# A Comparative Study of Nerve Growth Factor Level in the Follicular Fluid of Polycystic and Non-Polycystic Ovary Syndrome Women Undergoing ICSI: a Cross-sectional Study

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## Abstract

**Background :** Nerve growth factor is a member of a group of proteins called Neurotrophins (NTs), which are necessary for growth and differentiation of neurons in both central and peripheral nervous systems. In addition to this essential role, NGF has been suggested to play a possible role in ovarian functions. Thus, we proposed this study to evaluate the nerve growth factor level in the follicular fluid of women with polycystic ovary syndrome undergoing Intracytoplasmic sperm injection (ICSI) in comparison with the control group (non-polycystic women).

**Results :** Follicular fluid nerve growth factor levels of non PCOS women were significantly higher than these of PCOS group (245.399 ± 63.41pg/ml vs. 149.592 ± 64.86 pg/ml), *p*-value =0.0001*. Also, it was significantly correlated negatively with oocytes number (Correlation Coefficient r= -0.431, *P*-value = 0.01) and positively with FSH (r=0.370, *p* value = 0.029).

**Conclusion:** Nerve growth factor level in the follicular fluid of polycystic ovary syndrome women is lower than it in non-PCOS ones. Additionally, it correlates directly with cycle day two FSH and inversely with oocytes number.

***Keywords:*** *Follicular fluid, Polycystic ovary syndrome (PCOS), Nerve growth factor, Neurotrophins, subfertility.*

## Introduction

Nerve growth factor (NGF) is a member of a group of proteins called Neurotrophins (NTs), which are necessary for the growth and differentiation of neurons in both the central and peripheral nervous systems [1]. In addition to this essential role, NGF has been suggested to play a role in the ovarian function, folliculogenesis, oocyte development, and ovulation [2]. However, this NT is present in all compartments of the reproductive system with its high-affinity receptors trkA and p75, suggesting its physiologic role in ovarian functions. Different types of cells in the ovary, like theca cells, granulosa and cumulus cells ( CCs) have been found to produce NGF [3].

NGF promotes follicular maturation and increases cell responses to FSH via FSH receptors up-regulation in neonatal rat ovaries [4]. Similarly, in vitro studies on human GCs also showed FSH receptors expression up-regulation by NGF [5]. In addition to its effects on germ cells and GCs, NGF had been shown to have a role in promoting theca cells (TC) proliferation [6]. FSH enhances LH receptor expression in preantral oocyte granulosa cells and thus prepare oocyte for final maturation and further ovulation [7].

### Role of NGF in Ovulation

Dissen *et al.*, [8] postulated in his research in rats, that, both TrkA and NGF are increased in the follicular and ovarian interstitial cells, reaching their maximum level ~5 h prior to ovulation. Additionally, he found that in an in vivo study, administration of NGF antibodies leading to suppressed ovulation, showing the important role of

NGF in ovulation triggering in rodents. Furthermore, he stated that trkA receptors activation by NGF can disrupt cell to cell interaction by modulation of the connexin 43-based gap junction integrity, thus induce cellular dissociation of the follicular wall which occurs prior to ovulatory rupture in rats.

Nerve growth factor increases E2 prostaglandin (PGE2) secretion [8] and decreases granulosa-theca cells gap junction communication. Indeed, PGE2 is an LH mediator and it is a key molecular factor controlling the events of ovulation. Studies in animals have provided evidence that NGF has a role in the ovulation process. It was previously called ovulation-inducing factor (OIF) in animals . Barboni [9] in his research (Preovulatory rise of NGF in ovine follicular fluid) showed that the *in vitro* addition of NGF to cumulus-oocyte complex caused marked cumulus expansion and gradual cumulus-oocyte uncoupling. Additionally, it caused meiosis resumption in about 70% of oocytes. Streiter *et al*., [3], also stated that NGF is involved in the ovulation control. Moreover, studies in rats and hamsters, as well as sheep, showed an elevated level of NGF in the follicular fluid during the preovulatory phase [10,11]. Interestingly, an administration of NGF in different ways consistently induced ovulation in several induced ovulatory mammals, like llamas, camelids and alpacas [12-14]. In a research using a camel model, it was suggested that NGF in the seminal plasma was absorbed by the endometrium to the bloodstream and then reached to the hypothalamus where this NGF stimulated neurons to produce kisspeptin (a potent stimulator of GnRH), which eventually organized the preovulatory LH surge [12].

Some studies showed that abnormal NTs system may negatively disturb ovarian function, causing reproductive problems in form of reduced ovarian reserve, PCOS and endometriosis in addition to that, NTs defects may lead to female infertility and even ovarian cancer [2].

## Methods

### Study design and setting

This is a cross-sectional study, which had been approved by the Research Local Ethical Committee of the College of Medicine / University of Babylon.

Forty-four sub-fertile couples from the Fertility Center of Al- Najaf / Al-Sadr Medical City visitors were included in the study within the period from December 2019 to February 2020. Written consent had been taken from all couples participated in this study. Both partners had been evaluated by a gynecologist and an urologist. The evaluation based on medical history and physical examination including anthropometric measurements (height, weight and BMI). Cycle-Day 2 vaginal US for assessment of antral follicular count was performed, in addition to the hormonal assay; including luteinizing hormone (LH), Follicle stimulating hormone (FSH), prolactin and Estradiol (E2) as well as triggering time E2 were measured and recorded for all women participated [15].

### Selection criteria:

1. Women's age was between 20 and 40 years (mean ± SD = 28.57 ± 6.212). Women aged more than 40 years old had been excluded from the study.

2. Women with current untreated pelvic disease like endometriosis, uterine problems: including submucosal fibroids or polyps, malformations, ovarian cyst had been excluded.

3. Female partners were grouped into two groups:

**Group one**: females with polycystic ovary syndrome (n=20), Selected based on Rotterdam criteria, including a history of oligo/amenorrhea, clinical picture of high androgen level either in form of signs and symptoms or biochemical investigation (high testosterone level) as well as ovarian cystic appearance on transvaginal ultrasound (OS Guideline Development Group 2019). Male partners were either normal or having mild to moderate impairment of semen parameters.

**Group two**: females without PCOS (n=24), either normal females undergoing ICSI due to male factor infertility or females with tubal obstruction or unexplained infertility.

### Controlled ovarian hyperstimulation (COH)

Controlled ovarian hyper-stimulation was done based on ESHRE ovarian stimulation guidelines (OS Guideline Development Group 2019) by a gynecologist. It was performed via fixed GnRH antagonist protocol for both PCOS and non PCOS women to avoid the risk of

ovarian hyperstimulation syndrome (OHSS) (Behery *et al.* 2020) associated with close monitoring of follicles number, size, level of maturation as well as serial TVUS and serum E2 level.

Gonadotropins initial dosing was individualized for each patient and the doses was adjusted based on the patient's body mass index, age, ovarian reserve. Gonadotropin used is recombinant FSH (r-FSH) (Gonal-f and Follitropin). Drugs had been given on the basis of daily injection, usually either intramuscular or subcutaneous. The standard dose of Gonal-F was 150 IU / day which can be increased up to 450 IU/day for obese women. Ovulation trigger was done 36 hrs. before oocyte pick up by administration of low dose GnRH agonist (0.2 mg Decapeptyl) subcutaneously.

**Follicular fluid collection**

After oocytes scanning and saving, the follicular fluid of the first follicle aspirated (of 20-24 mm diameter) was collected in tubes, centrifuged for 5 min at 269.6g then the supernatant clear fluid was taken and divided into safe lock tubes and saved at ¯80 Cº in the deep freeze at the Kufa Medical College Center of Cancer researches until the time of the ELIZA test.

Nerve Growth Factor (NGF) level in follicular fluid measured by using enzyme immunoassay ELISA kit for human NGF from Bioassay Technology laboratory (BT) that have been validated for NGF level assessment in human follicular fluid, as per manufacturer's instructions. After thawing of FF samples, an accurate quantitative detection of NFG had been done by a sandwich ELISA principle.

## Statistics

Statistical Package for Social Sciences (SPSS) software had been used (version 21). An independent sample student t-test was used for comparing parameters between both groups. For categorical values, Chi-square was used. Bivariate analysis (Pearson's correlation test) was used to test the degree of association between the measured variables and other clinical parameters. $P \leq 0.05$ was considered as statistically significant.

## Results

Table 1 shows the demographic differences between PCOS and non PCOS groups. There was no significant statistical difference between the studied groups. while table 2 shows the insignificant statistical difference between both groups regarding cycle day two hormones, E2 at triggering time, LH/FSH ratio except for the oocytes number, there was a significant higher oocytes in PCOS group than non-PCOS one, *P* value = 0.033 (Tab. 1., 2.).

**Table 1: Comparison between both groups (PCOS and Non-PCOS) regarding age, body mass index (BMI) and infertility period.**

| Variable | PCOS (N = 20) Mean±SD | NON-PCOS (N = 24) Mean ±SD | P value |
|---|---|---|---|
| Age (years) | 26.6 ± 5.113 | 30.36 ± 6.680 | 0.490 |
| BMI (kg/m2) | 27.365 ± 3.91 | 27.35 ± 3.15 | 0.996 |
| Period of infertility (years) | 6.75± 4.179 | 6.98 ± 3.66 | 0.850 |

***Abbreviation: PCOS* polycystic ovary syndrome, *BMI* body mass index.**

**Table 2. Comparison between the studied groups regarding cycle day two hormones, E2 at triggering time and oocytes number.**

| Variable | PCOS Mean ±SD | NON-PCOS Mean ±SD | P value |
|---|---|---|---|
| E2 (pg/ml) | 34.20 ± 14.24 | 39.15 ± 16.42 | 0.305 |
| FSH (mIU/ml) | 4.87 ± 2.165 | 5.65 ± 2.413 | 0.276 |
| Prolactin (ng/ml) | 28.285 ±26.835 | 24.47 ± 10.158 | 0.539 |
| E2 (pg/mL) at triggering time | 2723.52 ± 1039.75 | 2108.27± 926.377 | 0.071 |
| Oocyte number | 11.35 ± 6.983 | 7.45 ± 4.228 | 0.033* |

***significant. Abbreviation: FSH* follicle stimulating hormone, *E2* estradiol.**

There was a significant statistical difference between the studied groups regarding follicular fluid NGF level (measured in picogram per milliliter), with a higher level in the non-PCOS group (245.399 ±63.41 n=24) than PCOS group (149.592 ± 64.86, n=20), *p* value =0.0001*

Although NGF correlated positively with LH, E2, FSH, prolactin but the difference was statistically insignificant only with FSH, it was significant, *P* value =0.029. Interestingly, NGF correlated negatively with E2 at triggering time and oocytes number, r = (-0.461 and - 0.431) respectively with a *p* value= 0.010 for both (Tab.3).

**Table.3. Correlation between follicular nerve growth factor and LH, FSH, E2, prolactin, E2 at triggering time and oocytes number**

| Hormones | | LH (mIU/mL) | FSH (mIU/mL) | E2 (pg/mL) | Prolactin (ng/mL) | E2 triggering time (pg/mL) | Oocytes number |
|---|---|---|---|---|---|---|---|
| NGF | r | 0.046 | 0.370* | 0.258 | 0.025 | -0.461* | -0.431* |
| | P value | 0.794 | 0.029 | 0.135 | 0.887 | 0.010 | 0.010 |

*** Correlation is significant at the 0.05 level (2-tailed). *Abbreviation: NGF* nerve growth factor, *FSH* follicle stimulating hormone, *LH* luteinizing hormone, *E2* estradiol.**

## Discussion

In the present study, there were no significant statistical differences regarding demographic characteristics including age, BMI, infertility type and infertility period between our study groups (PCOS and non PCOS). Additionally, there was a high specification of inclusion and exclusion criteria of other infertility causes and PCOS differential diagnosis that could affect the results. So the confounding factors were highly minimized.

The controlled ovarian hyperstimulation protocol used was GnRH antagonist protocol to reduce the risk of developing ovarian hyper-stimulation syndrome (OHSS) complication [16].

Table 2 compares between PCOS and non PCOS groups regarding cycle day2 hormones including FSH, E2, prolactin, E2 at triggering time and oocytes number. It shows no significant statistical difference between both groups. This can be explained as the PCOS is a WHO type 2 normogonadotropic anovulatory disorder [15].

The total retrieved oocytes number was significantly higher in PCOS women than non PCOS. These results are consistent with many previous studies (Falah Hassan and Zahir Al-yasiry; Kumar *et al.* 2013). This higher oocyte number in the PCOS group is attributed to the increased number of preantral follicles in polycystic women ovaries which are highly sensitive to iatrogenic ovarian stimulation done by COH [16].

Our results demonstrated that under conditions of ovarian hyperstimulation, the follicular fluid NGF levels were significantly higher in samples of non PCOS women than the PCOS group. These results are in agreement with the results stated by [1]. While Sadeu *et al*., [17] showed there was no difference in the FF NGF level between PCOS and non PCOS women. Other researches stated that there were higher FF NGF levels in PCOS women than non PCOS [18].

However, in the present study, we examine the correlation of NGF with collected oocyte number. It had a highly significant inverse correlation with retrieved oocytes number and the estradiol level at ovulation triggering time; in addition to its positive correlation with age and FSH, this may indicate its increase in lower responder women and this confirms Salas *et al.*, [5] and Buyuk and Seifer [1] opinions. Or it is presumed that, due to NGF high affinity-receptors (trkA) expression is predominated in the granulosa and theca cells of preantral and antral oocytes [6], NGF may haven't any impact on oocytes number and its role in the normal ovary is confined mainly to: Late follicular maturation (pre-antral and antral follicles) which is FSH dependent [6] and ovulation.

However, in human being the actual role of NGF in regulating the ovulation process still poorly defined and most information had been obtained from animals.

The main problem in PCOS subfertility is oligo / anovulation (Rotterdam ESHRE/ASRM-Sponsored PCOS Consensus Workshop Group Revised 2003 consensus) [19]. It considered as the commonest anovulation cause affecting females worldwide [15].

The lower follicular fluid NGF levels in PCOS women as compared to control group highlights the possible role of low NGF level in the PCOS anovulation problem. Therefore, the NGF role in human PCOS pathophysiology especially in events surrounding late oocyte maturation and ovulation needs further clarification.

## Conclusions

Follicular fluid NGF levels are significantly higher in non PCOS women than the PCOS group. Additionally, the NGF correlated positively with cycle day-2 FSH and negatively with oocytes number and E2 at triggering time. Thus NGF role in the ovary may be limited to its participation in preantral and antral oocyte maturation and ovulation. Indeed, this requires further study in human ovaries for confirmation.

**Financial Disclosure:** There is no financial disclosure.

**Conflict of Interest:** None to declare.

**Ethical Clearance:** All experimental protocols were approved under the Fertility center of Al- Najaf and all experiments were carried out in accordance with approved guidelines.

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