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Evaluation of Estradiol on the HCG Trigger Day in Predicting Pregnancy and Neonatal Outcomes of Patients Undergoing IVF/ICSI Treatment: A Retrospective Cohort Study

IVF/ICSI Tedavisi Yapılan Hastalarda Gebelik ve Yenidoğan Sonuçlarının Öngörülmesinde HCG Tetikleme Günündeki Estradiolün Değerlendirilmesi: Retrospektif Kohort Çalışması

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ÖZ

Amaç: Bu çalışmadaki amacımız, insan koryonik gonadotropin (hCG) tetikleme günündeki serum estradiolün (E2) gebelik sonuçları ve yenidoğan doğum ağırlığını tahmin etmedeki rolünü araştırmaktır.

Gereç ve yöntemler: Bu çalışma 22. gebelik haftasından sonra canlı doğum yapan kadınlar (n:417) ile kontrol grubunu oluşturan klinik gebelik tanısı almış kadınları (n:260) içermektedir.

Demografik özellikler kaydedildi. Klinik endikasyonlar, ovulasyon stimülasyon süresi, 3. gün (D3) E2, folikül uyarıcı hormon ve luteinize edici hormon düzeyleri, antral folikül sayısı, stimüle edici ajanların toplam dozları, tetikleme gününde progesteron ve estradiol düzeyleri, toplanan oosit sayısı, matür oosit sayısı, tetikleme ve oosit toplama gününde endometrial kalınlık iki grup arasında karşılaştırıldı. Canlı doğum ile sonuçlanamama riskine etki eden faktörleri belirlemek için Binary Logistic Regresyon, (Backward LR modeli) kullanıldı.

Bulgular: Toplam 677 gebe çalışmaya dahil edildi. Tetikleme gününde E2 düzeyi yüksek olan gebelerde canlı doğum oranı daha yüksek bulundu. Tetikleme günündeki E2 düzeyi ile yenidoğan doğum ağırlığı arasında istatistiksel olarak anlamlı bir ilişki izlendi. (p=0,005). Erkek faktörü ve kadın yaşının canlı doğum ile sonuçlanamama durumunu etkileyen en önemli parametreler olduğu görüldü (p<0.05). Kadın yaşının 1 birim artması ile canlı doğum ile sonuçlanamama riskinin %5 artacağı bulundu.

Sonuç: Tetikleme gününde E2 düzeyi arttıkça, canlı doğum oranı artarken yenidoğan doğum ağırlığı azalmıştır. Erkek faktörü ve kadın yaşının canlı doğum ile sonuçlanamama durumunu etkileyen önemli parametreler olduğu bulunmuştur.

Anahtar kelimeler: Estradiol, in vitro fertilizasyon, gebelik sonuçları, doğum ağırlığı

ABSTRACT

Aim: Our aim was to investigate the value of serum estradiol (E2) on human chorionic gonadotrophin (hCG) trigger day in predicting pregnancy outcomes and neonatal birth weight.

Materials and Methods: The study sample comprised a group of women who had live birth (labor after 22 gestational week) (n= 417) and a control group with clinical pregnancy (n=260).

Demographic characteristics were recorded. Clinical indications, duration of ovulation stimulation, day 3 (D3) E2, follicle stimulating hormone and luteinizing hormone levels, antral follicle count, total doses of stimulating agents, progesteron and estradiol levels on trigger day, number of oocytes retrieved, number of mature oocytes, endometrial thickness on trigger and oocyte pick-up day were compared between the two groups.

Binary Logistic Regression, (Backward LR model) was used to determine the factors affecting the risk of not having a live birth.

Results: Totally 677 pregnant women were included in the study. We found increased live birth rate in pregnant whose E2 level was higher on the trigger day. There was a statistically significant relation between E2 level on trigger day and newborn weight in live birth (p=0.005) It was identified that the male factor and age of the women are important parameters that impact not having a live birth (p<0.05). When the age of the woman increases by 1 unit, the risk of not having a live birth will increase by 5%.

Conclusion: The E2 level on hCG trigger day increased, the live birth rate increased whilst the newborn birth weight decreased. It was also found out that the male factor and age of the women are important factors that influence not having a live birth.

Keywords: Estradiol, In vitro fertilization, pregnancy outcomes, birth weight

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INTRODUCTION

Increase in assisted reproductive technologies (ART) has enabled the birth of more than 7 million children in the world (1). On the other hand, unfortunately perinatal complications such as low birth weight, small for gestational age, and obstetric complications are more common in in vitro fertilization (IVF) infants than in spontaneously conceived pregnancies (2). Recent studies have shown that supraphysiological estradiol (E2) levels are associated with poor perinatal outcomes due to abnormal placentation as a result of impaired implantation environment and insufficient trophoblastic invasion (3, 4). Although the pathogenesis of the relation between decreased birth weight and increased E2 level is not clearly known, it is thought that abnormal placentation causes this situation (5).

In a recent study by Pereira et al., E2 level > 2500 pg/mL on human chorionic gonadotrophin (hCG) trigger day was a predictor of low birth weight (4). Royster et al. revealed that placenta accreta and placenta previa were more common in patients with E2 level > 3000 pg/mL on the day of the trigger (6). In addition, superovulation can alter the phenotype and fetal development by affecting the epigenetics of the oocyte by imprinted DNA methylation and histone post-translational modifications (7, 8).

Based on the negative effects of supraphysiological E2 levels on implantation and placentation; our aim was to investigate the value of serum E2 on hCG trigger day in predicting pregnancy (clinical pregnancy, missed abortion, live birth) outcomes and neonatal birth weight.

MATERIALS AND METHODS

This was a retrospective study conducted at the in vitro fertilization clinics (IVF) of Etlik Zübeyde Hanım Women's Health Training and Research Hospital, and was carried out with a total of 677 women. The study protocol was approved by the Local Ethics Committee (Clinical study 19.11.2021/13/33). The study sample comprised a group of women who had live birth (labor after 22 gestational week) (n= 417) and a control group with clinical pregnancy (missed abort and anembryonic pregnancy) (n=260).

Exclusion criteria were as follows: controlled ovarian stimulation using mild or natural cycle protocols, freeze-thaw cycles, patients with multiple embryo transfers, multiple pregnancies, preimplantation genetic diagnosis, oocyte and embryo recipients, patients with moderate to severe ovarian hyperstimulation syndrome (OHSS), oocyte retrieval of more than 15 and a history of chronic disease.

Ovarian stimulation was initiated by using gonadotropins (recombinant follicle-stimulating hormone (FSH), Gonal-F® Merck, Germany) or human menopausal gonadotropin (hMG), Menopur®, Ferring Pharmaceuticals, Germany) between 150 to 450 IU daily. Gonadotropin releasing hormon (GnRH) antagonist (141 Cetrotide®, Merck, Germany) and GnRH agonist triptorelin (Gonapeptyl®, Ferring Pharmaceuticals, Germany) were used for preventing luteinizing hormone (LH) surge, dose and the type of ovarian stimulation protocols were determined according to patient characteristics or responses during previous cycles.

Ovarian stimulation was followed up with follicular growth and estradiol (E2) until hCG trigger day, hCG trigger was performed when at least three follicles over 17-18 mm in diameter were detected with ultrasonography. Oocyte retrieval (OPU) was performed 35-36 hours later, via transvaginal aspiration under ultrasound guidance and were inseminated using intracytoplasmic sperm injection. Luteal support was provided with intramuscular progesteron (100 mg daily) and oral dydrogesteron (10 mg 3 times a day) until 12 weeks gestational age.

Demographic characteristics i.e., maternal and paternal ages, body mass index (BMI), gravidity, abortion, and living child were recorded. Clinical indications, pregnancy outcomes (clinical pregnancy, missed abortion, live birth) and neonatal birth weight were compared between the two groups. Duration of ovulation stimulation, day 3 (D3) E2, FSH and LH levels, antral follicle count, total doses of stimulating agents, progesteron and estradiol levels on trigger day, number of oocytes retrieved, number of mature oocytes, endometrial thickness on trigger and OPU day were measured.

E2 and progesteron measurements on the day of hCG trigger were performed in our laboratory using the IMMULITE 2000 Immunoassay System (Siemens, Berlin, Germany). The E2 assay has a detectable range from 5 to 3000 pg/ml. The inter- and intra-assay coefficient of variation (CV) were < %10. The progesteron assay has a detectable range from 0.05 to 60 ng/ml. The inter- and intra-assay coefficient of variation (CV) were < %7.

Statistical Analysis

Statistical analyses were performed using a package program entitled, SPSS (IBM SPSS Statistics 24) and frequency tables and descriptive statistics were used to interpret the findings. Non-parametric methods were utilized for the measurement

values that did not conform to the normal distribution. In accordance with non-parametric methods, the "Mann-Whitney U" test method was exploited to compare the measurement values of two independent groups. Pearson-χ2 crosstabs were resorted to so as to examine the relationships between two qualitative variables. "Spearman" correlation coefficient was preferred to examine the relationship between two quantitative data that did not have a normal distribution. Binary Logistic Regression, (Backward LR model) was adopted to determine the factors affecting the risk of not having a live birth.

RESULTS

Totally 677 pregnant women were included in the present study. Demographic and obstetric characteristics of pregnant women are given in Table 1. We found no significant differences in living child and BMI between the two groups (p>0.05). Gravidity and abortus were significantly higher in control group than in study group (p<0.05). Paternal and maternal age were higher in control group compared with study group (p<0.05) (Table 1).

Table 1: Comparison of demographic characteristics

Variables	Live birth (n=417) Medyan [Min-Max]	Clinical Pregnancy (n=260) Medyan [Min-Max]	P value*
Gravidity	0,0 [0,0-7,0]	0,0 [0,0-7,0]	**0,009**
Abortion	0,0 [0,0-5,0]	0,0 [0,0-6,0]	**0,017**
Living child	0,0 [0,0-4,0]	0,0 [0,0-3,0]	0,177
Paternal age(years)	33,0 [25,0-58,0]	34,0 [21,0-55,0]	**0,014**
Maternal age(years)	30,0 [19,0-44,0]	31,0 [21,0-45,0]	**0,001**
BMI (kg/m^2)	25,5 [15,7-42,7]	25,5 [16,4-41,7]	0,712

* "Mann-Whitney U" test.

There was no significant difference in tubal factor, diminished ovarian reserve and unexplained infertility between the two groups (p>0.05). Male factor was significiantly higher in study group than control group (p<0.05) (Table 2).

Table 2: Comparison of clinical indications

Variables	Live Birth (n=417)		Clinical Pregnancy (n=260)		P value*
	n	%	n	%	
Male factor					
No	256	61,4	192	73,8	**0,001**
Yes	161	38,6	68	26,2	
Tubal factor					
No	385	92,3	246	94,6	0,320
Yes	32	7,7	14	5,4	
Diminished ovarian reserve					
Yes	91	29,9	65	32,7	0,518
No	213	70,1	134	67,3	
Unexplained infertility					
No	294	70,5	165	63,5	0,056
Yes	123	29,5	95	36,5	

* "Pearson-χ2" crosstabs.

There was no significant difference in duration of ovulation stimülation, dosage of gonadotropins, D3 FSH level, D3 LH level, D3 E2 level, D3 antral follicle count, progesteron level on trigger day, antral follicle count on trigger day, endometrial thickness on trigger day, endometrial thickness on OPU day, total oocytes retrieval and total MII oocytes between the groups (p>0.05). Duration of infertility was significantly longer, number of cycle was significantly higher in control group than in study group (p<0.05). E2 level on trigger day was significantly higher in study group than in control group (p<0.05) (Table 3).

Table 3: Outcome of patients undergoing in vitro fertilization

Variables	Live Birth (n=417)	Clinical Pregnancy (n=260)	P value*
	Medyan [Min-Max]	Medyan [Min-Max]	
Duration of infertility(months)	48,0 [2,0-288,0]	60,0 [1,0-240,0]	**0,006**
Duration of ovulation stimülation (days)	10,0 [7,0-15,0]	10,0 [6,0-14,0]	0,713
Number of cycle	1,0 [1,0-6,0]	2,0 [1,0-8,0]	**0,035**
Dosage of gonadotropins(IU)	2025,0 [600,0-4725,0]	2100,0 [775,0-52,50]	0,917
D3 FSH level (mIU/ml)	7,3 [0,5-21,7]	7,3 [1,3-26,9]	0,870
D3 LH level (mIU/ml)	4,6 [0,1-23,1]	4,8 [0,2-20,4]	0,314
D3 E2 level(pg/ml)	43,0 [5,0-286,0]	47,0 [1,1-261,0]	0,199
D3 Antral follicle count	12,0 [0,0-30,0]	11,0 [0,0-30,0]	0,497
Progesteron level on trigger day	9,9 [0,1-51,0]	9,0 [0,2-54,8]	0,103
E2 level on trigger day	2057,0 [127,0-8752,0]	1894,5 [193,0-8380,0]	**0,049**
AF count on trigger day	3,0 [0,0-18,0]	2,0 [0,0-23,0]	0,465
Endometrial thickness on trigger day	10,0 [4,3-16,4]	10,0 [6,0-20,0]	0,586
Endometrial thickness on OPU day	9,9 [0,9-18,8]	9,7 [1,2-18,0]	0,545
Total oocytes retrieval	10,0 [1,0-38,0]	9,0 [1,0-34,0]	0,310
Total MII oocytes	8,0 [1,0-31,0]	7,0 [0,0-26,0]	0,495

* "Mann-Whitney U" test.

We found no significant relation between progesterone level on trigger day and newborn weight in live birth (p>0.05). There was a statistically significant relation between E2 level on trigger day and newborn weight in live birth (p=0.005) (Table 4). We found negative correlation between the number of abortions and live birth rate. Maternal and paternal age were negatively correlated with live birth rate.

Table 4: The relation of E2 and progesteron levels on trigger day with newborn weight in live birth

Live Birth (n=417)	Newborn Weight	
Correlation*	*r*	*p*
Progesteron level on trigger day	-0,053	0,284
E2 level on trigger day	-0,137	**0,005**

* "Mann-Whitney U" test.

As a result of the Backward:LR logistic regression analysis based on the risk of not having a live birth, using all the predictive parameters that could have a significant effect in the univariate analysis; the optimal model is given in Table 5. In the current model; it was determined that the male factor is indeed pivotal that impacts the case of not having a live birth (p<0.05). Those with the male factor have a 59.1% higher risk of not having a live birth than those without male factors. It has been determined that the age of the woman carries gravity towards the status of not having a live birth (p<0.05). When the age of the woman increases by 1 unit, the risk of not having a live birth will increase by 5%.

Table 5: The Logistic Regression model based on the risk of not having a live birth

Variables	B	S.H.	Wald	sd	p	OR	95% Odds Ratio (OR)	
							Min	Max
Male factor*	0,464	0,179	6,748	1	**0,009**	1,591	1,121	2,258
Maternal age	0,049	0,018	7,389	1	**0,007**	1,050	1,014	1,088
Constant	-2,292	0,549	17,450	1	**0,000**	0,101		
*Reference category: yes			CCR=79,7%			$\chi^2_{(8)}$=3,566; p=0,894		

DISCUSSION

In the current study, we percevied that as the E2 level on hCG trigger day boosted, the live birth rate elevated while the newborn birth weight diminished. In addition to these findings, the progesterone level on trigger day was not statistically significant on these parameters.

There are a fair number of risk factors of pregnancy loss: uterin malformations, parental karyotype abnormalities, endocrine disorders, advanced maternal age, thrombophilia panel disorders (9). In a study by Ogasavara, when the number of previous miscarriages enhanced the live birth rate declined (10). In line with the relevant line of literature,

we figured out a negative correlation between the number of abortion and live birth rate. In present study, number of gravidity was lower in pregnant women who reached live birth, to which we ascribed the young maternal age.

Maternal age is verily of direct relation to aneuoploidy, which begins to rise after the age of 30 and escalates sharply after the age of 35 (11), as maternal age increases, the live birth rate decreases (12). Live birth rates for women < 35 years are 42.4%, for 38-40 years are 21.9% (13). The decrease in both quantity and quality of oocytes that related with advanced maternal age, manifested itself with a reduced live birth rate (14). Elder women presumably have longer duration of infertility. Although, there have been many studies that confirmed negative effect of maternal age on ART success, the effect of paternal age did not appear to have an effect along the same lines. In a study by Ghuman, as the sperm donor age increased, the live birth rate decreased (15). In present study, maternal and paternal age were negatively correlated with live birth rate which was consistent with the accumulated literature. In the present study, when the age of the woman increases by 1 unit, the risk of not having a live birth will increase by 5%.

In a study by Mang et al. preimplantation genetic testing for aneuploidy (PGT-A) was used for transferring euploid embryos to investigate whether live birth rates were associated with infertility diagnosis, but no significant differences were arrived at (16). As opposed to a previous study, for the etiology of infertility, the male factor was higher in live birth group in our study.

In tandem with the literature, duration of infertility correlated negatively with the live birth rate in present study, and increased female age could be considered as the reason for this (17). The number of unsuccessful embryo transfers for stopping are uncertain in IVF, commonly stopped after 3 or 4 cycles. As the number of cycle increased, the live birth rate within each cycle decreased which seemed compatible with the present study (18).

When we reviewed the bulk of literature, we have witnessed that the hyperestrogenic milieu exchanges uterine environment that lead to impaired implantation and placentation and adverse perinatal outcomes (19, 20). Differantial expression of the Grb 10 gene and GATA3 transcription factor were the causes of impaired placentation that induced by E2 (21, 22). In contrast, we found increased live birth rate in pregnant women whoose E2 level was higher on the trigger day. This contrast can be explained by excluding oocyte retrieval more than 15 oocytes in present study.

On the other hand, in this study, the negatively correlation between newborn birth weight and increased E2 level were alligned with the findings of the literature. Imudia et al. reported that pregnant with E2 level >3450 pg/ml in fresh IVF cycles, had an increased risk of low birth weight (3), while lower E2 levels of 2042.4 (±802.6) pg/ml did not (23). In the literature, plentiful studies exist on the value of E2 levels but not with progesterone. In present study, there was no correlation between live birth, clinical pregnancy and newborn birth weight with progesterone levels on the trigger day that was in agreement with a previous study (24).

In conclusion, E2 levels on the trigger day show signs of being effective in live birth rate and newborn birth weight howbeit the levels of progesterone do not. Studies of more comprehensive nature are required to be able to confirm the results of this study.

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