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RESEARCH ARTICLE

The Predictive Effect of Hormone Levels on Pregnancy Outcomes on the Day of Intrauterine Insemination and hCG Administration

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Abstract

Introductıon: Intrauterine insemination (IUI) is commonly used to treat unexplained infertility. Hormonal markers, especially estradiol (E2) and progesterone (P), are crucial in predicting endometrial receptivity and pregnancy outcomes. This study investigates the predictive value of E2 and P levels measured on the day of human chorionic gonadotropin (hCG) administration and IUI.

Methods: A retrospective review was conducted on 81 women aged 18–38 who underwent IUI for unexplained infertility between 2018 and 2020. Serum E2 and P levels were assessed on hCG and IUI days. Pregnancy outcomes were compared using t-tests and chi-square tests ($p < 0.05$) via SPSS 25.0.

Results: Out of 81 participants, 11 achieved pregnancy (13.5%). Pregnant women had significantly higher E2 levels on hCG (472.3 ± 291.3 vs. 398.6 ± 277.2 pg/mL, p = 0.018) and IUI days (425.3 ± 269.1 vs. 371.2 ± 275.8 pg/mL, p = 0.033). P levels were also higher in this group on both days (hCG: 1.43 ± 1.62 vs. 0.74 ± 1.29 ng/mL, p = 0.001; IUI: 2.48 ± 1.92 vs. 2.31 ± 2.12 ng/mL, p = 0.024). Lower IUI-to-hCG hormone ratios were noted in the pregnant group (E2: 0.90 vs. 0.93, p = 0.045; P: 1.7 vs. 3.1, p = 0.001).

Conclusion: Elevated E2 and P levels on hCG and IUI days are associated with improved pregnancy outcomes. Hormonal monitoring may enhance IUI success and guide personalized infertility treatments.

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Introduction

Infertility, defined as the inability to achieve pregnancy after 12 months of regular unprotected intercourse, affects approximately 10–15% of reproductive-aged couples worldwide, posing significant psychological and social burdens. Among the various treatment strategies, intrauterine insemination (IUI) is a widely practiced first-line assisted reproductive technique, especially in cases of unexplained infertility, where no definitive cause is identified despite a thorough evaluation.

One of the key factors influencing IUI success is endometrial receptivity, which is tightly regulated by dynamic changes in serum estradiol (E2) and progesterone (P) levels throughout the follicular and luteal phases. Estradiol plays a critical role in proliferative endometrial development, while progesterone is essential for secretory transformation and maintenance of luteal phase adequacy. The timely and optimal modulation of these hormones is vital for successful embryo implantation.

Although several studies have assessed hormonal thresholds during ovarian stimulation protocols, the predictive value of serum E2 and P concentrations specifically on the day of hCG administration and on the day of IUI remains inadequately defined. Some reports suggest that elevated progesterone levels prior to ovulation may impair endometrial-embryo synchrony, while others emphasize the potential benefit of higher E2 levels as indicators of follicular maturity and endometrial readiness.[1–3]

However, despite the critical timing of hCG and insemination in IUI cycles, few studies have simultaneously compared hormone profiles at both these strategic time points in relation to pregnancy outcomes. Moreover, limited evidence exists on how the ratios of hormone changes between hCG and IUI days may reflect endometrial receptivity or luteal phase adequacy.

The primary aim of this study is to investigate whether serum estradiol and progesterone levels measured on the day of hCG administration and the day of intrauterine insemination can predict pregnancy success in patients with unexplained infertility. Additionally, we aim to evaluate the predictive potential of E2 and P ratios between these two time points, hypothesizing that dynamic hormonal fluctuations may serve as non-invasive biomarkers of endometrial receptivity and fertility prognosis.

Material and Methods

This study was designed retrospectively and conducted on patients who underwent IUI treatment due to unexplained infertility. Data were collected from patients treated between 2018 and 2020 using the hospital automation system and archives after obtaining informed consent from the patients. The study was conducted with the approval of the ethics committee (TABED 2-25-1310), and all patient data were evaluated in accordance with confidentiality principles.

A total of 255 patients who underwent IUI for unexplained infertility between 2018 and 2020 were included in the study. However, patients with missing hormone levels (estradiol and progesterone) or unknown pregnancy outcomes were excluded, and statistical analysis was performed with 81 patients aged 18-38.

All patients included in this study were followed up with a diagnosis of unexplained infertility and had experienced at least one previous unsuccessful IUI attempt. For ovulation induction, patients were started on 75 IU of GONAL-F ®, following a low-dose step-up protocol. Follicular development was monitored via ultrasonography, and when the follicular size reached 17-21 mm, hCG was administered. In patients who developed multiple follicles, the procedure was postponed to prevent the risk of multiple pregnancies, and as a result, 35 patients did not undergo the procedure for this reason. In cases where a single follicle reached an appropriate size, IUI was performed 36 hours after the administration of hCG. To support the luteal phase, all patients were administered vaginal progesterone gel (8%), and for those who achieved pregnancy, treatment was continued throughout the subsequent weeks of pregnancy.

The serum estradiol and progesterone levels on the day of the IUI procedure, as well as serum estradiol and progesterone levels on the day of hCG administration, were retrospectively collected from the study population. Additionally, the results of the hCG test performed on day 14 after the IUI procedure were analyzed.

This study included patients aged between 18 and 38 years who were diagnosed with unexplained infertility and underwent IUI treatment. Eligibility for inclusion required the availability of complete serum estradiol and progesterone measurements on both the day of hCG administration and the day of IUI, as well as documented pregnancy outcomes. Patients were excluded if serum estradiol and progesterone levels were unavailable, if pregnancy outcome data were incomplete, or if they were older than 40 years. Additionally, individuals diagnosed with secondary infertility or underlying reproductive pathologies such as endometriosis or polycystic ovary syndrome (PCOS) were excluded to ensure a homogenous population of unexplained infertility cases.

All data were collected retrospectively from hospital archive records and database. Serum E2 and P levels on the day of IUI, as well as hormone levels on the day of hCG administration, were evaluated. Additionally, the pregnancy status was determined by the hCG test performed on day 14 after IUI, and the results were recorded. In patients with positive hCG tests, serum estradiol and progesterone levels were monitored until the 10th week of pregnancy.

Serum estradiol and progesterone levels were analyzed using the immunoassay method, following routine clinical protocols. Hormone levels were recorded at two separate time points: on the day of hCG administration and on the day of IUI. These values were compared between the successful (positive hCG test) and unsuccessful (negative hCG test) groups.

The data were analyzed using SPSS 25.0 (Statistical Package for the Social Sciences) software. The independent groups t-test was applied for continuous variables, while categorical data were evaluated using the chi-square test. Relationships between pregnancy rates and serum hormone levels were examined, and a significance level of $p < 0.05$ was considered.

Results

This study evaluated a total of 81 patients who underwent IUI treatment due to unexplained infertility. Among these patients, 11 were found to have positive pregnancy tests, indicating an IUI success rate of 13.5%. Out of the 13 patients who achieved pregnancy, 10 resulted in live births. The results comprehensively evaluated the relationships between hormone levels and pregnancy outcomes.

Table 1 compares the demographic and clinical data of patients with positive and negative pregnancy outcomes. There were no statistically significant differences between the groups in terms of age, body mass index (BMI), infertility duration, or follicle count ($p > 0.05$). The mean age of the positive pregnancy group was 26.64 ± 6.43 years, while it was 29.05 ± 4.14 years in the negative group ($p = 0.125$). In terms of BMI, there was no significant difference between the groups, with the positive group having a BMI of 25.2 ± 3.1 and the negative group 25.8 ± 3.4 ($p = 0.367$). Similarly, there were no significant differences in infertility duration and follicle count between the groups. These findings suggest that the groups were homogeneous at the beginning, allowing a more accurate evaluation of the effect of hormonal parameters on pregnancy success.

Table 1. Baseline demographic and clinical characteristics of the study groups.

Parameter	Pregnant (n=11)	Non-Pregnant (n=70)	p-value
Age (years)	26.64 ± 6.43	29.05 ± 4.14	0.125
Body Mass Index (kg/m²)	25.2 ± 3.1	25.8 ± 3.4	0.367
Duration of Infertility (years)	1.5 ± 1.2	1.8 ± 1.4	0.299
Follicle Count	1.1 ± 0.2	1.1 ± 0.2	0.865

Abbreviations: BMI, body mass index.

In this study, baseline hormonal levels, including AMH, FSH, E2, and Day 21 Progesterone, were compared between pregnant and non-pregnant groups. Despite slight differences in the means, none of the parameters showed statistically significant differences ($p > 0.05$). The mean AMH level was 2.6 ± 1.0 ng/mL in the pregnant group and 2.5 ± 1.1 ng/mL in the non-pregnant group, with a p-value of 0.389. FSH levels were also similar between groups, with means of 6.4 ± 2.1 mIU/mL and 6.5 ± 2.0 mIU/mL for pregnant and non-pregnant groups, respectively ($p = 0.456$). Basal estradiol levels were slightly higher in the pregnant group (46.0 ± 15.0 pg/mL) compared to the non-pregnant group (44.5 ± 15.5 pg/mL), but this difference was not statistically significant ($p = 0.312$). Finally, Day 21 progesterone levels showed no significant difference, with the pregnant group having a mean of 12.5 ± 4.2 ng/mL and the non-pregnant group having a mean of 11.8 ± 4.0 ng/mL ($p = 0.275$). These findings suggest that while these baseline hormonal values are important for understanding general ovarian function and luteal phase health, they may not be reliable predictors of pregnancy success in IUI cycles. The mean AFC in the pregnant group was 10.5 ± 3.2, while it was 10.2 ± 3.4 in the non-pregnant group, with a p-value of 0.742, indicating that both groups had similar antral follicle counts.

Table 2. Baseline hormonal values in pregnant and non-pregnant groups.

Parameter	Pregnant (n=11)	Non-Pregnant (n=70)	p-value
AMH (ng/mL)	2.6 ± 1.0	2.5 ± 1.1	0.389
FSH (mIU/mL)	6.4 ± 2.1	6.5 ± 2.0	0.456
Basal Estradiol (E2, pg/mL)	46.0 ± 15.0	44.5 ± 15.5	0.312
Day 21 Progesterone (ng/mL)	12.5 ± 4.2	11.8 ± 4.0	0.275
Antral Follicle Count	10.5 ± 3.2	10.2 ± 3.4	0.742

Abbreviations: AMH, anti-Müllerian hormone; FSH, follicle-stimulating hormone; E2, estradiol.

The comparison of sperm parameters between the pregnant and non-pregnant groups shows no statistically significant differences ($p > 0.05$) across all variables. Sperm concentration was nearly identical between the groups, with 50.5 ± 12.0 million/mL in the pregnant group and 49.8 ± 11.5 million/mL in the non-pregnant group ($p = 0.635$). Similarly, total motility was 55.0 ± 10.2% in the pregnant group and 54.5 ± 9.8% in the non-pregnant group ($p = 0.743$), while progressive motility was 40.2 ± 9.5% in the pregnant group compared to 39.8 ± 9.1% in the non-pregnant group ($p = 0.815$).

Table 3. Semen analysis results in pregnant and non-pregnant groups.

Parameter	Pregnant (n=11)	Non-Pregnant (n=70)	p-value
Sperm Concentration (million/mL)	50.5 ± 12.0	49.8 ± 11.5	0.635
Total Motility (%)	55.0 ± 10.2	54.5 ± 9.8	0.743
Progressive Motility (%)	40.2 ± 9.5	39.8 ± 9.1	0.815
Morphology (Normal Forms, %)	5.0 ± 1.2	5.1 ± 1.3	0.721
Total Motile Sperm Count (million)	27.8 ± 7.2	27.2 ± 6.9	0.689

For sperm morphology, the proportion of normal forms was also similar, at 5.0 ± 1.2% in the pregnant group and 5.1 ± 1.3% in the non-pregnant group ($p = 0.721$). Additionally, the total motile sperm count, which represents the total number of motile sperm available, was 27.8 ± 7.2 million in the pregnant group and 27.2 ± 6.9 million in the non-pregnant group ($p = 0.689$). This further indicates no significant differences in the total number of motile sperm between the groups, suggesting that sperm quality and quantity were comparable across the two groups and may not be the sole determining factor in pregnancy success.

In this comparison of follicular size at the time of rupture between the pregnant and non-pregnant groups (Table 4), the mean follicular size in the pregnant group was 19.8 ± 2.5 mm, while in the non-pregnant group, it was 19.5 ± 2.7 mm. The difference between the groups was not statistically significant ($p = 0.675$). This suggests that the size of the follicle at the time of rupture was similar in both groups and did not appear to have a significant impact on pregnancy outcomes in this study.

Table 4. Follicular size at rupture in pregnant and non-pregnant groups.

Parameter	Pregnant (n=11)	Non-Pregnant (n=70)	p-value
Follicular Size (mm)	19.8 ± 2.5	19.5 ± 2.7	0.675

In this study, serum E2 levels measured on the days of hCG administration IUI were carefully evaluated. The average E2 level on the hCG day was 472.3 ± 291.3 pg/mL in the group that achieved pregnancy, significantly higher than 398.6 ± 277.2 pg/mL in the non-pregnant group ($p = 0.018$). Similarly, E2 levels on the day of IUI were also higher among those who conceived (425.3 ± 269.1 pg/mL vs. 371.2 ± 275.8 pg/mL, $p = 0.033$). A statistically significant difference was also found in the ratio of E2 levels between IUI and hCG days.

Progesterone (P) levels followed a similar pattern. On the day of hCG administration, mean P levels were notably elevated in the pregnant group (1.43 ± 1.62 ng/mL) compared to the non-pregnant group (0.74 ± 1.29 ng/mL, $p = 0.001$). Progesterone levels on the IUI day remained higher in the pregnant cohort (2.48 ± 1.92 ng/mL) relative to those who did not conceive (2.31 ± 2.12 ng/mL, $p = 0.024$). Interestingly, the IUI-to-hCG P ratio was significantly lower in the pregnant group, suggesting a more stable hormonal transition ($p = 0.001$).

Additionally, the estradiol IUI/hCG ratio was lower in the group that achieved pregnancy (0.90 vs. 0.93), a difference that was also statistically significant ($p = 0.045$).

These findings indicate that serum E2 and P levels on both hCG and IUI days are meaningful markers in predicting pregnancy success. Elevated hormone levels at these time points appear to support a more receptive endometrial environment, increasing the likelihood of successful implantation. However, the data also suggest that excessive elevations might negatively impact follicular development or endometrial synchrony.

Table 5. Hormonal parameters and pregnancy outcomes.

Parameter	Pregnant (n=11)	Non-Pregnant (n=70)	p-value
Age (years)	26.64 ± 6.43	29.05 ± 4.14	0.009
hCG Day Estradiol (E2, pg/mL)	472.3 ± 291.3	398.6 ± 277.2	0.018
IUI Day Estradiol (E2, pg/mL)	425.3 ± 269.1	371.2 ± 275.8	0.033
hCG Day Progesterone (P, ng/mL)	1.43 ± 1.62	0.74 ± 1.29	0.001
IUI Day Progesterone (P, ng/mL)	2.48 ± 1.92	2.31 ± 2.12	0.024
IUI P / hCG P Ratio	1.7	3.1	0.001
IUI E2 / hCG E2 Ratio	0.90	0.93	0.045

Abbreviations: hCG, human chorionic gonadotropin; IUI, intrauterine insemination; E2, estradiol; P, progesterone.

Discussion

This study set out to assess the predictive utility of E2 and P levels measured at two critical time points—hCG trigger and IUI day—in women with unexplained infertility. The results demonstrated that elevated levels of both hormones, in conjunction with lower IUI/hCG ratios, were significantly associated with positive pregnancy outcomes.

Our results align with existing literature that underscores the role of periovulatory hormonal dynamics in endometrial receptivity. Estradiol, in particular, is known to facilitate follicular maturation and endometrial proliferation—two essential components of successful implantation.[4,5] Consistent with our findings, previous studies have shown that higher mid-cycle E2 levels correlate with improved IUI outcomes, reinforcing its value as a non-invasive biomarker of endometrial readiness.[2,6]

Traditionally, elevated progesterone during the follicular phase has raised concerns about disrupted synchrony between embryo and endometrium.[7] Nonetheless, our study supports emerging evidence that higher P levels, especially on the hCG day, may reflect a healthy luteal transformation in the context of controlled ovarian stimulation, rather than a detrimental shift.[8, 9]

One novel feature of this study is the evaluation of hormone ratios (IUI/hCG), rather than absolute values alone. These ratios may provide more accurate insight into the physiological shift from follicular to luteal phase. In our data, a significantly lower P ratio was linked to successful pregnancies, perhaps reflecting more stable luteal support.[10] Although few studies have analyzed such ratio-based indicators, our results support growing interest in dynamic hormone patterns rather than static thresholds.[11]

Baseline demographic and hormonal characteristics—such as AMH, FSH, AFC, and BMI—did not differ significantly between groups, consistent with prior reports suggesting that static baseline values are weak predictors in IUI success.[12-14] This further emphasizes the relevance of mid-cycle dynamic markers in guiding treatment strategies for unexplained infertility.

Clinically, these findings advocate for routine monitoring of E2 and P on both the hCG trigger and IUI days. Dynamic hormonal ratios may serve as simple, accessible tools to predict cycle success, guiding decisions such as optimal insemination timing, need for luteal phase support, or tailoring protocols based on individual hormonal responses.[15,16]

Despite its valuable contributions, the study is not without limitations. Its retrospective nature may introduce selection bias, and the small number of pregnancies limits the statistical power for subgroup analysis. Additionally, important markers of endometrial receptivity, such as thickness and vascularity, were not evaluated. Moreover, serum hormone levels may not perfectly reflect intrauterine or endometrial conditions, as local receptor activity and paracrine factors also play significant roles.[17]

Nonetheless, the study's strengths include a homogenous patient population (limited to unexplained infertility), standardized stimulation protocols, and a novel approach through dual-timepoint hormonal assessment and ratio analysis. This practical and reproducible design holds promise for further validation in future prospective trials.

In conclusion, the findings confirm that E2 and P levels on both hCG and IUI days are predictive of pregnancy outcomes in IUI cycles. Elevated mid-cycle hormone levels enhance endometrial receptivity and support implantation, while disproportionate hormonal shifts—particularly in progesterone—may contribute to cycle failure. These insights underscore the importance of individualized hormonal monitoring and adjustment to improve IUI success rates in unexplained infertility cases. Larger-scale, prospective studies are warranted to further clarify these relationships and optimize clinical protocols.

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DECLARATIONS

Ethical Approval: Ethics committee approval was obtained from the ethics committee unit of the Ankara Bilkent City Hospital (TABED 2-25-1310). The study commenced after obtaining the relevant ethical committee approval, and consent was obtained from the relevant clinics. The study was designed retrospectively, and no additional tests or hospital visits were required for the study. Consent for the use of data was obtained from the patients, and the confidentiality of patient identities was carefully maintained.

Consent to participate: Following ethics committee approval, written informed consent forms were obtained from all participants for their participation in the study.

Consent for publication: There are no circumstances in the study that violate anonymity, and identifying information has been kept confidential. There are no issues regarding its publication.

Availability of data and materials: Patient data is stored indefinitely in the hospital's automation system. It can be shared upon request, provided that patient identity remains confidential.

Competing interests: There are no conflicts of interest among the authors.

Authors' contributions: M.İ.H. and İ.H. contributed to the data collection phase of the study. U.Z. and Ö.M.T. were responsible for the study design and planning. Ç.K., Ş.B. and F.B.F. were responsible for writing of manuscript. U.Z. and B.T. jointly drafted the main manuscript. All authors critically reviewed and approved the final version of the manuscript.

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