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THE ROLE OF SERUM AND FOLLICULAR FLUID GHRELIN HORMONE IN POLYCYSTIC OVARY SYNDROME PATIENTS UNDERGOING ICSI

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ABSTRACT

Background: Ghrelin is a 28 amino acid gastric peptide hormone, discovered as being the endogenous ligand of growth hormone secretagogue receptor, mainly produced by a subset of stomach cells and also by the hypothalamus, the pituitary, and other tissues . The Ghrelin receptors can also be found in ovarian tissue, thus suggesting a possible reproductive function of this hormone. The objective of this study was to estimate the effect of ghrelin hormone level in serum and follicular fluid in PCOS patient at day of ova pick up in predicting pregnancy outcome after intra cytoplasmic sperm injection. A total of 90 infertile women(60 women with Poly cystic ovary syndrome and the other 30 women as a control)undergoing controlled ovarian hyperstimulation for intracytoplasmic sperm injection cycle were prospectively recruited for this study in center of fertility and in vitro fertilization at Kamal AL_Samarai Hospital (Baghdad/Iraq)during the period from September 2014 to the end of February 2015. All patients underwent a long standard gonadotrophin releasing hormone agonist protocol(GnRH-a). Serum and follicular fluid ghrelin hormone levels were measured on the day of oocyte retrieval by using Enzyme linked immuno sorbent assay. The concentration of serum ghrelin hormone was lower in poly cystic ovary syndrome patients than in control patients(6.81±0.44,12.09±0.28)pg/ml respectively. There was no significant correlation between serum and follicular fluid ghrelin hormone($r=0.04, p=0.660$).E2 level in both serum and follicular fluid were highly significant increase($p<0.001$)in PCOS than control group Regarding pregnancy rate it was higher in PCOS patients(23.33%) than in control patients(10%). We can conclude that the level of ghrelin hormone at day of oocyte

retrieval in both serum and follicular fluid, cannot be used as a strong test in predicting pregnancy after intra cytoplasmic sperm injection cycles**.**

KEYWORDS: ghrelin, PCOS, ICSI.

INTRODUCTION

Polycystic ovary syndrome (PCOS) is a common endocrine disorder in women of reproductive age with primary manifestations of infertility, menstrual dysfunction and clinical or biochemical hyperandrogenism (hirsutism, acne and elevated androgen).[1,2] The etiology of PCOS is multifactorial, including both genetic and environmental issues. Although hyperandrogenism, ovarian dysfunction, abnormalities in the hypothalamic-pituitary axis, and excess insulin activity are known to be responsible for pathogenesis of the syndrome, the exact etiology has yet to be discovered.[3,4] PCOS is associated with metabolic aberrations including dyslipidemia and impaired glucose tolerance.[5] Obesity is a very common clinical feature in women affected by PCOS. More than 50-60% of PCOS women are obese.[6] The diagnosis of PCOS must be based on the presence of two of the three following criteria: oligo and/or anovulation , clinical and/or biochemical signs of hyperandrogenism, polycystic ovaries on ultrasonography and exclusion of related disorders.[7] Several methods have been effective in ovulation induction and fertility treatment in PCOS patients[8], one of these recent methods is assisted reproductive techniques(ART), which according to WHO definition refers to all infertility treatments and procedures that involve in vitro handling of human oocyte and sperm or embryo for purpose of establishing pregnancy.[9]

Ghrelin is a 28-amino acid acylated peptide produced primarily by the endocrine cells in the stomach.[10] It stimulates GH secretion through its action as an endogenous ligand for the hypothalamic-pituitary GH secretagogue receptor. In addition, it is implicated as an important regulatory peptide in food intake, body weight regulation, endocrine pancreatic function, glucose metabolism, and ovarian function.[11,12] Low ghrelin levels were found during conditions of positive energy balance such as obesity[13,14] and accordingly, studies reported low ghrelin levels to be associated with insulin resistance and diabetes.[15] Ghrelin was found to be expressed in pancreatic ß cells and may possibly be able to inhibit insulin secretion.[16,17] Recent research indicates the significant role of this hormone in regulating reproductive functions in broad aspect. Ghrelin is a hormone that is secreted by the ovarian follicle and regulates the processes of steroidogenesis in the ovary stimulating the

proliferation of cells and showing anti apoptotic effect. In vitro researches suggest that ovaries may be important locations of ghrelin's activity as high concentration of ghrelin is found in human ovaries. Negative correlation between ghrelin's concentration and androgens in females' serum has also been noted.[18] The aim of study was to summarize the effect of ghrelin hormone on ovulation and pregnancy outcome in patient with PCOS undergoing IVF/ICSI cycle and to find correlation between its concentration in serum and follicular fluid.

PATIENTS AND METHODS

A total of 90 infertile women(60 PCOS and 30 control) undergoing controlled ovarian hyperstimulation(COH) for ICSI were prospectively recruited for this study in Kamal AL-Samarai Hospital, center of fertility and IVF (Baghdad/Iraq) during the period from September 2014 to the end of February 2015. Written informed consent was obtained from patients as well as control to participate in this study . The diagnosis of PCOS depend on Rotterdam' s criteria (2003)(7).

The inclusion criteria were:1) The patients who had diagnosed as PCOS in the presence of at least 2 of Rotterdam's criteria, based on Rotterdam consensus meeting (2003).
2) Patient age (18-40)years.
3)The patient's agreement to participate in the study. While the exclusion criteria were: 1) Evidence of endocrine abnormalities such as hyperprolactinemia, thyroid dysfunction, and hypogonadotropic hypogonadism.
2) Patients whose follicular fluid (FF) was bloody during the oocyte retrieval.
3) Cycles where no oocytes (empty follicles) were retrieved on the day of aspiration.
4) Patient aged more than 40years.

All patients underwent a long standard gonadotrophin releasing hormone agonist protocol(GnRH-a). For the current study the main outcome measure were:1) The ghrelin, and E2 concentration in 90 matched FF/serum pairs with basal FSH and LH serum level have been determined. 2) The relations of (ghrelin, E2, LH and FSH) hormones have been compared with the prognostic parameters and ICSI outcome data.

Methods of measuring hormones in serum and follicular fluid

Serum levels of LH and FSH on day 3 of the menstrual cycle were determined by using miniVIDAS system (bioMerieux, France). The assay principle combines an enzyme immunoassay sandwich method with a final fluorescent detection (enzyme linked fluorescent

assay(ELFA) technology).Serum and FF obtained on the day of oocyte retrieval were estimated for ghrelin levels by ELISA technique using diagnostic kit (CUSABIO, CSB-E13398h, China). On the other hand E2 was measured in the obtained sera and FF via enzyme-linked immunosorbent assay (ELISA) technique by using diagnostic kit was provided by(MonobindInc., USA).

Statistical analysis

The Statistical Analysis System- SAS (2012) , Version 9 was used to effect of different factors in study parameters. Numeric variables were expressed as mean + standard error (SE), while nominal variables were expressed as number and percentage. Least significant difference –LSD test was used to significant compare between means and Chi-square test was used to significant between percentage in this study. Estimate of correlation coefficients between some parameters study.

Pearson's correlation coefficient was used to evaluate correlation between numeric variables.

RESULTS

The 90 infertile women divided into 60 PCOS and 30 control groups. The percentage of pregnancy was higher in PCOS group(23.33%) than in control group(10%)as in Figure1. By comparing the level of ghrelin hormone between two groups there was high significant difference($p<0.001$) in the level of ghrelin in serum(lower level in PCOS),but there was no significant difference ($p>0.05$) in level of ghrelin hormone in FF between PCOS and control group(Figure2). The statistical analysis shows no significant difference($p>0.05$) in the level of ghrelin both in FF and serum between pregnant and non pregnant PCOS groups. According to the level of E2 both in serum and FF the statistical analysis shows highly significant difference($p<0.001$) in two groups(Figure 3). Clinical parameters (age, BMI, basal LH, and FSH) of this study with respect to the ICSI outcome, showed highly significant difference($p<0.001$) in the level of basal LH and FSH between two groups(LH higher in PCOS group while FSH higher in control group), also significant increase in total number of oocyte and number of MII oocyte in PCOS than in control(Table 1)

Pearson's correlation analysis of associations between the FF and serum ghrelin and the clinical(age, BMI, basal LH and FSH) and treatment (oocytes (total, MII), and fertilization rates)parameters(Table2), there was negative correlation between BMI, basal LH,

oocyte(total, MII),fertilization rate and number of embryo transferred with serum ghrelin, while with FF ghrelin there was only negative correlation with BMI, basal FSH and total number of oocyte. On the other hand there was positive correlation between serum ghrelin and basal FSH. According to correlation between ghrelin in serum and FF with level of E2 there was negative correlation in serum and FFghrelin and E2 in both compartment.

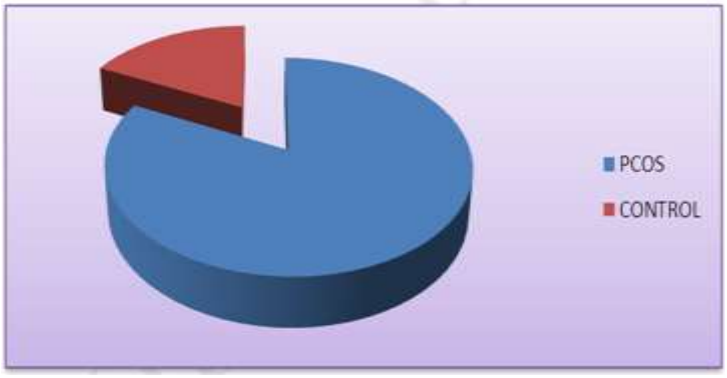

Figure 1: Percentage of pregnancy between PCOS and control group

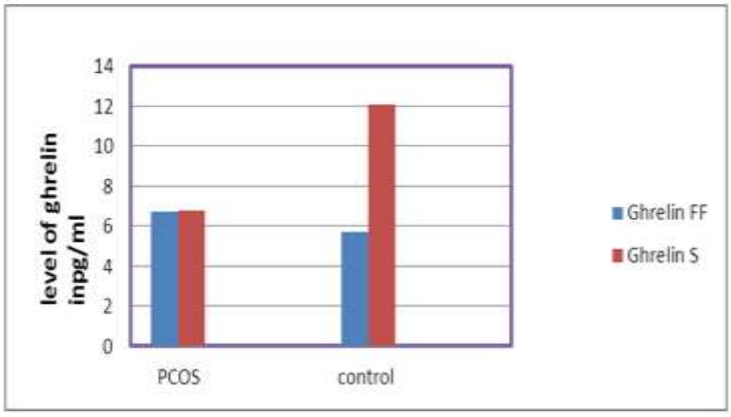

Figure 2: Comparison between the level of ghrelin hormones in FF and Serum both in PCOS and control groups.

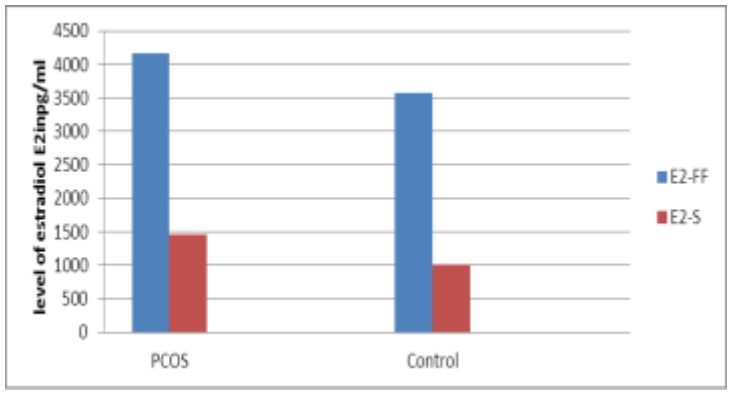

Figure 3: Comparison between the level of E2 hormones in FF and Serum both in PCOS and control groups.

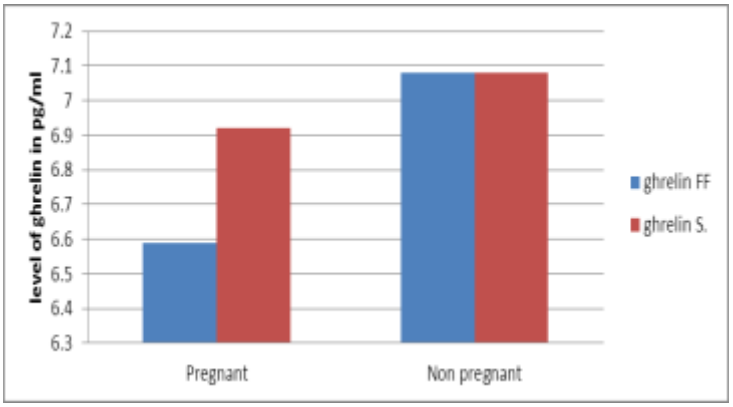

Figure 4: Comparison between pregnant and non pregnant in level of FF and serum ghrelin

Table 1: Comparison between PCOS and control in clinical and treatment parameters:

parameters	Mean ± SE		P-value
	PCOS	Control	
Age (years)	28.33±0.94	28.56±1.24	0.492 NS
BMI(kg/m^2)	28.69±1.05	28.33±0.95	0.562 NS
Basal LH in mIU/ml	6.40±0.47	2.81±0.22	0.0001**
Basal FSH in mIU/ml	4.36±0.26	6.14±0.37	0.0002**
Oocyte no.	10.78±0.88	7.33±0.87	0.0155*
No. of MII	7.26±0.62	5.27±0.63	0.046*
FR%	57.76±3.56	55.16±6.08	0.695 NS
No. of ET	2.65±0.17	2.23±0.22	0.152 NS

All values expressed by mean± SE(stander error). BMI: body mass index. PCOS: Poly Cystic Ovary Syndrom, LH: Luteinizing Hormone. FSH: Follicle Stimulating Hormone MII: Metaphase II oocyte. FR%:Fertilization Rate. ET: Embryo Transferred. **P<0.001:highly significant *p<0.05:significant.NS: Non significant.

Table 2: The correlation between follicular fluid (FF) and serum(S) Ghrelin with clinical and treatment parameters.

Parameters		Ghrelin. FF	Ghrelin. S
Age (years)	r	0.14 NS	0.17 NS
	p	0.186	0.101
BMI(kg/m^2)	r	-0.03*	-0.21*
	p	0.749	0.050
Basal-LH	r	0.10 NS	-0.34**
	P	0.337	0.0011
Basal-FSH	r	-0.10 NS	0.27*
	P	0.343	0.0097
Oocyte no.	r	-0.05 NS	-0.29**
	p	0.608	0.006

No. of MII	r	0.01 NS	-0.19*
	p	0.901	0.058
FR%	r	0.008 NS	-0.20*
	p	0.935	0.056
No. of ET	r	0.005 NS	-0.26**
	p	0.962	0.012
E2-FF	r	-0.12 NS	-0.68**
	P	0.256	0.0001
E2-S	r	-0.04NS	-0.24*
	P	0.641	0.022

FF: Follicular Fluid. S: Serum. BMI: Body Mass Index. LH: Luteinizing Hormone. FSH: Follicle Stimulating Hormone. MII: Metaphase II. FR: Fertilization Rate. ET: Embryo Transferred. E2:Estradiol r: Correlation Coefficients. **p<0.001:highly significant. *p<0.05:significant.NS: Non significant.

DISCUSSION

Although age is well known as one of the most important factors predicting IVF/ICSI outcome[19,20], in our study there was no significant differences(P>0.05) in mean age between PCOS group and control group**.** These results indicated that PCOS women had comparable age with control group to eliminate any variations that may affect the results of the measured biochemical parameters. Also there was no significant difference between pregnant and non pregnant**.** and this result is in agreement with the results of other studies suggested that although age is an essential factor in sub fertility, it is not very precise in predicting the reproductive potential, because some women will be incapable to conceive in their thirties, while others become pregnant in their forties. Clearly, there is an extensive range in the relationship between ovarian function and age, and ovarian reserve appears to be responsible for these differences.[21] Clinical observations on the effects of body weight during IVF are more controversial. Some studies found decreased implantation and pregnancy rates in obese and overweight women P[23,23]P. Whereas, others have not found such an effect for ART cycles in relation to obesity [24,25] P. In agreement of these results, results of this study which shows no significant differences between pregnant and non pregnant patients regarding BMI, also there was no significant difference in BMI between PCOS and control group(28.69kg/m2 ,28.33kg/m2)respectively. The similarity in the BMI between PCOS and control is not surprising since it has been reported that only one third of the PCOS patients are obese.[26] This had disagreement with other studies that said women suffering from PCOS have been shown to have higher amount of body fat compared to healthy women even when

they are of normal weight.[27] There were no significant differences between BMI and clinical and treatment parameters. This result is in agreement with the results found by Sathya *et al*[24] who concluded that the three groups of BMI (normal, overweight, and obese groups) were similar with respect to basal LH/FSH levels, oocyte quality, fertilization rate, number of good quality embryos transferred and clinical pregnancy rateP. In overall, this study shows that BMI has no adverse effects on IVF outcome. This could be explained by the fact obtained by the previous study that the embryo quality was not affected by BMIP.[24]

Ghrelin's role in reproduction was first suggested by its wide expression in many human reproductive tissues including its immune histochemical expression in the human ovary.[28] Centrally, ghrelin is known to suppress hypothalamic GnRH release and GnRH-induced gonadotropin secretion by the pituitary in animals and humans.[29] In our study, there was highly significant decrease in the level of serum Ghrelin in PCOSgroup than control group. These results were in agreement to results obtained by Christof Schofl *et al*[17] who found that serum levels of the recently characterized endogenous GH secretagogue, ghrelin, are significantly lower in women suffering from PCOS than in controls. Ghrelin levels are decreased in PCOS women and are highly correlated to the degree of insulin resistance. This indicates that ghrelin, apart from its role in the control of appetite and body weight could be linked to insulin resistance. According to clinical parameters there was significant negative correlation between serum ghrelin and BMI, total oocyte retrieved, MII oocyte, FR%, and number of ET. This in agreement with Liyun Li *et al*[30] who found that serum ghrelin correlated negatively with the oocyte maturation rate (MII/total oocytes), the number of viable cleavage-stage embryos, and the cleavage rate (cleavage stage embryos/2PN) but not with the fertilization rate, and BMI. While Dorte Glintborg *et al*[31] found that Ghrelin levels were significantly decreased in both patients and controls when BMI and waist circumference increased. also Daghestani *et al*.[32] showed a significant inverse relationship between ghrelin and BMI in both PCOS and healthy subjects. Regarding to FF ghrelin There was no significant difference in level of FF ghrelin between PCOS and control group. This in agreement with Hossein and Robert Normann[33] who found that ghrelin and GHS-R have been expressed in follicular cells of PCOS and non-PCOS patients. There were not significantly differences in ghrelin and GHS-R expression between PCOS and non-PCOS groups. Ghrelin and ghrelin receptors may not be considered risk factors for pathogenesis of PCOS. There was negative correlation between FF ghrelin and E2 both in follicular fluid and serum and with basal FSH. This agreed with Viani *et al*[34] who found that ghrelin is able to

significantly inhibit, in a dose-dependent manner, E2 production by granulosa-lutein cells(so decrease its concentration in FF) This suggests that ghrelin may serve an autocrine- paracrine role in the control of gonadal function and be part of a network of molecular signals responsible for the coordinated control of energy homeostasis and reproduction. While Liyun Li et al[30] found that FF ghrelin correlated positively with FF E2. On the other hand there was no significant correlation between FF ghrelin with clinical parameters, which demonstrate that ghrelin in FF do not reflect human oocyte quality or embryonic development. About the correlation between FF and serum ghrelin, our study showed no significant difference in the level of ghrelin both in FF and serum, this explained by some authors that FF ghrelin is not produced locally but could be a serum transudate.[30]

The evaluation of follicle maturation usually depend on serum estradiol(E2) concentration. This measurement is important to predict the timing of ovulation when treating infertility patients. As the date of ovulation approaches, the E2 level changes dynamically , particularly in natural cycle in vitro fertilization (IVF). In pre-menopausal women, E2 is mainly secreted by granulosa cells in the follicles. As these cells divide and proliferate within a follicle, increasing in number as the follicle grows, the E2 level also increases. Thus, the E2 concentration is a good index of follicular maturation.[35] The steroids are synthesized by follicular cells during the process of follicle maturation and accumulate in FF. Since the oocyte is in close contact with FF, an association is believed to exist between the hormonal content of FF and the quality and maturity degree of oocyte, and therefore FF hormonal content is believed to be related to fertilization, embryo development and implantation rate.[36] These observation explained the important role of E2 in the pregnancy outcome. Our study showed that both serum and follicular E2 highly significant in PCOS patients than control group(Figure2).These results agreed with results obtained by Yi-Ping Zhong *et al*[37] who found that compared to the control groups PCO and PCOS patients exhibited reduced duration of ovarian stimulation and total Gonadotropin dose, as well as increase serum and follicular E2 and number of collected oocyte. On the other hand there was no significant difference in both serum and follicular E2 among the pregnant and non pregnant group. While the results found by Westergard[38] revealed that the serum concentration of estradiol were similar in conception and non-conception cycles, and the estradiol level in the follicular fluid was higher but only with borderline significance.

In conclusion, the level of ghrelin hormone was lower in PCOS than in control group and there was no significant difference in its level both in serum and FF between pregnant and non pregnant group, so it cannot be used as a strong test in predicting pregnancy after intra cytoplasmic sperm injection cycles**.**

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