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The Predictive Value of Serum ß-HCG Levels in The Detection of Ectopic Pregnancy Sixteen Days after Embryo Transfer: A Cross-Sectional Study

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Abstract

Background: To detect the predictive value of beta human chorionic gonadotropin (β-hCG) levels 16 days post embryo transfer (ET) regarding detection of an ectopic pregnancy (EP) in assisted reproductive technology (ART) cycles.

Materials and Methods: In this cross-sectional study, we reviewed the database of Royan Institute from January 2011 to December 2014 and from January 2017 to December 2019 retrospectively. All cases with positive β-hCG levels sixteen days after ET were screened (n=4149). The pregnancies with oocyte or embryo donation and the multiple pregnancies based on the first ultrasound were excluded. All eligible singleton pregnancies with documented serum β-hCG levels at Royan institute laboratory (n=765) were included and then classified according to the type of pregnancy: EP (n=189) or non-EP (n=576). The data of the treatment cycle was extracted from the patients' files. A receiver operating characteristic (ROC) curve was used to detect the predictive power of the first measurement of β-hCG level in distinguishing EP from ongoing pregnancy in the ART and intrauterine insemination (IUI) cycles separately. Sensitivity, specificity, area under the ROC curve and 95% confidence intervals (CI) were calculated for each of the estimates.

Results: The mean levels of β-hCG 16 days after ET were remarkably higher in the ongoing pregnancy group than the EP group (1592.35 ± 87 IU/L vs. 369.69 ± 50.61 IU/L, P<0.001). The β-hCG thresholds predictive of ongoing pregnancy were 278 IU/L as the most suitable cut-off to predict viable pregnancy with a sensitivity of 72.8%, a specificity of 67.5%, a positive predictive value of 77.8%, standard error of 0.02, and a confidence interval of 73.8-81.7%. However, this relationship was not found in IUI cycles.

Conclusion: Based on these findings, if β-hCG levels 16 days after ET are below 278 IU/l, close follow-up is recommended, until either the diagnosis of EP or miscarriage is established.

Keywords: Beta Subunit, Chorionic Gonadotropin, Ectopic, Pregnancy, Reproductive Techniques Assisted

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Introduction

An ectopic pregnancy (EP) is a form of abnormal pregnancy occurs when a zygote implants and grows outside the main cavity of the uterus. EP constitutes 1-2% of all pregnancies and hemorrhage caused by EP because tubal rupture is the most common reason for maternal mortality in early pregnancy. Infertility affects 8-12% of couples in the whole world. There is a complex relationship between infertility and EP, as one of them could be a cause and the other a consequence. In pregnancies after infertility treatment, the rate of EP increases, which could be because of the effects of the treatment or the pre-existing disorder (1). A recent review has reported the rate of EP after assisted reproduction technologies (ART) cycles were between 2.1 and 8.6% of all clinical pregnancies (2).

The cause of the increased risk of EP in ART cycles is unclear; however, some risk factors such as tubal factor infertility, intracytoplasmic sperm injection (ICSI), assisted hatching, the fresh embryos transfer (ET) versus frozen ET,

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and the day of ET was reported in previous studies (3). EP rupture which occurred due to delay in its early diagnosis is responsible for more than a third of maternal mortality in the first trimester (4). Although unlike, a normal pregnancy, the day of ET in the *in vitro* fertilization (IVF) process is clear and follow-up such as assessment β- hCG titers and transvaginal sonography is performed routinely, Wang et al. (4), reported that delay in detection of EP following ART has occurred in 12.9% of cases.

Low levels of beta human chorionic gonadotropin (β-hCG) in early pregnancy can be associated with poor pregnancy outcomes such as miscarriage, ectopic pregnancy, which can be the result of abnormal placentation (5). Limited studies have shown that β-hCG levels in early pregnancy may be a predictor of IVF outcome (6), but the timing of β-hCG measurements varies in these studies (7), and ideally, each infertility center should provide its own data to determine the cut off for its center (8). Since early detection of pregnancy can reduce anxiety in couples and prevent dangerous consequences such as EP and considering the wide use of ART in the reproduction era. It is clinically valuable to determine EP in infertile patients as soon as possible, so high-risk women can be found at the early phase of abnormal pregnancy and be provided with targeted treatment to preserve their reproductive ability and lessen the incidence of ruptured EP. The present study was designed to detect the predictive value of β-hCG levels 16 days after ET regarding detection of ongoing pregnancy from an EP in ART cycles.

Materials and Methods

Study design

In this cross-sectional study, the Royan Institute database was reviewed that contained clinical and laboratory information on infertility treatment cycles carried out in the department of reproductive and endocrinology from January 2011 to December 2014 as well as from January 2017 to December 2019. The study protocol was approved by the Institutional Review Board and Ethics Committee of Royan Institute (IR.ACECR.ROYAN.REC.1398.156). The reason for selecting this period of time was that the patients' files were more complete based on the required information. The retrospective data was obtained from the registered database (Hakim and Rashen software). All cases with positive β-hCG levels (more than 10 mIU/mL), were screened (n=4149) sixteen days after ET. The pregnancies with oocyte or embryos donation and the multiple pregnancies were excluded from the study based on the first ultrasound. All eligible singleton pregnancies with documented serum β-hCG levels at Royan institute laboratory (n=765) were included and classified according to type of pregnancy (ectopic or ongoing). The diagnosis of all the ectopic pregnancies (EP group) (n=189) was confirmed by trans-vaginal ultrasound. All of eligible ongoing pregnancies (successful pregnancy with gestational age above 20 weeks) (n=576) were classified as non-EP group (Fig.1). The data of the treatment cycle was extracted from the patients' files and no patients' identifiable information was disclosed.

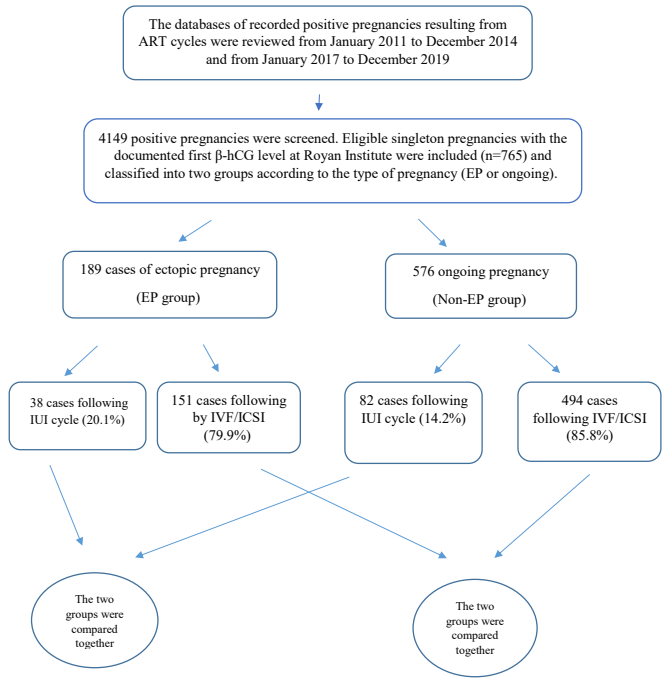

Fig.1: Sampling procedure and the distribution of the EP according to type of treatment cycles. EP; Ectopic pregnancies, ART; Assisted reproductive technology, β-hCG; Beta-human chorionic gonadotropin, IVF; *In vitro* fertilization, ICSI; Intra-cytoplasmic sperm injection, and IUI; Intrauterine insemination.

Treatment protocols and pregnancy follow up

The controlled ovarian stimulation (COS) protocol was selected based on the women's age, ovarian reserve, and previous cycle results. The standard ovarian stimulation protocols for patients included the standard gonadotropin-releasing hormone (GnRH) agonist protocol, GnRH antagonist. The descriptions of different COS protocols and standard procedures of IVF/ICSI as well as the qualification of ET cycles in Royan institute were stated elsewhere (9). Also, the verification method of embryo freezing and frozen ET cycles have been previously explained in detail (10). Luteal phase support in the fresh ET cycles was performed by a 400 mg vaginal progesterone suppository twice a day (Cyclogest®, Actavis, Barnstaple, UK) which was initiated on the evening of the oocyte retrieval day and continued until 10 weeks of pregnancy.

Because of FET cycles, endometrial preparation was done by using the artificial hormonal method with oral estradiol valerate (4-6 mg daily, Aboureihan Co., Iran) administrated from day 2-3 of the menstrual cycle, until the endometrial thickness reached ≥7 mm per two layers. Estradiol and progesterone treatment were prescribed until serum β-hCG level was checked and continued for 10 weeks in viable pregnancies. The serum β-hCG assay was performed routinely 16 days after ET and 17 days after IUI, respectively. It was measured by standard kits (Elecsys reagent kit, Roche Cobas). The demographic and infertility information such as body mass index (BMI), type of assisted reproduction method (IVF/ICSI or IUI), cause of infertility, type of infertility treatment protocol, number of transferred embryos, day of ET (cleavage or blastocyst), type of ET embryo (fresh or frozen) were entered into the statistical software.

Statistical analysis

The statistical analysis was performed by using SPSS Statistics Software (21.0, Statistical Package for the Social Sciences, Inc., Chicago, IL, USA). Also the power of study was measured by placing the mean and standard deviation of the first β-hCG in two groups in the post-hoc power calculator (ClinCalc software) and the analysis showed that the study has 90% power with α level 0.05. The distribution of variables was tested by the Kolmogorov Smirnov test. The Student's t test was used for variables with normal distribution. The comparison of proportions were carried out by using the Chi-square test. The presentation of continuous variables was done by mean ± standard deviation. A receiver operating characteristic (ROC) curve was applied to detect the predictive power of the first measurement of β-hCG level in distinguishing EP from ongoing pregnancy in the ART and IUI cycle that are determined separately. Sensitivity, specificity, positive and negative predictive values (PPV and NPV respectively), area under the ROC curve, and 95% confidence intervals (CIs) were computed for each of the estimates by MedCalc 11.1.1.0 (MedCalc® Statistical Software, Belgium). The multivariable logistic regression was applied for detection of significant predictive factors in the ectopic pregnancy.

Results

In this study, all cases with β-hCG levels >10 (mIU/mL), sixteen days after ET were determined in a specific period of time and then were divided into two groups on the basis of EP diagnosis. 189 pregnancies in the EP group and 3960 pregnancies in the non-EP group were identified. Out of 3960 who registered as ongoing pregnancies, data of 576 pregnancies were randomly matched in terms of maternal age compared with the EP group. Out of 576 cycles with ongoing pregnancy, 489 cycles (85.8%) were related to ART and 82 cycles (14.2%) were related to IUI. Out of the 189 cycles with EP, 151 cycles (79.9%) were ART, and 38 cycles (20.1%) were IUI (Fig.1).

The baseline and clinical characteristics of patients in the ART and IUI cycles were compared in two study groups in Tables 1 and 2, respectively. In 645 ART cycles, no statistically significant difference was observed between the two groups in terms of BMI, duration of infertility, duration of ovulation stimulation, endometrial thickness, number of previous IUI cycles, while the mean total dose of used gonadotropin in the ongoing pregnancy group was significantly lower than that of in the ectopic pregnancy group (P=0.052). The mean level of β-hCG 16 days after ET in the ongoing pregnancy group was statistically higher than that of the ectopic pregnancy group (P<0.0001). The previous history of EP was significantly higher in the ectopic pregnancy group compared to the control group (P=0.005). Regarding the type of ET, no statistically significant difference was noticed between groups (fresh or frozen) (P=0.07), fresh versus frozen. Most patients in the ongoing pregnancy group had primary infertility (76.3%) and there was a statistically significant difference between the two groups in the number of nulligravid cases (P<0.0001). Regarding the cause of infertility, a statistically significant difference was observed between the two groups (P<0.0001) and the number of patients with tuboperitoneal infertility in the EP pregnancy group (13.2%) was significantly higher than that of the ongoing pregnancy group (3.1%). In the ongoing pregnancy group, the number of blastocysts transferred was significantly higher than that of cleavage (77.6 compared to 22.4% respectively), whereas, in the ectopic pregnancy group, 56.2% of all transferred embryos were cleavage compared to 43.8% of the blastocyst transfers. Regarding the ovarian stimulation protocol between the groups under study, no statistically significant difference was seen (Table 1).

Table 1: The comparison of the baseline and clinical characteristics of patients between groups in ART cycles

Variables	Ectopic pregnancy (n=151)	Ongoing pregnancy (n=494)	P value*
BMI at the beginning of the treatment cycle (kg/m^2)	25.0 ± 3.5	25.6 ± 3.6	0.06
Duration of infertility (Y)	7.11 ± 4.44	6.64 ± 4.71	0.28
Duration of ovarian stimulation (Day)	11.05 ± 2.94	10.71 ± 2.54	0.27
Total dose of gonadotropin (IU)	2164.59 ± 144.65	1920.14 ± 80.52	0.052
Endometrial thickness on ET (mm)	9.47 ± 1.69	9.81 ± 1.97	0.14
Number of embryos transferred	2.3 ± 0.46	2.4 ± 0.45	0.1
Day of ET			0.0001>
Day 2 or 3 (cleavage stage)	85 (56.3)	112 (22.6)	
Day 4 or 5 (blastocyst stage)	66 (43.7)	382 (77.4)	
β-hCG level 16 days after ET (mIU/mL)	369.69 ± 61.50	1592.35 ± 87.00	0.0001>
Number of the previous IVF cycles	1.1 ± 0.11	0.78 ± 0.05	0.006
Number of previous IUI cycles	0.79 ± 0.09	0.70 ± 0.05	0.41
The number of previous miscarriages	0.3 ± 0.06	0.17 ± 0.02	0.017
Number of previous EP			0.0001>
No history	127 (84.1)	479 (96.9)	
1 time	19 (12.6)	12 (2.5)	
2 times	5 (3.3)	3 (0.6)	
Nulliparity			0.1
Yes	126 (83.4)	433 (87.7)	
No	25 (16.6)	61 (12.3)	
Nulligravidity			0.01
Yes	91 (60.2)	372 (75.3)	
No	60 (39.8)	122 (24.7)	
Type of embryo transfer			0.07
Fresh	76 (50.3)	290 (58.7)	
Frozen	75 (49.7)	204 (41.3)	
Type of infertility			0.0001>
Primary	91 (60.2)	376 (76.2)	
Secondary	60 (39.8)	118 (23.8)	
Causes of infertility			0.001
Ovulatory factor	48 (31.8)	179 (36.2)	
Tuboproteinal factor	20 (13.2)	15 (3.0)	
Unexplained factor	11 (7.3)	48 (9.7)	
Male factor	56 (37.1)	169 (34.3)	
Mixed factors	16 (10.6)	83 (16.8)	
Type of ovarian stimulation protocol			0.5
Long	97 (64.2)	254 (51.4)	
Antagonist	31 (20.5)	176 (35.6)	
Other protocols	23 (15.3)	64 (13)	

Data are presented as mean ± SD or n (%). ART; Assisted reproductive technology, BMI; Body mass index, ET; Embryo transfer, β-hCG; Beta-human chorionic gonadotropin, IVF; *In vitro* fertilization, IUI; Intra uterine insemination, EP; Ectopic pregnancy, and *; Obtained by independent sample t test and chi square test, statistically significant differences at 0.05.

Regarding the IUI cycles, there was no statistically significant difference between groups in terms of BMI, duration of infertility, duration of ovulation stimulation, gonadotropin dose, endometrial thickness, and the number of cycles. The significant differences were not observed in terms of the number of previous IUI cycles, abortion, and EP as well as the number of nulliparous and nulligravida cases between the two groups (Table 2).

Table 2: The comparison of the baseline and clinical characteristics of patients between groups in IUI cycles

Variables	Ongoing pregnancy (n=82)	Ectopic pregnancy (n=38)	P value*
BMI at the beginning of the treatment cycle (kg/m^2)	26.7 ± 3.2	26.2 ± 3.4	0.08
Duration of infertility (Y)	4.73 ± 3.97	5.7 ± 3.69	0.66
Duration of ovarian stimulation (day)	10.27 ± 2.49	10.31 ± 3.48	0.95
Total dose of gonadotropin (IU)	506.25 ± 71.26	702.2 ± 167.24	0.2
Endometrial thickness on IUI day (mm)	8.53 ± 1.18	8.23 ± 1.45	0.39
Level β-hCG 17 days after IUI (mIU/mL)	196.7 ± 58.1	215 ± 49.1	0.7
Number of the previous IUI cycles	0.62 ± 0.13	0.71 ± 0.17	0.67
The number of previous miscarriages	0.35 ± 0.09	0.35 ± 0.11	0.98
Number of previous EP			0.09
No history	82 (100)	35 (92.1)	
1 time	0 (0)	2 (5.2)	
2 times	0 (0)	1 (2.7)	
Nulliparity			0.41
Yes	68 (83)	34 (89.5)	
No	14 (17)	4 (10.5)	
Nulligravidity			0.81
Yes	54 (66.7)	25 (65.7)	
No	28 (33.3)	13(34.3)	0.88
Type of infertility			
Primary	58 (61.7)	24 (63.1)	
Secondary	24 (29.3)	14 (36.9)	
Causes of infertility			0.23
Ovulatory factor	30 (36.6)	15 (39.5)	
Unexplained factor	23 (28.1)	10 (26.3)	
Male factor	26 (31.7)	8 (21.1)	
Mixed factors	3 (3.6)	5 (13.1)	

Data are presented as mean ± SD or n (%). BMI; Body mass index, ET; Embryo transfer, β-hCG; Beta-human chorionic gonadotropin, IUI; Intra uterine insemination, EP; Ectopic pregnancy, and *; Obtained by independent sample t test and Chi square test, statistically significant differences at 0.05.

As shown in Figure 2, in ART cycles, the β-hCG threshold for distinguishing ongoing pregnancy from EP was 277 IU/l with a specificity of 72.9% (95% CI: 68.7-76.8) and a sensitivity of 67.5% (95% CI: 59. 5-74.9). The PPV was 40% (95% CI: 34.4-46.0) and the NPV was 93.8% (95% CI: 84.4-97.6). The accuracy of predicting the probability of ongoing pregnancy from non-ongoing pregnancy was 77.5% with a standard error of 0.02 and the confidence interval was 74.1 - 80.1% (P<0.0001, Fig.2).

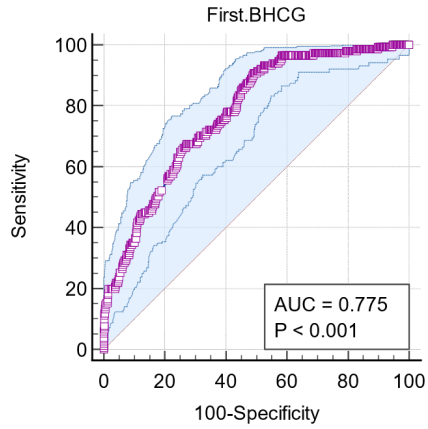

Fig.2: Receiver-operating characteristic curve of serum β-hCG levels 16 days after embryo transfer to distinguish between ongoing pregnancies and ectopic pregnancies in ART cycles [area under cure (AUC): 0.775, 95% CI: 0.74-0.80, P<0.001]. β-hCG; Beta-human chorionic gonadotropin and ART; Assisted reproductive technology.

In IUI cycles, the area under the curve was 0.57, which indicates the first measurement of β-hCG level had no significant power for differentiating ongoing pregnancy from EP (P=0.2, Cl: 0.45-0.69, Fig.3).

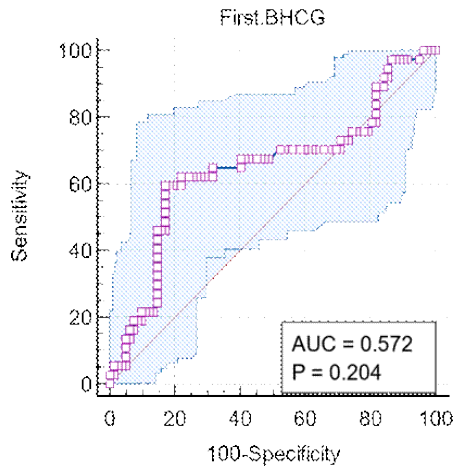

Fig.3: Receiver-operating characteristic curve of serum beta-human chorionic gonadotropin (β-hCG) levels 17 days after intrauterine insemination (IUI) to distinguish between ongoing pregnancies and ectopic pregnancies [area under curve (AUC): 0.57, 95% CI:0.55-0.73, P=0.2].

Discussion

Our results suggest that in IVF/ICSI cycles, the first detected β-hCG level (16 days after ET) can be predictive of ectopic pregnancy, and in cases with β-hCG level, less than 278 IU/L close follow-ups are recommended. However, this relationship was not found in IUI cycles.

β-hCG, as a sign of pregnancy progression, is detectable in the maternal blood serum as soon as day 8-9 of fertilization. Hence, detecting the level of β-hCG has been utilized as an index to note the IVF cycle outcome in order to ease the couple's anxiety and assist the physicians for assessing such high-risk pregnancies. The prevalence of pregnancy complications like ectopic pregnancy, blighted ovum, and missed abortion are more than that of

natural pregnancies (4, 11). It is well documented that the different hormonal milieu created by ovulation induction with gonadotropins may interfere with tubal function and embryo transport. Moreover, it is also confirmed that junctional zone contractions can be responsible for this as well. Lesny et al. (12) demonstrated that the contractions in the junctional zone and consequently the strong endometrial waves in the fundal area of the uterus caused by a difficult ET can shift mock embryos into the fallopian tubes in their studied oocyte donors.

The embryos are transferred directly into the uterus cavity through IVF treatment. In theory, the incidence of ectopic pregnancy after IVF treatment should be lower than that of natural pregnancies. Nevertheless, because of the higher rate of tubal infertility problems among those who are seeking IVF treatment, it is logical that the rate of EP is reported to be much higher in IVF cycles in comparison with spontaneous conception. IVF process by itself puts a lot of stress on couples. There is a challenging time between the initial positive pregnancy result and the first ultrasound revealing viable pregnancy for families. By the means of some serum markers or tests, we can predict the pregnancy outcome to help the clinician to make a better decision for the patient's situation and relieve the couple (7).

For the ongoing pregnancy, the threshold value to exclude patients with safety is 278 IU/L (sensitivity 72.8%, specificity 67.5%). The standard error of our study at this cut-off level was around 0.02 and its confidence interval was 73.8-81.7.% The study conducted by Kim have taken a cut-off value of serum β-hCG on day 11 after ET is significantly lower in the early pregnancy loss group, and 50 mIU/mL was considered a cut-off for the group with promising pregnancy results (11). Naredi et al. (13), showed that a satisfactory pregnancy outcome (beyond 12 weeks) can be achieved by higher levels of initial β-hCG while they were analyzing the outcomes of two different levels of β-hCG.

The present study is consistent with the worldwide experience and emphasizes the need for prognostic information to be given to patients as soon as pregnancies are diagnosed. In this study, we report evidence that day 16 hCG values routinely obtained on IVF-GIFT patients hold significant prognostic information. β-hCG shows placental functional activity and a low level of β-hCG is related to poor pregnancy outcomes. The comparison among causes of infertility in our study demonstrates that the number of patients with the diagnosis of tuboperitoneal infertility was significantly higher (13.2%) in the EP group compared with those of the ongoing pregnancy group (3.1%). This was consistent with the study of Ribic-Pucelj et al. (14) conducted on 8083 cycles, and the most frequent cause of ectopic pregnancies in IVF-ET cycles was (95.4%) tubal factor, followed by (2.3%) unexplained.

In the study of Bu et al. (15) in 2016, the risk factors for EP following IVF in 712 women were examined. They reported an odds ratio (OR) of 3.99 for women with tubal factor infertility compared to those with other infertility causes. Regarding the infertility category, the rate of ectopic pregnancy in tuboperitoneal group was higher than that of ongoing pregnancy. We presume that in such cases the most likely cause is the underlying pathology, which could not necessarily be diagnosed with routine diagnostic procedures such as HSG and laparoscopy.

It is possible that the freezing and thawing process may damage the trophoblast, thereby adversely affecting implantation, embryonic development, and β-hCG production. However, in comparison between FET and fresh ET, there was no statistically significant difference in β-hCG levels between ongoing and ectopic pregnancy groups in our study. Reljič et al. (16) performed a detailed analysis on 775 (51.5 %) cycles; 568 after fresh ET and 207 after FET in which statistics demonstrate comparable results in biochemical pregnancy, spontaneous abortion, and rate of ectopic pregnancy. On the other hand, the live birth rate was statistically higher after fresh ET than that of FET. There was no statistically significant difference at the level of mean β-hCG between these two groups. Moreover, 496 IU/L was determined as an ideal threshold level with good sensitivity and specificity for anticipating live birth post ET compared to 527 IU/L for the FET group (10). On the other hand, Poikkeus et al, compared the β-hCG level of 290 fresh versus 72 frozen embryo cycles that resulted in viable singleton pregnancies that was found no difference in median HCG values (114 versus 115 IU/I) between groups (17). Studies investigating β-hCG levels after fresh ET and FET cycles present inconsistent results so it is impossible to use these findings in predicting pregnancy outcomes after FET. It has been hypothesized that several factors may affect β-hCG level after transfer. In method of freezing (vitrification compared to slow freezing) in FET cycles, and treatment protocol (the effect of gonadotropin level on endometrial maturation) are some factors that may have an impact on β-hCG levels in a transfer cycle.

One time measuring of β-hCG on predicting pregnancy outcome has been investigated. The study conducted by Bjercke et al. (18) showed that the level of β-hCG of more than 150 IU/L, 14 days after ET, with a sensitivity and specificity of 79 and 78% respectively identifying between viable and non-viable pregnancies. Comparably, in the study conducted by Bjercke et al. (18), β-hCG levels more than 55 IU/l, 12 days after ET had a 90% association with viable pregnancy results.

The present study was the first study in Iran on this topic which was its strength. Our study is limited by its retrospective nature. Whether the population under study represents the general IVF women is not clear. However, there were considerable similarities in terms of demographics and clinical characteristics between the participants in our study compared to that of other studies. Moreover, it is assumed that the different assays we applied to measure the level of β-hCG may have an impact

on the β-hCG level reported. Therefore, physicians need to be aware of this in their consultations with patients. Prospective studies are needed to predict pregnancy outcomes for IUI and IVF/ICSI based on reliable cut-off values.

Conclusion

This study implies that the higher the initial production of β-hCG, the more biologically efficient and ultimately, a better chance to get as far as the stage of ongoing pregnancy, and we found that, a one-time measurement of β-hCG at an early stage can significantly aid to spot pregnancies that extend to a minimum 12 weeks of gestation. Based on our study, if β-hCG levels were above 278 IU/l on day 16, intensive follow-up is not required in the absence of danger signs, and if βHCG levels are below 278 IU/l on day 16, close follow-up is recommended, such as weekly β-hCG titers and an ultrasound evaluation until either the diagnosis of EP or miscarriage is established. This particular outcome can aid physicians with analyzing early βhCG, assist with calming stressed patients until they receive their IVF cycle treatment results, and help comfort couples about whether it is more likely for the pregnancy to be successful or not.

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Authors' Contributions

S.H.H., F.Gh.; The conception and interpretation of data. S.H.H., S.V., A.A.; Design of the work. Z.Z., S.V., S.K.; The acquisition and analysis. S.H.H., A.A., F.Gh.; Have drafted the manuscript and revised it. All authors have read and approved the manuscript.

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