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RESEARCH

Total fertilization failure in intracytoplasmic sperm injection: a retrospective comparative study

İntrasitoplazmik sperm enjeksiyonunda döllenme başarısızlığı: retrospektif karşılaştırmalı bir çalışma

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Abstract

Purpose: The aim of this study was to evaluate total fertilization failure (TFF), embryo transfers following TFF cycles, and recurrent TFF, and to define the factors that might cause TFF.

Materials and Methods: Cycles that resulted in TFF (group 1, n=109), embryo transfers after TFF cycles (group 2, n=30), and cases of recurrent TFF (group 3, n=15) were evaluated retrospectively.

Results: Peak estradiol was higher in group 2 than other groups, and the rate of sperm morphology below 4% was lower in group 2 when compared to groups 1 and 3. The total numbers of retrieved oocytes (5.3±4.1 vs 10.2±1.5 (Odds Ratio=1.639; 95% CI 1.267-2.122)) and MII oocytes (2.9±2.2 vs 6.8±1.8 (Odds Ratio=2.218; 95% CI 1.529-3.216)) were significantly higher in embryo transfer cycles when compared to previous TFF cycles. Retrieved and MII oocytes counts were higher in group 2 with a median fertilization rate of 46.42% when compared to groups 1 and 3.

Conclusion: With more retrieved and MII oocytes, normal sperm morphology can increase the fertilization rate of ICSI cycles following earlier TFF.

Keywords:. İnfertility, intracytoplasmic sperm injection, perinatal outcomes, total fertilization failure

Öz

Amaç: Bu çalışmada total fertilizasyon başarısızlığı (TFF), TFF siklusları sonrası embriyo transferleri ve tekrarlayan TFF'yi değerlendirmek ve TFF'ye neden olabilecek faktörlerinin belirlenmesi amaçlanmıştır.

Gereç ve Yöntem: TFF ile sonuçlanan sikluslar (grup 1, n=109), TFF siklusları sonrası embriyo transferleri (grup 2, n=30) ve tekrarlayan TFF olguları (grup 3, n=15) retrospektif olarak değerlendirildi.

Bulgular: Pik estradiol grup 2'de diğer gruplara göre daha yüksekti, %4'ün altında sperm morfolojisi oranı grup 2'de grup 1 ve 3'e göre daha düşüktü. Elde edilen toplam oosit sayısı (5,3±4,1 vs 10,2±1,5 (Odds Ratio=1,639; %95 CI 1,267-2,122) ve MII oositleri (2,9±2,2 vs 6,8±1,8 (Odds Ratio=2,218; 95) % CI 1,529-3,216;) embriyo transfer sikluslarında önceki TFF sikluslarına göre anlamlı derecede yüksekti. Elde edilen ve MII oosit sayısı medyan fertilizasyon oranı %46.42 ile grup 1 ve 3'e göre grup 2'de daha yüksekti.

Sonuç: Daha fazla toplanan ve MII oositlerle, normal sperm morfolojisi, erken TFF'yi takiben ICSI sikluslarının fertilizasyon oranını artırabilir.

Anahtar kelimeler: infertilite, intrasitoplazmik sperm enjeksiyonu, perinatal sonuçlar, total fertilizasyon başarısızlığı

INTRODUCTION

Despite developments in intracytoplasmic sperm injection (ICSI) treatment, average fertilization rates do not exceed 80%[1]. Total fertilization failure (TFF) is defined as the complete failure of all MII oocytes processed after pick-up and can occur at a rate of 5-10% in IVF cycles and can recur at a rate of 30% in the next treatment cycle[2,3]. This situation has negative psychological and financial effects on patients and can also create disappointment for the physician who arranges the treatment[4,5]. While the main problem in TFF is an oocyte activation defect, nonviable or

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abnormal sperm, chromosomal defects, and technical problems can also cause such failures[6].

There are three possible factors in the development of TFF, encountered in 1-3% of ICSI cycles, namely those relating to sperm, oocytes, and the technique itself. To minimize the negative effects of TFF, careful examination of the failure's etiology is required to optimize future treatment[2,6]. TFF following ICSI is primarily attributed to failed oocyte activation, but may be due to less common causes such as defective sperm DNA decondensation, abnormal pronuclear development, oocyte spindle defects, reduced oocyte yield and quality, severe forms of teratozoospermia such as globozoospermia, and technical problems. Therefore, identifying a sperm-related or oocyte deficiency is vital to management clinically of these couples. It has been suggested that not all patients will benefit from AOA, only those with sperm-related activation deficiency may benefit[7]. Since the etiopathogenesis of TFF has not been fully elucidated in the limited literature about the subject, the current study sought to evaluate TFF cycles, embryo transfers following TFF, and cases of recurrent TFF in detail, and to determine the factors that might cause failure of this kind.

MATERIAL AND METHODS

Sample

Data on patients with TFF who had undergone fresh ET-ICSI cycles at Ali Kemal Belviranlı Women's Health Research and Education Hospital, Reproductive Endocrinology Department were reviewed. The hospital is a tertiary referral centre in Konya for patients throughout the country, and it performs an average of 420 ICSI-IVF cycles per year. A total of 4192 ICSI cycles from between January 2007 and December 2017 were reviewed. Cycles that resulted in TFF (group 1, n=109), embryo transfers after TFF cycles (group 2, n=30), and cases of recurrent TFF (group 3, n=15) were evaluated retrospectively. Inclusion criteria were participants aged 20–44 years, body mass index (BMI) between 18 and 35 kg/m^2, orderly menstrual cycles, no uterine abnormalities in the ultrasonography (USG), and normal basal hormonal levels. Women were deported from the analyses if they were $\geq$45 years, BMI $\geq$35 kg/m^2, any significant systemic illness or metabolic disturbances, no retrieved sperm or oocyte, the history of ovarian or testicular surgery, and chemotherapy or radiotherapy history. The Ethical Committee of Necmettin Erbakan University Medical Faculty confirmed the study (2017/57). The participants read and signed the informed consent before the treatment for future use.

Controlled ovarian stimulation and ovulation trigerring

Pituitary down-regulation was achieved using leuprolide acetate in the GnRH agonist cycles. Recombinant FSH and leuprolide acetate daily together were used for microdose flare-up cycles. Cetrotide or Orgalutran was used in the GnRH antagonist cycles. The denuded oocytes were evaluated under light microscopy for the determination of their developmental stage and quality. Metaphase II (MII) oocytes were graded and scored based on their morphological criteria and ICSI resistance (Supplementary file 1- Table 5).

Sperm retrieval technique

Semen were obtained by masturbation after 2–3 days of abstinence, and then the samples were leaved to liquefy for at least 20-30 min at 37° C before analysis. The concentration, motility and morphology of the sperm were evaluated, followed by centrifugation.

ICSI, fertilization check and embryo grading

Fertilization controls were performed 18–19 h after the ICSI procedure. Only embryos that showed two pronuclei (2PN) were accepted normally fertilized. Day-2 and Day-3 embryos with less than 30% fragmentation, were taken into account to be transferable embryos. After the transfer of the Day-2 and Day-3 embryos, the remaining embryos were cultured further to Day-5 to form blastocysts.

Embryos were assessed according to the European Society for Human Reproduction and Embryology guidelines and categorized into four quality classifications[8]. The highest quality embryos were selected for embryo transfer on days 2, 3, and 5 after fertilization. The number of embryos transferred (two or fewer per patient) complied with national regulations in Turkey.

ET procedure

Two senior physicians performed the ETs accompanied ultrasonographic appearance using an embryo transfer catheter system. A sterile speculum

was placed into the vagina in the lithotomy position and the vagina and the cervix were cleaned using sterile cotton swabs. The embryos were loaded into a transfer catheter which was advanced to the ET physician who deposited the embryos approximately 10 mm from the uterine fundus under USG and then, the catheter was gently withdrawned after 10 seconds.

All catheters were immediately controlled for retained embryos, blood, and the woman stayed in the Trendelenburg position for about 15 minutes. Luteal phase support was provided by progesterone in the form of Crinone 8% gel (Serono, Istanbul, Turkey) at a daily dose of 90 mg. Baseline parameters and ICSI outcomes were compared between the groups. Biochemical pregnancy was detected with by hCG levels in venous blood tests performed 12-14 days after embryo transfer, and clinical pregnancy was accepted as those with a gestational sac accompanying fetal heart-beat on ultrasound examination at 4-5 weeks after embryo transfer. Live birth was defined as the birth of a live fetus after 22 weeks of gestational age. Baseline parameters, ICSI outcomes, and reproductive outcome parameters were compared among the groups.

Statistical analysis

The statistical analyses was performed by the SPSS 15.0 for Windows (SPSS, Chicago, IL, USA) programme. The Shapiro-Wilk test was performed to determine the normal or non-normal distributions for the continuous variables. The normally distributed continuous variables were compared by the one-way analysis of variance (ANOVA) and variables not normally distributed were compared by the Kruskal-Wallis test. Pearson's chi-square or Fisher's exact test was used for the categorical data. The normally distributed continuous variables were given as the mean±standard deviation (SD) and variables not normally distributed median and inter-quartile ranges. The categorical variables were given as the number of percentages. The Bonferroni-adjustment was used to control the type I errors for all possible multiple comparisons. Logistic regression analyses were used to evaluate the factors thought to affect embryo transfer following TFF cycles. A p value less than 0.05 was accepted as significant.

RESULTS

Out of 4,192 ICSI cycles over a 10 year period, 109 (2.6%) TFF cycles (group 1) were determined during first ICSI attempt. Fortyfive of these 109 participants were treated for a new ICSI cycle for the second time, and embryos were developed in 30 of these cycles, while 15 did not. Thirty embryos following TFF (group 2) were transferred for 45 new ICSI cycles performed among these cycles. Recurrent TFF occurred in 15 (33.3%) ICSI cycles (group 3). Enrollment and follow-up of the study subjects are presented in Figure 1.

Demographic and cycle characteristics of the participants is displayed in Table 1. Peak E_2 was significantly higher in group 2 (2264.80±559.66) than in groups 1 (1339.63±1014.50) and 3 (1502.87±812.70) ($p<0.001$ and $p=0.027$, respectively), and the rate of sperm morphology below 4% was lower in group 2 (36.7%) when compared to groups 1 (64.2%) and 3 (73.3%) ($p<0.011$ and $p=0.029$, respectively).

Logistic regression analysis of the factors thought to affect embryo transfer following TFF cycles in same couples are given in Table 2. When parameters in embryo transfer cycles are compared to parameters that resulted in TFF in the previous treatment cycle, there was no difference in female and male ages, day 3 hormone levels, antral follicle count, gonadotropin dose required, follicle count 10-14 mm on day hCG, progesterone levels on day hCG, endometrial thickness on hCG day, and transfer day ($p>0.05$). However, the total numbers of retrieved oocytes (5.3±4.1 vs 10.2±1.5 (Odds Ratio=1.639; 95% CI 1.267-2.122; $p<0.001$)) and MII oocytes (2.9±2.2 vs 6.8±1.8 (Odds Ratio=2.218; 95% CI 1.529-3.216; $p<0.001$)) were significantly higher in embryo transfer cycles when compared to previous TFF cycles.

Table 3 describes the laboratory and reproductive outcomes of the participants. The numbers of retrieved and MII oocytes were higher in group 2 with a median fertilization rate of 46.42% when compared to groups 1 and 3 ($p<0.001$ and $p=0.001$; $p<0.001$ and $p<0.001$, respectively). Out of 30 ICSI cycles resulting in embryo transfer, the biochemical and clinical pregnancy rates were 16.6% (n=5) and 13.3% (n=4) respectively. Out of 4 clinical pregnancies, 2 (6.6%) resulted in a live birth with healthy babies and 2 (6.6%) of them resulted in a miscarriage.

Flowchart of the study

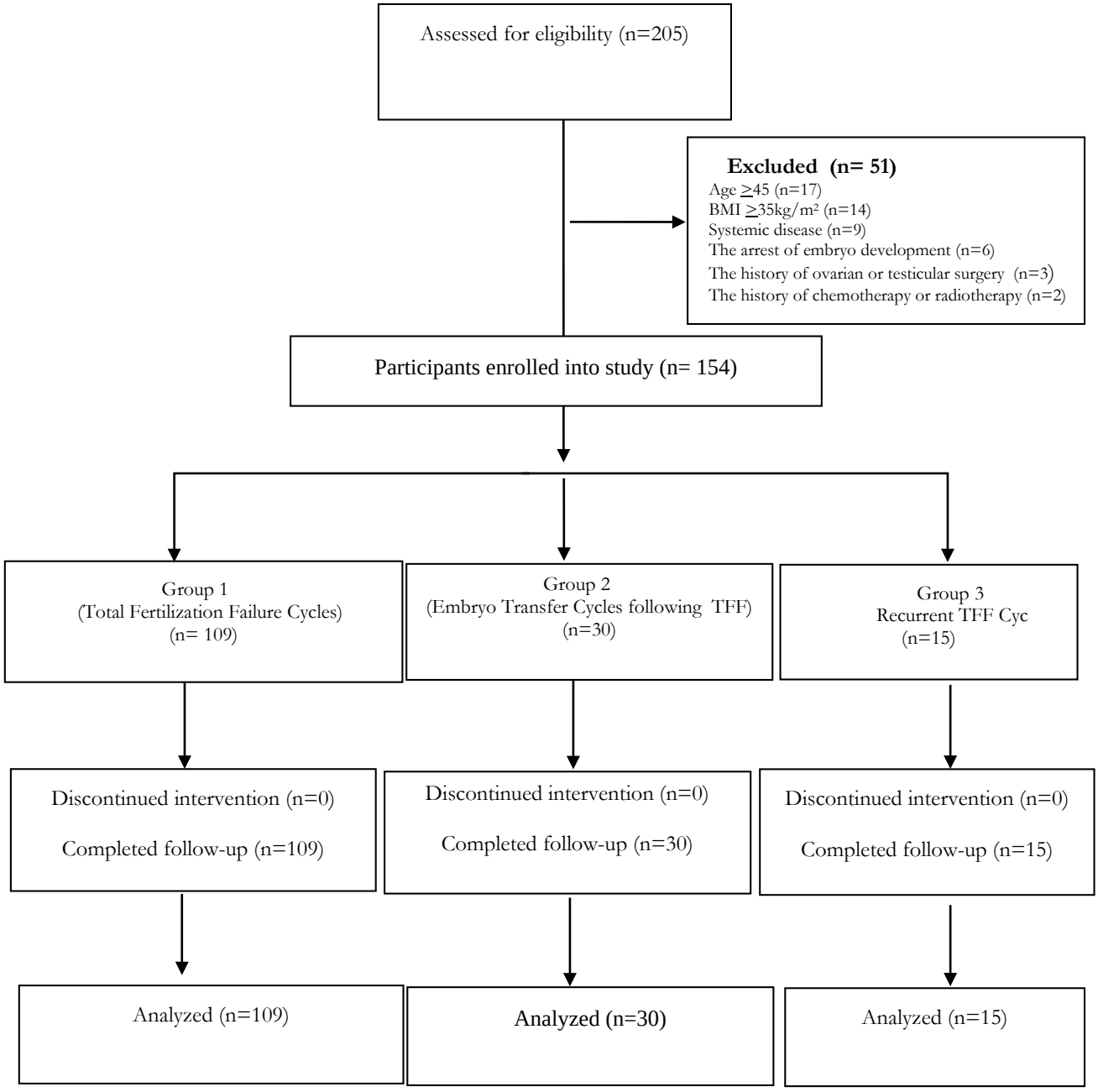

Fig 1. Enrollment and follow-up of the study subjects.

Table 1. Demographic and stimulation characteristics of the patients.

		Total Fertilization Failure cycles (Group 1) (n=109)	Cycles with Embryo Transfer (Group 2) (n=30)	Recurrent Total Fertilization Failure Cycles (Group 3) (n=15)	p		
					1 vs 2	1 vs 3	2 vs 3
[a]Age (years)		31.69±4.25	30.20±4.27	31.93±4.97	0.226		
[a]BMI (kg/m²)		26.18±4.50	25.44±4.94	26.70±3.41	0.620		
[c]Smoking rate (%)		22.9%	16.7%	20.0%	0.744		
[b]Duration of infertility (years)		5.5 (4.0-7.0)	5.0 (3.0-6.25)	6.0 (5.0-9.0)	0.525		
[c]Etiology of infertility (%)	Male factor	37.6%	46.7%	20.0%	0.361		
	Tubal factor	-	-	-			
	Unexplained	46.8%	33.3%	53.3%			
	Poor responder	15.6%	20.0%	26.7%			
[a]Baseline-FSH (IU/mL)		8.13±2.73	7.69±2.31	9.33±2.51	0.144		
[a]Baseline-LH (IU/mL)		6.62±2.41	5.56±2.18	6.49±2.38	0.108		
[a]Baseline-Estradiol (pg/mL)		46.22±17.49	41.65±22.94	48.61±14.64	0.370		
[a]Antral follicle count		8.47±2.60	9.33±2.96	7.87±2.10	0.169		
[a]TSH (μIU/mL)		2.07±0.98	1.97±0.74	2.25±0.83	0.623		
[a]Prolactin (ng/mL)		17.12±9.19	15.12±6.04	18.78±13.23	0.399		
[a]Male age (years)		36.08±4.33	34.50±4.63	36.40±3.26	0.178		
[a]TPMSC (million)		9.34±7.55	11.27±3.02	9.35±4.36	0.420		
Rate of participants with sperm morphology <4%		64.2%	36.7%	73.3%	0.011*	0.487	0.029*
[c]Stimulation protocol (%)	Long	12.8%	23.3%	13.3%	0.342		
	Antagonist	57.8%	43.3%	40.0%			
	Microdose	29.4%	33.3%	46.7%			
[b]Duration of stimulation (days)		10 (9-10)	9 (8-10)	10 (9-10)	0.123		
[a]Gonadotropin dose (IU)		2401.25±887.88	2237.91±771.05	2728.23±882.66	0.205		
[a]Follicle count ≥ 18 mm on day hCG		1.7±0.5	2.1±0.8	1.8±0.4	0.113		
[a]Follicle count 15 -17 mm on day hCG		2.6±0.7	3.1±0.6	2.6±0.5	0.125		
[a]Follicle count 10 -14 mm on day hCG		3.1±0.4	3.5±0.5	3.2±0.6	0.101		
[a]Estradiol levels on day hCG (pg/mL)		1339.63±1014.57	2264.80±559.66	1502.87±812.70	<0.001*	0.712	0.027*
[a]Progesterone levels on day hCG (pg/mL)		0.84±0.36	0.94±0.40	0.78±0.22	0.257		
[a]Endometrial thickness on day hCG (mm)		10.37±1.62	10.72±1.32	9.82±1.54	0.191		
[a]Endometrial thickness on transfer day (mm)		10.69±1.54	11.02±1.26	10.01±1.49	0.106		

BMI: body mass index; FSH: follicle stimulating hormone; LH: luteinizing hormone; TSH: thyroid stimulating hormone; TPMSC: total progressive motile sperm count; hCG: human chorionic gonadotropine

a Variables are expressed as mean + SD, b Variables are expressed as median and inter-quartile range, c (%),

*p<0.05 is significant

Table 2. Logistic regression analysis of the factors thought to affect embryo transfer following TFF cycles.

	Total Fertilization Failure cycles (n=30)	Cycles with Embryo Transfer after TFF cycles (n=30)	Odds Ratio	95% Confidence Interval	p
Age (years)	28.94±4.73	30.20±4.27	-	-	0.279
Baseline-FSH (IU/mL)	8.41±2.86	7.69±2.31	-	-	0.282
Baseline-LH (IU/mL)	6.40±1.73	5.56±2.18	-	-	0.105
Baseline-Estradiol (pg/mL)	46.12±17.75	41.65±22.94	-	-	0.398
Antral follicle count	8.21±2.29	9.33±2.96	-	-	0.167
Male age (years)	32.81±4.14	34.50±4.63	-	-	0.145
TPMSC (million)	7.63±5.13	11.27±3.02	1.260	1.048-1.515	0.014*
Rate of participants with sperm morphology <4%	64.5%	36.7%			0.041*
Gonadotropin dose (IU)	1958.12±506.60	2237.91±771.05			0.101
Follicle count ≥ 18 mm on day hCG	1.4±0.9	2.1±0.8	2.047	1.058-3.958	0.033*
Follicle count 15 -17 mm on day hCG	2.4±0.6	3.1±0.6	2.338	1.124-4.863	0.023*
Follicle count 10 -14 mm on day hCG	3.1±0.9	3.5±0.5	-	-	0.068
Estradiol levels on day hCG (pg/mL)	1308.78±1040.07	2264.80±559.66	-	-	<0.001*
Progesterone levels on day hCG (pg/mL)	1.01±0.39	0.94±0.40	-	-	0.558
Endometrial thickness on day hCG (mm)	10.21±1.07	10.72±1.32	-	-	0.101
Endometrial thickness on transfer day (mm)	10.53±1.24	11.02±1.26	-	-	0.136
Number of oocytes retrieved	5.3±4.1	10.2±1.5	1.639	1.267-2.122	<0.001*
Number of MII oocytes	2.9±2.2	6.8±0.8	2.218	1.529-3.216	<0.001*

FSH: follicle stimulating hormone; LH: luteinizing hormone; TPMSC: total progressive motile sperm count; hCG: human chorionic gonadotropine; * p<0.05 is statistically significant

Table 3. Laboratory and reproductive outcome parameters of the patients.

		Total Fertilization Failure cycles (Group 1) (n=109)	Cycles with Embryo Transfer (Group 2) (n=30)	Recurrent Total Fertilization Failure Cycles (Group 3) (n=15)	p		
					1 vs 2	1 vs 3	2 vs 3
[a]Number of oocytes retrieved		5.3±1.4	10.2±1.5	6.2±1.3	<0.001 *	0.641	0.001*
[a]Number of MII oocytes		2.9±0.4	6.8±0.8	3.1±0.7	<0.001 *	0.765	<0.001 *
2 Pronucleus		-	3 (2-4)	-			
Fertilization rate (%)		-	46.42 (33.33-60.0)	-			
Grade I embryo n (%)		-	4 (13.3)	-			
Embryo transfers n (%)	Single	-	7 (23.3%)	-			
	Multiple	-	23 (76.7%)	-			
The days of embryo transfer n (%)	2	-	6 (20.0%)	-			
	3	-	22 (73.4%)	-			
	5	-	2 (6.6%)	-			
The embryo transfer technique	Easy transfer with a soft catheter	-	17 (56.7%)	-			

n (%)	After external guidance transfer	-	9 (30.0%)	-	
	Difficult transfer with a stylet	-	4 (13.3%)	-	
Clinical pregnancy rate n (%)		-	4 (13.3%)	-	
Biochemical pregnancy rate n (%)			5 (16.6%)		
Live birth rate n (%)		-	2 (6.6%)	-	
Miscarriage rate (%)		-	2 (6.6%)	-	

BMI: Body mass index; [a] Variables are expressed as mean ± SD ; * $p<0.05$ is statistically significant

Table 5. (Supplementary file) Parameters used for oocyte grading.

Properties of oocytes	0	1
Cytoplasmic granulation	Central	Homogenous
Vacuole/inclusion	Positive	Negative
ICSI resistance	Positive	Negative
Polar body	Large fragmented bodies	Non fragmented (normal)
Perivitelline space	Presence of debris	Absence of debris
Zona pellucida	≥15 mm thickness and rough surface	≤15 mm thickness and smooth surface

*Good quality oocyte indicates the oocytes with higher than 3 points (>3).
*Poor quality oocytes indicates the oocytes with 3 or less than 3 points (≤3).
ICSI: Intra cytoplasmic sperm injection.

DISCUSSION

The current study found that fertilization and embryo transfer success in the ICSI cycle following TFF depends on the number of oocytes and MII oocytes and on sperm count with normal morphology. Although clinical and biochemical pregnancy rates are 13.3%, the live birth rate is relatively very low at 6.6%. ICSI was primarily developed to address oligoasthenoteratospermia following a successful pregnancy, and although all IVF indications are dependent on sperm parameters, ICSI indications have recently been considerably expanded[1,3,9]. Although the basic logic of ICSI is to prevent fertilization failure by bypassing negative conditions related to sperm, unfortunately the fertilization rate has not exceeded 80%[10]. In our study, a 2.6% rate of TFF was detected in the ICSI cycles reviewed, and this is similar to the existing literature.

It has been shown in some studies that sperm-head abnormalities and spermatozoic nuclear vacuoles in particular reduce fertilization rate and embryo quality[11,12]. According to the strict criteria, a 60% fertilization rate can be obtained with sperm of <4% morphology, increasing to 80% at >5% morphology[7]. Possible causes of the low fertilization rate in substandard sperm morphologies, and especially teratozoospermia, appear to be chromosomal abnormalities and/or increased DNA fragmentation[8]. The selection of spermatozoa with normal morphology is therefore vital to obtain high rates of fertilization, and various selection techniques have been developed, including the motile sperm organelle morphology examination and intracytoplasmic morphologically selected sperm injection[13]. To obtain spermatozoa with minimal DNA fragmentation and chromosomal anomalies, testicular rather than ejaculate sperm can be selected[1,8]. In the current study, the rate of abnormal spermatozoa was significantly lower in transfer cycles following TFF and the total motile progressive sperm count was higher. It is important to choose morphologically normal sperm to obtain the fertilization rate required for successful embryo transfer. This technique was not used by embyologists in this study.

For poor responders, the lower the number of oocytes collected means the more likely TFF becomes, and it has been determined that obtaining three or fewer MII oocytes is the most important risk factor for TFF[2,6]. It has been reported elsewhere that fewer than five oocytes makes embryo transfer particularly difficult with the probability of TFF in ICSI increasing from 1% to 35% if more than one or

five oocyte is retrieved, respectively[8]. As the number of immature oocytes increases with the decrease in the number of collected oocytes, the TFF rate increases indirectly; if the number of immature oocytes exceeds 25%, transfer is at risk and clinical pregnancy success decreases[2,3]. In our study, as the number of oocytes and MII oocytes increased, the rate of TFF decreased inversely in accordance with the literature. Additionally, the number of oocytes and MII oocytes collected following a TFF cycle increased by 1.6 and 2.2 times, respectively.

Fertilization is made possible by the maturation of the cytoplasm and nucleus of the oocyte and also depends on the timely receipt of the appropriate signal from the spermatozoa. The maturation of the oocyte is directly proportional to follicle diameter and requires a certain amount of time. While chromosomal structures are important in the oocyte's nuclear maturation, organelle and cytoskeleton development potential is key to cytoplasmic maturation. Abnormal structures and components occuring during this process can adversely affect fertilization[6,8]. It is known that the cytoplasmic appearance is important in ICSI success, and a dysmorphic appearance and inclusion formation can adversely affect oocyte competence which then reduces the fertilization rate, embryo quality, and, ultimately, clinical pregnancy rates[2]. This condition is particularly common in female infertility and becomes more pronounced after the age of 35[2,3]. It has elsewhere been shown that extracytoplasmic abnormalities such as dark zones or large perivitelline spaces are phenotypic deviations and do not affect fertilization rates[15]. Oocyte activation is a complex process induced by calcium releases from the endoplasmic reticulum accumulates when the sperm entry into the ooplasm. For oocyte activation that cannot be successful with artificial oocyte activation, methods aiming to increase calcium in oocyte cytoplasm with mechanical, chemical and electrical stimulation are defined[16].

There are contradictory results in the literature in terms of the laboratory and perinatal outcomes in ICSI cycles with AOA. Following 122 TFF-ICSI cycles, the activation of artificial oocyte in the new ICSI cycle showed that clinical pregnancy rates increased from 29.4% to 49% and live birth rate increased from 22.1% to 41.2%, and the authors highlighted the importance of AOA[9]. However, in a meta-analysis including four studies, although AOA increased fertilization rate, cleavage stage embryo and high grade quality emmryo rate, it was shown that it did not cause any statistical increase in implantation, clinical pregnancy and live birth rates[7].

Unexplained causes were the most common reason for infertility in a study[2], and this was also the case in the current review. Also in line with existing studies available in the literature, the GnRH antagonist was the most used stimulation protocol in embryo transfers following the TFF ICSI cycle. Clinical pregnancy rate decreases to 30.3% between the ages of 35 and 37, 25% between the ages of 35 and 37, and 10% over the age of 40. However, it should also be kept in mind that a diminished ovarian reserve reduces clinical pregnancy rates regardless of age. When Bologna criteria are used in infertile women with diminished ovarian reserves, the live birth rate varies between 6.8% and 8%[5,17]. This is considered an innate ovarian problem that has a more important prognostic role regardless of chronological age, and it was the third most common cause of infertility in our study.

The strength of this review lies in its prototypical sample from central Turkey from which the results can be generalized to most of the country's population. However, the study is limited in that it was conducted in a single tertiary care institution, the low number of cases, and is retrospective in design.

In conclusion, TFF in ICSI does not necessarily inhibit successful fertilization in the subsequent cycle if the causal factors are identified accurately and the appropriate treatment protocol implemented to retrieve adequate oocytes, MII oocytes, and morphologically normal sperm. Additional research is needed to establish the most appropriate treatment regimen in ICSI cycles that follow cases of TFF. Further studies with larger cohorts are now necessary to elucidate this issue.

Yazar Katkıları: Çalışma konsepti/Tasarımı: HAI ZOI; Veri toplama: HAI; Veri analizi ve yorumlama: ZOI; Yazı taslağı: HAI ZOI; İçeriğin eleştirel incelenmesi: HAI ZOI; Son onay ve sorumluluk: HAI ZOI; Teknik ve malzeme desteği: ZOI; Süpervizyon: HAI ZOI; Fon sağlama (mevcut ise): yok.
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