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Assessment of follicular fluid levels of PlGF and PlGF/sFlt on ICSI outcomes in polycystic ovary syndrome women

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ABSTRACT— Polycystic ovarian syndrome (PCOS) is characterized by increased ovarian angiogenesis and vascularity. Vascular endothelial growth factor (VEGF) is main angiogenic factor which increased in PCOS and may play an important role in these vascular changes. Placental growth factor (PlGF), a VEGF family member. PlGF and VEGFA that are expressed by both granulosa cells and theca cells, are required for ovulation, luteinization, and follicular angiogenesis. Considering potential roles of PlGF and its receptor (sflt-1) in ovarian function and embryo implantation, in the present study, the association of these factors with ICSI outcome among PCOS women during controlled ovarian stimulation have evaluated. This comparative cross sectional study include of 30 PCOS women (Rotterdam criteria) and matched 30 non-PCOS women undergoing controlled ovarian stimulation. Serum was collected on day 3 and day of oocyte retrieval. Follicular fluid (FF) was collected on retrieval day. PlGF and sFlt-1 concentrations were measured using ELISA. In the present study, at the measured time point of controlled ovarian stimulation the result observed no significant difference in the serum PlGF and sFlt levels between PCOS women compared with non PCOS women ($p<0.001$). As well as the FF PlGF was correlated positively with total number of oocyte retrieved, number of MII oocyte and the ovarian reserve marker antimullerian hormone (AMH) but it was correlated negatively with basal FSH level. PlGF bioavailability in FF was significantly greater in PCOS women compared with non-PCOS ($p < 0.001$). These data provide evidence that FF PlGF was correlated with ovarian stimulation and ICSI outcome. Moreover, its level is significantly increased in women with PCOs undergoing controlled ovarian stimulation. Thus that PlGF may be played a role in PCOs pathogenesis and its angiogenic dysregulation.

KEYWORDS: Placental growth factor (PlGF), Soluble Fms-like tyrosine kinase (sFlt-1), Polycytic ovarian syndrome (PCOs), Angiogenesis, Ovarian stimulation

1. INTRODUCTION

PCOS is the most common, yet complicated, endocrine condition that affects women during their reproductive years. PCOS affects about 5% to 10% of women [17]. Typical clinical features include hirsutism, irregular menses, chronic anovulation, and infertility. Impaired hypothalamic–pituitary feedback, LH hypersecretion, premature granulosa cell luteinization, aberrant oocyte maturation, and premature arrest of activated primary follicles are all linked to persistent hyperandrogenism [42].

Angiogenesis is the process of forming new blood vessels from existing ones, and it's crucial for tissue growth and development [14]. In adults, angiogenesis is usually linked to pathological circumstances such wound healing [22]. An exception is the female reproductive tract (i.e., ovary, uterus). The ovary undergoes cyclic changes in angiogenesis that are required for folliculogenesis, ovulation, Corpus luteum formation,

and maintenance of pregnancy [12]. Angiogenic factors control the formation and regression of the vasculature. The main angiogenic factor that promotes endothelial cell proliferation and migration and vascular permeability is Vascular endothelial growth factor (VEGF) [45].

The VEGF family includes several members that perform various functions: VEGF-A (which presents several isoforms), VEGF-B, VEGF-C, VEGF-D, VEGF- E,VEGF-F and the placenta growth factor (PlGF) [49], [52]. Placenta growth factor (PIGF) is homodimeric glycoprotein which is a member of VEGF family [9]. Vascular endothelial growth factor–A and PLGF are structurally related to each other, and they bind to the same receptors, Flt-1 [2]. However, although data are still relatively limited, higher levels of circulating PlGF have been linked to a variety of pathological conditions such as metastatic breast cancer [39], leukemia [59], rheumatoid arthritis [60], systemic lupus erythematosus [61], metabolic syndrome [51] type II diabetes [13], coronary artery disease [16], and neovascular age-related macular degeneration [19]. In addition, growing evidence has shown an important role of PlGF in regulating the reproduction process, starting from ovulation to placentation, implantation, and embryo development [6]. Consequently, imbalance in PlGF levels may lead to pregnancy complications like preeclampsia, giving birth of small for gestational age, preterm birth, and stillbirth [34]. These effects may arise from the pleiotropic effects of PlGF, as besides its proangiogenic activity, PlGF acts as an immune modulator by enhancing monocyte and macrophages activation, inhibiting dendritic cells differentiation and maturation [48], [29], [32], and regulating uterine NK cells proliferation and/or differentiation [55]. In addition, it skews the type1 T helper immune response to the Th2 phenotype [32]. PlGF produced within the ovulatory follicle in response to the ovulatory gonadotropin surge [6]. PlGF and VEGFA that are expressed by both granulosa cells and theca cells is required for ovulation, luteinization, and follicular angiogenesis in primates and it is necessary for both ovulation and development of the corpus luteum [6].

sFlt-1 is a soluble receptor for two angiogenic factors, PlGF and VEGF-A and it could inhibit PlGF and VEGFA angiogenic effects by binding them [10]. sFLT-1 belongs to the VEGF/VEGFR family; a family which plays a vital role in angiogenesis and vasculogenesis. sFLT-1 comprises the extracellular domains of the vascular endothelial growth factor receptor-1 (VEGFR-1), and is soluble, being present in the circulation.

Considering potential roles of PlGF and it's receptor (sflt-1) in ovarian function and embryo implantation, the present study have evaluated the association of these factors with ICSI outcome among PCOS women. So the aim of current study to investigate PIGF in serum and follicular fluid of women with PCOS as compare to women without PCOS during controlled ovarian stimulation and to evaluat the association of this factors with ICSI outcome.

2. Materials Subjects and Methods

This study was conducted at the High Institute of Infertility Diagnosis and Assisted Reproductive Techniques, Al- Nahrain University, Baghdad-Iraq. The duration of the study extended from October 2021 to September 2022. In the comparative cross sectional study. 60 women were selected and intentionally divided into 30 infertile women with polycystic ovarian syndrome (PCOS) and 30 infertile women without PCOS. Each group was included women that were subjected to ovarian stimulation by antagonist protocol. The indications for Controlled ovarian hyperstimulation (COS) in the PCOS group were male factor and tubal factor. Non-PCOS control women were undergone COS in preparation for ICSI and these group was matched to PCOS group by age, BMI prior to enrollment. Written informed consent was obtained from all participating women. Women were treated using a GnRH antagonist protocol to prevent premature ovulation. Ovarian stimulation was performed using a combination of recombinant FSH and or HMG. The

standard stimulation protocol was modified when there was risk of ovarian hyperstimulation or previous history of poor response. Considering potential roles of serum and follicular fluid sflt and PlGF in ovarian function, the present study, has evaluated the association of these factors and also PlGF/sFlt-1 ratio with the ovarian response and for ART outcome by dividing patients according to Rotterdam criteria. PCOS women were fulfilling at least two of the following three criteria based on the Rotterdam ESHRE/ASRMS sponsored PCOS consensus workshop group as an inclusion criteria: 2 out of 3 of the following: Polycystic ovary on ultrasound (at least 12 follicles 2-9 mm in diameter per ovary), chronic anovulation or oligovulation, clinical or biochemical Hyperandrogenism. The inclusion criteria include infertile women who diagnosed as PCOS in the presence of at least 2 of Rotterdam criteria, based on Rotterdam consensus meeting (2003). those who have history of recent administration of hormonal therapy, infertile women aged more than 40 years, infertile women with Cushing syndrome, thyroid disorders and other endocrine disease, diabetes mellitus, cardiovascular diseases, liver diseases, kidney diseases, cancer or had any conditions that might affect IVF outcomes like endometriosis, uterine fibroids, hydrosalpinx, adenomyosis, or autoimmune diseases, infertile women with three or more previous IVF failures, poor responders (Bologna criteria (Ferraretti et al., 2011), and those who were previously undergone unilateral oophorectomy all these women excluded from the study. When at least three follicles are 18 mm with serum E2 measured, 2 ampule of recombinant hCG 250 mg/0.5 ml prefilled syringe (Ovitrelle ® 500 mg product of MERK SERONON) were given and to trigger and induce maturation of oocyte. Then, 34-36 hours after the hCG injection, oocyte retrieval was conducted with transvaginal ultrasound guidance under general anesthesia or spinal anesthesia Embryo transfer was performed transcervically on a patient with a dorsal lithotomy, guided by ultrasound and the embryo was placed 15-20mm from the fundus. Clinical pregnancy was detected by performing B-hCG test after 2 weeks of embryo transfer, followed by a transvaginal ultrasound 2 weeks later to confirm the viability of the embryo.

2.1 Sample collection and storage

Blood samples and follicular fluid were collected from each women enrolled in ICSI procedure. Blood sample was obtained from the patients at day 2 of the menstrual cycle for hormones analysis (LH, FSH, E2, testosterone, prolactin) and AMH for infertile women aged 40 years and more assay by MiniVidas. At the day of pickup, the blood sample that was taken allowed to coagulate for 10 minutes then centrifuged at 3000 rpm for 20 min to separate the serum. The samples were aliquot and quickly frozen and stored at -20C° until assayed. For follicular fluid collection, follicles with a diameter of >16 mm were aspirated. Aspirating only the first clear follicular fluid associated with the presence of an oocyte, without blood or flushing solution, was used for analysis after removal of the oocyte, the fluid was centrifuged at 3000 rpm for 10 min to remove granulosa cells and debris. The supernatant was divided into aliquots and stored at −20°C until assayed.

2.2 Serum and Follicular fluid parameters assay

Serum and FF PlGF and sFlt-1 levels were determined by enzyme-linked immunosorbent assay (ELISA) kits This kit uses enzyme-linked immune sorbent assay (ELISA) based on the Biotin double antibody sandwich technology to assay the Human placental growth factor (PlGF) and the Human Soluble Vascular Endothelial Growth Factor Receptor 1 (sFlt-1) according to the manufacturer instructions. The sensitivities for PlGF and sFlt-1 were 2.54 and 0.25ng/ml respectively. Moreover, intraassay and interassay coefficients of variation (CV) were respectively <8% and <10% for PlGF and sFlt-1.

2.3 Statistical analysis

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 23.0 and Microsoft office 2007.The descriptive statistics including frequency, mean and standard deviation were measured to

describe the data .The groups were compared by applying independent sample t-test (unpaired t-test between two groups) and chi square (for non-continuous data or percentage) and the degree of association between continuous variables was calculated by Pearson's correlation coefficient (r) .The cut off value, sensitivity and specificity were calculated by using Receiver operative characteristics (ROC) curve and the results were considered statistically significant when p value was less than 0.05. (Daniel and Cross, 2014)

3. Results

There were no significant differences in mean patients age, BMI and duration of infertility type and cause of infertility (p=0.109,p=0.125,p=0.220,p=0.136,p=0.389) respectively between PCOS and non PCOS groups. The follicular fluid PlGF level and Follicular fluids PlGF/sFlt ratio were significantly higher in PCOS groups than non PCOS, 147.96 ± 7.40 versus 77.66 ± 3.69 and 0.0398 ± 0.013 versus 0.023 ± 0.006 respectively. On the other hand there ware non-significant lower serum and follicular fluids sFlt level in PCOS group ($p > 0.05$) as showed in table 1 follicular fluid PlGF protein concentration on day of oocyte retrieval was significantly higher in PCOS groups than non PCOS, 147.96 ± 7.40 versus 77.66 ± 3.69 and 0.0398 ± 0.013 versus 0.023 ± 0.006 respectively and follicular fluids PlGF/sFlt ratio was significantly higher in PCOS groups than non PCOS (0.0398 ± 0.013 versus 0.023 ± 0.006 respectively). No differences were observed between PCOS and non-PCOS women in serum levels of PlGF. On the other hand there was no significant lower serum and follicular fluids sFlt level in PCOS group ($p > 0.05$) as showed in table 1. Follicular fluids PlGF/sFlt ratio was significantly higher in PCOS groups than non PCOS (0.0398 ± 0.013 versus 0.023 ± 0.006 respectively). The correlation between serum and FF PlGF and sFlt with women's hormones were illustrated in table 2. Pearson correlation analysis was performed to evaluate the correlations of PlGF or sFlt-1 concentration with various stimulation cycle parameters. Serum PlGF was significantly positive correlated with total oocytes count, metaphase 2 oocytes, total number of embryos & grade 1 embryos (r=0.526, r= 0.452 r= 0.34, r=0.375 respectively). In addition, follicular fluid PlGF was significantly positive correlated with total oocytes count, metaphase 2 oocytes total number of embryos & grade 1 embryos (r=0.628, r=0.545 r=0.501,r=0.522 respectively). However there was significant positive correlation between grade 2 embryos with follicular fluid PlGF (r=0.522) as showed in table 3. Seven patients (23.3 %). Out of thirty in both PCOS and non PCOS groups became pregnant, so there was no significant difference in pregnancy rate between the two studied group (p=1.00) table 3.

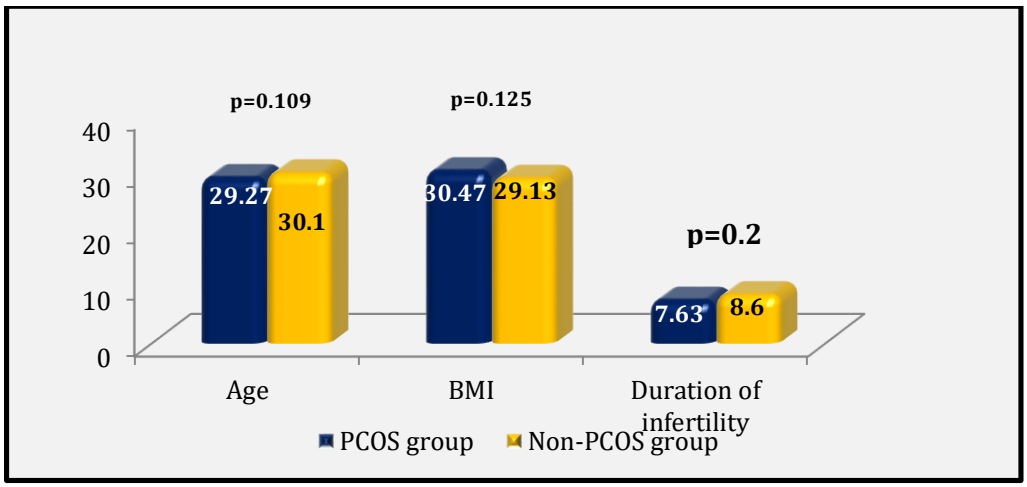

chart 1: Comparison of age, BMI and duration of infertility between PCOS and non PCOS groups

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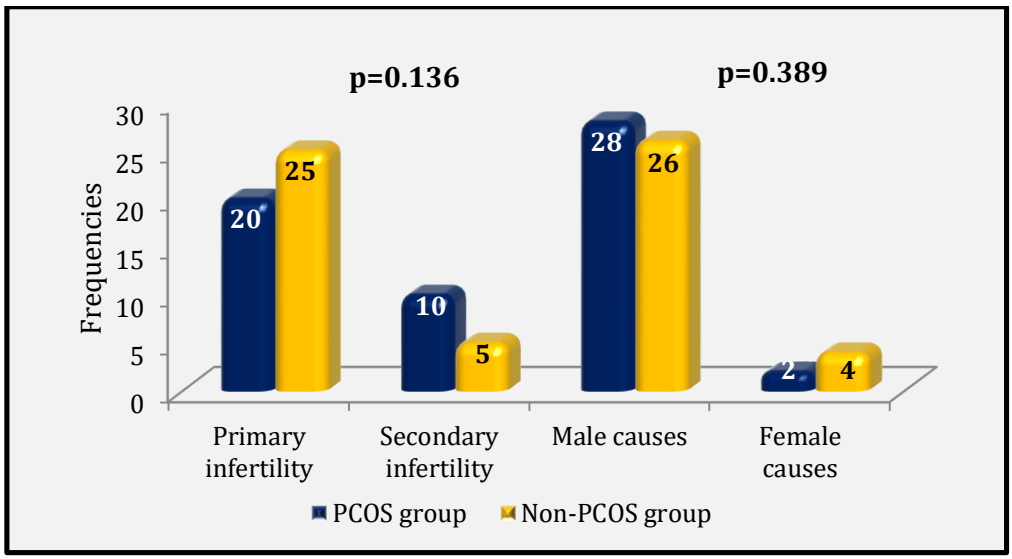

Chart 2: Comparison of type and causes of infertility between PCOS and non PCOS groups

Table 1: Comparison of PlGF & sFlt levels between PCOS & non PCOS patients

Parameter	PCOS group (Mean±SD)	Non-PCOS group (Mean±SD)	p value
Serum PlGF (ng/l)	135.9 ± 8.68	127.46 ± 2.86	0.131 NS
Follicular fluid PlGF(ng/l)	147.96 ± 7.40	77.66 ± 3.69	< 0.001 S
Serum sFlt (ng/ml)	3.29 ± 0.05	4.25 ± 0.48	0.055 NS
Follicular fluid sFlt (ng/ml)	3.40 ± 0.46	4.12 ± 0.40	0.086 NS
Follicular fluids PlGF/sFlt ratio	0.0398 ± 0.013	0.023 ± 0.006	< 0.001 S

PlGF: Placental growth factor; sFlt: soluble FMS-Like tyrosine kinase; NS: Not significant ($p > 0.05$); S: Significant ($p \leq 0.05$).

Table 2: Correlations between PlGF & sFlt with hormones

Parameters		Serum PlGF	Follicular fluid PlGF	Serum sFlt	Follicular fluid sFlt
LH (mIU/ml)	r	0.698*	0.782*	0.395*	0.352*
	p value	< 0.001	< 0.001	0.002	0.006
FSH (mIU/ml)	r	-0.345*	-0.333*	-0.136	-0.105
	p value	0.007	0.009	0.301	0.426
AMH (ng/ml)	r	0.588*	0.565*	0.144	0.108
	p value	< 0.001	< 0.001	0.272	0.410
Prolactin	r	0.147	0.143	0.004	0.013
	p value	0.263	0.275	0.978	0.921
TSH	r	-0.136	-0.240	-0.120	-0.078
	p value	0.300	0.065	0.362	0.553
E2 (pg/ ml) CD2	r	0.425*	0.555*	0.094	0.101

	p value	0.001	< 0.001	0.474	0.445
E2 at day of trigger (pg/ml)	r	0.650*	0.701*	0.087	0.061
	p value	< 0.001	< 0.001	0.507	0.645

PlGF: Placental growth factor; sFlt: soluble FMS-Like tyrosine kinase;*: Significant correlation; r :Pearson's correlation coefficient

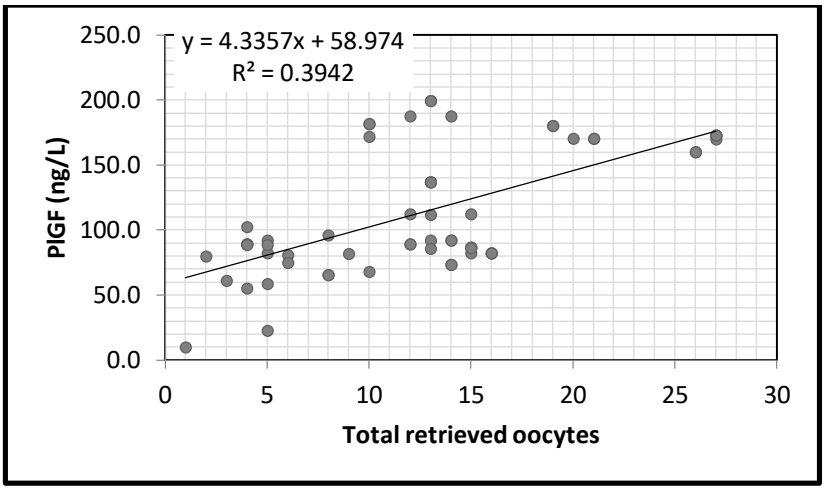

Figure 3: Correlations between follicular fluid PlGF & total oocytes

Table 3: Correlations between PlGF & sFlt with ICSI parameter

Parameters		Serum PlGF	Follicular fluid PlGF	Serum sFlt	Follicular fluid sFlt
Total oocytes count	r	0.526*	0.628*	-0.027	-0.032
	p value	< 0.001	< 0.001	0.840	0.810
Metaphase I oocytes	r	0.034	0.243	0.129	0.153
	p value	0.797	0.061	0.326	0.243
Metaphase II oocytes	r	0.452*	0.545*	-0.044	-0.036
	p value	< 0.001	< 0.001	0.737	0.787
Fertilization Rate	r	*0.066*	*0.181*	*0.154*	*0.183*
	p value	*0.619*	*0.169*	*0.243*	*0.164*
Total embryos	r	*0.34**	*0.501**	*0.112*	*0.129*
	p value	*0.008*	*0.000*	*0.395*	*0.325*
Grade 1 embryos	r	*0.375**	*0.522**	*0.126*	*0.141*
	p value	*0.003*	*0.000*	*0.336*	*0.281*
Grade 2 embryos	r	*0.238*	*0.300**	*0.041*	*0.054*
	p value	*0.067*	*0.020*	*0.753*	*0.684*
Grade 3 embryos	r	*-0.005*	*0.165*	*0.026*	*0.042*
	p value	*0.971*	*0.209*	*0.846*	*0.748*

PlGF: Placental growth factor; sFlt: soluble FMS-Like tyrosine kinase;* Significant correlation; r: Pearson's correlation coefficient

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Table 4: Comparison of PlGF & sFlt levels between pregnant & non pregnant females

Parameter	Pregnant female (n.=14)	Non-Pregnant female (n.=46)	p value
Serum PlGF (ng/l)	108.5 ± 9.43	106.17 ± 7.22	0.868 NS
Follicular fluid PlGF (ng/l)	117.92 ± 9.39	111.25 ± 7.52	0.650 NS
Serum sFlt (ng/ml)	3.46 ± 0.43	3.87 ± 0.32	0.493 NS
Follicular fluid sFlt (ng/ml)	3.34 ± 0.38	3.89 ± 0.27	0.266 NS
Follicular fluids PlGF/sFlt ratio	0.035 ± 0.009	0.030 ± 0.014	0.231 NS

PlGF: Placental growth factor; sFlt: soluble FMS-Like tyrosine kinase; NS: Not significant ($p \geq 0.05$).

4. Discussion

The follicular fluid PlGF level was significantly higher in PCOS group than non PCOS ($p < 0.001$). This result similar to the result of Tal.R etal. who found that FF PlGF levels were increased 1.5-fold in PCOS women compared with controls [54]. This data may be due to the presence of abundance of angiogenic NK cells in the FF of patients with PCOS [15]. Because recently have shown that the NK cells can be proangiogenic through the secretion of cytokines such as vascular endothelial growth factor, placental growth factor, and others [43] and other study confirmed that the increased activation of NK cells results in the constant secretion of cytokines. These cytokines may be involved in the onset of the signs and symptoms of PCOS, as it interferes in the hypothalamic-pituitary axis, possibly resulting in disturbances in ovulation [58]. So, from the above results that interpret and concurrent with the data of present study can conclude that the elevated level of FF PlGF in PCOS women may be due to presence of large number of angiogenic NK cell in follicular fluid of these women as well as the PlGF has overactivity role0in the 0pathogenesisof PCOS. Furthermore FF PlGF/sFlt ratio were significantly higher in PCOS group than non PCOS women ($p < 0.001$). This results agreed with [40] who found that the PlGF/sFlt-1 ratio was significantly higher in high-responders compared to poor-responders. Thus it can be conclude that the significant higher FF PlGF/sFlt-1 ratio in PCOS group than the non PCOS women may be due to increase PlGF/sFlt-1 ratio lead to increase the bioavailability of PlGF in women with PCOS.

In current study table 2 showed there was significant positive correlation between Luteinizing hormone with both serum PlGF($r=0.698$) and follicular fluid PlGF($r=0.782$). [6] Showed that PlGF produced within the primate ovulatory follicle is an important mediator of the ovulatory cascade and the follicular levels of mRNA and PLGF protein increased after the ovulatory gonadotropin surge. Granulosa cells and theca cells likely both contributing to the elevated follicular fluid levels of PlGF detected 36 hours after hCG administration, or just before ovulation [6]. Form this result current study suggest that the PlGF may have a role in oocyte development, maturation and selection of dominant follicle in the ovulatory process.

Current study also found that AMH level was significant positive correlated with both serum PlGF ($r=0.588$) and follicular fluid PlGF ($r=0.565$). These results concurrent with the result obtained by Tal R who found FF PlGF correlated positively with the ovarian reserve marker AMH [54]. Other study found the AMH secretion by granulosa cells of the antral follicles (4-6mm) and its secretion gradually decreases during follicular growth and cannot be distinguished in follicles larger than 8 mm [20], [21]. PlGF has been found to be expressed by both granulosa cells and theca cells.on the other hand PlGF levels in follicular fluid have demonstrated to be lowest before hCG. PlGF levels remained low until 36 hours after hCG

administration, when they surge sevenfold to reach peak levels [6]. Thus both PlGF and AMH produce from granulosa cell. Moreover, [56] demonstrated that VEGF and gonadotropins are involved in the regulation of expression of AMH [57]. Therefore, further study required to conform association between follicular fluid AMH and PlGF at day of trigger and to demonstrate the role for PlGF in the regulation of expression of AMH in growing follicles.

Furthermore table 2 demonstrated there was There was a positive correlations between basal E2 level and day of trigger E2 with follicular and serum PlGF. These results similar to that obtained by [22] who found that the mRNA expression of vascular endothelial growth factor (VEGF), VEGFR1, and placental growth factor (PIGF) increased by 4 h after E2-treatment [23]. On the other hand estrogens are one of the known factors within the cellular mechanisms that could initiate repairs to the damaged vascular tissues, since estrogens are known inducers of angiogenesis leading to this cellular regrowth. Research has also shown that this cellular regrowth is induced by vascular angiogenic growth factors via the estrogen receptors [5]. Based on the above information, present study hypothesized that PlGF may be regard as a mediater through which Estradiol induce angiogenesis and further research is required to determine the association of PlGF and E2 in ovarian pathology at molecular level. [3] found a significant positive correlation between 17B - Estradiol and Luteinizing hormone. On the other side LH stimulates the expression of PlGF from granulosa cell just before ovulation and there is a temporal patterns of PlGF and VEGFA accumulation in the follicle. Because VEGFA levels in follicular fluid peaked early in the ovulatory interval, whereas PlGF levels peaked just before ovulation [6] from these data give a suggestion0that PLGF may have important role in ovulation and ovarian0angiogenesis.

However in current study there is significant negative correlation between follicular stimulated hormone (FSH) with serum and follicular fluid PlGF (table 2). Recent study found the FF VEGF concentration was significantly positively correlated with basal FSH level and FF VEGF concentration has a negative association with ovarian reserve level and oocyte maturation rate in patients undergoing GnRH antagonist IVF protocols. Based on these data suggest that PlGF and VEGF, while belonging to the same VEGF family, may have different biological roles in the local ovarian microenvironment.

In current study there was a significant positive correlation between total oocytes count with serum and follicular fluid PlGF (table 3) this result concurrent with the result obtained by [54] who conclude that follicular fluid PlGF correlated positively with serum AMH and number of oocytes retrieved. [28] demonstrated that A strong positive correlation between serum AMH and the number of collected oocytes. Previously, serum AMH has been shown to correlate with ovarian response to ovarian stimulation during ART [35], [36]. Furthermore the Placental growth factor and VEGF-A have synergistic effects in the induction of angiogenesis that increased follicular vascularity may be a primary determinant of follicular dominance and that dominant follicles have an increased uptake of serum gonadotrophins [33], [46]. On the other hand a previous study demostrated that the increased ovarian stromal blood flow velocity may be associated with an increased delivery of gonadotrophins to the target cells for stimulation of follicular growth resulting in the production of more oocytes [4]. Based on these data the current study conclude that the PlGFis similar to AMH, which may be associated with ovarian response to stimulation and could be used as markers for determining the high-responder women and potentially may permit to identify patients at risk for OHSS only when OHSS is developed yet.

Present study found that metaphase 2 oocytes was significantly positive correlated with serum and follicular fluid PlGF (table 3). One of recent study showed a positive correlation between estradiol levels and the number of follicles, number of retrieved oocytes, and number of mature oocytes. As estradiol increased,

estradiol per follicle, oocyte, and mature oocyte increased but beyond a level of 5000 pg/ml, all these decreased. Furthermore E2 levels < 1000 pg/ml and > 5000 pg/ml had a negative impact on IVF outcome [37]. Similar findings were noted in the study by [29] who found the number of oocytes retrieved, and the number of mature oocytes increased as E2 level increased [30] on the other hand [24] found that the mRNA expression of vascular endothelial growth factor (VEGF), VEGFR1, and placental growth factor (PIGF) increased by 4 h after E2-treatment [25]. Moreover, numerous studies emphasized that a crosstalk between estrogen and angiogenic factors occurs and is mainly responsible for regulating the angiogenesis processes. VEGF and PlGF share similar signaling pathways with E2 (i.e., phosphatidylinositol-3 kinase/protein kinase B). In addition, the VEGF gene contains a variant estrogen receptor and E2 upregulates VEGF, PlGF and VEGF receptor 1 (VEGFR-1) expression [7], [8]. Based on the above data the current study can conclude that PlGF may be linked with estradiol and high level of PlGF and E2 result in high number of oocyte retrival and increase number of mature oocyte.

Current study also illustrated that there was significant positive correlation between total number of embryos and grade 1 embryos with serum and follicular fluid PlGF (table 1). Recent study found high number of oocytes was a strong predictor of multiple good-quality embryos [50] from this data give a suggestion that PlGF may be involved in oocyte maturation and oocyte quality. Furthermore in present study found no correlation between FF PlGF and serum PlGF level and the fertilization rate. Additionally no significant difference in the serum and follicular fluid PlGF level among pregnant and non-pregnant women (table 4) these findings concurrent with the finding obtain by [40].

sFlt-1 is a soluble receptor for two angiogenic factors, PlGF and VEGF-A and it could inhibit PlGF and VEGFA angiogenic effects by binding them [11]. In current study did not find any significant differences in serum and FF sFlt-1 levels among PCOS and nonPCOS group and also there was no correlation between sFlt-1 levels with the number of oocytes and number of MII, number of MI, total number of embryos, grade 1 embryo and grade 2 embryos. [47] have also obtained similar results. Only in study conducted by [41] an inverse relation between sFlt-1 levels and the number of oocytes has been observed. Since PlGF and VEGF have determinant role in FF sFlt-1 levels by binding to it, and [41] have obtained totally different levels of VEGF, this reason could cause the controversial results. In current study, there were no significant differences in levels of FF sFlt-1 between pregnant and non-pregnant groups. The same results have been achieved in previous study [47]. PlGF/sFlt-1 ratio in present study was statistically higher in PCOS than non PCOS group. High level of PlGF/sFlt-1 ratio in PCOS possibly leads to an increase in availability of PlGF and thus this factor exerts through VEGFR-1 (Flt-1).

5. Conclusions

The follicular fluid Placental growth factor (PlGF) level and PlGF / sFlt ratio were higher in PCOS groups than non PCOS. While they direct correlated with total oocytes count, metaphase 2 oocytes, and total number of embryos and grade 1 embryos while no significant difference of serum and follicular fluid PlGF level with fertilization rate. But there was no difference in serum and follicular fluid PlGF, sFlt levels and PlGF/sFlt ratio between pregnant and non-pregnant females.

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