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Serum and Follicular Fluid PEDF/VEGF Angiogenic Index as a Predictor of Embryo Quality and Pregnancy Rate in ICSI Cycles

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ABSTRACT

Objective: *To assess the angiogenic index of PEDF/VEGF in both serum and follicular fluid (FF), and to examine its relationship with oocyte maturation, embryo quality, and pregnancy outcomes in women facing infertility who underwent intracytoplasmic sperm injection (ICSI).* ***Method:*** *A prospective cohort study was conducted on ninety (90) women with infertility undergoing ICSI at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology, Baghdad, Iraq, from November 2023–April 2025. Serum and FF samples were collected on the day of oocyte retrieval. PEDF and VEGF levels were measured using ELISA, and the PEDF/VEGF index was computed. Oocyte maturation, fertilization rate, embryo grading, and pregnancy outcomes were evaluated.* ***Results:*** *No statistically significant differences were found in PEDF levels from serum and FF, VEGF levels, or the PEDF/VEGF index ($p > 0.05$). The PEDF/VEGF index did not correlate significantly with oocyte factors, including retrieval, metaphase II, and maturation rate. However, significant correlations were observed with early embryology parameters: pronuclei ($p = 0.021$), Grade 1 embryos ($p = 0.03$), and fertilization rate ($p = 0.04$). Serum and FF PEDF/VEGF values were higher in women who achieved pregnancy than those who did not ($p = 0.0001$).* ***Novelty:*** *The FF PEDF/VEGF index is the first predictive biomarker of ICSI outcomes in the Iraqi population. The index reflects ovarian angiogenic balance and may be a superior predictor of embryo quality and pregnancy rate compared to individual PEDF or VEGF levels. Total kata: ≈195.*

INTRODUCTION

Infertility is an increasing global concern, impacting millions of couples around the world. In Iraq, infertility rates are particularly significant, with an estimated 15% of couples of reproductive age being affected. Various factors contribute to this issue. The primary methods for restoring fertility include intrauterine insemination, in vitro fertilization, and intracytoplasmic sperm injection. Current demographic trends underscore an urgent need to enhance the predictability of treatment outcomes, as the ability to conceive, successfully implant, and carry a pregnancy to term is considered a crucial aspect of oocyte and embryo quality [1]. Folliculogenesis is the process of follicle formation and development that begins during fetal life. This process involves the assembly of primordial follicles, which consist of an oocyte and surrounding granulosa cells, the latter being somatic support cells. It concludes with the formation of fully mature pre-ovulatory follicles [2].

The ovarian follicle plays a crucial role in female reproduction and fertility, with a finite but adequate steady supply of primordial follicles formed during prenatal development. Within these follicles, oocytes progress through various meticulously

controlled stages of folliculogenesis. Based on the principles of follicular biology, there is significant interest in the complex microenvironment of the ovarian follicle and the crucial role that angiogenesis plays in development of follicles, oocyte quality, and the successful implantation of embryos. The process of ovarian folliculogenesis necessitates continuous support from the ovarian interstitial stroma [3]. Granulosa cells in the ovaries are responsible for the production and secretion of PEDF, a process that is regulated by hormonal signals and inversely related to the expression of vascular endothelial growth factor (VEGF). PEDF secretion is inversely related with the presence of Estradiol, luteinizing hormone (LH), and progesterone [4]. PEDF plays a significant role in folliculogenesis by interacting with essential components involved in the growth and development of follicles. It seems to control both angiogenesis and oxidative stress throughout the process of folliculogenesis [2]. The interplay of PEDF and VEGF influences the process of follicular angiogenesis, oocyte maturation, and successful ovulation [5], [6]. PEDF acts as a natural inhibitor of VEGF-mediated vascular permeability and neovascularisation, maintaining controlled angiogenic dynamics in the ovarian microenvironment [7]. Pigment Epithelium-Derived Factor (PEDF) has been shown to possess anti-angiogenic properties. Studies indicate that PEDF plays a vital role in maintaining ovarian angiogenic, inflammatory, and oxidative balance [8].

This potent physiological antiangiogenic activity results from various PEDF functions, including the induction of endothelial cell apoptosis, the inhibition of cell migration, the formation of new vessels, and the response to VEGF (vascular endothelial growth factor), the most potent proangiogenic factor, as well as the response to bFGF (basic fibroblast growth factor) [9], [10]. VEGF isoforms are synthesised in follicular cells and act on receptors in endothelial cells located in the theca layer to regulate follicular angiogenesis. They can also act directly on granulosa and theca cells to promote follicular growth. It is known that in the theca layers, there is higher mRNA and protein expression of both membrane and soluble VEGF receptors [11]. VEGF is directly involved in the regulation mechanisms of oocyte maturation and can serve as a marker of poor ovarian response, as well as a predictor of unsatisfactory outcomes in ovarian stimulation treatments for infertility using ART methods [12].

RESEARCH METHOD

Study Design and Population

This prospective cohort study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Technology, Al-Nahrain University, Baghdad, Iraq, from November 2023 to April 2025, involving 90 infertile couples undergoing intracytoplasmic sperm injection (ICSI). Approval was obtained from the local Ethics Committee, and informed consent was secured from all participants. On the second day of the menstrual cycle, female participants aged 18-44 years with a BMI of 19-30 kg/m² underwent comprehensive assessments including medical, physical, and gynaecological history, clinical examinations, body mass index (BMI) was calculated as weight in kilograms divided by height in meters squared, hormonal analyses (LH, FSH, E2, and

AMH), and transvaginal ultrasounds (TVUS) to evaluate ovarian reserve via antral follicle count (AFC) and helps identify ovarian pathology, adnexal masses, endometrial thickness (ET), and endometrial abnormalities. AFC quantifies small follicles measuring 2-6 mm in diameter in both ovaries Women diagnosed with polycystic ovary syndrome, pelvic inflammatory disease, endometriosis, sexually transmitted infections, autoimmune diseases, or neoplastic conditions were excluded. Male partners were assessed through seminal fluid analysis in accordance with WHO standards.

Ovarian Stimulation Protocol

Participants underwent a standardized GnRH antagonist protocol. Controlled ovarian stimulation was started on Day 2 or 3 of the cycle with different types of gonadotropins; human menopausal gonadotropin (HMG) in the form of in vitro fertilisation-Menotropin (IVF-M), LG Chem Ltd, Korea, 75- 150 IU (75 IU FSH + 75 IU LH), or recombinant FSH (rFSH) as Gonal–F, Merck KGaA Darmstadt, Germany, 75-300 IU. Doses were adjusted based on age and Antral Follicle Count (AFC). Regular assessments of follicular growth via transvaginal ultrasonography, along with serum estradiol (E2) levels, were performed on each patient starting from day 8. When follicular recruitment began and a sufficient number of follicles reached a size of 14 mm, pituitary down-regulation was initiated using a gonadotropin-releasing hormone (GnRH) antagonist; Cetrotide, Merck KGaA Darmstadt, Germany, 0.25 mg once daily subcutaneously (flexible protocol). Final oocyte maturation and ovulation were triggered by administering 250 µg (6500 IU) of recombinant human chorionic gonadotropin (r-hCG); Ovitrelle, Merck Global, Germany, subcutaneously, or 10 IU of hCG when at least three follicles reached a diameter of ≥18 mm. Oocyte retrieval was performed 35-36 hours' post-trigger via transvaginal ultrasound guidance under mild anaesthesia. Luteal phase support was initiated from the evening of the oocyte retrieval day using vaginal progesterone suppositories (Cyclogest 200, L.D. Collins & Co. Ltd., micrograms twice daily) and injectable progesterone (Primolute Depot, Bayer AG, Germany, 250 micrograms every three days), continuing until the day of a positive chemical pregnancy test.

Sample Collection Serum

Venous blood samples (5 mL) were collected on the day of oocyte retrieval. Serum was separated by centrifugation at 3000 rpm for 10 minutes and stored at -80°C. During oocyte pick-up (OPU), follicular fluid (FF) from the leading follicle was aspirated. Only blood-free samples were centrifuged and stored at −80°C.

Assessment of Reproductive Outcomes Oocyte Quality

A 38 hours after hCG, cumulus cells were removed mechanically and enzymatically to denudate cumulus–oocyte complexes (Sage In-Vitro Fertilization, Inc., Cooper Surgical, Trumbull, CT). The presence of the first polar body (MII stage) and morphological grading (cytoplasm homogeneity) were used to assess the maturity of all denuded oocytes. Mature oocytes were categorized as metaphase II (MII; containing one polar body), immature oocytes as metaphase I (MI; lacking a polar body), and germinal vesicle stage (GV) oocytes as immature. Forty hours after hCG had been administered,

all MII oocytes underwent ICSI. The resultant zygotes were examined under an inverted microscope sixteen to eighteen hours after ICSI and categorized as follows: properly fertilized oocytes displayed two pronuclei (PN) and a second polar body; Unfertilized oocytes had no PN, improperly fertilized oocytes displayed one or three PN, and lysed oocytes were also seen. In an embryo culture dish with continuous single culture media (Fujifilm Irvine Scientific Inc., Santa Ana, CA) under mineral oil, normally fertilized oocytes were cultivated at 37°C with 6% CO2, 5% O2, and 89% N2 in a humidified incubator until they reached the blastocyst stage. Gardner's grading method was used to evaluate blastocyst quality; blastocysts rated 4AA, 5AA, or 6AA were deemed to be of the highest caliber. All other grades were categorized as blastocysts of inferior quality. Regardless of quality, cycles with an embryo that successfully reached the blastocyst stage are referred to as successful blastocyst creation. Fresh blastocyst-stage embryo transfer was carried out five days following oocyte retrieval.

Biochemical analysis

VEGF and PEDF concentrations in serum and follicular fluid (FF) was performed using commercial Enzyme-Linked Immunosorbent Assay (ELISA) kits (R&D Systems, USA) according to the manufacturer's instructions. Calculation of the index: The PEDF/VEGF index was determined using the following formula:

Index = PEDF concentration (ng/mL) / VEGF concentration (pg/mL) × 100

Statistical analysis

Data were analyzed using SPSS version 25.0. Continuous variables are presented as Mean ± Standard Deviation (SD). Differences between pregnant and non-pregnant groups were analyzed using the independent Student's t-test or Mann-Whitney U test. A p-value of < 0.05 was considered statistically significant.

Continuous data are presented as mean ± SD (for normal data) or median [IQR] (for non-normal data), while categorical data are expressed as counts and percentages. Group comparisons were carried out using t-tests (for normal continuous data), Mann–Whitney U tests (for non-normal continuous data), chi-square tests, or Fisher's exact test (for categorical data).

RESULTS AND DISCUSSION

Results

In the present study, the demographic characteristics of the patients enrolled in this study are shown in Table 1. Significant differences were observed between women who achieved pregnancy and those who did not regarding age, duration of infertility, and total gonadotropin dose. The pregnant group was notably younger than the non-pregnant group (30.98 ± 5.81 vs. 34.22 ± 6.78 years, $p = 0.015$) and had a shorter duration of infertility (6.21 ± 3.12 vs. 8.22 ± 5.22 years, $p = 0.001$). Furthermore, women who did not conceive required a significantly higher total gonadotropin dose during ovarian stimulation (2784.66 ± 1712.89 vs. 2153.77 ± 292.31 IU, $p = 0.009$). In contrast, no significant differences were found between the two groups regarding BMI, AMH levels, or basal FSH concentrations (all $p > 0.05$).

Table 1. Baseline demographic and clinical characteristics of the study population

Variable	Pregnant (n =53) Mean ± SD	Non-pregnant (n=37) Mean ± SD	*p*- value
Age (years)	30.98±5.81	34.22±6.78	**0.015*S**
BMI (kg/m²)	29.33±4.10	28.64±4.40	0.48 NS
Duration of infertility (years)	6.21±3.12	8.22±5.21	**0.001*S**
AMH (ng/mL)	3.15 ±2.25	2.72 ±2.24	0.868 NS
Basal FSH (IU/L)	6.82 ±3.17	6.50 ±2.24	0.240 NS
Total gonadotropin dose (IU)	2153.77±292.31	2784.66±1712.89	**0.009 *S**

* Independent t-test. was used, SD: standard deviation; BMI: body mass index; AMH = anti-Müllerian hormone; FSH = follicle-stimulating hormone; * p value < 0.05 (significant); NS indicates p ≥ 0.05.

Comparison of serum and follicular fluid concentrations showed no statistically significant differences in PEDF, VEGF, or the PEDF/VEGF ratio, as detailed in Table 2. Serum PEDF levels (3.53 ± 1.25 ng/mL) were almost identical to those in follicular fluid (3.58 ± 1.27 ng/mL), with no notable variation between compartments. Similarly, VEGF concentrations were comparable in serum (1104.25 ± 191.32 pg/mL) and follicular fluid (1081.68 ± 215.64 pg/mL). Additionally, there was little variation in the PEDF/VEGF ratio between serum (0.0031 ± 0.0007) and follicular fluid (0.0032 ± 0.0008). These findings show that the angiogenic and anti-angiogenic factor balances in systemic and intrafollicular settings are similar, indicating little variance within the PEDF–VEGF regulation axis.

Table 2. Serum and follicular fluid PEDF, VEGF and PEDF/VEGF angiogenic index

Variable	Serum Mean ± SD	Follicular fluid Mean ± SD	P value
PEDF (ng/mL)	3.53 ±1.25	3.58 ±1.27	0.79 NS
VEGF (pg/mL)	1104.25±191.32	1081.68 ±215.64	0.46 NS
PEDF/VEGF index	0.0031±0.0007	0.0032±0.0008	0.37 NS

Independent t-test. was used, SD: standard deviation; PEDF= Pigment Epithelium Derived Factor; VEGF= Vascular Endothelial Growth Factor; * NS indicates p ≥ 0.05.

Correlation with Oocyte Quality

No statistically significant correlation was observed between the serum or follicular fluid PEDF/VEGF index and the number of retrieved oocytes, the number of metaphase II (MII) oocytes, or the oocyte maturation rate (p > 0.05 for all comparisons). Although the follicular fluid PEDF/VEGF index showed a marginally higher mean maturation rate

compared to serum values, this difference did not achieve statistical significance, as shown in Table 3.

Table 3. Association between PEDF/VEGF index and oocyte maturation

Variables	Retrieved oocytes (Mean ± SD)	MII oocytes (Mean ± SD)	Maturation rate (%)	P value
Serum PEDF/VEGF	0.0031±0.00069	0.0031±0.00069	77.994±16.6341	0.68 NS
FF PEDF/VEGF	0.0033±0.00075	0.0033±0.00075	78.855±19.8646	0.42 NS

Independent t-test. was used, SD: standard deviation; PEDF= Pigment Epithelium Derived Factor; VEGF= Vascular Endothelial Growth Factor; MII= Metaphase II; * NS indicates $p \geq 0.05$.

Correlations between serum and culture media ROS levels and embryo grading

The present findings demonstrate a statistically significant association between the Serum PEDF/VEGF index and Follicular Fluid PEDF/VEGF index with early embryological parameters, pronuclear formation (PN), Grade 1 embryos (G1E), and fertilization rate. However, no significant relationship was observed with later-stage embryo grades (G2E and G3E), as shown in Table 3.

Table 4. Relationship between PEDF/VEGF index and embryo quality

Embryo parameters	SPEDF/SVEGF Index Mean ± SD	FPEDF/fVEGF Index Mean ± SD	*p*-value
PN	0.0031±0.00069	0.0033±0.00075	0.021*S
G1E	0.0031±0.00070	0.0032±0.00076	0.03*S
G2E	0.0033±0.00058	0.0032±0.00078	N.S
G3E	0.0033±0.00063	0.0032±0.00079	N.S
Fertilization rate (%)	68.4±12.6	71.9±13.4	0.04*S

One-way ANOVA was used, SD: standard deviation; PEDF= Pigment Epithelium Derived Factor; VEGF= Vascular Endothelial Growth Factor; 2 PN; 2 Pronuclei, E; Embry, G; Grad *: p value < 0.05 (significant), * NS indicates $p \geq 0.05$.

The PEDF/VEGF index in serum and follicular fluid was compared between pregnant and non-pregnant women and PT outcomes, as illustrated in Table 5. The results demonstrate a highly significant association between both the serum PEDF/VEGF index and the follicular fluid (FF) PEDF/VEGF index with clinical pregnancy outcome ($p = 0.0001$ for both). Women who achieved pregnancy exhibited slightly higher mean PEDF/VEGF ratios compared with non-pregnant women in both compartments.

Table 5. PEDF/VEGF angiogenic index according to pregnancy outcome

Variables	Pregnant (= 37) Mean ± SD	Non-pregnant (n=53) Mean ± SD	P value
Serum PEDF/VEGF index	0.0032± (0.00075)	0.0031± (0.00061)	0.0001*S
Follicular PEDF/VEGF index	0.0033± (0.00076)	0.0032± (0.00073)	0.0001*S

Discussion

The microenvironment of the pre-ovulatory follicle is a complex milieu where the coordination of angiogenesis influences the fate of the oocyte. This study examined the PEDF/VEGF index, a ratio that reflects the "angiogenic balance," rather than analyzing these factors separately.

These study findings indicate that younger age, shorter duration of infertility, and lower gonadotropin requirements may be associated with higher chances of pregnancy, while ovarian reserve markers (AMH and basal FSH) and BMI did not appear to affect pregnancy outcomes in this cohort.

The current study indicated that the age of pregnant women aligns with many findings showing that female age is a strong, independent predictor of IVF/ICSI outcomes. Recent cohort studies and reviews firmly establish that clinical pregnancy and live birth rates decline consistently with increasing maternal age, especially after 35 years of age [13]. Another important finding was that the BMI difference is expected to be small and non-significant. Results from meta-analyses and studies using cohorts show that high BMI is linked with adverse pregnancy outcomes in women undergoing IVF/ICSI treatment [14]. In other research, it was found that high BMI (hesitant categories of overweight and obese individuals) was significantly correlated with reduced rates of clinical pregnancy and live births and increased rates of miscarriages following IVF/ICSI, thus useful in IVF patients having abnormal BMI in predicting pregnancy outcome (CLBR), when consulted concerning weight before undergoing IVF treatment [15].

The dose of gonadotropins was higher in the non-pregnant group, and this is almost significant at $p = 0.009$. Several clinical observations and studies demonstrated a negative association between increased doses of gonadotropins and reduced rates of clinical pregnancy and live births, interpreted as either (1) it's a marker of poor ovarian reserve/harder-to-stimulate patients who require a higher dose, or (2) it could represent a potential iatrogenic effect of overdosing on oocyte/embryo competence. Nevertheless, recent studies have demonstrated little to no independent association between dose and pregnancy outcomes, whereas other studies have reported a negative correlation. Xu *et al*., indicated that it is worth considering that the association of LBR with FSH dose was different for patients of different ages [16], Baker *et al.* demonstrated a statistically significant reduction in live birth rates with increased doses of FSH [17], which remained

important for patients with a favourable prognosis, and for women of all ages, except those aged >35years, with 1-5 oocytes resected.

In this study, serum and follicular fluid levels of PEDF, VEGF, and the PEDF/VEGF index were similar among GV, MI, and MII oocytes, suggesting that the balance of these angiogenic factors does not affect nuclear maturation or its stages. Although some studies associate high follicular VEGF levels with better vascularity and fertilization rates, contradictions remain. VEGF correlates to perifollicular vascularity, where increased blood flow enhances fertilization, embryo quality, and pregnancy rates, with potential predictions of oocyte viability. Additionally, VEGF is essential for ensuring proper blood supply and oxygen levels within follicles, particularly influencing oocyte quality in women under 35. Previous research has established a link between VEGF levels and IVF success, affirming its importance in developing vascular networks [18].

Other clinical studies also showed that the concentrations of VEGF were correlated with oocyte and embryo quality, but had no significant relationship [19]. *Kudsy et al.,* found that serum VEGF levels were not significantly different between pregnant and non-pregnant groups in both PCOS and control patients [20]. Additionally, there were observations that Elevated VEGF concentrations in FF and serum were associated with poor conception rates [21], [22].

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 [23]. 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 [4].

While the PEDF/VEGF index quantifies angiogenic profiles, its lack of variation across oocyte maturity categories suggests it may not be a reliable standalone marker for oocyte quality. However, the study indicates that the best-quality embryos show distinct angiogenic profiles with a favorable PEDF/VEGF ratio, which correlates with improved embryo quality. PEDF, an anti-angiogenic glycoprotein, and VEGF, a pro-angiogenic agent, together influence follicular vascularity and oocyte maturation. Early developmental stages may reflect differences in PEDF/VEGF levels, but for later stages, these biomarkers do not strongly indicate embryo quality. Overall, a well-vascularized environment seems essential for developing mature oocytes and high-quality embryos [18].

In the case of women undergoing IVF-ET, the VEGF level in the FF fluid showed a negative correlation with the response of the ovaries, and an inconsistent correlation with the quality of embryos and the outcome of pregnancies [24].

Angiogenesis and implantation processes rely on the Vascular Endothelial Growth Factor (VEGF) but due to the complicated process, mechanisms, and individual patient variability, the clinical utility of predicting IVF outcomes is limited [25]. In another study, the higher the VEGF in the follicular fluid, the lower the pregnancy rates post IVF/GIFT, suggesting that too much angiogenesis is detrimental, or the ovarian environment is unfavorable. In another IVF study, the VEGF content in the follicular fluid did not

correlate to embryo quality or the pregnancy outcomes [19]. In the most recent systematic review, including VEGF along with other biomarkers showed dispersed results and suggested the necessity of additional research to investigate the significance of VEGF as a clinical marker [23]. In a clinical study, some stimulation protocols altered the levels of PEDF and VEGF in the follicular fluid, suggesting that PEDF is dynamically modulated and may be a regulatory factor in angiogenesis, supporting that the PEDF/VEGF ratio may be important in the follicular microenvironment [26].

Embryonic angiogenesis regulation is a complicated process relies on genetic factors, hormonal levels and environmental culture conditions. Assessment of embryonic development through grading systems which evaluate morphological characteristics, shows limited accuracy because it looks at its shape and structure. To get a better understanding, we need to look at certain biochemical signs, like PEDF and VEGF, which are only useful in the early stages of development rather than throughout grading scores [27]. The connection between VEGF levels in follicular fluid are not always a reliable indicator of the embryo's quality, because different research studies reach different conclusions about their association with various groups. The reliable prediction of successful pregnancy outcome through blood flow measurement shows greater accuracy than using VEGF level assessment. The latest reviews require additional research to confirm these results before they can be used in medical practice.

The studied population showed no clinical pregnancy prediction value from angiogenic and anti-angiogenic factor measurements which were taken at the time of pickup. The results confirm previous research which found that these markers show inconsistent links to pregnancy success when measured in follicular fluid or serum during assisted reproductive technology (ART) procedures. Higher FF-VEGF levels has been connected to bad results which indicate harmful conditions in the follicular environment, yet other research studies maintain that FF-VEGF primarily affects ovarian response and embryo quality instead of pregnancy success. The function of PEDF in this situation stays unclear because researchers have not yet established it as a reliable pregnancy indicator despite discovering its role in follicular blood vessel growth and maintenance of oxidative stress balance. Previous studies demonstrated that elevated FF-VEGF levels lead to worse results because excessive VEGF production creates an extremely bad condition for the follicles. This thesis has been explored by Friedman et al.

The negative outcomes of higher FF-VEGF levels occur because these levels produce detrimental effects on follicles, while other studies show that FF-VEGF primarily influences ovarian response and embryo development rather than determining pregnancy outcomes.

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*., found that serum EG-VEGF correlates with pregnancy outcomes, while follicular VEGF relates to ovarian response and oocyte maturity [24]. Reproductive

aging diminishes ovarian function and egg quality. Pigment epithelium-derived factor (PEDF), produced by granulosa cells, affects ovarian blood supply and free radical balance. This study investigated PEDF's role in reproductive aging, concluding that it is a good reliable pregnancy marker. Some studies reported lower PEDF in follicular fluid after nitric oxide inhibitor treatments, linking PEDF levels and embryo outcomes to oxidative stress in the oocyte environment, yet the connection between PEDF levels and pregnancy post-ART remains unclear [26], [28].

Recent reviews emphesis that FF is complex. Even when looking at only one marker (VEGF or PEDF), study failure as a predictor 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, and ovarian response. The value of panels of multiple markers, or the inclusion of embryo evaluation parameters in the model increases the predictive capacity [29].

CONCLUSION

Fundamental Finding : The PEDF/VEGF index measured in both serum and follicular fluid emerges as a promising biomarker for predicting embryo quality and pregnancy outcomes in IVF, highlighting the value of evaluating angiogenic balance in reproductive medicine for personalized treatment planning in Iraqi infertile women. **Implication :** Integrating the assessment of angiogenic factors into clinical practice could enhance individualized IVF strategies and optimize embryo selection, potentially improving patient-specific reproductive outcomes. **Limitation :** The study's generalizability is restricted due to its single-center setting and modest sample size, the exclusive collection of follicular fluid from dominant follicles, the cross-sectional design that precludes causal inference, the lack of live birth and long-term embryo competence data, potential ELISA assay variability, and the exclusion of certain patient cohorts such as those with severe metabolic diseases. **Future Research :** Further studies should expand to multicenter designs with larger, more diverse populations, include longitudinal follow-up for live birth and embryo competence, examine smaller follicles, and validate assay reliability to strengthen clinical applicability of angiogenic biomarkers in IVF.

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