ZKTB ORIGINAL RESEARCH

Live Birth and Endometrial Thickness in Unexplained Infertility

Açıklanamayan İnfertilide Endometriyal Kalınlık ve Canlı Doğum

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ABSTRACT

Objective: *We aimed to demonstrate any possible relationship between endometrial thickness on the day of hCG trigger and live birth rates (LBRs) among women with unexplained infertility who underwent IVF/ICSI-ET cycles.*

Material and Methods: *We retrospectively collected data from Zeynep Kamil Women's and Children's Disease Training and Research Hospital, IVF Center archive. Cases between 2005 and 2013 were collected. Women aged between 23-39 years with a BMI <30 kg/m2 with fresh embryo transfers were included. Patients were divided into two groups based on their livebirth status (live birth: group 1, no live birth: group 2). Demographic characteristics, treatment regimens, and endometrial thickness on the day of hCG trigger were compared between the two groups. In addition, patients were divided into subgroups according to the endometrial thickness on the day of hCG trigger (≤7 mm, 8 mm, 9 mm, 10 mm, 11 mm, 12 mm, 13 mm, and ≥14 mm, respectively). LBRs were compared between these subgroups.*

Results: *Three hundred fifty-nine cycles (group 1: n=104, group 2: n=255) were included for statistical analysis. Other than estradiol level (pg/mL) on the day of hCG trigger (2517.2±1106.0, 2210.8±991.7, respectively; p=0.011), there were no statistically significant differences between the two groups. Among the subgroups based on endometrial thickness, the highest LBR was detected in the 13 mm subgroup (36.8%) and lowest LBR was detected in 12 mm subgroup (23.9%). However, LBRs were not statistically significant between the subgroups.*

Conclusion: *LBRs do not seem to be affected by endometrial thickness on the day of hCG trigger among couples with unexplained infertility.*

Keywords: *unexplained infertility, in-vitro fertilization, endometrial thickness, live birth rate*

ÖZET

Amaç: *Açıklanmayan infertilide human koryonik gonadotropin (hCG) günü ölçülen endometriyal kalınlık ile canlı doğum oranları arasında ilişki olup, olmadığını araştırmaktır.*

Gereç ve Yöntemler: *Bu araştırma 2005-2013 yılları arasında Zeynep Kamil Kadın ve Çocuk Hastalıkları Eğitim ve Araştırma hastanesinde tüp bebek tedavisi uygulanmış hastalarda yapılan retrospektif bir çalışmadır. Çalışmaya 23-39 yaş aralığında, vücut kitle indeksi 30 kg/m2 altında olan ve embriyoda herhangi bir işlemin yapılmadığı ve taze embriyo transferi yapılmış kadınlar dahil edildi. Hastalar canlı doğum yapıp (grup 1), yapmamalarına (grup 2) göre 2 gruba ayrıldı. hCG günü 2-boyutlu ultrason ile midsagittal planda ölçülen endometriyal doku kalınlığı, siklus tedavi karakterleri ve hastaya ait demografik karakterler gruplar arasında karşılaştırıldı. Ayrıca hCG günü ölçülen endometriyal doku kalınlığına göre; 7 mm ve altı, 8 mm, 9 mm, 10 mm, 11 mm, 12 mm, 13 mm ve 14 mm ve üstü olan subgruplar oluşturuldu. Bu subgruplar arasında canlı doğum yüzdeleri açısından karşılaştırmalar yapıldı. İstatistiksel karşılaştırmada devamlı veriler için student t-testi ve kategorik değerler için ki-kare testi kullanıldı.*

Bulgular: *Çalışmaya 359 hasta dahil edildi, 104 hasta grup 1 ve 255 hasta grup 2. Gruplar arasında hCG günü ölçülen estradiol seviyeleri (pg/ml) dışında fark yoktu (2517.2±1106.0 ve 2210.8±991.7, sırasıyla; p=0.011). Her bir endometriyal doku kalınlığından oluşturulan subgruplar arasında en yüksek canlı doğum oranı 13 mm de %36.8 iken, en düşük canlı doğum oranı 12 mm de %23.9 idi. Fakat subgruplar arasında canlı doğum oranları açısından istatistiksel olarak bir fark bulunamadı.*

Sonuç: *Açıklanamayan infertilitede canlı doğum oranları hCG günü ölçülen endometriyal doku kalınlığından bağımsızdır.*

Anahtar Kelimeler: *açıklanamayan infertilite, tüp bebek, endometriyal kalınlık, canlı doğum oranı*

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INTRODUCTION

Studies are continuing to increase sustainable success in in-vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI) treatment cycles, which are widely available. One of the important factors that affect success is the endometrium. Unfortunately, the molecular mechanisms are not yet clearly understood. It is difficult to investigate these due to technical and ethical reasons. The endometrium is important for implantation, and it is important that endometrial development is synchronized with fertilization and embryo development. In addition, the endometrium is also important in the nutrition of the embryo in the first few weeks until the placenta develops. The most frequently used method to assess the endometrium is through endometrial thickness measurement, which is simple, cheap, and atraumatic.

The reason for the thickening of the endometrium is estradiol (E2) secretion, which increases with oocyte maturation. For this reason, measurement of the endometrium in the highest E2 concentration moment, in other words, the endometrium on the human chorionic gonadotropin (hCG) trigger day is used frequently [1, 2]. However, in some studies, oocyte pick-up (OPU) or transfer day measurements were used [3, 4].

In some studies, it was shown that endometrial thickness had no clear relation with age and E2 values of women [5]. The success of IVF has been analyzed in many studies; however, the results were not consistent. Although some authors found certain endometrial thickness values and pregnancy rates to be higher, others reported contrasting results [6-8]. However, the negative effects of both extremes of endometrial thicknesses are known in terms of success [9]. It has also been shown that the endomet-

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rium could be affected negatively in coasting and ovarian hyperstimulation situations [10].

The aim of this study was to investigate the effect of endometrial thickness on hCG administration day in IVF/ICSI-ET cycles in couples with unexplained infertility (UEI) on live birth rate (LBR).

MATERIAL AND METHOD

Approval was received for this study from the Ethics Committee of Zeynep Kamil Education and Research Hospital (Reference number: 2018/22), and written informed consent was not required because of the retrospective nature of the study. We retrospectively collected data from Zeynep Kamil Women's and Children's Disease Training and Research Hospital, IVF Center archive. When couples that underwent UEI were selected, couples with normal spermiogram according to the World Health Organization (WHO) and the strict Kruger criteria, and normal couples in whom no low ovarian reserve was detected and who had normal hysterosalpingography were included. Women aged between 23-39 years who had normal regular menstrual cycles, and whose basal follicle-stimulating hormone (FSH) levels were <10 mIU/mL, and body mass index were <30 kg/m2 were selected. Only fresh transfers of these patients were included in the study. Women who had missing data, who did not have embryo transfer, who had thaw embryo transfer, who received assisted hatching, who received preimplantation genetic diagnosis, those with ovarian hyperstimulation syndrome, patients whose birth information could not be reached, and women who received coasting were excluded from the study.

The long-agonist and antagonist protocols were used in the women for IVF and for induction with gonadotropins [11]. The human menopausal gonadotropins (hMG) or recombinant follicle-stimulating hormones (rFSH) were started on the 2nd and 3rd days of the menstrual cycle. Leuprolide acetate was used as an agonist, and also cetrorelix or ganirelix were used as antagonists. Follicles and endometrial thickness were measured using a GE LOGIQ 200 Ultrasound System (GE Healthcare, Chalfont St. Giles, United Kingdom), using a 6.5-MHz endovaginal probe. Like in many other studies, the endometrial thickness was measured as the maximal distance between the echogenic interfaces of the myometrium and the endometrium, and was measured in the midsagittal plane using 2-dimensional transvaginal ultrasound on the day of hCG administration [2, 5, 9]. Ten thousand units of hCG were given when at least three follicles exceeded 18 mm in the measurements. Oocyte pick-up was performed after 35-38 hours. For fertilization, generally ICSI was applied. Embryo transfers were performed either on the 3rd or 5th days according to the embryo count and grading [12, 13]. One or two embryo transfers were made using transabdominal ultrasound guidance. Intravaginal progesterone (Crinone, Serono, USA) was started after embryo transfer and was continued until fetal heart activity. Serum hCG was measured in all patients after 10-12 days. Those who were positive were repeated after 48 hours. After 2 weeks, fetal cardiac activity and embryo were confirmed. Women with multiple gestations were determined. In this study, the birth data of the patients included were obtained either from the hospital records or by a direct phone call.

The patients were divided into two groups according to their live births: women with live births (group 1, n=104) and women with no live births (group 2, n=205). Each group was compared in terms of basal FSH, basal E2, induction treatment protocol, induction duration, total gonadotropin dose used, E2 levels measured on the hCG trigger day, endometrial thickness on the hCG trigger day, the oocyte count obtained from the OPU and mature (M2) oocyte count, and the blastocyst transfers performed.

The data were analyzed using SPSS version 14 software (Chicago, IL, USA). Continuous values were evaluated using Student's t-test, and categorical values were evaluated using the Chi-square test. Receiver operating characteristic (ROC) curve analysis was performed to assess the predictive value of endometrial thickness on LBR. A P value < .05 was taken as significant.

RESULTS

Ten thousand cases between 2005 and 2013 were collected. Seven hundred thirty-four of these were initially eligible for this research. In the end, 359 cycles were included in the analyses. One hundred four women with live births were included in group 1, and 255 women with no live births were included in group 2. The mean age of the patients who were included in the study was similar in both groups (Table 1). The characteristics of the patients, the treatment protocols used, and the findings are summarized in Table 1. As seen in the table, no statistically significant differences were found between group 1 and group 2.

Table 1: Characteristics of cycles and findings of ART among unexplained infertility cases.

	Group 1	Group 2	*P*
Age, years	31.6±3.8	31.2±4.1	.550[a]
FSH, mU/mL	6.5±1.4	6.5±1.5	.772[a]
Basal E_2, pg/mL	49.1±18.1	51.5±29.5	.436[a]
Treatment Protocol (Agonist/Antagonist)	78/26	169/86	.135[b]
Induction duration, day	8.8±1.4	8.8±1.3	.988[a]
Total Gn Dose, IU	2538.6±867.1	2408.2±786.9	.168[a]
Trigger E_2, pg/mL	2517.2±1106.0	2210.8±991.7	.011[a]
Endometrial Eco, mm	10.3±1.9	10.3±1.8	.943[a]
Oocyte count	10.6±5.1	10.1±4.8	.337[a]
M2 oocyte count	8.5±4.6	8.1±4.1	.470[a]
Blastocyst transfer	21/83	51/204	.967[b]

***Group 1**: Women with live births, **Group 2**: Women with no live births Mean and standard deviation values were used, **a**; Student's t-test and **b**; Chi-square test.*

The LBRs in each endometrial thickness levels are shown in Table 2. The highest endometrial thickness was 17 mm in the live births in our study. When the LBRs in each endometrial thickness were compared, it was determined that there were no statistically significant differences.

Table 2: Endometrial thicknesses and live birth / not live birth rates.

Endometrial thickness	Group 1/Group 2	LBR (%)
7 mm	3/8	27.3
8 mm	12/28	30
9 mm	19/53	26.4
10 mm	31/63	32.9
11 mm	15/41	26.8
12 mm	11/35	23.9
13 mm	7/12	36.8
≥14 mm	6/15	28.6

***Group 1**: Women with live births, **Group 2**: Women with no live births, Chi-square test.*

Even when the lowest LBR, which was 23.9% (12 mm) was compared with the highest LBR, which was determined as 36.8% (13 mm) the result was p=0.450 (Chi-square test). There was no difference between the groups in terms of endometrial thickness (10.3+1.9 mm and 10.3+1.8 mm; P = .943, respectively) (Table 1). The area under the ROC curve was 0.499 (95% CI: 0.433-0.564; P = .972). The ROC analysis endometrial thickness cut-off value was 9.5 mm (sensitivity 67.3%, specificity 34.9%).

When 7 mm and higher endometrial thickness values were compared using Fisher's exact test, it was determined that there were no significant differences between the LBRs (3/8 vs. 101/247; P = .999, respectively). When each endometrial thickness value was compared with consecutive higher values together with lower values, no significant results were found, as seen in Table 3 (Chi-square test).

Table 3: Live birth rates according to endometrial thicknesses.

	Subgroups											
	7 mm			8 mm			9 mm			10 mm		
Groups	≤7	>7	*P*	≤8	>8	*P*	≤9	>9	*P*	≤10	>10	*P*
1	3	101	.999*	15	89	.940**	34	70	.781**	65	39	.697**
2	8	247		36	219		89	166		152	103	
	Subgroups											
	11 mm			12 mm			13 mm and above					
Groups	≤11	>11	*P*	≤12	>12	*P*	≤13	>13	*P*			
1	80	24	.910**	91	13	.736**	98	6	.967**			
2	193	62		228	27		240	15				

***Group 1**: Women with live births, **Group 2**: Women with no live births, * Fisher's exact test, ** Chi-square test, **p**<0.05 indicates statistical significance.*

DISCUSSION

In order to evaluate ART success, endometrium thickness, structure and vascularization were evaluated using ultrasonography in many studies. Among these, endometrial thickness is preferred mostly [14]. The results are conflicting, however, because there are many confounding factors that affect the success of ART. Generally, pregnancy rates (PR) or ongoing pregnancy rates (OPR) are evaluated [5]. Very few studies have evaluated LBR [14]. However, in this study, the LBRs were compared.

In the literature, the LBR in UEI is 33.1% below the age of 35 years, whereas it is 12.5% in the 40-42 years age range. The American Society for Reproductive Medicine (ASRM), on the other hand, reported the LBR was 41.3% in patients aged under 35 years in whom 2 embryo transfers were performed. It was found that there was a decrease in spontaneous abortus rates with increased endometrium thickness [10]. Lui et al. did not investigate the number of embryos transferred when they performed a study on endometrial thickness [14]. In the present study, 359 patients who received one or multiple embryo transfers were included. The LBR in our series was 29.08%.

Weissman et al. found that the PR decreased at and above 14 mm, and the abortus rates were decreased at a significant level [7]. The rates were very low in this study with endometrial thicknesses of 14 mm. On the contrary, Chan et al. showed that the chances of pregnancy did not change at ≥14 mm [12]. We evaluated ≥14 mm as only the last group. In this study, the greatest endometrial thickness was 18 mm. In another study in which 1186 IVF cycles were evaluated, it was reported that as the endometrium thickness decreased, the PR decreased [15]. Liu et al. showed a decrease in LBR with less than 8 mm [14]. There was no endometrial thickness below 7 mm in our study group. Perhaps, if there were 4-5 mm values, the results would be different.

On the other hand, Noyes et al. found endometrial thickness to be greater in patients whose E2 levels were high and in younger patients [16]. In the current study, however, the ages were at similar average values. The other variables could be evaluated without the effect of age. Zhang et al. reported that age had no effect. However, they also found a positive relation between PR and peak E2 levels [5]. In the present study, similarly, the trigger E2 levels were a little higher in the live birth group.

It was considered that as the length of ovarian stimulation was prolonged, so would the endometrial thickness. It was shown with stepwise multiple regression analysis that there was no statistically significant relationship [5]. In the present study, consistent with this, there was no statistically significant relationship between stimulation duration and endometrial thickness.

If there are high quality embryos ready for transfer, the effects of the optimal endometrial thickness are less [5]. Statistically similar results were obtained in our study regarding LBR. The LBRs are similar in statistical terms in 7 mm and over endometrial thicknesses. We thought that endometrial thickness on hCG administration day had no effect on LBR in the UEI group.

There are some limitations to our study. Analog and antagonist cycles were evaluated together because there were many publications showing that the success rates in long-agonist or antagonist cycles were similar [12, 17]. Chan et al. showed that there was no relationship between endometrial thickness and biochemical pregnancy rates, miscarriage rates, and implantation rates [12]. In our study, we only studied LBR. In a meta-analysis by Martins et al., they found that there was no difference between cleavage and blast embryo transfers for LBR [18]. In addition, a systematic review by Glujovsky et al. also examined LBR after fresh transfer; blastocyst stage transfer showed a difference with low-quality

evidence compared with cleavage-stage transfer [19]. Therefore, in our study, we evaluated the cleavage and blast embryo transfer in the same groups due to the low number of patients; however, this creates great heterogeneity [13]. The retrospective nature of our study is another important limitation. A strength of our study is the exclusion of patients with ovarian hyperstimulation syndrome (OHSS), which would affect the endometrial receptivity and thus the LBR ratio.

It is difficult to make a suggestion about the significance of endometrial thickness because of the limited number of cases in this study. Accordingly, there should be more studies with larger sample sizes from single centers in this region to further verify the findings.

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