Predictive value of βhCG measured on day 11 after blastocyst embryo transfer for early pregnancy outcome

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Abstract

Objectives: *To study the value of βhCG on day 11 after embryo transfer in predicting the early outcome of pregnancy after in vitro fertilization.*

Materials and methods: *A prospective descriptive study was examined on 148 cycles that satisfied inclusion criteria that the βhCG concentration at day 11 after IVF was higher or equal to 25mIU/ml. This research was conducted at Hue center for Reproductive Endocrinology and Infertility from December 2019 to July 2022. The cycles that met the inclusion criteria were followed up to 12 weeks of gestation. Comparing the variation of the βhCG level between different groups: biochemical/clinical pregnancy, singleton/multiple pregnancy, and ongoing pregnancy/early fetal loss. ROC curves were used to calculate the serum βhCG cutoff levels with maximum sensitivity and specificity to predict early pregnancy outcomes.*

Results: *The mean concentration of serum βhCG of clinical pregnancy was significantly higher than that of biochemical pregnancy (631.4 ± 433.6 mIU/ml vs 150.9 ± 182.8 mIU/ml); that of multiple pregnancy was significantly higher than that of singleton pregnancy (935.6 ± 460.6 mIU/ml vs. 558.3 ± 388.1 mIU/ml); and that of ongoing pregnancy was significantly higher than that of early fetal loss (619.4 ± 407.8 mIU/ml vs. 327.0 ± 159.1 mIU/ml). The serum βhCG level on day 11 after embryo transfer had a good value in predicting clinical pregnancy at the cutoff of 212.4 mIU/ml (AUC = 0.900); a moderate value in predicting twin pregnancy at the cutoff of 537.8 mIU/ml (AUC = 0.774) and ongoing pregnancy at the cutoff of 501.3 mIU/ml (AUC = 0.742).*

Conclusions: *The serum βhCG level on day 11 after blastocyst transfer was a prognostic indicator for early pregnancy outcome after in vitro fertilization which included: clinical pregnancy, twin pregnancy and ongoing pregnancy.*

Keywords: *blastocyst transfer, βhCG level, early pregnancy, multiple pregnancy, predictive value.*

1. INTRODUCTION

In 1978, the first baby - Louise Brown was born from in vitro fertilization techniques. The advancement in assisted reproductive technology resulted in the increasing successful IVF rate and the indications of this technique were expanding. To 2018, over 8 million IVF children have been born, and over 2.5 million cycles are being performed every year, resulting in over 500,000 deliveries annually [1].

In vitro fertilization pregnancy has been a high-risk pregnancy. The spontaneous miscarriage in IVF was reported at 21%, higher than in natural cycles [2]. The early prediction of pregnancy outcomes following in vitro fertilization has played a crucial role in clinical practice. Although transvaginal ultrasound has been helpful in assessing early pregnancy, its role has been restricted with gestational age below 5 - 6 weeks. Some serum markers such as progesterone, inhibin and CA125 have been studied in relation to pregnancy but the exact predictive value has been controversial [3], [4].

Beta - human chorionic gonadotropin (βhCG) is the earliest biochemical marker in pregnancy. βhCG measurement is a simple and low-cost method. The important role of βhCG in predicting the early outcome of pregnancy following in vitro fertilization has been demonstrated but there was no consensus about its cutoff. Zhang Y (2022) found the excellent value of this marker in forecasting clinical pregnancy at the threshold of 241.1 mIU/ml and the moderate value in the prediction of ongoing pregnancy and multiple pregnancy at the cutoff of 585.9 mIU/ml and 981.1 mIU/ml, respectively [5]. Wang Z (2019) concluded that βhCG was an excellent prognostic indicator for clinical pregnancy and multiple pregnancy at the cutoff of 213.2 mIU/ml and 986.7 mIU/ml, respectively; and was a less predictive value in forecasting the ongoing pregnancy at the threshold of 270.6 mIU/ml [6]. However, most of the current studies investigated the value of βhCG in cleavage embryo transfer or both of cleveage and blastocyst embryo transfer.

In the in-vitro fertilization technique, embryo can be transferred in different stage (cleavage or blastocyst), which in relation to the exact time of measuring serum βhCG level [7]. The recent years have seen a steady

shift in in-vitro fertilization practice from cleavage stage embryo transfer to blastocyst stage embryo due to the advances in cell culture media. A Cochrane meta - analysis with data from 21 randomized controlled trials concluded that the live birth rate and clinical pregnancy rate in the blastocyst transfer group were higher that that of the cleavage transfer group [8]. The number of research about the role of βhCG in predicting the outcome of pregnancy following blastocyst transfer has been limited. Therefore, we conducted this study aimed to evaluating the predictive value of βhCG level in predicting the early pregnancy outcome following in vitro fertilization.

2. MATERIALS AND METHODS

Study design

A prospective descriptive study were conducted at Hue Center for Reproductive Endocrinology and Infertility from December 2019 to July 2022.

Subjects

All the IVF/ICSI cycles fulfilled the inclusion criteria as follows were eligible to enroll in this study: (1) serum βhCG levels were examined on day 11 after IVF embryo transfer (2) Blastocyst-stage embryo transfer (3) Ovarian stimulation protocol was GnRH antagonist protocol and (4) completed follow-up data to 12 weeks of pregnancy and infertile couples agreed to take part in this research. The exclusion criteria were as follows: (1) Donor egg cycles and (2) Cleavage-stage embryo transfer.

Sample size calculation

Sample size was calculated using the following formula:

$$n \geq Z^2_{1-\alpha/2} \frac{p(1-p)}{d^2}$$

In which: $Z_{1-\alpha/2}$ = 1,96 corresponding to a 95% level of confidence. p = 0.9, which was the clinical pregnancy rate in Singh's study [9]. d = 0.05 with the expected precision of 95%. The minimal sample size calculated by this formula was 139 IVF cycles. In our research, we collected data from 148 cycles that satisfied the inclusion and exclusion criteria.

Study procedures

The infertile couples were recorded some necessary information which included: sociodemographic characteristics, medical, surgical history, obstetric history and infertility history.

All female participants were clinically examined, measured height, weight, waist circumference, hip circumference, and evaluated volume and morphological characteristics of ovarian and uterine by gynecological ultrasound. The concentration of basal FSH, basal LH, basal Estradiol, AMH, and Prolactin were evaluated on day 2 or day 3 of the cycle. The male fertility was evaluated by semen analysis test.

Ovarian stimulation

The controlled ovarian stimulation protocol was GnRH antagonist protocol starting from day 2 or day 3 of cycles. The starting dose of recombinant FSH (rFSH, Gonal F 300UI, Merch – Serono, Italy) was determined individually for each patient. Preventing the release of LH by GnRH antagonist (Cetrotide 0,25 mg, Serono, Rockland, USA).

Triggering of final oocyte maturation when at least three follicles reached 17 mm and more than 50% of follicles ≥ 14 mm. Triggering of final oocyte maturation with recombinant hCG (Ovitrelle, 250µcg, Merch - Serono, Italy) or with GnRH agonist Triptorelin 0.3 mg in cases at risk of ovarian hyperstimulation syndrome. Oocyte pick up procedures were performed 36 hours after hCG injection by transvaginal ultrasound-guided aspiration.

Evaluating the embryo grade:

ICSI was carried out at 4 - 6 hours after oocyte retrieval. Assessment of the embryo stage based on the Istanbul consensus 2011 of Alpha Scientists in Reproductive Medicine and ESHRE Special Interest Group of Embryology [10].

+ Good: Prominent, easily discernible, with many cells that were compacted and tightly adhered together.

+ Fair: Easily discernible, with many cells that were loosely grouped together.

+ Poor: Difficult to discern, with few cells.

The good and fair embryos were directly transferred in cases of choosing fresh – embryo transfer method or preserved by vitrification for frozen – embryo transfer method if the infertile couples were not ready to become parents or the endometrium was not suitable.

Endometrium preparation and Embryo Transfer

There were two different techniques of embryo transfer: Fresh embryo transfer and Frozen embryo transfer. Considering the frozen embryo transfer method: exogenous Estradiol protocol was used for endometrium preparation starting from day 2 -3 of cycles with oral Estradiol (Valiera 2mg, Laboratorios Recalcine S.A, Chile). Usually, after 12 – 14 days of Estradiol administration, vaginal ultrasound examination was performed for endometrial thickness measurement. When the endometrial thickness ≥ 7mm, Luteal phase support with supplementing Progesterone microparticle 400mg twice daily, and timing of embryo transfer was scheduled after 5 days of supplementing Progesterone.

Embryo thawing by vitrification techniques at least 3 hours before transferring embryos. Assessment the quality of embryos after the thawing process. The number of embryos transferred depended on maternal age and the embryo quality, were performed by Tulip 4000 catheter (Gynetics, Belgium). This process was taken under abdominal ultrasound guidance, and required the patient to have a full bladder.

Luteal phase support with supplementing oral Estradiol, the dose was 8mg per day (Valiera 2mg, Laboratorios Recalcine S.A, Chile), combined with vaginal Progesterone microparticle 400mg twice daily (Utrogestan, 400mg). Luteal phase support was given until 8 weeks of pregnancy.

Measurement the serum βhCG level

Blood was drawn on day 11 after the blastocyst embryo transfer to determine the serum quantitative βhCG concentrations. βhCG was determined by the electrochemiluminscense immunoassay (ECLIA) on an automated Cobas 6000 machine (Roche, Switzerland). The measuring range for hCG was 0.0 - 15,000 mIU/ml. The cycles that satisfied the serum βhCG level ≥ 25mIU/ml was chosen in this study.

The variables of pregnancy outcome

Biochemical pregnancy was diagnosed by the only detection of serum βhCG level ≥ 25mIU/m without any gestational sac. Clinical pregnancy was diagnosed by ultrasonographic visualization of the gestational sac (including ectopic pregnancy). Singleton pregnancy was defined as the presence of one gestational sac and the definition of twin pregnancy was the presence of two gestational sacs. Ongoing pregnancy was defined as one that proceeded beyond 12 weeks gestation. Early fetal loss included stillbirth and spontaneous miscarriage.

Statistical analysis.

SPSS 20.0 and Excel 2016 were used for statistical analysis. The serum βhCG concentration was normal distribution. The Independent Samples t Test was used for comparing the means of βhCG level between different groups (biochemical/clinical pregnancy, singleton/multiple pregnancy, and ongoing pregnancy/early fetal loss). Receiver operating characteristic (ROC) curves were used to calculate the cutoff of serum βhCG level with maximum sensitivity and specificity to predict the early pregnancy outcome. The cutoff values were determined when the Youden index (sensitivity + specificity - 1) was the largest. The statistical significance of AUC as follows: 0.5 - 0.6: no predictive value, 0.6 - 0.7: less predictive value, 0.7 - 0.8: moderate predictive value, 0.8 - 0.9: good predictive value and > 0.9: excellent predictive value.

3. RESULTS

During the research period from December 2019 to July 2022, there were 148 cycles fulfilled the inclusion criteria, including 6 biochemical pregnancies and 142 clinical pregnancies. The group of clinical pregnancies contained 110 singleton pregnancies, 30 twin pregnancies and 2 ectopic pregnancies. Following up to 12 weeks of pregnancy recorded 116 ongoing pregnancies and 24 early fetal losses (Figure 1).

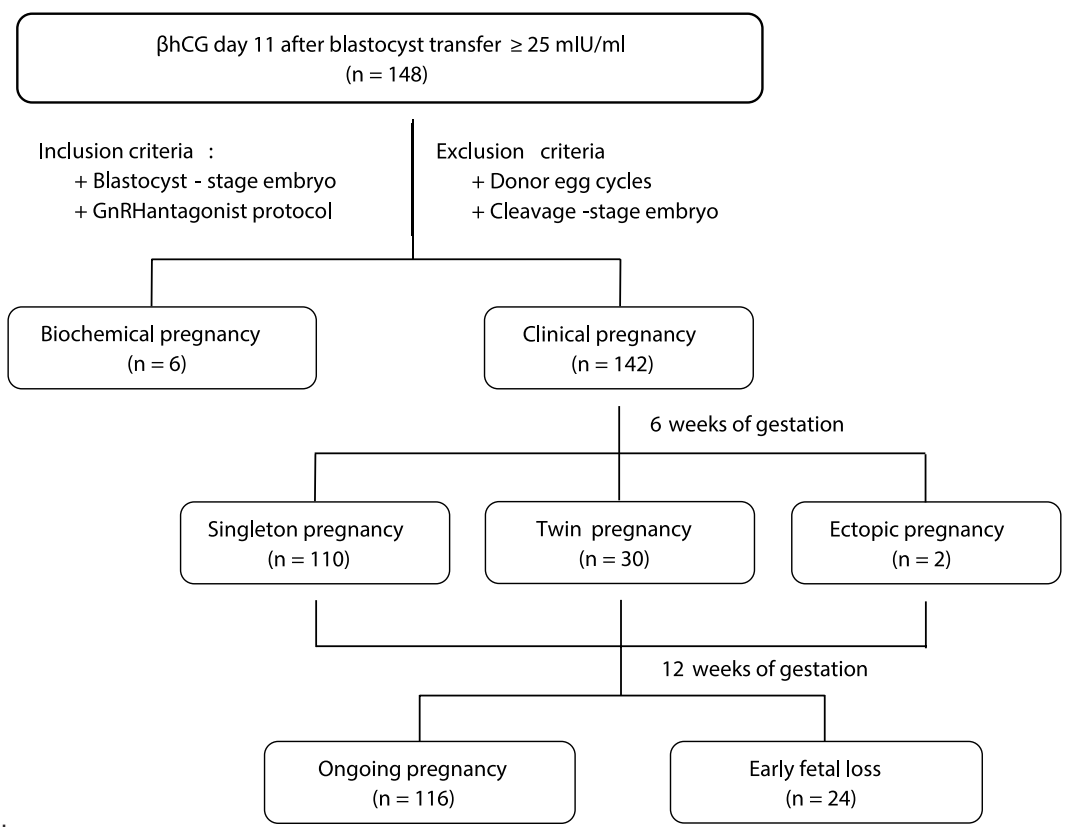

Figure 1. The flowchart of study

About the general characteristic of the included IVF cycles: the mean maternal age was 32.4 ± 3.9 years old

and the group of < 35 years old accounted for 17.3%. The mean duration of infertility was 5.4 ± 2.7 years. The most common cause of infertility was both male and female factors.

Table 1. General characteristic of included IVF cycles (n=148)

General characteristic		N	%
Age (years)	< 30	34	23.0
	30-34	73	49.3
	35-39	33	22.3
	≥ 40	8	5.4
	Mean	32.4 ± 3.9	
Type of infertility	Primary	73	49.3
	Secondary	75	50.7
Duration of infertility (years)	< 5	60	40.5
	≥ 5	88	59.5
	Mean	5.4 ± 2.7	
Causes of infertility	Only male factors	28	18.9
	Only female factors	13	8.8
	Both side factors	107	72.3
BMI (kg/m^2)	< 18.5	16	10.8
	18.5 - 22.9	102	68.9
	≥ 23	30	20.3
	Mean	20.9 ± 2.2	
The mean basal FSH concentration (mIU/mL)		6.4 ± 1.6	
Endometrial thickness on the embryo transfer day (mm)		9.3 ± 1.7	
Number of embryos transferred	01 embryo	50	33.8
≥ 02 embryos		98	66.2
Types of embryo transfer method	Fresh embryo transfer	5	3.4
Frozen embryo transfer		143	96.6

The serum βhCG level of multiple pregnancy was theoretically higher than that of singleton pregnancy. Therefore to compare the difference in βhCG level between the ongoing pregnancy and early fetal loss, we just analyzed the singleton pregnancy group (that contained 87 ongoing pregnancies and 23 early fetal losses) in order to reduce the confounding factors.

Table 2. Comparison of the mean serum βhCG level between the different groups: biochemical/clinical pregnancy, singleton/multiple pregnancy and ongoing pregnancy/early fetal loss

Pregnancy outcomes \ βhCG(mIU/mL)	N	Mean ± standard deviation	Min - Max	p
Biochemical pregnancy	6	150.9 ± 182.8	30 - 498	**0.008**
Clinical pregnancy	142	631.4 ± 433.6	60 - 2233	
Singleton pregnancy	110	558.3 ± 388.1	60 - 2233	**< 0.001**
Multiple pregnancy	30	935.6 ± 460.6	314 - 2205	
Ongoing pregnancy	23	327.0 ± 159.1	60 - 635	**< 0.001**
Early fetal loss	87	619.4 ± 407.8	68 - 2233	

In our study, the mean serum βhCG level of clinical pregnancy was statistically higher than that of biochemical

pregnancy (631.4 ± 433.6 mIU/ml vs. 150.9 ± 182.8 mIU/ml, p = 0.008); that of multiple pregnancy was statistically higher than that of singleton pregnancy (935.6 ± 460.6 mIU/ml vs. 558.3 ± 388.1 mIU/ml, p < 0.001); and that of ongoing pregnancy was statistically higher than that of early fetal loss (619.4 ± 407.8 mIU/ml vs. 327.0 ± 159.1 mIU/ml, p < 0.001).

ROC curve was used to determine the cutoff and the value of serum βhCG level in predicting the early pregnancy outcome. To determine the value of βhCG in predicting the multiple pregnancy, we just analyzed the clinical pregnancy and excluded the ectopic pregnancy (140 cycles); and with the prediction of ongoing pregnancy, we analyzed 110 singleton pregnancies.

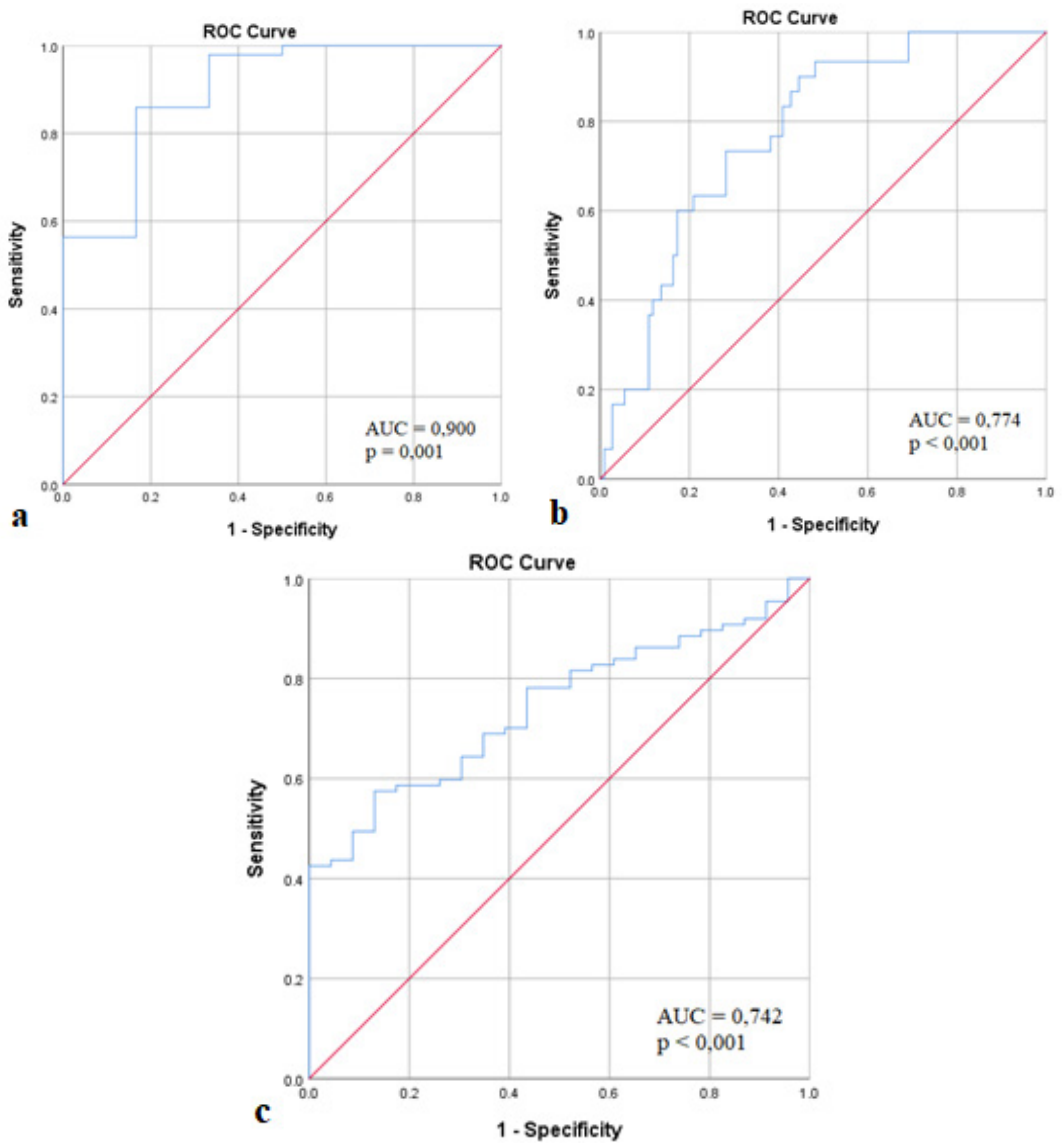

Figure 2: ROC curves of serum βhCG levels to predict clinical pregnancy (a), multiple pregnancy (b) and ongoing pregnancy (c).

	AUC (95%CI)	Cutoff	Se (%)	Sp (%)	p
Clinical pregnancy	0,900 (0.77 - 1.00)	212.4	85.9	83.3	0.001
Multiple pregnancy	0,774 (0.689 - 0.858)	537.8	90.0	55.5	< 0.001
Ongoing pregnancy	0,742 (0.647 - 0.837)	501.3	57.5	87.0	< 0.001

The cutoff of serum βhCG levels in predicting the clinical pregnancy, multiple pregnancy and ongoing pregnancy were 212.4 mIU/ml, 537.8 mIU/ml and 501.3 mIU/ml, respectively; and AUCs were 0.900, 0.774, and 0.742, respectively.

4. DISCUSSION

βhCG (Beta - human chorionic gonadotropin) has been the earliest hormone produced by cytotrophoblasts and syncytiotrophoblasts. Six days after fertilization, the trophoblastic cells secreted trace amount of βhCG, however serum βhCG could be detected only 10 days after fertilization [6]. The concentration of serum βhCG has been proportional with the number and the activity of the trophoblastic cell. The serum βhCG level was reported to be substantively higher than in several clinical circumstances: multiple pregnancy, erythroblastosis fetalis associated with fetal hemolytic anemia, gestational trophoblastic disease; and relatively lower in women with early pregnancy failure including ectopic pregnancy [11]. The role of βhCG in predicting the pregnancy outcome has been started to study on the basis of this theory. The day of measuring serum βhCG level was dependent on the stage of embryo transferred, in particular on day 14 after transfer with cleavage – stage embryo and on day 11 with blastocyst embryo. Our aim in this study is evaluating the predictive value of serum βhCG level for the early pregnancy outcome in the blastocyst transferred group.

About the general characteristics of the included IVF, the mean maternal age was 32.4 ± 3.9 years **(Table 1)**. This result was consistent with Løssl Kristine's finding which was 31.3 ± 4.0 years [12]. The duration of infertility was 5.4 ± 2.7 years **(Table 1)** which was higher than the result of Løssl Kristine's study (2.5 years) and Zhang's study (3 years) [5], [12]. The difference between these studies was due to the variation in the sample sizes and the centers where conducted research.

The value of serum βhCG level measured on day 11 after blastocyst transfer in predicting the clinical pregnancy

The mean serum βhCG level after blastocyst transfer of clinical pregnancy was 631.4 ± 433.6 mIU/ml significantly higher than that of biochemical pregnancy was 150.9 ± 182.8 mIU/ml **(Table 2)**. Kahyaoğlu İ (2017)'s study also found a significant difference in serum βhCG concentrations between biochemical pregnancy (77.4 ± 44.8 mIU/ml) and clinical pregnancy (207.7 ± 170.6 mIU/mL) [13]. The mean βhCG level of biochemical pregnancy in Zbořilová B (2018)'s study was 82 mIU/ml [14]. Even though there was a variation between different studies, the statistical difference in serum βhCG levels between clinical pregnancy and biochemical pregnancy was consistently reported.

In our study, the cutoff of βhCG level in predicting clinical pregnancy was 212.4 mIU/ml with sensitivity of 85.9% and specificity of 83.3%. Our cutoff was similar to Wang Z's finding which was 213.2 mIU/ml; and higher than the cutoff of Løssl Kristine was 123 mIU/ml and Kahyaoğlu İ was 86.6 mIU/ml [6], [12], [13]. The variation between these studies may be caused by the different threshold of serum βhCG levels in diagnosing biochemical pregnancy, specifically in Wang Z's research was > 6 mIU/ml and in the studies of Løssl Kristine và Kahyaoğlu İ was > 10 mIU/ml. The threshold for diagnosing biochemical in our research which based on consensus of The international committee for monitoring assisted reproductive technology (ICMART) in 2009 was 25 mIU/ml [15]. The predictive values of βhCG with clinical pregnancy in these studies was reported with the ranges of AUCs from 0.76 to 0.95 [6], [12], [13]. Therefore, our study was in line with these results with AUC was 0.9.

There were only two cases of ectopic pregnancy in a total of 148 included cycles in our study. We didn't evaluate the prognostic value in the ectopic pregnancy group due to the small number of cases.

The value of serum βhCG level measured on day 11 after blastocyst transfer in predicting the multiple pregnancy

In our study, the mean serum βhCG level day 11 after blastocyst transfer of singleton pregnancy was 558.3 ± 388.1 mIU/ml and that of multiple pregnancy was 935.6 ± 460.6 mIU/ml. The research of Kartha (2017) reported the mean serum βhCG concentration of singleton pregnancy was 272.7 ± 155.0 mIU/mL and that of multiple pregnancy was 683.3 ± 194.4 mIU/ml [16]. The mean βhCG level of multiple pregnancy in Zbořilová B (2018)'s study was 1070 mIU/ml [14]. Our finding was consistent with these findings which was a significant difference in βhCG levels between singleton and multiple pregnancy.

Multiple pregnancy was optimally predicted by a βhCG value of 537.8 mIU/ml with the sensitivity of 90% and specificity of 55.5% **(Figure 2)**. Our βhCG cutoff was lower than that of Wang Z's study which was 986.7 mIU/ml and Zhang's study which was 981.1 mIU/ml [5], [6]. The predictive value of βhCG day 11 after embryo transfer with AUC of 0.774 which was similar to that of Zhang's study with AUC of 0.774 and lower than Wang Z's study with AUC of 0.907 [5], [6]. There was a variation in the cutoffs and the predictive values of βhCG due to the difference in the sample size, the inclusion criteria, the technique and the exact day of measuring this marker.

The value of serum βhCG level measured on day 11 after blastocyst transfer in predicting the ongoing pregnancy

The serum βhCG concentration was variable

according to the status of pregnancy (biochemical pregnancy, ectopic pregnancy, or intrauterine pregnancy) and the number of gestational sac (singleton pregnancy or multiple pregnancy). Therefore we analyzed the association between serum βhCG level and the ongoing pregnancy just in the singleton group (110 cases) in order to reduce the confounding factors. There was a limitation regarding the characteristic of included cycles. Specifically, we just recorded one case of early twin pregnancy losses in 30 cases of twins followed up to 12 weeks of gestation. For that reason, we didn't investigate the value of serum βhCG level in predicting the outcome of twin pregnancy group at 12 weeks of gestation.

In our study, the mean serum βhCG concentration of ongoing pregnancy was statistically higher than that of early fetal loss (619.4 ± 407.8 mIU/ml vs. 327.0 ± 159.1 mIU/ml) **(Table 2)**. Singh N (2013)'s research also reported a significant difference in serum βhCG levels between these groups (600 mIU/ml vs. 178 mIU/ml) [9]. Hence, our finding was in line with result of Singh (2013)'s research.

Ongoing pregnancy was optimally predicted by a βhCG value of 501.3 mIU/ml with the sensitivity of 57.5% and specificity of 87%. Our cutoff of serum βhCG level was higher than that of Wang Z's study which was 270.6 mIU/ml and Singh N's study which was 347 mIU/ml [6], [9]. Different predictive values with AUCs ranging from 0.631 to 0.803 were reported in the related studies [6], [9], [12]. Therefore, our study was in line with these results with AUC was 0.742.

5. CONCLUSIONS

The mean serum βhCG level on day 11 after blastocyst transfer was an important indicator in predicting the early pregnancy outcome (clinical pregnancy, multiple pregnancy and ongoing pregnancy). And there was a significant difference in serum βhCG levels between two different groups specifically biochemical/clinical pregnancy, singleton/multiple pregnancy, and ongoing pregnancy/early fetal loss.

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