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RESEARCH ARTICLE

CORRELATION BETWEEN FOLLICULAR FLUID LEPTIN AND THE PREGNANCY RATE
IN WOMEN WHO UNDERWENT ICSI

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ABSTRACT

Background: The clinical significance of leptin hormone in predicting pregnancy after controlled ovarian hyperstimulation (COH) protocols for intracytoplasmic sperm injection (ICSI) is still poorly understood. Several reports exist on the prospects of hormonal profile in the luteal phase for the diagnosis of early pregnancy.

Objectives: This study was designed to investigate the role of follicular fluid leptin in predicting pregnancy in women underwent ICSI cycle.

Materials and Methods: This study included 54 women aged 16–44 years who underwent their first ICSI cycles in Baghdad IVF infertility centre in the period between December 2014 and May 2015, they have normal ovulatory cycles, and without any evident endometrial pathology. All patients received mid-luteal long-protocol down-regulation with GnRH- agonist. Each patient was monitored for ovarian follicular development by transvaginal sonography and series of serum E2 level. Women were given human chorionic gonadotropin (hCG), Serum E2 level was estimated on the day of hCG administration. Intracytoplasmic sperm injection procedure was done by the embryologist. Chemical pregnancy will be defined at the 14th day after embryo transfer by elevated serum β -hCG level, then, progesterone supplementation was continued up to 12 weeks' gestation. Evaluated Follicular fluid leptin at day of oocyte retrieval.

Results: The results of this study showed that 11 women achieved pregnancy (20.37%) and was defined as pregnant group, while 43 women failed in achieving pregnancy (79.63%) who defined as non pregnant group after they underwent their first ICSI –ET treatment cycles. There was no significant difference in follicular fluid Leptin at day of Oocyte retrieval between the pregnant and non-pregnant group. A significant positive correlation between follicular fluid leptin with BMI in pregnant patients. A significant positive correlation between follicular fluid leptin with BMI in non-pregnant women.

Conclusion: Evaluation of follicular fluid leptin level was not a promising diagnostic and prognostic parameter for the prediction of pregnancy in ICSI-ET treatment cycles. Follicular fluid leptin vary between the pregnant patients based on maternal BMI.

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INTRODUCTION

Infertility is the inability of a couple to achieve pregnancy after 12 months of regular unprotected intercourse. Approximately 10-15% of the couples experience some forms of infertility problems.¹ A female reaches peak fertility by the age of 25 years and fertility declines slightly after the age of 30. After age 40, fertility declines rapidly.² Significant improvements in fertility treatment have made it possible for many couples to conceive with medical assistance. In vitro fertilization-embryo transfer (IVF-ET) and more recently, intracytoplasmic sperm

injection (ICSI) were now commonly used for the treatment of infertility attributable to tubal factor, significant endometriosis, malefactor and also used to treat persistent unexplained infertility.³ Hence, after the application of in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI), both couples and the medical team are subjected to increased anxiety about the detection and prognosis of pregnancy. The early prediction of pregnancy and its outcome has therefore great importance.⁴ In humans, girls must reach a minimum weight or "critical fat mass" before maturation of the hypothalamic-pituitary-gonadal (HPG) axis and onset of puberty can occur.⁵ Leptin seems a good candidate to signal to the hypothalamus whether the amount of fat mass in the body is adequate for reproduction, and thus to trigger the activation of the HPG axis.⁶ During pregnancy, plasma leptin levels are

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elevated, and then they continue to increase. They drop sharply after delivery. This increase is correlated with gestational weight gain, and thus body fat accumulation. Absolute leptin levels are correlated with BMI.^{vii} Other factors that can contribute to this rise in leptin levels are the production of leptin by placental tissue and changes in other hormones that may influence leptin concentrations.^{viii} Several reports exist on the prospects of hormonal profile for the diagnosis of early pregnancy.^{ix} However, hormonal events of the luteal phase subsequent to various stimulation protocols remain controversial.^x It is evident that there is a lack of clear consensus on the role of hormones in the prediction of successful implantation after controlled ovarian hyperstimulation (COH) protocols in assisted reproductive techniques (ART).^{xi}

Aims of the study

This study aims to study the correlation between follicular fluid leptin and the pregnancy rate in Iraqi women who underwent ICSI.

Patients and Methods

This study included 54 infertile couples who were enrolled in assisted reproductive technology (ART) programs to enter their first ICSI cycle in Baghdad IVF infertility Centre in Baghdad, Iraq. All couples 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, and semen analysis. The average age of the included women ranged between 16 and 44 years. They had primary infertility with duration between 2 - 8.6 years. They have normal ovulatory cycles.

They were without any evident endometrial pathology, which were confirmed by the serial vaginal ultrasound and mid luteal phase progesterone level, early follicular phase FSH, LH, E2, thyroid and prolactin (PRL) hormone levels. They were done as part of the work up. Ultrasound, hysterosalpingography and/or laparoscopy were used for assessment of the uterine cavity. Patients with polycystic ovary syndrome were excluded. All patients were enrolled in long protocol type of IVF/ICSI cycle, which started on day 21 of the previous menstrual cycle. An ultrasound examination was performed in order to exclude those women with ovarian cyst and assess the endometrial thickness. After selection, women received mid-luteal long-protocol down-regulation with gonadotropin releasing hormone agonist (GnRH-agonist) triptorelin (Decapeptyl 0.1 mg, Ferring Co, Kiel, Germany)[®] by daily subcutaneous injection until the next menstrual cycle started.

The pituitary desensitization was completed by reaching the level of E2 < 50 pg/ml and endometrial thickness was ≤ 2-3 mm on ultrasound examination. The women received recombinant human follicle stimulating hormone (rhFSH) (Gonal F, Merck Serono)[®] containing 75 IU of FSH activity per ampoules by daily subcutaneous injection (2-4) ampoules in addition to the (GnRH- agonist). Transvaginal ultrasound was performed on cycle day 5 and subsequent scans were done every 2-3 days as required. The doses of (Gonal F)[®] and

follicle growth were monitored by serial serum E2 level and transvaginal U/S till the day of hCG administration (12-15 day of cycle). Then, ovulation was induced by administration of human chorionic gonadotropin (hCG), (Ovitrelle 6500 IU; Merck Serono)[®] subcutaneously when at least 3 follicles > 16mm in diameter were detected on ultrasound examination.

Oocytes were retrieved by transvaginal ultrasound-guided oocyte aspiration which was done by a gynecologist approximately 34–36 hours after hCG administration under general anesthesia. The aspirated follicular fluid was collected into a test tube which was rapidly transferred to the ICSI lab to search directly for oocyte by the embryologist. The Follicular fluid (FF) was centrifuged at 1500 rpm for 15 minutes and the supernatants were frozen at -70 °C for future analysis. In preparation for intracytoplasmic sperm injection, the cumulus corona cells were removed by a combined enzymatic and mechanical treatment. Each oocyte was carefully assessed, noting the presence or absence of a germinal vesicle or the first polar body. Only those ova that had extruded the first polar body (metaphase II) and were morphologically intact were suitable for microinjection.^{xii}

The sperm of their husbands were collected either by masturbation into a clean, dry and sterile plastic dish after 3-5 days of abstinence. The sample was transported to the laboratory immediately and placed in an incubator at 37°C for 30 minutes to allow liquefaction. Once collected, the sperm could be prepared by using swim-up or by density gradient centrifugation procedures. Sperm preparations were performed in human tubal fluid (HTF) medium.

^{xiii} The ICSI procedure was done under a microscope using multiple micromanipulation devices (micromanipulator, microinjectors and micropipettes). A holding pipette stabilized the mature oocyte with gentle suction with the polar body at the 12 or 6 o'clock position. From the opposite side a thin, hollow glass microinjector was used to collect a single sperm.^{xiv} After culturing in the laboratory, eggs were checked for evidence of fertilization (2 distinct pronuclei) on the following day (18-24) hours.

Good quality embryos were transferred to the uterus, after 2 (four cell embryo), 3 (six-eight cell embryo) early cleavage stages or 5 (blastocyst stage) days after egg removal. This was done by using a thin plastic catheter.

All infertile patients received luteal support progesterone therapy for 2 weeks in the form of actavis (Cyclogest, Barnstaple)[®] 400 mg transvaginal twice a day until a pregnancy test was performed.^{xv}

RESULTS

Fifty-four infertile women who were included in this study were classified into two groups according to the results of the pregnancy test (P.T) which was achieved after 14 days after embryo transfer in their first ICSI cycles

- Pregnant group which consisted of eleven women who succeeded in achieving pregnancy.

- Non pregnant group which consisted of forty three women who failed in achieving pregnancy.

Table (4-1) shows the results of ICSI treatment cycles where:

- Eleven (20.37 %) of them achieved pregnancy, while
- Forty- three (79.63 %) failed to achieve pregnancy.

Table 4-1. The results of ICSI treatment cycles

	All Patients	Pregnant	Non- Pregnant
Number	54	11	43
Frequency	100%	20.37%	79.63 %

Table (4-2) shows the demographic feature of the studied groups. The ages expressed in years of the total patients, pregnant, and non-pregnant group were (30.94 ± 6.71 , 32.18 ± 6.52 , and 30.63 ± 6.8 , respectively). The BMI expressed in (Kg/m²) (21.84 ± 2.60 , 22.55 ± 3.06 , and 21.55 ± 2.39 , respectively). The table also shows the mean \pm SD of the duration of infertility expressed by (years) (3.6 ± 1.5 , 3.4 ± 0.8 , and 3.8 ± 2.3) respectively. No significant difference was observed in age, BMI, and duration of infertility between the pregnant and non-pregnant group.

Table 4- 2. Demographics features of the patients undergoing IVF

	All Patients	Pregnant	Non Pregnant	*P
Age (years)	30.94 ± 6.71	32.18 ± 6.52	30.63 ± 6.8	NS
BMI (Kg/m ²)	21.84 ± 2.60	22.55 ± 3.06	21.55 ± 2.39	NS
Duration of Infertility (Years)	3.6 ± 1.5	3.4 ± 0.8	3.8 ± 2.3	NS

Table 4-3. Serum and follicular fluid leptin level throughout the IVF stimulation cycle in infertile patients who become pregnant or non-pregnant

Leptin Level ng/dl	Total No.	Pregnant Patients	Non- Pregnant Patients	*P value
Follicular fluid	54.81 ± 28.25	65.12 ± 35.97	50.44 ± 23.78	NS

Table (4-3) shows The follicular fluid leptin level expressed by (ng/dl) on day of Oocyte retrieval was (54.81 ± 28.25 , 65.12 ± 35.97 , and 50.44 ± 23.78 , respectively). No significant difference in follicular fluid Leptin between the pregnant and non-pregnant groups.

DISCUSSION

Follicular fluid provides a very important microenvironment for the development of oocytes. FF was a product of both the transfer of blood plasma constituents that cross the blood follicular barrier and of the secretory activity of granulosa and thecal cells.^{xvi} It was reasonable to think that some biochemical characteristics of the FF surrounding the oocyte may play a critical role in determining oocyte quality and the subsequent potential to achieve fertilization and embryo development.

The analysis of FF components may also provide information on metabolic changes in blood serum as the circulating biochemical milieu may be reflected in the composition of FF.^{xvii}

In the present study, there was no significant difference in follicular fluid leptin between the pregnant and non-pregnant groups (Table 4-3). Asimakopoulo *et al.*^{xviii} reported that FF leptin was significantly associated with the pregnancy outcome of ICSI cycles. Non-pregnant women had threefold higher FF total leptin levels than pregnant ones and this difference was statistically significant. This finding confirms the results of a previous study that showed lower concentrations of leptin in FFs were significantly associated with positive outcome of IVF cycles reported by Mantzoros, *et al.*^{xix}

Barroso, *et al.*^{xx} reported that leptin FF levels correlated negatively with the embryo quality in IVF cycles, suggesting that leptin, together with vascular endothelial growth factor, are markers of follicular hypoxia. Bu^{tz}ow, *et al.*^{xxi} found that a considerable increase in leptin levels during COH was related with reduced ovarian response. Mantzoros, *et al.*¹⁹ were the first who reported that women who became pregnant during IVF or gamete intrafallopian transfer (GIFT) cycles had significantly lower intrafollicular leptin concentrations than women who failed to become pregnant. After that, several investigators. Reported that either serum or intrafollicular high levels of leptin were associated with lower pregnancy rates in IVF cycles.^{xxii,xxiii,xxiv,xxv}

Byron,^{xxvi} opposes the notion that circulating or intrafollicular concentrations of leptin can serve as a prognostic factor for the outcome in ICSI cycles. According to our present results, neither circulating nor intrafollicular leptin concentrations were associated with the number of oocytes retrieved, the fertilization rate, and the achievement of pregnancy.

These results were consistent with several previous studies.^{xxvii,xxviii,xxix,xxx, xxxi, xxxii,xxxiii} Barroso, *et al.*^{xxxiv} found that follicular leptin levels were negatively-correlated with follicular pO₂ and positively-correlated with pCO₂. Thus, they suggested both cytokines as markers of follicular hypoxia and suboptimal embryo development.

In conclusion Evaluation of follicular fluid leptin levels were not a promising diagnostic and prognostic parameter for the prediction of pregnancy in ICSI-ET treatment cycles.

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