Blood Contamination of Embryo Transfer Catheter Does not Decrease ICSI Success

Embriyo Transfer Kateterine Kan Kontaminasyonu ICSI Başarısını Azaltmamaktadır

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ABSTRACT Objective: The aim of this study was to investigate the relationship between blood contamination of embryo transfer catheter (ETC) and its effect on clinical pregnancy rate in intra cytoplasmic sperm injection (ICSI) cycles. **Material and Methods:** The data of patients underwent controlled ovarian stimulation for ICSI cycles with standart stimulation protocols in Erciyes University Center of Assisted Reproductive Technology and Infertility were retrospectively analysed. On the day of human chorionic gonadotropin administration, endometrial thicknesses were measured by transvaginal ultrasonography and recorded. Endometrial thickness were grouped as the following; Group 1: ≤5,9 mm (n: 22), Group 2: 6-7,9 mm (n: 204), Group 3: 8-9,9 mm (n: 474), Group 4: 10-11,9 mm (n: 476), Group 5: 12-13,9 mm (n: 277), Group 6: ≥14 mm (n:139). After the completion of ultrasound guided embryo transfer procedure, catheter tips were inspected under the light microscope in order to observe the presence of blood. Main outcome measures were clinical pregnancy rate (CPR), endometrial thickness, blood contamination of ETC. **Results:** Tenaculum was not used in any patient and all the embryo transfer in this study were easy type. In cycles that resulted in pregnancy patients were younger (29±4.52 versus 30.53±5.11 years, p<0.001). E2 concentration on the day of hCG administration for trigger was significantly greater in cycles where pregnancy were achieved (2517.37±1241.94 versus 2264.84±1222.66 pg/mL (p<0,001)]. No statistically significant difference found between endometrial groups in terms of blood contamination of ETC (p= 0.179). When controlled for endometrial thickness, blood contamination of ETC did not decrease CPR. Contamination of blood on ETC was not an independent factor related to CPR. **Conclusion:** Blood contamination of embryo transfer catheter does not negatively affects ICSI cycles outcomes.

Key Words: Blood; catheters; embryo transfer; endometrium; sperm injections, intracytoplasmic

ÖZET Amaç: Bu çalışmanın amacı intrastoplazmik sperm injeksiyonu (ICSI) sikluslarında embriyo transferi sonrasında embriyo transfer kateterinde kan izlenmesinin klinik gebelik oranına etkisini araştırmaktı. **Gereç ve Yöntemler:** Erciyes Üniversitesi Yardımla Üreme ve İnfertilite Merkezinde ICSI için kontrollü ovaryan hiperstimulasyon uygulanan hastaların verileri retrospektif olarak incelendi.İnsan koryonik gonadotropin (hCG) uygulandığı gün endometriyal kalınlık transvajinal ultrasonografi ile ölçülüp kaydedildi. Endometriyal kalınlıklarına göre hastalar; Grup 1≤ 5,9 mm (n: 22), Grup 2: 6-7,9 mm (n: 204), Grup 3:8-9,9 mm (n: 474), Grup 4: 10-11,9 mm (n: 476), Grup 5: 12-13,9 mm (n: 277), Grup 6: ≥14mm (n:139) olarak gruplandırıldı. Ultrason rehberliğinde yapılan embriyo transferi sonrasında kateter ucunda kan bulaş varlığı ışık mikroskopu altında değerlendirildi. Ana sonuç ölçütlerimiz klinik gebelik oranı, endometriyal kalınlık ve embriyo transfer kateterinde kan izlenmesi idi. **Bulgular:** Hiçbir hastada embriyo trasferi sırasında tenakulum kullanılması gerekmedi. Bu çalışmadaki transfer tipi kolay transfer idi. Gebelikle sonuçlanan sikluslardaki hastalar daha genç idi (29±4,52 ve 30,53±5,11 yıl, p<0,001). Ovulasyon tetiklenmesi için hCG uygulandığı gün serum östradiol düzeyleri, gebelik elde edilen sikluslarda daha yüksekti (2517,37±1241,94 ve 2264,84±1222,66 pg/mL (p<0,001)]. Endometriyal gruplar arasında, embriyo transfer kateterinde kan bulaşı bakımından istatistiksel bir fark izlenmedi (p=0,179). Endometriyal kalınlık için kontrol edildiğinde embriyo transfer kateterinde kan bulaşı olmasının klinik gebelik oranını azaltmadığı ve embriyo transfer kateterinde kan bulaşının klinik gebelik oranına etki eden bağımsız bir faktör olmadığı izlendi. **Sonuç:** Embriyo transfer katetrinde kan bulaşı izlenmesi ICSI siklus sonuçlarını olumsuz yönde etkilememektedir.

Anahtar Kelimeler: Kan; kateterler; embriyo transferi; endometriyum; sperm enjeksiyonu, intrasitoplazmik

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From the day of its inauguration, intracytoplasmic sperm injection (ICSI), and embryo transfer (ET) has been the subject of continuing effort to optimize its results, namely, clinical pregnancy rate (CPR). For this purpose, variety of factors affecting the ICSI succesess had been defined, and the procedures by which these factors could be modified have been suggested. The main conclusion of this vast body of work is that successful implantation depends on a mutual interaction between blastocyst and the receptive endometrium.

Being a host for implanting, and developing embryo, endometrial characteristics are believed to have important role in the pregnancy success. It is a common notion in gynaecologic practise to assume that endometrial thickness is an indirect marker of endometrial perfusion, and proliferation, and thus, receptivity. Because of its non-invasiveness and low-cost, transvaginal ultrasonographyic (TVUSG) examination of endometrium is prefered over the other more invasive, and sophisticated methods for the evaluation of endometrial receptivity.[1-3] In order to delineate the predictive value of USG examination of endometrium for achieving pregnancy, relationship between endometrial thickness and CPR has been vigorously studied and presented in the literature with conflicting results.[4-9]

After the optimisation of stimulation protocols and culture conditions, and the determination of endometrial factor, investigators turned their attentions to the final step of IVF/ICSI cycles; Embryo transfer. Being an operator dependent procedure, ET is thought to cause endometrial trauma, and bleeding, resulting in defective interaction between blastocyst, and decidua. As an indicator of transfer related bleeding in the decidua, presence of mucous and/or blood on the catheter surface has been evaluated at ET studies.[10-13] Despite the existance of variety of studies investigating the effects of blood on catheter in different patient population, and with different ET technics, there is no study assessing the interaction between endometrial thickness and blood contamination of embryo transfer catheter (ETC) in ICSI cycles.[14-17]

The main aim of the present study was to clarify the relationship between endometrial thickness, and blood contamination of ETC, and the presumed effect of interaction of these two variables on the outcome of ICSI cycles.

MATERIAL AND METHODS

The study was a retrospective analysis of the prospectively collected data of all ultrasound guided ICSI-ET cycles that were performed at the Erciyes University, Reproductive Endocrinology and Infertility department between January 2005 and September 2011. This study in accordance with the principles of the Declaration of Helsinki 2008 have been made. Patients aged 42> years, those with baseline serum FSH (bFSH) levels ≥12 IU/mL on cycle day 3, and those who received cryopreserved cleaving embryos or blastocyst were excluded from the study. The patients who lost in follow up were excluded from the study as well. A total of 1592 ICSI, and ET procedures were included in the final analysis. This study was reviewed, and approved by the Institutional Review Board of Erciyes University.

Patient's selection, evaluation and monitoring of the ovarian stimulation, oosit retrival, and ET were performed by the two expert operators (YS or EMA). Based on patient's age, ovarian reserve, and previous treatment results, patients underwent controlled ovarian stimulation with the standard stimulation protocols. Ovarian stimulation was carried out with subcutaneous recombinant FSH (Gonal F; Serono, Istanbul, Turkey). Follicular response to stimulation was observed with TVUSG and estradiol (E2) measurement. When the leading two or more follicules reached ≥17 mm in size, 5 000-10 000 IU of IM human chorionic gonadotropin (hCG) (Pregnyl; Organon Istanbul, Turkey) were administered. On the day of hCG administration, endometrial thicknesses were measured by TVUSG. After a true longitudinal view of the uterus had been obtained, the endometrial thickness was measured as the maximum thickness between the highly reflective interfaces of the endometrial-myometrial junction. A triple line pattern was described as hypo-echogenic endometrium surrounded by hyper-echogenic zone. Endometrial thickness were grouped as the following; Group 1: ≤5.9 mm (n: 22), Group 2: 6- 7.9 mm (n: 204), Group 3: 8-9.9 mm (n: 474), Group 4: 10-11.9 mm (n: 476), Group 5: 12-13.9 mm (n: 277), Group 6: ≥14 mm (n:139). Approximately 36 hours after the hCG injection, transvaginal oocyte retrieval was performed. Patients were started on vaginal progesterone for luteal support until 8 weeks of gestation. Prior to starting an ART cycle, all patients underwent a mock embryo transfer to determine the patency, depth, and the direction of endocervical canal. Additionaly servical dilation was performed to those patients with cervical stenosis and/or hyperflexed uterus on day 6 of the controlled ovarian hyperstimulatin cycle. At the time of ET, patients placed in the dorsal lithotomy position with full bladder, and a sterile speculum was introduced into the vagina. The cervix was flushed with culture media to minimize contamination and excess mucus was aspirated. For embryo transfer, outher sheath of Wallace echogenic catheters (SureView Wallace Embryo Replacement Catheter; Smiths Medical, Hythe, Kent, UK) was advanced into the cervical canal under the USG guidance and left in place Tenaculum was not used in any patient and all the embryo transfer in this study were regarded as easy type. Afterwards, soft inner cathter was loaded with the day 3 embryos, and advanced through the sheath to the correct placement (Afterloading technic). Care was taken to avoid contact with the uterine fundus, and maintain a distance of 10 to 20 mm from the ETC tip. At least one Grade 1 or Grade 2 embryos were transferred to the patients. After the completion of transfer procedure, catheter tips were inspected under the light microscope for the presence or absence of embryo and/or blood. Blood contamination of outer surface of the ETC was not included in the analysis.

Blood pregnancy test was done 16 days after embryo transfer, and if positive, transvaginal ultrasound examination was performed two weeks after the embryo transfer to confirm intrauterine pregnancy. Only clinical pregnancies defined by the presence of fetal cardiac activity on transvaginal ultrasonography at 12 weeks of gestation or later were included in the final analysis.

STATISTICAL ANALYSIS

Categorical data were expressed as number and percentage, and numerical data as mean and standard deviation. Data were analyzed using ANOVA, chi-square and Student's t tests where applicable. If ANOVA testing detected a significant difference, post-hoc comparisons were performed using the Tukey honestly significantly different (hsd) test.

Binary logistic regression analysis, univariate, and multiple analyses using Backward-Wald method was performed to analyse whether blood contamination of ETC was an independent factor influencing CPR, and identify independent risk factors associated with clinical pregnancy. The following variables were entered into the model: duration of infertility, bFSF levels, E2 levels, cause of infertility, endometrial pattern at the day of hCG administration, and blood on catheter. Using Backward-Wald elimination, we excluded variables that were not significantly associated with clinical pregnancy. Odds ratio (OR) and their 95% confidence interval (CI) were calculated. Statistical significance was set at p.05. All analyses were performed using Statistical Package for Social Science (SPSS), version 15.0 (SPSS, Chicago, IL, USA).

RESULTS

The study included 1592 consecutive ICSI/ ET cycles performed at our clinic. Mean age of the study population was 30.8±4.96 (19-42) years and the mean duration of infertility 7.73±4.88 (1-25) years. CPR for the whole population was 29.3%.

Characteristics of patients, cycle data, ICSI outcomes, and the results of univariate analysis were presented in Table 1. In cycles that resulted in pregnancy patients were younger (29±4.52 versus 30.53±5.11 years, $p<0.001$). E2 concantration on the day of hCG administration was significantly greater in cycles where pregnancy were achieved [2517.37±1241.94 versus 2264.84±1222.66 pg/mL ($p<0,001$)]. Pregnant and non-pregnant groups were statistically identical in terms of duration of infertility, cause of infertility, endometrial triple line pattern, bFSH level, and the blood contamination of ETC. While the highest clinical pregnancy rate was seen in group 6 which was 39.6%, the lowest rate was seen in group 2 which was 20.1%. The statistically significant difference regarding CPR was only found between group 6 and 2 (p<0.001). Multiple logistic regression analysis revealed that E2 levels at the day of BhCG administration (OR 1.00; 95% CI 1.0-1.0; p<0.001), maternal age (OR 0.95; 95% CI 0.93-0.97; p<0.001) were independent variables significantly affecting CPR (Table 1). Contamination of blood on ETC was not an independent factor related to CPR (Table 1).

Table 2 shows patients characteristics, endometrial pattern, and blood contamination of ETC in the endometrial thickness groups. At subgroup analaysis, no statistically significant difference found between endometrial groups in terms of blood contamination (p=0.179). The only significant difference between endometrial thickness groups was seen at age. Woman in endometrial groups 2 and 3 were older than other groups (Group 2= 31.14±5.15. and Group 3= 30.72±4.88 years, p<0.001).

Clinical pregnancy rates were similar for the two operators. No statistically significant difference was found between the operators regarding blood contamination of ETC (136/940, 14.4%, and 117/652, 17.9%, p=0.072).

TABLE 1: Characteristics of patients, cycle data, ICSI outcomes and univariate-multiple binary logistic regresyon analysis of factors influencing clinical pregnancy rate.

	Clinical pregnancy			Univariate analysis		
Variables	**Positive $\bar{\chi}$±SD**	**Negative $\bar{\chi}$±SD**	**p**	**Odds ratio**	**95%CI**	**p**
Age (Years)	29.05±4.52	30.53±5.11	<0.001	0.94	0.92-0.96	<0.001
Duration of infertility (Years)	7.13±4.40	7.98±5.05	0.001	0.96	0.94-0.99	<0.001
bFSH (IU/mL)	6.20±2.05	6.47±2.01	0.016	0.94	0.89-0.99	0.016
E2 (pg/mL)	2517.37±1241.94	2264.84±1222.66	<0.001	1.00	1.00-1.00	<0.001
	n (%)	**n (%)**				
Cause						
Male	246 (28.5)	617 (71.5)				
Unexplained	148 (32)	314 (68.0)		1.18	0.93-1.51	0.181
PCOS	31 (36.0)	55 (64.0)	0.053	1.14	0.89-2.25	0.144
Female	41 (22.7)	140 (77.3)		0.74	0.50-1.07	0.110
Triple line pattern						
Present	94 (30.5)	214 (69.5)	0.592			
Absent	372 (29.0)	912 (71)		1.08	0.82-1.41	0.592
End group (mm)						
Group 6 (>14)	55 (39.6)	84 (60.4)				
Group 1 (<6)	8 (36.4)	14 (63.6)		0.87	0.34-2.20	0.775
Group 2 (6-8)	41 (20.1)	163 (79.9)	<0.001	0.38	0.24-0.62	<0.001
Group 3 (8-10)	123 (25.9)	351 (74.1)		0.54	0.36-0.80	0.002
Group 4 (10-12)	140 (29.4)	336 (70.6)		0.64	0.43-0.94	0.024
Group 5 (12-14)	99 (35.7)	178 (64.3)		0.85	0.56-1.29	0.446
Blood on ETC						
Present	385 (28.8)	954 (71.2)	0.296			
Absent	81 (32)	172 (68.0)		1.17	0.87-1.56	0.296
					Multiple analysis	
Age				0.95	0.93-0.97	<0.001
E2				1.00	1.00-1.00	<0.005
Endometrium				1.09	1.04-1.15	<0.001

†ICSI: Intracytoplasmic sperm injection; bFSH: baseline follicule-stimulating hormone; E2: estradiol; End. Group: Endometrial thickness groups; PCOS: Polycystic ovary syndrome; ETC: Embryo transfer catheter.

TABLE 2: Patients characteristics, endometrial pattern and blood contamination of embryo transfer catheter in endometrial thickness groups.

	Endometrium groups (mm) Group 1 ≤5.9 mm (n: 22)	Group 2 (6-7.9) mm (n:204)	Group 3 (8-9.9) mm (n:474)	Group 4 (10-11.9) mm (n:476)	Group 5 (12-13.9) mm (n:277)	Group 6 ≥14 mm (n:139)	p
Women age (Years)	30.45±5.79[ac]	31.14±5.15[a]	30.72±4.88[a]	29.63±4.87[bc]	29.47±4.98[bc]	29.22±4.93[bc]	<0.001
Basal FSH (IU/mL)	6.11±2.45	6.48±2.18	6.34±2.04	6.43±2.02	6.37±1.97	6.40±1.80	0.933
E2 (pg/mL)	2173.05±1042.11	2249.00±1279.45	2258.44±1194.78	2358.99±1243.93	2488.08±1261.21	2403.69±1214.08	0.153
Cause							0.095
Unexplained	6(1.3)	68 (14.7)	145 (31.4)	112 (24.2)	94 (20.3)	37 (8.0)	
PCOS	1 (1.2)	11 (12.8)	28 (32.6)	21 (24.4)	16 (18.6)	9 (10.5)	
Female	5 (2.8)	26 (14.4)	57 (31.5)	55 (30.4)	22 (12.2)	16 (8.8)	
Male	10 (1.2)	99 (11.5)	244 (28.3)	288 (33.4)	145 (16.8)	77 (8.9)	
Triple line pattern							0.457
Absent	6 (1.9)	44 (14.3)	87 (28.2)	82 (26.6)	57 (18.5)	32 (10.4)	
Present	16 (1.2)	160 (12.5)	387 (30.1)	394 (30.7)	220 (17.1)	107 (8.3)	
Blood on ETC							0.179
Absent	20 (1.5)	161 (12.0)	401 (29.9)	401 (29.9)	242 (18.1)	114 (8.5)	
Present	2 (0.8)	43 (17.0)	73 (28.9)	75 (29.6)	35 (13.8)	25 (9.9)	

Groups with different superscript letters were found to have statistically significant differences.
FSH: Follicle-stimulating hormone; E2: Estradiol at the day of human chorionic gonadotropin administration; PCOS: Polycystic ovary syndrome; ETC: Embryo transfer catheter.

Table 3 presents the effect of blood contamination of ETC on CPR according to endometrial thickness groups. CPR was found to be significantly higher in group 6 when blood contamination of ETC occured. [16/25 (64%) versus 39/124 (31.4%), p=0.011]. There was no statistically significant difference between other groups.

DISCUSSION

Mamalian endometrium undergoes series of changes that make it more suitable for implantation after ovulation and fertilization. The first maternal change in the implantation process is the increased vascularity, and vascular permeability in endometrium near the adherent embryo.[18] It was known that some of the mediators responsible for increased vascular permeability are also potent endometrial mitogens that cause endometrial proliferation.[3,19] While increasing endometrial thickness, these mediators may also cause capillary proliferation, tortuosity, and friability that make endometrium vulnerable to catheter induced bleeding.

TABLE 3: The effect of blood contamination of embryo transfer catheter on cinical pregnancy rate in the endometrial thickness groups.

	Endometrium groups (mm) Group 1 ≤5.9 mm (n: 22) Pregnancy		Group 2 (6-7.9) mm (n:204) Pregnancy		Group 3 (8-9.9) mm (n:474) Pregnancy		Group 4 (10-11.9) mm (n:476) Pregnancy		Group 5 (12-13.9) (n:277) Pregnancy		Group 6 ≥14 mm (n:139) Pregnancy	
Blood on ETC	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative	Positive	Negative
Positive	2 (100)	0 (0)	9 (21)	34 (79)	18 (25)	55 (75)	24 (32)	51 (68)	12 (34)	23 (66)	16 (64)	9(36)
Negative	6 (30)	14 (70)	32 (20)	129 (80)	105 (26)	296 (74)	116 (29)	285 (71)	87 (36)	155 (64)	39 (34)	75 (66)
p	0.121		1.000		0.898		0.691		0.997		0.011	

ETC: Embryo transfer catheter.

In order to delineate inter-relationship between endometrial thickness, blood contamination of ETC and CPR, we firstly analysed the endometrial pattern between pregnant, and non- pregnant groups. Our data demostrated that triple- line pattern of endometrium had no discernible effect on ICSI outcome. At endometrial thickness subgroup analysis, highest pregnancy rate was seen in group 6 and statistically significant difference was only seen between group 6 and 2. The estimated odd ratio for successful pregnancy with each additional milimeter of endometrial thickness was 1.09 but this trend did not reach statistical significance. Despite the large number of study concerning this issue, the prognostic values of ultrasonographic endometrial thickness or the appearance of the endometrium, in conception, and non-conception IVF cycles remain contraversial. Nevertheless, our results are consistent with more recent literature which showed that no lineer relationship between endometrial thickness and CPR.[7-9,20] In contrast to earlier reports relating elevated endometrial thickness with negative IVF outcome, in our study, the pregnancy rate in patient whose endometrium thicker than 14 mm was 39.6% which was consistent with recent studies.[21,22] In accordance with our results, Rinaldi et al. found that, unlike IVF cycles, endometrial thickness could not predict ICSI success as a result of higher fetilization ratio.[23] Our study showed that, at the other end of the spectrum, patients whose endometrium thinner than 6 mm achieved 36.4% CPR. Similiarly, Bozdag, and colleagues reported %35 CPR in patiens undergone ICSI whose endometrial thickness below 7 mm.[24] It seems reasonable that with more advanced laboratory, and stimulation methods, and with the use of ICSI, effects of endometrial thickness on outcome may have been obscured.

Though it has been used for nearly three decades for transferring embryo, interaction between ETC, and endometrium is not well understood. It was speculated that ETC may cause endometrial bleeding by way of direct contact and/or provoking uterine, and junctional zone contraction via prostaglandin release.[25] However, studies trying to test this finding yielded conflicting results. Edwards and associates, and Visser and co-workers did not observe a statistically significant effect of catheter contamination with blood on CPR.[10,11] These results were supported by Moragianni and collaborators.[26] However, Goudas et al., Awonuga et al., and Alvero et al. found that the presence of blood on the transfer catheter after ET was associated with decreased implantation rate and CPR.[12,13,17] Our results showed that presence of blood on ETC has no significant effect on CPR.

In order to test our first hypothesis suggesting that endometrial thickness may obscure negative effect of blood contamination of ETC, we further analysed the groups according to the catheter contamination, and CPR. Our results showed that in the first five groups, catheter contamination did not affect CPR, and patients in group 6 showed higher CPR when blood contamination of ETC had occurred. However, blood contamination of ETC was seen most commonly in group 3, and 4, and there was no lineer correlation between endometrial thickness, and blood contamination (Table 2). The presences of blood on the outer surface of the ETCs are thought to occur during the passage of ETC through cervical channel, and may not represent the endometrial trauma.[27] Accordingly, Marikinti et al., showed that multiple passes even with a soft catheter, use of an outer sheath and or tenaculum was frequently associated with mucosal bleeding from the endocervix but not from the endometrium.[28] Unlike the cervical mucosa, luteal phase endometrium is thick and oedematous with most of the superficial layers supplied by a subepithelial capillary plexus with sluggish blood flow.[29] According to our hypothesis, whereas bleeding from thin endometrium mainly results from endometrial vascular laceration, and consequently impairs implantation, and reduces CPR, bleeding from thick endometrium reflects well perfused, and congested endometrium and consequently does not affect implantation. However, our results did not support first side of this equation by showing that blood on ETC did not reduce CPR even in thinnest endometrium groups (Group 1, and 2). In Group 6, the positive correlation between blood contamination of ETC and CPR ratio reflects the fact that well perfused and congested endometrium facilitate implantation and also leads to blood contamination on ETC due to minor trauma to the dilated and congested vessels. Indeed, variety of factors may determine endometrial receptivity other than endometrial thickness. Endometrial perfusion, balance between endometrial proliferation, and apoptosis, and hormone receptor compartmantalization seem to exert their independent effects at given endometrial thickness.[27-32] Unfortunately, the relationship between these factors, endometrial thickness, and the role played by embryo transfer process is yet to be determined.

We acknowledge some limitations in our study. Firstly, the retrospective nature of the study, but it was based on detailed prospectively collected data. Secondly, the sample size was relatively small in group 1, a problem also occurred in previous studies.

Over the last decade, variety of study had been designed to investigate aformentioned variables supposedly affecting IVF/ICSI success. Majority of these trials showed conflicting results as a result of huge list of confounding factors such as different stimulation protocols, inadequate sample size, different assisted reproductive technics (IVF/ICSI), and catheter used, number of transferred embryos, baseline ovarian reserve, care provider, and transfer technics. Unfortunately, the results of these studies just permit vague generalisation regarding endometrium, transfer procedure, and the prediction of succesfull implantation. However, the present study does have some significant strength. First of all, ET is an operator dependent procedure; the major strength is that all ETs were performed by two expert operators. In addition, the present study has the advantage of inclusion of huge number of patients, standart application of oosit retrival, ICSI and the ET technics in the single center, and the high experienceses of two operators trained under the same institution performing all procedures. In these aspects, the study population may be regarded as an ideal group to investigate the topic. Additionally, a MEDLINE search using the terms *ICSI, endometrial thickness, and embryo transfer catheter* revealed that, to date, this is the first study which assessed the relationship between endometrial thickness, and blood contamination of ETC in ICSI cycles. Nontheless, when taken into account the conflicting results of the related studies in the literature, it seems that future focus of investigation for the prediction of successful implantation, and CPR will be divert from indirect methods such as measurement of endometrial thickness, and catheter examination to measurement of molecular markers, and secretomics.[2,3]

We conclude, therefore, that blood contamination of ETC is unrelated to endometrial thickness, and has no negative effect on ICSI results, even in patients with thin endometrium. Transvaginal ultrasonographic measurement of endometrial thickness has no reliable predictive value in ICSI cycles and lowest and higest values do not rule out the posibility of achieving pregnancy.

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