

**PREDICTIVE VALUE OF VASCULAR ENDOTHELIAL GROWTH
FACTOR IN WOMEN UNDERGOING INTRACYTOPLASMIC SPERM
INJECTION WITH HIGH RISK FOR OVARIAN
HYPERSTIMULATION SYNDROME**

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ABSTRACT

Background: Vascular endothelial growth factor (VEGF) is expressed and produced by granulosa-lutein cells and is released into the follicular fluid in response to human chorionic gonadotropin (hCG), increasing capillary permeability. In addition, plasma VEGF levels correlate with the clinical picture of ovarian hyperstimulation syndrome (OHSS). **Objective:** This study aimed to investigate the predictive value of serum VEGF at the day of hCG administration in women undergoing intracytoplasmic sperm injection (ICSI) cycle and in high risk of OHSS. **Study design:** Prospective cohort study. **Subjects and Methods:** This study was performed on eighty women with polycystic ovary syndrome with infertility and will undergo ICSI cycle. All women were subjected to routine practice of long protocol for controlled ovarian stimulation (COS). The eighty subjects included in this study were classified into two groups based on ovarian

response and E2 level before the day of hCG. Group A: Normal ovarian stimulation; thirty women with E2 less than 3000pg/ml before the day of hCG administration. Group B: Ovarian hyperstimulation (Coasting group); fifty women with E2 more than 3000pg/ml and count of large follicles more than 20 follicles in each ovary with diameter > 17mm before the day of

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hCG administration, then those women coasted to reach E2 level less than 3000pg/ml at the day of hCG administration (as a high risk group suspected to ovarian hyperstimulation during induction of ovulation). Vascular endothelial growth factor concentrations in serum at the day of hCG administration were determined by enzyme- linked immune sorbent assay (ELISA).

Results: The mean serum VEGF (pg/ml) for coasting and control groups was (296.9 ± 15.53 , 455.1 ± 44.26) respectively which was significantly lowered compared to control group ($p=0.0001$). There were significant positive correlations between VEGF and both of follicle number ($p=0.0133$, $r=0.4469$) and mean of follicle diameter (mm) ($p < 0.0001$, $r=0.7515$) in both ovaries of the control group. Also there were significant positive correlation between VEGF and both of follicle number ($p=0.0027$, $r=0.4153$) and mean of follicle diameter (mm) ($p < 0.0001$, $r=0.5271$) in both ovaries of the coasting group. **Conclusion:** Serum VEGF estimation after coasting at the day of hCG administration in high risk patient is recommended for early prediction of OHSS in addition to E2, follicle number and follicle diameter. Moreover, it can be used as an indicator of success of coasting. Further studies for measurement of total, free and soluble receptors of VEGF for clarification the role of VEGF in OHSS are recommended.

KEYWORDS: Infertility, Ovarian hyperstimulation, vascular endothelial growth factor.

INTRODUCTION

Ovarian hyperstimulation syndrome (OHSS) is the most serious iatrogenic complication of supraphysiologic ovarian stimulation that occurs during either the luteal phase or early pregnancy. The most common form occurs a few days after the induction of the follicular rupture following the administration of hCG when follicular growth stimulation has been medically induced by using exogenous gonadotropins, and is only rarely observed after spontaneous ovulation.^[1] OHSS imposes a physical, psychological and economical effect on patients as a consequence of hospitalization and fear of infertility or miscarriage.^[2]

The reported incidence of OHSS is highly variable according to different studies because various classifications are used. According to the Egyptian IVF registry report: ART in Egypt 2005, the incidence of OHSS was 1.8% which is higher than 1.1% reported in 2005 ART world results.^[3]

According to WHO report, the incidence of moderate and severe OHSS was 3–6% and 0.2–1%, respectively. While mild forms of OHSS are common, affecting up to 33% of in vitro

fertilization (IVF) cycles. The majority of cases of severe OHSS are seen following IVF treatment but the syndrome can occur after any form of supraphysiological ovarian stimulation, including clomifene and gonadotrophin ovulation induction.^[4] Severe OHSS viewed by gynaecologists as a relatively rare complication. However the incidence has almost surely increased over the years.^[5]

Some immune system products are implied in the pathophysiology of OHSS. A number of cytokines are associated with the inflammatory process that takes place during the late follicular maturation, ovulation, corpus luteum function, and embryo implantation. Increased expression of interleukin 6 is associated with increased vascular permeability, hemoconcentration, elevated plasma E2 concentration, and inhibition of hepatic albumin production.^[6]

OHSS is associated with extravascular protein-rich exudates which accumulated in the peritoneum, in the pleura and even in the pericardiac space is associated with intravascular volume depletion, activation of vasoconstrictor and anti-natriuretic factors, severe hypoalbuminemia.^[7] The cardiovascular effects include arterial hypotension, reduced fluid volume, low central venous pressure, tachycardia, low peripheral resistance, hemoconcentration and hyper coagulation. The associated hypovolemia can induce oliguria and electrolyte imbalance.^[8]

OHSS is an exaggerated response to controlled ovarian stimulation (COS) characterized by the shift of protein-rich fluid from the intravascular space to the third space, mainly the abdominal cavity that occurs when the ovaries become enlarged due to follicular stimulation. This shift in fluid is due to increased vascular permeability (VP) in response to stimulation with hCG.^[9] Prostaglandins, inhibin, the renin-angiotensin-aldosterone system and inflammatory mediators have all been implicated in the etiology of OHSS.

Vascular endothelial growth factor VEGF has been characterized as a heparin binding angiogenic growth factor displaying high specificity for endothelial cells (ECs). VEGF, also known as VEGF-A, is a protein with vascular permeability activity that was originally purified from a fluid secreted by a tumor. A few years later, a protein with angiogenic activity was independently purified and named VEGF. Molecular cloning, however, revealed that these 2 proteins were identical and encoded by a single gene.^[10]

The human gene for VEGF resides on chromosome 6p21.3. The coding region spans 14 kb and contains eight exons. The VEGF family is divided into five members having a homodimer structure. VEGF belongs to the platelet-derived growth factor (PDGF) family. It shares 20% amino acid sequence identity with PDGF. Members of the PDGF/VEGF family contain eight conserved cysteine residues and therefore have a common mode of dimerization and a similar structure within their receptor-binding domains.^[11]

VEGF plays a crucial role in building up the circulatory system under physiological conditions. Furthermore, angiogenesis is a requisite for the growth and persistence of solid tumors and their metastases and severe inflammatory diseases progress to a malignant stage associated with angiogenesis. VEGF serves as a major stimulator of pathological angiogenesis. Vascular endothelial growth factor has been implicated in virtually every type of angiogenic disorder including those associated with cancer, ischemia and inflammation.^[12]

Many cytokines and growth factors upregulate VEGF m-RNA or induce VEGF release including tumor necrosis factor(TNF- α), platelet derived growth factor (PDGF), transforming growth factor (TGF- α , TGF- β), fibroblast growth factor (FGF-4), keratinocyte growth factor (KGF), IL-1a, IL-1b, IL-6 and epidermal growth factor (EGF).^[13]

At ovulation, the follicle is converted into the corpus luteum. VEGF is important in luteal angiogenesis. VEGF mRNA or protein is detectable in the granulosa cells of primordial and primary follicles, as they progressively become localized to the granulosa surrounding the oocyte and theca cells of the pre-ovulatory follicle. After ovulation, VEGF mRNA and protein expression are observed in granulosa-derived luteal cells. VEGF expression in the corpus luteum appears highest early in the luteal phase and declines after the mid-luteal phase, with little or no expression in the late corpus luteum.^[14]

Gonadotrophic hormones, particularly LH, appear to be a major regulator of angiogenesis in the ovary. The LH-stimulated luteinization of granulosa cells at the time of ovulation is associated with enhanced VEGF-expression.^[14]

VEGF is a potential mediator in the development of OHSS because it has vasoactive properties and is thought to mediate redistribution of adherents junction proteins and the loss of the endothelial cell barrier architecture. In women who develop OHSS, VEGF is expressed and produced by granulosa-lutein cells and is released into the follicular fluid in response to

hCG, increasing capillary permeability. In addition, plasma VEGF levels correlate with the clinical picture of OHSS.^[15]

During OHSS, VEGF mediates increased VP and endothelial migration at least partly through modulation and rearranging endothelial junction proteins, including vascular endothelial (VE)-cadherin and claudin 5 function. It has been suggested that tyrosine phosphorylation may be involved in the loosening of cell-cell contacts in established vessels that modulates transendothelial permeability and allows sprouting and cell migration during angiogenesis.^[15]

This study aimed to investigate the predictive value of serum VEGF at the day of hCG administration in women undergoing ICSI cycle and in high risk of OHSS.

Subjects and Methods

This is cohort study including eighty polycystic ovary (PCO) patients with the same basal characteristic, where they were attended to ART unit in the International Islamic Center for Population Studies and Research, Al-Azhar University, for intracytoplasmic sperm injection (ICSI) in the period from 2013 to 2015. All patients had the following criteria, age < 35 years old, BMI <32, normal basal hormones, and pelvic ultrasound finding showed the picture of PCO. All patients were received the stimulation protocol in the form of mid luteal long protocol (triptoline acetate 0.1mg aqueous solution of D-Trp6-GnRH: Ferring, Hoofddorp, Netherland) daily from the day 21 of the cycle and for 14 days where E2 level estimates, when it reaches a level of < 50pg/ml, gonadotropin will be started (gonal f 150iu pen for s.c. injection, Merck Serono Egypt Pharmaceutical Company). The dose was modified according to ovarian response, E2 measurements were done serially with ultrasonic monitoring during folliculometry.

According to E2 level before day of hCG administration the patients were divided into coasting and control groups.

Group A: Normal ovarian stimulation; thirty women with E2 less than 3000pg/ml before the day of hCG administration.

Group B: Ovarian hyperstimulation (Coasting group); fifty women with E2 more than 3000 pg/ml and count of large follicles more than 20 follicles in each ovary with diameter > 17mm before the day of hCG administration, then those women coasted to reach E2 level less than

3000pg/ml at the day of hCG administration (as a high risk group suspected to ovarian hyperstimulation during induction of ovulation).

When patients were considered at risk of developing OHSS coasting protocol was started and applied as follows, the gonadotropins injections were stopped and GnRH-antagonist was administrated, duration of coasting is preferred to not exceed 4 days.

Daily serum E2 was estimated and hCG was given when serum E2 fell to ≤ 3000 pg/ml. Ovum retrieval was performed 36h after hCG administration.

Data were collected for baseline (age, day 3 hormones level) and stimulation (number of follicles, oocytes, mature oocytes, embryos available, embryos transferred, embryo quality) parameters and cycle outcome (implantation rate, clinical pregnancy rate) for coasting and control group.

In addition, in the coasting group “coasting parameters” (peak E2 level, E2 level after coasting, decrease in E2, days of coasting) were evaluated.

For VEGF: Blood sample (5ml) was drawn in the morning of hCG day administration and centrifuged for 10min within 1hour of collection, supernatant (serum sample) was then transferred into an eppendorf tube and immediately stored in a freezer at -40°C .

For other hormones: Blood sample (5ml) was collected on day 3 of menstrual cycle, centrifuged for 10min within 1 hour of collection, supernatant (serum sample) was then transferred into an eppendorf tube and measured for hormonal assay.

Vascular endothelial growth factor concentrations in serum at the day of hCG administration were determined by enzyme- linked immune sorbent assay (ELISA) by AviBion, Orgenium Laboratories' human VEGF ELISA kit.

Basal hormonal assays which include day 3 FSH, LH, Prolactin, TSH, E2 and serial E2 during folliculometry were measured by enzyme-linked fluorescent assay (ELFA) by BioMérieux SA, France, VIDAS Kits assay.

Statistical Methods

The statistical analysis of the data was performed using the GraphPad Prism (version 5) and Sigma Plot version 12.5. The data were presented as mean \pm standard error of the mean

(SEM) and percentages (%). The tests used were; Student's t test: for testing statistical significant difference between means of two samples. Chi- square test (χ^2): is used to study association between two or more qualitative variables. Pearson's correlation test (correlation coefficient r): to test a positive or negative linear relationship between two variables (one dependent and the other is independent variable). Significant result is considered if $p < 0.05$. Receiver operating characteristic (ROC) curve was used to detect the cut-off value for VEGF that predict patient at risk for development of ovarian hyperstimulation syndrome.

RESULTS

This study was conducted on 80 female Egyptian PCO subjects. They were classified into two groups, control and coasting groups according to E2 level before the day of hCG administration.

Table (1): Demographic data for control and coasting groups.

	Control n=30	Coasting n=50	p-value
Age (years) Mean \pm SEM	31.15 \pm 0.8714	28.34 \pm 0.6944	0.0144*
Duration of marriage (years) Mean \pm SEM	7.167 \pm 0.6982	6.290 \pm 0.5583	0.3337
BMI (Kg/m ²) Mean \pm SEM	31.66 \pm 1.344	26.22 \pm 0.7002	0.0002***

Table (1): Demographic data of the studied subjects showed that there was no significant difference in mean \pm SEM of marriage period for control and coasting groups. However, there was significant difference in mean \pm SEM of age and mean \pm SEM of BMI (Kg/m²) for control and coasting groups.

Table (2): Laboratory data for control and coasting groups.

	Control n=30	Coasting n=50	p-value
Day3 E2 (ng/ml) Mean \pm SEM	47.67 \pm 5.607	58.68 \pm 5.527	0.1915
Day3FSH (mIU/ml) Mean \pm SEM	6.180 \pm 0.3763	6.072 \pm 0.2233	0.7927
Day3 LH (mIU/ml) Mean \pm SEM	3.480 \pm 0.3045	4.804 \pm 0.3679	0.0148 *
Day3 TSH (μ U/ml) Mean \pm SEM	2.567 \pm 0.3165	2.624 \pm 0.2296	0.8811
Day3 prolactin(ng/ml) Mean \pm SEM	19.44 \pm 1.691	22.63 \pm 1.724	0.2213
VEGF (pg/ml) Mean \pm SEM	455.1 \pm 44.26	296.9 \pm 15.53	0.0001***

Table (2): Showed no significant difference in mean \pm SEM of day3 E2 (pg/ml), mean \pm SEM of day3 FSH (mIU/ml), mean \pm SEM of day3 TSH and mean \pm SEM of day3 prolactin (ng/ml) for control and coasting groups. However, there was significant difference in mean \pm SEM of day3 LH (mIU/ml) for control and coasting groups, where coasting group has higher value of day3 LH than control but both still within normal range. Moreover, there was significant difference in mean \pm SEM of VEGF (pg/ml) for coasting and control group; where the coasting group has lower VEGF level than the control group.

Table (3): E2 level before and at the day of hCG administration for control and coasting groups.

	Control n=30	Coasting n=50	<i>p</i> -value
E2 level before day of hCG administration Mean \pm SEM	1715 \pm 120.3	6021 \pm 187.9	< 0.0001***
E2 level at the day of hCG administration Mean \pm SEM	1715 \pm 120.3	2505 \pm 87.80	<0.0001***

Table (3): E2 level before and at the day of hCG administration of the studied groups showed significant difference in mean \pm SEM. Although coasting has been done, the coasting group still has higher E2 level than control group.

Table (4): Number and diameter of follicles for control and coasting groups.

	Control n=30	Coasting n=50	<i>p</i> -value
<i>Follicle number in both ovaries</i> Mean \pm SEM	12.30 \pm 0.8525	31.86 \pm 0.7651	< 0.0001***
<i>Mean of follicle diameter (mm) in both ovaries</i> Mean \pm SEM	11.5 \pm 0.3768	20.74 \pm 0.2387	< 0.0001***

Table (4): Number and diameter of follicles which were periodically determined, patients were considered at actual risk of developing OHSS when they have large number of follicles (≥ 20) on both ovaries and the majority of follicles being (>17 mm in mean diameter).

Table (5): Correlations between VEGF and other clinical data of studied groups.

VEGF	Control		Coasting	
	<i>r</i>	<i>p</i> -value	<i>r</i>	<i>p</i> -value
Age	0.09469	0.6187	0.09979	0.4905
Duration of marriage	0.1691	0.3718	0.2896	0.0414*
BMI	0.04360	0.8190	0.04095	0.7777

Table (5): Showed that there were no significant correlation between VEGF and any of the clinical parameters. While there was significant positive correlation between VEGF and duration of marriage ($p=0.0414^*$, $r=0.2896$) in coasting group.

Table (6): Correlations between VEGF and other laboratory data of control and coasting groups.

VEGF	Control		Coasting	
	<i>R</i>	<i>p</i> -value	<i>R</i>	<i>p</i> -value
Day3 E2	-0.01483	0.9380	0.07895	0.5858
Day3 FSH	-0.1420	0.4543	0.2796	0.0492*
Day3 LH	-0.2406	0.2003	0.02929	0.8400
Day3 TSH	-0.1462	0.4409	0.1292	0.3713
Day3Prolactin	0.1786	0.3450	-0.06983	0.6299
E2 at the beginning of coasting	-----	-----	0.1501	0.2981
E2 at the day of hCG administration	0.05227	0.7838	-0.05728	0.6927
Follicle number of both ovaries	0.4469	0.0133*	0.4153	0.0027**
Mean of follicles diameter of both ovaries	0.7515	<0.0001***	0.5271	<0.0001***

Table (6): As shown in this table, there was significant positive correlation between VEGF and FSH ($p=0.0492^*$, $r=0.2796$) in coasting group. While no significant correlations between VEGF and any other day3 hormonal levels for control and coasting groups were recorded. There were no significant correlations between VEGF and E2 level before and at the day of hCG administration for coasting and control groups. There were significant positive correlations between VEGF and both of follicle number ($p=0.0133^*$, $r=0.4469$) and mean of follicle diameter (mm) ($p < 0.0001^{***}$, $r=0.7515$) in both ovaries of the control group. Also there were significant positive correlation between VEGF and both of follicle number ($p=0.0027^{**}$, $r=0.4153$) and mean of follicle diameter (mm) ($p < 0.0001^{***}$, $r=0.5271$) in both ovaries of the coasting group.

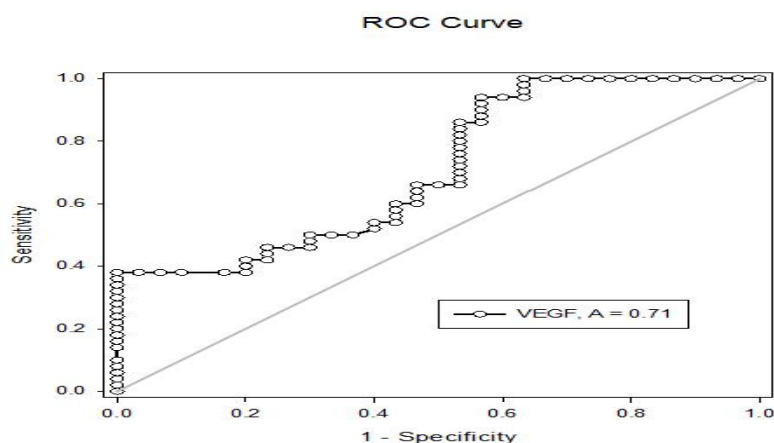


Figure (1): ROC curve of VEGF in coasting and control groups.

Figure (1): Showed ROC analysis of the levels of VEGF between coasting group and control group to determine its diagnostic accuracy. It was found that area under the curve (AUC) of the ROC curve was 0.7110. The cut-off was 222.50pg/ml which yielded sensitivity (38%), positive predictive value (PPV) (100%), this mean increasing the sample number will increase sensitivity up to (100%), specificity(100%), negative predictive value (NPV) (96.8400%) and ($p=0.001659$).

DISCUSSION

Ovarian hyperstimulation syndrome encountered in practice as an iatrogenic complication of controlled ovarian stimulation (COS). COS is aimed at producing multiple ovarian follicles during assisted conception cycles in hope of increasing the number of oocytes available for collection. OHSS, however, is characterized by an exaggerated response to this process.^[16]

It is characterized by ovarian enlargement caused by multiple ovarian cysts and the shift of intravascular fluid to the extracellular compartments, mostly into the abdominal cavity. Granulosa cell-derived VEGF has been implicated as the main factor in the pathogenesis of iatrogenic OHSS. By binding to the vascular endothelial growth factor receptor-2(VEGFR-2) on endothelial cells, VEGF increases vascular permeability, loosens intercellular junctions, leading to fluid extravasation. In granulosa cells, hCG binding upregulates the hypoxia inducible factor 1 α (HIF-1 α) and its target gene VEGF via cyclic adenosine monophosphate/protein kinase A (cAMP/PKA).^[17]

Traditionally, high or rapidly rising serum E2 levels on the day of the hCG trigger, denoting oversensitivity to hCG, was used as a predictor of OHSS. However, high E2 levels alone are poor predictors of OHSS. The number of follicles in combination with serum E2 levels predicts OHSS with high sensitivity and specificity.^[18]

In the present study there was significant difference with regard to age, the mean age of control and coasting groups was 31.5 and 28.34 respectively. This was in agreement with Delvigne and Rozenberg^[4] who reported that in most studies the younger women have an increased propensity to develop OHSS. In another study carried out by Lyons et al^[19] the age distributions were 29.7 ± 1.8 years for OHSS versus 33.9 ± 0.15 years for controls, while in a large population of 128 cases of OHSS the mean age was 30.2 ± 3.5 years for OHSS versus 32.0 ± 4.5 years for 256 controls.^[20]

A plausible explanation is that the ovaries of younger women are more responsive to gonadotrophins because they possess a higher density of gonadotrophin receptors or a larger number of follicles that are able to respond to gonadotrophins. But this small difference between the means combined with the wide spread, expressed by relatively large standard deviation, permits the concern that a usable cut-off point for age as a risk factor is not available.^[21]

In the current study there was significant difference with regard to BMI (Kg/m²), the mean BMI of control and coasting groups was 31.66 and 26.22 respectively. This was supported by ASRM, (2008) whom mentioned low BMI as a risk factor for OHSS. Also, Navot and his colleagues^[22] described a positive correlation between low BMI and OHSS. A plausible explanation is that low BMI women have increased sensitivity to ovulation induction, which makes them prone to develop ovarian hyperstimulation syndrome with relatively low-dose human menopausal gonadotropin.

In our study there was an elevation of LH/FSH ratio (>2) recorded in 16% of coasting group. This was in agreement with Delvigne et al^[20] who recorded an elevated LH/FSH ratio (>2) in 17% of OHSS patients.

It might be related to an increased frequency of premature LH surge in cases stimulated with purified FSH Mizunuma et al^[23] and/or to a longer initial period of LHRH analogue treatment as described by Smits et al.^[24] Bodis et al^[25] suggested that LH dominance leads to disturbed androgen-oestrogen conversion and to a higher propensity for OHSS. While many women with polycystic ovarian syndrome(PCOS) still have LH and FSH still within the 5-20 mIU/ml range, their LH level is often two or three times that of the FSH level.

PCOS appeared to be the major predisposing factor for OHSS in a large number of studies. An LH: FSH ratio >2 has also been considered as a risk factor for OHSS, even in the absence of other signs of PCOS.^[4]

In the current study there was significant difference in mean of E2 level at the beginning of coasting (6012±187.9) and E2 level at the day of hCG administration (2505± 87.8) for coasting group, $p < 0.0001$. This was in agreement with Elter et al^[26] and Gustofson et al^[27] who reported that women who had been switched from a GnRH-agonist in a down-regulation protocol to a GnRH-antagonist for the prevention of OHSS had a significant decrease in

serum E2 levels within 24 hours of starting the antagonist without coasting and may avoid cycle cancellation.

In the present study there was a highly significant difference in VEGF mean for coasting and control groups (p -value < 0.0001), where the coasting group has lower VEGF level than the control group. The mean \pm SEM of VEGF for coasting and control groups was 296.9 ± 15.53 and 455.1 ± 44.26 pg/ml respectively. This was in agreement with Garcia-Velasco et al^[28] who reported that the serum VEGF concentrations were significantly lower on the day of hCG administration for coasting than the control group (152 ± 60 versus 273 ± 34 pg/ml, $P < 0.05$).

Possible mechanisms to explain the decreased VEGF level in coasting group are; First: In the presence of a high E2 level in a COS cycle, most VEGF may be bound to tissue receptors, resulting in decreased detectable VEGF in follicular fluid and serum. VEGF combined with receptors may result in decrease free VEGF which increased VP and triggers OHSS.^[29]

Second: The ovary has been shown to be the primary source of VEGF secretion responsible for development of OHSS.^[30] It seems that there is a direct action of GnRH-antagonist on the ovary, which may lead to a decline of serum VEGF levels, as well as estradiol, progesterone and ovarian volume, suggesting a luteolytic effect.^[1] GnRH-antagonist has been shown to inhibit the expression of VEGF in human granulosa luteal cell cultures, supporting the hypothesis of direct action of on the ovary.^[31] Kosaka et al^[32] demonstrated that coculture of granulosa lutein cells with a GnRH-agonist or GnRH-antagonist for 48 h revealed that the concentration of VEGF in the GnRH-antagonist group was significantly lower compared with the GnRH-agonist group, and that the VEGF level is closely associated with the manifestation of OHSS.

Third: Coasting reduces VEGF gene expression and secretion by increasing granulosa-lutein cell necrosis/apoptosis, especially in small and medium (<14 mm) follicles. During the coasting period, the smaller immature follicles, which are less receptive to FSH, will undergo developmental arrest and enter necrosis/apoptosis. As a consequence, steroidogenesis will be reduced and stopped in the granulosa cells of these follicles, as reflected by declining E2 concentrations, and, similarly, vasoactive mediators, such as VEGF.^[28] This fact confirmed by observation that VEGF gene expression was also reduced in coasted granulosa-lutein cells, a decline that was even lower in small/ medium follicles when compared with mature follicles.^[33]

Fourth: It has been reported that plasma levels of soluble VEGF receptor-1 may determine the onset of early and late OHSS, and the ability to secrete soluble vascular endothelial growth factor receptor-1 (sVEGFR-1) and bind VEGF seems to be the determining factor in OHSS. Therefore, an assay to measure total and free VEGF or endocrine gland derived-VEGF and their soluble receptors synchronously for may help clarify some of the contradictions among studies.^[29]

More studies have been done to understand the real role of the different types of VEGF receptors. In some studies, the gap between VEGF and receptors is necessary for developing OHSS, and the VEGFR-2 inhibitor, like the dopamine agonist, can reverse hCG action on vascular permeability, and can also act to prevent and treat OHSS. However, other studies have associated the lower serum levels of soluble VEGFR-2 with OHSS occurrence, and the severity of the disease increases with a drop in soluble VEGFR-2. The amount of free, biologically active VEGF-A is modulated by binding to soluble VEGFR-2. Therefore, women with a large amount of soluble VEGF-R2 have a smaller quantity of free VEGF and are, therefore, at lower risk of developing severe OHSS. In contrast, women with a smaller amount of soluble VEGFR-2 exhibit higher free VEGF levels and are, therefore, at increased risk of developing OHSS.^[34]

Another study carried out by Ding et al^[35] revealed that the mean plasma VEGF concentration in women with GnRH-agonist withdrawal treatment was significantly lower than that of the control group (agonist continued) in the hCG administration day. The expression of VEGF mRNA in granulosa cells was measured by qRT-PCR showing that VEGF mRNA concentration in granulosa cells from women with GnRH-agonist withdrawal treatment was 16.5% lower compared with that from women in the control group ($p < 0.05$).

The Lainas et al^[1] study demonstrates that luteal administration of GnRH-antagonist for the management of established severe early OHSS is associated with a significant and rapid decline of high serum VEGF levels starting as early as 2 days after GnRH-antagonist administration.

VEGF plays a key role in the increase of vascular permeability, so the aim to target VEGF synthesis, bioavailability or downstream signaling is an attempt to prevent development of OHSS.^[36] The dopamine agonist cabergoline^[37] and more recently quinagolide^[38] have been shown to block VEGF signaling by inhibiting VEGFR-2 phosphorylation, leading to reduced

incidence and severity of OHSS.^[39] In addition, reduced VEGF gene and protein expression was demonstrated following coasting in women who were at high-risk for OHSS partly explaining why coasting may be effective in the prevention of OHSS^[28]

On the other hand, our results are incompatible with Gao et al^[29] who recorded no significant difference in serum VEGF concentrations. This finding may be due to the fact that VEGF is not produced only by the ovary, but also is expressed in various tissues. Because serum VEGF concentration is affected by its release from other tissues, it is impossible to reflect the primary effects of the ovary in the etiology of OHSS. Also our results do not agree with Agrawal et al^[40] study which showed that serum VEGF concentration rose significantly in all the women during ovarian stimulation and was higher on the day of hCG administration, oocyte collection and embryo transfer (ET) than in the early follicular phase (i.e., at the beginning of the IVF cycle), serum VEGF concentration rose further after hCG administration. Women in whom OHSS developed had consistently higher serum VEGF concentrations before and throughout their IVF cycles. The rise in the serum VEGF concentration that occurred between the day of hCG administration and the day of oocyte collection (subsequently referred to as the “VEGF rise”) proved to be a good marker of the development of OHSS.

In the present study there was positive correlation between VEGF and FSH of the coasting group. This was in agreement with Agrawal et al^[40] who stated that the concentrations of VEGF were elevated in serum and follicular fluid of women who had PCOS compared with women with normal ovaries.

Incubation with FSH, LH, and hCG significantly increased VEGF release by luteinized granulosa cells at all concentrations studied, so FSH stimulates VEGF production to a similar degree compared with hCG. Pituitary secreted FSH was reported to stimulate the expression of endothelial mitogen VEGF in granulosa cells.^[41]

In our study there was positive correlation between VEGF and follicle number in both ovaries of the coasting and control groups. A prompt explanation might be that VEGF mRNA and protein are expressed by human ovarian granulosa and theca cells late in follicle development and, subsequent to ovulation by granulosa and theca lutein cells. Therefore, VEGF is ideally positioned to provoke the increased permeability of thecal blood vessels that occurs shortly before ovulation.^[42]

The present study showed negative correlation between age and follicle number in both ovaries of the coasting group. This was in agreement with Kupesic et al^[43] who reported that all three-dimensional ultrasonographic variables (antral follicle count, total ovarian volume, and mean ovarian stromal flow) negatively correlated with chronologic age; in fifty-six consecutive women 22 to 43 years of age with normal basal serum FSH concentrations who were undergoing their first IVF cycle. Lass et al^[44] concluded that increasing age showed a strong negative correlation with follicular density (no./mm³) and ovarian volume ($r = -0.46$, $p = 0.0003$ and $r = -0.43$, $p = 0.0016$ respectively) in a group of 60 infertile women aged 19–45 years.

Age-related decline of fertility in women is the result of the decline in both quantity and quality of the resting ovarian follicle pool. Damage might accumulate with age. The age-related changes in the mitochondria, smooth endoplasmic reticulum, and Golgi complex suggest a role for oxidative damage. Age-related cellular damage might induce quality decline and atresia (it is accompanied by specific morphological changes in the oocyte and granulosa cells).^[45]

The proportion of resting follicles that undergo atresia is small in young women. This cellular mechanism for the qualitative decline of follicles, together with rapidly declining follicle numbers, may be responsible for declining fertility in the fourth decade of life in women.^[45]

CONCLUSION AND RECOMMENDATION

Serum VEGF estimation after coasting at the day of hCG administration in high risk patient is recommended for early prediction of OHSS in addition to E2, follicle number and follicle diameter. Moreover, it can be used as an indicator of success of coasting. Further studies for measurement of total, free and soluble receptors of VEGF for clarification of the role of VEGF in OHSS are recommended. Other studies compare coasting with and without GnRH-antagonist for more clarification the role of GnRH-antagonist on VEGF level are recommended.

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