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Original Article

Serum Follistatin and Its Role in Intracytoplasmic Sperm Injection Outcomes

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Abstract

Background: progress in assisted reproductive technology (ART) has enabled the clinicians to treat many types of infertility. **Objectives:** The ultimate goal of all these procedures is to get a viable intrauterine pregnancy as a step to get a healthy baby. Intracytoplasmic sperm injection (ICSI) refers to the technique of assisted reproduction, including injecting a single sperm into the center (cytoplasm) of the egg. **Materials and Methods:** A prospective study included 45 women who were enrolled in ART programs in infertility center for *in-vitro* fertilization (IVF) in International Center/Kirkuk, Iraq. All women were subjected to the basic fertility workup at the infertility center which consists of history taking, physical examination, ovulation detection, evaluation of tubal patency, and uterine cavity. The average age of the included women ranged between 20 years and 42 years of age. All women were enrolled in short protocol type of IVF/ICSI cycle, and they had normal menstrual cycles. Serum samples were stored for estimation of follistatin level by ELISA technique and for estimation of luteinizing hormone, follicle-stimulating hormone, estrogen, and progesterone by VIDAS technique. **Results:** The present study showed that 31.1% of women underwent ICSI technique became pregnant and 68.9% were nonpregnant. The highest mean of age were recorded among pregnant women compared with nonpregnant women (32.21 ± 6.68 vs. 31.80 ± 5.38 years) although the result was nonsignificant ($P > 0.05$). The highest mean of body mass index were recorded among nonpregnant women compared with pregnant women (23.92 ± 1.55 vs. 25.36 ± 1.99 kg/m^2); the result was significant. The study showed highest mean level of serum follistatin present in nonpregnant compared with pregnant women (0.75 ± 0.32 vs. 0.62 ± 0.24 ng/ml) although the result was nonsignificant ($P > 0.05$). **Conclusions:** There was no relation of serum follistatin with pregnancy after ICSI.

Keywords: Intracytoplasmic sperm injection, pregnancy, serum follistatin

Introduction

Infertility is a disease of the reproductive system defined by the failure to achieve a pregnancy after at least 1 year of regular unprotected sexual intercourse; it affects about 10%–15% of couples.[1] According to WHO definition, a couple is considered infertile if, after 2 years of regular sexual intercourse, without contraception, the woman has not become pregnant (there is no other reason, such as breastfeeding or postpartum amenorrhea).[2] Infertility classified as primary (no pregnancy in the past) or secondary (pregnancy has occurred in the past not necessarily leading to live birth).[3] Nowadays, progress in assisted reproductive technology (ART) has enabled the clinicians to treat many types of infertility.[4]

Assisted reproduction is a complicated process involving multiple stages such as ovarian stimulation, ovum pick up, then fertilization of these oocytes, embryo cleavage, and implantation.[5] The ultimate goal of all these procedures is to get a viable intrauterine pregnancy as a step to get a healthy baby. Intracytoplasmic sperm injection (ICSI) refers to the technique of assisted reproduction, including injecting a single sperm into the center (cytoplasm) of the egg.[6,7]

ICSI is a well-established treatment for most types of infertility, including long-standing infertility due to tubal disease, endometriosis, unexplained infertility, and even some mild forms of male factor infertility and cases of failure with respect to *in-vitro* fertilization (IVF) cycles.[8,9] Follistatin is a glycoprotein, was originally identified

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in porcine follicular fluid, and received its name because it suppresses synthesis and secretion of follicle-stimulating hormone (FSH) from the pituitary gland.[6] For this reason, the activities of follistatin on the oocyte maturation depend on their autocrine and paracrine effects in follicular fluid than their serum levels.[7]

Follistatin not only worked as an activin-binding protein but also regulator of hormone secretion from the pituitary gland and degraded the activin from the circulation.[10] Because it localized within the human fallopian tube, endometrium, and placental tissues, these proteins have been proposed as potential sensitive and specific markers to monitor the progress and outcome of pregnancy.[11] Follistatin levels increased during the course of pregnancy and decreased rapidly postpartum; this mean increasing maternal serum FST levels are associated with healthy pregnancies and it could be altered in a status of failed pregnancy.[7,11]

The aim of the study was to evaluate serum follistatin level and correlate the results with pregnancy outcome in women undergoing ICSI.

Materials and Methods

A prospective study carried out in Kirkuk city from January 15, 2019 to April 10, 2019, included 45 women who were enrolled in ART programs in infertility center for IVF in International Center/Kirkuk, Iraq. All women were subjected to the basic fertility workup at the infertility center which consists of history taking, physical examination, ovulation detection, evaluation of tubal patency, and uterine cavity. The average age of the included women ranged between 20 years and 42 years of age. All women were enrolled in short protocol type of IVF/ICSI cycle, and they had normal menstrual cycles.

Five ml of blood sample was taken at 12–13 days of menstrual cycle. The samples were placedinto sterile test tubes, centrifuged at 3000 rpm for 15–20 min and the obtained serum was aspirated using mechanical micropipette and transferred into clean test tubes which labeled and stored in deep freeze at −20°C for biochemical measurement. After approximately 2 weeks of embryo implantation, another blood samples 2 ml was taken to assess pregnancy status.

Statistical analysis

Computerized statistically analysis was performed using Minitab version 18.0 statistic program (LLC, State College, Pennsylvania, USA). Comparison was carried out using Chi-square and *t*-test for determination of the *P* value ($P < 0.05$: significant).

Ethical consideration

The study was conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki. It was carried out with the patient's verbal and analytical approval before the sample was taken. According to this approval, all the samples were collected and the tests were carried out. A copy of the results of the tests was then given to the patients.

Results

The present study showed that 31.1% (14 of 45) of women underwent ISCI technique became pregnant and 68.9% were nonpregnant (31 of 45) [Table 1].

Figure 1 shows that the highest mean of age were recorded among pregnant women compared with nonpregnant women (32.21 ± 6.68 vs. 31.80 ± 5.38 years) although the result was nonsignificant ($P > 0.05$). The highest mean of body mass index (BMI) were recorded among nonpregnant women compared with pregnant women (23.92 ± 1.55 vs. 25.36 ± 1.99 kg/m^2); the result was significant.

The study showed highest mean level of serum follistatin present in nonpregnant compared with pregnant women (0.75 ± 0.32 vs. 0.62 ± 0.24 ng/ml) although the result was nonsignificant ($P > 0.05$) [Table 2].

The study revealed that there was negative correlation between serum follistatin level and age of pregnant women and positive correlation between serum follistatin level and age of nonpregnant women [Figures 2 and 3].

The study revealed that there was negative correlation of serum follistatin level with FSH in pregnant and nonpregnant women ($r = -0.05, -0.19$), respectively [Figures 4 and 5].

The study showed that there was negative correlation of serum follistatin level with estrogen level in pregnant women ($r = -0.06$). There was positive correlation of serum follistatin level with E2 in nonpregnant women ($r = 0.044$) [Figures 6 and 7].

Discussion

Data presented in this study included 45 women under IVF process and yield two groups (successful and nonsuccessful pregnancy). The overall pregnancy rate for all subject of current study was 31.1% In international IVF Center in Kirkuk, Iraq. In consistent with our result, Al-Ubodi *et al*.[12] found that the pregnancy rate of women after ICSI was 28.89%. Several study also indicated that pregnancy rates were only 30%–40%.[13,14]

Table 1: Distribution of women in the study according to pregnancy after intracytoplasmic sperm injection

	All patients	Pregnant	Nonpregnant
Number	45	14	31
Frequency (%)	100	31.1	68.9

Table 2: Level of serum follistatin in pregnant and nonpregnant women

Follistatin level (ng/ml)	Pregnant	Nonpregnant	*P*
In serum	0.62±0.24	0.75±0.32	NS

NS: Not significant

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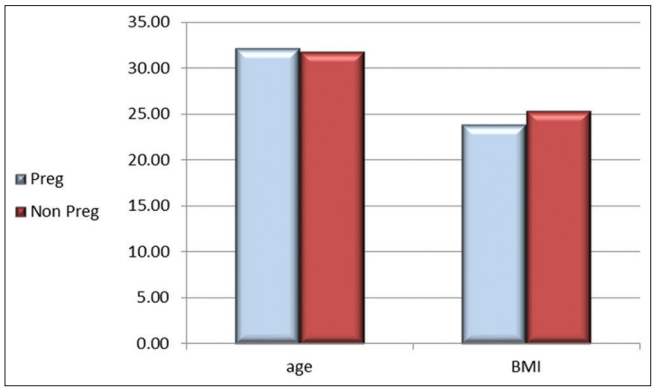

Figure 1: Mean of age and body mass index in pregnant and nonpregnant women

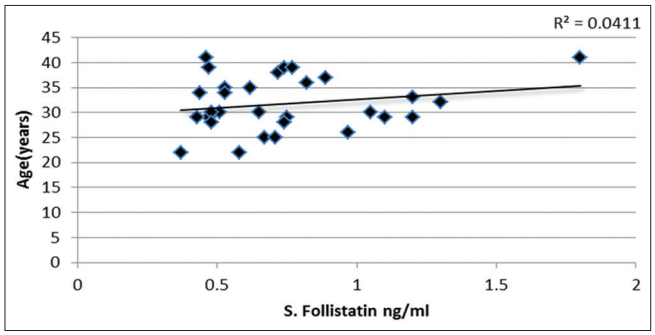

Figure 3: Correlation of serum follistatin level with age in nonpregnant women

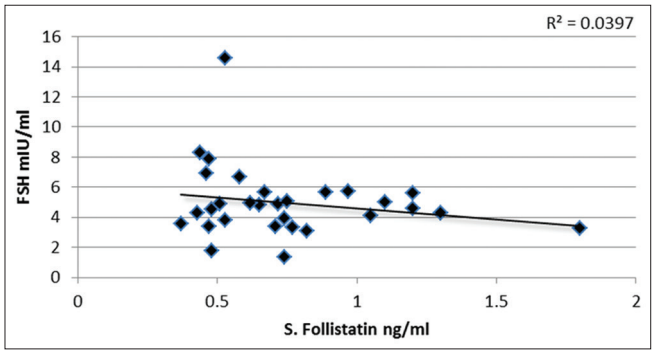

Figure 5: Correlation of serum follistatin level with follicle-stimulating hormone in nonpregnant women

Furthermore, the pregnancy rate by Orvieto *et al*.[15] was 31.25%. This difference between these findings may be related to the environmental condition such as the level of air pollution.[7]

In the current study, the highest mean of age was recorded among pregnant women compared with nonpregnant women (unsuccessful ICSI) (32.21 ± 6.68 vs. 31.80 ± 5.38 years) although the result was nonsignificant ($P > 0.05$) and the highest mean of BMI were recorded among nonpregnant women compared with pregnant women (25.36 ± 1.99 vs. 23.92 ± 1.55 kg/m^2). These findings were close to that reported Ahmeid,[9] who found that mean age of pregnant women was 32.18 years and 30.36 years for nonpregnant women; his

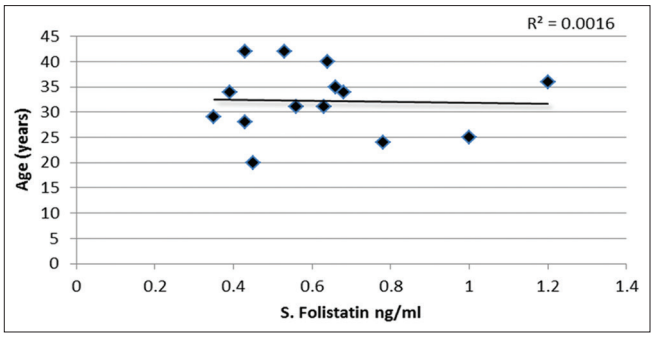

Figure 2: Correlation of serum follistatin level with age in pregnant women

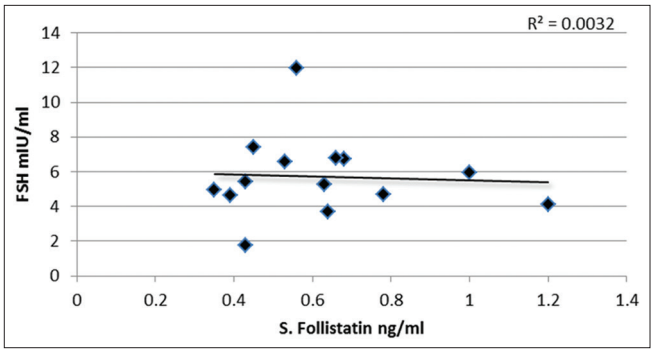

Figure 4: Correlation of serum follistatin level with follicle-stimulating hormone in pregnant women

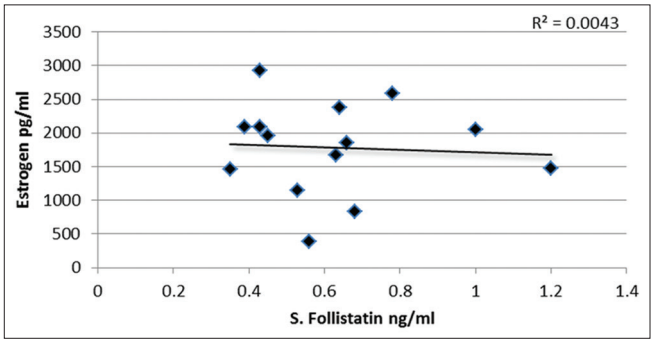

Figure 6: Correlation of serum follistatin level with estrogen in pregnant women

study also found that the mean of BMI was 22.9 (kg/m^2) for nonpregnant and for the pregnant group was 22.55 (kg/m^2). Al-Dujaily *et al*.[6] also found that there was no significant statistical difference between the mean age of pregnant (31.5 yeas) and nonpregnant women (31.0 years). In addition, Gultekin[16] showed that the mean age women under IVF a was 31.3 years. Many women choose to get pregnant later in life, waiting until their mid-30 or later to begin trying.[4] Due to the younger age of women seeking IVF in developing countries, including Iraq, could be explained in the context of social habits where most families have the desire to have children immediately after marriage.[9]

Most studies to date report decreased pregnancy success in obese women treated with IVF due to BMI has an adverse effect on reproduction.[17] In agreement with our findings,

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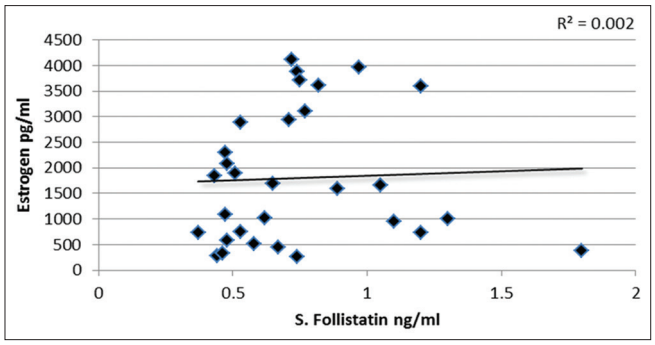

Figure 7: Correlation of serum follistatin level with estrogen in nonpregnant women

Hussein *et al*.[18] found that positive pregnancy occurred in women BMI 22.55 (kg/m^2) whereas negative pregnancy was 22.9 (kg/m^2) with a significant differences between pregnant and nonpregnant women ($P < 0.05$). Lauritsen *et al*.[19] showed that obese women had a significantly longer mean period of infertility and more an ovulatory infertility ($P < 0.01$) compared to normal weight women. In assisted reproduction, obesity affects on oocyte quality, embryo development, lower number of mature oocytes, lower implantation, and pregnancy rate.[20]

The current study showed highest mean level of serum follistatin present in nonpregnant compared with pregnant women (0.75 ± 0.32 vs. 0.62 ± 0.24 ng/ml) although the result was nonsignificant ($P > 0.05$). This showed agreement with study done by Alwan and Al-Dujaily[7] who showed no statistical significant difference was recorded between the values of follistatin hormone through the menstrual cycle between the pregnant and nonpregnant women. Also agreed with studies done by Muttukrishna *et al*.,[21] Welt *et al*.,[22] and Erickson *et al*.[23] they showed that there was no significant difference in the serum follistatin level during menstrual cycle in normal and polycystic ovarian syndrome women and women undergoing IVF. The similarity in follistatin concentration between pregnant and nonpregnant women during menstrual cycle indicated that extra gonadal follistatin production and serum follistatin does not reflect ovarian activity.

Our study disagrees with study done by Al-Dujaily *et al*.[6] who found that there was a statistical difference between the follistatin level of pregnant and nonpregnant women ($P = 0.004$), with nonpregnant women having higher levels. This may be due to the fact that circulating FST not only produced by the ovary but also other organs such as pituitary, uterus, muscle, liver, pancreas, and placenta. In addition, the overexpression of follistatin in nonpregnant women may be expected to lead to increased ovarian androgen production and reduction in circulating FSH levels so arrest follicular development and block folliculogenesis and become infertile.[24]

The current study revealed that there was negative correlation of serum follistatin level with FSH in pregnant and nonpregnant women ($r = -0.05, -0.19$), respectively. Our study agreed with study done by El-Shafey *et al*.[25] who found that follistatin concentrations were negatively affected with FSH ($r = -0.355$).

Our findings also agreed with study done by Mubarak *et al*.[26] and Suganthi *et al*.[27] They indicated that the relationship between serum follistatin and FSH for nonpregnant women study was negatively correlated. This confirmed that follistatin leads to increase production of ovarian androgens and reduce FSH levels in the blood due to blocking FSH stimulation by neutralization of activins, and subsequently results in defect in follicular development and block folliculogenesis, reduced fertility, and early loss of reproduction.[26]

The current study showed that there was weak negative correlation of serum follistatin level with E2 level in pregnant women, ($r = -0.07$) and positive correlation of serum follistatin level with E2 in nonpregnant women, ($r = 0.044$). In agreement with our findings, Mubarak *et al*.'s[26] and Muttukrishna *et al*.'s[21] study showed negative correlation between FST and estrogen in missed and recurrent miscarriages. Furthermore, Eldar-Geva *et al*.[28] found that there was negative correlation between FST and E2 in obese nonobese PCOS. This negative correlation of FST with E2 may be due to that follistatin inhibits FSH and estrogen synthesis. In addition to that granulosa cell LH receptors may also lead to decrease enhancement of oocyte maturation.

The current results disagree with study done by Fujiwara *et al*.,[29] who found that there was a positive correlation of E2 with FST during pregnancy. This may be due elevation of serum estradiol and follistatin levels after treatment with a GNRH agonist and gonadotropin.

Conclusions

There was no relation of serum follistatin with pregnancy after ICSI.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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