Unexplained Total Fertilization Failure after Intracytoplasmic Sperm Injection Cycles: A Case-Control Study on Predictive Factors and Retreatment Prognosis

Parisa Mostafaei, M.D.1, Firouzeh Ghaffari, M.D.1, Zahra Zolfaghari, M.Sc.2, Samira Vesali, M.Sc.2

1. Department of Endocrinology and Female Infertility, Reproductive Biomedicine Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran
2. Reproductive Epidemiology Research Center, Royan Institute for Reproductive Biomedicine, ACECR, Tehran, Iran

Abstract

Background: The aim of our study was to detect the rate of unexplained total fertilization failure (TFF) after intracytoplasmic sperm injection (ICSI) and identify its risk factors and retreatment prognosis.

Materials and Methods: In this retrospective case-control study, we searched the computerized database of the Royan Institute (Tehran, Iran) from March 2015 to March 2019 and retrieved all cases diagnosed with TFF after ICSI. TFF cases that did not have any recognized risk factors were classified as unexplained (subgroup A). Cases with recognized risk factors were classified as subgroup B. The control group was randomly selected from infertile couples who underwent ICSI cycles with fertilization of at least one oocyte during the same time interval. Characteristics and treatment outcomes of the cases with unexplained TFF (subgroup A) were compared to the control group, and to the other TFF cases (subgroup B).

Results: Out of 18,750 couples who underwent ICSI cycles, 296 (1.58%) experienced TFF for the first time. Of these, 49 (16.5%) couples were diagnosed as unexplained TFF (subgroup A) and 247 (83.5%) were placed in subgroup B, TFF with expected risk factors. Multivariable logistic regression analysis showed that the total number of mature oocytes (P<0.001), duration of infertility (P=0.043), and women’s body mass index (BMI, P<0.001) were significant predictive factors for unexplained TFF. In the ICSI cycle after TFF, clinical pregnancy and live birth rates in subgroup A were higher than subgroup B. Although differences between these groups were not statistically significant (P=0.14 and P=0.07, respectively), this finding could be clinically important.

Conclusion: Unexplained TFF following ICSI is a rare event significantly related to a lower number of mature oocytes, longer duration of infertility and higher female BMI. It has a good prognosis in retreatment cycles in comparison with expected TFF cases. Clinicians should take this into consideration for patient counseling and management.

Keywords: Case-Control Study, Fertilization, Intracytoplasmic Sperm Injection, Retreatment

Introduction

Total fertilization failure (TFF) is a problem for patients and a major challenge for physicians (1). Fertilization via in vitro fertilization (IVF) is the result of complex molecular reactions between sperm and oocytes, and any breakdown in this process leads to TFF, which occurs in 5-10% of IVF cycles (2) and in 1-3% of intracytoplasmic sperm injection (ICSI) cycles (3, 4).

ICSI was initially used to bypass all physiological sperm screening mechanisms. This procedure can result in fertilization in couples diagnosed with severe male factor infertility. Despite recent advances in ICSI, the fertilization rate remains around 50-70% and does not differ from standard IVF in non-male factor cases (2). It is proposed that factors other than sperm binding and penetration are involved in limiting the fertilization rate (5). Significant risk factors for TFF include total immotility of spermatozoa, azoospermia and other surgically retrieved sperm conditions (6), oocyte activation deficiencies that can be caused by both sperm or oocyte related factors, cytoplasmic immaturity and spindle abnormalities (7, 8) as well as low oocyte yield, oocyte aneuploidy, fragile oocytes, defects in the in vitro sperm/oocyte medium (9), and operator proficiency for ICSI (5).
Prediction of TFF is complex and sometimes one good quality oocyte and one retrieved sperm from a globozoo-
spermia case can result in successful fertilization; how-
er, TFF can also occur in couples with apparently normal
gametes (5). Although many researchers have examined
the rate of TFF and its related risk factors, no study has
compared the prognosis after a retreatment ICSI cycle
in expected versus unexplained TFF cases. We designed
the present study to evaluate the occurrence rate of unex-
plained TFF after an ICSI cycle in patients treated at the
Royan Institute (Tehran, Iran) and to identify its risk fac-
tors and prognosis in one subsequent cycle.

Materials and Methods

The Scientific Council and Ethics Committee of the
Royan Research Institute approved this retrospective
study protocol (IR.ACECR.ROYAN.REC.1398.112). We
searched a computerized database of 18,750 ICSI cycles
performed at the Royan Institute from March 2015 to
March 2019 for cases diagnosed with TFF. The following
cases were excluded: donor oocyte (donor sperm is illegal
in our center); embryo transfer (ET) failure due to arrest
of embryo development (uncleaved embryo); and cycles
that lacked sperm or oocyte retrieval. Finally, a total of
296 cycles with TFF (the absence of two pronuclei [2PN]
embryos 16-18 hours after all injected oocytes) were
retrieved and subsequently placed in two subgroups. The
TFF cases without any recognized risk factors were clas-
sified as unexplained TFF (subgroup A) while the other
TFF cases with recognized risk factors (azoospermia,
women over 40 years of age, and less than five retrieved
oocytes) were placed in subgroup B. The control group
was randomly selected from infertile couples who under-
went ICSI cycles with fertilization of at least one oocyte
during the same time interval. Patients diagnosed with
azoospermia were not included in the control group. The
occurrence rates of unexplained TFF and its risk factors
were assessed. In addition, the outcome of ET cycles and
the recurrent rate of TFF following a second ICSI cycle
were compared in subgroups A and B among TFF cases
readmitted for subsequent ICSI cycles.

Characteristics of the couples in the study included age
and body mass index (BMI); cause, type, and duration of
infertility. Data on baseline follicle stimulating hormone
(FSH), luteinizing hormone (LH) and anti-Müllerian hor-
mone (AMH) were collected from the patients’ records.

Controlled ovarian hyperstimulation (COH) was per-
formed using long gonadotropin releasing hormone
(GnRH) agonist or antagonist protocols. Details of these
protocols have been reported previously (10). Oocyte
pick up (OPU) procedures were performed by transvagi-
nal ultrasound-guided aspiration 34-36 hours after the fi-
nal oocyte triggering. Semen samples were collected by
masturbation after 3-4 days of abstinence on the day of
the OPU. For each semen sample, separate gradients for
each 1.5 ml volume were prepared with 1.5 ml of each
custom-made SupraSperm® solutions (100% lower and
50% upper, Origio A/S). Sperm concentration and motil-
ity were assessed after mixing. A total of 1.5 ml of the
liquefied semen sample from the top of the prepared gra-
dient was dispensed, and the gradient was centrifuged at
525 RFC for 10 minutes. The supernatant was removed
and re-suspended in 5 ml Ham’s F10 Alb (10%) plus me-
dium for 5 minutes. Next, the supernatant was aspirated.
This washing procedure was repeated once more before
we determined sperm concentration and motility in the
washed sample.

The final pellet was used for ICSI. Sperm morphol-
ology was evaluated by Papanicolaou staining according
to World Health Organization (WHO; 2010) guidelines
for male infertility workup and strict criteria on sperm
morphological assessment (11). Routine oocyte and
embryo quality assessments at our institute have been
explained elsewhere in detail (12). The oocyte maturity
rate was defined as the number of metaphase II (MII)
oocytes divided by the total number of retrieved oocytes
per patient. ICSI was performed according to a standard
protocol by the same team of embryologists who used
the same technique and culture conditions for all pa-
ients. Fertilization was defined as the presence of 2PN
and two polar bodies at 16-18 hours after ICSI. ET was
performed on the second, third or fifth day after ICSI.
Vaginal progesterone suppositories (Cyclogest, 400 mg)
were administered twice per day for luteal phase sup-
port. ICSI cycle characteristics included ovarian stimu-
lation duration; total dose and type of gonadotropin; time
from triggering to ovum pickup; and ICSI performance.
Total number of retrieved and MII oocytes, total sperm
count, sperm morphology, and progressive motility were
obtained from patients’ charts. Chemical pregnancy was
determined by serum beta-human chorionic gonadotro-
ipin (β-hCG) levels (Elecsys reagent kit, Roche Cobas)
performed 14 days after ET. Clinical pregnancy was de-
defined as the presence of a gestational sac with a fetal
heartbeat observed by ultrasound four weeks after ET.
Live birth was defined as the delivery of a live fetus, ir-
respective of the duration of the pregnancy.

Statistical analysis

Data analysis was carried out using SPSS software,
version 21 (SPSS Inc., Chicago, IL, USA). Normality
distributions for continuous variables were determined
using the Kolmogorov-Smirnov test. Data are presented
as mean ± standard deviation (SD) or median (min-max),
where applicable. Mean differences between the unex-
plained TFF and control groups and between TFF sub-
groups A and B were compared using the student’s t test.
The Mann-Whitney U test was applied for the comparison
of median values. Both univariate and multivariable lo-
gistic regression analyses were performed to detect pre-
dictive factors for unexplained TFF. The odds ratio (OR)
and 95% confidence interval (CI) for significant variables
were also calculated. The Hosmer-Lemeshow test was
used to confirm the goodness-of-fit of the logistic regres-
sion model. P<0.05 were considered to be statistically
significant.
Results

In total, 18,750 ICSI cycles were performed between March 2015 and March 2019 at the Royan Institute. Of these, 296 (1.58%) couples experienced TFF for the first time; 49 (16.5%) were diagnosed with unexplained TFF (subgroup A) and 247 (83.5%) with TFF with recognized risk factors (subgroup B).

A comparison of basic characteristics between patients with unexplained TFF and control patients showed no significant differences with respect to age and baseline hormone levels (FSH, LH and AMH); however, women’s BMI (P<0.001), infertility duration (P=0.02), and cause of infertility (P=0.03) were significantly different between the groups (Table 1). Despite a similar distribution of standard COH protocols between the two groups, both total gonadotropin doses and duration of stimulation were higher in women with unexplained TFF than in the controls. Moreover, the number of mature oocytes and the oocyte maturity rate in the unexplained TFF group were significantly lower than in the control group. In terms of sperm characteristics, although total sperm count (P=0.21) and progressive motility (P=0.22) did not differ significantly between groups, the level of normal sperm morphology (P=0.050) in the unexplained TFF group was lower than in the controls. A further investigation compared mean time intervals between oocyte triggering to puncture and between oocyte retrieval to sperm injection between the groups. No statistically significant differences in oocyte triggering to puncture (P=0.9) and oocyte retrieval to sperm injection (P=0.3) were observed (Table 1).

Multivariable logistic regression analysis using stepwise backward selection demonstrated that the total number of MII oocytes (P<0.001), duration of infertility (P=0.043) and women’s BMI (P<0.001) were significant risk factors for unexplained TFF, but cause and duration of infertility, total gonadotropin dose, total number of MII oocytes, and normal sperm morphology were not (Table 2). A low chi square value (1.28, df: 8, P=0.99) from the Hosmer-Lemeshow test showed that the model was a satisfactory fit for the data.

| Characteristics                        | Unexplained TFF group (n=49) | Control group (n=100) | P value | OR* (95% CI) |
|----------------------------------------|------------------------------|-----------------------|---------|--------------|
| Female age (Y)                         | 33.4 ± 4.3                   | 33.1 ± 1.9            | 0.66    | 1.02 (0.91-1.15) |
| BMI (kg/m²)                            | 25.7 ± 3.27                  | 22.8 ± 2.7            | <0.001  | 1.39 (1.21-1.60) |
| Basal LH (IU/L)                        | 5.820 ± 4.339                | 5.2 ± 3.4             | 0.36    | 1.04 (0.95-1.13) |
| Basal AMH (ng/mL)                      | 3.444 ± 3.015                | 3.5 ± 1.3             | 0.89    | 0.98 (0.82-1.18) |
| Basal FSH (IU/L)                       | 6.195 ± 2.372                | 6.2 ± 3.2             | 0.94    | 0.99 (0.88-1.11) |
| Causes of infertility                  |                              |                       |         |              |
| Ovulatory factor                       | 1 (2.0)                      | 25 (25)               |         | Reference group |
| Tuboperitoneal factor                  | 3 (6.1)                      | 3 (3)                 | 0.12    | 0.01-1.14     |
| Unexplained factor                     | 13 (26.5)                    | 15 (15)               | 3.16    | 0.50-20.0     |
| Male factor                            | 26 (53.0)                    | 38 (38)               | 2.74    | 0.84-8.93     |
| Mixed (both female and male factors)   | 6 (12.2)                     | 19 (19)               | 2.16    | 0.76-6.15     |
| Duration of infertility (Y)            | 6.6 ± 4.0                    | 5.03 ± 3.2            | 0.020   | 1.14 (1.03-1.25) |
| COH protocol                           |                              |                       |         |              |
| Standard long GnRH agonist             | 31 (63.2)                    | 67 (67)               | 0.65    | Reference group |
| GnRH antagonist                        | 18 (36.7)                    | 33 (33)               | 1.17    | 0.57-2.40     |
| Duration of stimulation (days)         | 11.7 ± 2.4                   | 10.7 ± 2.3            | 0.01    | 1.20 (1.03-1.39) |
| Total gonadotropin dose (IU)           | 2216 ± 905                   | 1912 ± 796            | 0.03    | 1.0 (1.00-1.002) |
| Total retrieved oocyte count           | 8.9 ± 3.9                    | 9.6 ± 3.0             | 0.25    | 0.93 (0.84-1.04) |
| Number of MII oocytes                  | 5.6 ± 3.5                    | 8.0 ± 2.8             | <0.001  | 0.75 (0.66-0.86) |
| Oocyte maturity rate                   | 0.6 ± 0.3                    | 0.8 ± 0.1             | <0.001  | 0.037 (0.007-0.17) |
| Total sperm count (million)            | 47.9 ± 29.8                  | 41.8 ± 25.3           | 0.21    | 1.007 (0.99-1.02) |
| Normal sperm morphology (%)            | 4.2 ± 2.9                    | 5.3 ± 3.0             | 0.050   | 0.25 (0.14-0.46) |
| Progressive motility (%)               | 20.0 ± 10.1                  | 23.6 ± 18.8           | 0.22    | 1.02 (0.99-1.04) |
| Time interval between oocyte retrieval and sperm injection (hours) | 1.21 ± 0.71 | 1.10 ± 0.6 | 0.34 | 0.60 (0.13-2.77) |

Data are presented as mean ± SD or n (%). OR: Odds ratio, CI: Confidence interval, *: These results were obtained from univariate logistic regression analysis, TFF: Total fertilization failure, BMI: Body mass index, LH: Luteinizing hormone, AMH: Anti-Müllerian hormone, FSH: Follicle stimulating hormone, COH: Controlled ovarian hyperstimulation, GnRH: Gonadotropin releasing hormone, and MII: Metaphase II.
Table 2: Multivariable logistic regression analysis with unexplained TFF as the outcome of interest

| Risk factors               | OR    | 95% CI          | P value |
|---------------------------|-------|-----------------|---------|
| Number of mature oocytes  | 0.72  | 0.603, 0.874    | <0.001  |
| Women’s BMI               | 1.54  | 1.281, 1.862    | <0.001  |
| Duration of infertility   | 1.15  | 1.004, 1.322    | 0.043   |

TFF; Total fertilization failure, BMI; Body mass index, OR; Odds ratio, and CI; Confidence interval.

In the follow-up, out of 49 patients diagnosed with unexplained TFF, 17 patients were referred again for an ICSI treatment cycle. Of these, 5 (29.4%) had recurrent TFF. Out of the 12 patients who had ET, 3 (25.8%) had clinical pregnancies and live births. By comparison, out of the 247 couples diagnosed with expected TFF, 61 patients were referred for an ICSI treatment cycle. Of these, 20 (32.8%) had recurrent TFF and 41 patients had ET. The analysis indicated that the recurrence rate was similar between the groups (P=0.79, Table 3).

Table 3: Comparison of readmission and recurrence rates between TFF subgroups

|                          | Unexplained TFF (n=49) | Expected TFF (n=247) | P value |
|--------------------------|------------------------|----------------------|---------|
| Readmission for ICSI cycle after TFF | 17 (34.7)       | 61 (24.7)            | 0.10    |
| TFF recurrence rate      | 5/17 (29.4)           | 20/61 (32.8)         | 0.79    |

Data are presented as n (%). TFF; Total fertilization failure and ICSI; Intracytoplasmic sperm injection.

Table 4: Comparison of basic and controlled ovarian stimulation cycle characteristics of patients who underwent ART cycle after the first TFF

| Characteristics                        | Unexplained TFF (subgroup A) (n=17) | Expected TFF (subgroup B) (n=61) | P value |
|----------------------------------------|-------------------------------------|---------------------------------|---------|
| Female age (Y)                         | 32.3 ± 5.2                          | 34.4 ± 5.0                      | 0.14    |
| Male age (Y)                           | 35.2 ± 5.2                          | 39.3 ± 6.7                      | 0.02    |
| Female BMI (kg/m²)                     | 26.9 ± 2.51                         | 27.19 ± 4.5        | 0.85    |
| Male BMI (kg/m²)                       | 26.3 ± 6.1                          | 28.9 ± 6.5                      | 0.04    |
| Male smoker                            | 5 (29.4)                            | 19 (31.1)                      | 0.57    |
| Basal LH (IU/L)                        | 3.8 ± 1.7                           | 4.9 ± 4.1                      | 0.28    |
| Basal AMH (ng/mL)                      | 1.9 ± 1.8                           | 2.4 ± 3.9                      | 0.60    |
| Basal FSH (IU/L)                       | 5.8 ± 2.5                           | 7.0 ± 3.6                      | 0.21    |
| Cause of infertility                   |                                     |                                 | <0.0001 |
| Ovulatory factor                       | 2 (12)                              | 13 (21.4)                      |         |
| Tuboperitoneal factor                  | 0 (0)                               | 1 (1.6)                        |         |
| Unexplained factor                     | 8 (47)                              | 4 (6.5)                        |         |
| Male factor                            | 6 (35)                              | 21 (34.4)                      |         |
| Mixed (both female and male factors)   | 1 (6)                               | 22 (36.1)                      |         |
| Duration of infertility (Y)            | 5.7 ± 3.8                           | 7.7 ± 6.1                      | 0.19    |
| COH protocol                           |                                     |                                 | 0.88    |
| Standard long GnRH agonist             | 6 (35.3)                            | 24 (39.3)                      |         |
| GnRH antagonist                        | 11 (64.7)                           | 37 (60.7)                      |         |
| Duration of stimulation (days)         | 10.7 ± 2.0                          | 10.6 ± 2.83                    | 0.83    |
| Total gonadotropin dose (IU)           | 2053 ± 807                          | 1747 ± 1086                    | 0.29    |
| Total retrieved oocyte count           | 8.0 ± 4.2                           | 4.8 ± 4.4                      | 0.01    |
| Number of MII oocytes                  | 5.6 ± 3.334                         | 3.9 ± 3.8                      | 0.10    |
| Oocyte maturity rate                   | 0.9 ± 0.2                           | 0.7 ± 0.2                      | 0.02    |
| Oocyte activation (yes)                | 3 (17.6)                            | 7 (11.4)                       | 0.37    |
| Total sperm count (million)            | 52.7 ± 29.9                         | 35.6 ± 33.1                    | 0.06    |
| Normal sperm morphology (%)            | 2.0 ± 1.1                           | 1.2 ± 1.1                      | 0.01    |
| Progressive motility (%)               | 32.1 ± 17.3                         | 18.7 ± 16.2                    | 0.004   |
| Physiological ICSI                    | 1 (5.88)                            | 2 (3.27)                       | 0.52    |
| Number of transferred embryos          | 2.6 ± 2.5                           | 1.5 ± 1.6                      | 0.14    |
| Positivity β-hCG/ET                    | 4/12 (33.3)                         | 6/41 (14.6)                    | 0.20    |
| Clinical pregnancy/ET                  | 3/12 (27.3)                         | 4/41 (10)                      | 0.14    |
| Live birth rate/ET                     | 3/12 (27.3)                         | 3/41 (7.5)                     | 0.07    |

Data are presented as mean ± SD or n (%). TFF; Total fertilization failure, BMI; Body mass index, LH; Luteinizing hormone, AMH; Anti-Müllerian hormone, FSH; Follicle stimulating hormone, COH; Controlled ovarian hyperstimulation, MII; Metaphase II, ICSI; Intracytoplasmic sperm injection, GnRH; Gonadotropin releasing hormone, β-hCG; Beta-human chorionic gonadotropin, and ET; Embryo transfer.
A comparison of baseline characteristics and COH cycle outcomes for subgroup A and B patients who had a second ICSI cycle after the first TFF is presented in Table 4. The analysis showed no significant difference between the unexplained TFF (subgroup A) and expected TFF (subgroup B) in terms of female age and BMI, basal serum LH, FSH and AMH levels, duration of infertility, COH protocol, and total dose of gonadotropins used. Notably, mean male age (P=0.02) and BMI (P=0.04) in subgroup A were significantly higher than in subgroup B. The majority of couples in subgroup A had unexplained factor infertility compared to mixed factor infertility in subgroup B (P<0.001). The couples in subgroup A had significantly higher numbers of retrieved and MII oocytes, as well as higher levels of progressive motility and normal sperm morphology. The rates of oocyte activation and physiological ICSI did not significantly differ between the two subgroups. Finally, ET outcomes were compared between the TFF subgroups. Although clinical pregnancy and live birth rates in the unexplained TFF (subgroup A) were higher than in the expected TFF (subgroup B), these differences were not statistically significant between the groups (P=0.14 and P=0.07, respectively, Table 4).

Discussion

The results of the present study indicated that TFF occurred in 1.58% of all ICSI cycles during the four-year study period. This finding is in line with previous reports, range 1 to 5%, and those reported by Goksan Pabuccu et al. (13) 4.3%, Esfandiari et al. (14) 3.6%, and Bhattacharya et al. (4) 1%. The TFF recurrence rate (32%) in the current study was slightly higher than those previously reported, 15 to 30% (2, 5, 13). As not all the patients with a diagnosis of TFF in our study were referred for a second treatment cycle, the calculated recurrence rate may have been slightly under or overestimated. In addition, we note that unexplained TFF was rare (0.26%). Shinar et al. (15) reported that the rate of TFF in patients under 40 with at least five total retrieved oocytes was 0.7%. It is interesting that the rates of readmission for a second treatment cycle and repeated TFF in both subgroups of patients (unexplained and expected) were similar (range: 25 to 35%). The results of a previous study showed that repeated TFF occurred in 13% of treatment cycles during the second ICSI attempt. Although the rate of referral for a second cycle in patients with expected TFF was lower in the unexplained group (24 vs. 34%) and was not statistically significant, this finding could be clinically significant. Most couples who experience TFF following an ICSI cycle with azoospermia or where the woman is of advanced age and diminished ovarian reserve are reluctant to begin retreatment.

In the present study, male factors, unexplained factors and longer durations of infertility were more common among couples with unexplained TFF compared to controls. These results are comparable to those reported by Shinar et al. (15). The higher mean BMI in patients with unexplained TFF might explain the higher level of gonadotropin consumption and longer duration of ovarian stimulation in these patients compared to controls. The results of a large cohort study indicated that, in comparison with women of normal weight, overweight women (BMI>25＜30 kg/m²) had significantly fewer retrieved oocytes (16). In another large cohort study, overweight women (BMI>25＜30 kg/m²) had significantly lower fertilization rates compared to women of normal weight (16, 17). These data suggest that weight reduction may be advisable for patients with unexplained TFF to improve the outcomes of subsequent ICSI treatments.

Pregnancy results in patients who underwent a second ICSI/ET cycle in both TFF subgroups were similar; however, differences between the two groups in terms of live birth rates were clinically significant. Rates in the unexplained TFF group were three times higher than in the expected TFF group. In our opinion, the results of the present study give us the opportunity to make better decisions in counseling and in selection of an appropriate clinical approach to these patients.

Our results indicate that total sperm count and progressive motility rate had no significant relationship with unexplained TFF, whereas sperm morphology was significantly related in univariate regression analysis. In agreement with our findings, previous research has indicated that total sperm motility has no effect on ICSI results; however, fertilization is decreased or fails when few spermatozoa are available for injection, particularly when nonviable sperm are injected (6). The effects of semen origin on fertilization have been extensively studied, with no significant differences observed in fertilization and clinical outcomes of ICSI cycles (18). Univariate analysis in the current study showed that patients with unexplained TFF had a lower level of normal sperm morphology compared to the control group. However, in the multivariate logistic regression model, these sperm characteristics were not significant predictors of unexplained TFF following ICSI. Recently, Krog et al. (19) concluded that female factors (low number of retrieved oocytes, female smoking, and non-tubal infertility) and an apparently minor sperm factor (low number of progressive motile spermatozoa) were independent predictors of TFF.

Some researchers suggest that ICSI success rates are independent of typical semen analysis and largely depend on the total number of retrieved oocytes (20). In the present study, the number of MII oocytes was the most important factor in predicting unexplained TFF following ICSI. Esfandiari et al. (14) found that ≤2 total MII oocytes was one of the most significant risk factors for TFF. Melie et al. (21) reported that the risk of TFF increased when the number of retrieved oocytes was less than two. Flaherty et al. (3) showed that the risk of TFF decreased from 37 to 0.8% when the total number of retrieved oocytes increased from one to more than five. We therefore consider that less than five total retrieved oocytes is a major risk factor for TFF. Interestingly, although the total number of retrieved oocytes in the unexplained TFF group was similar to the
control group, the number of MII oocytes in this group was significantly lower.

Most TFF cases can be explained by factors such as sperm abnormalities (azoospermia, nonviable, immotile) and oocyte abnormalities (number, maturity, and morphology). However, in some TFF cases, there is no observed abnormality. In these unexplained TFF cases, technical conditions are reported to be involved. One of the most common technical causes of TFF is the failure properly to insert sperm into the oocyte cytoplasm; therefore, choosing the right injection site may increase the number of euploid embryos (22). In the present study, we evaluated the first occurrence of unexplained TFF following ICSI using the same embryologist team at the Royan Institute. We evaluated technical factors, time intervals (hours) between oocyte triggering and puncture, and between oocyte retrieval and sperm injection. We found no relationship between these factors and unexplained TFF. Interestingly, Zhang et al. (23) evaluated the relationship between general and partial time intervals, from serum β-hCG to sperm micro-injection, and ICSI outcomes (fertilization rate, available embryo rate and clinical pregnancy rate). Similar to our findings, they found no relationship between the time interval from oocyte pickup to ICSI and fertilization and clinical pregnancy rates. As a new finding, they reported that an extended time interval between denudation (DN) and ICSI was associated with a higher rate of fertilization than a short DN-ICSI interval, but there was a significant decrease in the clinical pregnancy rate when the interval was over four hours (24). Further studies are warranted to confirm this finding.

Based on recent studies, some couples who undergo ICSI are unable to achieve successful fertilization due to unexplained reasons. In these challenging cases, the ICSI cycles are generally associated with fertilization rates of 70 to 80% and TFF rates of 1 to 3% (24). In the current study, we found that couples with unexplained TFF who undergo an ICSI cycle after the first TFF have higher levels of oocyte maturity, normal morphology, and progressive motile sperm compared to those with expected TFF, which may explain the better pregnancy outcomes in this group. A strength of our study is that this is the first study to compare two subgroups of patients with TFF. To strengthen our conclusions, we suggest that more prospective studies should be undertaken in this area.

Conclusion

The results of the current study show that unexplained TFF following ICSI is a rare event that is significantly related to a lower number of mature oocytes, longer duration of infertility and higher female BMI. It has a good prognosis in retreatment ICSI cycles when compared with expected TFF cases. Clinicians should take this into consideration during patient counseling and management.

Acknowledgements

We express our appreciation to our colleagues at Royan Institute, especially to Mrs. Arezoo Arabipoor, for their assistance with this study. There are no financial support and conflict of interest in this study.

Authors’ Contributions

P.M., F.Gh.; Designed the study, drafted and revised the manuscript, participated in the conception of the study, and data interpretation. Z.Z.; Contributed to data acquisition and drafted and revised the manuscript. S.V.; Performed data analysis and revised the manuscript. All authors read and approved the final manuscript.

References

1. Kahyaoglu I, Demir B, Turkkani A, Cınar O, Dilbaz S, Dilbaz B, et al. Total fertilization failure: is it the end of the story? J Assist Reprod Genet. 2014; 31(9): 1155-1160.
2. Mahutte NG, Arici A. Failed fertilization: is it predictable? Curr Opin Obstet Gynecol. 2003; 15(3): 211-218.
3. Fisherty SP, Payne D, Matthews CD. Fertilization failures and abnormal fertilization after intracytoplasmic sperm injection. Hum Reprod. 1998; 13 Suppl 1: 155-164.
4. Bhattacharya S, Hamilton MP, Shaaban M, Khalaf Y, Seddler M, Gobara T, et al. Conventional in-vitro fertilisation versus intracytoplasmic sperm injection for the treatment of non-male factor infertility: a randomised controlled trial. Lancet. 2001; 357(9274): 2075-2079.
5. Sarikaya E, Eryilmaz OG, Deveer R, Dogan M, Mallamahmutoglu L. Analysis of 232 total fertilization failure cycles during intracytoplasmic sperm injection. Iran J Reprod Med. 2011; 9(2): 105-112.
6. Nasr-Esfahani MH, Razavi S, Tavalaeae M. Failed fertilization after ICSI and spermogenic defects. Fertil Steril. 2008; 89(4): 892-898.
7. Sang G, Li B, Kuang Y, Wang X, Zhang Z, Chen B, et al. Homozygous mutations in WEE2 cause fertilization failure and female infertility. Am J Hum Genet. 2018; 102(4): 649-657.
8. Yeste M, Jones C, Amdani SN, Patel S, Coward K. Oocyte activation deficiency: a role for an oocyte contribution? Hum Reprod Update. 2016; 22(1): 23-41.
9. Fisherty SP, Dianna P, Swann NJ, Matthews CD. Aetiology of failed and abnormal fertilization after intracytoplasmic sperm injection. Hum Reprod. 1995; 10(10): 2623-2629.
10. Madani T, Ashrafi M, Mohammad Yeganeh L. Comparison of different stimulation protocols efficacy in poor responders undergoing IVF: a retrospective study. Gynecol Endocrinol. 2012; 28(2): 102-105.
11. Cooper TG, Noonan E, Von Eckardstein S, Auger J, Baker HWG, Behre HM, et al. World Health Organization reference values for human semen characteristics. Hum Reprod Update. 2010; 16(3): 231-245.
12. Ashrafi M, Karimian L, Eftekari-Yazdi P, Hasani F, Arabipoor A, Bahmanabadi A, et al. Effect of oocyte dysmorphisms on intracytoplasmic sperm injection cycle outcomes in normal ovarian responders. J Obstet Gynaecol Res. 2015; 41(12): 1912-1920.
13. Goksan Pabuccu R, Sinem Caglar G, Dogus Demirkiran O, Pabuccu R. Uncommon but devastating event: total fertilisation failure following intracytoplasmic sperm injection. Andrologia. 2016; 48(2): 164-170.
14. Esfandiarl R, Javed MH, Götlible L, Casper RF. Complete failed fertilization after Intracytoplasmic sperm injection-analysis of 10 years’ data. Int J Fertil Womens Med. 2005; 50(4): 187-192.
15. Shinar S, Almog B, Levin I, Shwartz T, Amit A, Hasson J. Total fertilization failure in intra-cytoplasmic sperm injection cycles-classification and management. Gynecol Endocrinol. 2014; 30(8): 593-596.
16. Zhang D, Zhu Y, Gao H, Zhou B, Zhang R, Wang T, et al. Overweight and obesity negatively affect the outcomes of ovarian stimulation and in vitro fertilisation: a cohort study of 2628 Chinese women. Gynecol Endocrinol. 2010; 26(5): 325-332.
18. Yang C, Zhou ZH, Zheng DN, Xu XF, Huang J, Lian Y, et al. Sperm origins and concentration do not impact the clinical outcomes in intracytoplasmic sperm injection cycles. Asian J Androl. 2018; 20(5): 454-458.

19. Krog M, Prior M, Carlsen E, Loft A, Forman J, Pinborg A, et al. Fertilization failure after IVF in 304 couples—a case-control study on predictors and long-term prognosis. Eur J Obstet Gynecol Reprod Biol. 2015; 184: 32-37.

20. Khatun A, Rahman MS, Pang MG. Clinical assessment of the male fertility. Obstet Gynecol Sci. 2018; 61(2): 179-191.

21. Melie NA, Adeniyi OA, Igbinedewka OM, Ajayi RA. Predictive value of the number of oocytes retrieved at ultrasound-directed follicular aspiration with regard to fertilization rates and pregnancy outcome in intracytoplasmic sperm injection treatment cycles. Fertil Steril. 2003; 80(6): 1376-1379.

22. Fleming SD, King RS. Micromanipulation in assisted conception. Cambridge: Cambridge University Press; 2003.

23. Zhang Y, Ma Y, Fang Z, Hu S, Li Z, Zhu L, et al. Performing ICSI within 4 hours after denudation optimizes clinical outcomes in ICSI cycles. Reprod Biol Endocrinol. 2020; 18(1): 27.

24. Cardona Barberán A, Boel A, Vanden Meerschaut F, Stoop D, Heindryckx B. Diagnosis and treatment of male infertility-related fertilization failure. J Clin Med. 2020; 9(12): 3899.