WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 7.523
Volume 6, Issue 14, 6-17. Review Article ISSN 2277–7105

CORRELATION BETWEEN SERUM AND FOLLICULAR FLUID ACTIVIN AND CLINICAL PREGNANCY RATES IN SUBFERTILE WOMEN UNDERGOING INTRACYTOPLASMIC SPERM INJECTION

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Article Received on
04 Sept. 2017,

Revised on 25 Sept. 2017,
Accepted on 15 October 2017

DOI: 10.20959/wjpr201714-9869

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ABSTRACT

Background: Successful pregnancy requires an intricate succession of molecular and genetic interactions through implantation process. The "window of implantation" or "window of receptivity is a limited time that reciprocal interactions between the embryo and the uterus must be executed within it. **Aim of the study:** to evaluate the correlation between serum and follicualr fluid activin A and preganancy rates in subfertile women undergoing intracytoplasmic sperm injection(ICSI)**. Pateints and methods:** This study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Techniques, Al-Nahrain University and Kamal Al-Samarayi IVF Center-Ministry of Health in Baghdad-Iraq. The duration of the study extended from November 2015 to September 2016.The study was designed to be prospective cohort study. The sample size was 100 sub-fertile undergoing in ICSI. Serum and follicular fluid activin A were estimated for all women and then the correlation with implantation and pregnancy rates was studied**. Results:** serum cycle day 2 (CD2) activin A, serum activin A at day of ova pickup (OPU), follicular activin A at day of ova pickup (OPU). All these variables were highly significantly($P<0.001$) lower in polycystic ovary syndrome (PCOS) groups in comparison with non-PCOS group. serum CD activin A, serum OPU activin A, FF OPU activin A, were all significantly ($P<0.05$) increment in women with positive pregnancy outcome than those with negative pregnancy outcome. **Conclusions:** Serum and follicular fluid activin A are good predictors of better pregnancy outcome in subfertile women.

KEYWORDS: Serum Activin A, Subfertile Women, PCOS, ICSI.

INTRODUCTION

The ovarian cycle lasts for 28 days and it is a series of events in the ovaries that occur during and after the maturation of the oocyte. It involves both oogenesis and preparation of the uterus to receive a fertilized ovum.[1] The principal events of it are under control by Hormones secreted by the hypothalamus, anterior pituitary gland and ovaries. The release of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the anterior pituitary gland are stimulated by Gonadotropin releasing hormone (GnRH), secreted by the hypothalamus. Follicular growth and the secretion of estrogens by the growing follicles are in turn initiated by FSH.[2] LH promotes the formation of the corpus luteum and stimulates the production of estrogens, progesterone, relaxin and inhibin by the corpus luteum and stimulates further development of ovarian follicles and their full secretion of estrogens, brings about ovulation.[3]

Successful implantation requires an intricate succession of molecular and genetic interactions. The “window of implantation” or “window of receptivity is a limited time that reciprocal interactions between the embryo and the uterus must be executed within it.[4] During this time any breach in the communication between the endometrium and the embryo leads to implantation failure. An important cause of infertility is implantation failure.[5] The implantation process is controlled by number of molecules like ovarian hormones, cytokines and growth factors which play an important role in regulating trophoblast differentiation and invasion.[6]

Activin hormone is polypeptide growth factor, member of the transforming growth factor-beta superfamily, Activin is disulphide-linked dimeric glycoproteins of the TGF-ß superfamily. The activin protein complexes are dimeric in structure constitute of two identical beta subunits and the two monomers in each complex are joined by a single disulfide bond.[7]

Activin is produced at both the ovarian and the pituitary level, in addition to placenta, and other organs. It plays an autocrine/paracrine role in the regulation of ovarian follicle development and it is considered as an important modulator of FSH synthesis and secretion in the pituitary.[8] Only activin A and activin Bforms are involved in the control of FSH.There is an important interplay between activin and estrogen signaling because, activin has an

important role in inducing the expression of estrogen receptors (ERs) in the ovary. Activin were played a role in oocyte maturation, as higher concentrations of activin A produced by good quality oocyte–cumulus complexes. The net issue of Activin is dependent on the available receptors, the amount of bioavailable ligand, and the comparative amounts present.[9,10]

The folliculostellate cells of the anterior pituitary at specific points on their surface secrete follistatin (FS), (activin binding protein). Gonadotropin secretion, in particular of FSH, is regulated by Follistatin released from the c folliculostellate cells. The granulosa cells are the type of cell in the ovary responsible for producing and secreting follistatin in most species.[11]

The granulosa cells in antral follicles and luteinized granulosa cells within the ovaryare the main sites that express follistatin mRNA and protein, whereas other structures appear to be devoid of follistatin mRNA, such as the theca cells, stroma and oocytes. Depends on the developmental stages of follicles in the ovary, the level of follistatin mRNA express. Within the antral follicles, Follistatin mRNA expression increases as follicular maturation progresses and declines during the atretic process. Furthermore, in only the selected dominant follicles, the follistatin protein appears to be present. It is not expressed in the primordial and primary follicles, so it is not involved in the initial stage of recruitment of follicles.[12]

The role of Follistatin in female physiology is regulating FSH levels through blocking activin actions. Premature ovarian failure is the net result of FSH regulation failure. The highest concentration of FS has been found to be in the female ovary, followed by the skin. The macrophages and monocytes circulating within the whole blood, the endothelial cells lining of blood vessels may be determined as the sources of follistatin in circulating blood plasma due to its autocrine nature. All circulating follistatin was activin-bound free indicated by follistatin assay. At the onset of puberty there is a significant change in serum concentrations of FSH-regulatory peptides.[13]

On the other hand, polycystic ovary syndrome is the association of increase androgen level with chronic an ovulatory cycles in women without specific underlying diseases of the adrenal or pituitary glands. The recent application of modern, high-resolution diagnostic ultrasonography has again tipped the balance toward a more morphologically based diagnosis; however, there is great match between clinical biochemical and radiological findings.[14]

Abnormal secretion of estrogen, in women with the PCOS, is the major result of chronic anovulation. For the early follicular and mid-follicular phases of the menstrual cycle, (both total and free)S. estradiol is in the the normal ranges, there is no preovulatory or mid-luteal rise in estradiol levels sothere is differs in the pattern of secretion from that of normal menstrual cycle. A lack of cyclical progesterone secretion make the action of estradiol on the hypothalamic–pituitary axis and on the endometrium is unopposed. In obese women these events may be compounded by increased serum concentrations of estrone deriving from the extra glandular transition of androgens by adipose tissue.[15]

The aim of the present study was to evaluate the role of Activin, measured in serum and follicular fluid in prediction of pregnancy outcome in subfertile women with and without PCOS.

PATIENTS AND METHODS

Study design

This study was conducted at the High Institute for Infertility Diagnosis and Assisted Reproductive Techniques, Al-Nahrain University and Part of sample collection was done at Kamal Al-Samirayi IVF center-Ministry of Health in Baghdad-Iraq. The duration of the study extended from November 2015 to September 2016.The study was designed to be prospective cohort study. The sample size was 100 sub-fertile undergoing intracytoplasmic sperm injection (ICSI), a convenient sample. The selected 100 women were intentionally divided, according to the cause of infertility, into 50 infertile women with polycystic ovary syndrome (PCOS) and 50 infertile women without PCOS. Each group would include women stimulated long GnRH agonist and women stimulated by antagonist protocol. Patients with elevated FSH levels and endocrine pathology were excluded from the study.

History and physical examination

Each couple underwent a complete history with physical examination (systemic and local, as outlined in the ASRM 2006 Practice Committee Opinion) in attempt to find the factors that could be the cause of impairing fertility. For women participating in the present study an early follicular phase FSH and LH levels were assessed. To ensure the patient has normal ovarian reserve, antimullerian hormone (AMH) was measured in those who are more than 35 years old. In addition serum prolactin, testosterone, progesterone and thyroid function test (T3,T4,and TSH) were performed were performed on day 2-3 of the cycle (CD2) for assessment of the hypothalamus-pituitary function and to exclude premature menopause.

Also serum E_2 (on CD2) and progesterone (on CD21) were measured for assessment of ovarian function, activin A was measured on early follicular phase of the cycle (CD2-3) and at the day of ovarian pick up. Hysterosalpingography (HSG) was performed to exclude tubal blockage. Ultrasound examination was performed. For male partners, standard seminal fluid examination was carried out.

Ovarian stimulation medication used included Gonal-F(75IU/vial, Merck-Serono), Menogon(75 IU/vial Ferring), Ovitrelle (6500IU/vial, Merck- Serono) and Pregnyl(5000IU/vial Organon, Netherland) injection. Controlled ovarian hyperstimulation protocols included both GnRH agonist and GnRH antagonist protocols. O'ocyte retrieval was carried out 34 - 36 hours after the hCG injection under general anesthesia. oocytes were harvested by needle aspiration through theposterior fornix with a transvaginal ultrasound transducer guidance. Intracytoplasmic sperm injection (ICSI) was done according to standard procedure.[16]

Embryo transfer was done on day 2 and 3 of embryonic development with or without assisted hatching, mostly two or three embryos were transferred depending on the recommendation of the couple and the quality of the embryos. The day -3 embryos were graded morphologically as good embryos (less than 20% fragmentation and an even blastomere) or poor embryos (more than 20% fragmentation or an uneven blastomere). It is also classified according to the number of blastomere (The normal cleavage 7-8 cells).

Activin A is measured using quantitative sandwich enzyme immunoassay technique; the detection range was 53pg/ml-2000. HB-EGF kit uses enzyme-linked immune sorbent (ELISA) based on biotin double antibody sandwich technology to assay Human heparin-binding epidermal growth factor (HB-EGF). Human ACV-A ELISA kits of Hcusabio products allow for the *in vitro* quantitative determination of human ACV-A (pg/ml) concentrations in serum and follicular fluid.

Statistical analysis

Data involved in the present study were collected, summarized, analyzed and presented using the following software programs: Statistical Package for Social Sciences (SPSS 22.0), Microsoft Office Excel 2010 and Medcalc 2010. The statistical analysis was done according to the nature of data. Categorical variables were presented as number and percentage whereas numeric variables were expressed as mean, standard deviation, median and inter-quartile

range. The level of significance was considered at P-value of equal or less 0.05 and highly significant level at p-value equal or less than 0.01.

RESULTS

Mean age of the entire women was (32.10 ±5.66) years and there was no significant (P>0.05) difference in the mean age between non-PCOS group and PCOS group, (31.72 ±5.45 versus 32.48 ±5.89 years, P=0.523). The number and percentage of women having primary infertility were slightly less in the group of non-PCOS than in the group of PCOS, 34(66%) versus 34(68%), respectively. On the other hand, number and percentage of women complaining of secondary infertility were slightly more in the non-PCOS group than in the PCOS group, 17 (34%) versus 16(32%), respectively; however, the difference was not statistically significant (P=0.832). Overall, the main cause of infertility observed in the entire sample was male factors, occurring in 38(38%) of women. In the non-PCOS group, the main cause of infertility was male factor followed by unexplained cause and then tubal factor, 26(52%), 17(34%) and 7(14%), respectively; While the main cause of infertility in the PCOS group was tubal factor, followed by unexplained factor and then male factor, 21(42%), 17(34%) and 12(24%), respectively. Overall, the difference in the distribution of causes of infertility among non-PCOS and PCOS group was statistically different (P =0.002). However, the occurrence of unexplained factor was the same in both groups, thus the present study fixed this factor and treated it as a reference in order to compare the two other factors, male factor and tubal factor, between the non-PCOS and PCOS groups. The percentage of women having tubal factor was significantly greater in the PCOS group than in the non-PCOS group (P=0.044). Although, male factor was more frequent in the non-PCOS group than in the PCOS group, the difference did not reach statistical significance (P =0.112), table 1.

Table 1: Mean age and age range in subfertile women classified into PCOS and non-PCOS groups.

Characteristic	Non-PCOS (*n* = 50)	PCOS (*n* = 50)	Total (*n* = 100)	P-value
Mean age ±SD (years)	31.72 ±5.45	32.48 ±5.89	32.10 ±5.66	0.523* NS
Infertility type				
Primary, *n* (%)	33 (66)	34 (68)	67 (67)	0.832 † NS
Secondary, *n* (%)	17 (34)	16 (32)	33 (33)	
Infertility cause				
Unexplained, *n* (%)	17 (34)	17 (34)	34 (34)	Reference
Tubal, *n* (%)	7 (14)	21 (42)	28 (28)	0.044 S
Male, *n* (%)	26 (52)	12 (24)	38 (38)	0.112 NS

PCOS: polycystic ovary syndrome; *n*: number of cases; SD: standard deviation; IQR: interquartile range; %: percentage; * Mann Whitney U test; † Chi-square test; NS: not significant.

The means of total oocytes number, number of M1 oocytes and number of GV oocytes were in PCOS group were highly significantly (P <0.001) greater than that in non-PCOS group. Whereas no significant difference concerning mean ofM2 oocyte number (P =0.796) was recorded between the two women groups.In addition to that, PCOS group showed no significant differencecompare to non-PCOS groupregardingto thenumber of total embryos, number of transferred embryos,number of grade 1 (G1) embryos andnumber of blastocyst stage (P=0.388), as shown in table (2).

Table 2: Characteristics of ova and embryos in non-PCOS and PCOS groups.

Characteristic		Non-PCOS (*n* = 50)	PCOS (*n* = 50)	Total (*n* = 100)	P *
Ova	Number of oocyte (Mean ±SD)	8.34 ±2.75	11.16 ±3.70	9.75 ±3.54	<0.001 HS
	M2 (Mean ±SD)	4.26 ±1.56	4.70 ±2.53	4.48 ±2.10	0.796 NS
	M1 (Mean ±SD)	2.60 ±1.14	3.36 ±1.12	2.98 ±1.19	<0.001 HS
	GV (Mean ±SD)	1.48 ±0.81	3.08 ±1.41	2.28 ±1.40	<0.001 HS
Embryo	Number of embryos (Mean ±SD)	5.70 ±2.54	6.44 ±2.42	6.07 ±2.50	0.061 NS
	Number of embryo transfer (Mean ±SD)	2.34 ±0.75	2.24 ±0.74	2.29 ±0.74	0.448 NS

	G1 (Mean ±SD)	2.28 ±0.69	2.21 ±0.70	2.24 ±0.69	0.618 NS
	Blastocyst (Mean ±SD)	2.88 ±0.35	2.70 ±0.48	2.78 ±0.43	0.388 NS

PCOS: polycystic ovary syndrome; *n*: number of cases; SD: standard deviation; *Mann Whitney U test; NS: not significant; S: significant; HS: Highly significant.

Hormones and growth factor levels in PCOS and non-PCOS groups

Highly significant differences were encountered between PCOS and non-PCOS groups regarding estradiol (E_2), serum cycle day 2 (CD2) activin A, serum activin A at day of ova pickup (OPU), follicular activin A at day of ova pickup (OPU). All these variables were highlysignificantly(P<0.001) lower in PCOS groups in comparison with non-PCOS group, table (3).

Table 3: Comparison of hormones and growth factor between PCOS and non-PCOS groups.

Hormone	Non-PCOS (*n* = 50)	PCOS (*n* = 50)	Total (*n* = 100)	P *
	Mean± SD	Mean ± SD	Mean ± SD	
E_2	1428.10 ±570.83	2057.60 ±578.82	1742.90 ±653.59	<0.001 HS
Serum CD 2 activin A	2.88 ±1.70	7.62 ±2.46	5.25 ±3.18	<0.001 HS
Serum OPU activin A	5.23 ±1.85	16.23 ±5.54	10.73 ±6.89	<0.001 HS
FF OPU activin A	5.22 ±1.82	16.14 ±5.83	10.68 ±6.97	<0.001 HS

*Mann Whitney U test; *n*: number; SD: standard deviation; E2: estradiol; CD2: cycle day 2; HS: highly significant; FF: follicular fluid; OPU: oocyte pickup.

Receiver operator characteristic curve was carried out to calculate cutoff values that predict a positive diagnosis of PCOS and it the results are shown in table 4.

Table 4: Cutoff values that identify PCOS group.

Variable	Cutoff value (> or =)	AUC	P	Sensitivity	Specificity
E2	1679.00	0.782	<0.001	72	72
Serum CD activin A	5.59	0.945	<0.001	82	94
Serum OPU activin A	9.97	0.985	<0.001	78	100
FF OPU activin A	9.87	0.980	<0.001	76	100

Regarding PCOS group, mean estradiol (E2) was significantly (P=0.018) higher in women with positive pregnancy outcome(2298.80±475.73) than those with negative pregnancy outcome(1911.00 ±582.12). In addition to that, serum CD activin A, serum OPU activin A, FF OPU activin A, were all significantly (P<0.05) increment in women with positive pregnancy outcome than those with negative pregnancy outcome, as illustrated in table (5).

Table 5: Hormone levels according to biochemical pregnancy outcome in PCOS group.

Characteristic	Positive pregnancy (n =21)	Negative pregnancy (n = 28)	P
	Mean ± SD	Mean ± SD	
E2	2298.80 ±475.73	1911.00 ±582.12	0.018
Serum CD2 activin A	8.78 ±2.14	6.91 ±2.28	0.028
Serum OPU activin A	18.09 ±4.43	15.23 ±5.76	0.024
FF OPU activin A	18.16 ±4.55	15.04 ±6.09	0.032

Regarding non-PCOS group, mean estradiol (E2) was significantly (P=0.010)higher in women with positive pregnancy outcome than those with negative pregnancy outcome, 1703.90 ±618.75versus 1286.70 ±508.77, in addition Serum CD activin A, Serum OPU activin A, FF OPU activin A were all significantly (P<0.05) higher in women with positive pregnancy outcome than those with negative pregnancy outcome, as demonstrated in table (6).

Table 6: Hormone levels according to biochemical pregnancy outcome in non-PCOS group.

Characteristic	Positive pregnancy (n =16)	Negative pregnancy (n = 33)	P
	Mean ± SD	Mean ± SD	
E2	1703.90 ±618.75	1286.70 ±508.77	0.010
Serum CD 2 activin A	3.63 ±1.77	2.48 ±1.59	0.006
Serum OPU activin A	6.07 ±1.94	4.80 ±1.70	0.022
FF OPU activin A	6.02 ±1.91	4.83 ±1.70	0.025

DISCUSSION

Hormones levels in PCOS and non-PCOS groups

The present study showed highly significant differences between PCOS and non-PCOS groups regarding serum cycle day 2 (CD2) activin A, serum activin A at day of ova pickup (OPU), follicular activin A at day of ova pickup (OPU); all these variables were higher in PCOS groups in comparison with non-PCOS group.

The present study showed that both serum and follicular activin A levels were significantly higher in the PCOS group than in control non-PCOS group. The controversy about the serum and follicular level of activin A is still present in the vast majority of published literatures. Some authors found no significant difference in mean serum and follicular activin A concentration between PCOS and control groups.[17, 18,19,20] Whereas other authors stated that serum activin A level was significantly lower in PCOS group than control group.[18] Teede *et al*., in 2013 observedthat the mean of serum activin A was nearly double when measured in PCOS women in comparison with healthy women of comparable age, and this finding support our finding. The high serum and follicular fluid activin A may be due to obesity which well defined in PCOS patientsor may be due to ovarian stimulation.[17]

It was supposed that low activin A level is crucial for lack of further development of ovarian follicles in PCOS, however our findings and the findings of other authors[21] made this hypothesis untrue. We believe that the ratio of follistatin to activin A, and not just the absolute activin concentration, is the main player in the pathogenesis of PCOS and that high follistatin to activin A ratio, as stated by most of published literatures[22], is the one that should be blamed because follistatin will bind and deactivate activin A and will not reduce its level.

Biochemical pregnancy outcome in relation to serum E_2 and serum follicular fluid activin A.

In the present study, mean of bothserum estradiol (E2) and serum and follicular fluid activin A were significantly higher in women with positive biochemical pregnancy than women with negative biochemical pregnancy in both PCOS and non-PCOS groups. In both groups serum estradiol was shown to be a fair predictor for positive pregnancy outcome since area under the curve was more than 0.7[22]; however it gave rise to poor sensitivity, 57.1 % and 56.2% for PCOS and non-PCOS groups respectively. Serum cycle day 2 activin A was better predictor for pregnancy in PCOS than in non-PCOS group, where as serum activin at day of ova pickup was better predictor of positive pregnancy outcome in non-PCOS group than in PCOS group, because area under the curve was > 0.7 in non-PCOS group and < 0.7 in PCOS group. In both groups, serum activin A has poor sensitivity for positive pregnancy outcome at variable levels of specificity. Follicular fluid activin A was poor predictor for positive pregnancy outcome in both groups, because area under the curve was < 0.7.

According to Al-Dujaily and Alwan in 2013[23], It has been shown that activin A can predict pregnancy and that the best cut off predictive value is ≥ 397.5ng/ml. The failure of obtaining a sensitive cutoff value for activin A that predict pregnancy in the present study might be due to small sample size or may be due to the fact that our sample included patients with PCOS in whom the biochemistry of active is different from other causes of infertility.

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