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ASSISTED REPRODUCTION TECHNOLOGIES

Total fertilization failure: is it the end of the story?

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Abstract
Purpose To study parameters that could predict in-vitro fertilization (IVF) success in patients who experienced total fertilization failure (TFF) with intracytoplasmic sperm injection (ICSI) in their previous cycles.
Methods Cycle characteristics of patients with TFF (Group I, n=136 cycles), cycles resulting in embryo transfer (ET) following TFF (Group II, n=36 cycles) and recurrent TFF (Group III, n=25 cycles) and were studied retrospectively. Demographic features, cycle characteristics of three groups were compared.
Results Follicle count measuring 15–17 mm was significantly higher in group II when compared to group I (p=0.02). Total number of retrieved oocytes and mature oocytes were significantly higher in group II when compared to groups I and III (p=0.001). Estradiol level at oocyte pick up (OPU) day was significantly higher in group II when compared to group I (p=0.02). When the characteristics of ET cycles and preceding TFF cycles of the same patient were compared, total number of retrieved oocytes (5.11±0.72 (95 % CI 3.69–6.52) vs. 11.44±1.60 (95 % CI 5.29–17.59)) and mature oocytes (3.26±3.66 (95 % CI 2.04–4.47) vs. 6.92±5.61 (95 % CI 5.09–8.75)) were found to be significantly lower in TFF cycles (p=0.001). Five biochemical and 5 clinical pregnancies occurred while only 2 healthy babies were born, corresponding to a live birth rate 5.5 %.
Conclusions Increasing the number of retrieved and mature oocytes may increase the success of fertilization in patients with a history of previous failed fertilization. However, live birth rate is still low in embryo transfer cycles.

Keywords Total fertilization failure · Intracytoplasmic sperm injection · Mature oocyte · Poor responder · Diminished ovarian reserve

Capsule Increasing the number of retrieved and mature oocytes may increase the success of cycles in patients with previous history of total fertilization failure.

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Introduction

With tremendous improvements in assisted reproductive technologies and use of sophisticated facilities for in-vitro fertilization (IVF) laboratories, fertilization rates approach 70–80 % [1]. However, fertilization failure still exists as a frustrating experience. Not only are the consequences devastating to the patient both financially and emotionally, but it also poses a difficult challenge to the clinician.

Total fertilization failure (TFF), which is the failure of fertilization in all oocytes, occurs in 5–10 % of IVF cycles [2]. Following intracytoplasmic sperm injection (ICSI), human oocytes still fail to fertilize almost 30 % of the time and TFF occurs in 2–3 % of ICSI cycles [3, 4]. Fertilization failure in IVF is mostly related to sperm abnormalities [2, 5], whereas in ICSI oocyte activation defects are the most frequent cause.

If a couple experiences fertilization failure, the likelihood of recurrence in subsequent cycles is approximately 30 % [6] suggesting that, to some extent, it is not random and could be predicted [2]. Understanding the etiology of fertilization

failure is of critical importance to assist in patient counseling and optimizing treatment.

To date studies regarding the failed fertilization consist of small number of cases only, and did not compare the characteristics of recurrent and successful cycles. Therefore, the aim of this study was to investigate the parameters that would be useful in predicting IVF success in patients who experienced failed fertilization with ICSI.

Materials and methods

Medical records of 2,030 treatment cycles from March 2007 through August 2013 at the Etlik Zubeyde Hanım Women's Health Teaching and Research Hospital, Center of Assisted Reproduction were reviewed using a computer based database. Cycles with total TFF (Group I), cycles that resulted in embryo transfer following TFF cycles (Group II) and recurrent TFF cycles (Group III) were included in this study. Cycles with no sperm or oocyte retrieval, cycles failing to undergo embryo transfer due to arrest of embryo development, and cycles with embryo transfer but without a history of total fertilization failure were excluded from the study. The approval of the local ethics committee was obtained at the beginning of the study (29.08.2013/Number:168).

Age, body mass index (BMI), basal serum follicle stimulating hormone (FSH), luteinizing hormone (LH) and estradiol (E2) values, antral follicle count (AFC), infertility etiology, duration of infertility, total progressive motile sperm count (TPMSC) and sperm morphology using Kruger's criteria and stimulation characteristics were recorded from the charts.

Controlled ovarian hyperstimulation was performed using long GnRH agonist, microdose flare or antagonist protocols. The type of gonadotropin used was either pure recombinant follicle-stimulating hormone (FSH) or human menopausal gonadotropin (hMG). Gonadotropin doses were individualized for each patient. Cycles were monitored by serial transvaginal ultrasound evaluation and serum estradiol levels. Recombinant human chorionic gonadotropin (hCG) (Ovitrelle, Serono, Istanbul, Turkey) was administered when at least three follicles showed a mean diameter of 17 mm. Oocyte pick up (OPU) procedures were performed by transvaginal ultrasound-guided aspiration 35.5–36 h after the hCG injection. Following retrieval, cumulus oophorus was removed from oocytes by incubation in solution containing hyaluronidase (Vitrolife, Sweden). The remaining cells were removed mechanically using commercial denuding pipettes. Morphologically evaluated oocytes were scored as described by Ozdegirmenci et al. [7]. Denuded oocytes were cultured in G-IVF (Vitrolife, Sweden) medium at 37 C in a humidified atmosphere of 5 % CO2-95 % air, until used for ICSI. As a policy of our clinic, ICSI is the procedure done routinely for all our patients, whereas classic IVF is only reserved for cases when the number of retrieved oocytes exceeds 20. In these cases, IVF is the performed procedure for half of the oocytes and ICSI is performed with the other half. All IVF or ICSI procedures were performed by the same team. Fertilization was checked for signs of fertilization (presence of two pronuclei and two polar bodies) 16–18 h after ICSI. Embryo transfer (ET) was performed on the second, third or fifth day after ICSI. Luteal phase support was given by vaginal progesterone (Crinone 8 % gel, Serono, UK) twice daily. Pregnancy was determined by β-hCG levels in blood tests performed 12 days after embryo transfer and clinical pregnancy was defined as the presence of a gestational sac with accompanying fetal heartbeat by ultrasound 4 weeks following the ET procedure.

Statistical analysis was performed by using IBM SPSS Statistics Software (21.0, SPSS Inc., Chicago, IL, USA). Shapiro-Wilks test was used to test the distribution of variables. Analysis of variance (ANOVA) test and Kruskal-Wallis test was used for multiple comparisons. Post-hoc analysis was done by using Bonferroni test. Data are presented as mean± standart error (SE). Statistical significance was assumed with a probability error of $p<0.05$.

Results

A total of 136 cycles of 125 patients in which TFF had occurred were selected for the study (Group I). 41 patients were admitted for 61 new cycles, 31 patients (36 cycles) underwent embryo transfer (Group II) and 10 patients (25 cycles) resulted in fertilization failure again (Group III).

There were no statistically significant differences regarding female age, BMI, day 3 hormonal profile, AFC or duration of infertility. Regarding sperm parameters, TPMSC was comparable among the groups. The rate of morphologically normal spermatozoa was significantly lower in group II when compared to group I and III ($p=0.007$ and $p=0.0001$, respectively) (Table 1).

With respect to stimulation parameters, follicle count measuring 15–17 mm was significantly higher in group II when compared to group I ($p=0.02$). Total number of retrieved oocytes and mature oocytes were significantly higher in group II when compared to group I and III ($p=0.001$). Estradiol level at OPU day was significantly higher in group II when compared to group I ($p=0.02$) (Table 2).

When cycle characteristics between TFF cycles and their own controls were compared, no significant difference was found with respect to age, BMI, baseline hormone levels, AFC, duration of infertility or sperm count, motility or morphology. However, total number of retrieved oocytes (5.11± 0.72 (95 % CI 3.69–6.52) vs. 11.44±1.60 (95 % CI 5.29–17.59)) and mature oocytes (3.26±3.66 (95 % CI 2.04–4.47) vs. 6.92±5.61 (95 % CI 5.09–8.75)) were significantly lower

Table 1 Demographic characteristics of patients

Characteristic	TFF cycles (*n*=136)	Embryo transfer cycles (*n*=36)	Recurrent TFF cycles (*n*=25)	*p-value*
Female age (years)	33.2±0.5	32.7±0.9	32.7±0.8	0.91
BMI (kg/m^2)	25.9±0.4	25.8±3.6	25.6±0.7	0.86
Day 3 LH (IU/L)	5.1±0.2	5.2±0.5	4.9±0.3	0.96
Day 3 E2 (pg/mL)	45.5±2.3	48.2±4.8	39.5±6.7	0.08
Day 3 FSH (IU/L)	8.6±0.4	7.9±0.5	9.6±1.9	0.99
Indication				
Unexplained	72 (52.9)	12 (33.3)	16 (64.0)	**
Male Factor	47 (34.5)	15 (41.7)	2 (8.0)	
DOR	9 (6.5)	8 (22.2)	5 (20.0)	
Tubal Factor	7 (5.1)	1 (2.8)	2 (8.0)	
AFC	8.7±0.6	11.1±1.2	9.1±1.6	0.08
Duration of infertility (months)	83.5±5.1	72.6±7.9	89.8±13.2	0.65
Male age (years)	35.6±0.53	35.0±0.9	35.8±1.0	0.062
TPMSC (million)	11.6±1.3	6.1±1.3	14.6±2.3	0.09
Sperm morphology (%)	2.6±0.3	1.7±0.7	5.2±1.5	0.013

TFF, Total fertilization failure; *BMI*, Body mass index; *LH*, Luteinizing hormone; *FSH*, Follicle stimulating hormone; *E2*, Estradiol; *AFC*, Antral follicle count; *DOR*, Decreased ovarian reserve; *hCG*, Human chorionic gonadotropin; *TPMSC*, Total progressive motile sperm count

* Data is presented as mean±Standard error, n(%)

** *p*-value is not presented because the number of cases in some of the cells are below 5

in TFF cycles when compared to transfer cycles (p=0.001). Two or less mature oocytes were retrieved in 60 % of the TFF cycles, whereas in 81 % of ET cycles 3 or more mature oocytes were retrieved (p=0.001).

Out of 36 cycles that resulted in embryo transfer, 5 (13.9 %) clinical pregnancies and 5 (13.9 %) biochemical pregnancies were obtained. Out of 5 clinical pregnancies, 2 resulted in miscarriage, and missed abortus occurred in one.

Table 2 Controlled ovarian stimulation parameters of the groups

	Total TFF cycles (*n*=136)	Embryo transfer cycles (*n*=36)	Recurrent TFF cycles (*n*=25)	*p-value*
Protocol				
Antagonist	53 (39.0)	13 (36.1)	10 (40.0)	0.11
Microdose	52 (38.2)	8 (22.2)	11 (44.0)	
Long	31 (22.8)	15 (41.7)	4 (16.0)	
Duration of stimulation (days)	9.4±0.2	9.5±0.4	8.8±0.6	0.75
Total gonadotrophin dose (IU)	2,939.9±134.2	2,520.2±194.7	2,780.0±380.3	0.36
E2 at day 7–8 (pg/mL)	715.1±46.9	1,123.8±165.1	803.3±110.5	0.04
E2 on hCG day (pg/mL)	1,277.4±93.9	1,815.8±282.3	1,722.9±319.9	0.06
Follicle count >17 mm on the day of hCG	1.8±0.1	2.3±0.4	2.24±0.4	0.62
Follicle count 15–17 mm on the day of hCG	2.3±0.2	3.6±0.5	3.1±0.7	0.02
Follicle count 10–14 mm on the day of hCG	4.1±0.4	4.7±0.6	3.9±0.8	0.17
Endometrial thickness on hCG day (mm)	9.3±0.2	8.9±0.3	9.9±0.5	0.17
E2 at OPU day (pg/mL)	1,070.7±88.9	1,562.4±195.3	1,363.4±277.3	0.02
P level at OPU day (ng/mL)	4.1±0.4	5.8±1.1	4.5±0.7	0.37
Total oocyte count	5.7±0.4	11.4±1.6	6.8±1.2	0.001
Number of MII oocytes	2.9±0.3	6.9±0.9	3.1±0.8	0.001
Oocyte quality index	4.6±0.1	4.9±0.2	4.3±0.3	0.26

TFF, Total fertilization failure; *E2*, Estradiol; *hCG*, Human chorionic gonadotropin; *MII*, Metaphase II; *OPU*, Oocyte pick up; *P*, Progesterone

* Data is presented as mean±SE; *n*(%)

Only two healthy babies were born, corresponding to a delivery rate of 5.5 % (Table 3).

Discussion

The presented study demonstrated that the chance of obtaining viable embryos for transfer for a couple with previous fertilization failure was improved when the number of retrieved oocytes and mature oocytes was increased. According to our results, 60 % of the TFF cycles resulted in retrieval of 2 or less mature oocytes while 81 % of ET cycles resulted in retrieval of 3 or more mature oocytes. Although pregnancy occurred in 27.8 % of the cycles, the live birth rate was still low (5.5 %).

Retrieval of few oocytes from a poor responder is the major contributor to poor ART outcome and total fertilization failure. Esfandiari et al. reported that retrieval of three or less MII oocytes is an important risk factor for failed fertilization [8]. In line with this study, Melie et al. also showed a higher risk of having no embryo transfer when the number of retrieved oocytes was less than 5 [9]. In the study of Flaherty et al., it was shown that risk of failed fertilization is most frequent in cycles in which one or two oocytes were injected in ICSI. The risk of failed fertilization was reduced from 37 % when only one oocyte was injected to 0.8 % when five or more oocytes were injected [3]. Our data support the previous reports documenting reduced risk of failed fertilization with increased number of injected oocytes with ICSI. Comparison of cycles with embryo transfer and cycles with fertilization failure revealed that the number of retrieved oocytes and mature oocytes are significantly higher in successful cycles. Also, when patients were used as their own controls, the number of retrieved and mature oocytes was found to be three times higher in transfer cycles when compared to fertilization failure cycles, which is in line with the above mentioned studies.

A decrease in the number of retrieved oocytes also increases the risk of being immature, which is also another factor in successful fertilization [10]. As the number of immature oocytes exceeds 25 % of the retrieved oocytes, successful fertilization with clinical pregnancy is greatly reduced. Approximately 8.6 % to 15.2 % of all infertility patients produce at least one meiotically incompetent oocyte [10]. It is an acceptable loss when a few of several oocytes fail to fertilize, however it is a catastrophic event when only a few oocytes are retrieved. Successful fertilization depends on cytoplasmic as well as nuclear maturation of the oocytes. Although these are the crucial steps for oocytes to obtain the ability to respond to signals from spermatozoa at the time of fertilization, oocyte maturity cannot be assessed with classical IVF techniques [11]. Maturity of oocytes is usually inferred from follicular size, however it is not an absolute relation [9]. Oocyte maturation is a long process that includes nuclear maturation and cytoplasmic maturation. Nuclear maturation mainly involves chromosome segregation, whereas cytoplasmic maturation involves proper spatial and temporal dynamics of organelles and cytoskeleton to acquire high developmental potency required for fertilization and subsequent embryo development [12]. Studies on unfertilized oocytes in IVF/ICSI cycles have revealed the presence of abnormal spindle and interphase microtubules, indicating deficiencies in ooplasmic and nuclear components, which may be a cause of failed fertilization [5, 13, 14]. The cytoplasm of the oocyte is of special interest as it is thought to be predictive of treatment success in IVF. The occurrence of specific cytoplasmic dysmorphic phenotypes in oocytes has been suggested to reflect intrinsic defects that may negatively influence oocyte competence [15–17]. Significantly lower fertilization rates, embryo cleavage rates, and lower embryo quality were reported for a group of oocytes with cytoplasmic inclusions when compared with a group of oocytes with normal cytoplasm. The incidence of the oocytes with cytoplasmic inclusions was significantly higher for female factor infertility compared with male factor infertility patients. The appearance of cytoplasmic inclusions significantly increased in women aged >35 when compared with women aged <35 [16]. However, there are conflicting data regarding the effect of extracytoplasmic morphological deviations. Although previous studies showed that embryos with an intact first polar body were associated with increased blastocyst formation compared to fragmented first polar body embryos [18], recent studies challenged this hypothesis [19, 20] demonstrating that changes in morphology grade of polar bodies occurred depending on the duration of time passed in in-vitro culture [21]. Also irregular shape of the oocyte, dark zona, or large perivitelline space were not associated with decreased fertilization rate [22, 23]. It was concluded that these types of oocyte dysmorphisms are considered as phenotypic deviations rather than abnormalities [21].

Diminished ovarian reserve corresponded to lower pregnancy rate irrespective of the woman's age among infertility reasons. SART data revealed lower pregnancy rates per cycle compared to other indications of IVF, demonstrating rates of 30.3 %, 24.9 %, 17.3 % and 10.5 % for women below 35 years old, 35–37 years, 38–40 and 41–42 years old, respectively [24]. However, a recent study by Polyzos et al. demonstrated lower live birth rates among different age groups (≤35 years, 36–39 years and ≥40 years) ranging from 6.8 to 7.9 % for poor

Table 3 Pregnancy outcomes of embryo transfer cycles who had TFF in their previous cycles

	n (%)
hCG positivity/ET	10/36 (27.8 %)
Clinical pregnancy rate/ET	5/36 (13.9 %)
Live birth rate/ET	2/36 (5.5 %)

hCG, Human chorionic gonadotropin; *ET*, Embryo transfer

responders when more strict Bologna criteria were used for the diagnosis of diminished ovarian reserve [25]. It is speculated that, irrespective of age, poor response was a result of an inherent ovarian problem that these patients share, referred to as ovarian aging, and has a prognostic role more important than chronological age [26]. However, there are conflicting reports in the literature regarding the importance of age. In their study, de Sutter et al. reported that rates of pregnancy and miscarriage in young poor and normal responders do not differ, provided that embryos of similar quality are transferred [27]. In contrast, older women had a lower pregnancy rate and a higher miscarriage rate, even when two good-quality embryos were available. Others also demonstrated higher pregnancy rate and live birth rates in younger cycling patients with high FSH when compared to older women with normal FSH [28].

According to our results, the most commonly used treatment protocol in recurrent TFF cycles was a microdose flare protocol (44 %); this reflects that those patients had diminished ovarian reserve. In embryo transfer cycles, a long luteal protocol was the protocol mostly used (41.7 %), indicating that patients with successful cycles have better ovarian response. Previous studies demonstrate that prematurely declining ovarian function occurs in approximately 10 % of infertile females and has been suggested to occur at an even higher prevalence in women with so called unexplained infertility [29, 30]. Complete fertilization failure, or poor fertilization, occurs more frequently in unexplained infertile patients undergoing IVF compared with patients with tubal factor infertility [31]. Results of the present study also support this finding that unexplained infertility was the most frequent indication in total TFF cycles (52.9 %) and cycles with recurrent TFF (64 %). On the other hand, among the patients with successful cycles, the most prevalent indication is male infertility (41.7 %). Data suggests that morphologically abnormal sperm have a negative impact on fertilization and embryo quality, even when ICSI is performed [32]. Also, several reports support this hypothesis, suggesting that the morphological quality of spermatozoa used for ICSI plays an important role in fertilization, implantation and pregnancy [33, 34]. Abnormal morphology of the sperm head and presence of nuclear vacuoles have been associated with inferior laboratory and clinical outcomes following ICSI procedures [35–37]. According to our results there was no statistically significant difference in TPMSC among the groups. The rate of morphologically normal spermatozoa was significantly lower in embryo transfer cycles. These results may reveal that selection of morphologically normal sperm in ICSI procedures ameliorates the fertilization rate. Sperm selection for ICSI is usually done under an optical magnification that enables observation of major sperm morphological defects, whereas minor morphological defects, which seem to be related to the ICSI outcome, are often not identified [38]. However, selection of sperm with better morphology via intracytoplasmic morphologically selected sperm injection (IMSI) showed an increase in implantation (25 % versus 5.9 %) and pregnancy rates (20 % versus 7 %) over conventional ICSI for patients with previous failed ICSI attempts [39].

In conclusion, diminished ovarian reserve is a risk factor for fertilization failure. Fertilization failure in one cycle does not preclude successful fertilization in another cycle. Prognosis may be more encouraging with increasing total number of retrieved oocytes and mature oocytes. A treatment protocol where the best response is anticipated should be selected for the new cycle.

Conflict of interest The authors declare that they have no conflict of interest.

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