**DOI:** 10.4274/forbes.galenos.2023.02359
Forbes J Med 2023;4(3):250-8

**Original Article / Orijinal Araştırma**

# Human Chorionic Gonadotropin Trigger Day Progesterone to Oocyte Ratio Predicts Pregnancy Outcomes of In Vitro Fertilization Cycles: A Retrospective Cohort Study and Literature Review

## *İn Vitro Fertilizasyon Sikluslarında, İnsan Koryonik Gonadotropini Tetikleme Günü Progesteron-oosit Oranı ile Gebelik Sonuçlarının Tahmini: Retrospektif bir Kohort Çalışması ve Literatürün Gözden Geçirilmesi*

**Onur İNCE[1,2,3], Bülent YILMAZ[2,4]**

[1]Kütahya Health Sciences University Faculty of Medicine, Department of Obstetrics and Gynecology, Kütahya, Türkiye
[2]University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital, Clinic of Obstetrics and Gynecology, İzmir, Türkiye
[3]Middle East Technical University, Faculty of Arts and Science, Department of Statistics, Ankara, Türkiye
[4]Recep Tayyip Erdoğan University Faculty of Medicine, Department of Obstetrics and Gynecology, Rize, Türkiye

**Cite as:** İnce O, Yılmaz B. Human Chorionic Gonadotropin Trigger Day Progesterone to Oocyte Ratio Predicts Pregnancy Outcomes of In Vitro Fertilization Cycles: A Retrospective Cohort Study and Literature Review. Forbes J Med 2023;4(3):250-8

## ABSTRACT

**Objective:** This retrospective cohort study investigated trigger day progesterone to total oocyte count ($P/O_{Total}$) and progesterone to MII oocyte count ($P/O_{MII}$) as potential predictors of live birth rate (LBR).

**Methods:** First cycles of 1,258 infertile patients who attended to the İzmir Tepecik Training and Research Hospital in vitro fertilization unit (IVF) between March 1, 2010 and November 1, 2016 and underwent intracytoplasmic sperm injection treatment following controlled ovarian stimulation + GnRH antagonist cycles with fresh embryo transfer were investigated.

**Results:** LBR for cycles with P levels ≤0.5, 0.5-1.5 and >1.5 ng/mL were 16.3% (50/307 cycles), 23.0% (180/783), and 19.6% (33/168), respectively. LBR was significantly lower in ≤0.5 ng/mL P level group than the 0.5-1.5 ng/mL group. After the exclusion of cycles with ≤0.5 ng/mL P level, $P/O_{Total}$ and $P/O_{MII}$ ratios were found to have significant area under the curve values (0.544, p=0.049; 0.552, p=0.022, respectively) for predicting LBR, whereas P levels (0.509, p=0.686) on its own was not a significant predictor. In ROC models adjusted for cycle characteristics, $P/O_{Total}$ and $P/O_{MII}$ ratio values showed neither better nor weaker prediction performance (0.608, p<0.001; 0.610, p<0.001, respectively) than using P and 1/O or P and 1/MII as separate parameters (0.610, p<0.001; 0.611, p<0.001, respectively).

**Conclusion:** The predictive performance of $P/O_{Total}$ and $P/O_{MII}$ ratios for LBRs are similar when O, P, and MII are used as separate parameters. Consequently, these ratios can be used as feasible clinical markers and have the advantage of simpler interpretation.

**Keywords:** Progesterone, oocyte, ratio, MII, live birth rate, GnRH antagonist, ICSI, IVF

**Received/Geliş:** 19.03.2023
**Accepted/Kabul:** 16.06.2023

**Corresponding Author/ Sorumlu Yazar:**

**Onur İNCE MD**

Kütahya Health Sciences University Faculty of Medicine, Department of Obstetrics and Gynecology, Kütahya; Middle East Technical University, Faculty of Arts and Science, Department of Statistics, Ankara; University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital, Clinic of Obstetrics and Gynecology, İzmir, Türkiye

**Phone:** +90 274 265 22 86

onurincemd@gmail.com

**ORCID:** 0000-0003-2263-8956

## ÖZ

**Amaç:** Bu retrospektif kohort çalışma, canlı doğum oranının (CDO) potansiyel öngörücüleri olarak tetikleme günü progesteron/toplam oosit sayısı ($P/O_{Total}$) ve progesteron/MII oosit sayısı ($P/O_{MII}$) oranlarını araştırmaktadır.

**Yöntem:** 1 Mart 2010 ile 1 Kasım 2016 tarihleri arasında Sağlık Bilimleri Üniversitesi, İzmir Tepecik Eğitim ve Araştırma Hastanesi in vitro fertilizasyon (IVF) kliniğine başvuran ve GnRH antagonisti eşliğinde kontrollü over stimülasyonu sonrasında intrasitoplazmik sperm enjeksiyonu tedavisi uygulanan 1.258 infertil hastanın ilk siklusları incelenmiştir.

**Bulgular:** Serum P düzeyleri ≤0,5, 0,5-1,5 ve >1,5 ng/mL olan sikluslar için CDO sırasıyla %16,3 (50/307 siklus), %23,0 (180/783) ve %19,6 (33/168) olarak bulunmuştur. CDO, P düzeyi ≤0,5 ng/mL olan grupta, 0,5-1,5 ng/mL grubuna göre önemli ölçüde daha düşüktü. P düzeyi ≤0,5 ng/mL olan sikluslar hariç tutulduktan sonra, $P/O_{Total}$ ve $P/O_{MII}$ oranlarının CDO'yu öngörmek için eğrinin altında kalan alan değerleri (sırasıyla 0,544, p=0,049; 0,552, p=0,022) anlamlı olarak saptanmıştır. Tek başına P düzeyi (0,509, p=0,686) anlamlı bir öngörücü olarak saptanmamıştır. Siklus özelliklerine göre düzenlenen ROC modellerinde, $P/O_{Total}$ ve $P/O_{MII}$ oranı değerleri (sırasıyla 0,608, p<0,001; 0,610, p<0,001), P ve 1/O ya da P ve 1/MII gibi, parametreleri ayrı ayrı kullanmaktan daha güçlü veya daha zayıf bir öngörü performansı göstermemiştir (sırasıyla 0,610, p<0,001; 0,611, p<0,001).

**Sonuç:** CDO için $P/O_{Total}$ ve $P/O_{MII}$ oranlarının öngörü performansı, ayrı parametreler olarak O, P ve MII kullanımıyla benzerdir. Sonuç olarak, bu oranlar uygun klinik belirteçler olarak kullanılabilir ve daha basit bir yorumlamaya sahip olma avantajına sahiptir.


**Anahtar Kelimeler:** Progesteron, oosit, oran, MII, canlı doğum oranı, GnRH antagonisti, ICSI, IVF

## INTRODUCTION

Progesterone (P) hormone, the terminal end product of human ovarian steroidogenesis, is essential for the establishment of pregnancy in the luteal phase of the menstrual cycle.[1] It also plays an important physiological role via a direct pituitary effect at relatively low concentrations, regulating the secretion of luteinizing hormone (LH) and follicle-stimulating hormone (FSH).[2] High P levels, on the other hand, appear to impair pregnancy outcomes by affecting endometrial receptivity, arguably inducing an asynchrony between the endometrium and the embryo in midcycle.[3]

The evidence on whether late follicular P elevation on the day of human chorionic gonadotropin (hCG) may adversely affect pregnancy outcomes in in vitro fertilization (IVF)/intracytoplasmic sperm injection (ICSI) treatment cycles is mixed.[4] Some studies find an adverse effect in assisted reproductive technologies cycles when serum P level on the day of hCG trigger exceeds 1.5 ng/mL.[5-12] Some more recent studies suggest increasing trigger day serum P levels thresholds for increasing ovarian responder levels.[13-15] There are also studies that take things forward and suggest that the ratio of trigger day P to total oocyte count (O) collected at oocyte pick-up ($P/O_{Total}$) or the ratio of P to metaphase II (MII) oocyte count ($P/O_{MII}$) can be good predictors for live birth rates (LBR).[16-18] Santos-Ribeiro et al.[19] (2014) pointed out that both low and high levels of P might be detrimental to LBRs, which provides an alternative view on the issue. Despite the other strong studies that chose high starting cut-off values for binning P levels (after 1 ng/mL), Santos-Ribeiro et al.[19] sequenced the serum P levels starting from 0 ng/mL with 0.25 ng/mL intervals and concluded that serum P levels lower than 0.5 ng/mL may also hinder the live birth results.

Up to now, alternative ratios such as $P/O_{Total}$, $P/O_{MII}$, P to follicle, or P to estradiol ratios have been investigated to determine how powerful they are in predicting cycle outcome. However, these studies assumed a decreasing linear relationship between increasing P levels and cycle outcomes. They did not present pregnancy outcomes for each P sequence in detail by binning, and their analysis included cycles that had low levels of P that arguably hindered LBRs. This approach may create a bias against the use of these ratios and avoid finding significant upper thresholds for these ratios.

The aim of this study was two fold: first, to evaluate the impact of $P/O_{Total}$ and $P/O_{MII}$ ratios on LBR in patients undergoing gonadotropin-releasing hormone (GnRH) antagonist/ICSI cycles with fresh autologous embryo transfer after the exclusion of cycles with low hCG day serum P levels and second, to review the literature on $P/O_{Total}$ and $P/O_{MII}$ ratios.

## METHODS

This retrospective cohort study was approved by the Local Ethics Committee at İzmir Kâtip Çelebi University (decision no: 329, date: 28.12.2016) and was conducted in the IVF Center of University of Health Sciences Türkiye, İzmir Tepecik Training and Research Hospital between March 1, 2010 and November 1, 2016. The patients in the sample underwent controlled ovarian stimulation (COS) + GnRH antagonist cycles with fresh embryo transfer followed by ICSI treatment. Patients with low ovarian reserve, tubal factor, polycystic ovary syndrome, unexplained infertility, mild to moderate male factor, endometrioma (only including patients that have diagnosis confirmed by previous surgery or obvious endometrioma appearance on transvaginal ultrasound) and aged between 20 and 40 years (for women) were included in the study. To create a more homogenous cohort, couples with genetic abnormalities, those who underwent testicular sperm extraction for azoospermia, and those who underwent frozen-thawed embryo transfer were excluded from the sample. Cycles in which a hormonally active follicle, diagnosed by an estradiol

level greater than 80 pg/mL at the start of the cycle, were excluded from the study.

The data collected from the patients included ultrasonographic examination and embryo development records, age and body mass index, IVF indication, basal and trigger day serum P, estradiol, LH, and FSH levels. To clarify, ultrasonographic examination and embryo development records refer to the measurements and evaluations of follicular development, endometrial thickness, and the number and quality of retrieved oocytes and developed embryos, as well as the timing of embryo transfer and any relevant notes on the embryonic development process.

COS was started on the second or third day of the menstrual cycle using recombinant (r) FSH (Gonal-F; Serono, Rome, Italy or Puregon; Organon, The Netherlands) and/or highly purified human menopausal gonadotropin (HP-hMG) (Menopur, Ferring, Sweden or Merional, IBSA, Switzerland) or HP-FSH (Fostimon, IBSA, Italy). The initial doses varied between 75 and 375 IU based on the estimated ovary response. From the fifth to seventh day of stimulation, the doses were adjusted on the basis of the ovarian response, the number and size of the follicles, and serum E2 levels. Doses were administered in the morning or evening as subcutaneous injections.

Transvaginal ultrasonographic monitoring and ovarian stimulation were maintained until the day of hCG trigger, and when the largest follicle reached a size of approximately 12 mm or on the sixth day, treatment with GnRH antagonists (Orgalutran, Merck Sharp and Dohme Ltd., Greece or Cetrotide, Merck Sharp and Dohme Ltd, Greece) was initiated. Before stimulation and on follicle follow-up visits and hCG days, serum E2, P, LH, FSH, and hCG levels were measured using Beckman Coulter hormone kits (Beckman Coulter Inc., Brea, CA, USA) and UniCel DxI 800 (Access Immunoasssay System, Brea, CA, USA) immune analyzers.

When the size of the largest follicle exceeded 17 mm and the number of follicles exceeded two, urinary hCG (10,000 IU) (Pregnyl amp, Organon, Türkiye) or 250 mgr recombinant hCG (r-hCG) (Ovitrelle, Serono, Italy) was used to trigger ovulation. Approximately 36 h after administration of hCG, follicles with a size greater than 12 mm were aspirated. For luteal support, crinone gel (8%) (P 8%, Serono, Italy) was administered on the oocyte pick-up day. On the second, third, or fifth day of oocyte pick-up, one or two high-quality embryos were transferred to the uterine cavity. In Turkish legislation, only one embryo can be transferred in the first two cycles of a woman younger than 35 years. In the third and later trials, a maximum of two embryos are allowed. For women older than 35 years, a maximum of two embryos can be transferred. This regulation is intended to prevent multiple pregnancies and limits the pregnancy rates in IVF cycles compared with those in other countries.[20]

If treatment resulted in pregnancy, vaginal P was recommended until the 8th-12th gestational week. A chemical pregnancy was defined as serum-hCG positivity following embryo transfer. Clinical pregnancy was confirmed by the observation of an intrauterine gestational sac with a fetal heartbeat six weeks after transfer. An ongoing pregnancy was defined as the presence of at least one live fetus at the end of the 11th week.

The primary outcome measure was LBR. Live birth predictor ratios were calculated for each cycle. The notation P, identified the P level on the day of trigger with hCG. The $P/O_{Total}$ ratio was calculated as trigger day P (ng/mL) divided by the total oocyte count collected during oocyte pick-up. Similarly, the $P/O_{MII}$ ratio was calculated as trigger day P level divided by the total MII oocyte count collected during oocyte pick-up.

## Statistical Analysis

Data were analyzed using the Statistical Package for the Social Sciences (SPSS), version 23.0 (SPSS Inc., Chicago, IL). For statistical significance, a two-tailed p value of 0.05 was used. Shapiro-Wilk and Levene's tests were used to test the normality of distributions and the homogeneity of the variances between the groups. The Mann-Whitney U test was used for non-normal variables and variables that had different distributions for live birth and non-live birth groups. The two-sample Z-test was used to test the difference between the proportions when comparing the etiology of infertility and embryo transfer day between cycles with and without live birth. The Pearson chi-square test was used to compare the LBR for the cycles with P level ≤0.5, 0.5-1.5, and >1.5 ng/mL. Pairwise comparisons were adjusted using Bonferroni correction. LBR was calculated for each P, $P/O_{Total}$, and $P/O_{MII}$ interval. The LBR trend for sequentially greater concentrations of P, $P/O_{Total}$, and $P/O_{MII}$ ratios was calculated with trend analysis using the Extended Mantel-Haenszel chi-square for the linear trend test. Receiver operating characteristic (ROC) curve analysis was used to calculate area under the curve (AUC) values for P, $P/O_{Total}$, and $P/O_{MII}$. Adjusted AUC values were calculated for these markers by post-estimation using the bivariate logistic regression model with confounders.

## RESULTS

Figure 1 presents the study flowchart. Of 1,602 patients with their first IVF cycles, 1,258 with routinely measured trigger day P levels were included in the study. Of the 344 excluded cycles, most had been performed before 2013, when blood tests for P on the trigger day were not

routinely performed. Table 1 summarizes the demographic and stimulation characteristics of the patients.

For the evaluation of P concentrations and $P/O_{Total}$ and $P/O_{MII}$ ratios using adjusted ROC analysis (stated below), cycle characteristics were compared between live birth positive and live birth negative groups. The parameters that had statistically different effects on LBRs were age (p=0.014), infertility time (p=0.012), total FSH dosage (p=0.001), total LH dosage (p=0.029), follicle counts larger than 12 mm (p=0.029) and 16 mm (p=0.045), trigger day endometrial thickness (p=0.023), trigger day estradiol level (p=0.033), total oocyte count (p=0.002), MII oocyte count (p=0.001) and ratio of patients with low ovarian reserve (p=0.002) and male factor (p=0.030) as an etiology of infertility.

Correlation analysis was performed to evaluate the effect of the total FSH dosage on the trigger day P levels and $P/O_{Total}$ and $P/O_{MII}$ ratios. There was a significant positive correlation between $P/O_{Total}$ and total FSH (rs=0.247, p<0.001) and between $P/O_{MII}$ ratio and total FSH (rs=0.215, p<0.001). However, the correlation between P and total FSH (rs=0.015, p=0.605) was not significant.

In our study, sample cycles with ≤0.5 ng/mL P level had LBRs of 16.3% (50/307 cycles) with 95% confidence level of (12.5-20.7). For the 0.5-1.5 ng/mL group, the LBR was 23.0% (180/783) (20.1-26.0%) and for >1.5 ng/mL group it was 19.6% (33/168) (14.2-26.1%). LBR was significantly lower in ≤0.5 ng/mL P level group [LBR: 16.3% (50/307)] than the 0.5-1.5 ng/mL group [LBR: 23.0% (180/783), p=0.044]. The

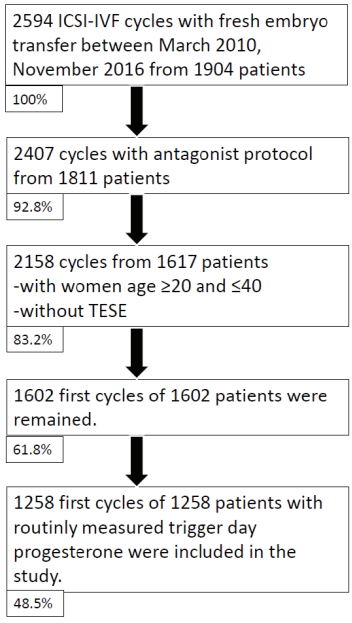



**Figure 1.** Flow chart of the IVF-ICSI cycles investigated in the study

*IVF-ICSI: In vitro fertilization-intracytoplasmic sperm injection*

LBR for the cycles with P level >1.5 was 19.6% (33/168) and not significantly different from the other groups.

The LBRs for the sequential intervals of trigger day P level, $P/O_{Total}$, and $P/O_{MII}$ ratios are presented in Figures 2, 3, and 4, both with crude values and adjusted means by the potential confounders that statistically significantly differ between live birth present and live birth absent groups. In the first ROC analysis of the 1,258 cycles, the AUC values of P concentrations (0.531, p=0.123), $P/O_{Total}$ (0.484, p=0.412) and P/F (0.478, p=0.264) were not significant for predicting live birth. After the exclusion of cycles with P level ≤0.5 ng/mL, an increase in serum P was not associated with a decrease in LBRs (p=0.667). However, for $P/O_{Total}$ and $P/O_{MII}$ values, a decreasing trend was statistically significant (extended Mantel-Haenszel chi square for linear trend=4.74 and 6.62, p values=0.030 and 0.010, respectively).

To investigate the detrimental upper limit of $P/O_{Total}$ and $P/O_{MII}$, cycles with P level ≤0.5 ng/mL were once

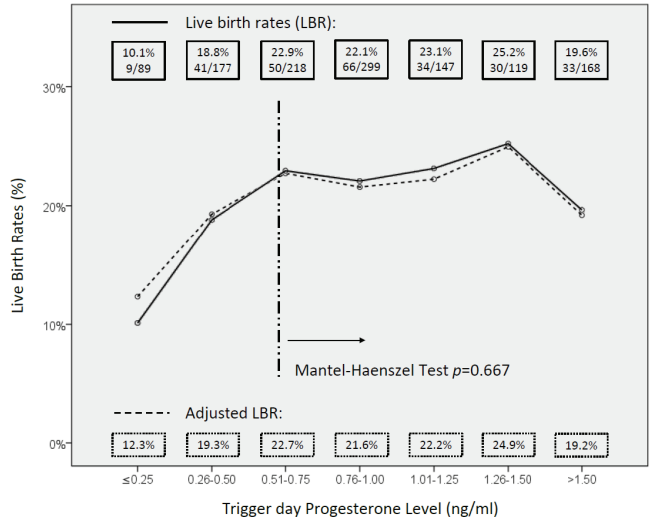



**Figure 2.** Live birth rates of cycles for the sequential intervals of trigger day progesterone level. Solid line presents the mean plot of LBR for the sequential intervals of trigger day P level. The live birth count and rates are shown in solid boxes. Dashed line presents LBR for the sequential intervals of trigger day P level after the adjustments for age, duration of infertility, total FSH and LH dosage, trigger day estradiol, number of embryos transferred, embryo transfer day, trigger day endometrial thickness, aetiology of infertility and total oocyte count. Adjusted mean LBR are shown in dashed boxes with percentages. Extended Mantel-Haenszel chi-square test for linear trend was conducted for the cycles with P level >0.5 (presented in the figure to the right of the vertical dashed line)

*LBR: Live birth rates, P: Progesterone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone*

again excluded from the sample. The AUC calculations of the crude and adjusted ROC models by adding cycle characteristics to the model are presented in Table 2.

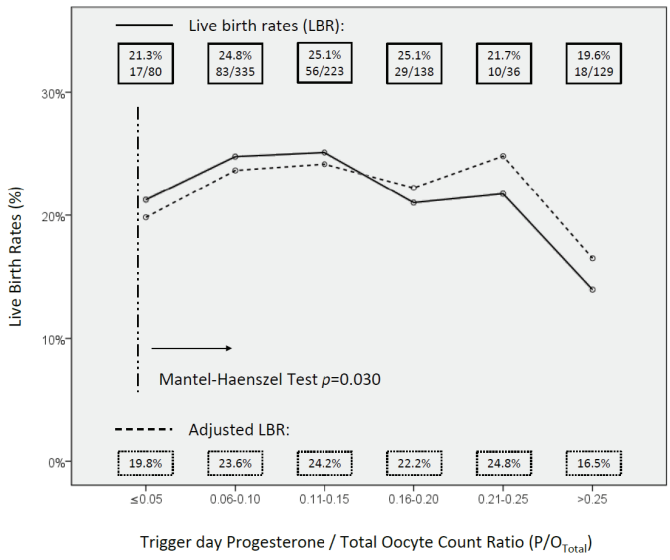
## DISCUSSION

This study investigated the predictive power of the $P/O_{Total}$ and $P/O_{MII}$ ratios for LBR in GnRH antagonist ICSI cycles. The evidence suggests that after the exclusion of cycles with low hCG day serum P levels, $P/O_{Total}$ and $P/O_{MII}$ ratios change into good predictors of LBRs, improving prediction based solely on serum P levels. At the same time, they do not provide a better prediction than a multivariate model including P and O or MII separately, suggesting that the detrimental effect of P on LBR is not oocyte count dependent.

**Table 1. Basal demographic, clinical and laboratory characteristics and pregnancy outcomes of patients**

| Parameter | Value | |
|---|---|---|
| Age, (years) | 31.4±4.5 | 31.0 (28.0; 35.0) |
| Body mass index, (kg/m$^2$) | 26.0±5.0 | 25.3 (22.5; 29.0) |
| Infertility period, (years) | 6.7±4.0 | 6.0 (4.0; 8.5) |
| In vitro fertilization indication, n (%) | | |
| Unexplained | 345 | 27.4% |
| Low ovarian reserve | 204 | 16.2% |
| Endometrioma | 7 | 0.6% |
| Hypogonadotropic hypogonadism | 8 | 0.6% |
| Polycystic ovary syndrome | 249 | 19.8% |
| Tubal factor | 64 | 5.1% |
| Male factor | 225 | 17.9% |
| Multiple causes | 155 | 12.3% |
| Basal FSH, (IU/L) | 8.6±4.1 | 7.9 (6.1; 10.5) |
| Basal E2, (pg/mL) | 39.7±29.4 | 34.0 (21.0; 52.0) |
| Stimulation period (days) | 8.7±2.0 | 8.0 (7.0; 10.0) |
| Total FSH (IU) | 2462±1259 | 2100 (1575; 3000) |
| Total LH (IU) | 782±964 | 525 (0; 1200) |
| Trigger-day follicle ≥12 mm, n | 9.5±4.9 | 9.0 (6.0; 12.0) |
| Trigger-day follicle ≥16 mm, n | 4.1±2.6 | 4.0 (2.0; 5.0) |
| Trigger-day follicle ≥18 mm, n | 1.2±1.4 | 1.0 (0.0; 2.0) |
| Trigger-day endometrial thickness, (mm) | 10.2±2.2 | 10.0 (8.8; 11.6) |
| Trigger-day estradiol level, (pg/mL) | 2275±1513 | 1927 (1189; 2931) |
| Trigger-day progesteron level, (ng/mL) | 0.95±0.67 | 0.85 (0.52; 1.20) |
| Total oocyte count, (n) | 9.6±6.2 | 8.0 (5.0; 13.0) |
| Progesterone/Oocyte count ratio | 0.14±0.16 | 0.10 (0.06; 0.16) |
| MII oocyte count, (n) | 7.9±5.1 | 7.0 (4.0; 10.0) |
| Progesterone/MII count ratio | 0.18±0.20 | 0.12 (0.08; 0.20) |
| Fertilization rate, n (%) | 0.64±0.25 | 0.67 (0.50; 0.83) |
| No. of embryos transferred, (n) | 1.3±0.5 | 1.0 (1.0; 2.0) |
| Embryo transfer day, n (%) | | |
| 2$^{nd}$ day | 733 | 58.3% |
| 3$^{rd}$ day | 327 | 26.0% |
| 5$^{th}$ day | 198 | 15.7% |
| Pregnancy rate, n (%) | 453 | 36.0% |
| Clinical pregnancy rate, n (%) | 331 | 26.3% |
| Ongoing pregnancy, n (%) | 272 | 21.6% |
| Live birth, n (%) | 263 | 20.9% |

Data are presented as mean±SD, median (interquartile range) or number (percentage).
FSH: Follicle-stimulating hormone, SD: Standard deviation, LH: Luteinizing hormone



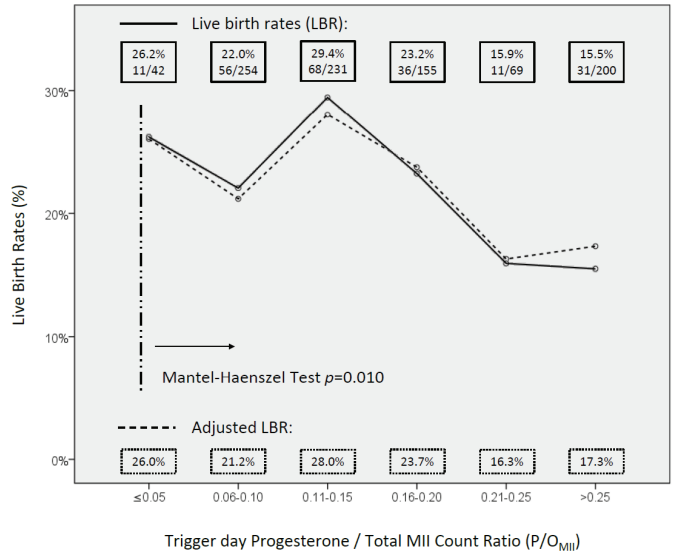




**Figure 3.** Live birth rates of cycles for the sequential intervals of trigger day progesterone/total oocyte count ratio ($P/O_{Total}$). Solid line shows the mean plot of LBR for the sequential intervals of trigger day $P/O_{Total}$ ratio. The live birth count and rates are given in solid boxes. Dashed line shows LBR for the sequential intervals of $P/O_{Total}$ ratio after the adjustments for age, duration of infertility, total FSH and LH dosage, trigger day estradiol, number of embryos transferred, embryo transfer day, trigger day endometrial thickness, and aetiology of infertility. Adjusted mean LBR are shown in dashed boxes with percentages. Extended Mantel-Haenszel chi-square test for linear trend was conducted for the cycles with P level >0.5 (presented in the figure to the right of the vertical dashed line)

*LBR: Live birth rates, P: Progesterone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone*

**Figure 4.** Live birth rates of cycles for the sequential intervals of trigger day progesterone/MII oocyte count ratio ($P/O_{MII}$). Solid line shows the mean plot of LBR for the sequential intervals of trigger day $P/O_{MII}$ ratio. The live birth count and rates are given in solid boxes. Dashed line shows LBR for the sequential intervals of $P/O_{Total}$ ratio after the adjustments for age, duration of infertility, total FSH and LH dosage, trigger day estradiol, number of embryos transferred, embryo transfer day, trigger day endometrial thickness, and aetiology of infertility. Adjusted mean LBR ware shown in dashed boxes with percentages. Extended Mantel-Haenszel chi-square test for linear trend was conducted for the cycles with P level >0.5 (presented in the figure to the right of the vertical dashed line)

*LBR: Live birth rates, P: Progesterone, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone*

**Table 2. Receiver operating characteristic curves for the trigger day P, $P/O_{Total}$ and $P/O_{MII}$ ratios**

| | AUC (95% CI) | p value | Adjusted AUC (95% CI)[a] | p value |
|---|---|---|---|---|
| P | 0.509 (0.465-0.553) | 0.686 | 0.605 (0.563-0.648) | <0.001* |
| $P/O_{Total}$ | 0.544 (0.502-0.586) | 0.049* | 0.608 (0.566-0.650) | <0.001* |
| $P/O_{MII}$ | 0.552 (0.510-0.593) | 0.022* | 0.610 (0.568-0.651) | <0.001* |
| P and 1/O | 0.550 (0.509-0.592) | 0.026* | 0.610 (0.568-0.652) | <0.001* |
| P and 1/MII | 0.556 (0.514-0.597) | 0.013* | 0.611 (0.570-0.652) | <0.001* |

The ROC analysis of the markers only included the cycles with P level >0.5 ng/mL.

[a]AUC values for the models after the adjustment for age, duration of infertility, total FSH and LH dosage, trigger day estradiol, number of embryos transferred, embryo transfer day, trigger day endometrial thickness, and aetiology of infertility.

*Statistically significant values.

P: Trigger day progesterone level, $P/O_{Total}$: Trigger day progesterone/total oocyte count ratio, $P/O_{MII}$: Trigger day progesterone/MII oocyte count ratio, FSH: Follicle-stimulating hormone, LH: Luteinizing hormone, CI: Confidence interval, ROC: Receiver operating characteristic, AUC: Area under the curve

The literature has proposed several threshold levels for elevated late follicular phase P in stimulated ART cycles ranging from 0.8 to 2.0 ng/mL.[3] The most common cut-off level is 1.5 ng/mL.[6] Griesinger et al.[7] reported that the incidence of late follicular P rise increases with ovarian response, and elevated P at a threshold of 1.5 ng/mL is independently associated with a decreased chance of pregnancy in poor and normal responders but not in high responders.

Another meta-analysis of over 60,000 IVF cycles using a GnRH agonist showed that elevated P levels on the day of hCG trigger had an adverse effect on pregnancy rates at levels of 1.75 ng/mL for the overall population, and the correlation became stronger with increases in P levels.[8] This adverse effect was detected only when the level of serum P reached 1.5 and 2.25 ng/mL for poor and high responders, respectively. Moreover, a recent prospective multicenter cohort study found that both low and high serum P levels in the early luteal phase decrease the probability of pregnancy in ART cycles with fresh embryo transfer.[11] Similarly, Santos-Ribeiro et al.[19] (2014) found that trigger day serum P levels lower than 0.5 ng/mL and higher than 1.5 ng/mL are detrimental to LBRs in a sample of 2,723 antagonist fresh embryo transfer cycles. Overall, there is no consensus on the trigger day serum P threshold level for predicting pregnancy outcomes.

There is a growing literature that investigates indicators that can predict pregnancy outcomes of patients undergoing IVF/ICSI treatment cycles other than late follicular phase elevated P levels. Among others, the association of hormone levels and follicle and oocyte numbers with pregnancy outcomes has been investigated. Our study builds on the insight that continuous predictor markers such as P/F (P to follicle count), P/E (P to hCG trigger day estradiol ratio), or P/O might be alternatives to calculating different P threshold levels for different discrete responder categories. A continuous ratio such as P/O might avoid information loss due to the disadvantages of a discrete analysis that divides the cycles into limited groups according to the collected oocyte counts. This insight is motivated by earlier studies that suggest that the hCG trigger day P threshold for adverse pregnancy rates is higher in cycles of patients with better ovarian response.[7,8] Dividing P levels by different measures of response rates such as oocyte count, follicle count, or hCG day estradiol gives the P level per oocyte, follicle, or unit estradiol level. These ratios might be better and more feasible indicators for pregnancy rates than P itself or less feasible models including P and response rate indicators (oocyte or follicle count) separately.

To the best of our knowledge, there are five studies reporting conflicting results regarding the $P/O_{Total}$ and $P/O_{MII}$ ratios, as shown in Table 3.[16-18,21,22] We identified these studies by searching for terms such as "progesterone/oocyte ratio", "progesterone per oocyte ratio", and "P/O ratio assisted reproductive technology" in PubMed. In one of the studies, Burns et al.[21] (1994) assessed 112 GnRH agonist-pretreated IVF cycles, and multiple logistic regression analysis showed a significant inverse relation of serum P level on the day after hCG trigger and $P/O_{Total}$ ratio with clinical and ongoing/delivered pregnancy rates. The second study of 378 cycles using both GnRH agonist and antagonist reported that the $P/O_{MII}$ ratio with a cut-off value of >0.32 is a better predictor of the clinical pregnancy rate (CPR) than the absolute P value and P/E ratio.[18]

**Table 3. Summary of the current literature investigating prediction of pregnancy outcome in IVF cycles using $P/O_{Total}$ ratio**

| Author (reference no) | Year | Number of cycle | Pituitary suppression by GnRH | Outcome measure | $P/O_{Total}$ ratio (ng/mL/oocyte) | AUC (p value) | Serum P predicts pregnancy outcome | $P/O_{Total}$ ratio predicts pregnancy outcome |
|---|---|---|---|---|---|---|---|---|
| **Burns et al.[21]** | 1994 | 114 | Agonist | OPR/LBR | 0.04±0.01 | NA | Yes | Yes |
| **Aflatoonian et al.[18]** | 2014 | 378 | Agonist and antagonist | CPR | NA | NA | No | Yes |
| **Singh et al.[22]** | 2016 | 687 | Agonist | CPR | 0.24±0.27 | 0.58 (p<0.001) | Yes | Yes |
| **Hill et al.[16]** | 2017 | 7608 | Agonist and antagonist | LBR | NA | 0.597 (p>0.05) | Yes | No |
| **Grin et al.[17]** | 2018 | 2311 | Antagonist | LBR | 0.16±0.23 | 0.680 (p<0.001) | No | Yes |
| **Present study*** | - | 951 | Antagonist | LBR | 0.16±0.17 | 0.544 (p=0.049) | No | Yes |

*After exlusion of cycles with P level ≤0.5 ng/mL.

IVF: In vitro fertilization, P: Progesterone, $P/O_{Total}$: Trigger day progesterone/total oocyte count ratio, AUC: Area under the curve, GnRH: Gonadotropin releasing hormone, NA: Not available, LBR: Live birth rate, OPR: Ongoing pregnancy rate, CPR: Clinical pregnancy rate

The third retrospective, single center, cohort study in 687 fresh IVF/ICSI treatment cycles with a long agonist protocol by Singh et al.[22] concluded that increases in P and $P/O_{Total}$ ratios are inversely associated with the probability of pregnancy, and $P/O_{Total}$ ratio with a cut-off value of >0.15 may be considered as a better tool to predict pregnancy outcomes than serum P levels alone. In a recent retrospective study, Grin et al.[17] investigated 2,311 fresh GnRH antagonist IVF/ICSI cycles by multivariate logistic regression analysis and found that $P/O_{Total}$ is inversely associated with LBR as in the previous three studies.[18,21,22]

Lastly, Hill et al.[16] conducted a large retrospective cohort study of 7,608 fresh GnRH agonist/antagonist IVF/ICSI cycles. Multivariate generalized estimating equation models and ROC curves were used to analyze the ability of trigger day serum P level, oocyte number, and the $P/O_{Total}$ ratio to predict LBR. The $P/O_{Total}$ ratio is not significantly associated with LBR after adjustment for covariates, whereas P has a significant negative association with LBR.

The current study is the sixth to evaluate the $P/O_{Total}$ ratio as a predictor of LBR and investigates 1,258 first cycles of patients who underwent an antagonist protocol with fresh embryo transfer. According to the first results of the ROC analysis, trigger day P, trigger day $P/O_{Total}$, and $P/O_{MII}$ ratio were not found to be significant predictors of live birth outcomes. The analysis by binning serum P concentrations and comparison of intervals showed that cycles with P lower than 0.5 ng/mL had significantly lower LBRs than the 0.5-1.5 ng/mL interval group. This lower threshold was consistent with that of Santos-Ribeiro et al.[19] (2014), who used thresholds of 0.5 ng/mL and 1.5 ng/mL for low and high P levels and found that both diminish LBRs.

A related question is whether excluding cycles with low P levels changes the results. These ratios assume that increasing P levels and decreasing oocyte counts hinder LBR. However, similar to the study of Santos-Ribeiro et al.[19], the cycles with P level ≤0.5 ng/mL in our study had low pregnancy rates. Adjusting the LBRs for other cycle characteristics does not change the result. Adjusted LBRs of P intervals 0-0.25 and 0.25-0.5 ng/mL still have relatively lower LBRs compared with the other intervals, as shown in Figure 2. When these cycles are excluded or canceled the alternative ratios might be good predictors of live birth because they would meet the assumption stated. Consistent with this idea, after exclusion of cycles with P level ≤0.5 ng/mL, LBRs have a negative relationship with $P/O_{Total}$ and $P/O_{MII}$. However, the relationship was not significant with P levels. AUC values for the combination of P and 1/O or 1/MII were statistically significant again for predicting live birth, but the model based solely on P was not. These results suggest that the ratios are better indicators than the P level when they are used without taking covariates into account.

ROC analysis was conducted to determine the probability of multivariate logistic regression models including cycle characteristics affecting LBRs. AUC values of all models were statistically significant and close to each other. These results indicate that $P/O_{Total}$ and $P/O_{MII}$ ratios do not offer a superior prediction than a model including P combined with 1/O or 1/MII parameters. However, as these models show similar prediction success rates (similar adjusted AUC values), the results indicate that O and MII parameters combined with P levels in the form of ratio do not lose their predictive power and can be a feasible marker for clinicians instead of alternative combined models that are harder to interpret.

### Study Limitations

There are several limitations in this study. The embryo grading scores and related adjustments were not feasible because they were not recorded. The retrospective design of the study was a limitation due to the risk of residual bias. At the same time, the main purpose of the study was to analyze associations, and retrospective cohort studies are appropriate for this purpose. The strengths of the study include the large sample size, the multivariate logistic regression model that controls for possible confounders, the prediction of LBRs instead of other pregnancy outcomes, and the inclusion of only the first cycle of patients. Binning of the levels of alternative ratios and analyzing data according to P level over 0.5 ng/mL was another powerful and important strength of the present study compared with the five previous studies on the same subject. Moreover, this study also included a detailed review of the literature on studies evaluating the $P/O_{Total}$ ratio to predict ART outcomes.

## CONCLUSION

In conclusion, the results of this study demonstrated that the use of hCG day $P/O_{Total}$ ratio and $P/O_{MII}$ ratio is clinically feasible to predict LBR in patients undergoing GnRH antagonist ICSI cycles with fresh autologous embryo transfer with hCG day serum P level >0.5 ng/mL. Further studies with a larger sample size and different protocols are needed to confirm the findings and better gage the feasibility of predicting IVF outcomes.

### Ethics

**Ethics Committee Approval:** This retrospective cohort study was approved by the Local Ethics Committee at İzmir Kâtip Çelebi University (decision no: 329, date: 28.12.2016).

**Informed Consent:** Retrospective cohort study.

**Peer-review:** Internally peer-reviewed.

## Authorship Contributions

Surgical and Medical Practices: B.Y., Concept: O.İ., B.Y., Design: O.İ., B.Y., Data Collection or Processing: O.İ., B.Y., Analysis or Interpretation: O.İ., B.Y., Literature Search: O.İ., B.Y., Writing: O.İ., B.Y.

**Conflict of Interest:** No conflict of interest was declared by the authors.

**Financial Disclosure:** The authors declared that this study received no financial support.

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