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The effect of hCG day progesterone in 1318 cycles on pregnancy outcomes: ongoing discussion

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ABSTRACT

Objectives: To investigate the effect of human chorionic gonadotropin day progesterone (hCG-P) level on pregnancy outcomes in *in vitro* fertilization (IVF) cycles.

Material and methods: This study is an analysis of a cohort of 1318 fresh IVF- embryo transfer cycles, including 579 agonists and 739 antagonists, performed at a single IVF center between 2007 and 2018. For fresh cycles, we performed Receiver Operating Characteristic analysis (ROC) to calculate the threshold value of hCG-P, which affects pregnancy outcomes. We divided patients below and above the determined threshold value into two groups, then, correlation analysis and we performed logistic regression analysis.

Results: According to ROC curve analysis of hCG-P, AUC was 0.537 (95% CI: 0.510–0.564, $p < 0.05$) for LBR, and the threshold value for P was 0.78. The hCG-P threshold value of 0.78 proved to be significant in relation to BMI, type of drug used during induction, the hCG day E2, the total number of oocytes, the number of oocytes and the subsequent pregnancy outcome between the two groups ($p < 0.05$). However, the model we built, which accounted for hCG-P, total number of oocytes, age, BMI, induction protocol, total dose of gonadotropin used in induction did not prove significant in terms of its effect on LBR.

Conclusions: The threshold value of hCG-P that we found to have an effect on LBR was quite low compared with the p value generally recommended in the literature. Therefore, further studies are needed to determine an accurate p-value that reduces success in managing fresh cycles.

Key words: intracytoplasmic sperm injection; *in-vitro* fertilization; progesterone; hCG day; IVF/ICSI outcome; pregnancy rate; progesterone levels; live birth rate

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INTRODUCTION

During cycles of *in vitro* fertilization/intracytoplasmic sperm injection and embryo transfer (IVF/ ICSI-ET), there are significant changes in the hormonal profile of the menstrual cycle. The premature increase in luteinizing hormone (LH) and the development of premature luteinization due to changes in estradiol (E2) levels induced by exogenous gonadotropins have been greatly reduced with the introduction of gonadotropin-releasing hormone (GnRH) analog and antagonist cycles. Despite low LH levels, an early increase in progesterone (P) may occur in 2 to 35% of cycles, regardless of the stimulation protocols used in IVF cycles [1]. However, the definition of this condition as early luteinization is controversial. This is because the definition of true luteinization involves the conversion of follicles to the corpus luteum and the exit of granulosa cells from the cell cycle [2]. However, in IVF cycles, an increase in human chorionic gonadotropin (hCG) day P (hCG-P) appears to occur as a result of excessive ovarian stimulation [3], and the impact of this situation on pregnancy outcomes is still under debate. Studies have arbitrarily chosen the threshold value for hCG-P to affect pregnancy outcomes, and many different threshold values ranging from 0.8 to 2.0 ng/mL have been proposed for high P [4, 5]. Bosch et al. [2] and Venetis et al. [6] showed a significant decrease in sustained pregnancy rates at serum levels of hCG-P above 1.5 ng/mL. However, there is still no consensus in the literature on the threshold level of hCG-P whose negative effects on cycle outcome have been clearly demonstrated.

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Another point of contention among these studies is the differences in the measurements used to assess the specific circulating P concentration. It is used in routine clinical practice for P measurement, ovulation detection, and differential diagnosis of ectopic pregnancy and is optimized for high values. However, very small P intervals are considered when evaluating the effect of IVF treatments [7, 8]. In addition, one of the most debated issues in studies is that the number of oocytes may alter the effect of hCG-P elevation on pregnancy rates, which is a source of confusion among clinicians. Bosch et al. [2] argued that hCG-P elevation decreases pregnancy rates regardless of the magnitude of the ovarian response. In contrast, Xu et al. demonstrated that higher hCG-P levels have a deleterious effect in high-responders [9]. Since the number of hormonally active follicles may be related to the P level, the P-follicle index was proposed as a new measurement parameter [10]. However, because the number of follicles seen on ultrasonography (USG) may vary from observer to observer, other investigators have suggested the P-aspirated oocyte index, which uses the number of oocytes retrieved as a more objective parameter [11]. The fact that the likelihood of pregnancy after IVF was no lower in women who received oocytes from donors with high hCG-P levels than in women who received oocytes from donors without high hCG-P levels [6] suggested that elevated hCG-P affects the endometrial milieu and thus endometrial receptivity rather than oocyte quality. This is also supported by studies showing changes in endometrial gene expression following hCG-P elevation [12].

We conducted a noninvasive, retrospective observational study of patients in our clinical practice to demonstrate the effects of hCG-P levels on pregnancy rates. The primary aim of this study was to determine the relationship between hCG-P level and live birth rate (LBR), defined as the birth of a live child at 24 weeks of gestation. The secondary aim was to investigate the relationship between hCG-P level and clinical pregnancy rate (CPR) with an amniotic sac and implantation rate (IR) with a positive beta-hCG test. In this study, we also aimed to determine the threshold above which an elevated hCG-P level detrimental effect LBR and possible factors associated with an elevated hCG-P level.

MATERIAL AND METHODS

This retrospective cohort study was conducted between September 2007 and June 2018 at the IVF clinic of Etlik Zübeyde Hanım Women's Health Training and Research Hospital. Only cycles with long GnRH agonists and antagonists were included in the study. Exclusion criteria for the study were: Patients with known endocrine disorders (e.g., thyroid dysfunction, hyperprolactinemia, pituitary adenoma), advanced endometriosis, decreased ovarian reserve [advanced maternal age (≥ 40 years) or other risk factors for decreased ovarian reserve, patients with at least two of the following conditions: Poor response to a previous conventional stimulation protocol (≤ three oocytes), an abnormal ovarian reserve test (antral follicle count: 5–7 follicles or AMH: 0.5–1.1 ng/mL)], and freeze-thaw cycles and cycles without embryo transfer. A total of 1318 IVF/ICSI-ET cycles aged 20–40 years with male factor, unexplained infertility, and infertility due to tubal factor that met these criteria were included in the study. Stimulation was initiated by individually adjusting the initial gonadotropin dose depending on the patient's age, basal follicle-stimulating hormone (FSH) level, body mass index (BMI), presence of polycystic ovaries, and ovarian response in the previous IVF cycle. In the presence of polycystic ovaries and/or in patients diagnosed with polycystic ovary syndrome and in patients who had ovarian hyperstimulation syndrome in the previous cycle, the antagonist protocol was used.

The research project and protocols were approved by the study's Institutional Review Board (12/21/2018/ issue 90057706-799). All data used in the study were collected from patients who underwent routine and standard IVF treatment at an approved center without additional interventions. All human study methods were performed in accordance with relevant guidelines and regulations. Because this was a retrospective study, formal informed consent was not required.

The long GnRH agonist protocol was started with leuprolide acetate (Lucrin, Abbot, Turkey) in the middle of the luteal phase of the previous cycle. After the onset of menstrual bleeding, when satisfactory pituitary desensitization was achieved (serum E2 level 50 pg/mL, endometrial thickness 5.5 mm, serum LH 5 IU/mL), daily stimulation with recombinant (rc) FSH (Gonal F; Merck Serono, Istanbul, Turkey or Puregon, Organon, Istanbul, Turkey) was started. In the GnRH antagonist protocol, gonadotropins were administered from Day 2 of the cycle, and a GnRH antagonist, 0.25 mg ganirelix (MSD Organon, Netherlands) or 0.25 mg cetrorelix (Merck-Serono, Geneva, Switzerland), was initiated when the follicular diameter reached 12 mm or the E2 level was 250 pg/mL (flexible protocol) [13]. Cycles were accompanied by serial transvaginal USG examinations and serum determinations of E2, P, and LH. The change in P level in the late follicular phase was calculated from the P levels measured on hCG day and before hCG day. A nonsignificant change in P level in the late follicular phase was considered a stable change. When three follicles were 17 mm, 10,000 IU hCG (Pregnyl, Schering-Plow, Turkey) or 250 µg rc hCG (Ovitrelle®, choriogonadotropin alfa, Serono) or 5000 IU urinary hCG were administered if there was a risk of ovarian hyperstimulation syndrome. Oocytes were retrieved 34–36 hours after hCG administration under intravenous sedation and using a transvaginal USG (General Electric Logiq A5, USA).

Sperm processing was performed by the gradient method. 1–3 of the good quality embryos obtained by the standard ICSI method, preferably grade 1, were transferred to the uterine cavity 3–5 days after oocyte retrieval under transabdominal USG guidance.

Luteal support was given either as vaginal P (Crinone 8% gel, Serono, Istanbul) twice daily or as vaginal P and 100 mg intramuscular P (Progestan, Koçak, Istanbul) from the day of ET until pregnancy testing and, in the case of pregnancy, until the 10th to 12th gestational week. In blood tests performed 12 days after ET, pregnancy was determined by beta-hCG level.

The primary endpoint of this study was LBR, defined as the birth of a live baby at 24 weeks' gestation, and the secondary endpoint was clinical pregnancy rate (CPR) with an amniotic sac and implantation rate (IR) with a positive beta-hCG test. Serum levels of P, LH, and E2 were measured on days 2 to 3 of the cycle, during stimulation, and on the morning of the hCG trigger day.

Statistical analysis

The area under the receiver operating characteristic (ROC) curve was calculated to assess the threshold value of hCG-P, which affects pregnancy outcomes. $P < 0.05$ was considered statistically significant. The MedCalc (Medcalc Software, Ghent, Belgium) program was used for this analysis. The distribution of continuous variables was presented as mean and standard deviation (SD), whereas categorical variables were presented as ratios and percentages of the total. Comparison of continuous variables between groups was performed using Student's t-test or Mann-Whitney U test, depending on the normality of the distribution, and comparison of categorical variables was performed using Pearson's chi-square test or Fisher's exact test. In the model built using logistic regression analysis to evaluate the factors that might have a regulating effect on the relationship between hCG-P increase and LBR, the total number of oocytes retrieved, the total dose of gonadotropin used in stimulation, hCG day E2, BMI, GnRH protocol used, and hCG day endometrial thickness were included in the analysis and evaluated.

Statistical analysis was performed using the Statistical Program for the Social Sciences version 20.0 (SPSS, Chicago, IL, USA). The significance level was $p \leq 0.05$ for all statistical tests.

RESULTS

A total of 26 of the 1344 available IVF cycles were excluded because ET could not be performed. The remaining 1318 cycles of agonists (n = 579) and antagonists (n = 739) that underwent ET were retrospectively analyzed for hCG-P levels. We performed a ROC analysis to find the hCG-P threshold value that affected the ratios of LBR, CPR, and IR. Of these values, the AUC value that affected only LBR was statistically significant [AUC: 0.537 (p = 0.036, 95% confidence interval (CI) 0.510–0.564)]. For CPR, AUC: 0.517 and for IR, AUC: 0.520 were not statistically significant (p = 0,300). The significant threshold value for sensitivity (39.84%) and specificity (67.91%) found for LBR was 0.78 ng/mL (Fig. 1–3).

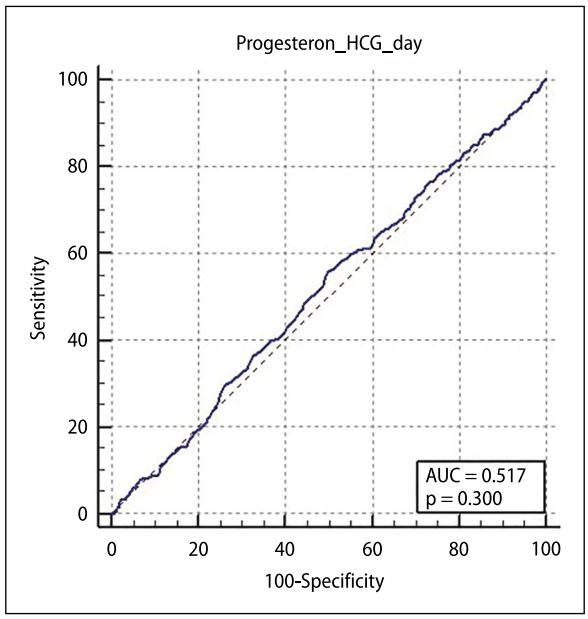

Figure 1. Receiver operating characteristic curve for progesteron on the day of hCG for prediction of clinical pregnancy rate (CPR) (AUC: 0.517, p = 0.300). Receiver operating characteristic curves of the prediction models of CPR and live birth rate (LBR), including hCG-P

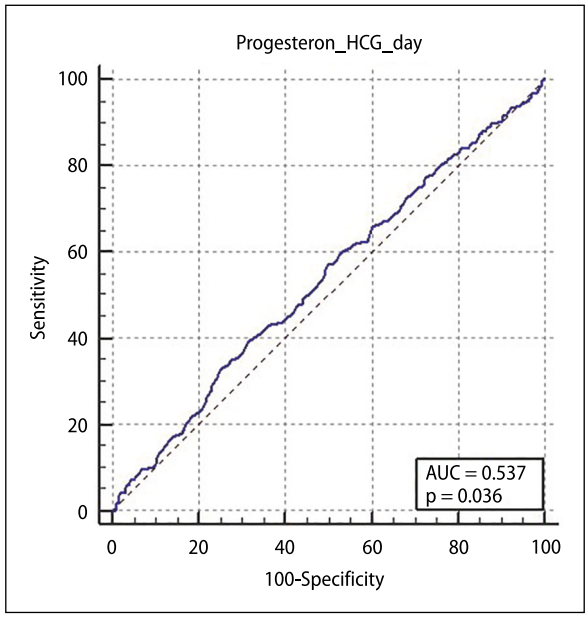

Figure 2. Receiver operating characteristic curve for progesteron on the day of hCG for prediction of live birth rate (LBR) (AUC: 0.537, p = 0.036)

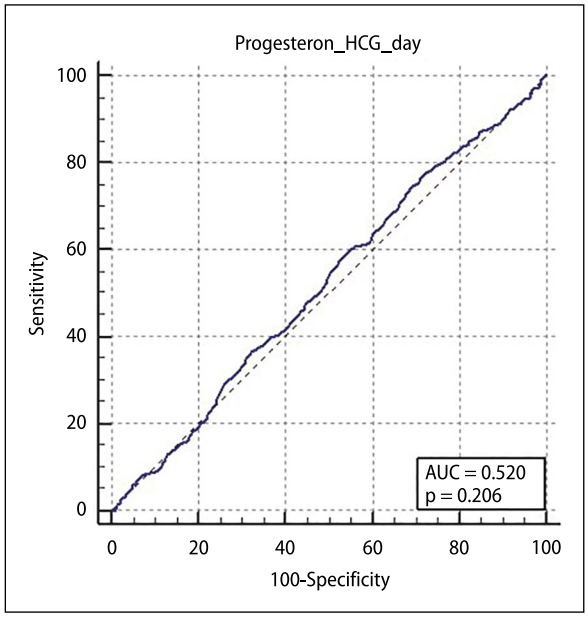

Figure 3. Receiver operating characteristic curve for progesteron on the day of hCG for prediction of implantation rate (IR) (AUC: 0.520, p = 0.206).

We divided the patients who underwent ET into two groups, Group 1 with hCG-P levels of 0.78 ng/mL and below and Group 2 with hCG-P levels above 0.78 ng/mL, and controlled for differences between these two groups.

Of the 380 cycles of ET, the outcome was live birth (28.1% per ET). Clinical pregnancy (36.9% per ET) was achieved in 497 patients and implantation in 578 patients (42.9% per ET). Distribution of transfer days: transfer on Day 2, 3.2%; on Day 3, 52.7%; on day 4, 1.7%; on day 5, 39.5%; and on day 6, 0.7%. Patient demographics were as follows: Age: 29.34 ± 4.51 (mean ± SD), BMI: 26.51 ± 4.95 (cm/m^2), and baseline FSH: 6.89 ± 2.04 (IU/mL), mean total gonadotropin dose: 2014.82 ± 728.66 (IU), and stimulation duration: 9.68 ± ± 1.5 days. On the day of hCG administration, the ovarian response parameters of the patients included the following: hCG day E2: 2882.55 ± 1652.39 pg/mL (mean ± SD), hCG-P: 1.04 ± 0.52 ng/mL, number of retrieved oocytes (> 15 mm): 13.65 ± 6.99, endometrial thickness: 10.11 ± 2.02 mm.

Inhibition of early LH increase was achieved with either GnRH agonists (n = 579) or GnRH antagonists (n = 739). Most patients (n = 779, 57.8%) were stimulated with rc FSH (follitropin-alpha or follitropin-beta), while 520 patients (38.6%) were stimulated with rc FSH+ HMG (human menopausal gonadotropin), 16 patients (1.2%) with HMG only, and 3 patients (0.2%) with rc FSH+ rc LH. The number of cycles with hCG-P > 0.78 ng/mL was 865 (65.6%), and there was no significant difference in P elevation between cycles with GnRH agonists and antagonists (43.1% and 56.9%, respectively) (p = 0.414) (Tab. 1).

Consistent with the analysis of ROC, the difference between the two groups in terms of LBR was significant. While the rate of live births in Group 1 was 33.6%, it was 26.4% in Group 2. The rate of non-live births was 66.4% in Group 1, but 73.6% in group 2 (p = 0.006). The difference between the number of retrieved oocytes was also significant (p < 0.001). While LBR was higher in Group 1, the rate of patients with high response (> 15 oocytes retrieved) was higher in Group 2 (Group 1: 21.9%, Group 2: 36.9%). Between groups 1 and 2, the difference between ongoing pregnancy outcomes was significant (p = 0.030). Specifically, the rate of abortion was higher in Group 2 (20.6%) than in group 1 (12.6%) (Tab. 1). Comparing groups 1 and 2, the total number of antral follicles, E2 level on hCG day, P level on Day 2 and 3, total number of retrieved oocytes, duration of antagonist administration, number of mature oocytes, number of oocytes used for ICSI, number of 2 pronuclei (PN), ET day P, and P level in cycles with increased P were significantly higher (respectively p = 0.02, < 0.001, < 0.001, < 0.001, < 0.001, < 0.001, < 0.001, < 0.001, p= 0.046). However, there was no difference between the two groups in age, duration of infertility, basal FSH level at Day 3 and basal E2 level, initial stimulation dose at Day 3, duration of ovarian stimulation, hCG day endometrial thickness, and total stimulation dose. Although a similar number of cycles resulted in embryo transfer in groups 1 and 2, embryo transfer at Day 5 was higher in Group 2 (p = 0.005). In addition, there was no significant difference between the two groups in terms of oocyte quality index and the number of 1st to 4th-grade embryos (p > 0.05) (Tab. 2).

Multivariate logistic regression analysis was performed to create predictive models for LBR. The following clinical parameters were included in the created model: hCG-P, total number of oocytes, age, BMI, induction protocol, total dose of gonadotropin used in induction, E2 on hCG day, and endometrial thickness on hCG day. However, the model was not significant (p > 0.05) (Tab. 3).

DISCUSSION

The negative impact of hCG-P elevation on IVF outcomes was first described by Schoolcraft et al. in 1991 [14], and the debate continues. While CPR is the most commonly studied condition, LBR has been investigated in only a few studies [15–17]. Based on the results of a meta-analysis of more than 60,000 fresh IVF cycles, there is evidence that high hCG-P is associated with a lower likelihood of pregnancy. However, the results are not strong enough to predict clinicians' treatment outcomes because of the heterogeneity of the population studied and confounding factors. Another reason for these conflicting results could be the different threshold values (ranging from 0.8 to 3.0 ng/mL) determined by different authors, which are close to the sensitivity

Table 1. Comparison of IVF cycle results for groups 1 and 2

Parameter[a]		Group 1 (hCG- P ≤ 0.78) n: 451	Group 2 (hCG- P > 0.78) n: 865	p value[b]
LBR	Yes	152 (33.6%)	228 (26.4%)	**0.006**
	No	301 (66.4%)	637 (73.6%)	
Type of drug used during induction	rcFSH+HMG	193 (42.6%)	327 (37.8%)	**0.001**
	rcFSH	247 (54.5%)	532 (61.5%)	
	HMG	12 (2.6%)	4 (0.5%)	
	rcFSH+rcLH	1 (0.2%)	2 (0.2%)	
Ovarian stimulation protocol	Agonist long protocol	206 (45.5%)	373 (43.1%)	0.414
	Antagonist protocol	247 (54.5%)	492 (56.9%)	
Embryo transfer day	2	14 (3.1%)	29 (3.4%)	0.154
	3	262 (57.8%)	448 (51.9%)	
	4	7 (1.5%)	17(1.9%)	
	5	165 (36.4%)	367 (%42.5%)	
	6	5 (1.1%)	4 (0.5%)	
Total number of retrieved oocytes	0-5	53 (11.7%)	42 (4.9%)	**< 0.001**
	6–15	301 (66.4%)	504 (58.3%)	
	> 15	99 (21.9%)	319 (36.9%)	
CPR	Yes	182 (40.2%)	315 (36.4%)	0.181
	No	271 (59.8%%)	550 (63.6%%)	
IR	Yes	212 (46.8%)	366 (42.3%)	0.108
	No	240 (53%%)	419 (57.7%%)	
Ongoing pregnancy outcomes	Ongoing one pregnancy	30 (16.5%)	55 (17.5%)	**0.030**
	Ongoing twin pregnancy	3 (1.6%)	5 (1.6%)	
	Abortion	23 (12.6%)	65 (20.6%)	
	İntrauterin exitus	2 (1.1%)	10 (3.2%)	
	Medical termination	2 (1.1%)	0	
	Ectopic pregnancy	3 (1.6%)	4 (1.3%)	
	One term pregnancy	96 (52.7%)	132 (41.9%)	
	One preterm birth	18 (9.9%)	21 (6.7%)	
	Twin preterm birth	1 (0.5%)	5 (1.6%)	
	Ongoing triple pregnancy	1 (0.05%)	0	
	Twin term birth	2 (1.1%)	9 (2.9%)	
	Blighted ovum	0	7 (2.2%)	
	Postpartum exitus	1 (0.5%)	2 (0.6%)	
BMI	< 20	28 (6.3%)	61 (7.2%)	0.89
	20–25	162 (36.2%)	346 (40.9%)	
	25–30	141 (31.5%)	268 (31.7%)	
	> 30	116 (26%)	170 (20.1%)	

[a]Data are presented as mean ± SD, median [interquartile range] or number (percentage); [b]Pearson Chi- Square test; BMI — body mass index; rcFSH — recombinant follicle-stimulating hormone; HMG — human menopausal gonadotropin; rc LH — rekombinant luteinizing hormone; LBR — live birth rate; CPR —clinical pregnancy rate; IR — implantation rate; IVF — in vitro fertilization; ET — embryo transfer

of the tests used [6]. The most commonly used threshold value in the literature is 1.5 ng/mL [2], and an hCG-P level above 1.5 ng/mL also seems to affect the endometrial gene expression profile [18]. However, this threshold is now being questioned again because there are many factors that influence P levels. These include factors such as the type of stimulation protocol, the type and dose of gonadotropin used, the age of the patient, and the number of follicles [6]. There is agreement that the detrimental effect of elevated hCG-P is due to an adverse effect on the endometrium, as no adverse effects due to high hCG-P have been observed in freeze-thaw cycles or donation oocyte cycles [6]. Studies

Table 2. Comparison of IVF cycle results for groups 1 and 2

Parameter[a]	Group 1 (hCG- P ≤ 0.78) n: 451	Group 2 (hCG- P > 0.78) n: 865	p value[b]
hCG-P	0.54 ± 0.18	1.30 ± 0.44	**< 0.001**
Age	29.62 ± 4.55	29.19 ± 4.49	0.108
BMI	27.08 ± 5.10	26.20 ± 4.85	**0.002**
Basal FSH, IU/L	6.90 ± 2.24	6.88 ± 1.93	0.886
Duration of infertility, (months)	73.51 ± 50.51	70.82 ± 48.66	0.357
Total number of antral follicles	15.38 ± 7.73	16.43 ± 7.78	**0.020**
Duration of antagonist administration	5.11 ± 1.31	5.47 ± 1.25	**< 0.001**
İnitial stimulation dose at day 3	217.91 ± 103.51	212.37 ± 60.07	0.294
ET- P	72.89 ± 45.51	88.61 ± 80.06	**0.046**
Days of stimulation	9.66 ± 1.66	9.69 ± 1.49	0.786
Total gonadotrophin dose, IU	2025.75 ± 772.62	2009.11 ± 704.95	0.702
Basal estradiol, pg/ ml	46.39 ± 47.22	45.13 ± 55.02	0.726
Estradiol on HCG day, pg/mL	2194.18 ± 1207.30	3203.22 ± 1732.76	**< 0.001**
Basal progesterone (ng/mL)	0.42 ± 0.26	0.75 ± 2.58	**< 0.001**
Endometrial thickness on hCG day	10.04 ± 1.83	10.14 ± 2.10	0.473
Oocytes retrieved	11.62 ± 6.22	14.72 ± 7.15	**< 0.001**
Number of mature oocytes	8.92 ± 4.96	10.85 ± 5.48	**< 0.001**
Number of oocytes used for ICSI	9.56 ± 5.18	11.28 ± 5.50	**< 0.001**
Oocyte quality index	5.16 ± 0.73	5.10 ± 0.71	0.127
Number of 2 pronuclei	4.92 ± 3.34	5.75 ± 3.75	**< 0.001**
Number of grade 1 embryo	0.65 ± 0.66	0.61 ± 0.61	0.247
Number of grade 2 embryo	0.38 ± 0.52	0.42 ± 0.57	0.201
Number of grade 3 embryo	0.18 ± 0.42	0.17 ± 0.41	0.704
Number of grade 4 embryo	0.05 ± 0.27	0.03 ± 0.18	0.143
Blastocyst transfer	0.34 ± 0.58	0.45 ± 0.62	**0.005**

[a]All values are presented as mean (SD); [b]Student's t-test or Mann–Whitney U-Test for differences between normal and elevated progesterone groups; hCG-P— human chorionic gonadotropin day progesterone; BMI — body mass index; ET — embryo transfer; IVF — in vitro fertilization; E2 — estradiol; ICSI — intracytoplasmic sperm injection; PN — Pronucleus

Table 3. Regression analysis

Independent variable	p value	OR	95% CI
Age, years	0.769	1.005	0.972–1.039
Progesterone on HCG day (ng/mL)	0.299	1.186	0.860–1.636
BMI	1.000	1.000	0.970–1.031
Ovarian stimulation protocol (1)	0.812	0.964	0.711–1.307
Retrieved oocytes	0.730	1.005	0.977–1.033
Endometrial thickness on HCG day, mm	0.351	0.966	0.898–1.039
Total gonadotrophin dose, IU	0.974	1.000	1.000–1.000
Estradİol on HCG day (pg/mL)	0.300	1.000	1.000–1.000
Constant	0.409	1.940	

hCG-P — human chorionic gonadotropin day progesterone; BMI — body mass index; E2 — Estradiol; GnRH — gonadotrophin-releasing hormone

in which an anti-progestin (RU-486) was administered during final oocyte maturation in mice showed that it affected endometrial receptivity and not embryo development [19]. In a study of agonist cycles, a ROC analysis was performed for the hCG-P threshold, but no threshold value was defined for predicting pregnancy [20]. Given the data from recent meta-analyses [15], we decided to reevaluate the effect of hCG-P on LBR, which is the true target for IVF. The aim of the study was to determine whether there is a value for hCG-P that predicts live birth rate and to investigate whether the methods recommended in the literature can be used because of such an effect. In our study, the area under the ROC curve for LBR was significant, but this result seems insufficient to use P values to predict pregnancy (AUC: 0.537, p < 0.036, 95% confidence interval (CI) 0.510–0.564). Sali et al. [21] also came to the same conclusion in their study, in which

they examined only GnRH-analogous cycles. In our study, the number of patients was much higher. According to our ROC analysis, the threshold value for hCG-P we found was 0.78, which is much lower than the value of 1.5 ng/mL, the most commonly used threshold value in the literature [6]. Between Group 1 ($p \leq 0.78$) and Group 2 ($p > 0.78$), the difference between the LBR and the number of oocytes retrieved was significant ($p = 0.006$ and < 0.001, respectively). The LBR was higher in Group 1, but in Group 2, the percentage of patients with a high response (> 15 oocytes retrieved) was higher (Group 1: 21.9%, Group 2: 36.9%). Martinez et al. [16] performed a ROC analysis in their study, examining a total of 1900 cycles after applying a logarithmic correction for the p value, and the AUC was 0.496.

In the group treated with both agonists and antagonists, there was no association between hCG-P levels and pregnancy and LBR, and no threshold value for hCG-P was detected [16]. This result is also consistent with the results of some other studies [16, 22–25]. Our results also support the idea that hCG-P is a very weak marker and plays a relatively minor role when considering all factors that have a significant impact on the final outcome of IVF, such as the age of the woman, the number of retrieved oocytes, the type and intensity of stimulation. Several reasons have been proposed to explain the contradictory results obtained by different authors: Most studies are retrospective, there are different tests, different stimulation protocols or differences in the population studied, and it is possible that individual and biased decisions were made for each patient. In the study by Venetis et al. [17], live birth rates did not differ significantly between cycles with and without elevated hCG-P.

It is well known that the number of retrieved oocytes is an important parameter for predicting pregnancy [26]. The most important question is whether this situation protects against the detrimental effects of hCG-P elevation in a cycle with a large number of retrieved oocytes. When we look at the studies in the literature, we come to contradictory results. Venetis et al. [17] proposed and applied multivariate regression analysis to eliminate the influence of confounding factors. In their analysis, the detrimental effect of hCG-P elevation was observed only in the intermediate group (6–18 retrieved oocytes). However, even in this analysis, where the data are ten years old and there may be both unmeasurable and residual factors, they reported that the results were insufficient to determine whether, in the case of hCG-P elevation, the fresh cycle should be aborted and switched to freeze-thaw [17]. In the study by Bosch et al. [2] in which they examined 4000 IVF cycles, the detrimental effect of high hCG-P was independent of the magnitude of the ovarian response. However, Griesinger et al. [24] suggested that elevated hCG-P did not affect pregnancy rates in patients with a high ovarian response. Xu et al. [9] reported that the detrimental effect occurred at higher levels of hCG-P in patients with a high ovarian response. In two studies using the number of follicles with a diameter of > 14 mm on the day of hCG administration, it was observed that a higher number of follicles could be protective against high hCG-P and decrease the P/follicle ratio [10]. However, in the study by Hill et al., more follicles or oocytes did not protect against the adverse effects of hCG-P on LBR [27].

In our study, when we performed a logistic regression analysis to understand the relationship between the number of oocytes retrieved and LBR and hCG-P levels, the results were not significant. Presumably, the presence of additional factors affecting LBR, the heterogeneity of the study group, and the presence of unpredictable and unexplained factors, as well as the examination of data dating back nearly a decade, influenced the results.

A globally accepted threshold value for hCG-P that confirms the negative association between hCG-P elevation and pregnancy probability will influence clinicians' decisions to freeze all embryos and delay fresh ET for the next freeze-thaw cycle. However, some clinicians and patients still prefer fresh ET. Therefore, reducing the likelihood of elevated hCG-P may be a more attractive strategy [28]. Alternatively, in patients who had elevated hCG-P levels during a previous IVF cycle, administration of hCG before the rise of P in the follicular phase may be beneficial [29]. It is known that the likelihood of a hCG-P increase is lower in patients with fewer retrieved oocytes [30]. However, the probability of live birth decreases with the number of retrieved oocytes [26]. Venetis et al. suggested using such a strategy in patients with a higher probability of hCG-P rise and using the baseline p value on the third day for prediction [28]. In our study, the mean p value on the third day was 0.42 ng/mL in Group 1, whereas it was 0.75 ng/mL in Group 2 and significantly higher in group 2 ($p < 0.001$). This result seems to support the proposal of Venetis et al. [28].

In most of the above studies, the threshold value for hCG-P seems to have been chosen arbitrarily. In our study, hCG-P had no predictive value for LBR according to multivariate logistic regression analysis results. Therefore, our results do not indicate a specific threshold value.

Oktem et al. [31], in their study investigating the effect of ovarian stimulation intensity on P synthesis in granulosa cells before hCG induction, reported that this effect could be responsible for premature P production without luteinization, with a direct stimulatory effect on the expression and enzymatic activity of 3β-hydroxysteroid dehydrogenase in granulosa cells, depending on the dose of gonadotropin used [31]. In our study, the difference between the two groups with respect to the total gonadotropin dose used was not significant ($p = 0.702$).

Although the effect of hCG-P on pregnancy outcomes is mainly explained by its effect on the endometrium [6], a negative correlation between high hCG-P and embryo quality has also been recently reported [32]. Retrospective data on patients using the GnRH antagonist protocol showed that high serum hCG-P levels were associated with a lower number of best quality embryos at Day 5 [33]. Impaired embryo quality can negatively affect both the fresh and freeze-thaw cycles. In this case, not only endometrial receptivity but also embryo quality is compromised, increasing embryo loss and decreasing success [34]. Therefore, it is doubtful that freeze-thaw improves success in these patients, and the potential efficacy of this strategy has not been demonstrated in randomized controlled trials. In our study, the difference between the two groups in terms of oocyte quality index and number of grade 1 to 4 embryos was not significant ($p > 0.05$). It was also found that blastocyst transfer was significantly higher in Group 2 ($p > 0.05$). The result of our study supports the hypothesis that hCG-P affects endometrial receptivity rather than embryo and oocyte quality.

Several strategies have been proposed to counteract the potentially adverse effects of high hCG-P on the likelihood of pregnancy, including postponing day ET [35], earlier administration of hCG [36], and even freezing whole oocytes or embryos [37]. Although some authors have suggested the use of highly purified menotropin (HP-HMG) to reduce the occurrence of high late follicular phase P [2], Santos-Ribeiro et al. [38] used HP-HMG for this purpose, this time with late harmful low hCG. They reported that this might lead to P levels, and therefore, it would be more appropriate to maintain hCG-P levels between 0.5 and 1.5 ng/mL to keep pregnancy outcomes at an optimal level [38]. A small retrospective study with close P monitoring before reaching high hCG-P levels (> 1.0 ng/mL) resulted in higher IR [29].

CONCLUSIONS

Our study was performed at only one IVF center and only long cycles with agonists and antagonists were analyzed. The threshold value of hCG-P we found for LBR was quite low compared with the hCG-P threshold often recommended in the literature. In the logistic regression model established with the total number of oocytes, the model was not significant because of the many possible factors affecting LBR and the heterogeneity of the study group. For most treatment cycles, the end goal of LBR is important because pregnancies can result in miscarriage if hCG-P is high, even if implantation occurs. Although the model did not prove significant in our study, the fact that cycles were not discontinued because of hCG-P level in the studied patient group and that LBR was examined increases the power of the study. Because we have not determined detrimental hCG-P threshold values in our population, our approach to date is to make an individual decision for each patient based on the ovarian response in the cycle. Another important point is that in IVF patients with unexplained recurrent implantation failure, despite a high or good ovarian response and transfer of quality embryos, it is important to consider the hCG-P threshold value, which has a significant detrimental effect on endometrial receptivity, and to perform specific clinical trials for this group. Further studies and evidence are needed to demonstrate conclusively that these recommendations presented in the literature are universal and persuasive to IVF professionals, especially when deciding to discontinue fresh cycles.

Limitations of the study

Because our study is a retrospective study conducted over an 11-year period, the presence of bias cannot be ignored. One of the main limitations of our study is the lack of data on ongoing pregnancies and LBR. In addition, there are only 22 patients with serum hCG-P levels above 2.53 ng/mL whose cycles were not discontinued. Twelve of them were pregnant and 10 of them had a live birth. Interestingly, the highest p value was 2.98 ng/mL, and the patient had a live birth. Since there are no patients with hCG-P levels above 3 ng/mL, no statement can be made about the possible adverse effects on these levels.

Article information and declarations

Conflict of interest

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