ART Outcomes

Sperm origins and concentration do not impact the clinical outcomes in intracytoplasmic sperm injection cycles

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In the present study, we evaluated the impact of sperm origins and concentration on the clinical outcomes of intracytoplasmic sperm injection (ICSI) cycles. A total of 1201 ICSI cycles were retrospectively analyzed for male azoospermia or oligozoospermia between January 2015 and December 2015 in the Peking University Third Hospital. Patients were divided into three groups (Group 1 vs Group 2/3; surgically extracted sperm vs ejaculated sperms): Group 1 included 343 ICSI cycles and Group 2 analyzed 388 cycles on semen with sperm concentration <5 × 10^6 ml^-1 (severe oligozoospermia group). Group 3 included 470 cycles with sperm concentration between 5 × 10^6 ml^-1 and 15 × 10^6 ml^-1 (mild oligozoospermia group). Fertilization rates, clinical pregnancy rates, and live birth rates were analyzed and compared among groups of different semen origins and concentrations on the oocyte retrieval day. Group 2 showed a lower fertilization rate than Group 3 (62.9% ± 21.6% vs 66.8% ± 22.1%, P < 0.05). There were no statistically significant differences in clinical pregnancy rate per transfer (51.3%, 46.7%, and 50.0%, respectively), live birth rate per transfer (44.4%, 40.9%, and 41.4%, respectively), accumulative live birth rate (58.3%, 51.0%, and 52.1%, respectively), twin birth rate (18.4%, 10.6%, and 12.6%, respectively), and birth defects rate (0, 0.3%, and 0.2%, respectively) among three groups. The results of this study indicated that sperm origins and concentration do not impact the clinical outcomes in ICSI cycles. Asian Journal of Andrology (2018) 20, 454–458; doi: 10.4103/aja.aja_27_18; published online: 25 May 2018

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INTRODUCTION

Infertility is defined as the failure to conceive after 12 months of unprotected intercourse and is estimated to affect between 8% and 12% of reproductive-aged couples worldwide. In some regions of the world, the rates of infertility are much higher, reaching 30% in certain populations. Males are found to be solely responsible for 20%–30% of infertility cases and account for 50% of cases overall. Clinically, semen quality, including sperm concentration, number, motility and morphology are used to identify sub fertile and infertile in males. In recent years, there have been numerous reports indicating a decline in the semen quality. In 2010, the World Health Organization (WHO) changed their guidelines for semen analysis to diagnose infertility in men, which were lower than their previous established reference values. Men are increasingly acknowledging infertility and seeking intracytoplasmic sperm injection (ICSI) treatment, which results in higher fertilization rates per oocyte compared with conventional in-vitro fertilization (IVF) treatment. Abnormal semen result makes infertile men concern about their clinical outcomes. Theoretically, ICSI technique requires a single-motile sperm, even from infertile patients with azoospermia through testicular sperm extraction (TESE) or Micro-TESE (MESE). Previous studies either focused on the outcomes and safety of ICSI with sperms from different sources or were solely concerned about the clinical outcomes of oligozoospermia. However, there is a lack of conclusive data on ICSI outcomes based on the semen retrieval method and sperm concentration in males with azoospermia to mild oligozoospermia. We retrospectively collected 1201 ICSI cycles performed due to male azoospermia or oligozoospermia in males from January 2015 to December 2015. Fertilization rates, clinical pregnancy rates, and live birth rates were analyzed and compared on the oocyte retrieval day between groups of different semen origins and concentrations.

PATIENTS AND METHODS

Patients

Between January 2015 and December 2015, 2132 ICSI cycles were conducted for male oligozoospermia or azoospermia in Peking University Third Hospital. In the present study, all female partners were younger than 35 years of age. Other causes of female infertility, including endometriosis, premature ovarian failure (POF), congenital reproductive tract malformation, and abnormal karyotyping, were ruled out. Patients were also excluded from the study if the male partners were diagnosed with teratozoospermia according to the WHO manual on semen analysis (manual 5th edition, 2010) on the day of oocyte retrieval. A total of 1201 qualified...
ICSI cycles (1120 couples) were divided into three groups on the basis of sperm origin and concentration (Table 1). We defined severe oligozoospermia as sperm concentration <5 × 10^6 ml⁻¹ in raw semen and mild oligozoospermia as sperm concentration (raw semen) between 5 × 10^6 ml⁻¹ and 15 × 10^6 ml⁻¹. This study was approved by the Institutional Review Board of Peking University Third Hospital. Written informed consent was obtained from all participants before the commencement of the treatment.

**Semen examination and grouping**

Ejaculated sperm were analyzed and processed according to WHO manual 5 (2010). Patients with azoospermia, or who’s encountering temporary ejaculation failures (TEFs) on the day of oocyte retrieval were treated with TESE or MESE by experienced urologists. The surgery was performed as described by Miller et al. and Schlegel. Sperm retrieval using surgery was used in 343 ICSI cycles (Group 1). Group 2 was defined as having sperm concentration <5 × 10^6 ml⁻¹ (severe oligozoospermia group). Patients in Group 3 had sperm concentration between 5 × 10^6 ml⁻¹ and 15 × 10^6 ml⁻¹ (mild oligozoospermia group) (Table 1). The sperm concentration was manually counted with Makler counting chamber under an inverted microscope (NIKON, Tokyo, Japan) with a microscopic objective of ×10 (100 HP magnification) by an experienced embryologist.

**Protocols for ovarian stimulation and ICSI treatment**

A short protocol, long protocol, antagonist protocol, or a super long protocol (Table 2) was used for ovarian stimulation, as previously described. In Group 1, the surgically extracted sperms were collected by centrifugation at 500 g for 10 min in a Falcon tube (352003, Becton Dickinson, US). In Group 2, the sperm were treated with the “wash method.” Briefly, the sample was rinsed with HEPEs-HTF media and centrifuged to concentrate spermatozoa. The small pellet was resuspended and prepared for a direct injection.

In Group 3, with relatively better sperm quality, a swim-up was prepared (WHO 2010). The ICSI procedure was performed as described by Ou et al.

**Assessment of fertilization and embryo grading**

Fertilization was evaluated 16–18 h after ICSI. Normal fertilization was defined as zygotes with two pronuclei (2PN). The embryos were assessed on day 3 and day 5. We defined the cleavage stage embryo (D3 embryo) as top quality if the embryo had seven or eight cells on day 3, with no more than 10% cytoplasmic fragments. Day-5 embryos (blastocyst) were assessed with Gardner’s score. The day-5 embryos with full blastocysts and the inner cell mass with numerous tightly packed cells and trophoderm with many cells forming a cohesive epithelium were defined as top-quality embryos. One or two embryos were transferred on day 3 or day 5 and the rest were frozen.

**Clinical outcomes**

The fertilization rate is the ratio of the 2PN oocytes to the injected metaphase II (MII) oocyte. We defined biochemical pregnancy

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**Table 1: Frequencies of intracytoplasmic sperm injection cycles and couple characteristics in three groups**

| Parameter                        | Group 1     | Group 2     | Group 3     | P        |
|----------------------------------|-------------|-------------|-------------|----------|
| Cycles (n)                       | 343         | 388         | 470         |          |
| Sperm origins                    | TESE/MESE   | Ejaculation | Ejaculation |          |
| Age of female (year), mean±s.d. | 28.5±3.4    | 29.3±3.3\a  | 29.9±3.2\c  | <0.01    |
| BMI of female (kg m⁻²), mean±s.d.| 22.2±3.3    | 22.7±3.7\c  | 22.8±3.6\c  | 0.04     |
| Duration of infertility (year), mean±s.d. | 3.6±2.6 | 3.8±2.8    | 4.0±2.4\a  | 0.05     |
| Female FSH (mIU ml⁻¹), mean±s.d. | 6.5±2.0     | 6.5±2.2\a  | 6.6±2.1\a   | 0.95     |
| Female LH (mIU ml⁻¹), mean±s.d.  | 4.8±5.1     | 4.4±3.8    | 4.7±3.6\b   | 0.50     |
| Female E2 (pmol l⁻¹), mean±s.d. | 175.1±89.4  | 169.1±98.0 | 169.6±133.4 | 0.77     |
| Age of male (year), mean±s.d.   | 30.3±4.6    | 31.1±5.0\c  | 31.5±4.4\c  | 0.02     |
| BMI of male (kg m⁻²), mean±s.d. | 25.0±4.1    | 25.5±3.8    | 25.9±4.5\d  | 0.01     |
| Previous sperm concentration\b (×10^6 ml⁻¹), median (range) | 0 (0–14.4) | 1.5 (0–23.5)\b | 5.7 (0.5–20.3)\b | <0.01 |

\(\text{\a}\) P<0.05, compared with age of female in Group 1; \(\text{\b}\) P<0.05, compared with BMI of female in Group 1; \(\text{\c}\) P<0.05, compared with age of male in Group 1; \(\text{\d}\) P<0.05, compared with BMI of male in Group 1; \(\text{\e}\) P<0.05, compared with data of previous sperm concentration in Group 1; \(\text{\f}\) P<0.05, compared with data of previous sperm concentration in Group 3.

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**Table 2: Protocols for ovarian stimulation and intracytoplasmic sperm injection outcomes**

| Treatment protocol (n)   | Group 1 | Group 2 | Group 3 | P        |
|--------------------------|---------|---------|---------|----------|
| Short protocol, n (%)    | 7 (2.0) | 19 (4.9) | 16 (3.4) | 0.39     |
| Long protocol, n (%)     | 101 (29.4) | 110 (28.4) | 140 (29.8) | 0.39     |
| Super long protocol, n (%) | 17 (5.0) | 26 (6.7) | 23 (4.9) | 0.39     |
| Antagonist protocol, n (%) | 218 (63.6) | 233 (60.0) | 291 (61.9) | 0.39     |
| Number of oocytes retrieved (mean±s.d.) | 14.1±6.7 | 13.3±6.9 | 13.8±7.5 | 0.35     |
| Number of oocytes injected (mean±s.d.) | 11.2±5.3 | 10.3±5.4 | 10.8±6.1 | 0.10     |
| Number of oocyte fertilized (mean±s.d.) | 7.3±4.3 | 6.6±4.2\a | 7.5±5.1 | 0.02     |
| Fertilization rate (mean±s.d.) | 63.9±20.3% | 62.9±21.6% | 66.8±22.1% | 0.02     |
| Cycles with no transferable embryo, n (%) | 8 (2.3) | 18 (4.6) | 12 (2.6) | 0.13     |

\(\text{\a}\) P<0.05, compared with number of oocyte fertilized in Group 1; \(\text{\b}\) P<0.05, compared with number of oocyte fertilized in Group 3; \(\text{\c}\) P<0.05, compared with fertilization rate in Group 3.

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as the serum HCG positive, which was performed 14 days after embryo transfer. A clinical pregnancy was defined as the presence of a gestational sac, with or without a fetal heart beat as examined by transvaginal ultrasound examination 2 weeks postserum HCG testing. Delivery was defined as live birth. Pregnancy loss before 12 complete gestation weeks was defined as early miscarriage, and if it happened after 12 weeks and before 28 weeks of gestation, it was defined as late miscarriage. The cumulative live birth rate is the ratio of cases with live-birth babies to the ovarian stimulation cycles in each group. Birth defect is defined as malformation, as reported by Reefhuis et al.22 Twin birth rate and birth defect rate are the ratio of respective number of cases to the ovarian stimulation cycles in each group. Birth of children was recorded after telephonic conversation.

Statistical analysis
All statistical calculations and analyses were carried out using IBM SPSS 21.0 software (SPSS Inc., Chicago, IL, USA). Categorical data are represented as frequency and percentage. Differences between the study groups were analyzed by Pearson Chi-square analysis with the use of Fisher’s exact test for expected frequencies of <5. Continuous outcomes are represented as mean ± standard deviation (s.d.) and one-way ANOVA was used to analyze the differences between groups. We also used the logistic regression models to adjust for the effect of baseline characteristics. Differences were considered statistically significant when P < 0.05.

RESULTS
We retrospectively analyzed the 1201 ICSI cycles from 1120 couples, representing 949 fresh transfer cycles and 577 frozen embryo transfer cycles. Baseline characteristics of the patients in the three groups are summarized in Table 1. The average age of females at ICSI in Group 1 was lower than in the Group 2 and Group 3 (P < 0.05), but no statistical difference was found in the average age between Group 2 and Group 3. The BMI of females in Group 3 was higher than Group 1 (22.8 ± 3.6 vs 22.2 ± 3.3, P < 0.05). There were no significant differences between groups in female FSH, LH, E2, and duration of infertility. The average age of males in Group 1 was also lower than in Group 2 and Group 3 (P < 0.05), but there was no statistical difference in male age between Group 2 and Group 3. The Group 3 males’ BMI was higher than the males’ BMI in Group 1 (25.9 ± 4.5 vs 25.0 ± 4.1, P < 0.05). Previous sperm concentrations of the male partners also matched based in the grouping methods.

The oocyte retrieval number, injected MII oocyte number, and 2PN rate are summarized in Table 2. There were no statistically significant differences between the three groups in oocyte retrieval number and MII oocyte injected number. Group 2 had the lowest fertilization rate (2PN) among the three groups and was significantly lower than Group 3 (62.9% ± 21.6% vs 66.8% ± 22.1%, P < 0.05).

We further analyzed the clinical outcomes of each embryo transfer (Table 3). The percentages of cleavage stage embryo transfer (D3) and blastocyst transfer (D5), fresh embryo transfer and frozen embryo transfer, single embryo transfer and two embryo transfer, and the number of at least one top embryo transferred were comparable between the three groups. There were no statistically significant differences between the three groups for biochemical pregnancy rates, clinical pregnancy rates, ectopic pregnancy rates, late miscarriage rates, and live birth rates. However, Group 2 had a lower early miscarriage rate than Group 3 (3.3% vs 6.9%, P < 0.05). Highest percentage of two embryos transfer (83.7%) in Group 2 may account for lower early miscarriage rate in this group.

The cumulative clinical outcomes of each ovarian stimulation cycles were calculated and are summarized in Table 4. Three groups have identical rates of live birth rate and birth defect rate. Group 1 showed a significantly higher rate of twin birth than Group 2 and Group 3 (P < 0.05), but no statistically significant differences were observed between Group 2 and Group 3. For female age, BMI, and male age may influence the clinical outcomes, and we further used the logistic regression model to adjust for the effect for cumulative live birth and twin birth (Table 5). The result demonstrated that female age, BMI, and male age had substantial effects on live birth. However, all the factors shown in Table 5 had no effect on the twin birth rate.

DISCUSSION
In the present study, we compared the reproductive outcomes of ICSI cycles grouped based on sperm origin and concentration. Using comparatively large samples of ICSI cycles in a single IVF center, we analyzed the clinical outcomes of infertile patients with azoospermia and oligozoospermia by established highly reliable laboratory techniques and rigorous clinical processes.

Table 3: Outcomes of per transfer (n=1526)

|                  | Group 1 | Group 2 | Group 3 | P       |
|------------------|---------|---------|---------|---------|
| Patients undergoing embryo transfer, n (%) | 354 (78.7) | 407 (84.1) | 486 (82.1) | 0.10   |
| D3               | 96 (21.3) | 77 (15.9) | 106 (17.9) | 0.10   |
| Patients undergoing embryo transfer, n (%) | 268 (59.6) | 316 (65.3) | 365 (61.7) | 0.19   |
| Fresh transfer   | 182 (40.4) | 168 (34.7) | 227 (38.3) | 0.19   |
| Frozen embryo transfer | 88 (19.6) | 79 (16.3) | 106 (17.9) | 0.44   |
| 1                | 362 (80.4) | 405 (83.7) | 486 (82.1) | 0.44   |
| Number of at least one top-quality embryos transferred, n (%) | 219 (48.7) | 213 (44.0) | 286 (48.3) | 0.27   |
| Number of biochemical pregnancy, n (%) | 262 (58.2) | 251 (51.9) | 321 (54.2) | 0.14   |
| Number of clinical pregnancy, n (%) | 231 (51.3) | 226 (46.7) | 296 (50.0) | 0.34   |
| Number of early miscarriage, n (%) | 23 (5.1) | 16 (3.3) | 41 (6.9) | 0.03   |
| Number of ectopic pregnancy, n (%) | 4 (0.9) | 4 (0.8) | 2 (0.3) | 0.47   |
| Number of late miscarriage, n (%) | 4 (0.9) | 8 (1.7) | 8 (1.4) | 0.59   |
| Number of live birth rate, n (%) | 200 (44.4) | 198 (40.9) | 245 (41.4) | 0.49   |

*P<0.05, compared with number of early miscarriage in Group 3
In recent years, a growing number of studies have suggested that sperm sources used in ICSI cycles can impact clinical outcomes, resulting in several conflicting results. Some investigators have cited that sperm ejaculated from men with severe oligozoospermia is inferior to sperm extracted by surgical techniques. Suganuma et al. have postulated that sperm are susceptible to damage while passing through the male reproductive tract. Furthermore, infertile men with oligozoospermia were observed to have significantly higher oxidative parameters in semen. Inhibition of sperm motility and sperm membrane integrity, the role it may play in clinical outcomes, and therefore, systematic studies would need to evaluate the role of sperm morphology on birth outcomes in conditions involving teratozoospermia.

As for clinical outcomes between severe oligozoospermia and mild oligozoospermia, several studies have concluded that sperm concentration positively affects the likelihood of achieving pregnancy. However, our results agree with the findings of Zheng et al. whereby decreased sperm concentration and motility retarded ICSI fertilization rates; however, no differences in live birth rates were observed. We discovered that fertilization rates were significantly lower in the group with severe oligozoospermia when compared to the group with mild oligozoospermia. In the mild oligozoospermia group, we used the swim-up procedure. However, as the sperm concentration was low in this group, a small sperm pellet was resuspended for ICSI procedures. With a higher sperm recovery rate, the "wash method" was observed to produce more white blood cells, bacteria, and dead spermatozoa responsible for oxygen stress, all of which negatively influence the egg fertilization ability of normal spermatozoa. In addition, differences between sperm preparation techniques may influence fertilization results. Therefore, further large randomized controlled trials are warranted to conclude the influence of sperm preparation techniques on fertilization rates.

In our study, the transfer cycles containing at least one top-quality embryo were comparable among three groups. The composition of cleavage stage or blastocyst stage embryo transfer, fresh or frozen embryo transfer, and embryo transfer numbers were similar among the three groups. As the three groups had identical chances with regard to live birth rate, this suggested that sperm retrieval methods and concentrations used in ICSI cycles had insignificant impacts on prospective clinical outcomes. The logistic regression analysis of cumulative live births demonstrated that female age and BMI were negatively associated with cumulative live birth, which is consistent with earlier studies. Interestingly, our results also demonstrated that male age is positively associated with birth outcome. Only a few studies have investigated this association, specifically that male age has a positive impact on fertility outcomes. Recently, a cohort study revealed that sperm telomere length (STL), which is a biomarker for sperm quality, increased with male age, and that this is positively associated with embryo quality in IVF. However, more studies have concluded that paternal age is not a prognostic factor for pregnancy, or that paternal age has negative effects on pregnancy outcomes. As the present study did not focus on male age and fertility, additional studies are required to determine the effect of male age on ICSI outcomes.

We further analyzed the birth defect rates among the three groups. We observed no birth defects in Group 1, a girl born with congenital heart disease in Group 2 (severe oligozoospermia group), and a boy born with hydrocephaly in Group 3 (mild oligozoospermia group). There were no differences among the three groups. Previous studies have demonstrated that infants conceived through ICSI or IVF have double the risk of a major birth defect when compared to naturally conceived infants. However, no risk difference exists for major birth defects in children conceived through IVF or ICSI. Congenital cardiac anomalies are the most common birth defect affecting nearly 1% of all live births naturally, and hydrocephaly has reported incidence rate of 0.48–0.81 per 1000 live births. In the present study, we could not determine if the birth defects observed for Group 2 and 3 were sporadic or in any manner linked to treatments.

Table 4: Outcomes for cumulative live birth, twin birth and birth defects

| Factors                        | Group 1 | Group 2 | Group 3 | P  |
|--------------------------------|---------|---------|---------|----|
| Cumulative live birth, n (%)   | 200 (58.3) | 198 (51.0) | 245 (52.1) | 0.11 |
| Number of twin birth, n (%)    | 63 (18.4) | 41 (10.6) | 59 (12.6) | 0.02 |
| Number of birth defect, n (%)  | 0 (0.0)  | 1 (0.2)  | 0 (0.0)  | 0.50 |

Table 5: Logistic regression analysis of cumulative live births and twin births

| Factors                        | Group 1 | Group 2 | Group 3 | P  |
|--------------------------------|---------|---------|---------|----|
| Female age (year)              | 0.87 (0.80–0.95) | <0.01 | 0.94 (0.83–1.08) | 0.38 |
| Female BMI                     | 0.93 (0.87–0.98) | <0.01 | 1.04 (0.95–1.13) | 0.46 |
| Male age (year)                | 1.07 (1.00–1.14) | 0.03 | 1.02 (0.94–1.11) | 0.64 |
| Male BMI                       | 1.05 (1.00–1.10) | 0.05 | 1.02 (0.94–1.10) | 0.64 |
| Previous sperm concentration   | 1.02 (0.97–1.08) | 0.41 | 0.96 (0.86–1.08) | 0.49 |

In the present study did not focus on male age and fertility, additional studies are required to determine the effect of male age on ICSI outcomes.

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AUTHOR CONTRIBUTIONS
JH and YL carried out the semen analysis. QJ conceived the study, CY collected and collated the data, CY and DNZ performed the statistical analysis, and CY drafted the manuscript. ZHZ, XFX, and JQ supervised the project and revised the manuscript. All authors read and approved the final manuscript.

COMPETING INTERESTS
All authors declared no competing interests.

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