Asian Journal of Andrology (2022) 24, 299–304
www.asiaandro.com; www.ajandrology.com

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ORIGINAL ARTICLE

Male Infertility

Comparison of intracytoplasmic sperm injection (ICSI) outcomes in infertile men with spermatogenic impairment of differing severity

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The extent of spermatogenic impairment on intracytoplasmic sperm injection (ICSI) outcomes and the risk of major birth defects have been little assessed. In this study, we evaluated the relationship between various spermatogenic conditions, sperm origin on ICSI outcomes, and major birth defects. A total of 934 infertile men attending the Center for Reproductive Medicine of Ren Ji Hospital (Shanghai, China) were classified into six groups: nonobstructive azoospermia (NOA; *n* = 84), extremely severe oligozoospermia (esOZ; *n* = 163), severe oligozoospermia (sOZ, *n* = 174), mild oligozoospermia (mOZ; *n* = 148), obstructive azoospermia (OAZ; *n* = 155), and normozoospermia (NZ; *n* = 210). Rates of fertilization, embryo cleavage, high-quality embryos, implantation, biochemical and clinical pregnancies, abortion, delivery, newborns, as well as major birth malformations, and other newborn outcomes were analyzed and compared among groups. The NOA group showed a statistically lower fertilization rate (68.2% *vs* esOZ 77.3%, sOZ 78.0%, mOZ 73.8%, OAZ 76.6%, and NZ 79.3%, all *P* < 0.05), but a significantly higher implantation rate (37.8%) than the groups esOZ (30.1%), sOZ (30.4%), mOZ (32.6%), and OAZ (31.0%) (all *P* < 0.05), which was similar to that of Group NZ (38.4%). However, there were no statistically significant differences in rates of embryo cleavage, high-quality embryos, biochemical and clinical pregnancies, abortions, deliveries, major birth malformations, and other newborn outcomes in the six groups. The results showed that NOA only negatively affects some embryological outcomes such as fertilization rate. There was no evidence of differences in other embryological and clinical outcomes with respect to sperm source or spermatogenic status. Spermatogenic failure and sperm origins do not impinge on the clinical outcomes in ICSI treatment.

Asian Journal of Andrology (2022) **24**, 299–304; doi: 10.4103/aja202151; published online: 15 October 2021

Keywords: intracytoplasmic sperm injection; nonobstructive azoospermia; normozoospermia; obstructive azoospermia; oligozoospermia

INTRODUCTION

Infertility is a prevalent condition affecting about 70 million people in the world. It is estimated that 9% of couples worldwide are facing fertility problems and that males contribute to 50% of them, according to the World Health Organization (WHO).[1] The most recent publication reported a prevalence of infertility of 12.5% in women and 10.1% in men, from a population of 15 162 men and women in UK.[2] Many couples with varying severity of male infertility have relied on intracytoplasmic sperm injection (ICSI) as the first line of treatment to achieve biological parenthood. Even men diagnosed with nonobstructive azoospermia (NOA), categorized as an intrinsic testicular impairment of sperm production, they also have the opportunity to have their own children through ICSI combined with testicular sperm extraction, with a retrieval rate of 40.3%.[3]

Despite the dramatic increase in ICSI-related pregnancies, concerns about the safety of the processes that ICSI treatment entails exist. Oligozoospermia is one of the more common causes of male factor infertility, with the extent of sperm concentration decrease reflecting the severity of spermatogenic impairment, with NOA being the extreme category. Whether the severity of spermatogenic impairment affects reproductive viability and developmental competence of the embryo is not known. Children conceived after ICSI by spermatozoa from fathers with severely abnormal spermatogenesis are poorly studied, although the underlying defects in spermatozoa derived from spermatogenic dysfunction may be related to the risks of potential imprinting defects.[4,5]

Previous studies have provided conflicting results regarding the associations between ICSI and major birth defects.[6–8] Large population-based studies suggest that underlying male factors and the severity of male factor infertility increase the risk of mental retardation and autism in offspring.[9] There are few conclusive data on ICSI outcomes related to different spermatogenic conditions, ranging from NOA, extremely severe oligozoospermia (esOZ; defined as sperm concentration $<2 \times 10^6$ ml^{-1}), severe oligozoospermia (sOZ; sperm concentration $>2 \times 10^6$ ml^{-1} and $\leq 5 \times 10^6$ ml^{-1}), mild oligozoospermia (mOZ; sperm concentration $>5 \times 10^6$ ml^{-1} and $\leq 15 \times 10^6$ ml^{-1}), to normozoospermia.[10] Consequently, whether spermatozoa from various degrees of spermatogenesis dysfunction have an impact on the ICSI outcome and health of the offspring remains to be explored.

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Received: 26 January 2021; Accepted: 09 July 2021

In this retrospective study, we analyzed ICSI outcomes in couples with male infertility, including NOA, esOZ, sOZ, mOZ, obstructive azoospermia (OAZ), and normozoospermia (NZ) from January 2018 to December 2019 in the Center for Reproductive Medicine, Ren Ji Hospital (Shanghai, China). Detailed embryological, clinical, and newborn outcomes, including major birth malformations, were analyzed and compared, to provide solid evidence to guide clinical decision-making.

PATIENTS AND METHODS

Patients

In this retrospective study, we included 934 patients with oligozoospermia, azoospermia, and NZ who underwent 934 ICSI cycles between January 2018 and December 2019. It was the first time for all the couples in this cohort to undergo ICSI treatment, and all the parameters were analyzed and calculated in one oocyte-retrieval cycle. The entire cohort was divided into six groups according to sperm concentration on the day of ICSI as described above, comprising Group NOA (84 patients), Group esOZ (163 patients), Group sOZ (174 patients), Group mOZ (148 patients), Group OAZ (155 patients), and Group NZ as control (210 patients). All patients had their first ICSI cycle conducted in the Center for Reproductive Medicine, Ren Ji Hospital, Shanghai Jiao Tong University School of Medicine. This study has been approved by the ethics committee and did not violate the ethical principles. All patients signed informed consent forms for ICSI and embryo transfer. All patients agree to provide informed consent to conduct relevant scientific research on their own data. Our data collection was approved by the Ethics Committee of Ren Ji Hospital, School of Medicine, Shanghai Jiao Tong University (Approval No. 2018072612).

Ejaculated spermatozoa were collected and analyzed on the day of oocyte retrieval by masturbation after 2–7 days of sexual abstinence. Both NOA and OAZ needed to meet the conditions that no spermatozoon was found in three consecutive semen analyses after extended centrifugation. The differentiation between NOA and OAZ was distinguished by history, physical examination, reproductive hormonal profiling, ultrasound examination, genetic testing (karyotype and Y microdeletion), and testicular histological assessment. Of 84 patients in Group NOA, 25 patients presented with hypospermatogenesis, 6 patients with maturation arrest, and 53 patients with Sertoli cell-only syndrome. We excluded patients with abnormal karyotypes and Y microdeletion. All female partners were younger than 35 years and were without abnormal karyotypes or any factors of female infertility other than Fallopian tube damage or blockage.

Sperm-retrieval technique

For patients with OAZ, testicular sperm aspiration (TESA) was performed on the same day of ICSI. For patients with NOA, microsurgical testicular sperm extraction (micro-TESE) was performed via an operating microscope as reported by Schlegel.[11] The procedure began on the right testis if there was no difference in size between the two, or on the obviously larger testis. After a midline incision was made in the scrotum, the testis was delivered and the tunica vaginalis was opened. Testicular parenchyma was observed directly at 15×–20× magnification to locate and collect the larger and more opaque seminiferous tubule with the higher chance of harboring spermatozoa. Successful retrievals were defined as the detection of spermatozoa.

Sperm processing

Ejaculated semen was liquefied at 37°C. Spermatozoa extracted by TESA in Group OAZ were collected in human tubal fluid (HTF; SAGE, CooperSurgical, Inc., Malov, Denmark) on a warm plate at 32°C–33°C. In Group NOA, testicular fragments were washed in Earl's Balanced Salt Solution (EBSS; Sigma-Aldrich, St. Louis, MO, USA) to remove blood, then finely minced, and directly examined for the presence of spermatozoa. If oocyte collection was not arranged on the same day, spermatozoa were routinely cryopreserved in 0.5 ml straws for later use. Testicular tissue was mixed 1:1 with sperm freezing medium (Origio 10670010F, CooperSurgical, Inc.) in 1 ml labeled straws (Cro.s^{TM} 123277, Greiner, Frickenhausen, Germany), suspended for 30 min in liquid nitrogen vapor before being stored in liquid nitrogen. If oocytes were collected on the same day, fresh testicular sperm samples were used after centrifuging. In all groups, a carefully extended sperm examination was made to find the most morphologically normal motile spermatozoon for ICSI.

Oocyte recovery and ICSI procedure

Women underwent controlled ovarian hyperstimulation with a long or short gonadotropin-releasing hormone (GnRH) antagonist protocol. Gonadotropin dosage was determined on the basis of the patient's age, body mass index (BMI), and assessment of ovarian reserve (antral follicle count and anti-Müllerian hormone [AMH] levels). Exogenous gonadotropins (Gonal-F, Merck Serono, Geneva, Switzerland) were administered daily. Serum estradiol (E_2) levels and transvaginal ultrasounds were performed frequently to monitor the response to stimulation. Endogenous gonadotropins were suppressed with either GnRH-antagonist (Cetrotide, 0.25 mg; Merck-Serono) or GnRH agonist (leuprolide acetate, Abbott, North Chicago, IL, USA). The trigger with human chorionic gonadotropin or GnRH-agonist (leuprolide acetate) was administered when ≥2 follicles reached 18 mm. Oocyte retrieval was performed under ultrasound guidance 35–37 h after triggering.[12,13]

ICSI was performed 3–6 h after ultrasonographically controlled vaginal ovum pick-up. Gamete and embryo handling were performed with culture medium (G-Series, Vitrolife, Goteborg, Sweden). Microinjection was performed in an inverted microscope (Ti-V, Nikon, Tokyo, Japan) with Narishige micromanipulators (NT-88-V3, Narishige, Tokyo, Japan), using micropipettes from Sunlight Medical (Jacksonville, FL, USA). ICSI was performed using strong disruption of the cytoplasm.[14–16] On failure of sperm retrieval, ICSI would be carried out with donor spermatozoa for couples accepting sperm donation. Fertilization was evaluated 16–18 h after microinjection. Normal fertilization was defined as zygotes with two pronuclei (2PN). Two to three zygotes were selected for culture in cleavage medium (G-1 PLUS, Vitrolife) until day 3 for embryo transfer (ET).

Embryo grading and transfer

The grade of embryos was evaluated on day 3 according to morphology, fragmentation, and blastomeres. High-quality embryos were defined by the presence of 4–5 blastomeres on day 2, or 7–8 blastomeres on day 3, symmetry of cleavage, and a fragmentation rate <10%.[17] One or two top-quality embryos were transferred on day 3 after oocyte retrieval. The remaining embryos were followed for blastocyst formation. Blastocysts were assessed by Gardner's score.[18] High-quality blastocysts were morphologically good, with an inner mass presenting numerous tightly packed cells and a trophectoderm exhibiting many cells forming a squamous epithelium. One or two embryos were transferred on day 3 or day 5, and the supernumerary embryos were frozen.

Luteal supplementation began the next day of oocyte retrieval with daily intravaginal administration of 90 mg progesterone from a sustained-release vaginal gel (Fleet laboratories Ltd., Watford, UK). Biochemical pregnancy was confirmed by a rise in serum β-human chorionic gonadotropin (β-HCG) 12–14 days after embryo transfer. Clinical pregnancy was established by ultrasound at 6 weeks of

gestation. Progesterone was maintained until β-HCG serum assays, and if positive, continued until 9–10 weeks of gestation.

Clinical outcomes

Clinical pregnancy was defined as the presence of a visible gestational sac examined by transvaginal ultrasound examination 2 weeks after serum HCG testing. The live birth rate is the ratio of cases with live birth babies to the ovarian stimulation cycles in each group.[19] Birth of children was recorded after telephone conversations. Preterm is <37 weeks' gestation; very preterm is <32 weeks' gestation; and extremely preterm is <28 weeks' gestation. Low birth weight is <2500 g; very low birth weight is <1500 g; and extremely low weight is <1000 g. We also included major birth defects that were identified before the end of the neonatal period (within 28 days). Congenital anomalies (defined as structural or functional disorders that occur during intrauterine life and that can be identified prenatally, at birth or later in life), including trisomy 13, 18, and 21, neural tube defects, congenital heart disease, cleft lip, excessive numbers of fingers or toes, and hydrocephalus, were recorded. Clinical diagnosis of a congenital anomaly was defined according to the International Classification of Diseases, revision 10 criteria.[20]

Statistical analyses

Calculations were analyzed using SPSS 19.0 software (SPSS Inc., Chicago, IL, USA). Continuous variables were presented as mean ± standard deviation (s.d.). Frequencies were expressed as percentages. One-way analysis of variance (ANOVA) was used to analyze the difference between groups, whereas χ^2 test was used for comparison of proportions. Comparison of mean values among more than three groups was performed using analysis of variance test. Differences between the values were considered statistically significant when $P < 0.05$.

RESULTS

We retrospectively analyzed 934 first ICSI cycles from 934 couples. The baseline characteristics of the patients in the six groups are summarized in **Table 1**. No statistical difference was found in the average age of females at ICSI in any group ($P > 0.05$). There were no significant differences between groups in female follicle-stimulating hormone (FSH), AMH, E_2, duration of infertility, HCG dose, and follicle number (all $P > 0.05$). There was no statistical difference in male age among the groups ($P > 0.05$).

The oocyte retrieval number, mature oocyte number, injected MII oocyte number, 2PN number, fertilization rate, embryo cleavage rate, D3 grade A/B rate, the number of transferred embryos, biochemical and pregnancy rates per cycle, implantation rates, ectopic pregnancy rates, and abortion rates are summarized in **Table 2**; newborn number, newborn malformation, and other newborn outcomes are presented in **Table 3**. There were no statistically significant differences among the six groups in the parameters above, except that Group NOA had a significant reduction in fertilization rate compared with other groups ($P < 0.05$), and the number of transferred embryos in Group NOA was the fewest of all the groups ($P < 0.01$). However, Group NOA had a significantly higher implantation rate than that of Group esOZ, Group mOZ, Group sOZ, or Group OAZ ($P < 0.05$), but very similar to that of Group NZ. Additionally, except Group NA, Group NOA had the highest biochemical ($P = 0.15$) and clinical ($P = 0.13$) pregnancy rate, and the lower abortion rate, but the differences was not statistically significant ($P = 0.44$). There were two cases of major birth defects (both were patent foramen ovale): one in Group NOA and the other in Group NZ ($P = 0.34$). There were no significant difference in gestation age ($P = 0.11$) and new born weight ($P = 0.07$).

DISCUSSION

Over nearly past 20 years, ICSI has clearly become the method to overcome untreatable severe male factor infertility, which is most commonly caused by various extents of spermatogenic failure, clinically noted as oligozoospermia or azoospermia.

Although ICSI is mainly used for male factors, studies in male infertility are much fewer than those in female infertility. Whether low numbers of spermatozoa and severe spermatogenic impairment affect ICSI outcome is still a matter of debate.[21] To provide the best care for males with severe infertility, especially NOA, we would like to know not only their likelihood of successful retrieval of spermatozoa, but also the live birth rate and the health of the resulting offspring.

In the present study, we compared the reproductive outcomes of ICSI cycles among Group NOA, Group esOZ, Group mOZ, Group sOZ, Group OAZ, and Group NZ, from samples in a single *in vitro* fertilization (IVF) center. All the clinical outcomes of patients were analyzed by established highly reliable laboratory techniques and processes.

Sperm concentration can reflect the condition of male fertility and testicular function. For clinical outcomes, several studies have concluded that sperm concentration is positively related to the pregnancy rate.[22,23] However, our results showed no difference in rates of fertilization, high-quality embryo, implantation, clinical pregnancy, abortion; the number of embryos transferred to achieve pregnancy, transferable embryos; low birth weight, premature birth rate, or major birth malformations among Group mOZ, Group sOZ, and Group esOZ, which is consistent with the results of Yang *et al.*[24]

In recent years, a growing number of studies have suggested that ejaculated spermatozoa are inferior to testicular spermatozoa extracted by surgery. Suganuma *et al.*[25] have postulated that ejaculated spermatozoa are susceptible to damage while passing through the reproductive tract. Furthermore, Mehta *et al.*[26] have reported that the level of terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick-end labeling (TUNEL)-positive ejaculated spermatozoa was significantly higher than that in testicular spermatozoa. However, Plouvier *et al.*[27] have observed no significant difference in fertilization rates, implantation rates, or the percentage of top-quality embryos and clinical pregnancy rates per embryo transferred between ejaculated and testicular spermatozoa. Our results are consistent with these studies with respect to fertilization rates, clinical pregnancy rate, and live birth rate between groups of ejaculated sperm and testicular sperm of OAZ. However, for spermatozoa retrieved by micro-TESE in NOA, a lower fertilization rate has been reported,[21] which is consistent with the results in our cohort. Our observations indicate that there may exist differences in spermatozoa of NOA and OAZ patients, since the spermatogenic efficiency of the two categories differs greatly. For implantation rate, our results showed that Group NOA had significantly higher implantation rates than Groups OA and sOZ, very similar to Group NZ. Consistent with our finding, a recent retrospective analysis showed that although abnormal semen quality compromised embryo development, it did not negatively affect the rate of euploid blastocysts obtained or their implantation potential.[28]

For NOA patients, the most severe kind of male infertility, performing ICSI is often a challenge, as it is often difficult to find sufficient suitable spermatozoa (motile and morphologically normal) in these patients.[29] Although both NOA and esOZ are derived from extreme spermatogenic failure, there are significant differences in andrological profile between esOZ and NOA groups.[27] Our data highlight the reduced two-pronuclear zygote rate of NOA compared

Table 1: Demographic and stimulation characteristics

Variable	*NOA*	*esOZ*	*sOZ*	*mOZ*	*OAZ*	*NZ (control)*	*P*
Patients/oocyte-retrieval cycle (*n*)	84/84	163/163	174/174	148/148	155/155	210/210	
Female age (year), mean±s.d.	30.57±3.72	30.11±3.78	30.80±3.93	29.82±3.44	29.78±3.40	30.60±2.97	0.18
Male age (year), mean±s.d.	31.84±5.81	31.90±4.73	32.93±6.04	31.74±5.45	31.57±4.30	31.27±2.58	0.10
Time infertility (year), mean±s.d.	2.26±1.48	3.00±1.58	2.36±1.60	2.76±1.52	2.83±1.47	2.78±1.79	0.74
Male factor, *n* (%)	75 (89.3)	140 (85.9)	149 (85.6)	128 (86.5)	137 (88.4)	20 (17.4)	<0.01
Female factor, *n* (%)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	60 (52.2)	
Mixed factors, *n* (%)	9 (10.7)	23 (14.1)	25 (14.4)	20 (13.5)	18 (11.6)	35 (30.4)	
bFSH (mIU ml^{-1}), mean±s.d.	6.89±1.56	7.46±1.66	5.86±2.28	5.63±2.10	5.98±1.32	7.17±1.18	0.06
AMH (ng ml^{-1}), mean±s.d.	4.99±2.63	3.54±2.10	4.62±1.81	4.17±3.13	3.39±2.09	3.60±2.50	0.54
Follical number (*n*), mean±s.d.	15.45±5.82	12.83±4.39	17.18±2.23	13.54±3.83	15.27±4.22	12.36±3.98	0.74
Total dose (IU), mean±s.d.	1344.32±457.76	1422.92±451.92	1414.77±408.46	1695.45±577.47	1743.18±729.91	1655.68±695.82	0.42
Stimulation time (day), mean±s.d.	8.09±1.14	8.67±0.98	8.00±1.26	8.54±1.29	8.82±1.83	9.64±1.50	0.08
Estradiol (pg ml^{-1}), mean±s.d.	3156.91±1862.74	2062.08±882.56	3040.64±1700.06	2121.55±980.15	1813.90±712.07	2164.36±1064.70	0.07
HCG dose (IU), mean±s.d.	3727.27±2370.27	5250.00±2094.36	4000.00±2408.32	4818.18±2182.96	4818.18±2561.96	5272.73±1489.36	0.46

P<0.05 indicates a significant statistical difference; *P*<0.01 indicates extremely significant statistical difference. NOA: nonobstructive azoospermia; esOZ: extremely severe oligozoospermia; sOZ: severe oligozoospermia; mOZ: mild oligozoospermia; OAZ: obstructive azoospermia; NZ: normozoospermia; s.d.: standard deviation; AMH: anti-Müllerian hormone; HCG: human chorionic gonadotropin; bFSH: basal follicle-stimulating hormone

Table 2: Embryological and clinical outcomes

Variable	*NOA*	*esOZ*	*sOZ*	*mOZ*	*OAZ*	*NZ (control)*	*P*
Oocyte-retrieval cycle (*n*)	84	163	174	148	155	210	
COC number (*n*), mean±s.d.	11.98±5.03	11.74±7.33	11.85±5.96	10.55±4.62	10.63±3.62	10.57±3.67	0.06
MII number (*n*), mean±s.d.	9.74±3.76	9.71±5.32	8.87±5.53	8.66±4.77	8.50±4.00	8.46±3.19	0.08
Maturity rate (MII/COC), %	81.3	82.8	74.9	82.1	80.0	80.0	0.08
2PN number (*n*), mean±s.d.	6.64±4.62	7.51±5.17	6.92±4.47	6.39±3.65	6.51±3.14	6.71±3.12	0.07
Fertilization rate (2PN/MII), %	68.2	77.3	78.0	73.8	76.6	79.3	0.01
Cleaved embryos number (*n*), mean±s.d.	5.51±4.56	6.21±4.97	5.91±4.41	5.19±3.30	5.40±2.99	5.83±2.69	0.06
Embryo cleavage rate (D3/2PN), %	83.0	82.7	85.4	81.2	83.0	86.9	0.25
Day 3 Grade A/B embryos number (*n*), mean±s.d.	3.76±3.23	4.01±3.11	3.97±2.89	3.55±2.40	3.36±2.29	3.61±2.01	0.19
Day 3 Grade A/B rate (AB/D3), %	68.2	64.6	67.2	68.4	62.2	61.9	0.06
ETC (*n*)	104	225	238	168	197	273	
Transferred embryos number (*n*), mean±s.d.	1.58±0.88	2.11±1.06	2.10±1.16	2.12±0.79	2.08±0.64	1.70±0.48	<0.01
Biochemical pregnancy[a], % (*n*)	59.6 (62)	56.9 (128)	55.5 (132)	50.8 (97)	58.4 (115)	60.4 (165)	0.15
Clinical pregnancy rate[a], % (*n*)	57.7 (60)	55.6 (125)	53.8 (128)	48.7 (93)	56.89 (112)	59.0 (161)	0.13
Sacs, *n*	62	143	153	116	127	178	
Implantation rate[a], % (*n*)	37.8 (62)	30.1 (143)	30.4 (153)	32.6 (116)	31.0 (127)	38.4 (178)	0.03
Twins, % (*n*)	6.90 (4)	14.4 (18)	13.3 (24)	16.1 (23)	13.4 (15)	10.6 (17)	0.23
Ectopic pregnancy rate[a], % (*n*)	1.7 (1)	2.4 (3)	0 (0)	1.1 (1)	1.8 (2)	0.6 (1)	0.55
Abortion rate[a], % (*n*)	3.3 (2)	7.2 (9)	3.9 (5)	7.5 (7)	2.7 (3)	6.2 (10)	0.44
Clinical pregnancy rate[b], % (*n*)	71.4 (60)	76.7 (125)	73.6 (128)	62.8 (93)	72.3 (112)	76.7 (161)	0.07
LBDR, % (*n*)	70.2 (59)	69.3 (113)	66.9 (123)	57.4 (85)	69.0 (107)	71.4 (150)	0.08
NB (*n*)	61	134	147	101	119	167	
Male (/NB), % (*n*)	50.8 (31)	56.0 (75)	51.7 (76)	57.4 (58)	65.8 (65)	53.9 (90)	0.94
Female (/NB), % (*n*)	49.2 (30)	44.0 (59)	48.3 (71)	42.6 (43)	34.2 (54)	46.1 (77)	
NB malformations, % (*n*)	1.6 (1)	0 (0)	0 (0)	0 (0)	0 (0)	0.6 (1)	0.34
Gestation age (week), mean±s.d.	38.63±2.16	38.40±2.05	38.09±2.68	38.43±1.81	37.76±2.49	37.94±2.07	0.11

[a]This index was calculated according to each embryo tranfer cycle; [b]this index was calculated according to each oocyte retrieval cycle. *P*<0.05 indicates a significant statistical difference; *P*<0.01 indicates extremely significant statistical difference. NOA: nonobstructive azoospermia; esOZ: extremely severe oligozoospermia; sOZ: severe oligozoospermia; mOZ: mild oligozoospermia; OAZ: obstructive azoospermia; NZ: normozoospermia; s.d.: standard deviation; COC: cumulus-oocyte complexes; MII: mature oocytes; 2PN/2PB: 2 pronuclei and 2 polar bodies; LBDR: live birth delivery rate; ETC: embryo transfer cycle; NB: newborn; D3: day 3

with that of other groups; this could be due to the limited availability of spermatozoa suitable for ICSI, which could lead to the selection of suboptimal spermatozoa for ICSI. Another reason for lower fertilization rate of NOA may lie in the increased aneuploidy rate in testicular spermatozoa, which carry deficiencies in genetic material to affect fertilization.[30]

Since ICSI can circumvent all the natural barriers to fertilization, deriving from poor sperm motility and ability to cross the zona pellucida and fuse with the oocyte, it is possible that chromosomal abnormalities in the gametes used in ICSI influence the risk of congenital malformations: innate structural or functional abnormalities.[31] Few studies to date have investigated the effects of male infertility on the risks of major birth defects following ICSI,[32] and there are reasons for concern as risks of congenital malformations and epigenetic disorders. Their origin may be genetic or multifactorial, but in most cases, the cause is difficult to ascertain. This study attempted to reveal the

Table 3: Newborn outcomes

Variable	*NOA*	*esOZ*	*sOZ*	*mOZ*	*OAZ*	*NZ (control)*	*P*
Oocytes retrieval cycle (*n*)	84	163	174	148	155	210	
Embryo transfer cycles (*n*)	104	225	238	168	197	273	
Newborn (*n*)	61	134	147	101	119	167	
Gestation age (week), mean±s.d.	38.63±2.16	38.40±2.05	38.09±2.68	38.43±1.81	37.76±2.49	37.94±2.07	0.11
Term, % (*n*)	93.4 (57)	88.8 (119)	88.4 (130)	91.1 (92)	84.9 (101)	94.0 (157)	0.46
PT, % (*n*)	3.3 (2)	10.5 (14)	10.9 (16)	8.9 (9)	13.5 (16)	5.4 (9)	0.11
Very PT, % (*n*)	1.6 (1)	0 (0)	0 (0)	0 (0)	1.7 (2)	0.6 (1)	0.29
Extremely PT, % (*n*)	1.6 (1)	0.8 (1)	0.7 (1)	0 (0)	0 (0)	0 (0)	0.54
Weight (g), mean±s.d.	3098.30±741.68	3135.28±512.16	3135.28±564.78	3147.39±548.98	3029.19±541.56	3189.34±617.73	0.07
Normal weight, % (*n*)	90.2 (55)	90.3 (121)	91.2 (134)	90.0 (91)	89.9 (107)	94.6 (158)	0.68
LW, % (*n*)	8.2 (5)	9.7 (13)	8.8 (13)	7.9 (8)	10.1 (12)	5.4 (9)	0.73
Very LW, % (*n*)	1.6 (1)	0 (0)	0 (0)	2.0 (2)	0 (0)	0 (0)	0.06
Extremely LW, % (*n*)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	

P<0.05 indicates a significant statistical difference; *P*<0.01 indicates extremely significant statistical difference. NOA: nonobstructive azoospermia; esOZ: extremely severe oligozoospermia; sOZ: severe oligozoospermia; mOZ: mild oligozoospermia; OAZ: obstructive azoospermia; NZ: normozoospermia. s.d.: standard deviation; PT: preterm; LW: low weight

relationship between low fertility in men, ICSI treatment itself, and the risk of major birth defects in offspring. In our study, the live birth rate per cycle and birth defect rate did not differ among Group NOA, Group OAZ, and groups with various sperm concentrations. Several causes for these observations can be postulated. Unknown maternal factors could influence fetal growth and lead to a heterogeneous outcome.[33] An observational longitudinal cohort study of 1219 ICSI cycles showed that severe male factors impair embryonic competence in terms of fertilization rate and blastocyst rate per fertilized oocyte, but the euploidy rate and implantation potential of the obtained blastocysts are independent of sperm quality.[29] Although the degree of damage varied among spermatozoa, it could still result in embryos with good enough quality for successful implantation and clinical pregnancy. Our results suggested that sperm sources and the degree of spermatogenic failure had insignificant impacts on prospective clinical outcomes.

There are some limitations in our study. There was no prospective study on this topic. Our retrospective cohort studies have some inevitable limitation on methodology and only represented a local population from a single IVF center.

CONCLUSIONS

Our study indicated that NOA, as an extreme example of spermatogenic failure, does negatively affect some embryological outcomes of ICSI treatment, such as fertilization rate, but has a significantly higher implantation rate than ICSI in cases of OAZ and oligozoospermia. There is no evidence of differences in other embryological and clinical outcomes such as embryo cleavage rate, high-quality embryo rate, biochemical and clinical pregnancy rates, abortion rate, delivery rate, major birth malformations, and other newborn outcomes with respect to sperm source and different severities of spermatogenic failure. These results clarify that different sperm sources, and different extents of spermatogenic dysfunction, had limited effect on the live birth rate after the embryo had initiated a clinical pregnancy. We also report that risks of low weight, premature delivery, and congenital malformations are irrelevant for the above factors.

AUTHOR CONTRIBUTIONS

PP and ZZ participated in collection and assembly of data, data analysis and interpretation, and manuscript writing. YM and SSZ participated in critical discussion and manuscript writing. XFC participated in conception and design, administrative support, and manuscript writing. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

We are very thankful regarding the great assistance of physicians of our IVF clinic and we here acknowledge their valuable support. This work was supported by the National Natural Science Foundation of China (Grant No. 81671511 to PP, and No. 81871199 and No. 81501310 to YM).

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