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Effects of Activin A Hormone on Fertilization Capacity of PCOS Women Undergoing ICSI Cycle in Sample of Iraqi Women.

Ghofran Khalial [1], Wasan Adnan [1], Saad S. Al-Dujaily [1]

[1] **High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.**

mahdikhalial2@gmail.com

ABSTRACT

Activin is a member of the transforming growth factor-beta superfamily and is a polypeptide growth factor. Activin regulates follicle growth, gonadotropin responsiveness, steroidogenesis, oocyte maturation, ovulation, and corpus luteum function in the ovary via a local autocrine/paracrine effect. Activin measurement A level in serum and follicular fluid during the preovulatory stage in PCOS women undergoing an IVF/ICSI cycle to predict fertilization capacity earlier Fifty infertile couples, 25 with PCOS and 25 without PCOS, who attended Al Nahrain University in Baghdad, Iraq's High Institute of Infertility Diagnosis and Assisted Reproductive Technologies. The females that participated were between the ages of 18 and 40. On the day of oocyte retrieval, the level of Activin A in serum and follicular fluid was measured. Activin A levels in PCOS women's serum (activin A=1274 77.9) and follicular fluid (activin A =1038 50.3) compared to non-PCOS women's serum (activin A=453 25.74) and follicular fluid (Activin A=450 23.73). The study shows no correlation between activin A hormone with oocytes characteristics and fertilization rate in PCOS women with a significant increase of activin A hormone in the serum and FF of PCOS women compared to non PCOS women. Activin A level can predict a diagnosis of PCOS in addition to positive predictive pregnancy but not indicator of fertilization capacity in PCOS women.

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KEYWORDs

Infertility, Activin-A, pregnancy outcome, PCOS, ICSI.

1. Introduction

Infertility is defined as the inability of a sexually active, non-contracting couple to conceive within a year (Duffy et al., 2020 [1]). As one of the reasons for infertility, polycystic ovarian syndrome (PCOS) includes a wide range of symptoms and signs, ranging from mild to severe disruptions in reproductive, endocrine, and metabolic function. PCOS is the most prevalent cause of oligovulation and anovulation, which are symptoms of persistent ovarian dysfunction (Escobar-Morreale., 2018 [2]). Oocytes with morphological characteristics associated with the cumulus cells, polar body, and cytoplasm are chosen based on their quality (Coticchio et al., 2018 [3]). In the 1980s, activin was found as a gonadal protein in ovarian follicular fluid, and it was discovered to have a role in oocyte maturation, with higher levels of activin A produced by high-quality oocyte–cumulus complexes (Ando, 2020 [4]). Because the factor synthesis was increased, this finding shows that elevated activin A levels in serum and follicular fluid may play a role in PCOS folliculogenesis. The mean of serum activin A was almost d when PCOS women were compared to healthy women of comparable age.

2. Materials and Methods

This study was conducted on 50 infertile couples who attended Al-Nahrain University in Baghdad, Iraq's High Institute of Infertility Diagnosis and Assisted Reproductive Technologies. The study's duration was extended from November 2020 to August 2021. The couples went through the fertility clinic's basic fertility work-up, which included a case history, physical examination, ovulation detection, tubal and uterine cavity assessment, and seminal fluid analysis. Activin A in serum and follicular fluid is measured using an ELISA kit (My BioSource, USA). Cetrorelix, 0.25mg (GnRH antagonist), was administered subcutaneously to the

infertile females who participated in this research. All 50 infertile women had a normal ovarian reserve based on their serum AMH level and antral follicle count (AFC), which are commonly tested in infertility clinics on day 1 or 2 of the menstrual cycle (follicular phase). Polycystic ovarian syndrome was found in 25 women and was diagnosed using the Rotterdam criteria (2003) (oligo-and/or anovulation, hyperandrogenism symptoms, and polycystic ovary morphology on ultrasound).

On the day of oocyte retrieval, blood samples were obtained from each infertile woman via venepuncture. They were centrifuged at 1000 g for 10 minutes. The serum was taken out and kept at -20 °C or -80 °C. To avoid contamination of the blood and flushing media, the follicular fluid (FF) was taken from the first extracted follicle. It was frozen for about 20 minutes at -20 °C before being analyzed. An enzyme-linked immunosorbent assay was used to determine the levels of Activin A in serum and follicular fluid. Oocyte quality and maturation were assessed. The ICSI program was done as described (Faramarzi et al., 2017 [5]). The fertilization was evaluated for evidence of fertilization after 16-17 hours of the ICSI technique. The embryo was transferred using a flexible catheter on day three (it was a six-cell embryo) (Cook-Ireland Ltd). Progesterone medication in the form of Cyclogest (Actavis, Barnstable, UK) ® 200-400 mg twice a day was administered to all women for luteal phase support, starting on the day of oocyte retrieval and continuing until a pregnancy test was conducted. 14 days after the embryo transfer, a pregnancy test was done

3. Statistical analysis

Statistical Package for Social Sciences (SPSS) version 22.0 and Microsoft Office 2007 were used to analyze the data. The variables were

described using statistical data such as frequency, mean, and standard deviation. The groups were compared using the independent sample t-test (unpaired t-test between two groups) and Chi-square (for non-continuous data or percentage), and Pearson's correlation coefficient (r) was used to determine the degree of relationship between continuous variables. When the p-value was less than 0.05, the results were considered statistically significant (Cronk, 2019 [6]).

3. Results

The study groups were classified as follows: A total of 50 infertile women were enrolled in this cross-sectional comparative study, 25 of whom were diagnosed with polycystic ovarian syndrome (PCOS group) and 25 of whom were not.

Table (1): Comparison of demographic characteristics between PCOS and non-PCOS groups

Demographic characteristics	PCOS patients	Non-PCOS patients	P-value
Age (years)	30.00 ± 1.116	30.90 ± 1.22	**0.609**
BMI (Kg/m²)	28.29 ± 0.962	28.02 ± 0.732	**0.835**
Duration of infertility (years)	7.47 ± 1.052	8.23 ± 0.718	**0.655**
Type of infertility n. (%)	Primary 20 (80 %)	Primary 17 (68 %)	**0.508**
	Secondary 5 (20 %)	Secondary 8 (32 %)	

The values are expressed as Mean ± SE n=25 per group
*: p-value < 0.05 (significant); BMI: Body mass index.

Table (2): Comparison of hormonal levels between PCOS & Non-PCOS groups

Hormones levels	PCOS group	Non-PCOS group	p-value
FSH (mIU/ml)	6.91 ± 1.66	6.04 ± 0.506	**0.246**
LH (mIU/ml)	8.43 ± 1.374	4.02 ± 2.09	**0.025***
AMH (ng/ml)	5.02 ± 0.738	2.63 ± 0.914	**0.113**
E2 (pg/ ml) basal	45.44 ± 1.69	37.27 ± 2.56	**0.036***
E2 at day of trigger (pg/ ml)	1582 ± 119.8	1412 ± 135.4	**0.460**

The values are expressed as Mean ± SE

FSH: Follicle stimulating hormone; LH: Luteinizing hormone; E2: Estradiol; AMH: AntiMullerian hormone; *: p-value < 0.05 (significant).

Table (3): Comparison of serum and follicular fluid activin-A levels between PCOS & non-PCOS groups

Parameter (pg/ml)	**PCOS group**	**Non-PCOS group**	**P-value**
Serum activin-A	1274 ± 77.9	453 ± 25.74	**< 0.001***
Follicular fluid Activin-A	1038 ± 50.3	450 ± 23.73	**< 0.001***

Table (4): Comparison of oocyte quality and fertilization rate between PCOS & non-PCOS groups

Parameters	PCOS group	Non-PCOS group	P-value
Total oocytes count	13.77 ± 0.978	10.74 ± 1.07	**0.021***
Germinal vesicles (GV)	1.91 ± 0.18	1.67 ± 0.16	**0.605**
Metaphase I	1.89 ± 0.796	1.56 ± 0.224	**0.127**
Metaphase II	8.19 ± 0.802	5.53 ± 0.826	**0.039***
Abnormal oocytes	1.56 ± 0.106	1.83 ± 0.266	**0.577**
Fertilization rate	42.00 %	69.02 %	**0.001***

values are expressed as Mean± SE.*: p-value < 0.05 (significant)

Table (5): Correlations between activin-A with fertilization capacity in the PCOS group

Fertilization capacity		Serum activin-A	F.F. activin-A
Total oocyte count	r	-0.335	-0.143
	p-value	0.172	0.524
GV	r	-0.434	-0.247
	p-value	0.183	0.465

Abnormal oocytes	r	0.214	0.226
	p-value	0.581	0.559
MI	r	-0.158	-0.015
	p-value	0.686	0.969
MII	r	0.269	0.294
	p-value	0.239	0.195
Fertilization rate	r	-0.004	0.112
	p-value	0.987	0.649

r: Pearson`s correlation coefficient

4-Discussion

According to the findings, there was no significant difference in mean age (p=0.609), BMI (p=0.835), duration (p=0.655), or type of infertility (p=0.508) between the two groups. When comparing hormone levels between PCOS and non-PCOS women, it was discovered that PCOS women had significantly greater LH levels (p=0.025) and basal E2 levels (p=0.036) than non-PCOS women. However, there was no significant difference in FSH, AMH, or E2 levels between PCOS and non-PCOS groups (p > 0.05) on the day after triggering ovulation (P>0.0). As a result, the current study found that PCOS women have higher LH concentrations than non-PCOS women. Approximately 60% of women with PCOS have an increase in LH concentrations as a result of this. (Ranjzad et al., 2011 [7]). The current study discovered highly significant differences in estradiol (E2) in serum at cycle day 2 (CD2), serum, and FF activin A at the day of ova pickup between PCOS and non-PCOS groups (OPU). The level of serum

estradiol (E2) in PCOS women is higher than in non-PCOS women, according to several studies (Khandgawi et al., 2016 [8]). One possible explanation for the results in which the PCOS group's estradiol was far higher Than the control groups is that ovarian stimulation by more follicles with medication causes the granulosa cells of patients with PCOS to be functionally robust and exhibit increased estrogen responses to FSH stimulation compared to those of normal women, which may, in part, explain why the PCOS group's estradiol was far higher than the control group's (Altun et al., 2011 [9]).

The findings showed a higher significant (p 0.001) difference between PCOS and non-PCOS women in the levels of Activin A assessed in serum and follicular fluid. As factor production was increased, this finding shows that elevated activin A levels in serum and follicular fluid may play a role in PCOS folliculogenesis. The following were the findings of the current study: The PCOS group had a considerably higher mean total oocyte number (P0.001) and a significantly higher mean MII oocyte number (P0.001) than the control group. The mean number of MI, GV, and abnormal oocytes, on the other hand, did not change statistically significantly. Our findings are similar to those of (Nikbakht et al., 2020 [10]; Al-Dujaily and Shukry et al., 2019 [11]). Infertile women with PCOS had a considerably higher number of total recovered oocytes than infertile women for other reasons. According to a study by (Rajani et al., 2012 [12]), the mean of mature oocytes (MII) was considerably lower in PCOS, which contradicts our findings (Sigala et al., 2015 [13]). Despite the fact that PCOS patients in this study produced more oocytes, they had poor fertilization capacity, resulting in a considerably (p=0.001) lower fertilization rate in PCOS women (42% vs. non-PCOS women (69.02%), which is consistent with other research (Nikbakht et al., 2020 [10]; Al-Dujaily

and Shukry, et al., 2019 [11]). This result could lead to decreased rates of cleavage and implantation, as well as a higher rate of miscarriage (Qiao & Feng,2011 [14]). As a result, the current study discovered no link between activin A hormone levels and the quality of oocytes or the rate of fertilization in PCOS women. Many hormones and factors produced by follicular cells have been associated to high oocyte quality, including FSH, LH, estrogen, Activin, epidermal growth factors (GFs), and oocyte-derived factors, as well as bone morphogenetic protein 1 (BMP1) (Puttabyatappa & Padmanabhan, 2018 [15]). Poor oocyte quality has been associated to androgens, AMH, cytokines IL6, IL12, and IL13, as well as corticotrophin-releasing hormones (Qiao & Feng, 2011 [14]). Thus, elevated levels of the activin A hormone are not the only factor influencing oocyte quality or the fertilization rate in PCOS patients.

6- Conclusion

In conclusion, despite the lack of a strong link between activin A and fertility, women with PCOS had considerably greater Activin levels in their blood and follicular fluid than non-PCOS women. As a result, measuring Activin A can be used to predict PCOS.

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Author Contribution

Ghofran Khalial performed the study, and Wasan Adnan and Saad S. Al-Dujaily supervised the work .

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The Ethical Approval Committee approved the study.

Financial Disclosure

There is no financial disclosure.

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