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Growth Differentiation Factor-8 (GDF-8) as a predictor of clinical pregnancy in GnRH-agonist ICSI cycles: a prospective cohort study

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ABSTRACT

Objective. The objective of this study was to assess the reliability of serum growth differentiation factor-8 (GDF-8) as a predictor of clinical pregnancy after IVF-ET and to investigate the relationship between serum GDF-8 and progesterone (P4) for more understanding of the function of GDF-8 in controlling serum P4 during controlled ovarian stimulation (COS).

Materials and Methods. We conducted a prospective cohort study, recruiting 42 women from a single fertility centre in Alexandria, Egypt, who underwent intracytoplasmic sperm injection (ICSI) from May 2023 to January 2024. The conventional long agonist protocol was used for COS, and the serum levels of P4 and GDF-8 were measured at different time points. Serum human GDF-8 ELISA kits were used in accordance with the manufacturer's protocol.

Results. GDF-8 and P4 serum levels on the day of trigger administration and 2 weeks after embryo transfer (ET) showed a highly statistically significant negative correlation ($p < 0.001$), and there was a highly significant positive correlation between the decline in GDF-8 and the rise in P4 serum levels (from the day of hCG to the day of oocyte retrieval). A GDF-8 level ≥ 3.9 ng/ml on the trigger day has a sensitivity of 94.3% in predicting pregnancy following ICSI-ET.

Conclusions. During controlled ovarian stimulation, GDF-8 level exhibits a dynamic pattern in conjunction with serum progesterone level. High serum levels of GDF-8 (≥ 3.9 ng/ml), followed by a decline in this level (by ≥ 1.35 ng/ml), might be a reliable predictor of pregnancy after ICSI-ET.

INTRODUCTION

During controlled ovarian stimulation (COS), variations in the blood levels of progesterone (P4) and estradiol (E2) are frequently seen. These variations

may have an impact on the chance of conception in individuals receiving assisted reproductive technology (ART) therapy [1, 2].

In consequence of the phenomenon known as "premature progesterone rise" (PPR) or late follicular

P4 rise, early endometrial exposure to P4 during ART has been proposed as the reason for lower implantation rates because of embryo-endometrial desynchronization [3]. This PPR has an adverse effect on the molecular structure of endometrial genes and protein expression, which affects the window of implantation (WOI), which is a critical and brief time [4, 5]. However, the effect of PPR was found to be not harmful as historically cited; instead, it was found that early P4 rise > 1.5 ng/ml was associated with faster early embryological development [6].

Many factors have been investigated as possible predictors of the successful outcome of ART treatment in terms of conception rates. These variables include the woman's age, ovarian reserve indicators, the number of retrieved oocytes, endometrial receptivity, and various hormone levels [7, 8]. Moreover, a number of autocrine and paracrine pathways, through a range of growth factors, have one or more regulatory mechanisms influencing the ovarian response to stimulation, implantation, and the success of an IVF cycle [9, 10].

The growth factor family known as transforming growth factor- β (TGF- β) has attracted significant interest because of its extensive expression in the endometrium, ovarian follicles, decidua, and the maternal-fetal interface. Consequently, it has a major impact on a variety of components of the female reproductive process [9, 11-13].

Furthermore, compared to non-pregnant women, pregnant women's follicular fluid has much higher expression of TGF- β 1 [14, 15].

A crucial component of the TGF- β family, GDF-8 is sometimes referred to as "myostatin". Notably, in the human reproductive system, GDF-8 is expressed in trophoblasts, follicular fluid, and granulosa cells. Moreover, new biological functions of GDF-8 in steroidogenesis regulation and human cumulus growth have been identified [16-19].

In ovarian granulosa cells, GDF-8 was supposed to boost E2 synthesis and reduce P4 production by downregulating the expression and activity of steroidogenic acute regulatory protein (StAR) and upregulating aromatase activity in human granulosa cells [16, 20].

This study aimed to assess the reliability of serum GDF-8 as a predictor of clinical pregnancy after IVF-ET and to investigate the relationship between serum GDF-8 and P4 for more understanding of the function of GDF-8 in controlling serum P4 during COS.

PATIENTS AND METHODS

Study design and settings

This study is a prospective, non-controlled cohort study that was conducted from May 2023 to January 2024 on 42 women experiencing their first ICSI-ET cycle. The ladies were recruited from a single fertility clinic in Alexandria, Egypt.

Using one correlation power analysis, which achieves 80% power with a goal significance level of 5% and a precision level of 8%, the sample size was determined using the NCSS 2004 and PASS 2000 computer programs. The Ethics Committee of the Faculty of Medicine, Alexandria, Egypt, granted approval for the study protocol in February 2023 (IRB No. 00012098, approval number 0107574).

Study participants

Women aged 20-35 years, with a body mass index (BMI) ranging from 19-29.9 kg/m², regular menstrual cycles, and undergoing their first-time ICSI-ET cycles owing to male and/or tubal causes or unexplained infertility were included in the study population.

Women diagnosed with endometriosis, couples with azoospermic males, women with inadequate ovarian reserve [anti-mullerian hormone (AMH) < 1.2 ng/dl and/or antral follicle count (AFC) < 5], and women with medical comorbidities such as thyroid problems, diabetes, and hyperprolactinemia have been excluded in our sample. Written informed consent was obtained from each participant prior to their involvement in the study.

Ovarian stimulation, ovum pickup, and embryo transfer protocol

In the mid-luteal phase, patients started treatment according to the traditional long agonist protocol. A subcutaneous injection of 0.1 mg (one prefilled syringe) of the GnRH-agonist triptorelin was given daily, commencing from day 21 of the cycle. When pituitary desensitization (menstruation commenced, serum E2 < 50 pg/dl, and endometrial thickness < 5 mm) was reached [21], daily doses of 150-300 IU of recombinant follicle-stimulating hormone (FSH) and 75-150 IU of human menopausal gonadotropins (hMGs) were given, and the dose of triptorelin was reduced to half a syringe (0.05 mg). Follow-up of ovarian stimulation was carried out by serial ultrasonographic scans and serum E2 levels every 2-3 days. A bo-

lus of 10,000 IU human chorionic gonadotrophin (hCG) is used as the final oocyte maturation trigger when at least three follicles reach 18 mm [22]. Thirty-six hours following the trigger injection, ovum pick-up (OPU) was performed using transvaginal ultrasonography, and mature (metaphase II) oocytes were fertilized using ICSI. To support the luteal phase, 400 mg of vaginal progesterone twice a day and 50 mg of intramuscular progesterone in oil once daily were administered starting from the evening of the OPU day.

Five days after OPU, one- or two-day 5 embryo(s) were transferred according to the woman's age and embryo availability [23].

A biochemical pregnancy was identified 14 days after embryo transfer (ET) based on the level of β -hCG in the blood. The identification of a gestational sac at 28-35 days after ET was considered clinical pregnancy [24].

Measurement of serum hormones (E2 and P4) levels and GDF-8

Venipuncture was used to take blood samples three times during and after ovarian stimulation. On the day of trigger injection, on the day of OPU, and 14 days after embryo transfer.

On the day of trigger injection, serum estradiol levels were determined. At each of the following points, the levels of GDF-8 and serum progesterone were analysed. First, on the day of trigger administration; second, on the day of oocyte retrieval; and lastly, 14 days after embryo transfer. On the appointed day, samples were collected at about 8:00 a.m. The serum was stored at -80°C until analysis upon collection. GDF-8 ELISA kits were utilized in compliance with the manufacturer's instructions [8].

Statistical analysis of the data

The Statistical Package for Social Sciences, version 23.0 (SPSS Inc., Chicago, Illinois, USA), was used to analyse the data collected. When the distributions of the quantitative data were parametric (normal), they are displayed as means \pm standard deviations and ranges; nonparametric (nonnormally distributed) variables are reported as medians with interquartile ranges (IQRs). Qualitative variables are further represented as percentages and figures. The Shapiro-Wilk and Kolmogorov-Smirnov tests were used to examine the data for normality. The subsequent statistical analyses were employed:

- The independent-samples t test of significance was used when comparing two means.
- Chi-square test: for categorical variables, to compare between different groups.
- Fisher's Exact or Monte Carlo correction: correction for chi-square when more than 20% of the cells have an expected count less than 5.
- Repeated-measures analysis of variance (ANOVA) was used to compare multiple within-group measures.
- *Post-hoc* test: Tukey's test was used for multiple comparisons between different variables.
- Pearson's correlation coefficient (r) test was used to assess the degree of association between two sets of variables.
- A Logistic regression model was constructed within the statistically significant variables.

RESULTS

We conducted our study with 42 eligible women to further understand the roles of GDF-8 in regulating progesterone levels during COS in women undergoing IVF/ICSI-ET and evaluate its influence as a predictor of the pregnancy rate.

Descriptive analysis

Table 1 displays the study cohort's baseline characteristics, specifics, and outcomes of controlled ovarian stimulation. 32 (76.2%) and 10 women (23.8%), respectively, had P4 levels < 1.5 ng/dl and P4 levels > 1.5 ng/dl on the day of hCG injection among the 42 participants. In our cases, there were no instances of OHSS.

Regarding the pregnancy rates in our cohort, 8 patients (19%) failed to achieve clinical pregnancy, whereas 34 women (81%) in the study group were clinically pregnant.

Serum progesterone levels

The mean P4 serum level (ng/ml) was 0.79 ± 0.55 on the day of trigger injection, 10.99 ± 3.81 on the OPU day, and 23.47 ± 7.88 14 days following embryo transfer. RM-ANOVA test was used to compare the serum P4 levels between the study groups at each of the three points. **Table 2** demonstrates that there was a highly significant ($p < 0.001$) difference in serum P4 levels between time points; the mean P4 serum level was lowest at the time of trigger administration, increased on OPU day, and then increased again 14 days after embryo transfer.

Table 1. Baseline characteristics, details, and outcomes of ovarian stimulation in the study cohort.

Baseline characteristics	
Mean Age ± SD in years	30.11 ± 4.44
Mean Weight ± SD in kg	77.57 ± 7.56
Gravidity	
Mean ± SD	0.60 ± 1.55
Median (IQR)	0 (0-1)
Parity	
Mean ± SD	0.26 ± 0.45
Median (IQR)	0 (0-1)
Previous abortion	
Mean ± SD	0.33 ± 1.41
Median (IQR)	0 (0-1)
Total gonadotropin dose (IU)	
Range	2475-4500
Mean ± SD	3763.10 ± 503.68
Days of stimulation	
Range	10-14
Mean ± SD	11.93 ± 1.18
Estradiol level on day of hCG (pg/dl)	
Range	1240-5187
Mean ± SD	3884.69 ± 797.14
Number of oocytes	
Range	8-47
Mean ± SD	25.10 ± 7.39
Number of metaphase-II(MII) oocytes	
Range	2-40
Mean ± SD	16.38 ± 7.94
Number of embryos on day 5	
Range	1-20
Mean ± SD	9.79 ± 5.47

1,533 women with diagnosed uterine fibroids across 8 countries were asked about the impact of their symptoms on their daily life in the last 12 months. Those women who reported a mild to severe impact of symptoms were additionally asked which activities were negatively affected by their symptoms (Zimmermann *et al.*, 2012 [5]).

Serum GDF-8 levels

The mean serum GDF-8 level (ng/ml) at the trigger “hCG day” was 2.76±0.86; it was 1.15±0.15 on the OPU day, and it was 2.44±0.70 at time point 3, which was 14 days following embryo transfer. RM-ANOVA test was used to compare the serum GDF-8 levels in our group at each of the three points. **Table 3** demonstrates that there is a statistically significant difference in the GDF-8 serum level (ng/ml) across the time points. The highest mean value of the serum GDF-8 level was recorded at the time of the hCG trigger, which was followed by a decrease on the OPU day and an increase 14 days after embryo transfer.

Table 2. Comparisons of serum P levels among the study patients at the three time points.

Serum P4 level (ng/ml)	Range	Mean ± SD
At time of trigger “hCG day”	0.24-1.96	0.79 ± 0.55
Oocyte pick up day (OPU day)	6.82-21.92	10.99 ± 3.81
14 days after embryo transfer	13.83-37.34	23.47 ± 7.88
RM-ANOVA		57.386
P-value		0.001 (HS)

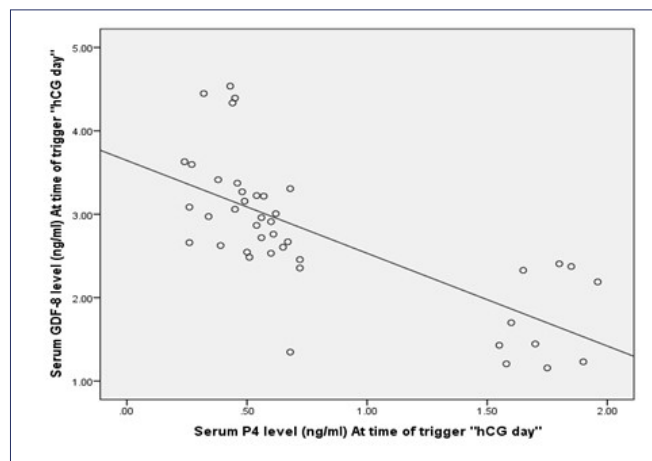


Figure 1. Scatter plot showing a negative correlation between GDF-8 and P4 serum levels on “hCG day”.

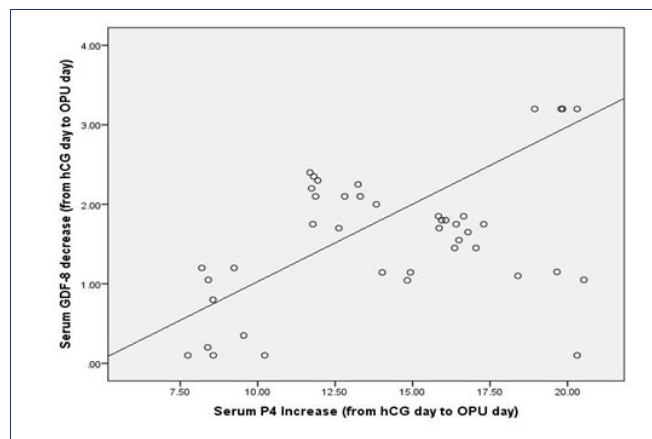


Figure 2. There was a strong positive correlation between the decrease in serum GDF-8 and the increase in serum P4 from the day of hCG to the day of OPU.

Correlation between GDF-8 and progesterone serum levels

GDF-8 and P4 serum levels (ng/ml) at the time of hCG trigger administration showed a highly statistically significant negative correlation, as shown in **Figure 1** (r = -0.713 & p < 0.001).

A highly statistically significant positive association (r = 0.648 with p < 0.001) was seen on the day of ovum pickup between the rise in serum P4 level (ng/ml) from the day of hCG to OPU day and the decline in serum GDF-8 level (**Figure 2**).

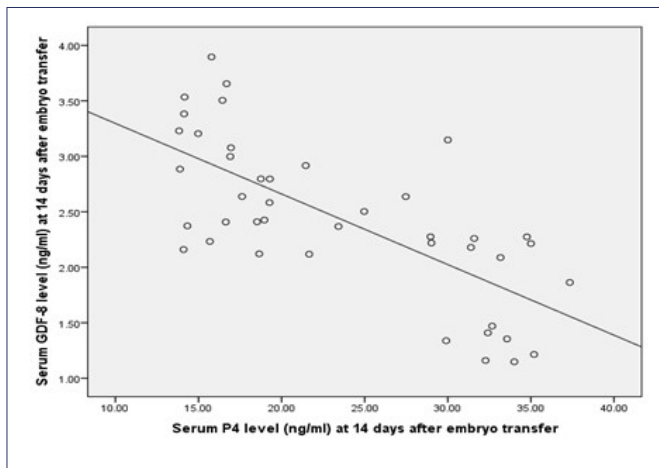


Figure 3. Scatter plot of GDF-8 and P4 serum levels (ng/ml) at 14 days after embryo transfer

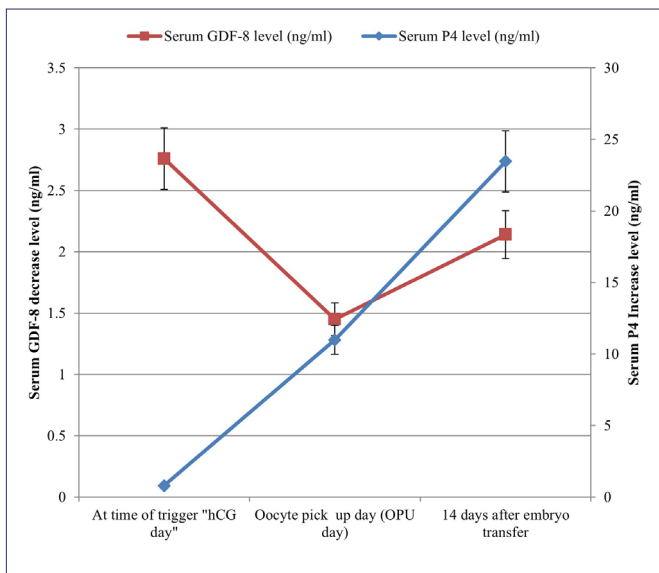


Figure 4. The decrease in GDF-8 levels was accompanied by an increase in P4 levels in the serum at the indicated time points. The expression data are presented as the means ± SDs.

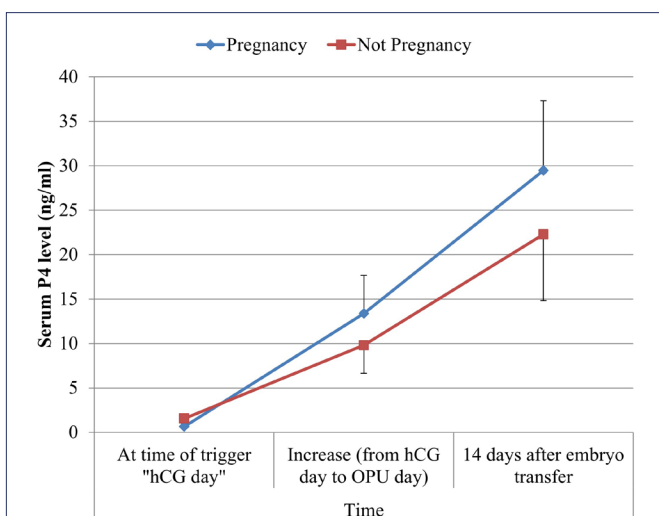


Figure 5. Comparison between the pregnant and nonpregnant groups according to P4 serum level (ng/ml).

Table 3. Comparisons of serum GDF-8 levels among the study patients at the three time points.

Serum GDF-8 level (ng/ml)	Range	Mean ± SD
At time of trigger "hCG day"	1.16-4.54	2.76 ± 0.86A
Oocyte pick up day (OPU day)	0.87-1.56	1.15 ± 0.15B
14 days after embryo transfer	1.15-3.90	2.44 ± 0.70A
RM-ANOVA		5.556
P-value		0.019 (S)

Table 4. Comparison between the pregnant and nonpregnant groups according to the P4 serum level (ng/ml).

Serum P4 level (ng/ml)	Outcome		t test	P-value
	Pregnant (n = 34)	Non-Pregnant (n = 8)		
At the time of trigger administration "hCG day"	0.64 ± 0.39	1.55 ± 0.45	-5.529	0.001*
Increase from the day of hCG to the day of OPU)	13.37 ± 4.26	9.80 ± 3.18	-2.560	0.014*
14 days after embryo transfer	29.46 ± 7.83	22.27 ± 7.43	-2.315	0.026*

Statistically significant.

GDF-8 and P4 serum levels (ng/ml) at 14 days following embryo transfer exhibit a highly statistically significant negative connection ($r = -0.663$ and $p < 0.001$), as shown in Figure 3.

The correlations between the serum levels of GDF-8 and progesterone (ng/ml) at the three time points are shown in Figure 4.

Comparison of the serum levels of GDF-8 and P4 between pregnant and nonpregnant women

Pregnant women (n = 34) and nonpregnant women (n = 8) had significantly different serum P4 levels at each of the three time points; P4 levels were significantly lower on the trigger day for pregnant women, and significantly higher in the latter two instances (Table 4, Figure 5).

As anticipated, there were substantial differences in the mean GDF-8 serum level between pregnant and nonpregnant women at each time point (Table 5, Figure 6).

Multivariate logistic regression analysis

Logistic regression analysis was conducted between clinical pregnancy (as the dependent variable) and different P4 and GDF-8 measurements at different time points.

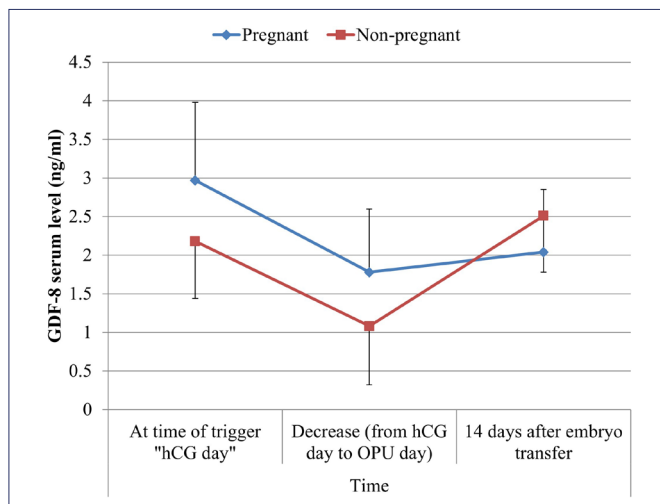


Figure 6. Comparison between the pregnant and nonpregnant groups according to the GDF-8 serum level (ng/ml).

Table 5. Comparison between the pregnant and nonpregnant groups according to the GDF-8 serum level (ng/ml).

GDF-8 Serum level (ng/ml)	Outcome		t test	P-value
	Pregnan-cy (n = 34)	Non-Pregnant (n = 8)		
At the time of trigger administration "hCG day"	2.97 ± 1.01	2.18 ± 0.74	2.212	0.033
Decrease (from the day of hCG to the day of OPU)	1.78 ± 0.82	1.08 ± 0.76	2.897	0.015
14 days after embryo transfer	2.04 ± 0.73	2.51 ± 0.81	2.835	0.019

Statistically significant.

Table 6 shows that serum GDF-8 at the day of trigger and at 14 days post ET were independent variables of clinical pregnancy. Nevertheless, serum progesterone levels at the three time points were shown to be insignificant predictors of clinical pregnancy on logistic regression.

GDF-8 cutoff values and correlation with pregnancy outcome at two time points

The pregnancy rate was found to be statistically significantly correlated with the GDF-8 serum cutoff level of ≥ 3.9 ng/ml compared to a cutoff of < 3.9 ng/ml, at the time of hCG trigger administration. Pregnancy and the drop in GDF-8 ≥ 1.35 from the trigger day to the OPU day were shown to be statistically significantly associated. When the drop in serum GDF-8 between the day of hCG injection and the day of OPU is compared using a threshold value of ≥ 1.35 or < 1.35 ng/ml, about 94% of pre-

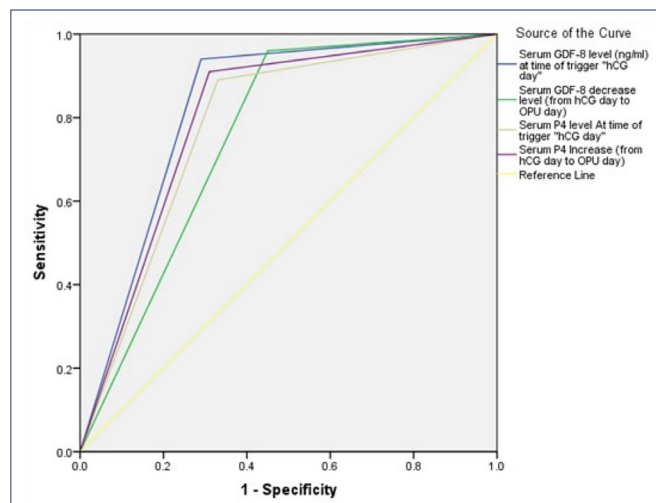


Figure 7. Receiver operating characteristic (ROC) curve for the prediction of pregnancy using GDF-8 and P4 serum levels (ng/ml).

Table 6. Logistic regression between clinical pregnancy and different measurements of GDF-8.

Variables	Multivariate logistic regression analysis		
	OR	95%CI	P-value
Serum GDF-8			
On the trigger day	20.45	4.26 - 1379489.7	0.01*
On the OPU day	10.34	0.012 - 9045.3	0.494
14 days after ET	0.017	0.0005 - 0.55	0.02*

OR: odds ratio; CI: confidence interval; *statistically significant; OPU: ovum pickup; ET: embryo transfer.

gnant women had a drop in serum GDF-8 of ≥ 1.35 . ($p < 0.001$).

GDF-8 serum level as a predictor of pregnancy in the study group

We examined the GDF-8 serum levels at two distinct points during COS (the hCG day and the OPU day) in an effort to identify a trustworthy predictor of pregnancy in the study group. **Table 7** shows that if the blood level of GDF-8 was ≥ 3.9 ng/ml on the day of trigger injection, then the clinical pregnancy of the cohort could be predicted with a sensitivity of 94.3% and an overall accuracy of 90.5%. Furthermore, a 94% sensitivity was demonstrated in predicting clinical pregnancy when there was a ≥ 1.35 drop in GDF-8 serum level between the trigger day and the OPU day.

Figure 7 displays a receiver operating characteristic (ROC) curve for the various serum levels of GDF-8 and P4. The highest area under the curve (AUC) was seen with a serum GDF-8 concentration of ≥ 3.9 ng/ml at the trigger point. This was followed

Table 7. Sensitivity and specificity of the GDF-8 and P4 serum levels (ng/ml) for the prediction of pregnancy.

Groups	Cutoff	Sen.	Spe.	PPV	NPV	Accura-cy%
Serum GDF-8 level (ng/ml)						
Serum GDF-8 level (ng/ml) at trigger "hCG" day	≥3.9	94.3%	71.4%	94.3%	71.4%	90.5%
Serum GDF-8 de-crease level (from hCG day to OPU day)	≥1.35	93.9%	55.6%	88.6%	71.4%	85.7%
Serum P4 (ng/ml)						
Serum P4 level at time of trigger "hCG day"	<1.5	88.8%	67.2%	88.8%	67.2%	87.2%
Serum P4 increase (from hCG day to OPU day)	>16.03	91.5%	69.3%	91.5%	69.3%	87.8%

Sens.: Sensitivity; Spec.: specificity; PPV: positive predictive value; NPV: negative predictive value.

by a rise in serum P4 concentration of > 16.03 ng/ml from the trigger day to the OPU day.

DISCUSSION

The current prospective study's findings imply that, for women undergoing COS-ICSI, the serum GDF-8 levels may be a reliable indicator of clinical pregnancy. On the day of trigger injection, serum GDF-8 and serum P4 levels were found to be significantly correlated negatively. When serum P4 levels rise from the trigger day to the OPU day, serum GDF-8 levels substantially drop.

The findings of Fang *et al.* [8], who were the first to record a dynamic trend between serum GDF-8 and serum P4 levels during COS, are supported by the results presented here. Based on previous reports, GDF-8 controls granulosa cell steroidogenesis capacity during COS and at different points of the menstrual cycle. This is based on the finding that GDF-8 inhibits the expression of StAR in granulosa cells, which lowers P4 production [20].

The purpose of this study was to look at the association between serum P4 and serum GDF-8 during COS and how it could affect the rate of pregnancy. According to our findings, women with high blood levels of GDF-8 also have low P4 levels on the day of trigger injection. This enhances the chance for implantation by preventing the endometrium from being prematurely exposed to an increase in P levels. Thus, high levels of GDF-8 on day of trigger injection may serve as a preventive measure against PPR and its documented adverse effects on endometrium, embryo quality, and live birth rate [25]. Conversely, decreased GDF-8 levels following hCG injection may be essential for the initial embryo implantation process, as they maintain greater P4 levels. Therefore, this dyna-

mic interaction between GDF-8 and P4 is required for pregnancy to occur.

Furthermore, a significant downregulation of StAR expression in hGL (human granulosa cell) caused by overexpression of GDF-8 in the follicles is consistent with PCOS women. This is linked to lower progesterone levels that hinder implantation, which in turn is linked to a lower pregnancy rate in PCOS women compared to non-PCOS women in the same study [26].

Intriguing research contrasted a number of growth factors between women who had poor ovarian response and those who had normal and high response. Compared to women who responded normally, those who responded poorly had significantly higher concentrations of GDF-8 in their cellular receptors, blood, and follicular fluid. In the same study, it was identified that GDF-8 had a negative correlation with LH and E2 levels, as well as the antral follicle count. Because of its impact on steroidogenesis, the results of this study indicate that GDF-8 may be a potential predictor of poor ovarian response. Nevertheless, there was no significant correlation between GDF-8 and hyper-response [27].

From our evaluation of serum GDF-8 levels as a predictor of the pregnancy rate after IVF, we found that levels ≥ 3.9 ng/ml on the day of trigger and a decrease in GDF-8 levels of more than 1.35 ng/ml were associated with significantly better outcomes in terms of clinical pregnancy after ICSI-ET.

These results are close to those of Fang *et al.* [8], who found that the pregnancy rate was considerably higher in women whose GDF-8 serum level was 4.7 ng/ml or higher on the day of hCG injection than in those whose level was less than 4.7 ng/ml. Moreover, the pregnancy rates were considerably higher in women whose serum GDF-8 level dropped by more than 1.3 ng/dl between the trigger day and the OPU day than in those whose

level dropped by less than 1.3 ng/dl. Notably, only 19 women participated in this study.

Our study was limited by the relatively small sample size, the fact that the study population comprised young women with adequate ovarian reserve and a generally favorable prognosis of IVF treatments, and that no long-term follow-up was continued after the detection of clinical pregnancy. We recommend further research on a larger scale of participants and for different ovarian response status (poor and hyper-responders).

CONCLUSIONS

Based on the findings of the present study, we may infer that during controlled ovarian stimulation, serum GDF-8 levels exhibit a dynamic pattern in conjugation with serum progesterone.

Pregnancy after IVF-ET may be predicted by a high blood level of GDF-8 on the day of trigger administration together with a decline in this level from the day of trigger to the day of oocyte pickup. However, to derive more broadly applicable conclusions, larger-scale research including women with both poor and high ovarian response is necessary.

COMPLIANCE WITH ETHICAL STANDARDS

Authors' contributions

A.S.: Investigation, Methodology, Formal Analysis, Writing – original draft. S.A.H.: Conceptualization, Supervision, Writing – review & editing, Validation. S.T.: Data curation, Investigation, Methodology. D.H.: Supervision, Writing – review and editing.

Funding

None.

Study registration

Trial registration number: NCT06529627 (retrospectively registered on 31/07/2024).

Disclosure of interests

The authors declare that they have no conflict of interests.

Ethical approval

The study was conducted according to the declaration of Helsinki and ethical approval of the study protocol

was obtained from the Ethics Committee of the Faculty of Medicine, Alexandria, Egypt, on 16/02/2023 (IRB No. 00012098, approval number 0107574).

All procedures and measurements in the study were performed in accordance with the recommended guidance and regulations.

Informed consent

All study participants were told about the nature of the study and its aims, with a comprehensive explanation of the procedures it entails, its expected risks and complications, then a written informed consent was obtained from every patient.

Data sharing

Data are available under reasonable request to the corresponding author

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