Clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) using extracted testicular sperm or ejaculated extreme severe oligo-astheno-teratozoospermia sperm: a comparative study

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Objective: To evaluate the clinical outcomes and development of children born after intracytoplasmic sperm injection (ICSI) with extracted testicular sperm or ejaculated extreme severe oligo-astheno-teratozoospermia (OAT) sperm.
Design: Retrospective study.
Setting: Infertility clinic at Chang Gung Memorial Hospital.
Patient(s): A total of 126 ICSI cycles were performed using extracted testicular sperm from men with azoospermia and 65 ICSI cycles using fresh ejaculated sperm from men with extreme severe OAT.
Intervention(s): Retrospective analysis of clinical outcomes and development of children born after ICSI with extracted testicular sperm or ejaculated extreme severe OAT sperm.
Main Outcome Measure(s): Fertilization rates, number of grade 1 zygotes and number of embryos produced, implantation rate, clinical pregnancy rate, abortion and live birth rate per transfer, perinatal outcomes, and birth defects.
Result(s): The demographic and clinical factors, including age, E_2 level on hCG day, number of oocytes retrieved, normal fertilization rate, zygote grade 1 score distribution, number of top-quality embryos transferred, clinical pregnancy rate per transfer, chemical pregnancy rate per transfer, implantation rate, live birth rate per transfer, and abortion rate per transfer, were similar between the groups. Sixty live births resulted from 48 extracted testicular sperm cycles and 21 live births from 19 extreme severe OAT. The obstetric and perinatal outcomes were similar between the groups, and children conceived by using ICSI were healthy and without major psychomotor or intellectual development retardation. One case of tetralogy of Fallot occurred in each group.
Conclusion(s): There is no evidence of differences in the clinical outcomes and development of children result after ICSI with extracted testicular sperm or ejaculated extreme severe OAT sperm. (Fertil Steril® 2011;96:567–71. ©2011 by American Society for Reproductive Medicine.)

Key Words: ICSI, TESE, severe oligoasthenoteratozoospermia, clinical outcome

Infertile men with severe oligo-astheno-teratozoospermia (OAT) or azoospermia are uncommon, but not rare. Most of these men are healthy, and the cause of impaired spermatogenesis is rarely identified with certainty (1). Now, even men with no sperm in the ejaculate (azoospermia), whether from obstruction or defects of sperm production (nonobstructive azoospermia), may have a chance to father a biological child by using intracytoplasmic sperm injection (ICSI) (2).

There is, however, concern that the quality of spermatozoa in terms of DNA damage or maturation when collected from ejaculated semen may differ from that collected from the testis. It is also questioned whether sperm of different origins will affect the outcome and safety of ICSI. Many studies have shown conflicting results when ICSI is performed with sperm from different sources (3–10). Also, the relationship between poor-quality semen and the development of embryos as well as clinical outcomes in patients undergoing ICSI is still uncertain (10–12). It can be reasoned that because spermatozoa were of limited number and good-quality sperm were chosen for ICSI, similar results might have been found after ICSI with different semen qualities (6, 13–18).

Although ICSI was initially developed, and has been shown to be an effective treatment for male factor infertility (19), because of the lack of natural selection of sperm in the ICSI procedure, researchers and clinicians have reason to be concerned about its use and the health of the children born using this technique (20–23).

The clinical outcomes and development of children born after ICSI with extracted testicular sperm or ejaculated extreme severe OAT sperm has not been thoroughly assessed or compared. The goal of this study is address this issue.

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TABLE 1

Comparison of ICSI using testicular biopsy sperm from men with azoospermia vs. fresh ejaculate sperm from men with extreme severe OAT.

Transfer	Testicular biopsy sperm	Fresh ejaculate extreme severe OAT sperm	*P* value
No. of cycles	126	65	
Age of female partner (y)	31.6 ± 5.0	32.1 ± 4.7	.484
Age of male partner (y)	36.4 ± 5.6	35.1 ± 4.9	.104
Body mass index of female partner	22.1 ± 3.6	22.0 ± 3.0	.928
Duration of infertility (y)	3.6 ± 2.6	4.7 ± 3.7	**.031**
Days of FSH treatment	9.2 ± 2.3	9.2 ± 1.6	.948
Ampoules of 75 IU FSH	32.5 ± 14.0	30.7 ± 10.9	.378
Endometrial thickness on hCG day (mm)	1.4 ± 0.3	1.4 ± 0.3	.917
E_2 (pg/mL) on hCG day	1876.6 ± 1327.1	2321.6 ± 1747.3	.055
No. of oocytes retrieved	7.3 ± 4.1	8.2 ± 3.6	.115
No. of MII oocytes injected	784	447	
Normal fertilization rate	91.3% (716/784)	90.8% (406/447)	.767
Zygote score:			
Z1	47.9% (343/716)	48.0% (195/406)	.968
Z2	35.5% (254/716)	35.5% (144/406)	.998
Z3	14.8% (106/716)	16.0% (65/406)	.589
Z4	1.8% (13/716)	0.5% (2/406)	.064
No. of top-quality embryos transferred	1.5 ± 0.8	1.6 ± 0.9	.717
Clinical pregnancy rate per transfer	46.6% (55/118)	39.7% (25/63)	.371
Chemical pregnancy rate per transfer	4.2% (5/118)	3.2% (2/63)	.724
Implantation rate	26.2% (80/305)	22.7% (35/154)	.427
Live birth rate per transfer	40.7% (48/118)	30.2% (19/63)	.197
Abortion rate per transfer	7.6% (9/118)	9.5% (6/63)	.778

Note: Values are mean ± SD or percentage. Boldface indicates significance. MII = metaphase II.

Tsai. TESE vs. extreme severe OAT in ICSI. Fertil Steril 2011.

MATERIALS AND METHODS

Data were collected from consecutive infertile couples who underwent ICSI and transcervical fresh ET from January 2001 to April 2009 at our institute. A detailed chart review was conducted of 126 ICSI cycles using extracted testicular sperm from men with azoospermia and 65 ICSI cycles using fresh ejaculated sperm from men with extreme severe OAT. Ejaculates were investigated at least two times on different occasions, according to the guidelines of the World Health Organization (24). We defined infertile men as having a sperm count <1 million/mL, with all spermatozoa immotile or only nonprogressively motile widely defined as extreme severe oligozoospermia and/or severe asthenozoospermia (extreme severe OAT, defined as total sperm count <1 million/mL and/or <5% rapid progressive type A motility and/or <4% morphologically normal spermatozoa in the fresh semen). The azoospermic men selected for ICSI were counseled to undergo testicular sperm extraction (TESE) by the urological services of the hospital. Formal scrotal exploration was performed before surgical extraction. The techniques were in accordance with those described in previous reports (25).

The laboratory facilities, clinical strategy, and protocol for controlled ovarian hyperstimulation followed the standard down-regulation regimen we published previously (26, 27). A single team of embryologists coordinated all procedures, thereby ensuring that both the culture protocols and embryo assessments were standardized. This study was approved by the Ethics Committee of Chang Gung Memorial Hospital. Approval from the Institutional Review Board was obtained for analysis of this series.

Assessment of Fertilization, Embryo Culture, and Zygote and Embryo Grading

For ICSI, the procedure of immobilization of the motile spermatozoa, done by pressing the tail on the glass dish with an injection pipette, was completed without the help of polyvinylpyrrolidone (PVP), as described elsewhere (20). Gametes were fertilized in universal IVF medium (Medi-Cult), and fertilization was evaluated 16–18 hours after ICSI. Normal fertilization was defined as zygotes with two pronuclei (2PN) after ICSI. After the ICSI procedure, oocytes were cultured and assessed for the presence of pronuclei after 16–18 hours of incubation. The zygotes were scored according to the Z-score scoring system (27). The system takes into account nuclear size and alignment and nucleoli number and distribution. G1.2TM medium (Scandinavian IVF Science) was used for culture of embryos on days 1–3, and G2.2TM medium (Scandinavian IVF Science) was used for culture of embryos from days 3–5 or 6. On day 3, all transferable embryos were assessed for blastomere number and regularity as well as presence and volume of cytoplasmic fragmentation. After 2 days of culture in G2.2 medium, blastocyst formation was evaluated. The scoring assessment for blastocysts was based on the expansion state of the blastocyst, and on the consistency of the inner cell mass and trophectoderm cells. Z1 zygotes had equal numbers of nucleoli aligned at the pronuclear junction (the absolute number was not counted, but it was between three and seven). Grade 1 day 3 embryo morphologies (eight cells, blastomeres of equal size, and no cytoplasmic fragments) or day 5 blastocysts (full blastocysts onward; development of the inner cell mass with numerous, tightly packed cells; and trophectoderm, with many cells forming a cohesive epithelium) were considered as "top-quality" embryos.

Establishment and Follow-Up of Pregnancy

In our program, we have routinely offered blastocyst transfer to patients with more than three 8-cell embryos on day 3. Luteal-phase supplementation of micronized P (Utrogestan, 800 mg intravaginally daily; Piette International Laboratories) was begun on the day of oocyte retrieval, and 5000 IU of hCG was administered on day 6 after oocyte recovery in all patients. Pregnancy was confirmed on detecting hCG in the urine 2 weeks after transfer. Clinical pregnancy was determined by means of transvaginal ultrasonography on identifying a gestational sac at 7 weeks' gestation. If conception had occurred, micronized P supplementation was provided for an additional 4 weeks.

TABLE 2

Demographic characteristics of maternal and live birth ICSI children: TESE cycles versus extreme severe OAT cycles.

	TESE	Extreme severe OAT	*P* value
No. of cycles	48	19	
Age of female partner (y)	30.9 ± 5.4	33.7 ± 4.1	.037
Body mass index	22.3 ± 3.6	23.0 ± 3.8	.610
Cesarian section, n (%)	29 (60.4%)	13 (68.4%)	.588
Multiple pregnancy	13 (27.1%)	6 (31.5%)	.768
High-order pregnancy with fetal reduction	5 (10.4%)	1 (5.3%)	.505
Vanishing twin	0	2 (10.5%)	.077
Pregnancy complications, n (%)	6 (12.5%)	2 (10.5%)	.882
All deliveries	60	21	
Female/male, n (%)	30 (50.0%)/30 (50.0%)	4 (19.0%)/17 (81.0%)	.014
Parity: first-born, n (%)	41 (85.4%)	16 (84.2%)	.845
Birth parameters			
Gestational age (range), wk	37.4 ± 2.5 (30–40)	36.6 ± 2.8 (27–40)	.261
Birth weight (range), g	2707.0 ± 652.1 (930–4020)	2868.0 ± 686.8 (870–4050)	.340
Prematurity, n (%) (gestational age <37 wk)	17 (28.3%)	6 (28.5%)	.983
Birth weight <2500 g, n (%)	23 (38.3%)	5 (23.8%)	.292
Apgar 1 min <5 or 5 min <7, n (%)	5 (8.3%)	1 (4.7%)	.591
Neonatal respiratory distress syndrome, n (%)	9 (15.0%)	5 (23.8%)	.503
Children's age at follow-up (range)	5.6 ± 2.0 (1–8)	4.4 ± 2.4 (1–8)	.050
Minor congenital anomalies	5 (8.3%)	1 (4.8%)	.591
Heart	3	1	
Musculoskeletal system	1	0	
Urogenital system	1	0	
Major congenital anomalies Heart (TOF)	1 (1.7%)	1 (4.7%)	.454

Note: Values are mean ± SD or percentage. TESE = testicular sperm extraction; TOF = tetralogy of Fallot.

Tsai. TESE vs. extreme severe OAT in ICSI. Fertil Steril 2011.

Maternal, Infant, and Children Outcome Survey

Adverse maternal outcomes examined in this study included preeclampsia, eclampsia, gestational diabetes, preterm labor, and cesarean delivery. A detailed review of the medical records of the live births from TESE-ICSI cycles or extreme severe OAT-ICSI cycles was conducted. Adverse infant outcomes examined in this study included major birth defects, fetal death, preterm birth, fetal growth restriction, Apgar score <7 at 5 minutes, intracranial hemorrhage, seizures, sepsis, and the need for mechanical ventilation. Only birth defects with major morphologic or functional importance were considered as anomalies. Birth defects were grouped into the following categories: congenital heart defects, gastrointestinal anomalies, musculoskeletal anomalies, and chromosomal anomalies. Preterm birth was defined as birth at a gestational age <32 completed weeks and fetal growth restriction (small-for-gestational age) was defined as a birth weight smaller than the third percentile in the corresponding standard population stratum. Fetal death was defined as intrauterine death at a gestation age greater than 20 weeks or birth weight greater than 500 g.

The children's developmental outcomes were evaluated using a preschool developmental screening table developed for children in Taiwan. The table design varies depending on the age of the child, and specific tables have been created for the ages 6, 9, 12, 18, 24, 30, 36, 42, 48, 54, and 60 months. Table was completed by the child's pediatrician and it included detailed questions for assessment of the following: [1] feeding and sleeping behavior, [2] posture, [3] coordination, [4] memory, [5] problem-solving skills, [6] language skills, and [7] socialization. The pediatrician rated the results according to operationally defined degrees of normalcy.

Statistical Analysis

SPSS 10.0 computer software (SPSS, Inc.) was used for data analysis. Continuous data were summarized as the mean ± SD. The clinical outcome comparison included the Mann-Whitney rank-sum test for the comparison of means, and the Fisher's exact test for proportions. All *P* values were two-sided, and $P < .05$ was considered statistically significant.

RESULTS

We first compared the baseline characteristics between ICSI using extracted testicular sperm or ejaculated extreme severe OAT sperm. The age of the female subjects (31.6 ± 5.0 vs. 32.1 ± 4.7 years) and their male (36.4 ± 5.6 vs. 35.1 ± 4.9 years) partners, E_2 (pg/mL) on hCG day (1876.6 ± 1327.1 vs. 2321.6 ± 1747.3), numbers of oocytes retrieved (7.3 ± 4.1 vs. 8.2 ± 3.6), normal fertilization rate (91.3% vs. 90.8%), zygote grade 1 score distribution (47.9% vs. 48.0%), numbers of top-quality embryos transferred (1.5 ± 0.8 vs. 1.6 ± 0.9), clinical pregnancy rate per transfer (46.6% vs. 39.7%), chemical pregnancy rate per transfer (4.2% vs. 3.2%), implantation rate (26.2% vs. 22.7%), live birth rate per transfer (40.7% vs. 30.2%), and abortion rate per transfer (7.6% vs. 9.5%) were comparable between the two groups (Table 1).

We further analyzed perinatal outcomes and followed up the live-birth children following ICSI (age 1–7 years) (Table 2). There were 60 live births from 48 TESE-ICSI cycles and 21 live births from 19 extreme severe OAT-ICSI cycles. The obstetric and perinatal outcomes between the two groups were comparable. The general health of children conceived by using ICSI was satisfactory and no major psychomotor or intellectual development retardations were noted. However, one major congenital heart anomaly (tetralogy of Fallot) was observed in each group, and attention deficit/hyperactivity disorder was observed in a child born from an extreme severe OAT-ICSI cycle.

DISCUSSION

This study showed no evidence of differences in clinical outcomes and development of children after ICSI with extracted testicular sperm or ejaculated extreme severe OAT sperm. An ejaculated semen sample can present varying degrees of sperm abnormalities from absence of sperm to severe alterations in all spermatic parameters, as in cases of OAT. In our study, we strictly define extreme severe OAT to re-mark the effect of semen quality and attempted to elucidate whether origin or quality affects the clinical outcome. Our data suggest that the effect is comparable, and this is not unexpected because ICSI uses a limited number of spermatozoa, and choosing good-quality sperm is always of the highest priority. Moreover, lowering the number of embryos transferred to avoid high-order pregnancy rates is a worldwide assisted reproductive technology treatment tendency. It is difficult to determine the influence of spermatic defects on clinical ICSI outcome. Our study suggests that outcomes of ICSI are not affected by sperm from different origins.

However, we still agree that the importance of selecting good-quality sperm for oocyte injection, especially in cases involving very extreme severe OAT, must be emphasized. Our finding of no remarkable sperm effect on ICSI outcome can be due to our definition of severe OAT criteria, which needs to be further restricted. Indeed, in rare cases in which the semen quality is very poor, it is difficult to select sperm satisfactory for ICSI. When selecting the best sperm for ICSI, sperm parameters plus ultramorphology of subcellular organelles, chromosomal stability, and nuclear integrity associated with ICSI outcomes have been discussed. On the one hand, it has been proposed to verify whether microinjection of motile spermatozoa with morphologically normal nuclei—strictly defined by using high-power light microscopy (×6600)—into retrieved oocytes improves the ICSI pregnancy rate in couples with repeated ICSI failures (28–31). On the other hand, using hyaluronic acid may optimize ICSI outcome, and physiological selection of sperm without DNA fragmentation and with normal nucleus have also been discussed (32, 33). Our study of ICSI did not use these sperm selection methods, and thus we cannot verify if these methods will influence the outcome or difference in azoospermia and extreme severe OAT group.

The effect of sperm quality on embryo development after ICSI is another concern (5, 10–12). Overall, a negative relationship has been observed between semen quality and embryo development, even before activation of the embryonic genome, suggesting that sperm quality can affect embryogenesis from a very early stage (34–36). However, in our study there was comparable zygote distribution and fertilization conditions between the groups. We think this finding is worth noting.

Some studies have shown that children born after assisted reproductive technology are at increased risk of birth defects (37–40), whereas other studies suggest that there is no concern about children conceived using ICSI with ejaculate, epididymal, or testicular sperm (21, 41–43). On the basis of an analysis of data in a number of large and reliable surveys, Van Steirteghem et al. (41) concluded that children conceived using ICSI do not have a higher rate of malformations compared with naturally conceived children. In our study, we found comparable obstetric and perinatal outcomes between children conceived after ICSI with extracted testicular sperm or ejaculated extreme severe OAT sperm.

However, our study confirmed earlier findings that the risks of prematurity and low birth weight are both higher than those observed in the general population (44, 45). We suggest that higher incidence of multiple pregnancies, and intrinsic factors, in subfertile couples predispose women to having smaller infants.

When we analyzed the gender of offspring from extreme severe OAT-ICSI cycles and compared it with offspring from TESE-ICSI cycles, we found a significant sex-ratio imbalance toward male (P=.014). Our previous data show a similar trend in favor of a higher number of male offspring following ET after day 3 culture and day 5 sequential blastocyst culture. However, the overall female-to-male ratio of offspring resulting from day 3 embryos was not significantly different from the ratio of offspring resulting from blastocyst transfer (46). We suspect that male-biased sex ratio is the result of selective ET favoring the top-quality embryos on fresh transfer (47). Tracing back the live birth cycles in this study, we found that the percentage of top-quality embryos transferred had a higher trend, but no statistically significant difference, for the extreme severe OAT cycles compared with the TESE cycles (69.8% vs. 54.0%, respectively). This is in accordance with our previous report.

However, one major congenital heart anomaly, TOF, occurred in each group and cannot be dismissed (Table 2). Tetralogy of Fallot occurs once in approximately 400 million live births (48). In our study period, we had 227 live births after 490 fresh ICSI cycles and 53 thaw ICSI cycles, and two TOF cases occurred—one in an extreme severe OAT cycle and one in a TESE cycle. Bonduelle et al. (49) conducted a medical follow-up study of 300 five-year-old ICSI children, and found one with TOF. TOF has long been considered a congenital disorder that occurs as a result of environmental alterations during gestation or is associated with severely mutated genes (50). Surgical repair has remarkably improved the survival of patients with TOF. In addition, TOF due to microdeletions are sporadic and are frequently also present in trisomy 21 patients (50). However, Y chromosome microdeletion testing of both severely oligozoospermic and nonobstructive azoospermic men has become routine (1). We have not encountered any report describing a relationship between Y chromosome microdeletions and TOF.

In our study, children conceived by using ICSI were healthy without major psychomotor or intellectual developmental retardation. We found one attention deficit/hyperactivity disorder in an extreme severe OAT-ICSI child and cannot determine if it is sporadic or treatment related. In the report by Bonduelle et al. (49), the authors reported one attention deficit/hyperactivity disorder in 266 spontaneous conceptions that served as a control group.

Our design was not a prospective longitudinal study and lacked rigorous assessment scales for psychomotor and intellectual development in ICSI children. In addition, our study design with respect to statistical power and case number recruitment could be improved on (e.g., according to our preliminary data, a sample size of at least 600 cases in each group would be needed to detect a twofold difference in TOF). The study represents a local population from a single treatment center. Nevertheless, we believe that this study suggests no evidence of differences in clinical outcomes.

In summary, we conclude that there is no evidence of differences in clinical outcomes and development of children born after ICSI with extracted testicular sperm or ejaculated extreme severely OAT sperm. Our study may provide information for consultation for ICSI treatment in these patients.

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