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FOLLICULAR FLUID CONCENTRATION OF VASCULAR ENDOTHELIAL GROWTH FACTOR, INHIBIN A AND INHIBIN B IN IVF CYCLES: ARE THEY MARKERS FOR OVARIAN RESPONSE AND PREGNANCY OUTCOME?

By

Mohamed Hatem Helmy Hassan El-Husseiny, Yehia Abd El-Salam Wafa, Ahmed Samy Soliman and Wael Refaat Abd El-Hamid Hablas*

Departments of Obstetrics & Gynecology and Clinical Pathology*, Faculty of Medicine,

Al-Azhar University

***Corresponding author:** Mohamed Hatem Helmy Hassan,

E-mail: mohamedhatemhelmy16@gmail.com

ABSTRACT

Background: Vascular endothelial growth factor (VEGF) is a protein produced by many different tissues. In the ovary. VEGF is produced both by granulosa and theca cells. The granulosa cells increase production of VEGF in response to FSH, LH-hCG, as well as hypoxia. VEGF concentrations of FF at the time of oocyte retrieval for in vitro fertilization (IVF) are significantly higher in older women.

Objective: To measure concentrations of vascular endothelial growth factor (VEGF), inhibin A and inhibin B in follicular fluid (FF) of women undergoing to in-vitro fertilization (IVF) cycles, and to determine their relationship with ovarian response and pregnancy.

Patients and methods: This was a prospective randomized comparative study that was carried out at Al-Azhaar University Hospitals from January 2020 till October 2020. The study was conducted at Gynecology outpatient clinic of Al-Hussein Hospital of Al-Azahar University over 58 women were divided into two groups, based on reproductive outcome: Group (A): Women who became pregnant after embryo transfer, and Group (B): Non-pregnant women.

Results: Among the studied cases, according to pregnancy, there were 28(48.3%) pregnant, 30(51.7%) non-pregnant, according to single or multiple. There were 18(64.3%) single and 10(35.7%) twins. Among total cases, the fertilized rate was 299/527 (56.7%). According to quality; there were 243/299 (81.3%) grade 1, 56/299 (18.7%) grade 2, the number of embryos transferred were115/299 (38.5%). Among group A, the fertilized rate was 185/307 (60.3%), according to quality; there were 160/185 (86.5%) grade 1, 25/185 (13.5%) grade 2, and the number of embryos transferred were 64/185 (34.6%). Among group B, the fertilized rate was 114/220 (51.8%), according to quality; there were 83/114 (72.8%) grade 1, 31/114 (27.2%) grade 2, and the number of embryos transferred were 51/114 (44.7%). There was a statistically significant difference between studied cases as regard serum VEFG, FF VEGF, serum inhibin A, FF inhibin A, serum inhibin B and FF inhibin B.

Conclusion: Lower concentrations of serum and FF VEGF, higher concentrations of FF inhibin A and B may serve as a reliable predictive marker for pregnancy, in women undergoing IVF. All of these parameters allowed the recognition of cycles predetermined to fail, and this information may contribute to the criteria for cryopreservation of embryos to be used in future transfers.

Keywords: Follicular fluid concentration, Vascular endothelial growth factor, Inhibin A and inhibin B, IVF cycles.

INTRODUCTION

Folliculogenesis defines the progress of a primordial follicle to a mature follicle. It is a complex and well-organized process, which includes dynamic and endocrine changes. The antral follicle contains the outermost thecal layers, which contain vasculature and steroidogenic cells and synthesize and secrete androgen *(Vural et al., 2016)*. The inner granulosa cells aromatize androgen to produce estrogen. They also produce other protein hormones and secrete proteoglycan to produce an osmotic gradient and fluid-filled cavity *(Rao et al., 2019)*.

The resulting capillary network mediates the transport of oxygen, nutrients, and precursor substances. Vascularization is the primary essential step in follicular growth, and the follicular microenvironment is an essential factor in oocyte growth. A variety of parameters, including hypoxia, aging, paracrine factors, and autocrine factors, modulate angiogenesis *(Savchev et al., 2010)*.

Vascular endothelial growth factor (VEGF) (referred to also as VEGF-A) is a 45 kD heparin-binding, homodimeric glycoprotein. Alternative splicing of the VEGF gene yields four different isoforms, having 121, 165, 189 and 206 amino acids. In the ovary VEGF is expressed in granulosa and theca cells. Among the four VEGF isoforms, mRNAs encoding VEGF165 and VEGF121 are dominant in normal human ovaries *(Kudsy et al., 2016)*.

VEGF is secreted in the premenopausal human ovary in a cyclic manner and regulated by gonadotropin secretion during the menstrual cycle. Indeed, VEGF plays an important role in angiogenesis, follicular vascularization, dominant follicle selection, corpus luteum development and intrafollicular oxygenation *(Qiao and Feng, 2011)*.

Vascular endothelial growth factor is expressed in many types of tissues and is upregulated during development, tissue remodeling, wound healing, and in several human disease states *(Ranjbaran et al., 2019)*.

Follicular granulosa cells secrete two different types of inhibins, inbibin-A and inhibin-B, belonging to the transforming growth factor beta family. These inhibins have diverse actions, and their concentrations vary throughout the menstrual cycle. The level of inhibin-B increases from the luteal phase to the follicular, reaching maximum levels in the midfollicular phase. Inhibin-B reflects granulosa cell activity and follicular development. The level of inhibin-a increases in the late follicular phase. Inhibin-A is secreted by mature follicles and reflects follicular maturity *(Vural et al., 2016)*.

The aim of the present work was to measure concentrations of vascular endothelial growth factor (VEGF), inhibin A and inhibin B in follicular fluid (FF) of women undergoing to in-vitro fertilization (IVF) cycles, and to determine their relationship with ovarian response and pregnancy.

PATIENTS AND METHODS

This was a prospective randomized comparative study that was carried at Al-Azhar University Hospitals from January 2020 till October 2020. Over 58 women divided into two groups, based on

reproductive outcome: **Group (A):** Women who became pregnant after embryo transfer, and **Group (B):** Non-pregnant women.

Follicular fluid was collected from 58 patients undergoing oocyte retrieval for IVF. Ovulation was induced with GnRH analogues and gonadotropins. Follicular fluids of mature follicles (>17 mm) were aspirated and pooled for each patient. Follicular fluid steroid hormone levels (E2, P) and VEGF, inhibin A, inhibin B concentrations were studied. The serum levels of E2, P and VEGF were also assessed on the day of the oocyte retrieval. These parameters and characteristics of the cycles were compared between the pregnant (group 1) and non-pregnant (group 2) patients.

Sampling technique: This study was performed on systematic random sampling technique.

The researcher introduced himself to all participants included in this study and asked them to participate after illustrating the goal of the study. All participants received comprehensive information regarding objective and the expected benefit of the study. All of the patients have signed written informed consents to participate in the study, and the local ethics committee approved the experimental design.

All patients were subjected to:

Complete history was taken with special emphasis on:

- **Personal history:** Age, marital status, parity, address, occupation and any special habits.
- **Complaint of each woman in the study:** Period of infertility, type of infertility whether primary or secondary, hirsutism and acne.
- **Menstrual history:** with emphasis on menstrual dating and regularity.
- **Obstetric history:** History of similar condition (recurrent abortion); number of abortions, induced or spontaneous, followed by surgical evacuation or not, and if there was any post-abortive complications.
- **Contraceptive history:** (Types and duration).
- **Past history of any medical problem:** as {hypertention, diabetes mellitus and deep venous thrombosis (DVT)}, history of blood intake, allergy to certain drugs and any previous operations including cesarean section (CS).
- **Family history:** of infertility or consanguinity.

Clinical examination:

- **Physical examination**: included general examination (Weight, Height, BMI), abdominal examination, and local (pelvic) examination.
- **Investigations:**
- **General**: (CBC, urinalysis, and random blood sugar) when needed.
- **Specific:** Levels of luteinizing hormone (LH), thyroid-stimulating hormone (TSH), follicle-stimulating hormone (FSH), prolactin (Prl), estradiol (E2), and testosterone (T), were measured on days 2–4 of spontaneous menstrual cycle or gestagen-induced menstruation-like

reaction. Hormonal assays were carried out by electro- and immunochemiluminiscent methods on the automatic analyzers Cobas e 411 (F. Hoffmann-La Roche, Basel, Switzerland), Immulite 2000, and Immulite 1000 (Siemens, Los Angeles, USA) using reagents of the same companies. The immunoassays were standardized via mass spectrometry assays (isotope dilution-gas chromatography/mass spectrometry (ID-GC/MS)) according to the instructions by Roche Diagnostics. Concentrations of AMH were measured by the enzyme-linked immunoassay on the DYNEX DSX System analyzers and using the Diagnostic Products Corporation (DPC) system on the Immulite device (DYNEX Technologies, VA, and USA).

- **Ultrasound examination:** was done using Mindray 2200 plus. The women were scanned in the lithotomy position with an empty bladder.

For each individual from whom FF samples were available, only FF from a single cycle was analyzed of all in-vitro fertilization (IVF) procedures were retrospectively reviewed to collect the following data: age, duration of stimulation, duration of infertility, total ampoules of gonadotropins administered, estradiol and progesterone concentration on the day of hCG administration, number of follicle, number of oocytes retrieved, fertilization rate and pregnancy rate. Forty-three women did not become pregnant after IVF (group 2).

Pregnancy was defined by significant HCG concentrations and the observation of embryonic cardiac activity during the transvaginal ultrasound examination was performed 4 weeks or more after follicular aspiration.

All samples were run in duplicate and the sensitivity of the test was 9 pg/ml. The intra-assay and inter-assay coefficients of variation were 3, 5 and 7%, respectively. Intra and interassay coefficients of variation in serum samples were 7, 1 and 9.2%, respectively. In FF, these values were 5, 4 and 7.5%, respectively. Estradiol, progesterone and HCG concentrations in serum and FF were measured by radioimmuno-assay (RIA) (CoatA-Count DPC, Diagnostic Products Corporation, Los Angeles, USA.)

Statistical analysis:

Analysis of data was done using Statistical Pakage for the Social Science version 20 (SPSS Inc., Chicago, IL, USA). Quantitative data were described using range (minimum and maximum), mean, standard deviation, median and interquartile range (IQR). Qualitative variables were described as number and percent. In order to compare parametric quantitative variables between two groups, Student t test was performed. Qualitative variables were compared using chi-square (X2) test or Fisher's exact test when frequencies were below five. Pearson correlation coefficients were used to assess the association between two normally distributed variables. The Kolmogorov-Smirnov test was used to verify the normality of distribution. Mann-Whitney test was use for abnormally distributed quantitative variables, to compare between two studied groups. P value < 0.05 was considered significant.

RESULTS

Among the studied cases, according to pregnancy, there were 28(48.3%) pregnant, 30(51.7%) non-pregnant, according to single or multiple. There were 18(64.3%) single, 10(35.7%) twins **(Table 1)**.

Table (1): Distribution of the studied cases according to pregnancy (n = 58)

	No.	%
Pregnancy		
Pregnant	28	48.3
Non-pregnant	30	51.7
Single or Multiple (n = 28)		
Single	18	64.3
Twins	10	35.7

Among total cases, the mean of age was 32.17 (± 5.48 SD) with range (20.0 – 39.0). Among group A, the mean of age was 30.64 (± 5.71 SD) with range (20.0 – 39.0). Among group B, the mean of age was 33.60 (± 4.94 SD) with range (22.0 – 39.0). There was a statistically significant difference between studied cases as regard age **(Table 2)**.

Table (2): Comparison between the two studied groups according to age

	Total (n = 58)	Group A (n = 28)	Group B (n = 30)	p
Age (years)				
Min. – Max.	20.0 – 39.0	20.0 – 39.0	22.0 – 39.0	0.039
Mean ± SD.	32.17 ± 5.48	30.64 ± 5.71	33.60 ± 4.94	
Median (IQR)	33.0 (29.0 –37.0)	30.50(27.0 –35.0)	35.0 (31.0 –37.0)	

IQR: Inter quartile range, SD: Standard deviation, t: Student t-test
p: p value for comparing between the studied groups
Group A: Women pregnant after embryo transfer
Group B: Non-pregnant women

Among total cases, according to CASA, 35(60.3%) were normal, 15(25.9%) were asthenospermia, 7(12.1%) were teratospermia, 15(25.9%) were oligospermia, 1(1.7%) were azoo, according to cause of infertility, as regard age there was 1(1.7%), as regard male there were 25(43.1%), as regard tubal, there were 6(10.3%), as regard PGD, there were 8(13.8%), as regard unexplained, there were 13(22.4%), as regard multifactorial, there were 3(5.2%), as regard anovulation, there were 3(5.2%), as regard Luteal phase defect, there were 7(12.1%).

Among group A, according to CASA, 16(57.1%) were normal, 9(32.1%) were asthenospermia, 4(14.3%) were teratospermia, 7(25%) were oligospermia, and 0(0%) were Azoo, according to cause of infertility, and as regard age there was 0(0%), as regard male there were 13(46.4%), as regard tubal, there were 3(10.7 %), as regard PGD, there were 4(14.3%), as regard unexplained, there were 7(25%), as regard multifactorial, there were 0(0%), and as regard

anovulation, there were 1(3.6%), as regard luteal phase defect, there were 5(17.9%).

Among group B, according to CASA, 19 (63.3%) were normal, 6(20%) were asthenospermia, 3(10%) were teratospermia, 8(26.7%) were oligospermia, and 1(3.3%) were azoo, according to cause of infertility, as regard age there was 1(3.3%), as regard male there were 12 (40%), as regard tubal, there were 3(10 %), as regard PGD, there were 4(13.3%), as regard unexplained, there were 6(20%), as regard multifactorial, there were 3(10%), as regard anovulation, there were 2(6.7%), and as regard luteal phase defect, there were 2(6.7%).

There was no statistically significant difference between studied cases **(Table 3)**.

Table (3): Comparison between the two studied groups according to CASA and cause of infertility

Groups / Parameters	Total (n = 58)		Group A (n = 28)		Group B (n = 30)		p
	No.	%	No.	%	No.	%	
CASA							
Normal	35	60.3	16	57.1	19	63.3	0.630
Asthenospermia	15	25.9	9	32.1	6	20.0	0.291
Teratospermia	7	12.1	4	14.3	3	10.0	^{FE}p=0.701
Oligospermia	15	25.9	7	25.0	8	26.7	0.885
Azoo	1	1.7	0	0.0	1	3.3	^{FE}p=1.000
Cause of infertility							
Age	1	1.7	0	0.0	1	3.3	^{FE}p=1.000
Male	25	43.1	13	46.4	12	40.0	0.621
Tubal	6	10.3	3	10.7	3	10.0	^{FE}p=1.000
PGD	8	13.8	4	14.3	4	13.3	^{FE}p=1.000
Unexplained	13	22.4	7	25.0	6	20.0	0.648
Multifactorial	3	5.2	0	0.0	3	10.0	^{FE}p=0.238
Anovulation	3	5.2	1	3.6	2	6.7	^{FE}p=1.000
Luteal phase defect	7	12.1	5	17.9	2	6.7	FEp=0.246

χ2: Chi square test, FE: Fisher Exact

p: p value for comparing between the studied groups

Group A: Women pregnant after embryo transfer

Group B: Non-pregnant women

There was a statistically significant difference between studied cases as regard hormonal scan **(Table 4)**.

Table (4): Comparison between the two studied groups according to basic hormonal scan

Groups / Basic hormonal scan	Total (n = 58)	Group A (n = 28)	Group B (n = 30)	p
FSH (mlu/ml)				
Min. – Max.	4.0 – 8.90	4.20 – 7.20	4.0 – 8.90	0.157
Mean ± SD.	5.93 ± 1.08	5.72 ± 0.85	6.12 ± 1.24	
Median (IQR)	5.90 (5.20 –6.50)	5.75 (5.20 –6.30)	5.90 (5.50 –7.0)	
LH (mlu/ml)				
Min. – Max.	2.90 – 8.70	2.90 – 7.50	3.20 – 8.70	0.047
Mean ± SD.	5.76 ± 1.49	5.36 ± 1.32	6.13 ± 1.56	
Median (IQR)	5.70 (4.70 –6.60)	5.30 (4.35 –6.30)	5.75 (5.10 –7.0)	
E2 (mlu/ml)				
Min. – Max.	15.0 – 49.0	15.0 – 49.0	18.0 – 43.0	0.417
Mean ± SD.	31.29 ± 8.91	32.29 ± 9.56	30.37 ± 8.33	
Median (IQR)	31.50(22.0 –39.0)	32.0(25.50–39.50)	31.50(22.0 –37.0)	
PRL (mlu/ml)				
Min. – Max.	11.0 – 52.0	12.0 – 45.0	11.0 – 52.0	0.602
Mean ± SD.	30.40 ± 10.39	31.14 ± 10.09	29.70 ± 10.78	
Median (IQR)	28.0 (24.0 –41.0)	29.50(23.0 –42.0)	27.50(24.0 –39.0)	

IQR: Inter quartile range, SD: Standard deviation
p: p value for comparing between the studied groups
Group A: Women pregnant after embryo transfer
Group B: Non-pregnant women

There was a statistically significant difference between studied cases as regard dose and duration of stimulation **(Table 5)**.

Table (5): Comparison between the two studied groups according to dose and duration of stimulation (days)

Groups / Parameters	Total (n = 58)		Group A (n = 28)		Group B (n = 30)		p
	No.	%	No.	%	No.	%	
Dose (HMG/D)							
150	6	10.3	1	3.6	5	16.7	$^{MC}p=$ 0.017
225	20	34.5	15	53.6	5	16.7	
300	14	24.1	5	17.9	9	30.0	
325	1	1.7	0	0.0	1	3.3	
375	14	24.1	7	25.0	7	23.3	
450	3	5.2	0	0.0	3	10.0	
Min. – Max.	150.0 –450.0		150.0 –375.0		150.0 –450.0		0.297
Mean ± SD.	284.91 ± 81.78		273.21 ± 68.36		295.83 ± 92.40		
Median (IQR)	300.0 (225.0 –375.0)		225.0 (225.0 –337.5)		300.0 (225.0 –375.0)		
Duration of stimulation (days)							
Min. – Max.	9.0 – 13.0		9.0 – 12.0		9.0 – 13.0		0.017
Mean ± SD.	10.66 ± 0.93		10.36 ± 0.73		10.93 ± 1.01		
Median (IQR)	11.0 (10.0 –11.0)		10.0 (10.0 –11.0)		11.0 (10.0 –12.0)		

IQR: Inter quartile range, SD: Standard deviation

$\chi 2$: Chi square test, MC: Monte Carlo

p: p value for comparing between the studied groups

Group A: Women pregnant after embryo transfer

Group B: Non-pregnant women

There was astatistically significant difference between studied cases as regard number of oocytes and quality **(Table 6)**.

Table (6): Comparison between the two studied groups according to number of oocytes

Parameters \ Groups	Total (n = 58)	Group A (n = 28)	Group B (n = 30)	p
No. of oocytes				
Min. – Max.	1.0 – 22.0	4.0 – 22.0	1.0 – 18.0	0.004
Mean ± SD.	9.09 ± 5.0	10.96 ± 4.86	7.33 ± 4.52	
Median (IQR)	8.0 (6.0 –13.0)	10.0 (7.0 –14.0)	6.50 (5.0 –9.0)	
Quality				
M1				
Min. – Max.	0.0 – 10.0	0.0 – 10.0	0.0 – 6.0	0.152
Mean ± SD.	2.93 ± 2.38	3.39 ± 2.51	2.50 ± 2.19	
Median (IQR)	2.0 (1.0 –5.0)	3.0 (2.0 –5.0)	2.0 (1.0 –4.0)	
M2				
Min. – Max.	0.0 – 15.0	2.0 – 15.0	0.0 – 12.0	0.004
Mean ± SD.	5.95 ± 3.78	7.29 ± 3.71	4.70 ± 3.45	
Median (IQR)	5.50 (3.0 –8.0)	7.0 (4.0 –9.0)	3.50 (2.0 –6.0)	
GV				
Min. – Max.	0.0 – 2.0	0.0 – 2.0	0.0 – 1.0	0.755
Mean ± SD.	0.21 ± 0.55	0.29 ± 0.71	0.13 ± 0.35	
Median (IQR)	0.0 (0.0 –0.0)	0.0 (0.0 –0.0)	0.0 (0.0 –0.0)	

IQR: Inter quartile range, SD: Standard deviation
p: p value for comparing between the studied groups
Group A: Women pregnant after embryo transfer, Group B: Non-pregnant women

There was a statistically significant difference between studied cases as regard quality (grade 1) and number of embryos transferred. There was a statistically significant difference between studied cases as regard fertilized rate **(Table 7)**.

Table (7): Comparison between the two studied groups according to embryos in day 3

Embryos in day 3	Total (n = 58)	Group A (n = 28)	Group B (n = 30)	P
Fertilized rate				
Min. – Max.	1.0 – 14.0	2.0 – 14.0	1.0 – 10.0	0.001
Mean ± SD.	5.16 ± 3.30	6.61 ± 3.17	3.80 ± 2.85	
Median (IQR)	4.0 (2.0 –7.0)	6.0 (4.0 –8.0)	3.0 (2.0 –6.0)	
Quality				
Grade 1				
Min. – Max.	0.0 – 11.0	2.0 – 11.0	0.0 – 9.0	<0.001
Mean ± SD.	4.19 ± 2.97	5.71 ± 2.68	2.77 ± 2.51	
Median (IQR)	3.50 (2.0 –6.0)	6.0 (3.0 –8.0)	2.0 (1.0 –4.0)	
Grade 2				
Min. – Max.	0.0 – 4.0	0.0 – 3.0	0.0 – 4.0	0.215
Mean ± SD.	0.97 ± 1.01	0.89 ± 1.13	1.03 ± 0.89	
Median (IQR)	1.0 (0.0 –1.0)	0.50 (0.0 –1.0)	1.0 (1.0 –1.0)	
No. of embryos transferred				
Min. – Max.	1.0 – 3.0	2.0 – 3.0	1.0 – 3.0	<0.001
Mean ± SD.	1.98 ± 0.63	2.29 ± 0.46	1.70 ± 0.65	
Median (IQR)	2.0 (2.0 –2.0)	2.0 (2.0 –3.0)	2.0 (1.0 –2.0)	

IQR: Inter quartile range, SD: Standard deviation
p: p value for comparing between the studied groups
Group A: Women pregnant after embryo transfer, Group B: Non-pregnant women

There was a statistically significant difference between studied cases as regard quality grade 1 and 2 **(Table 8)**.

Table (8): Comparison between the two studied groups according to embryos in day 3

Groups / Embryos in day 3	Total (n = 58)	Group A (n = 28)	Group B (n = 30)	p
Fertilized rate	299/527 (56.7%)	185/307 (60.3%)	114/220 (51.8%)	0.054
Quality				
Grade 1	243/299 (81.3%)	160/185 (86.5%)	83/114 (72.8%)	0.003
Grade 2	56/299 (18.7%)	25/185 (13.5%)	31/114 (27.2%)	0.003
No. of embryos transferred	115/299 (38.5%)	64/185 (34.6%)	51/114 (44.7%)	0.080

$\chi 2$: Chi square test
p: p value for comparing between the studied groups
Group A: Women pregnant after embryo transfer, Group B: Non-pregnant women

There was a statistically significant difference between studied cases as regard serum VEFG, FF VEGF, serum inhibin A, FF inhibin A, serum inhibin B and FF inhibin B **(Table 9)**.

Table (9): Comparison between the two studied groups according to follicular fluid and serum concentrations of different markers

	Total (n = 58)	**Group A (n = 28)**	**Group B (n = 30)**	**p**
Serum VEFG (pg/ml)				
Min. – Max.	176.4 – 1463.9	176.4 – 715.1	227.6 – 1463.9	0.041
Mean ± SD.	465.1 ± 270.9	382.8 ± 152.0	542.0 ± 331.7	
Median (IQR)	374.5 (265.6 –565.1)	365.1 (230.0 –518.8)	468.9 (277.4 –697.3)	
FF VEGF (pg/ml)				
Min. – Max.	125.2 – 4318.6	125.2 – 3301.9	328.6 – 4318.6	0.038
Mean ± SD.	1561.2 ± 1048.9	1270.4 ± 863.1	1832.7 ± 1144.9	
Median (IQR)	1165.2 (877.4 –2126.3)	1007.0 (722.4 –1592.4)	1658.6 (1004.1 –2421.9)	
Serum inhibin A (pg/ml)				
Min. – Max.	106.6 – 1343.1	304.2 – 1343.1	106.6 – 921.3	0.037
Mean ± SD.	503.7 ± 227.2	561.6 ± 232.7	449.8 ± 211.7	
Median (IQR)	445.4 (361.5 –582.1)	489.0 (416.1 –660.6)	421.8 (324.3 –543.5)	
FF inhibin A (pg/ml)				
Min. – Max.	559.0 – 15641.0	1319.0 – 13340.0	559.0 – 15641.0	0.048
Mean ± SD.	5479.1 ± 3677.3	6099.9 ± 3309.2	4899.6 ± 3957.8	
Median (IQR)	4361.5 (2404.0 –8026.0)	5640.0 (3452.0 –8031.5)	2593.0 (2234.0 –6683.0)	
Serum inhibin B (pg/ml)				
Min. – Max.	121.4 – 1563.0	216.2 – 1352.1	121.4 – 1563.0	0.045
Mean ± SD.	502.2 ± 382.3	516.7 ± 263.9	488.6 ± 471.2	
Median (IQR)	373.6 (238.4 –592.4)	459.5 (320.6 –643.9)	315.0 (175.3 –503.8)	
FF inhibin B (pg/ml)				
Min. – Max.	521.6 – 387233.5	6701.5 – 387233.5	521.6 – 127612.3	0.008
Mean ± SD.	26165.9± 51772.4	35751.7± 70160.1	17219.1± 22668.3	
Median (IQR)	14781.0 (9701.0 – 24949.7)	19258.2 (13279.7 – 33012.2)	11980.8 (9434.5 –15249.7)	

IQR: Inter quartile range, SD: Standard deviation
p: p value for comparing between the studied groups
Group A: Women pregnant after embryo transfer
Group B: Non-pregnant women

DISCUSSION

The present study showed that among the studied cases, according to pregnancy, were 48.3% pregnant, 51.7% non-pregnant, according to single or multiple, there were 64.3% single, and 35.7% twins.

From the results of this study, among total cases, according to CASA, 60.3% were normal, 25.9% were asthenospermia,

12.1% were teratospermia, 25.9% were oligospermia, 1.7% was azoospermia. There was no statistically significant difference between studied cases.

Semen analysis is an imperfect tool but remains the cornerstone of the investigation of male infertility. It must be performed to a consistently high standard in order to evaluate descriptive parameters of the ejaculate *(Vasan, 2011)*.

The results demonstrated that according to cause of infertility, as regard age there was 1.7%, as regard male there were 43.1%, as regard tubal, there were 10.3%, as regard PGD, there were 13.8%, as regard unexplained, there were 22.4%, as regard multifactorial, there were 5.2%, as regard anovulation, there were 5.2%, as regard luteal phase defect, there were 12.1%. There was no statistically significant difference between studied cases. Our results agree with those of *Siristatidis et al. (2020)* who reported that among infertile couples, male factor was the most common cause (51%), followed by unexplained infertility, tubal factor infertility and anovulation.

In this study, among group A, according to FSH, the mean was 5.72, according to LH, the mean was 5.36, according to E2, the mean was 32.29, according to PRL, the mean was 31.14. Among group B, according to FSH, the mean was 6.12, according to LH, the mean was 6.13, according to E2, the mean was 30.37, according to PRL, the mean was 29.70. There was a statistically significant difference between studied cases as regard LH.

Our results were supported by another study where, according to FSH, the mean was 5.9 and 7.0, according to LH, the mean was 3.8 and 5.0, according to E2, the mean was 41.6 and 48.9, according to PRL, the mean was 10.4 and 14.0 for pregnant and non-pregnant groups respectively *(Vural et al., 2016)*.

In clinical practice, serum or plasma estradiol is an established variable of follicular development. A close correspondence has been reported between circulating inhibin and estradiol concentrations in women undergoing ovulation induction in the IVF program suggesting that both circulating inhibin and estradiol can be used as a monitoring indicator of ovarian response during ovulation induction *(Pan et al., 2015)*.

Although serum estradiol has been used to monitor the follicular growth during controlled ovarian stimulation, estradiol levels in FF may not play a role in predicting the quality of oocytes and embryos *(Pan et al., 2015)*. This is supported by the results from the present study that estradiol levels in FF were not statistically different between pregnant and non-pregnant women.

The current study results showed that among group A, according to dose of HMG, the mean was 273.21, according to Duration of stimulation, the mean was 10.36. Among group B, according to dose of HMG, the mean was 295.83, according to duration of stimulation, the mean was 10.93, and there was statistically significant difference between studied cases as regard dose and duration of stimulation.

In contrast, there was no statistically significant difference between studied cases as regard dose and duration of stimulation as according to dose of HMG. The mean was 2311 and 2904. According

to duration of stimulation, the mean was 10 and 10.6 for pregnant and non-pregnant groups respectively *(Vural et al., 2016)*.

Among total cases, according to number of oocytes, the mean was 9.09. the mean was 9.88 and the mean was 11.33 *(Savchev et al., 2010)*. *Ranjbaran et al. (2019)* among total cases, according to number of oocytes.

Among group A, according to number of oocytes, the mean was 10.96. Among group B, according to number of oocytes, the mean was 7.33.

There was a statistically significant difference between studied cases as regard number of oocytes and quality.

This result agreed with what of *Ocal et al. (2011)* who stated that there was a statistically significant difference between the total number of oocytes retrieved in the two groups for the pregnant women versus for the non-pregnant women.

There was a statistically significant difference between studied cases as regard fertilized rate [Among group A, and among group B]. This finding was similar to *Vural et al. (2016)* who stated, there was a statistically significant difference between the fertilization rate in the two groups.

In contrast, there was no statistically significant difference between the fertilization rate in the two groups *(Ocal et al., 2011)*.

There was a statistically significant difference between studied cases as regard embryo quality grade 1. In contrast, there was no statistically significant difference between the embryo quality grade 1 in the two groups *(Ocal et al., 2011)*.

There was statistically significant difference between studied cases as regard number of embryos transferred.

Our results were supported by another study where there was a statistically significant difference between the total numbers of embryos transferred *(Ocal et al., 2011)*.

There was a statistically significant difference between studied cases as regard serum VEFG, FF VEGF, serum inhibin A, FF inhibin A, Serum inhibin B and FF inhibin B.

Our finding was in agreement with *Orief et al. (2014)* and *Kudsy et al. (2016)*, who found lower FF VEGF levels in the group of pregnancy than those from the group of no pregnancy in both polycystic ovary syndrome (PCOS) and control women, and to *Asimakopoulos et al. (2012)*, who found that elevated concentrations of VEGF in follicular fluid correlate negatively with conception rates in assisted reproductive technologies.

In contrast, *Kudsy et al. (2016)* found that serum VEGF levels were not significantly different between pregnant and non-pregnant group in both PCOS and control patients.

Elevated VEGF concentrations in FF and serum were associated with poor conception rates. Pooled FF from multiple follicles was used to negate the reported variation in VEGF and inhibin concentrations in individual follicles as well as to be consistent with the use of ‘‘pooled embryo transfers’’ in determining pregnancy outcome *(Ocal et al., 2011)*.

Under these conditions, elevated FF VEGF concentrations were associated with fewer follicles, fewer oocytes retrieved, fewer mature oocytes, and fewer embryos and then reduced pregnancy rates. These findings consist with ovarian aging or decreased ovarian reserve *(Hafner et al., 2018)*.

Previous study showed that inhibin A and inhibin B reflect ovarian function in assisted reproduction but are less useful at predicting outcome. In addition, changes in FF inhibin B levels correlate closely with the pattern of circulating inhibin B, FF inhibin B may reflect the ovarian response and further predict the quality of embryo in women undergoing stimulation of ovulation for IVF programs *(Enskog et al., 2010)*.

CONCLUSION

Lower concentrations of serum and FF VEGF, higher concentrations of FF inhibin A and B may serve as a reliable predictive marker for pregnancy in women undergoing IVF. All of these parameters allowed the recognition of cycles predetermined to fail, and this information may contribute to the criteria for cryopreservation of embryos to be used in future transfers.

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تركيزات السائل الجريبي لعامل نمو البطانة الوعائية، الإنهيبين A والإنهيبين B في دورات التلقيح الاصطناعي: هل هي علامات من أجل استجابة المبيض ونتائج الحمل؟

محمد حاتم حلمي حسن الحسيني، يحي عبدالسلام وفا، أحمد سامى سليمان،

وائل رفعت عبدالحميد حبلص*

قسمي أمراض النساء و التوليد و الباثولوجيا الاكلينيكية*، كلية الطب، جامعة الازهر

E-mail: mohamedhatemhelmy16@gmail.com

خلفيـــة البحـــث: عامـــل النمـــو البطـــاني الوعـــائي هـــو بـــروتين تنتجـــه العديـــد مـــن الأنســـجة المختلفـــة. فـــي المبـــيض، يـــتم إنتـــاج عامـــل النمـــو البطـــاني الوعـــائي بواســـطة كـــل مـــن الخلايـــا الحبيبيـــة وخلايـــا ثيكـــا. تزيـــد الخلايـــا الحبيبيـــة مـــن إنتـــاج عامـــل النمـــو البطـــاني الوعـــائي اســـتجابةً للهرمـــون المحفـــز لتكـــوين الحويصـــلات، و هرمـــون المحفـــز للجســـم الأصـــفر، وهرمـــون الحمـــل، ونقـــص الأكســـجة. وأظهـــرت العديـــد مـــن المجموعـــات البحثيـــة تركيـــزًا عاليًـــا جـــدًا مـــن عامـــل النمـــو البطـــاني الوعـــائي داخـــل الســـائل الجريبـــي بعـــد تحفيـــز الجونـــادوتروبين وإدارة هرمـــون الحمـــل. عـــلاوة علـــى ذلـــك، فـــإن عامـــل النمـــو البطـــاني الوعـــائي مفيـــد فـــي تحويـــل الجريـــب اللاوعـــائي نســـبيًا قبـــل التبـــويض إلـــى واحـــد مـــن أكثـــر أعضـــاء الأوعيـــة الدمويـــة، وهـــو الجســـم الأصـــفر. وتركيـــزات عامـــل النمـــو البطـــاني الوعـــائي مـــن الســـائل الجريبـــي فـــي وقـــت إســـترجاع البويضـــات مـــن أجـــل الإخصاب في المختبر أعلى بشكل ملحوظ عند النساء الأكبر سنًا.

الهـــدف مـــن البحـــث: قيـــاس تركيـــزات عامـــل النمـــو البطـــاني الوعـــائي، وإنهيبـــين A و B فـــي الســـائل الجريبـــي للنســـاء اللـــواتي يخضـــعن لـــدورات الإخصـــاب فـــي المختبـــر وتحديـــد علاقتها مع استجابة المبيض و حمل.

المريضـــات وطـــرق البحـــث: هـــذه دراســـة مقارنـــة عشـــوائية مســـتقبلية أجريـــت فـــي مستشـــفيات جامعـــة الأزهـــر مـــن ينـــاير 2020 حتـــى أكتـــوبر 2020, وتـــم تقســـيم 58 ســـيدة إلـــى مجمـــوعتين. علـــى أســـاس النتيجـــة الإنجابيـــة: **المجموعـــة (أ):** النســـاء اللـــواتي حملن بعد نقل الأجنة، و**المجموعة (ب):** النساء غير الحوامل.

نتائج البحث: من بين الحالات التي تمت دراستها، وبحسب الحمل، كان هناك 28 (48.3٪) حامل، 30 (51.7٪) غير حامل، حسب فردية أو متعددة، وكان هناك 18 (64.3٪) فرداً، 10 (35.7٪) توأم. من بين مجموع الحالات، كان متوسط العمر 32.17، وكان هناك فرق ذو دلالة إحصائية بين الحالات المدروسة فيما يتعلق بالعمر. لم يكن هناك فرق ذو دلالة إحصائية بين الحالات المدروسة حسب CASA وسبب العقم. بين المجموعة (أ) كان المعدل المخصب 307/185 (60.3٪) حسب الجودة. كان هناك 185/160 (86.5٪) درجة 1، 185/25 (13.5٪) درجة 2، عدد الأجنة المنقولة كان 185/64 (34.6٪). وفي المجموعة ب كان معدل التخصيب 220/114 (51.8٪) حسب الجودة. كان هناك 114/83 (72.8٪) درجة 1، 114/31 (27.2٪) درجة 2، عدد الأجنة المنقولة كان 114/51 (44.7٪). وتوجد فروق ذات دلالة إحصائية بين الحالات المدروسة فيما يتعلق بالجودة في الصفين الأول والثاني. وكان هناك فرق ذو دلالة إحصائية بين الحالات المدروسة فيما يتعلق بمصل عامل النمو البطاني الوعائي و السائل الجريبي عامل النمو البطاني الوعائي و مستوي الإنهيبين A في الدم و السائل الجريبي الإنهيبين A و الإنهيبين A و B و السائل الجريبي الإنهيبين B.

الاستنتاج: هناك تركيزات منخفضة من عامل نمو بطانة الأوعية الدموية في المصل والسوائل الجريبية، وقد تكون التركيزات الأعلى من إنزيم السائل الجريبي A و B بمثابة علامة تنبؤية موثوقة للحمل، وعند النساء اللائي يخضعن للإخصاب في المختبر. وتسمح كل هذه المعلومات بالتعرف على الدورات المحددة مسبقًا للفشل، وقد تساهم هذه المعلومات في معايير حفظ الأجنة بالتبريد لاستخدامها في عمليات النقل المستقبلية.

الكلمات الدالة: تركيز السائل الجريبي، عامل نمو بطانة الأوعية الدموية، إنهيبين A و B، دورات أطفال الأنابيب.

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