Preimplantation Genetic Testing for Aneuploidies Improve the Clinical Pregnancy Outcome of Patients With AZFc Microdeletion

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Short report

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Abstract

Background

Microdeletions of AZF are the most common factor causing male infertility except Klinefelter syndrome. AZF patients are able to father babies through intracytoplasmic sperm injection (ICSI) and in vitro fertilization (IVF), and the ICSI embryos for IVF can be scored based on morphological criteria. However, the clinical pregnancy rate and the live birth rate are unsatisfactory.

Results

Here, we investigated the outcomes of using preimplantation genetic testing for aneuploidies (PGT-A) in families with AZFc microdeletions. A total of 26 intracytoplasmic sperm injection (ICSI) cycles were performed in 22 families, 25 ICSI cycles were performed, and 81 embryos underwent PGT-A. Among them, 48 were euploid embryos (23 females and 25 males), 30 were aneuploid embryos, and three embryos did not meet the quality control standards. Thirteen ICSI cycles with female euploid embryos and one ICSI cycle with male euploid embryos entered the stage of embryo implantation. Finally, the clinical pregnancy rate was 100% (14/14), and the live birth rate was 85.7% (12/14).

Conclusion

Compared with other published results, PGT-A increased the chance of fertility in AZFc microdeletion patients.

Background

Azoospermia factor (AZF) is a region located on the long arm of the Y chromosome (Yq11), including the subgroups AZFa, AZFb and AZFc. Microdeletions of AZF are the most common factor causing male infertility except Klinefelter syndrome, and AZFc microdeletion has the highest frequency, accounting for approximately 80% of all AZF microdeletions [1]. It is generally believed that patients diagnosed with AZFc microdeletion have residual spermatogenesis ability, and AZFc microdeletion patients can become fathers through intracytoplasmic sperm injection (ICSI) [2–4].

Generally, the embryos obtained by ICSI were scored based on morphological criteria [5], and the best embryos were selected for transfer [6]. However, it has been reported that ICSI possesses higher aneuploidy rates than conventional IVF procedures [7–9]. Moreover, the fertilization rate and embryo quality of AZF microdeletion patients were significantly decreased after ICSI [10, 11]. Therefore, when AZFc microdeletion patients receive ICSI, we should pay greater attention to the embryos for transfer. Preimplantation genetic testing for aneuploidy (PGT-A) could exclude aneuploid embryos, enable clinicians to select euploid embryos for embryo transfer, and improve the pregnancy rate and live birth rate [12].

The use of PGT-A in AZFc microdeletion patients has not been reported. Here, this study summarized the clinical outcomes of oligospermia patients diagnosed with AZFc microdeletion who received PGT-A before embryo transfer in our centre (Department of Reproductive Medicine, General Hospital of Northern Theater Command) in recent years. We found that the application of PGT-A improved the pregnancy rate and live birth rate compared to conventional ICSI methods.

Results

Among 22 couples, the men were 27–43 years old, and the women were 26–38 years old. Twenty-six ICSI cycles were carried out, of which 19 couples conducted one ICSI cycle, two couples conducted two ICSI cycles, and one couple conducted three ICSI cycles. A total of 248 fertilized oocytes were obtained from the 26 ICSI cycles, and 81 blastocysts from 25 cycles were detected by PGT-A. Among the blastocysts detected, 23 (28.4%) were female euploid blastocysts, 25 (30.9%) were male euploid blastocysts, 30 (37.0%) were aneuploid blastocysts, and the remaining 3 (3.7%) blastocysts failed to pass the quality control after genome amplification (Table 1).
Among the 25 cycles, female euploid blastocysts were detected in 14 cycles, only male euploid blastocysts were detected in 8 cycles, and no euploid embryos were detected in the other 3 cycles. For the 14 cycles in which euploid female embryos were detected, euploid female blastocysts were transferred in 13 cycles, and one cycle was delayed due to coronavirus disease 2019 (COVID-19). For the 8 cycles in which only euploid male embryos were detected, one couple chose to transfer one male euploid blastocyst. The clinical pregnancy rate of 14 cycles of blastocyst implantation was 100%. Finally, early abortion occurred in 2 cycles (abortion rate of 14.3%), normal delivery occurred in 12 cycles (one cycle for monozygotic female twins, one cycle for males, and 10 cycles for females), and the live birth rate was 85.7% (Table 1).

**Table 1**

| Family | Cycle | Date of consultation | Age of wife & husband | Sperm concentration (x10^6/mL) | Fertilized oocytes | Blastocyst biopsies | Female euploid | Male euploid | Implantation date | Clinical pregnancy | Early abortion | Libbi |
|--------|-------|---------------------|-----------------------|-------------------------------|-------------------|-------------------|---------------|--------------|-----------------|------------------|--------------|-------|
| 1      | 1     | 2015/1/5            | 37&37                 | 0.001                         | 4                 | 1                 | 1             | 0            | 2015/4/13       | √                | ×            | √    |
| 2      | 2     | 2015/3/13           | 33&35                 | 0.1                           | 22                | 2                 | 1             | 1            | 2015/6/30       | √                | ×            | √    |
| 3      | 3     | 2015/9/25           | 28&33                 | 0.1                           | 5                 | 1                 | 1             | 0            | 2015/11/25      | √                | ×            | √    |
| 4      | 4     | 2015/11/23          | 33&32                 | 1                             | 7                 | 1                 | 1             | 0            | 2016/5/15       | √                | √            | ×    |
| 5      | 5     | 2015/12/1           | 30&43                 | 0.01                          | 8                 | 6                 | 2             | 2            | 2016/3/4        | √                | ×            | √    |
| 6      | 6     | 2015/12/27          | 28&32                 | 1                             | 3                 | 1                 | 0             | 0            | 2016/6/6        | √                | ×            | ×    |
| 7      | 7     | 2016/3/6            | 30&29                 | 0.01                          | 9                 | 5                 | 2             | 1            | 2016/6/6        | √                | ×            | ×    |
| 8      | 8     | 2016/7/6            | 32&34                 | 19                            | 7                 | 2                 | 0             | 0            | 2016/11/14      | √                | ×            | ×    |
| 9      | 9     | 2016/7/7            | 35&41                 | 12                            | 4                 | 0                 | 0             | 0            | 2017/1/11       | √                | √            | ×    |
| 10     | 10    | 2016/9/5            | 34&36                 | 3                             | 7                 | 3                 | 3             | 0            | 2017/9/30       | √                | ×            | √    |
| 11     | 11    | 2016/11/2           | 38&39                 | 3                             | 11                | 6                 | 3             | 0            | 2017/11/11      | √                | √            | ×    |
| 12     | 12    | 2017/5/21           | 26&30                 | 0.5                           | 17                | 4                 | 0             | 2            | 2017/9/30       | √                | ×            | √    |
| 13     | 13    | 2017/6/1            | 29&32                 | 0.05                          | 9                 | 2                 | 1             | 0            | 2017/8/1        | √                | ×            | ×    |
| 14     | 14    | 2017/6/3            | 32&31                 | 0.1                           | 10                | 3                 | 2             | 0            | 2017/9/5        | √                | ×            | √    |
| 15     | 15    | 2018/4/13           | 37&38                 | 1                             | 6                 | 3                 | 0             | 1            | 2018/4/17       | √                | ×            | ×    |
| 16     | 16    | 2018/9/2            | 37&38                 | 0.01                          | 7                 | 4                 | 0             | 1            | 2019/5/31       | √                | ×            | √    |
| 17     | 17    | 2018/12/5           | 37&38                 | 0.1                           | 9                 | 4                 | 0             | 4            | 2019/4/17       | √                | ×            | ×    |
| 18     | 18    | 2019/1/8            | 32&32                 | 2                             | 9                 | 6                 | 1             | 2            | 2019/5/31       | √                | ×            | √    |
| 19     | 19    | 2019/1/18           | 30&29                 | 0.5                           | 22                | 4                 | 2             | 1            | 2019/4/17       | √                | ×            | ×    |
| 20     | 20    | 2019/4/30           | 36&36                 | 1                             | 3                 | 1                 | 0             | 1            | 2019/5/31       | √                | ×            | ×    |
| 21     | 21    | 2019/10/19          | 36&37                 | 0.5                           | 7                 | 3                 | 1             | 1            | 2019/10/29      | √                | ×            | ×    |
| 22     | 22    | 2019/5/29           | 36&36                 | 15                            | 12                | 6                 | 0             | 5            | 2019/10/29      | √                | ×            | ×    |

F: female; FF: female twins; M: male.

**Discussion**

In this study, PGT-A was performed on ICSI embryos of AZFc microdeletion patients. Finally, the overall pregnancy rate and live birth rate were 100% (14/14) and 86% (12/14), respectively, which were much higher than those in previous reports [13–15]. In fact, PGT-A should be carried out for ICSI embryos due to severe male factors to exclude aneuploid embryos [16]. However, PGT-A is commonly used in patients with severe male factors such as **DANH1, PAG6** and others[17, 18], and PGT-A has not been reported in patients with AZF microdeletions.

Female age is a prominent factor affecting the outcome of ICSHIVF [19, 20]. However, male age had no significant effect on the rate of aneuploidy embryos [21]. The female age in this study ranged from 26 to 37, of which 16 were under 35 years old and 6 were over 35 years old. Among the 48 embryos under 35 years old, 28 (58.3%) were aneuploid embryos (16 were female, and 12 were male). Among the 33 embryos over 35 years old, 20 (60.6%) were aneuploid embryos (7 were female, and 13 were male). Overall, there was no significant correlation between aneuploidy frequency and female age, which may be because the eldest female age was 37 years old or the sample size was not large enough, and the influence on embryos was not prominent in this study.
Male offspring of patients with AZF microdeletions using ART inherit AZF microdeletions, which may lead to similar or serious reproductive problems in adulthood [2–4]. In this study, after full genetic consultation with patients, for the 14 families who obtained female euploid embryos, most of them (13/14) chose to transfer female embryos, and one family was delayed due to COVID-19; for the 8 families who only obtained male euploid embryos, one family chose to enter the embryo transfer phase, and the other families chose to forgo embryo transfer.

Compared to ICSI using microTESE and ejaculatory sperm, the outcome of later sperm performed better [13, 14, 22]. In this study, our results show that PGT-A can significantly improve clinical outcomes after ICSI with ejaculated sperm in AZFc patients. However, the outcomes of PGT-A in AZFc patients undergoing ICSI with microTESE need further observation.

Conclusions
For patients with AZFc microdeletions, ICSI-IVF with ejaculatory spermatozoa and NGS-based PGT-A can significantly improve the chance of obtaining offspring.

Patients And Methods

Patients and AZF microdeletion detection
From 2015 to 2019, among the male patients who were diagnosed with AZFc microdeletion using a Y chromosome microdeletion detection kit (Tellgen, China) in the General Hospital of the PLA Northern Theater Command, 22 patients received assisted reproduction technologies (ART) with ICSI and PGT-A. The sperm concentration was evaluated according to the recommended method of the WHO Laboratory Manual for the Examination and Processing of Human Semen (5th edition). Twenty-six semen samples from 22 patients were analysed, and 25 semen samples were oligozoospermia (< 20 million/mL), while the sperm concentration of the other semen samples was ≥ 20 million/mL (Table 1).

Oocyte retrieval and oocyte insemination by ICSI
The patients’ wives received 11 days with daily injection of 150 IU recombinant follicle stimulating hormone (Merck Serono). On day 6, 0.25 mg gonadotropin-releasing hormone antagonist (Merck Serono) was given every daily until oocyte maturation by 4 mg of gonadotropin-releasing hormone agonist (triptorelin acetate for injection, Beijing Biote Pharmaceutical) on day 12. Thirty-six hours later, the oocytes were retrieved and cultured in Quinn’s Advantage fertilization medium (Origio) at 37°C, 6.0% CO2, 5% O2 and 89% N2. For ICSI, cumulus cells were removed by using hyaluronidase (Origio) four hours after oocyte retrieval, and metaphase II oocytes were injected 5 h after retrieval.

Quality assessment of fertilized embryos and blastocyst biopsy
Fertilization status was assessed 18 h after ICSI, and normal fertilization was characterized by two distinct pronuclei and two polar bodies. The embryos developed to the blastocyst stage, and 5-10 trophoblastic cells were collected and transferred into 200-μL sterile PCR tubes for PGT-A; the blastocysts after biopsy were cryopreserved for frozen embryo transfer.

A total of 5 -10 trophoblast cells were used for whole genome amplification (WGA) according to the documentation of the SurePlex DNA Amplification System (Illumina, America), and WGA products were purified and recovered using AMPure XP beads (Beckman Coulter, America). The purified WGA products were used for library preparation using the VeriSeq™ DNA Library Prep Kit (Illumina, America) and sequenced on the NextSeq 550 platform. The data were analysed by Peking Medriv Academy of Genetics and Reproduction.

Considering that sons of AZFc microdeletion patients will also inherit the affected Y chromosome [2-4], the patients were informed of not only the euploid condition but also the sex of the embryos. After full genetic consultation with the patients, the patients chose the embryo for implantation.

List Of Abbreviations

AZF: Azoospermia factor
ICSI: Intracytoplasmic sperm injection
IVF: In vitro fertilization
PGT-A: Preimplantation genetic testing for aneuploidies
ART: Assisted reproduction technologies

Declarations

Ethics approval and consent to participate
This research was approved by the Ethics Committee of General Hospital of Northern Theater Command. This was a retrospective study without any identifiers related to patients. All patients participating in the study provided informed consent.

Consent for publication
All patients in this study provided their consent for publication. A copy of the written consent is available for review by the Editor of this journal.

Availability of data and material
The datasets generated and analysed during the current study are not publicly available due to a concern to protect individual patient confidentiality, but are available from the corresponding authors on reasonable request.

Competing interests
The authors declare that they have no conflicts of interest.

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Authors’ Contributions
XW, FJ and YY designed the study. XW, YW, KH, DH, JZ, CT and WW performed the experiments. XW and YW drafted the manuscript. All authors reviewed and approved the manuscript.

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