Outcomes of 13 ICSI-PGD cycles with ejaculated spermatozoa in patients with Klinefelter syndrome

Tian-Xiang Ni1,*, Jun-Hao Yan1,2,3,*, Bo Wang1,4,*, Yue-Ting Zhu1, Hong-Chang Li1, Hong-Qiang Xie1, Wen-Jie Jiang1, Zi-Jiang Chen1,2,3

Asian Journal of Andrology (2016) 18, 498–499; doi: 10.4103/1008-682X.161238; published online: 11 September 2015

Dear Editor,

Klinefelter syndrome (KS) is the most frequent genetic cause of infertility in men. Paternity can be achieved through intracytoplasmic sperm injection (ICSI) with spermatozoa recovered from ejaculated semen if exist, or testes with testicular sperm extraction (TESE). Though the proportion of spermatozoa and embryos with abnormal sex chromosomes and autosomes, especially chromosomes 13, 18 and 21, is higher in KS patients as reported,1 which suggests the need of preimplantation genetic diagnosis (PGD), the application of PGD becomes questionable with more and more normal babies born with ICSI without PGD. This article aims to report the results of 13 ICSI-PGD cycles performed in 13 KS patients with ejaculated spermatozoa in the authors’ reproductive center and to discuss the necessity of application of PGD in comparison with the outcomes of ICSI with or without PGD in KS patients in literature (P < 0.05 was considered to be statistically significant).

The mean (±s.d.) age of 13 male partners is 28.62 ± 5.33 (maximum age 44 years), while the mean (±s.d.) age of female partners is 27.94 ± 5.37 (maximum age 43 years). Twelve patients were karyotyped with 47, XXY; the other one was mosaic with 47, XXY [74]/46, XY [26]. They all were characterized with microopenis and small atrophic testes, along with elevated Follicle-Stimulating Hormone (FSH) and Luteinizing Hormone (LH) (FSH 30.00 ± 14.29 IU·L⁻¹, LH 15.92 ± 5.80 IU·L⁻¹) and lower or relatively lower or lower or relatively normal testosterone (251.77 ± 157.40 ng·dl⁻¹). Three of them had unapparent prominentia laryngea and sparse pubic hair, while other ones were normal. All patients presented oligoasthenozoospermia, from slight to severe. As semen analysis on the day of oocyte retrieval showed, only one or two spermatozoa with low motility can be found in each high power field in six of them. Another one was evaluated with the presence of sperm cells after high-speed centrifugation (1800 g). Sperm concentration of the others was from 1 to 7 million·ml⁻¹ and the sperm motility was from 3% to 15%.

There were total 24 embryos transplanted in 14 embryo transfer cycles (1.47 ± 0.75 embryos per transfer). There were 12 biochemical pregnancies obtained (85.71% per transfer cycle), 14 embryos implanted (58.33% per transferred embryo), and there were 11 clinical pregnancies with births of 3 healthy boys and 8 healthy girls and 3 ongoing clinical pregnancies (78.57% per transfer cycle). And, no patient underwent prenatal diagnosis which was recommended strongly or karyotype detection of newborns. There are 90 embryos with two sex chromosomal signals among 103 embryos with clear diagnosis (87.38%), and the number of those with XX is 49, slightly more than those with XY (41). 13 abnormal embryos are with one, three, or four sex chromosomal signals (3 with X, 1 with XYY, 3 with XXY, 4 with XXX, and 2 with XXYY).

In 2003, Staessen et al. demonstrated their embryonic detection outcomes with Fluorescence In Situ Hybridization (FISH) that the proportion of normal embryos (54%) is significantly lower than controls (77.2%), who are with X-linked disease in need of PGD to determine gender. Among the abnormal embryos, the rate of sex chromosomal abnormalities is 13.2%, much higher than controls (3.1%). The other study in 2003 found that in embryos detected for chromosomes 13, 18, 21, and X and Y, 14 out of 23 embryos were diagnosed as normal (60.9%), and 5 in 28 (17.8%) were diagnosed as abnormal with sex chromosomal abnormalities.2 In our study, embryos were analyzed only for sex chromosomes, and the proportion of abnormal embryos is 12.62% (13 in 103) and, there is no statistically significant difference between our outcomes and those we have mentioned.1–3 (Chi-square test, in Table 1). Considering one patient is mosaic, the proportion of abnormal embryos is 12.62% if we take him into consideration (13 in 103); while it is 11.76% if we exclude him (10 in 85). There is also no statistically significant difference compared with the historical data (Chi-square test: P = 0.752, P = 0.615, respectively). As both historical studies above were almost those with TESE, the comparison result also implies that the proportion of abnormal embryos from ejaculated spermatozoa is comparable with those from TESE, at least for sex chromosomes.

In addition, excluding the couple in our study beyond 35 years old, the proportion of abnormal embryos is 11.76% (10 out of 85), much higher than the embryonic aneuploidy rate of sex chromosomes (3.4%, 235 out of 6819) in the population with infertility under 35 as mentioned.3

In spite of the higher aneuploidy rate of embryos in KS patients, there are at least 200 normal babies born with ICSI without PGD.

1Center for Reproductive Medicine, Shandong Provincial Hospital Affiliated to Shandong University, Shandong Provincial Key Laboratory of Reproductive Medicine, Jinan 250021, China; 2National Research Center for Assisted Reproductive Technology and Reproductive Genetics, Jinan 250021, China; 3The Key Laboratory for Reproductive Endocrinology of Ministry of Education, Jinan 250021, China; 4Department of Anesthesiology, Shandong Provincial Hospital Affiliated to Shandong University, Jinan 250021, China.

*These authors contributed equally to this work.

Correspondence: Dr. ZJ Chen (chenzijiang@hotmail.com)

Received: 03 March 2015; Revised: 15 May 2015; Accepted: 09 June 2015
Table 1: Outcomes of ICSI(-PGD) with ejaculated spermatozoa in KS patients from reports in history

| References          | PGD | Embryos detected | Karyotype of detected embryos | Cycle | ETC | TE | Clinical pregnancy | Implanted embryos | Newborn | Ongoing clinical pregnancy |
|---------------------|-----|------------------|-------------------------------|-------|-----|----|--------------------|-------------------|---------|-----------------------------|
| Hinney et al.⁴⁵      | No  | -                | -                             | 1     | 1   | 3  | 1                  | 1                 | 0       | 0                           |
| Bourne et al.⁶      | No  | -                | -                             | 1     | 2   | 4  | 1                  | 2                 | 2       | 0                           |
| Ron-el et al.⁷      | No  | -                | -                             | 1     | 1   | 3  | 1                  | 1                 | 1       | 0                           |
| Kitamura et al.⁸     | No  | -                | -                             | 1     | 3   | 10 | 1                  | 2                 | 1       | 0                           |
| Cruger et al.⁹      | No  | -                | -                             | 1     | 1   | 2  | 1                  | 1                 | 1       | 0                           |
| Tachdjian et al.³    | No  | -                | -                             | 1     | 1   | 2  | 1                  | 2                 | 2       | 0                           |
| Komori et al.¹⁰      | No  | -                | -                             | 2     | 2   | 4  | 2                  | 2                 | 2       | 0                           |
| Data from SDU       | Yes | 103              | XX (49); XY (41); Abnormal (13)⁶⁷    | 13    | 14  | 24 | 11                | 14                | 11      | 3                           |
| Staessen et al.¹⁸    | Yes | 113              | Normal XX or XY (98); Abnormal (15)⁶    | 32    | 26  | 41 | 5                  | 5                 | 4       | 0                           |
| Kahraman et al.²⁸    | Yes | 33               | Normal XX or XY (23); Abnormal (5)²⁸  | 8     | 8   | 19 | 4                  | 6                 | 3       | 2                           |
| Total without PGD with ejaculate |       | 8                | 11                            | 28                    | 11 | 9 | 0                  |                   |         |                             |
| Total with PGD with ejaculate |       | 14              | 15                            | 26                        | 12 | 15 | 12 | 3                           |                   |         |                             |

⁴ Data from Staessen et al. contain only 1 cycle with fresh ejaculate (1 transfer cycle, 2 embryos transferred, and clinical pregnancy was achieved with 1 embryo implanted and 1 baby born), 21 cycles with fresh sperm from TESE, 10 cycles with frozen-thawed sperm from TESE; ⁵ Data from Kahraman et al. was the outcomes of ICSI-PGD with spermatozoa from testes in KS patients; ⁶ Chi-square test, P=0.887; ⁷ Chi-square test, P=0.686; ⁸ ET: embryos transferred cycle; TE: transferred embryos; ICSI: intracytoplasmic sperm injection; PGD: preimplantation genetic diagnosis; TESE: testicular sperm extraction; KS: Klinefelter syndrome; SDU: Shandong University reproductive center.

This work was supported by the Science Research Foundation Item of No-earnings Health Vocation (2014020804) and Science and Technology Development Planning of Shandong (2013GGE247001).

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worldwide as reported to date. And, most of these children are proved as chromosomally normal. One possible reason is that endometrial selectivity would usually automatically pick up normal embryos without chromosomal aberration for implantation or ongoing pregnancy. And comparison of pregnancy outcomes with ejaculated spermatozoa between ICSI without and with PGD in KS patients is shown in Table 1. The implantation rates are 39.29% and 57.69% respectively, and clinical pregnancy rates are 72.73% and 80.00% respectively. No statistically significant difference exists (Chi-square test: P = 0.176; P = 1.00, respectively).

In conclusion, though all evidence indicate that there is a higher rate of abnormal embryos in KS couples, but its impact on pregnancy outcomes is rather limited. But, our data are still limited by far, thus, more cases and follow-ups are needed to evaluate the benefits and potential risks of all ART methods for KS patients.

AUTHOR CONTRIBUTIONS

TN extracted and analyzed the data, summarized the literature, and drafted the manuscript; JY and BW conceived the idea, participated in the study design, and reviewed the manuscript; YZ, HL, HX, and WJ performed the ICSI and FISH procedures. All authors read and approved the final manuscript.

COMPETING INTERESTS

All authors declare no competing interests.

ACKNOWLEDGMENTS

The authors would like to thank Keliang Wu and other personnel in the IVF laboratory for their help and precious participation in laboratory procedures.