Factors Affecting Initial Beta-HCG Values in Pregnancies Achieved by Assisted Reproductive Techniques

ART Gebeliklerinde Başlangıç Beta-HCG Değerlerini Etkileyen Faktörler

Müberra NAMLI KALEM,[a] Ziya KALEM,[b] Timur GÜRGAN[b]

[a]Clinic of Obstetrics and Gynecology, Liv Hospital,
[b]Gürgan Clinic Women's Health and IVF Center, Ankara

ABSTRACT

Objective: The purpose of this study is to investigate the parameters of female, male and cycles that could affect post-ART (assisted reproductive techniques)-pregnancy initial beta hCG value. **Material and Methods:** In this retrospective study, data of 3056 individuals who had become pregnant with IVF/ICSI at a private IVF center and between 2006 and 2016 and whose pregnancies continued for over 12 weeks were evaluated. Initial beta-hCG values of these pregnancies were compared with parameters of mothers, fathers and cycles. **Results:** It was seen in this study that there is a negative correlation between the initial beta-hCG values of pregnancies achieved by assisted reproductive techniques and mothers BMI values and a positive correlation between those beta-hCG values and the embryo transfer day. **Conclusion:** The data obtained in the study need to be supported with larger series in the future. It is also thought that these data may contribute to the literature which tries to achieve a β-hCG cut-off value in the determination of pregnancy outcome.

Key Words: HCG-beta (109-145); fertilization in vitro; body mass index; infertility, female

ÖZET

Amaç: Bu çalışmanın amacı ART gebeliklerinde başlangıç beta-hcg değerlerini etkileyebilecek olan kadın,erkek ve siklusa ait parametrelerin araştırılmasıdır. **Gereç ve Yöntemler:** Bu amaçla, özel bir IVF kliniğinde 2006-2016 yılları arasında yapılan ART uygulaması sonrası gerçekleşen ve 12 haftadan daha uzun süre devam eden 3056 gebeliğe ait veriler retrospektif olarak incelenmiştir. **Bulgular:** Çalışmanın sonucunda ART gebeliklerindeki başlangıç beta hcg değerlerinin annenin vücut kitle indeksi ile negatif korelasyonu olduğu ve embryo transfer günü ile pozitif korelasyonu olduğu izlenmiştir. **Sonuç:** Bu çalışmadaki verilerin gelecekte daha geniş kapsamlı çalışmalar ile desteklendiği takdirde literatürde gebeliğin akıbetini belirlemek için sürdürülen β-hCG cut-off belirleme çalışmalarına katkısı olabileceğini düşünmekteyiz.

Anahtar Kelimeler: HCG-beta (109-145); in vitro fertilizasyon; beden kitle indeksi; infertilite, kadın

β-hCG (beta-human chorionic gonadotrophin) is a pregnancy-associated molecule produced by implanted blastocysts and trophoblastic cells. β-hCG as one of the earliest embryonal signals may be a major regulator in the paracrine embryo-endometrial communication.[1,2]

β-hCG measurement in serum is a method used routinely for pregnancy test after the IVF (invitro fertilization) procedure.[3] Serial measurements of β-hCG is a marker which is also used for distinguishing pregnancies running their course from ectopic pregnancy or spontaneous pregnancy losses in early pregnancy.[4-6] In increasing number of recent studies, it has become a current issue that β-hCG levels in early

pregnancy may be a marker for designating the outcome in future phases of the pregnancy.[7-9] Cut-off studies are also conducted so the post-IVF initial β-hCG value can be used in the prediction of pregnancy success.[8,9]

Processes of implantation, achieving pregnancy and maintenance of pregnancy are ensured with sufficient trophoblastic activity in the beginning of the pregnancy.[10] Negative impacts of factors such as age and BMI on sperm-oocyte-embryo quality and endometrial receptivity may cause insufficiency in the trophoblastic activity.[11,12]

This study was conducted in consideration of the fact that changes in the trophoblastic activity may reflect on β-hCG values in early pregnancies. The purpose of this study is to investigate the parameters of female, male and cycles that could affect post-ART (assisted reproductive techniques) -pregnancy initial β-hCG value.

MATERIAL AND METHOD

In this retrospective study, data of 3056 individuals who had become pregnant with IVF/ICSI at a private IVF center and between 2006 and 2016 and whose pregnancies continued for over 12 weeks were evaluated. Initial β-hCG values of these pregnancies were compared with parameters of mothers, fathers and cycles. Those who had OHSS during the controlled induction of ovulation, patients who were applied with the IVF/ICSI with single oocyte, patients to whom embryos were transferred after having been accumulated in a few cycles and frozen-thawed cycles were not included in the study.

On the 12th day following the embryo transfers, β-hCG values of the patients were accepted to be the initial β-hCG values. The formulation "patient's weight (kg)/height * height (m²)" was used for the calculation of the body mass index (BMI). FSH on the second or third day of the treatment cycle was accepted to be the basal FSH. Endometrial thickness on the day when hCG would be applied for ovulation induction was taken as basis and the estradiol level on the same day was accepted to be E_2 max.

STATISTICAL ANALYSES

Results of 3056 individuals were evaluated in the study. The study was designed as follows: The study was divided into three titles: singleton, twin and triplet pregnancies, and separate analyses were performed under those titles. Shapiro-Wilks tests was applied as the normality test to the initial β-hCG value, which is the continuous parameter and it was decided which parametric or non-parametric tests would be applied on the basis of groups.

Female age, male age, numbered of transferred embryos, male factor and whether there was PCOS were compared with the β-hCG parameter on the basis of the group with parameters on the embryo transfer day in the Kruskal-Wallis non-parametric test. Dual-comparison Bonferroni correction test was applied to the parameter which was found to be significant. It was compared in the Mann Whitney U test if the outcome parameter significantly differed by female age older and younger than 35 years of age, male age older and younger than 39 years of age, BMI, endometrial thickness, basal FSH and E_2 max. A correlation analysis was performed between the β-hCG parameter and female age, male age, BMI, endometrial thickness, basal FSH and E_2 max values and a Spearman's Rho Coefficient was assigned.

IBM SPSS Statistics 21.0 (IBM Corp. Released 2012. IBM SPSS Statistics for Windows, Version 21.0. Armonk, NY: IBM Corp.) software program was used for the statistical analyses and the calculations.

RESULTS

It was found that 63.8% (n=1952) of the participants had a singleton pregnancy, 32.2 (n=987) had a twin pregnancy, and 3.82% (n=117) had a triplet pregnancy.

RESULTS FOR SINGLETON PREGNANCIES (n= 1952):

When evaluating median and IQR (Inter-Quartile Range) values of singleton pregnancies' initial β-hCG results on the basis of female age, male age, number of transferred embryos, presences/absence of male factor, presence/absence of PCOS, they did

TABLE 1a: Comparison of β-hCG values by the parameters.			
Parameters	β-hCG value	Test Statistics	
Female Age Group	Median (IQR)	Z; *	p
Younger than 35 years of age (n=1363)	200.50 (195.25)	0.098	0.922
Older than 35 years of age (n=589)	207.00 (197.00)		
Male Age Group			
Younger than 39 years of age (n=1405)	203.00 (195.00)	0.393	0.694
Older than 39 years of age (n=547)	202.50 (195.25)		
Number of transferred embryos			
1 embryo (n=1174)	196.00 (195.25)	4.421	0.620
2 embryo (n=671)	200.00 (197.00)		
3 and more embryos (n=107)	215.00 (188.75)		
Male factor			
Yes (n=859)	175.50 (249.25)	0.782	0.434
No (n=1093)	203.00 (194.00)		
PCOS			
No (n=1585)	171.00 (172.00)	0.303	0.762
Yes (n=367)	203.00 (196.00)		
Day of embryo transfer			
Day 2 (n=71)	128.00 (126.75)	137.789	*<0.001*
Day 3 (n=1003)	198.00 (179.00)		
Day 4 (n=95)	267.00 (224.00)		
Day 5 (n=780)	375.50 (410.00)		
Day 6 (n=3)	293.00 (-)		

*Mann Whitney U and Kruskal-Wallis non-parametric tests were performed.

TABLE 1b: Post-hoc test for the β-hCG parameter which was found to be significant by transfer day.	
β-hCG* Transfer Day	**p**
Days 2-3	*<0.001*
Days 2-4	*<0.001*
Days 2-6	1,000
Days 2-5	*<0.001*
Days 3-4	*0.003*
Days 3-6	1,000
Days 3-5	*<0.001*
Days 4-6	1,000
Days 4-5	*0.031*
Days 6-5	1.000

*Post-hoc dual-comparison Bonferroni correction was applied.

not differ statistically and significantly (p=0.922, p=0.694, p=0.620, p=0.434 and p=0.762 respectively) (Table 1a).

A statistically significant difference was found among medians of β -hCG results values on the basis transfer days (p=0.001) (Table 1a). The groups of transfer day which caused the difference were found to be days 2-3, days 2-4, days 2-5, days 3-4, days 3-5 and days 4-5 (p<0.001, p<0.001, p<0.001, p=0.003, p<0.001, p=0.031 respectively) (Table 1b).

A correlation analysis was conducted between the β -hCG values of the singleton pregnancies and the parameters given in Table 2 and a negative, weak and linear relationship was found only between BMI and the β-hCG parameter (p<0.001) (Table 2).

TABLE 2: The correlation analysis between the β-hCG parameter and the parameters stated.		
Parameters * β-hCG	**Spearman's Rho Coefficient**	**p**
Male age	0.006	0.821
Female age	-0.015	0.570
BMI	-0.132	*<0.001*
Endometrial thickness	0.003	0.908
Basal FSH	0.000	0.994
E2 max	-0.016	0.651

*Correlation analysis was performed.

TABLE 3a: Comparison of β-hCG values by the parameters stated.			
Parameters	β-hCG value	Test Statistics	
Female Age Group	Median (IQR)	Z; *	p
Younger than 35 years of age (n=741)	429.00 (378.00)	0.389	0.697
Older than 35 years of age (n=246)	407.00 (410.50)		
Male Age Group			
Younger than 39 years of age (n=768)	428.50 (388.75)	0.924	0.356
Older than 39 years of age (n=219)	416.00 (376.00)		
Number of transferred embryos			
2 embryo (n=829)	464.00 (401.50)	6.558	0.143
3 and more embryos (n=158)	438.50 (400.75)		
Male factor			
Yes (n=267)	383.00 (422.50)	0.221	0.825
No (n=720)	428.00 (384.50)		
PCOS			
Yes (n=196)	552.00 (445.00)	1.234	0.217
No (n=791)	421.00 (384.50)		
Day of embryo transfer			
Day 2 (n=34)	276.00 (266.25)	87.720	*<0.001*
Day 3 (n=576)	415.00 (363.25)		
Day 4 (n=59)	544.00 (435.00)		
Day 5 (n=312)	733.50 (559.00)		
Day 6 (n=6)	1000.50 (1535.00)		

*Mann Whitney U and Kruskal-Wallis non-parametric tests were performed.

TABLE 3b: Post-hoc test for the β-hCG parameter which was found to be significant by transfer day.	
β-hCG* Transfer Day	p
Days 2-3	*<0.001*
Days 2-4	*<0.001*
Days 2-5	*<0.001*
Days 2-6	*<0.001*
Days 3-4	0,957
Days 3-6	*0,010*
Days 3-5	*<0.001*
Days 4-5	0,060
Days 4-6	0,146
Days 5-6	1.000

*Post-hoc dual-comparison Bonferroni correction was applied.

RESULTS FOR TWIN PREGNANCIES (n= 987)

When evaluating median and IQR values of singleton pregnancies' initial β-hCG results on the basis of female age, male age, number of transferred embryos, presences/absence of male factor, presence/absence of PCOS, they did not differ statistically and significantly (p=0.697, p=0.356, p=0.143, p=0.825 and p=0.217 respectively) (Table 3a).

A statistically significant difference was found among medians of β-hCG result values on the basis transfer days (p=0.001) (Table 3a). The groups of transfer day which caused the difference were found to be days 2-3, days 2-4, days 2-5, days 2-6, days 3-5 and days 3-6 (p<0.001, p<0.001, p<0.001, p=0.001, p<0.010, p=0.001 respectively) (Table 3b).

A negative, weak, linear and statistically significant relationship was found between participants' BMI values and their β-hCG values (p=0.023). As BMI values increase/decrease, β-hCG value decreases/increases (rho: -0.084).

A negative, weak, linear and statistically significant relationship was found between participants' E_2 max values and their β-hCG values (p=0.009). As E_2 max values increase/decrease, outcome increases/decreases, too (rho: 0.113) (Table 4).

TABLE 4: The correlation analysis between the β-hCG parameter and the parameters stated.

Parameters * β-hCG	Spearman's Rho Coefficient	p
Male age	-0.025	0.493
Female age	-0.027	0.472
BMI	-0.084	*0.023*
Endometrial thickness	0.026	0.482
Basal FSH	-0.064	0.283
E2 max	-0.113	*0.009*

*Correlation analysis was performed.

RESULTS FOR TRIPLET PREGNANCIES (n = 117)

In the triplet pregnancy group, β-hCG median was 530.00 (IQR=491.75) for the female participants who were younger than 35 years of age and 445.00 (IQR=646.00) for the female participants who were older than 35 years of age. The female participants who were younger than 35 years of age had higher β -hCG median, but it did not differ statistically and significantly because the number of subjects in the groups was imbalanced (p=0.545) (Table 5a).

β-hCG median was 417.00 (IQR=280.75) for the male participants who were older than 39 years of age and 546.00 (IQR=617.00) for the male participants who were younger than 35 years of age. β -hCG values caused a statistically significant difference on the basis of the male age group (p=0.031). In the group of the male participants who were older than 39 years of age had low β-hCG median than the other group (Table 5a).

Outcome medians of the individuals were found to be 457.00 (IQR=580.25) for those whose transfer days were day 2, 453.00 (IQR=387.00) for those whose transfer days were day 3, 622.00 (IQR=498.50) for those whose transfer days were day 4 and 1535.00 (IQR=1296.00) for those whose transfer days were day 5 respectively. A statistically significant difference was found among medians of outcome values on the basis transfer days (p=0.001) (Table 5a). The groups of day which caused this difference were days 3-5 and days 2-5 (p<0.001, p=0.012 respectively) (Table 5b).

A negative, weak, linear and statistically insignificant relationship was found between participants' BMI values and their β-hCG values (p=0.687) (Table 6).

TABLE 5a: Comparison of β -hCG values by the parameters.			
Parameters	Outcome	Test Statistics	
Female Age Group	Median (IQR)	Z; *	p
Younger than 35 years of age (n=98)	530.00 (491.75)	*0.606	*0.545
Older than 35 years of age (n=19)	445.00 (646.00)		
Male Age Group (39 years old and older)			
Younger than 39 years of age (n=93)	546.00 (617.00)	2.157	*0.031*
Older than 39 years of age (n=24)	417.00 (280.75)		
Number of transferred embryos			
2 embryos (n=9)	495.50 (534.75)	1.758	0.881
3 and more embryos (n=108)	548.00 (504.75)		
Male factor			
Yes (n=42)	528.50 (598.25)	0.099	0.921
No (n=75)	507.00 (504.50)		
PCOS			
Yes (n=39)	416.50 (699.75)	0.540	0.589
No (n=78)	524.00 (509.00)		
DAYET (Transfer Day)			
Day 2 (n=6)	457.00 (580.25)	22.674	*<0.001*
Day 3 (n=86)	453.00 (387.00)		
Day 4 (n=6)	622.00 (498.50)		
Day 5 (n=19)	1535.00 (1296.00)		

*Mann Whitney U and Kruskal-Wallis non-parametric tests were performed.

TABLE 5b: Post-hoc test for the β-hCG parameter which was found to be significant by transfer day.

β-hCG* Transfer Day	p
Days 2-3	1.000
Days 3-4	0.891
Days 3-5	*<0.001*
Days 2-4	1.000
Days 2-5	*0.012*
Days 4-5	0.942

*Post-hoc dual-comparison Bonferroni correction was applied.

TABLE 6: The correlation analysis between the β-hCG parameter and the parameters stated.

Parameters * β -hCG	Spearman's Rho Coefficient	p
Male age	-0.087	0.349
Female age	0.007	0.937
BMI	-0.038	0.687
Endometrial thickness	-0.055	0.557
Basal FSH	0.069	0.658
E2 max	0.129	0.236

*Correlation analysis was performed.

TABLE 7: The correlation analysis between the BMI values β-hCG values in all groups.

	Spearman's Rho Coefficient	p
BMI * β-hCG	-0.049	*<0.001*

The relationship between the BMI values of all the female participants in the study and their β -hCG values was examined and it was found to be a statistically significant relationship. It was determined that there was a negative, weak and linear relationship between the two parameters (Table 7).

DISCUSSION

It was seen in this study that there is a negative correlation between the initial β-hCG values of pregnancies achieved by assisted reproductive techniques and mothers' BMI values and a positive correlation between those β-hCG values and the embryo transfer day. The correlation between BMI and β-hCG was observed to be statistically signifi-

cant in singleton and twin pregnancies, but it was not found to be statistically significant in triplet pregnancies. A negative relationship was observed between female age and β-hCG values, but it was not found to be statistically significant. No relationship was revealed between β-hCG values and presence of male factor, presence of PCOS, number of transferred embryos, endometrial thickness, FSH and E_2 max values.

Initial β-hCG implantation is indicated in ART pregnancies and it is stated to be associated with trophoblastic tissue activity.[13] β-hCG levels may be very high in uncontrolled trophoblastic invasion and low initial β-hCG levels may indicate that pregnancies cannot be implanted.[7,14] In the light of this information, the fact that as BMI increased, hCG levels decreased in this study shows parallelism with the negative effect of BMI on implantation. There are studies which stated that endometrial receptivity and reproductive outcome are broken as BMI increases.[15-17] The fact that the correlation between BMI and β-hCG is broken in the triplet pregnancies in this studies may be caused by the insufficient number of the patients in that group.

It is known that implantation rates decrease as female age and male age decrease and uterine receptivity declines with advanced age.[18,19] In this case, a correlation between female and male age and β-hCG could have been expected; however, this relationship could not be observed in this study. This implies that trophoblastic activity is not affected by age. The same applies to presence of male factor and presence of PCOS. Both are conditions which have been reported to affect implantation negatively.[20,21] β-hCG value and male factor and PCOS could not be associated in this study; likewise, these conditions may not be hindering the trophoblastic activity.

Kathiresan et al. (2011) showed that initial β-hCG level has a prognostic role in pregnancy and it is associated with the embryo transfer day.[22] Oron et al. (2015) revealed that initial hCG levels were higher in blastocyst transfer than cleavage embryo transfers in single embryo transfers and reported that hCG level has a predictive role in pregnancy

achievement.[23] In this study, too, it is seen that as embryo transfer day increased, β-hCG levels increased statistically and significantly.

There are studies revealing the relationship of endometrial thickness and maximum estradiol levels with implantation.[24-26] Nevertheless, no relationship was found in this study between endometrial thickness and maximum estradiol levels and β-hCG value.

Initial β-hCG value and β-hCG increase has been previously associated with early pregnancy outcome. Barnhart et al. (2004) and Fong et al. (2005) revealed that there is a relationship between early pregnancy outcome and β-hCG levels.[27,28] Forat et al. (2007) examined the relationship between long-term pregnancy outcome and hCG.[29] In the following years, studies reporting that initial β-hCG value is in direct proportion to live birth rates have been published.[30,31]

In this study, it is a limitation that the relationship between pregnancy outcome and β-hCG value was not investigated. This study aimed at investigating the factors which can affect initial β-hCG value independently from pregnancy. It is though that it is a first in this sense. The fact that twin and triplet pregnancies as well as single pregnancies were evaluated in separate groups in this study makes it more valuable.

In conclusion, it was shown in the study that initial β -hCG value is in a negative correlation with BMI and in a positive correlation with transfer day in ART pregnancies. A negative relationship was observed between mother's age and β-hCG values, but it was not found to be statistically significant. The data obtained in the study need to be supported with larger series in the future. It is also thought that these data may contribute to the literature which tries to achieve a β-hCG cut-off value in the determination of pregnancy outcome.

Conflict of Interest

No conflict of interest was declared by the authors.

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