

Original Article

Survey of Relation between Midcycle Progesterone Level with IUI Cycles Success Rate

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ABSTRACT

Introduction: Assisted reproductive technology is used routinely for treatment of infertile spouses. Previous studies reported conflicting results regarding effect of progesterone rise at the HCG injection day. The aim of current study is to evaluate the effects of mid cycle progesterone levels in IUI cycles. **Methods:** In this analytical cross-sectional study, the outcome of treatment in 200 IUI cycles were evaluated. Patients' demographic findings, progesterone level at the day of HCG injection, number of follicles >16 mm, endometrial thickness and clinical pregnancy rate were recorded. For better evaluation, progesterone levels were divided to <0.5, 0.5-1.5 and >1.5 ng/dl. **Results:** Patients' mean age was 29.70±4.38 years. The progesterone levels of <0.5, 0.5-1.5 and >1.5 ng/dl was detected in 24.5%, 56% and 19.5% of patients, respectively. The rate of clinical pregnancy was 27.5%. Cases with positive pregnancy had significantly lower progesterone levels (0.88±0.31 vs.1.11±0.80, p=0.04). The highest pregnancy rate was in progesterone levels 0.5-1.5 compared to levels <0.5 and >1.5 ng/dl (42% vs. 12.2% vs. 5.2%, p<0.001). There was no significant correlation between progesterone levels with endometrial thickness (r=-0.130, p=0.06) and number of follicles >16 mm (r=0.02, p=0.77). **Conclusion:** The results of current study showed that the increase in progesterone levels at the day of HCG injection accompanies with lower pregnancy rate. However, this increase has no correlation with number of mature follicles and endometrial thickness.

BACKGROUND

Due to the different reasons, some infertile couples use In-Vitro Fertilization methods (IVF) for pregnancy. However, implantation rate even with transfer of embryos that seem to be healthy is still low (1).

One of the oldest therapeutic methods for infertility treatment is artificial sperm insemination into the uterus that includes methods in which sperm will be entered into different parts of female reproductive system to provide more gametes inside uterus. Sperm insemination into the uterus (IUI) is the most common artificial insemination and is applicable in different infertility centers (2,3).

Results of pregnancy rate by IUI are limited and completely different, and has an approximate 10% to 25% value (4), which this success will be increased with enhancing the number of total inseminated sperms. Moreover, the success probability in the case of using infertile men's sperm in IUI, is relatively correlated to the severity of the disorder of semen. Motility, number, and morphology of sperm are effective on the rate of success (5).

IVI is often done with some form of ovarian superovulations. One result of this low pregnancy level is LH surge earlier than the usual time for treatment of infertility, and early folli-

cle luteinization at the end of ovary stimulation. Early release of LH occurs in 25-10% of IUI cycles (6), and theoretically, is involved in the IUI time, results and failure of treatment. The role of progesterone enhancement is not clearly studied well.

Progesterone has an essential role in the implantation process, as causes marked alterations in the endometrium that is required for invasion and attachment of embryo. Progesterone level, during enhancement of LH wave, is correlated with LH. Some studies indicated that increased progesterone level may be a result of high dose of FSH (7). Moreover, patients with a high-level estradiol have an increased progesterone concentration (1). This study proposes that at least one of the mechanisms that can cause progesterone to increase during follicular phase, are correlated with ovarian response during ovulation stimulation (1).

Few studies regarding effects of progesterone on implantation during IUI and IVF cycles was done (8,9). However, contradicted results are expressed about the progesterone level at the middle of luteal phase and implantation rate. Most of studies showed that lower levels of mid-luteal phase progesterone is correlated to the failure of treatment (10,11).

Although the effect of premature rise of progesterone in IVF cycled were clarified, its role in IUI is not clear. Proges-

terone rise could have a bad effect on cycle and decrease the rate of pregnancy despite appropriate size of follicle (12). The aim of the present study was to assess mid-cycle level with the success rate in the IUI cycles.

MATERIALS AND METHODS

In this analytical cross-sectional study, 200 infertile women who were candidate for IUI were entered based on the exclusion and inclusion criteria of this research. Inclusion criteria were 18 to 35 years old women who had primary or secondary infertility, they had at least an open tube based on ultrasonography or laparoscopy results, and were candidate for IUI. Women with the experience of receiving Contraceptive Pills, molar pregnancy, ovarian cysts (with performing basic sonography), ovarian cancer, CAH (based on history and hormonal normal levels), and cardiac diseases were excluded from the study. Moreover, patients that had incomplete recorded information in their files and those who could not be followed up via their address or phone number, or those who had not regular referral, were also excluded from the study.

After basic sonography in days 1-3 of the cycle, from the third day of the cycle, 2 clomiphene and/or letrozole pills per day were prescribed for 5 days, and in 6th, 8th, and 10th days, a gonadotrophin ampule was prescribed, and vaginal sonography performed 5 days after the end of the pills. In the case of presence of at least one dominant follicle (over 18 mm), HCG was prescribed, and at the same day before injection of HCG, serum progesterone and LH levels were assessed. 36 hours after injection of HCG, IUI was performed and 14 days after IUI, pregnancy test was performed and blood HCG was assessed. If HCG was positive, sonography was performed after two weeks on pregnant patients. Pregnancy rate was compared in different levels of serum progesterone.

In the day of HCG injection, serum sample was obtained to measure progesterone level. Then, serum samples were transferred to the biochemical laboratory of Tabriz Alzahra Hospital, and serum values of progesterone were measured using Randex Company's kit.

Statistical Analysis

All studied data were analyzed using SPSS 24 statistical software. Descriptive statistical methods were used for statistical evaluation (frequency, percent, mean \pm SD). For comparing qualitative findings, Chi-square statistical test was used, and for comparing quantitative findings, Independent T-test and Mann-Whitney U statistical tests were used. Besides, Pearson's correlation test was used to assess the correlation between quantitative variables. In this study, p value <0.005 were considered to be statistically significant.

RESULTS

In the present study, 200 IUI cycles were evaluated. Mean age of women was 29.70 ± 4.38 years, and mean age of their husbands was 32.05 ± 6.41 years.

Mean duration of marriage was 6.28 ± 3.77 years, and mean infertility duration was 4.26 ± 2.95 years. Mean BMI

of the women was 24.67 ± 4.76 Kg/m². Infertility type in 160 cases was primary (80%), and in 40 cases was secondary (20%). Only 40 patients had the experience of previous pregnancy.

Menstrual cycles in 150 cases (75%) was regular, and in 25 cases (12.5%) was oligomenorrhea, and in 25 cases (12.5%) was hypermenorrhea.

Endometrium thickness at the injection time was 7.83 ± 0.67 mm, number of follicles over 18 mm was 2.99 ± 0.91 , and progesterone level was 1.04 ± 0.71 ng/dl.

For exact evaluation of progesterone level, based on the previous studies, we divided progesterone level as under 0.5 ng/dl, 0.5-1.5 ng/dl, and over 1.5 ng/dl groups that were 49 cases (24.5%), 112 cases (56%), and 39 cases (19.5%), respectively.

Clinical pregnancy was detected in 55 cases (27.5%).

Table 1 shows basic findings between cases with or without positive pregnancy test. Cases with or without pregnancies has similar status regarding mother age and endometrium thickness. Moreover, in the pregnant group, number of follicles over 18 mm was higher, though this difference was not statistically significant. There was significantly lower mid-cycle progesterone level in whom become pregnant in comparison to the group without pregnancy.

According to Figure 1, the highest pregnancy rate in progesterone levels was significantly occurred in 0.5-1.5 ng/dl group ($p < 0.001$). Moreover, in comparison of levels of progesterone below 0.5 ng/dl and over 1.5 ng/dl groups, positive pregnancy rate in <0.5 ng/dl progesterone level was higher; however, the difference was not statistically significant ($p = 0.29$).

Table 1. Basic findings between cases with or without successful cycles

	Pregnancy	No pregnancy	p-value
Mother age	29.87 ± 4.20	29.63 ± 4.46	0.73
Endometrial thickness	7.89 ± 0.73	7.81 ± 0.65	0.49
Follicles >18 mm	2.98 ± 0.87	3.00 ± 0.92	0.12
Progesterone level	0.88 ± 0.31	1.11 ± 0.80	0.04

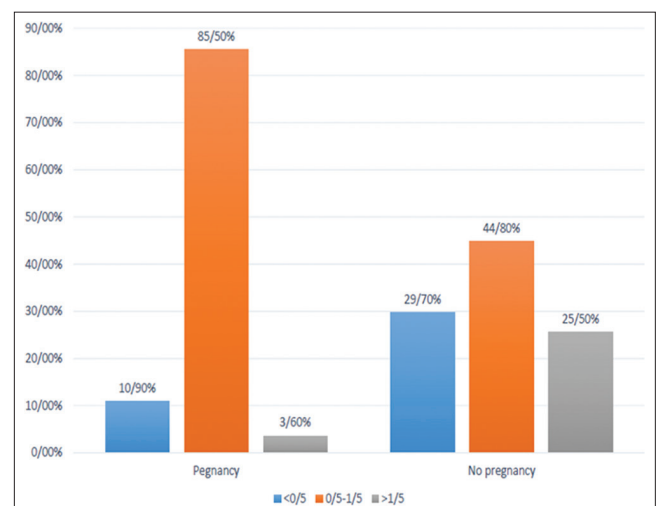


Figure 1. Pregnancy rate according to progesterone levels

Using Pearson's correlation test, the correlation between progesterone level with endometrium thickness ($p=0.06$, $r=-0.130$), and number of follicles with a size more than 16 mm ($p=0.44$, $r=-0.05$) was assessed that showed no statistically significant correlation.

DISCUSSION

The role of progesterone is to provide implantation in an endometrium during normal or induced cycles. Regarding results of previous studies, it is possible that progesterone level in the HCG injection day could be effective on success rate of IUI. At the present study, we have assessed the correlation of mid-cycle progesterone level with success rate in 200 IUI cycles. Clinical pregnancy occurred in 27.5% of mothers. Cases with positive pregnancy tests had a significantly lower level of progesterone in comparison to the group without success.

Until now, several studies have performed that assessed the correlation between increased progesterone with pregnancy rate, which resulted in contradicted consequences. Increased progesterone levels in early stages of follicular phase is accompanied with reduced pregnancy potential in women, which their ovaries are stimulated (13-15); however, other studies found no correlation between these two variables (16,17). A recent meta-analysis published in 2013, has reported that harmful effects of increased progesterone level on pregnancy rate are seen in the 0.8-1.1 ng/dl range (18).

In the present study, we evaluated the correlation between mid-cycle progesterone levels with success rate, and showed that the pregnancy rate is reduced as the level of progesterone increases; so that, the highest pregnancy rate is shown in the 0.5-1.5 ng/dl progesterone level, and the lowest rate is seen in the levels over 1.5 ng/dl.

Similarly, Ashmita *et al.* (19) indicated that clinical pregnancy rate in patients with under 1.5 ng/ml progesterone level was significantly higher than patients with increased levels of progesterone to over 1.5 ng/ml (33.3% against 12.9%). This study proposes that early increase in progesterone during ART cycles is most probably accompanied with clinical pregnancy rate.

In another study by Riberio *et al.* (20), it was shown that the clinical pregnancy rate and live birth rate in both patients with progesterone levels under 0.5 ng/ml and over 1.5 ng/ml were lower. Mutlu *et al.* (21) also observed that patients with positive pregnancy test have a significantly lower progesterone level in comparison to the non-pregnant patients.

In contrary to the above findings, Lu *et al.* (22) reported no statistically significant difference in the retrieval rate of oocyte, implantation rate, insemination rate, clinical pregnancy rate, and live birth rate, between different groups of progesterone levels. This shows that increased progesterone levels have no negative effects on the final consequence of the treatment.

Saleh *et al.* (23) also shown that the progesterone level in the HCG injection day is not a predictive factor for pregnancy consequences. Kutlu *et al.* (24) in assessment of patients under IUI treatment showed no difference regarding progesterone level at the time of HCG injection, between cases with or without positive pregnancy.

Although previous studies (14, 25) indicated a reverse correlation between pregnancy rate and progesterone level at the HCG injection day (especially when reaches to 1.5 ng/ml threshold), this threshold could not be absolutely considered, as from follicular phase to luteal phase in the natural cycle is changing (26).

In the present study, no correlation was also found between increased levels of progesterone with endometrium thickness and number of follicles with a size higher than 16 mm.

A positive correlation has been reported between number of mature follicles and progesterone release in late follicular phase, in the early studies (27). In the study of Singh *et al.* (28) an increasing pattern for progesterone level was seen with a statistically significant difference based on the number of oocyte. This shows that increased level of progesterone is an indication of number of follicles and is not a result of early luteinization.

Moreover, in the study of Lu *et al.* (22), number of oocytes in patients with increased progesterone level was higher than patients with progesterone level under 1 ng/ml.

Regarding results of studies performed on patients under IVF-ICSI, it seems that progesterone has a negative effect on quality of oocyte (29,30); so that, a significant reduction in clinical consequences could be due to incomplete endometrium receptivity because of abundance of progesterone or early ovulation, which increases duration of oocyte presence at female reproductive tubes and increases early aging before insemination.

It is also proposed that ovarian response may play a medium role on effect of increase of progesterone in the pregnancy rate. Recently, a meta-analysis has performed on 6 clinical trials with an approximate 1890 cycles by Griesinger *et al.* (31) showed that over 1.5 ng/ml progesterone level will not worsen the consequence of pregnancy in patients with more than 18 oocytes. On the other hand, Bosch *et al.* (14) showed that harmful effects of progesterone rise on pregnancy rate is separated of the ovarian response rate. Similarly, a study that evaluates the effect of progesterone enhancement on different ovarian responses shows the negative effect of progesterone rise on the pregnancy rate isolated from the ovarian response rate (30).

The exact mechanism that causes progesterone enhancement is a controversial subject among researchers (14,18,32). Some of them think that high steroidogenic activity of ovary due to stimulation of several oocytes with exogenous FSH, is the main source of progesterone rise (14,33).

However, alternative causes such as production of increased LH or alteration of LH receptor sensitivity, are introduced in some populations like those with low ovarian response (33-40).

CONCLUSION

Results of the present study showed that increased level of progesterone in the HCG injection day is accompanied with lower pregnancy rate. However, this enhancement has no correlation with the number of mature follicles and thickness of endometrium.

DECLARATIONS

Ethics Approval and Consent to Participate

The Ethical Committee of Tabriz University of Medical Sciences approved the study and written informed consents were obtained from all participants.

Consent for Publication

Not applicable.

Availability of Data and Materials

The datasets used and/or analyzed during the current study are available from the corresponding author upon a reasonable request.

Authors' Contributions

PR and HRF were responsible for the conception and design of the study. PR, HRF, FI and SA were responsible for acquisition and analysis of data. Furthermore, SA was in charge of statistical analysis. PD and SA drafted the manuscript and the final approved version. All authors read and approved the final manuscript.

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