Assessment of Follicular-Fluid Neurotrophin Levels as Predictor for Ovarian Reserve in Women Undergoing Assisted Reproductive Technology for Different Etiologies of Infertility

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ABSTRACT
Background: brain-derived neurotrophic factor (BDNF) is a27-kDa polypeptide that belongs to the neurotrophin family binding with high-affinity protein kinase receptors (Trk) and the unselective p75NGFR receptor. The BDNF gene has a complex structure with multiple regulatory elements and four promoters that are differentially expressed in the central or peripheral tissue. **Aim of the Work:** this study aimed to detect affection of follicular fluid neurotrophin level in different types of infirtility and its using as peredictor for ovarian reserve.
Patients and Methods: this crosssectional study was carried out in Ain Shams University, Maternity Hospitals Assisted Reproductive Technology Unit (ART unit) in the period from Septemper2016 to June 2017.
Results: the present study showed a cut-off value of BDNF for the patients who underwent this study was 2.7 pg/ml with sensitivity of 74.2%, specificity 95.5%, PPV 85.2% and NPV 91.4%. **Conclusion:** FF-BDNF measurement on the d-OPU in women with different types of infertility underwent COH for IVF-ET, had positive correlation with ovarian reserve and positive predictor of pregnancy which occurred in 25.8% of cases. **Recommendations:** BDNF has positive correlation with ovarian reserve and positive correlation with pregnancy outcome so we recommend to measure serum BDNF before starting IVF cycle as predictive factore and cost effective.
Keywords: follicular-fluid neurotrophin, predictor, ovarian reserve, ART, infertility.

INTRODUCTION

Growing evidence indicated that Brain-Derived Neurotrophic Factor (BDNF) was a27-kDa polypeptide that belonged to the neurotrophin family binding with high-affinity protein kinase receptors (Trk) and the unselective p75NGFR receptor. The BDNF gene has a complex structure with multiple regulatory elements and four promoters that are differentially expressed in central or peripheral tissues [1]. It is well known to play an important role in the survival, differentiation and outgrowth of select peripheral and central neurons during development and in adulthood [2,3].

Neurotrophins are a family of growth factors that are involved in the development of the central and peripheral nervous system [4]. Although they were initially thought to be restricted to the nervous system, it is well known that they affect non-neuronal cells, as cells of the endocrine system [5]. Brain-derived neurotrophic factor (BDNF), is a major member of the neurotrophin family and together with its receptor, it is found in both rodent and mammalian (including human) ovaries. It has a wide range of functions in the ovary, from support of early survival of germ cells to control of steroidogenesis and extrusion of polar bodies, as well as ovulation [6]. Moreover, it has been shown that *in vitro* BDNF treatment of bovine oocytes leads to the development of more parthenogenetic embryos compared to the case of controls [7]. Similarly, BDNF has been shown to be important in mouse oocyte development into preimplantation embryos because it promotes the

nuclear and cytoplasmic maturation of oocyte [8]. These findings demonstrate that neurotrophins are expressed in human ovaries and strongly suggest that they play a role in folliculogenesis and cytoplasmic competence of oocytes [6].

This is further supported by a study which showed that plasma BDNF levels change during the menstrual cycle and that concentrations fall steadily after menopause [9].

Follicular fluid BDNF levels are different for each etiological factor of infertility as patients with a history of endometriosis had significantly lower mean levels of follicular fluid BDNF compared to the control group. Patients with diminished ovarian reserve had lower levels of BDNF compared to the control group. Follicular fluid BDNF levels were not different between the control groups either in women with PCOS or unexplained infertility [10].

AIM OF THE WORK

This study aimed to detect affection of follicular fluid neurotrophin level in different types of infirtility and its using as peredictor for ovarian reserve.

PATIENTS AND METHODS

This cross-sectional study was carried out in Ain Shams University Maternity Hospitals, Assisted Reproductive Technology Unit (ART unit) in the period from Septemper 2016 to June 2017.

Protocol approval by ethical committee

Before the beginning of the study and in accordance with the local regulations followed, the protocol and all corresponding documents were declared for Ethical and Research approval by the Council of Obstetrics and Gynecology Department, Ain Shams University.

Sample size justification

Sample size was calculated using EpiInfo® version 6.0, setting the power (β) at 80% and the significance level (α) at 0.05. Data from a previous study [10], indicated that patients with a history of endometriosis had significantly lower mean levels of follicular-fluid BDNF compared with the control group. Patients with diminished ovarian reserve had lower levels of BDNF compared to the control group. Calculation according to these values produced a minimal sample size of 106 cases. Therefore, the total sample size was approximately be 120 cases.

Inclusion criteria

1- Age between: 25-40 years.
2- 1ry infertility: inability to conceive after 1year of continuous marital life without the use of any contraceptive method.
3- Women undergoing assisted reproductive technology for 1st time.
4- Both ovaries present deprived of morphological abnormalities and adequately visualized in TV U/S scan.

Exclusion criteria

1- Any growth lesion found in the ovaries and uterus as visualized by TV U/S scan.
2- Signs or symptoms of disturbed endocrinological functions (as hyperandrogenism, hypo or hyperthyroidism, hyperprolactinemia).

Patient were classified to two groups

1- Group I (60 patient).
Women with male-factor infertility and they were diagnosed by abnormal semen analysis and no other causes.

2- Group II (60 patient).
Women with different etiologies of infertility eg. unexplained infertility which was diagnosed when routine tests for infertility workup didn't reveal any abnormality, patients with history of endometriosis were diagnosed by laparoscopy and patients with history of PCO were diagnosed by abnormal hormonal profile and TV U/S.

For each patient in the study the following was done:

1) Informed consent was taken after explaining the nature of procedure.

2) Full history taking: including personal history, past medical and surgical history (addendium I), with special emphasis on:

- Indication for ART.
- History of previous courses of induction.
- Previous history of ART trials.
- History (past or present) of uterine, or ovarian anomalies, or endocrinopathies.

3) Physical examination:

a- **General examination**: for weight, height, BMI, signs of PCOD, hypo or hyperthyroidism.

b- **Abdominal** and **pelvic** examination (addendum I).

4) Baseline trans-vaginal (2D) ultrasonography:

- Done on cycle day 2.
- Using a machine: Mindary DP 8800 Plus (using 5-9 MHZ multifrequency transvaginal probe).
- Aim is to measure detect morphological changes in ovary and uterus to evaluate the number and size of early antral follicles and to calculate the mean ovarian volume (MOV). Follicles measuring from 2 to 10 mm in mean diameter in both ovaries were counted. Ovarian volume was calculated according to the formula for an ellipsoid (0.526 x length x height x width).

5) Baseline hormonal profile:

- On cycle day 2-3: a sample of 3 ml of blood was extracted from each patient
- The blood sample was used for assay of FSH, LH, TSH, E2 and PRL.

6) Protocol for controlled ovarian hyperstimulation (COH):

Patients were induced according to the long GnRH-agonist protocol (the protocol followed in ART Unit Ain Shams University) as follows:

- Long GnRH agonist protocol started in the mid-luteal phase, in the 7th days after ovulation (CD 21 of the previous cycle), daily S.C. injections with triptoreline acetate (Decapeptyl. 0.05 mg/day; Ferring pharmaceuticals, Kiel, Germany) were started.

- On day 3 of the next cycle, ovarian hyperstimulation was started with daily S.C. injections of a dose of 150-225 IU HMG (Menogon 75 lU/ampoule; Ferring pharmaceuticals. Kiel, Germany). The starting dose of the gonadotrophins was prescribed according to the age, body built of the subjects. Then the dose was adjusted according to the ovarian response detected by folliculometry.

7) Follow up folliculometry and subsequent management

Transvaginal sonography (TVS) was started on day 9 and repeated every other day till the moment when the leading follicle reached a diameter of 16 mm then was performed daily till the largest follicle reached a diameter of >18 mm. The maximum duration of HMG administration was not allowed to exceed 16 days. When the largest follicle reached a diameter of >18 mm, Menogon and Decapeptyl were discontinued and 10.000 1U of hCG (Pregnyl. 10.000 IU/ampoule: Organon, Oss, Netherlands) were administered. On the

day of hCG TVS was performed to count all follicles >10 mm (expressed as the total number of follicles). The protocol was approved by ART unit in Ain Shams University Maternity Hospital.

8) Ovum retrieval

All patients attend Assisted Reproduction Unit for the planned oocyte retrieval 36 hours after hCG injection, the d-(OPU).

Steps:

- Patient was fasting.
- The patient, emptied the urinary bladder, positioned in lithotomy position.
- A venous indwelling cannula was placed for intravenous administration of fluids and medications.
- General anesthesia was used in all patients.
- The vagina was washed by physiological saline.
- The transducer was connected to the ultrasound system.
- The direction of the guide beam was checked. The puncturing needle was connected to an aspiration/ flushing apparatus attached by a fixation ring to the front and rear ends of the vaginal transducer, thereby defining the direction of puncture corresponding to the guide beam on the ultrasound image.
- The aspiration/washing apparatus was checked using test tubes.
- The uterus, both ovaries and the iliac vessels were identified by the visualization in both planes. The distance between the upper pole of the vagina and ovary was closely evaluated (care was taken to avoid vascular or intestinal interposition).
- Depth localization of the nearest accessible follicle was done.
- Needle was pushed forcefully forward to the center of the follicle.
- Needle tip ridges are shown in the ultrasound image.
- While keeping the needle in place, the adjacent follicles were punctured without retracting the needle from the ovary between aspirations.
- The contents of the follicle were aspirated using an automatic suction apparatus.
- The aspirated follicular fluid (FF) was assessed (serous, blood tinged, bloody).
- No flushing was done as not to dilute follicular fluid.
- The tube with the follicular fluid were closed and passed on to the biologist. Ultrasound monitoring was done for any intra-abdominal bleeding.
- Speculum is introduced to evaluate any bleeding from the posterior vaginal fornix. The patient was monitored for 2 hours (risk of bleeding).

9) Sampling for BDNF: the aspirated fluid was subjected to:

a. Centrifugation: centrifuged at 3000 x g for 15 min at 4º C to eliminate cellular elements.

b. Freezing at (-80 ºC) for centralized hormonal analysis. Time elapsed between follicular aspiration and FF cryo preservation not exceeded 30 min.

c. Put in aliquots for analysis.

10) Then FF-BDNF was measured by

a. Using the Immunatech Enzyme Immune Assay kit (Bechman-Caulter, France) as described by the manufacturer. All samples were assayed at the same time to minimize intra-assay variation.

b. The results of the ELISA measured in ng/ml

c. With normal reference range (2.0 - 6.8 pg/ml) were analyzed in the National Research Inistitude.

11) Fertilization and embryo grading

The oocytes were placed in culture medium and intracytoplasmic sperm injection was performed using an Olympus (CK40 inverted phase microscope and micromanipulating equipment. The injected oocytes were incubated at $37 \circ C$. fertilization was diagnosed by the presence of two pronuclei in the injected oocytes.

- After insemination IVF embryo grading: Approximately 48-72 hrs after insemination/ injection, the cell number and morphology of each embryo by using the protocol of embryo evaluation in ART unit Ain Shams University.

The embryo inspections were routinely performed in daily intervals, 40-44 hours and 64-68 hours following the insemination or fertilization by ICSI. Embryos were categorized into classes, depending on several morphological parameters which were evaluated at that time. Beside the number of cells, the appearance of blastomeres and the presence of cytoplasm defects or fragmentation are the most often criteria used. The results of scoring were usually coded. Our laboratory had applied the following coding system.

• First symbol (digit) was corresponded to the number of cells.

• Second symbol (letter) corresponds to the structure of blastomeres: A- Symmetric blastomeres; B- Distinctly asymmetric blastomeres; and C- Defects of cytoplasm.

• Third symbol (digit) was referrered to fragmentation: 1- No fragmentation; 2- Fragmentation less than 20%; 3- Fragmentation between 20-50%; and 4- Fragmentation above 50%.

The good quality embryo had many blastomeres and no, or negligible fragmentation. The optimal numbers of blastomeres are 4 to 6 on the second day and 8-12 on the third day of culture. For example, the optimal quality embryos are described as 4A1 on the second day and 8A1 on the third day of culture.

12) Embryo transfer

- Day 2 or day 3 embryos were transferred to respective patients by using Sydney-IVF embyo

transfer (Cook, Limeric, Ireland ltd, USA) set according to the guidelines of the American Society of Reproduction. Excess good quality embryos were cryopreserved.

- The luteal phase was supported by daily IM injection of Progesteron (100 mg of Prontogest; IBSA, Switzerland) beginning on the day of embryo transfer.
- A positive pregnancy test was defined by >50 mIU/mL of plasma β-hCG (RIA) on day 14 after embryo transfer. An ultrasound scan 3 weeks after a positive pregnancy test confirmed a clinical pregnancy.

Statistical Method

Data were analyzed using Data were analyzed using Stata® version 14.2 (StataCorp LLC, College Station, TX, USA).

Normality of numerical data distribution was examined using the Shapiro-Wilk test. Non-Normally distributed numerical data were presented as median, interquartile and intergroup differences were compared using the Wlicoxon rank sum test (for two-group comparison) or the Kruskal-Wallis test (for comparison of multiple groups). The Dunn test was used for post hoc comparison with application of the Bonferroni correction for multiple pairwise comparisons.Categorical data were presented as number and percentage and differences were compared using Fisher's exact test (for nominal data) or the chi-squared test for trend (for ordinal data. Correlations were tested using the Spearman rank correlation. The correlation coefficient (Spearman rho) is interpreted as follows [11].

Correlation coefficient (Spearman rho)	Strength of correlation
<.2	Very weak
.2 – .39	Weak
.4 – .59	Moderate
.6 – .79	Strong
.8 – 1	Very strong

P-value <.05 was considered statistically significant.

Multivariable binary logistic regression analysis was used to determine the relation between BDNF concentration and ovarian response or chemical pregnancy as adjusted for other confounding factors.

- **Description** of quantitative variables as mean, SD and range.
- **Description** of qualitative variables as number and percentage.
- **Chi-square** test was used to compare qualitative variables between groups.
- **Fisher exact test** was used instead of chi-square when one expected cell less than or equal to 5.
- **Unpaired t-test** was used to compare quantitative variables, in parametric data (SD<50% mean).
- **Pearson Correlation** co-efficient test was used to rank variables versus each other positively or inversely.
- **ROC Curve** (receiver operator characteristic curve was used to find out the best cut off value, and validity of certain variable).
- Sensitivity = true ve +/true +ve + false –ve = ability of the test to detect positive cases.
- Specificity = true -ve/true-ve+ false +ve = ability of the test to exclude negative cases.
- PPV (positive predictive value) = true+/true+ve +false +ve,= % of true +ve cases to all positive.
- NPV = true-/true-ve + false –ve,= % of the true –ve to all negative cases.
- P value >0.05 insignificant.
- P<0.05 significant.
- P<0.01 highly significant.

The study was approved by the Ethics Board of Ain Shams University.

RESULTS

Our study had been conducted on 120 women with different types of infertility. They were all candidates for IVF-ET in ART unit of Ain Shams University Maternity Hospital. Their mean age was 31+5 years (selected) and mean duration of infertility was 4years ±2.

Table 1. Descriptive statistics for the whole study population: numerical variables

Variable	Range	Of mean	SD
Age (years)	(25-39)	32	5
FSH (mIU/l)	(4.2-25.0)	14.1	4.4
LH (mIU/l)	(3.00-11.50)	6.70	2.01
E2 (pg/ml)	(9.5-72.0)	31.7	11.9
PRL (ng/ml)	(7-40.0)	15.6	4.7
TSH (mIU/ml)	(1.10-4.80)	1.80	0.74
BDNF (pg/ml)	(1.8-9.6)	2.9	1.4
Number of retrieved oocytes	(2-10)	6	2

Table 2. Descriptive statistics for the whole study population: categorical variables

Variable		*n*	*%*
Ovarian reserve	Poor ovarian response	58	48.3%
	Good ovarian response	62	51.7%
Outcome of IVF	Negative pregnancy test	89	74.2%
	Positive pregnancy test	31	25.8%

Table 3. Relation between BDNF and cause of infertility

Variable	Cause of infertility				p-value¶
	Male factor (n=60)	**PCO (n=20)**	**Endo-metriosis (n=13)**	**Unexplained (n=27)**	
BDNF (pg/ml)	2.6 (2.5 – 2.8)	2.2 (2.1 – 2.45)†	2.2 (2.1 – 2.3)‡	2.7 (2.53 – 3.80)	**<.0001**

Data are median (interquartile range).

¶Kruskal-Wallis test.

†Statistically significant difference versus Male factor group (z=4.2483) and Unexplained infertility group (z = 4.7250) by Dunn's post hoc test (Bonferroni-corrected z > 2.6383 for p < .05).
‡Statistically significant difference versus Male factor group (z=3.2525) and Unexplained infertility group (z=3.8275) by Dunn's post hoc test (Bonferroni-corrected z > 2.6383 for p < .05).
BDNF was significant lower in endometrioses group and PCO group compared with male factor infertility group and unexplained infertility group.

Table 4-A. Comparison of patients with poor or good ovarian response: categorical variables.

Variable		Poor ovarian response (n=58)		Good ovarian response (n=62)		p-value¶
		n	%	n	%	
Cause of infertility	Unexplained	11	19.0%	16	25.8%	.073
	Male factor	25	43.1%	35	56.5%	
	PCO	12	20.7%	8	12.9%	
	Endometriosis	10	17.2%	3	4.8%	

¶Fisher's exact test.

Table 4- B.

Pregnancy test	egative	51	87.9%	38	61.3%	**.001**
	ositive	7	12.1%	24	38.7%	

Table 5. Relation between BDNF and ovarian response.

Variable	Poor ovarian response (n=58)	Good ovarian response (n=62)	p-value¶
BDNF (pg/ml)	2.5 (2.2 – 2.6)	2.65 (2.5 – 3.4)	**.0001**

Data are median (interquartile range).

¶Wilcoxon rank sum test.

There was a positive correlation between BDNF levels and ovarian reserve.

Table 6. Comparison of patients with negative or positive chemical pregnancy: categorical variables

Variable		Negative pregnancy test (n=89)		Positive pregnancy test (n=31)		p-value¶
		n	%	n	%	
Cause of infertility	Unexplained	19	21.3%	8	25.8%	.846
	Male factor	44	49.4%	16	51.6%	
	PCO	15	16.9%	5	16.1%	
	Endometriosis	11	12.4%	2	6.5%	

¶Fisher's exact test.

Table 7. Comparison of patients with ovarian response: categorical variables

Ovarian response	Poor ovarian response	51	57.3%	7	22.6%	**.001**
	Good ovarian response	38	42.7%	24	77.4%	

Table 8. Comparison of patients with poor or good ovarian response: numerical variables

	Poor ovarian response (n=58)		Good ovarian response (n=62)		
Variable	Median	IQR	Median	IQR	p-value¶
Age (years)	31	28 – 34	31	28 – 35	.477
FSH (mIU/l)	6.5	5.4 – 8.2	7.3	6.2 – 8.2	.153
LH (mIU/l)	6.20	5.10 – 7.20	7.05	5.40 – 8.30	.091
LH/FSH ratio	0.882	0.800 – 1.016	0.953	0.839 – 1.125	.156
E2 (pg/ml)	32.5	28.0 – 35.0	32.0	22.1 – 36.2	.394
PRL (ng/ml)	16.1	12.0 – 18.2	14.8-	12.0 – 17.1	.163
TSH (mIU/ml)	1.55	1.30 – 2.20	1.60	1.20 – 2.20	.661
BDNF (pg/ml)	2.5	2.2 – 2.6	2.7	2.5 – 3.4	**<.001**

Data are median and interquartile range (IQR).
¶Wilcoxon rank sum test.

Table 9. Relation between BDNF and chemical pregnancy

Variable	Negative pregnancy test (n=89)	Positive pregnancy test (n=31)	p-value¶
BDNF (pg/ml)	2.5 (2.2 – 2.6)	3.6 (2.825– 3.8)	**<.0001**

Data are median (interquartile range).
¶Wilcoxon rank sum test.

Table 10. Cut-off value of BDNF.

BDNF	Cut off value	2.7 pg/ml
	Sensitivity	74.2%
	Specificity	95.5%
	Positive predictive value	85.2%
	Negative predictive value	91.4%

Table 11. Multivariable binary logistic regression analysis for prediction of good ovarian response

Variable	Regression coefficient	SE	p-value	Odds ratio	95% CI
AGE	0.034	0.044	.445	1.035	0.948 to 1.129
LH/FSH ratio	1.201	0.884	.174	3.323	0.587 to 18.796
E2 (pg/ml)	-0.021	0.019	.274	0.980	0.944 to 1.017
PRL (ng/ml)	-0.084	0.046	.070	0.919	0.840 to 1.007
TSH (mIU/ml)	0.296	0.295	.317	1.344	0.754 to 2.397
BDNF (pg/ml)	0.396	0.197	**.044**	1.486	1.010 to 2.184
Constant	-1.823				

SE, standard error; 95%   CI, 95% confidence interval.

Table11 showed the results of multivariable binary logistic regression analysis for prediction of good ovarian response.

BDNF concentration was an independent predictor of good ovarian response (odds ratio = 1.486, 95% CI = 1.010 to 2.184, p-value = .044).

Table 12. Multivariable binary logistic regression analysis for prediction of chemical pregnancy

Variable	Regression coefficient	SE	p-value	Odds ratio	95% CI
Age (years)	-0.249	0.076	**.001**	0.780	0.672 to 0.905
E2 (pg/ml)	-0.017	0.028	.551	0.984	0.931 to 1.039
BDNF (pg/ml)	1.880	0.575	**.001**	6.556	2.124 to 20.243
Good ovarian response	1.479	0.671	**.028**	4.387	1.179 to 16.330
Constant	0.511				

SE, standard error; 95%   CI, 95% confidence interval.

Table 12 showed the results of multivariable binary logistic regression analysis for prediction of chemical pregnancy.

BDNF (odds ratio = 6.556, 95% CI = 2.124 to 20.243, p-value = .001), age (odds ratio = 0.780, 95% CI = 0.672 to 0.905, p-value = .001), and good ovarian response (odds ratio = 4.387, 95% CI = 1.179 to 16.330, p-value = .028) were independent predictors of chemical pregnancy.

DISCUSSION

BDNF, a member of the neurotrophin family, is initially recognized to be important for neuronal survival and differentiation in central and peripheral nervous system and is also found in non-neuronal tissues. Growing evidence showed that BDNF was essential for the oocyte maturation [6,12]. **Kawamura et al.** [8] had observed that BDNF enhanced the first polar body emission and promoted oocyte development into preimplantation embryos. Treatment with BDNF during the *in vitro* maturation (IVM) of bovine oocytes could produce more parthenogenetic embryos and the authors speculated BDNF could improve oocyte cytoplasm maturation [7]. This effect was also observed in porcine oocytes, which showed that BDNF could improve both nuclear and cytoplasmic maturation through autocrine and/or paracrine signal pathways [13]. A study demonstrated that BDNF could promote human oocyte maturation and early embryo development [14]. Our study had been conducted on 120 women with different types of infertility. They were all candidates for IVF-ET in ART Unit of Ain Shams University Maternity Hospital. Their mean age was 31 ± 5 years (selected) and mean duration of infertility was 4years ±2. The present results also showed that FF-BDNF levels among the mentioned age group in our selected patients was ranged from 1.8 pg/ml to 9.6 pg/ml with mean 2.9 pg/ml and standard deviation was 1.4 pg/ml.

Pregnant cases (25.8%) of the studied group had higher FF-BDNF level (3.6 ± 0.4 pg/ml) compared to non pregnant group (2.5 ± 0.7 pg/ml) with statistical significant difference in between by using unpaired-t test (p-value <0.05) and had higher embryo grade than non pregnant cases by using the same test (p-value <0.001).

This study aimed to detect follicular fluid neurotrophin levels in women underwent assisted reproductive technology for different etiologies of infertility. Follicular fluid was collected during routine egg retrieval from 106 consecutive women underwent controlled ovarian stimulation in preparation for IVF or ICSI. Women with male-factor infertility and no other causes constituted the control group (47 women). Polycystic ovary syndrome (PCOS) was diagnosed according to Rotterdam criteria (13 women) [10]. Unexplained infertility was diagnosed when routine tests for infertility workup did not reveal any abnormality (22 women). Diminished ovarian reserve was diagnosed with a day 3 FSH level of >10 mIU/mL, with an antral follicle count of <5 in one ovary or of <10 in both ovaries and when five or fewer eggs were retrieved after a routine controlled ovarian hyperstimulation (17 women). Patients with a history of endometriosis were diagnosed by laparoscopy (7 women).

Follicular-fluid BDNF levels for each etiologic factor are shown in patients with a history of endometriosis had significantly lower mean levels of follicular-fluid BDNF compared with the control group (274 ±96 pg/mL vs. 148± 42 pg/mL, P<.005). Patients with diminished ovarian reserve had lower levels of BDNF compared to the control group, but this difference did not achieve significance (P=.22). Follicular-fluid BDNF levels were not different between the control group and either women with PCOS or unexplained infertility.BDNF levels were lower in endometriosis and diminished ovarian reserve groups, whereas they were higher in group with PCOS compared to the control. There is a correlation of this study with our study as regard of occurrence of clinical pregnancy in 25% of cases and positive correlation between FF-BDNF level and ovarian reserve, but the difference that the previous mentioned study endometriosis had lower level of BDNF compared to the control group and Follicular-fluid BDNF levels were not different between the control group and either women with PCOS or unexplained infertility. In our study, FF-BDNF level was lower in both endometriosis and PCO compared

to the control group. Also, in our study we detected that cut -off value of BDNF concentration was > 2.7 pg/ml and this could predict a good ovarian reserve with a sensitivity of 40.3% and specify 89.7% and also could predict chemical pregnancy with sensitivity 74.2% and specify 95.5% which not mentioned in the previous study. Our study aimed to detect FF-BDNF level in different types of infertility and its using as peredictor for ovarian reserve. (120 patients selected for IVF). There was a significant direct positive correlation between the FF-BDNF level with number of total picked up oocytes, mature oocytes, embryo transfer and fertilization with the occurrence of pregnancy in 25% of the cases, also this study correlated the FF-BDNF level as a predictor of pregnancy by sensitivity 74.2% and specificity 95.5%, with cut-off point for FF-BDNF level according to presence or absence of pregnancy was 2.7 pg/ml. There was a correlation of this study with our results as regard positive correlation between FF-AMH level measured on the d-(OPU) and embryo grading (cell number and morphology) and occurrence of clinical pregnancy in 25.9% of the cases, but the difference that the previous mentioned study measured BDNF in serum on cycle day 3 and we measured in follicular fluid on the d-(OPU).

Study of **Ramer *et al.*** [14] included 226 women underwent IVF cycle in Reproductive Medicine and Infertility at Weill Cornell Medicine. Sera from 226 women were collected on day 2 of their menstrual cycle before initiation of any stimulation mediations. BDNF levels in ng/ml were 7.3 (3.5–13.1) in women with an ectopic pregnancy, 5.5 (2.6–11.3) in women who did not become pregnant, 4.2 (2.6–7.0) in women who had a spontaneous abortion, 3.8 (1.7–5.5) in women with a biochemical pregnancy and 3.6 (1.0–6.5) in those with a live term birth. The differences between groups were significant (p < 0.0001, Kruskal-Wallis Test). The serum BDNF concentration in women with an initial positive pregnancy test was significantly lower than in women who did not become pregnant (p < 0.0001, Mann-Whitney Test). By ROC curve analysis, a BDNF concentration of 5.2 ng/ml predicted the occurrence of an ectopic pregnancy in women with an initial positive pregnancy test with an AUC of 0.925 (0.866, 0.984) (p < 0.05), a sensitivity of 0.853 (0.689, 0.950), a specificity of 0.949 (0.897, 0.979), a positive predictive value of 0.806 (0.661, 0.933) and a negative predictive value of 0.963 (0.908, 0.985). Including women who did not become pregnant in the ROC analysis the AUC for ectopic pregnancy was 0.853 (0.788, 0.919), with a sensitivity of 0.853 (0.689, 0950), specificity of 0.815 (0.752, 0.867), positive predictive value of 0453 (0363, 0.732) and a

negative predictive value of 0.969 (0.922, 0.979). Women with an extrauterine ectopic pregnancy after embryo transfer also had a significantly higher BDNF level prior to cycle initiation than did women who had an intrauterine implantation (live birth, spontaneous abortion, biochemical pregnancy) (p < 0.0001, Mann-Whitney test). Prediction of a failure to conceive by determination of serum BDNF yielded an AUC of 0.845 (0.788, 0.902) by ROC analysis. There were no differences in BDNF levels between women with different causes of infertility (p = 0.1130). The individual median (range) values were 5.2 (3.9–12.4) for endometriosis, 4.8 (3.2–9.3) for uterine anomaly, 4.6 (3.7–12.0) for tubal occlusion, 4.6 (1.0–13.1) for advanced maternal age, 4.0 (3.0–8.5) for idiopathic, 3.9 (2.3–10.3) for male factor and 3.3 (2.7–4.9) for an ovulation/ polycystic ovaries. An elevated BDNF concentration in sera obtained prior to initiation of an IVF cycle was highly predictive of a subsequent extra-uterine implantation in women with a positive pregnancy test or with implantation failure.Women included in our study were 120.

Ramer *et al.* [14] study included 297 women who underwent an IVF cycle at The Center for Reproductive Medicine and Infertility at Weill Cornell Medical College. They collected serum BDNF from days 24 and 28 of individual IVF cycle responding to 1 week after ET. The study population consisted of 74 women with a subsequent live birth (delivery of a live-born infant), 49 who had an ectopic pregnancy (ultrasound documentation of a gestation sac or fetal pole in the adnexa, or surgical/pathological confirmation), 46 with a spontaneous abortion (nonviable fetus following observation of a uterine gestational sac), 60 with a biochemical pregnancy [transient rise and fall of human chorionic gonadotropin (hCG)], and 68 who did not become pregnant (negative hCG on day 28). Lower number of oocytes were fertilized in women who did not become pregnant (5.5) compared to the women who had a live birth (7.2) (P = 0.0225). The causes of infertility in our subjects were 36.9% advanced maternal age, 21.6% male factor, 14.4% tubal factor, 12.6% endometriosis, 5.1% idiopathic, 4.6% anovulatory, and 4.6% uterine problem.

In the current study, median serum BDNF concentrations in samples were obtained on days 24 and 28 and subsequent IVF outcome. On both days, BDNF levels were different between outcome categories (P < 0.0001, Kruskal–Wallis Test). Comparing median BDNF concentrations on day 24 in women with a subsequent live birth (932 pg/mL) with each of the other outcomes demonstrated that levels were reduced in women with adverse peri- or postimplantation outcomes, that was ectopic

pregnancy (738 pg/mL, P < 0.0001), spontaneous abortion (740 pg/mL, P < 0.0001) and biochemical pregnancy (776 pg/mL, P = 0.0004), but not in women who did not become pregnant following embryo transfer (904 pg/mL, P = 0.1678).

In the present study, BDNF levels on both days did not differ between women who had a live birth and those who did not become pregnant. This indicated that BDNF was probably not involved in the success or failure of pre-implantation events preceding or following embryo transfer and that only peri-implantation (biochemical pregnancy) or post-implantation (spontaneous abortion) failure may be influenced by the BDNF concentration. Another possible option was that peri- and postimplantation events may determine the level of BDNF because BDNF concentration was similar in both ectopic pregnancy and intra-uterine pregnancy loss. In agreement with this conjecture, we did not observe any association between the circulating BDNF concentration and oocyte parameters or *in vitro* embryo quality. However, we cannot rule out a possible role for BDNF in these events because levels in the systemic circulation might not be reflective of local concentrations in the ovary or embryo culture medium. Further studies on follicular fluid and embryo culture media were needed to further explore this relationship. Unexpectedly, BDNF levels were also found to be significantly lower on days 24 and 28 in sera from women who subsequently developed an ectopic pregnancy compared to those with an intra-uterine gestation followed by a live birth.We measured FF-BDNF during ovum retrieval in IVF cycle not serum sample

In our study, FF-BDNF level was lower in both endometriosis and PCO compared to the control group. Also, we did not follow the pregnancy outcome (ectopic, abortion, live birth) as done in the previous study. But, there was correlation between **Ramer *et al*.** [15] and our study that lower level of BDNF in serum an in FF associated with peri implantation failure.

In study of **Monteleone *et al*.** [16] 23 consecutive patients underwent their first IVF cycle between January and November 2006 at the Centre of Reproductive Physiopathology of the Pisa University Hospital. Mean age of women was 34.8±4.4 years and average body mass index (BMI) was 21.74±2.71. Mean basal FSH levels were 7.92±2.77. Indications for IVF included tubal factor (n=14) severe male factor (n=9). IVF or IVF with intracytoplasmic sperm injection and embryo transfer were performed as appropriate. Blood sampling for BDNF and estradiol was performed for all subjects on day 1 (D1), day 8 (D8), day of HCG administration (DHCG) and day of oocyte retrieval.

(DOR) after overnight fasting, between 8 am and 9 am in order to minimize the effects of a possible circadian variation of plasma BDNF concentrations.

Our study showed a strong positive correlation between BDNF and estradiol values was persisted even after the subdivision in the pregnant and nonpregnant patients. In pregnant patients and nonpregnant patients, the values of BDNF, unlike those of estradiol, grew significantly only between D8 and DHCG and remained constant until DOR. No statistical significance was found between DHCG and DOR values for estradiol as well within each of the studied groups. Between-group comparisons showed no statistically significant differences in both BDNF and estradiol values at D1, D8, DHCG and DOR the IVF cycle.

A serial plasma quantification of BDNF throughout an IVF cycle was done. Our results indicated that plasma BDNF, like estradiol, exhibited dynamic changes during controlled ovarian stimulation for IVF. In fact these two circulating factors showed a positive correlation throughout the controlled ovarian stimulation in both women who became pregnant and those who did not. BDNF plasma concentrations were not seemingly predictive of IVF outcome, this neurotrophin was obviously highly correlated to estradiol levels and seemed to be an important factor especially in the periovulatory period. Some drawbacks were detected in this study. Firstly, the small number of patients may not allow significant differences in number of total retrieved oocytes, number of mature oocytes and number of embryos available for transfer, to emerge between pregnant and nonpregnant patients. Secondly, pregnant patients were on average 3 years younger and this may explain their better chance in achieving a pregnancy. We measured FF-BDNF not serum and we didn't study the correlation between E2 and BDNF.We also measure correlation between BDNF and IVF outcome.

According to **Wang *et al*.** [17] a total of 59 women undergwent ICSI at the Reproductive Center of the Peking Union Medical College Hospital between June 2010 and March 2011.FF of the punctured follicles was collected in each patient after removal of the oocyte. The concentration of BDNF and E2 were expressed in pg/ml and P was expressed in ng/ml. Values were expressed as mean ± standard deviation (SD). Their data were done between pregnant and non-pregnant groups. The concentrations of FF BDNF, E2 and P were 242.0± 51.6 pg/ml, 1172644.0±408260.0 pg/ml and 27658.8± 9922.2 ng/ml, respectively. No significant difference was found in the concentration of BDNF, E2 and P between pregnant and non-pregnant groups. The concentration of BDNF in FF showed a

significant correlation with E2 (r=0.301, P=0.021), but there was no correlation with P (r=−0.066, P=0.621).

There was no correlation between age, blastocyst formation rate and BDNF in FF (r=0.071, P=0.593 and r=−0.001, P=0.993 respectively). However, the rate of mature oocytes collected and cleavage rate were strongly correlated with FF BDNF (r=0.41, P=0.001and r=0.312, P=0.016 respectively).The study evaluated the correlation between BDNF in FF and IVF outcome. BDNF in FF was positively correlated with the rate of mature oocytes collected and cleavage rate, but not with blastocyst formation rate. It could be speculated that BDNF in FF was important for the development of oocytes because it was positively associated with oocyte development. This study did not find any difference of BDNF in pregnant between that in non-pregnant groups. Therefore, the IVF outcome could not be predicted by BDNF value in FF.Our study is different from the previous study that there were positive correlation between BDNF and IVF outcome and this may be due to smaller sample size of previous study.

CONCLUSION

In our study, we concluded that FF-BDNF measurement on the d-(OPU) in women with different types of infertility underwent COH for IVF-ET, had positive correlation with ovarian reserve and positive predictor of pregnancy which occurred in 25.8% of cases.

RECOMMENDATIONS

1. BDNF showed positive correlation with ovarian reserve and positive correlation with pregnancy outcome so we recommend to measure serum BDNF before starting IVF cycle as predictive factore and cost effective.

2. Other studies are needed to assess relation between BDNF and pregnancy outcome in patient undergo IVF cycle.

3. Other studies are needed to asses BDNF receptors in the endometrium and fate of pregnancy.

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