Original Article

# Luteal phase serum progesterone level: a potential predictive marker for pregnancy rate in intracytoplasmic sperm injection.

**Shaimaa S. Gumaa*** **MBChB**
**Zaid A. Almadfa**** **PhD Physiology.**
**Nada F. Al-Aboudy***** **MBChB, DOG, HD.ART**

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***Summary:***

**Background:** It is evident that there is a lack of clear consensus on the role of luteal phase serum Progesterone (P) level in the prediction of early pregnancy after controlled ovarian hyperstimulation (COH) protocols in assisted reproductive techniques (ART).

**Objective:** We conducted this study in order to investigate the potential value of luteal phase serum progesterone measurement, in women undergoing ICSI treatment cycles and receiving progesterone supplements, in relation to pregnancy rate.

**Patients:** A total of 68 women aged 20-40 years undergoing their first intracytoplasmic sperm injection (ICSI) cycles in fertility and I.V.F center of Kamal Al samrai hospital.

**Methods:** women consecutively treated by ICSI had Estimation of luteal phase serum Progesterone levels on days 0 and 14 of embryo transfer (ET).

**Results:** 10 pregnancies were achieved (14.7%). Age, basal FSH level, peak E2 level, number of oocytes retrieved, and number of embryos transferred were comparable between pregnant and nonpregnant women. Day-0 and day-14 luteal phase serum Progesterone levels were significantly different between the two groups.

**Conclusion:** Luteal phase serum Progesterone concentration seems to be a promising marker for prediction of early pregnancy in intracytoplasmic sperm injection.

**Key Words:** Luteal phase, progesterone, pregnancy, ICSI.

## Introduction:

The clinical significance of luteal phase hormones such as serum progesterone (P) 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 (1). However, hormonal events of the luteal phase subsequent to various stimulation protocols remain controversial (2). It is evident that there is a lack of clear consensus on the role of luteal phase serum estradiol(E2) and progesterone(P) in the prediction of successful implantation after COH protocols in assisted reproductive techniques (ART) (3).

## Patients and Method:

Each patient was adequately informed about the aim of the study before she accepted to be enrolled. The study was approved by the Scientific Research Committee of kamal Al-Samarai Hospital in which the study was done.

This study included 68 women aged 20–40 years who underwent their first ICSI cycles in fertility and I.V.F. center of kamal Al-Samarai hospital in the period between December 2011 and May 2012,

Ovulatory cycles, and without any evident of endometrial pathology, which were confirmed by the hospital they have normal serial vaginal ultrasound and mid luteal phase progesterone level, early follicular phase FSH, LH, E2, thyroid and prolactin (PRL) hormone levels which was 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 GnRH- a: triptorelin (Decapeptyl 0.1 mg, Ferring Co, Kiel, Germany)® by daily Subcutaneous injection until the next menstrual cycle start and 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 ampoule by daily subcutaneous injection (2-4) ampoules in addition to the (GnRH- agonist). Transvaginal ultrasound was performed on cycle day 5 and subsequent scan 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

**Ministery of health, Diyala health Directorate.*
***College of medicine, University of Baghdad.*
****Kamal Al-Samarai hospital.*

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, the leading follicle of the three reached (18-20 mm) in diameter and endometrial thickness 7- 11mm on U/S examination. Serum E2 level was estimated on the day of hCG administration (peak E2).

Oocytes were retrieved by transvaginal ultrasound-guided oocyte aspiration approximately 34–36 hours after hCG administration. ICSI procedure was done. Embryo transfer was performed 2-5 days after oocyte retrival at four- to eight-cell cleavage stages in all patients. All patients received 400 mg micronized Progesterone; actavis (cyclogest, Barnstaple)® transvaginally twice daily until a pregnancy test was performed, and if the test results were positive, P supplementation was continued up to12 weeks' gestation. Chemical pregnancy will be defined at the 14th day after embryo transfer by presence of +ve pregnancy test by serum β-hCG estimation.

Serum P levels were evaluated on the day of ET (D0) and on the day of serum β-hCG estimation 14$^{th}$ day after embryo transfer (D14). Pregnant women were followed up by a transvaginal ultrasound scan at 8 weeks gestation.

**Blood Sampling and Hormonal Assays:**

Peripheral blood samples were collected by venipuncture, allowed to clot and centrifuged to separate the serum that stored in aliquots at -40◦C.

Blood sample collection was done in two separate days as below:

- Three milliliters of blood sample were collected from each infertile woman on day of embryo transfer (D0) for serum progesterone (P) level evaluation.
- Another blood sample was also collected from each infertile woman on day 14 after embryo transfer (D14) was used to evaluate serum progesterone (P) level at that day.

Determination of serum P levels was done by Radioimmunoassay (RIA). The device used in the lab was from (BERTHOLD TECHNOLOGY, LB 2111 Multi Crystal, Gamma Counter, KOMM. No.1007190).

Determination of serum Progesterone (P):

Principle of the assay: The Radioimmunoassay for the determination of progesterone in human serum is a competition assay. This assay is based on the competition between unlabeled target antigen (samples and calibrators) and a fixed quantity of 125I –labeled target antigen (as tracer) for a limited number of binding sites on its specific antibody. Samples and calibrators are incubated one hour with 125I -labeled target antigen in (antibody – coated tubes). After that, the content of the tubes is aspirated and bound radioactivity is measured. The radioactivity in the antibody bound fraction is inversely proportional to the unlabeled antigen concentration. By utilizing several different serum references of known antigen concentration in calibrators (standards), a calibration curve is established from which the antigen concentration of an unknown can be ascertained. The Kit was provided by (IMMUNOTECH SAS, France; IM1188) for RIA PROGESTERONE.

**Statistical Analysis:**

The data were analyzed using Statistical Package for Social Sciences (SPSS) version 17.0 (SPSS Inc, Chicago, IL, USA). When there were two independent groups, they were compared by Student's t- test or Mann Whitney U test, depending on the distributions of continuous data. Nominal data were compared with Chi-square test. The degree of association between continuous variables was calculated by Pearson's correlation coefficient (r). A P value < 0.05 was considered to be statistically significant, P value <0.01 was considered to be statistically highly significant.

**Results:**

The results of this study showed that 10 women achieved pregnancy(14.7%) and were defined as pregnant group, all of them had a viable intrauterine pregnancy at 8 weeks gestation, while 58 women failed in achieving pregnancy(85.3%) who were defined as non pregnant group after they underwent their first ICSI –ET treatment cycles. No statistically significant differences were observed in the average age, basal FSH level, peak E2, number of oocytes retrieved, and number of embryos transferred when comparing the pregnant with the nonpregnant group (Table 1).

**Table (1) Comparison of clinical data between pregnant and non pregnant women underwent ICSI cycles.**

| Parameters (mean) | Pregnant group (n=10) | Non pregnant group (n=58) | p- value p>0.05 |
|---|---|---|---|
| Age (year) | (30.70 ± 6.14) | (31.07±5.51) | 0.848 |
| BMI (kg/m²) | (24.80 ± 2.08) | (25.77± 3.21) | 0.423 |
| Basal FSH (mIU/ml) | (6.02 ± 1.99) | 5.74 ±1.54) | 0.661 |
| Peak E2(pg/ml) | (2142.69±884.63) | (1929.93±818.32) | 0.455 |
| No. of oocytes retrieved | (8.20±4.46) | (8.67±4.42) | 0.756 |
| No. of embryos transferred | (2.90±0.73) | (2.41±0.99) | 0.144 |

***• Values were expressed as mean± SD.***

***• P-values less than 0.05 were considered as statistically significant.***

A significant higher serum Progesterone concentration of pregnant group on day of embryo transfer ET (D0) was observed when comparing the mean values (±SD) of serum P level of pregnant women (47.15 ± 1.96 ng/ml), with non pregnant group (37.45 ± 21.01 ng/ml, p <0.05 ). Table (2)

**Table (2) Comparison of luteal phase serum P-D0 level between pregnant and non pregnant group**

| Hormone | Pregnant group | Non pregnant group | P-value P<0.05 |
|---|---|---|---|
| SerumP-D0 (ng/ml) | (47.15±1.96) | (37.45±21.09) | 0.001 |

• Values were expressed as mean± SD.
• P-values less than 0.05 were considered as statistically significant.

A similar trend was noticed on D14, with a highly significant increase in the level of P in pregnant women as compared with non pregnant women (58.28 ±11.86 ng/ml, 17.96±19.65 ng/ml, respectively; P<0.05). Table (3)

**Table (3) Comparison of luteal phase serum P-D14 level between pregnant and non pregnant group**

| Hormone | Pregnant group | Non pregnant group | P-value P<0.05 |
|---|---|---|---|
| Serum P-D14 (ng/ml) | (58.28±11.86) | 17.96±19.65) | 0.001 |

***• Values were expressed as mean± SD.***
***• P-values less than 0.05 were considered as statistically significant.***

**Discussion:**

It is well known that a functional corpus luteum secreting specific hormones is a major prerequisite for successful implantation. It is also suggested that the corpus luteum rescue after 7 to 8 days of oocyte retrieval could be an early sign of pregnancy and may be used as a diagnostic marker of pregnancy (1). This study revealed significantly higher mean values of serum P level of pregnant group than that of non pregnant group, on both D0 (early luteal phase ) and on D14 (late luteal phase), table 1&2.

This confirms the finding of previous studies of Elson in 2003, Ioannidis in 2005and Gruber in 2007 (4, 5, 6). Progesterone is sometimes called the "hormone of pregnancy", and it has many roles relating to the facilitation of implantation, and maintaining pregnancy: Progesterone converts the E2-prepared endometrium to its secretory stage to prepare the uterus for implantation (7). It also decreases contractility of the uterine smooth muscle. The uterine-relaxing properties of progesterone were supported by a study of IVF embryo transfer outcome by Fanchin in 2001(8) in which Results indicated that a high frequency of uterine contractions on the day of embryo transfer hindered transfer outcome, possibly by expelling embryos out of the uterine cavity. A negative correlation between uterine contractions frequency and progesterone concentrations was detected, underlining the benefits of progesterone in IVF (8, 9).

In addition to exogenous progesterone supplementation, serum progesterone levels were significantly elevated, from as early as 4 weeks gestation, in pregnant women. While women who failed to conceive had very low serum progesterone levels at this date despite exogenous supplementation.

**Conclusion:**

Evaluation of luteal phase serum Progesterone (P) level is a promising diagnostic and prognostic parameter for the prediction of pregnancy in ICSI-ET treatment cycles.

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