Correlation between Elevated Serum Progesterone in the Day of Hcg Injection, Metaphase II Oocytes and Their Impact on The Success of ICSI

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Abstract

Objective: In this study we tried to understand the effect of progesterone level at the time of ovulation trigger on intracytoplasmic sperm injection (ICSI) outcome. We expected identifying cut off values for serum progesterone level the day of hCG injection (P-hCG) & P-hCG per mature oocyte ratio (P-hCG / MII), as predictive tests of ICSI success. Methods: 200 women 18-40 years old and attending IVF unit of Kasr el Aini hospital for management of infertility were included in the study. They were scheduled for ICSI after controlled ovarian induction with GnRH- agonist protocol. Inclusion criteria were tubal factor of infertility, unexplained infertility, polycystic ovarian syndrome and BMI $\leq$ 30 kg/m$^2$. Exclusion criteria were severe male factor infertility, hyperprolactinemic patients, high basal FSH $> 11$ IU/L, frozen embryo transfer cycles, uterine anomalies or synechia. Primary outcome parameter included correlation of serum progesterone on day HCG administration, number of MII oocytes and calculated progesterone/ MII oocyte ratio with success of ICSI.

Results: 193 cases underwent embryo transfer, 7 cases cancelled. Clinical pregnancy was reported in 52 (27.9\%) cases. Higher P-hCG was observed in cases who didn’t get pregnant compared to those who got pregnant ($p=0.01$) with cut off value of 1 ng/ml correlated well with the clinical pregnancy rate after ICSI with 61\% sensitivity and 59\% specificity ($p=0.004$). A significantly lower P-hCG/MII ratio was found in the pregnant women group compared to that found in the non-pregnant group ($p=0.001$) and cut off value of 0.18 correlated best with clinical pregnancy after ICSI with sensitivity 70.5\% and specificity 61.2\% ($p<0.001$). Conclusion: We demonstrated negative correlation between P-hCG and P-hCG/MII and clinical pregnancy rate after ICSI.

Keywords: ICSI; Infertility; P-hCG/MII oocytes ratio;

Introduction

Futile infertility treatment is associated with psychological and financial burdens. Thus, several studies were conducted to search for an accurate marker for ICSI success (eg. D3 FSH/LH ratio, serum progesterone level on the day of hCG injection (P-hCG), number of mature oocytes retrieved …etc.) which would allow suitable selection of stimulation protocols and appropriate counseling before attending the stressful and expensive course of IVF. This is particularly important for patients belonging to lower and middle socioeconomic classes and cannot afford multiple cycles of IVF.

Outcome of IVF depends on number and quality of oocytes and endometrial receptivity. Moderate increase in serum progesterone in the peripheral circulation occurs in most super-ovulated cycles on the day of HCG injection\textsuperscript{1}. Ubaldi et al (1996) concluded the higher FSH exposure in the IVF cycles and the subsequent higher FSH-induced LH receptivity in granulosa cells might be one of the factors inducing premature luteinization but this didn’t cause adverse effects on IVF outcome.\textsuperscript{2}
Bourgain & Devroey (2003) concluded that “the endometrium of IVF cycles showed premature secretory changes in post-ovulatory phase of IVF cycles followed by dys-synchronous glandular and stromal differentiation in the mid-luteal phase.” This suggests a profound modification of luteal endometrial in stimulated cycles.3

The time of maximal endometrial receptivity “the implantation window” is characterized by the expression of endometrial products, among which pinopodes, integrins and leukemia inhibitory factors are most described. Premature expression of pinopodes and integrins are observed with precocious luteal transformation and increased progesterone level following ovarian stimulation.3

There is debate about origin and significance of elevated serum progesterone in stimulated cycles. It has been believed that increased LH in late follicular phase cause increased progesterone and the use of gonadotropin-releasing hormone (GnRH) agonists and antagonists to prevent the rise of LH and premature luteinization was tried. Results of this premature elevation of serum progesterone on IVF outcome is controversial.4

Several authors did not find any negative effect of this on IVF outcome.5,6,7 Other authors reported that pregnancy rate has been inversely related to P-hCG.8,9,10

In this study we tried to understand the effect of progesterone level at the time of ovulation trigger on intracytoplasmic sperm injection (ICSI) outcome. We expected identifying cut off values for P-hCG & P-hCG per mature oocyte ratio (P-hCG /MII), as a better predictive tests of ICSI success.

Materials and Methods

Following ethical approval of scientific committee of OB/GYN department, Kasr Al-Aini hospital, Cairo University, Egypt, 200 women aged 18-40 years old and attending the IVF unit of Kasr el Aini hospital (2015-2018) for management of infertility were included in the study after obtaining informed consent. All women were scheduled for ICSI after controlled ovarian induction. Inclusion criteria were normal serum prolactin level, tubal factor of infertility, unexplained infertility, polycystic ovarian syndrome and BMI ≤ 30 kg/m2. Exclusion criteria were severe male factor infertility, hyperprolactinemic patients, high basal FSH > 11 IU/L, frozen embryo transfer cycles, uterine Anomalies or synchia, repeated implantation failure in ICSI (more than 3 failed trials), thyroid dysfunction, history of ovarian surgery.

All women included in the study were subjected to careful history taking and general and local examination, AFC & AMH were recorded and BMI calculation was done. All patients were tested for basal serum FSH, LH, & E2 levels on the third day of a spontaneous cycle. Patients was subjected to testing of basal serum progesterone on day 3 of the down regulated cycle (E2<50 pg/ml) before stimulation with HMG and then repeated again on day of hCG administration to determine possible deleterious effect arising from elevated LH levels on pregnancy rates. Serum FSH, LH, E2 and Progesterone were measured using chemiluminescent enzyme immunoassay (Immulate 2000 Siemens Medical Solutions Diagnostic). Endometrial thickness on the day of hCG injection was determined using Sonoace x4 ultrasound machine (Samsung Medison Co., Ltd. Seoul, South Korea), duration of stimulation, total dose of gonadotropins used, number of retrieved, fertilized oocytes, embryo grading, number of transferred good quality embryos, & cancellation rates per cycle were determined.

The standard long GnRH agonist protocol was used for patients with predicted normal response based on clinical & hormonal profile; 1 mg of leuprolide acetate daily s.c injection (Lucrin ®; Abbott, Hoofddorp, The Netherlands) was applied from the mid luteal phase onward till the day of hCG injection. Gonadotropins in the form of HMG (Merional ®, IBSA, Institut Biochimique SA, Lugano, Switzerland) was given IM from the 2nd day of menstruation. The starting dose range from 150 to 450 IU depending on the basal FSH level, AFC, Patient’s age and BMI. In all protocols, stimulation was monitored by Tran- vaginal ultrasonography and serial E2 measurements starting from day 7 of the cycle and the gonadotropin dose was adjusted individually according to follicular response. After the development of at least three leading follicles≥18 mm, 10,000 unit of HCG (Choriomon, IBSA, Institut Biochimique SA) was given IM, and transvaginal ultrasound-guided oocyte retrieval was performed 36 hours later. Progesterone pessaries
400 mg twice daily (Cyclogest 400mg ® Actavis plc. Dublin, Ireland) was given as a luteal support starting from the day of embryo transfer and continued for 16 days after. Pregnancy was defined as the occurrence of a positive β-HCG >10 IU on day 12 after embryo transfer and a second higher value 2 days later, followed by ultrasonography confirmation of cardiac activity at 6 weeks gestation (for clinical pregnancy).

**Primary outcome parameter included** Correlation of P-hCG and calculated P-hCG/ MII ratio with Success of ICSI and ROC Analysis to determine accuracy of these tests in predicting clinical pregnancy in ICSI Cycles. **Secondary outcome parameters included** cut off levels for P-hCG and P-hCG/MII in predicting successful IVF outcome (clinical pregnancy).

**Statistical analysis:** Data were statistically described in terms of mean ± standard deviation ±SD), median and range, or frequencies (number of cases) and percentages when appropriate. Comparison of numerical variables between the study groups was done using Student t test for independent samples in comparing 2 groups of normally distributed data and Mann Whitney U test for independent samples for comparing not-normal data. For comparing categorical data, Chi-square (x²) test was performed. Exact test was used instead when the expected frequency is less than 5. Accuracy was represented using the terms sensitivity, and specificity. Receiver operator characteristic (ROC) analysis was used to determine the optimum cut off value for the studied diagnostic markers. p values less than 0.05 was considered statistically significant. All statistical calculations were done using computer program IBM SPSS (Statistical Package for the Social Science; IBM Corp, Armonk, NY, USA) release 22 for Microsoft Windows.

**Results**

Two hundreds patients metting the inclusion criteria included in the present study divided into 2 groups, 193 cases underwent embryo transfer, 7 cases cancelled (all in group A (FSH/LH ≥3) due to failure to yield mature follicle for fertilization. Clinical pregnancy was reported in 52 (27.9%) cases. Primary infertility was the type of infertility in 148 patients (74%) and it was secondary in 52 patients (26%). The indications for ART treatment included male factor infertility (40.5% n=81), PCO (24.5% n=49), unexplained infertility (16% n=32) and tubal factor infertility (19% n=38).

**Demographic characteristics:** The mean age was 29.83±4.43 years old, the mean BMI was 26.5±2.9 kg/m², the mean duration of infertility was 4.63±2.59, the mean FSH was 6.55±1.54 mIU/ml, the mean LH was 4.77±1.5 mIU/ml and the mean FSH/LH ratio was 1.57±0.8. The mean E2 was 36.13±10.1 pg/ml, the mean AMH was 3.9±3.15 ng/ml, the mean AFC was 13.7±SD 5.9. The mean basal serum progesterone was 0.76±0.25 ng/ml while the mean serum progesterone in the day of hCG injection was 1.4±0.9. Table 1 shows ovarian stimulation parameter.

| Table (1): Parameters of Ovarian stimulation and ICSI cycle. |
|-----------------------------------------------|-----------------|----------|----------|
| Stimulation Duration/day                      | 12.3±2.05       | Maximum | Minimum  |
| Total Gonadotropin dose/IU                    | 3525.3±1150.1   | 6750    | 1500     |
| Trigger day Endometrial Thickness/ml          | 11.6±2.21       | 17      | 8        |
| No of Retrieved oocytes (196 cases)           | 10.67±5.34      | 23      | 1        |
| No of MII oocytes (196 cases)                 | 7.3±3.7         | 15      | 0        |
| P-hCG/MII (193 cases)                         | 0.27±0.28       | 2.63    | 0.03     |
| Fertilized oocytes (196 cases)                | 6.15±3.05       | 13      | 0        |
| Fertilization ratio (193 cases)               | 0.87±0.2        | 2       | 0.28     |
| No of Transferred embryos (193 cases)         | 2.88±0.61       | 4       | 1        |
\( P-hCG/MII = \text{serum progesterone level the day of hCG injection per mature oocyte ratio} \)

- Studying Clinical pregnancy:

Table (2) studies the difference in the selected parameters between patients who got pregnant to those who didn’t. There was only a significant statistical difference in \( P-hCG \) (\( p=0.01 \)) and \( P-hCG/MII \) ratio (\( p=0.001 \)), while FSH/LH ratio, basal progesterone, and endometrial thickness on the day of hCG trigger were all non-significant (\( p=0.05 \), \( p=0.7 \), and \( p=0.4 \) respectively).

**Table (2): The impact of study parameters on clinical pregnancy rate.**

<table>
<thead>
<tr>
<th>Study parameters</th>
<th>Clinical pregnancy YES (n=54)</th>
<th>Clinical pregnancy NO(n=139)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FSH/LH</td>
<td>1.37±0.59</td>
<td>1.59±0.8</td>
<td>0.05</td>
</tr>
<tr>
<td>P basal</td>
<td>0.77±0.24</td>
<td>0.75±0.26</td>
<td>0.71</td>
</tr>
<tr>
<td>P-hCG</td>
<td>1.23±0.76</td>
<td>1.57±0.98</td>
<td>0.01*</td>
</tr>
<tr>
<td>P-hCG/MII</td>
<td>0.19±0.17</td>
<td>0.3±0.3</td>
<td>0.001*</td>
</tr>
<tr>
<td>Trigger day End. Thickness</td>
<td>11.83±2</td>
<td>11.56±2.2</td>
<td>0.4</td>
</tr>
</tbody>
</table>

*Values are given as mean ± SD.

* = statistically significant. \( P \) basal = serum progesterone before stimulation, \( P-hCG \) = serum progesterone in day of hCG injection, End = endometrial

- Diagnostic accuracy of the studied parameters:

ROC analysis was performed to determine cut off values for FSH/LH ratio, P-hCG/MII ratio and P-hCG that may correlate with clinical pregnancy. The following table (3) and figures shows the results with specificity and sensitivity for each. The cut off value of \( P-hCG \) (Figure 1) was 1 ng/ml with 61% sensitivity and 59% specificity (\( p=0.004 \)), while the best cut off value for \( P-hCG/MII \) ratio (Figure2) was 0.18 with sensitivity 70.5% and specificity 61.2% (\( p<0.001 \)).

**Table (3): Cut-off values for predicting IVF success (clinical pregnancy?).**

<table>
<thead>
<tr>
<th></th>
<th>Cut Off value</th>
<th>Sensitivity</th>
<th>Specificity</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-hCG</td>
<td>1</td>
<td>61%</td>
<td>59%</td>
</tr>
<tr>
<td>P-hCG/MII</td>
<td>0.18</td>
<td>70.5%</td>
<td>61.2%</td>
</tr>
</tbody>
</table>

\( P-hCG = \text{serum progesterone level the day of hCG injection} \), \( P-hCG/MII = P-hCG \) per mature oocyte ratio.
Figure (1): ROC curve for P-hCG as a predictor of clinical pregnancy after ICSI in our study patients (AUC =0.632 and p=0.004). At a P-hCG threshold of 1 ng/ml sensitivity was 61% and specificity 59%.

Figure (2): ROC curve for P-hCG/MII ratio as a predictor of clinical pregnancy after ICSI in our study patients (AUC =0.71 and p<0.001). At a P-hCG/MII ratio threshold of 0.18 sensitivity was 70.5% and specificity 61.2%.
Discussion

In our study, we tried to evaluate the role of serum progesterone in day of hCG injection in predicting ICSI outcome. We were also able to determine the optimal cut off value for P/MII oocyte ratio which might give us better prediction of ICSI success.

Higher P-hCG was observed in cases who didn’t get pregnant compared to those who got pregnant (1.57±0.98 vs 1.23±0.76 respectively, P= 0.01) and cut off value for P-hCG of 1 ng/ml correlated well with the clinical pregnancy rate after ICSI with 61% sensitivity and 59% specificity (p=0.004).

There has been an ongoing debate regarding the impact of preovulatory P on IVF outcome. Its clinical influence has been highly controversial for many years.

Schoolcraft et al., 1991, showed that the premature progesterone elevation was related to a lower pregnancy rate when using GnRH agonist for pituitary suppression. Progesterone levels more than 0.5 ng/ ml were associated with a lower rate of pregnancy (20%) compared with less than 0.5ng/ml (54%). These results showed that ovarian hyperstimulation might cause advanced luteinization and an adverse cycle outcome even with low-LH levels.12

Venetis et al. (2007) published an early systematic review and meta-analysis and revealed a lower pregnancy rate in patients with high serum progesterone, but the difference was not statistically significant.13 However, Kolibianakis et al.,(2012) in a more recent analysis found that women undergoing ovarian hyperstimulation using GnRH antagonists and gonadotropins, progesterone rise on the day of hCG injection is significantly associated with a lower possibility of clinical pregnancy.14

In an analysis on the outcomes of 2,566 cases after their first IVF/ICSI cycles managed with long or short protocols of GnRH agonist it was found that a premature progesterone elevation negatively correlated with live birth rate in fresh embryo-transfer (ET) cycles. However, live birth rates in frozen-thawed ET cycles showed no significant difference between case with or without progesterone rise implying that progesterone rise in stimulated cycles may have deleterious effects on endometrial receptivity.15 In a big analysis of more than 4,000 cycles, it was found that clinical pregnancy rates following IVF/ICSI cycles were inversely associated with serum progesterone levels on the day of hCG injection, regardles the GnRH analogue used. In particular, patients with serum progesterone levels < 1.5ng/ml had significantly higher clinical pregnancy rates than those with progesterone levels >1.5ng/ml.16

Although many studies have showed an adverse relation between elevated progesterone levels and IVF/ICSI pregnancy outcomes, the precise endocrinological mechanism is not clear. It has been theorized that progesterone in the late follicular phase might influence endometrial development which may lead to an asynchrony between the endometrium and the implanted embryo.17 Li et al in 2011 from micro RNA and microarray analysis of endometrium suggested dissimilar endometrial changes in patients with high progesterone levels on the day of hCG injection, and had poor pregnancy rates.8

On the other hand, several authors failed to demonstrate any negative effect of progesterone rise on IVF outcome.18,19,20,21 Using a previously described breakpoint in serum progesterone concentration of 0.9 ng/ ml in an earlier study, it was found that an elevated serum progesterone level on the day of hCG does not adversely affect the quality of oocytes or resulting embryos. The results suggest that the pregnancy rate in the elevated serum progesterone group is at least equal to the observed rate in the low progesterone group.22

A later investigation demonstrated that in the presence of an adequate response to ovarian stimulation progesterone levels > 0.9 ng/ml were not associated with lower pregnancy rates, indicating that good embryo quality may compensate for the adverse endometrial effects of elevated progesterone. However, when the response to ovarian stimulation was weak, premature progesterone elevation led to drastically reduced pregnancy rates.19,20

Another study found that there is no association between late follicular serum progesteron concentration on the day of hCG and the biochemical and clinical pregnancy rates obtained after ovarian stimulation for IVF/ICSI. Instead, a strong significant association was found between the number of follicles/oocytes and serum progesteron concentration, suggesting that each individual follicle contributes to the collective concentration observed in the circulation. Paradoxically,
the highest pregnancy rate in the study was found in the group of patients who had the highest late follicular progesterone concentrations (i.e. 47 nmol/l) and thus developed many follicles.18

Some authors concluded that serum progesterone cutoff value that negatively affect the outcome of IVF should be considered according to the ovarian response; which can be “normally higher” (about 1.75 ng / ml) in the cycles with ovarian hyper responsiveness.23,24

Based on these findings, It seems that evlated serum progesteron concentrations are a frequent event in GnRH analogue treated cycles and this elevation seems to be directly related to the number of oocytes obtained. According to various studies because the main sources of progesterone are follicles, stimulated by FSH, the level of progesterone produced per mature oocyte would be a better predictor for IVF outcome.4

Thus, we tried to evaluate the impact of the ratio between P-hCG and the number of mature oocytes retrieved on ICSI outcome. We found that significantly lower **P-hCG/MII ratio** (mean=0.19±0.17) was found in the pregnant women group compared to that found in the non-pregnant group (mean=0.3±0.3) (P=0.001). Cut off value of 0.18 correlated best with clinical pregnancy after ICSI with sensitivity 70.5% and specificity 61.2% (p<0.001).

**Conclusion**

Our study showed significant difference in P-hCG and P-hCG/MII oocyte ratio among patients with clinical pregnancy and those who didn’t get pregnant, however these tests have low diagnostic accuracy in predicting clinical pregnancy.

Using the above data, larger groups of patients can be analyzed to confirm our findings in larger studies. This study is also limited by its non-randomized design; future larger randomized trials are required to evaluate the role of P-hCG/MII oocyte ratio as a valuable prognostic tool in IVF over P levels alone.

**Conflict of Interest:** The authors have no conflicts of interest.

**Source of Funding:** Personal fund.

**Ethical Committee Approval:** Ethically approved by the department.

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