

# Estimation the Level of Follicular Fluid Follistatin in Women Undergoing Intracytoplasmic Sperm Injection

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## Abstract

**Objectives:** A prospective study carried out in Kirkuk city from 15<sup>th</sup> of January 2019 to 10<sup>th</sup> of April 2019, included 45 women who were enrolled in assisted reproductive technology (ART) programs in infertility Centre /Kirkuk, Iraq. **Patients and method:** All women were subjected to the basic fertility work-up 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 and 42 years old. All women were enrolled in short protocol type of IVF/ICSI cycle and they had normal menstrual cycles. Nearly 5 ml of follicular fluid was obtained from all women undergoing ICSI for estimation of follistatin level by ELISA technique. **Results :** The present study showed that 31.1% (14 of 45) of women underwent ISCI technique became pregnant and 68.9% were non-pregnant (31 of 45). The highest mean of age were recorded among pregnant women compared with non-pregnant women ( $32.21 \pm 6.68$  v.s  $31.80 \pm 5.38$  year) although the result was non-significant ( $P > 0.05$ ). The highest mean of BMI were recorded among non-pregnant women compared with pregnant women ( $23.92 \pm 1.55$  v.s  $25.36 \pm 1.99$  kg/m<sup>2</sup>), the result was significant. The highest mean level of follicular fluid follistatin evaluated in pregnant compared with non pregnant ( $10.20 \pm 0.30$  v.s  $9.83 \pm 0.58$  ng/ml) although the result was high significant difference ( $p < 0.05$ ). The study showed that there was negative correlation of follicular fluid follistatin level with age in pregnant women ( $r = -0.3$ ), with positive correlation of with age in non-pregnant women. **Conclusions:** There was a highly significant relation of follicular fluid follistatin with pregnancy outcomes after ICSI.

**Keyword:** Follistatin; Follicular Fluid; ICSI; Infertility; Pregnancy

## Introduction

Infertility is a disease of the reproductive system. According to the World Health Organization definition, it is defined as the failure to achieve a clinical pregnancy after two years of regular unprotected sexual intercourse without contraception, the woman has not become pregnant (there is no other reason, such as breastfeeding or postpartum amenorrhea) <sup>(1)</sup>. The chance of having a baby is 85%-90% in a year and the rest(10-15)% is infertile because it depends on the length of sexual exposure, frequency of coitus, and couple's age <sup>(2)</sup>. Nowadays, progress in assisted reproductive technology (ART) has enabled the clinicians to treat many types of infertility. Assisted reproduction is a complicated process involving multiple stages like ovarian stimulation, ovum

pick up, then fertilization of these oocytes, embryo cleavage and implantation<sup>(3)</sup>. Intra-cytoplasmic sperm injection (ICSI) refers to the technique of assisted reproduction, include injecting a single sperm into the center (cytoplasm) of the egg <sup>(4)</sup>. 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<sup>(5,6)</sup>. Follistatin (FSH-suppressing protein) is a cysteine-rich monomeric (single chain) glycoprotein with molecular weight of 39-kDa and is an important binding protein for inhibins and activins<sup>(7)</sup>. 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<sup>(8)</sup>. 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<sup>(9)</sup>. Because it localized within the human fallopian tube, endometrium and placental tissues, Therefore this proteins have been proposed as potential sensitive and specific markers to monitor the progress and outcome of pregnancy<sup>(10)</sup>. 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<sup>(11,12)</sup>. The aim of the study was to evaluate follistatin level in follicular fluid and correlate the results with pregnancy outcome in women undergoing intra cytoplasmic sperm injection (ICSI).

**Material and Method**

A prospective study carried out in Kirkuk city from 15th of January 2019 to 10th of April 2019, included 45 women who were enrolled in assisted reproductive technology (ART) programs in infertility Centre for IVF in International center/Kirkuk, Iraq. All women were subjected to the basic fertility work-up 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 and 42 years old. All women were enrolled in short protocol type of IVF/ICSI cycle and they had normal menstrual cycles.

Nearly, 5 ml of follicular fluid was obtained during ovum pick up from all women undergoing ICSI, Follicular fluid was collected from the dominant follicles (>18 mm) after( 34 to 36) hr. of HCG hormone administration. The samples were placed into sterile test tubes, centrifuged at 3000 rpm for 15\_20 minute and the obtained follicular fluid 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 two weeks of embryo implantation 2ml of blood sample was taken to assess pregnancy status.

**Statistical Analysis**

Computerized statistically analysis was performed using Mintab ver 18.0 statistic program. Comparison was carried out using Chi-square (X<sup>2</sup>)and T test for determination of the P. value (P<0.05: significant).

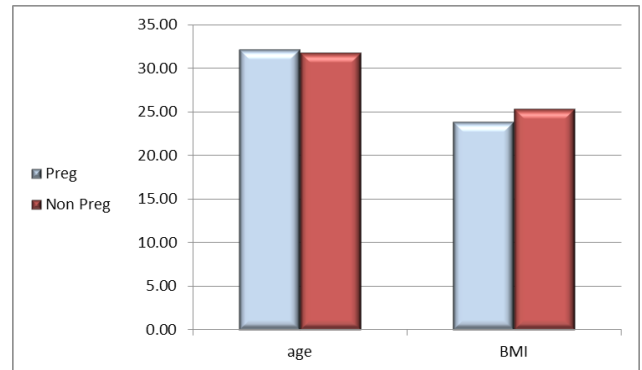
**Findings**

The present study showed that 31.1% (14 of 45) of women underwent ICSI technique became pregnant and 68.9% were non-pregnant (31 of 45), Table 1.

**Table 1: Distribution of women in the study according to pregnancy after ICSI**

	All patients	Pregnant	Non pregnant
Number	45	14	31
Frequency	100%	31.1%	68.9%

Figure 1 shows that the highest mean of age were recorded among pregnant women compared with non-pregnant women (32.21± 6.68 v.s 31.80±5.38 year) although the result was non-significant (P: > 0.05). The highest mean of BMI were recorded among non-pregnant women compared with pregnant women (23.92 ± 1.55 v.s 25.36±1.99 kg/m<sup>2</sup>), the result was significant.



**Figure 1: The mean of age and BMI of pregnant and non-pregnant women.**

The study showed that the highest mean level of follicular fluid follistatin was found in pregnant compared with non-pregnant (10.20±0.30 v.s 9.83±0.58 ng/ml), the result was high significant difference (p: < 0.05). Table 2.

**Table 2: The level of follicular fluid follistatin in pregnant and non-pregnant women.**

Follistatin level (ng/ml)	Pregnant	Non pregnant	P. value
In follicular fluid	10.20±0.30	9.83±0.58	< 0.05



Iraq, could be explained in the context of social habits where most families have the desire to have children immediately after marriage (8).

Most studies to date report decreased pregnancy success in obese patients treated with in vitro fertilization (IVF). In agreement with our findings, Hussein et al (20) found that IVF results showed that positive pregnancy occurred in women BMI 22.55 (kg/m<sup>2</sup>) whereas negative pregnancy was 22.9 (kg/m<sup>2</sup>) with a significant differences between pregnant and non-pregnant women (P <0.05). Sarais et al (21) showed that overweight and obese BMI women have a statistically significant lower live birth rate. Lauritsen et al (22) 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. Our findings were disagree with Rhodes et al (23) who revealed that pregnant group mean BMI was 25.1 (kg/m<sup>2</sup>) and non-pregnant group was 25.0 (kg/m<sup>2</sup>) with no significant difference (P >0.05). Due to body mass index (BMI) has an adverse effect on reproduction, overweight women have a higher incidence of menstrual dysfunction and anovulation, possibly because of altered secretion of gonadotropin releasing hormone, sex hormone binding globulin, ovarian and adrenal androgen, and luteinizing hormone and also because of altered insulin resistance (24). In assisted reproduction, however, there are many reports on the effect of obesity on oocyte quality, embryo development, lower number of mature oocytes, lower implantation and pregnancy rate (25) .

In agreement with the current results, studies done by Gultekin (19) and Köninger et al (26) found that the level of intrafollicular follistatin was higher pregnant women rather than non pregnant women after ICSI process. As well as, Lau (27) and Jeppesen et al (28) revealed that follistatin showed highly significant elevation in follicular fluid of pregnant women. Chang et al (29) showed that follistatin levels increase in the follicular fluid with the increasing growth of the follicle, a mechanism for decreasing activin activity. Level of FST increased in good quality oocyte compared with poor quality due to follistatin play role in folliculogenesis, oocyte maturation and embryogenesis(embryo development to oocyte) (30). The follistatin had a role on the oocyte grade and it was necessary for the oocyte maturation. As a result, the level of the follistatin directly affected the oocyte and embryo quality(31). In vitro study done by Jorgez et al (32) on the mouse showed that the foliculogenesis was paused and the infertility was appeared by the lack

of follistatin. Wakatsuki et al (33) found that porcine follicular fluid contained extremely high follistatin level (5.6 mg/L) due to follistatin originally isolated from porcine ovarian follicular fluid.

## Conclusions

There was a highly significant relation of follicular follistatin and pregnancy after ICSI.

**Conflict of Interest:** Nil

**Source of Findings:** Self

**Ethical Clearance:** This research 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

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