs G1E) and not necessarily across the full range of grading scores.

Certain IVF studies indicated that VEGF concentrations within follicular fluid are associated with vascularization and

potentially with the development of embryos or quality (Lee et al., 2012). However, these associations are variable and not strong or consistent enough across all populations to be used as standalone markers of embryo quality. In certain studies, VEGF is not a predictor of pregnancy, and blood flow indices may outperform VEGF alone as a prognostic indicator. Recent reviews also emphasize the heterogeneity of findings and the need for further validation before clinical adoption.

4.6 Comparison of Serum and Follicular Fluid PEDF and VEGF Levels at Oocyte Pickup Between Pregnant and Non-Pregnant

In the current study, serum and follicular fluid PEDF and VEGF concentrations and the PEDF/VEGF indices were not significantly different between women who became pregnant after ICSI and those who did not (all $p > 0.05$) for all of these parameters. These findings indicate that, in the studied population, these specific angiogenic and anti-angiogenic factors measured at the day of pickup were not useful tools for predicting clinical pregnancy. Our results agree with those studies that have showing inconsistent or absent associations between single angiogenic markers in follicular fluid/serum and final pregnancy after ART. Some studies have associated an increase in FF-VEGF with undesirable outcomes, indicative of a non-desirable, hyper-permeability, or inflammatory follicular environment, while others have associated the parameter only with ovary response and embryos quality rather than pregnancy outcomes. Regarding PEDF, there are fewer studies that indicate it plays a complex role in follicular angiogenesis and oxidative homeostasis, with PEDF alone has not been shown consistently as a robust predictor of pregnancy. Some older works noted that higher levels of VEGF in the follicular fluid correlated with worse outcomes (i.e. elevated FF-VEGF in non-pregnant or poor outcome groups), some see excess VEGF as a sign of a “hostile” follicular environment. This thesis has been explored by Guzmán et al. etc., who saw negative correlations between FF-VEGF and pregnancy (Guzmán et al., 2023).

Other reports say serum or follicular VEGF may simply connect to ovarian response, oocyte maturation or embryo quality but are not ever reliable enough to predict final pregnancy, some identify a positive correlation of specific serum angiogenic markers with embryo quality (but not pregnancy) but report a predictive value only in subgroups. Gao et al. (2012) reported that serum EG-VEGF was associated with pregnancy outcome, whereas follicular VEGF was linked with ovarian response and oocyte maturity.

Reproductive aging occurs when ovaries do not work as well and when the egg cells lose quality and produce less. Pigment epithelium-derived factor (PEDF) is an important factor that influences the blood supply to the ovary and the balance of free radicals within the ovary. It is made by granulosa cells and may help to keep the follicles developing normally. In this study, we examined whether PEDF is involved in reproductive aging. Our findings were similar to what others found before-We did not find that PEDF is a good marker of pregnancy. There are some reports of lower PEDF in the follicular fluid (FF) after stimulation with nitric oxide inhibitors (letrozole and similar agents). There are also reports that fertilization rate, embryo quality, and miscarriage are associated with levels of PEDF and/ or the oxygen stress in the extracellular environment of the oocyte in FF. The link of systemic or isolated FF PEDF levels with pregnancy after ART is not yet clear (Haas et al., 2018).

Recent reviews emphasize that FF is complex. Even when looking at only one marker (VEGF or PEDF), study failure as predictors of pregnancy must be used with caution. Many parameters influence the efficacy of an embryo to implant: increased oxygen stress in the Folliculate Milieu (FM), hormones, markers of inflammation, ovarian response. The value of panels of multiple markers, or the inclusion of embryo evaluation parameters in the model increases the predictive capacity (Moreira et al., 2023).

4.7 Frequency Distribution of Positive vs. Negative Pregnancy Outcomes

The pregnancy rate of 58.9% observed in current study aligns with contemporary ICSI success rates reported in recent international ART cohorts. Several studies show that the pregnancy rate generally varies within a range of 45–60%; this variation depending on the number of eggs retrieved, quality of embryos and heterogeneity in patient characteristics, (such as status of ovarian reserve, uterine factor, endocrine or immune conditions that now known to have an influence on outcomes. In general, current literature is still developing a clearer idea of factors that play a role in outcomes in ICSI. J. Chen et al. (2025), found that for patients with very poor ovarian reserve (retrieval of one to two eggs), have significantly lower fertilization and pregnancy rates compared with patients with higher oocyte yield, reinforcing the influence of ovarian reserve on clinical success.

In addition, contemporary studies highlight the impact of non-ovarian factors; for example, Kang and Qiao (2024) retrospective analysis showed that congenital uterine anomalies adversely affect reproductive outcomes following IVF/ICSI embryo transfer. Additionally, autoimmune thyroid disease affects cumulative live birth rates after IVF/ICSI cycles (Huang et al., 2024).

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