Y chromosome AZFc microdeletion may not affect the outcomes of ICSI for infertile males with fresh ejaculated sperm

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Abstract

Purpose To explore whether the presence of a Y chromosome AZFc microdeletion confers any adverse effect on the outcomes of intracytoplasmic sperm injection (ICSI) with fresh ejaculated sperm.

Methods A total of 143 oligozoospermia patients with Y chromosome AZFc microdeletion in ICSI cycles in a five-year period were studied. Infertile men with normal Y chromosome in ICSI at the same time-frame were used as controls matched to the study group for age of female, female's body mass index, male's age, infertility duration and number of oocytes retrieved. Retrospective case–control study was used. **Results** There were no significant differences between groups in clinical outcomes of endometrial thickness, transferred embryos, good embryo rates, implantation rates, biochemical pregnancy rates, clinical pregnancy rates, ectopic pregnancy rates, miscarriage rates, preterm birth rates, the ratio of male

and female babies, newborn body height, newborn weight, low birth weight and birth defects ($P>0.05$). Patients with Y chromosome AZFc microdeletion had a lower fertilization rate (61.8 % vs. 67.8 %, $P<0.05$) and higher cleaved embryo rate (94.0 % vs. 88.1 %, $P<0.05$).

Conclusions ICSI clinical outcomes for oligozoospermic patients with Y chromosome AZFc microdeletion are basically comparable to that of infertile patients with normal Y chromosomes. The results of ICSI were not affected by the AZFc deletion. Preimplantation genetic diagnosis (PGD) before ICSI for Y chromosome AZFc microdeletion may not be a justifiable regular procedure if the couples didn't care the vertical transmission of Y chromosome deletion.

Keywords Y chromosome microdeletion · AZF · ICSI · Male infertility · Clinical outcome · PGD

Capsule The object of this study is to explore whether the presence of a Y chromosome AZFc microdeletion confers any adverse effect on the outcomes of intracytoplasmic sperm injection (ICSI). The results show that there is no difference in ICSI clinical outcomes between oligozoospermic patients with Y chromosome AZFc microdeletion and that with normal Y chromosomes.

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Introduction

Up to 30 % of male infertility is caused by sperm production disorder due to certain genetic defects [1]. Y chromosome microdeletion is the most important genetic etiology of male infertility. Among infertile men, the prevalence of Y microdeletion is approximately 7 %, with a range of 1–35 % according to published reports [2, 3]. The variation in the reported rate is likely caused by the difference in study design and patient cohort selection [4]. The initial cytogenetic observation of an azoospermia factor (AZF) present on Yq was made in 1976, which enabled molecular analysis of Yq microdeletion in infertile men for the first time [5–8]. Subsequently, the long arm of the Y chromosome was shown to contain three AZF regions, namely AZFa, AZFb, and AZFc, from proximal to distal Yq [8]. The presence of AZF locus, containing one or more genes necessary for normal spermatogenesis, was then proposed as useful for fertility and mapped to deletion Yq-interval 6 (see Fig. 1). The AZF deletions are

Fig. 1 Diagram of the Y chromosome depicting the location of azoospermic factor (AZF) regions **a**, **b**, and **c**. SRY, sex-determining region of the Y chromosome

known to be associated with various spermatogenetic alterations including Sertoli cell-only syndrome (SCOS), maturation arrest, and hypospermatogenesis [9, 10]. The development of intracytoplasmic sperm injection (ICSI) has allowed patients with severe oligozoospermia to have children. However, the inheritance of Y chromosomal microdeletions from father to son through ICSI is a risk of concern [11, 12]. Only a handful studies about whether Y chromosome microdeletion has any adverse effects on the outcomes of ICSI have been published so far [13, 14]. The results of these studies have been controversial in terms of fertilization rate and quality of embryos.

One of the critical questions related to Y chromosome microdeletion that has not been addressed is whether the offspring of men with Y chromosome microdeletion who undergo ICSI treatment carry any heightened risk of birth defect. Presently, preimplantation genetic diagnosis (PGD) enables couples undergoing ICSI treatment to select female embryos for transfer [15]. However, the actual procedures of PGD might have adverse effects on the embryo development and long-term health of the offspring. This renders the PGD for Y chromosome microdeletion in ICSI a doubtful

practice, without clear evidence of whether this genetic defect seriously influences the clinical outcomes for ICSI. Here, we address this problem by comparing the men with AZFc microdeletion with matched normal Y chromosome men who are oligozoospermic to determine whether there are any significant differences in clinical outcomes between the two groups. The parameters include demographic and general clinical records. This study will provide robust insight into whether Y chromosome AZFc microdeletion confers any adverse effect on the outcomes of ICSI treatment and whether PGD is a necessary and worthwhile screening intervention for Y chromosome microdeletion. However, prior studies are limited to relatively small numbers of AZFc-deleted men. Here, we are reporting the follow-up results of infertile men with or without Y chromosome AZFc microdeletions (143 oligozoospermic cases for each group).

Materials and methods

Patients

The 143 men included in the study were consulted for infertility from January 2007 to December 2011 at the Center for Reproductive Medicine, Peking University Third Hospital. All the patients were screened for microdeletion in AZF a, b and c regions of the Y chromosome. They all have a Y chromosome AZFc microdeletion and were assigned to group Y-deleted. Semen analyses were performed according to the World Health Organization manual (4th Edition). A general physical examination with particular attention to testis volume was performed. Hormonal status reflective of the spermatogenic axis (FSH) and the androgenic axis (LH, testosterone) were drawn. Conventional chromosomal karyotype analysis was conducted to analyze chromosome abnormalities via peripheral blood. One hundred forty three subfertile men who applied for ICSI treatment because of oligospermia without Y chromosome microdeletion were selected as the control group. The control patients were matched to Y-deleted patients by female's age, female's body mass index (BMI), male's age, infertility duration and number of oocytes retrieved (Table 1). A fresh transplantation cycle for each couple was conducted for both groups. Patient consent was obtained and the study was approved by the hospital ethics committee.

Table 1 Matching data of Y-deleted and control group

Matching parameters	Y-deleted	Control	<i>t</i>	<i>P</i>
Female's age (yr)	30.24±3.973	30.23±3.975	-0.446	0.656
Female's BMI	22.02±3.311	21.72±2.825	-1.130	0.261
Male's age (yr)	31.78±4.268	31.73±4.595	-0.229	0.819
Infertility duration (yr)	4.90±3.541	4.90±3.381	-0.069	0.945
Number of oocytes retrieved	15.71±8.644	15.70±8.441	-0.057	0.955

Y chromosome microdeletion testing

Y chromosome microdeletion analysis was performed with a multiplex polymerase chain reaction (PCR) technique on DNA extracted from peripheral leukocytes. Three different AZFa, b, c regions were analyzed as previously described by Akinin-Seifer et al. [16]. Six sequence-tagged site (STS): SY84 and SY86 (AZFa), SY127 and SY134 (AZFb), SY254 and SY255 (AZFc) were tested in accordance with recommendations of the Guidelines of the European Academy of Andrology (EAA) [17].

Genetic counseling

All Y chromosome microdeletion patients were referred to a genetic consultant and informed about the possibility of their male offspring having the same subfertility problem if ICSI treatment was selected. Options such as sex selection by PGD or artificial insemination by donor (AID) were provided to the patients during the genetic counseling.

Origin of sperm

Semen samples were collected by masturbation after 3–5 days of abstinence on the day of oocyte retrieval. The preparation for ICSI was performed following the World Health Organization standard protocol [18].

Protocols for ovarian stimulation and ICSI treatment

A prolonged GnRH agonist (GnRH-a) protocol or a short GnRH agonist protocol was used for pituitary down-regulation. Briefly, GnRH-a was started on day 21–23 in the previous cycle or commenced on day 2 of the treatment cycle in both groups, and ovarian stimulation was performed with urinary and/or recombinant gonadotropins. Prior to administration of the first gonadotropin dose, the total number of antral follicles of 2–8 mm was counted in both ovaries. Ovarian stimulation was started on day 3 with 225 IU daily of rFSH (Gonal-F 75IU; Serono, Italy). Two days prior to administration of GnRH-a, serum FSH, estradiol and progesterone concentrations were assessed and rFSH dose was then adjusted according to a step-up protocol. Patients were evaluated with ultrasonography daily from day 8 until the day of HCG administration. HCG 10,000 IU (Profasi 5000 IU per ampoule; Serono) was administered i.m. when two or more follicles reached 18 mm in diameter and oocyte retrieval was scheduled 36 h later. Two or three embryos were transferred on day 3 according to the patients' age and the rest were kept frozen. According to the Code of Practice for Assisted Reproductive Technology established by the Ministry of Health of the People's Republic of China, the maximum number of

embryos transferred per cycle is three. In the luteal phase, all patients received progesterone supplementation with 60 mg i.m. (progesterone injection 20 mg; Shanghai General Pharmaceutical Company Ltd., China) post-oocyte retrieval. To assess treatment outcomes, serum β -HCG was measured at day 14 post-transfer and repeated 7 days later. A rise in serum β -HCG (>30 IU/l) indicated pregnancy. A clinical pregnancy was confirmed by ultrasound observation of fetal cardiac activity 30 days after embryo transfer.

Definitions

Embryo quality: The excellent embryo had at least 5 cells, embryos with $<30\%$ fragmentation in Day 3, derived from 2 PN.

A biochemical pregnancy is defined as the serum HCG positive which performed 14 days after the embryo transfer.

A clinical pregnancy is sonographic confirmation of fetal cardiac 30 days after embryo transfer.

Delivery: the birth of a live born or stillborn infant.

Early miscarriage (<12 week): pregnancy loss before 12 completed weeks of gestation.

Late miscarriage (>12 week): pregnancy loss after 12 weeks and before 28 weeks of gestation.

Ectopic pregnancy refers to the implantation of a fertilized ovum outside the uterine corpus. The most common sites of implantation are the fallopian tubes, with less frequent occurrence in the ovaries, cervix, and other abdominal areas. The diagnosis of an ectopic pregnancy was confirmed by laparoscopy.

Premature birth: birth between 28 and 37 weeks of gestation.

Low birth weight (LBW): an infant weighing less than 2,500 g at birth.

Birth defect: any congenital malformation defined in the International Classification of Disease (Chapter 17 in ICD-10).

Statistical analysis

Data are presented as the mean $\pm$ standard deviation (SD), median, and percentage. Continuous variables were compared using Student's *t*-test for normally distributed variables and Wilcoxon's rank sum test for abnormally distributed variables. The Pearson's chi-square test or Fisher's exact test was used to compare categorical variables. All statistical calculations and analysis were carried out using SPSS 16.0 software. Values of $P < 0.05$ were considered to be statistically significant.

Results

Baseline characteristics of Y-deleted (patients with Y chromosome AZFc microdeletion) and control groups are shown in Table 2. There was no statistically significant difference

Table 2 General data of Y-deleted and control groups

Variables	Y-deleted	Control	<i>t/Z</i>	<i>P</i>
Semen volume	2.80±1.573	2.70±1.571	0.544	0.588
Endometrial thickness (mm)	10.22±1.446	10.32±1.595	−0.554	0.580
Transferred embryos ^a	2	2	−0.711	0.477
Mean sperm concentration ^a	1.10	10.55	−8.739	0.000
Sperm motility a + b (%) ^a	5.57	12.41	−2.234	0.025
Newborn weight (singleton)	3513.04±455.825	3435.71±539.709	−0.515	0.609
Newborn weight (twin)	2449.17±495.259	2560.71±369.083	0.657	0.517
Newborn height (singleton)	50.47±1.463	50.25±1.215	−0.428	0.672
Newborn height (twin)	47.67±1.862	48.10±1.729	0.472	0.644

^aAbnormally distributed continuous variables, Wilcoxon test was used

in semen volume, endometrial thickness on HCG injection day, transferred embryos, and newborn body height and weight of singleton or twin babies between group Y-deleted and control patients. The mean sperm concentration, and sperm motility was lower in the Y-deleted group compared with controls, and the difference was of statistical significance ($P<0.05$).

The outcomes of ICSI treatment in group Y-deleted and control patients are shown in Table 3. Fertilization rate in the control group was 67.8 %, significantly higher than that in the Y-deleted group, which was 61.8 % ($P<0.05$). The rate of cleaved embryos in the Y-deleted group was 94.0 %, higher than the 88.1 % in the control group and the difference was of statistical significance ($P<0.05$). 14 boys and 21 girls were born in the Y-deleted group, 15 boys and 22 girls were born in the control group. None of the newborns had any birth defects. There were no statistically significant

differences between the two groups in terms of good embryo rate, embryo implantation rate, biochemical pregnancy rate, clinical pregnancy rate, delivery rate, early miscarriage rate (<12 week), late miscarriage rate (>12 week), ectopic pregnancy rate, preterm birth rate, low birth weight rate, male/female baby, and birth defect rate.

Karyotype analysis was available for 124 (86.7 %) of the 143 male AZFc microdeletion patients. Abnormal karyotypes were found in 9 cases (7.26 %). All abnormal karyotypes, with two exceptions, were extreme oligozoospermia (lower than $1 \times 10^6/\text{ml}$). There were 3 with 46,X,Yqh+ karyotype polymorphism. The results and details of patients' karyotypes are summarized in Table 4. Karyotype analysis of control men whose is available was all 46, XY.

No differences were seen in the AZFc-deleted oligospermic group compared with randomly selected Y-intact men: FSH, 9.58 versus 7.65 mIU/ml; LH, 5.47 versus

Table 3 Clinical outcomes of Y-deleted and control groups

Variables	Y-deleted	Control	χ^2	<i>P</i>
Fertilized oocyte rate ^a	61.8 (1133/1834)	67.8 (1153/1700)	14.118	0.000
Cleaved embryos rate ^b	94.0 (1203/1280)	88.1 (1144/1299)	27.569	0.000
Good embryo rate	73.4 (832/1133)	72.7 (838/1153)	0.165	0.685
Embryo implantation rate ^c	17.6 (42/239)	19.6 (48/245)	0.326	0.568
Biochemical pregnancy rate ^d	43.6 (48/110)	40.2 (47/117)	0.280	0.597
Clinical pregnancy rate ^d	32.7 (36/110)	33.3 (39/117)	0.009	0.923
Delivery rate	26.4 (29/110)	25.6 (30/117)	0.015	0.901
Early miscarriage rate (<12 week)	8.3 (3/36)	10.3 (4/39)	0.000	1.000
Late miscarriage rate (>12 week)	0	2.6 (1/39)	–	1.000 ^f
Ectopic pregnancy rate	11.1 (4/36)	10.3 (4/39)	0.000	1.000
Preterm birth rate (singleton)	4.3 (1/23)	4.3 (1/23)	–	1.000 ^f
Preterm birth rate (twin)	66.7 (8/12)	42.9 (6/14)	–	0.267 ^f
Low weight birth rate (singleton)	0	4.3 (1/23)	–	1.000 ^f
Low weight birth rate (twin)	50.0 (6/12)	42.9 (6/14)	–	1.000 ^f
Male/female baby ^e	0.67 (14/21)	0.68 (15/22)	0.002	0.963
Birth defect rate	0	0	–	–

^aPercentage of the total number of metaphase II oocytes injected

^bPercentage of the total number of embryos

^cDefined as the ratio between the number of gestational sacs and the number of transferred embryos

^dDefined as the ratio between the number of biochemical/clinical pregnancies and the number of embryo transfers

^eFisher's exact test is used

Table 4 Karyotype analysis of Y chromosome AZFc microdeletion infertile men

Karyotype	<i>n</i>	%
46, XY	115	80.4
46, X,Yqh+	3	2.1
45, XY,rob(13;22)	1	0.7
46, X,Yqh-	1	0.7
46, XY,del(Y)(q12)	1	0.7
46, XY,inv(Y) (p11q11)	1	0.7
46, XY,Y > 18	1	0.7
mos47, XXY[3]/46,XY[97]	1	0.7
N/A	19	13.3
Total	143	100.0

N/A not available

5.32 mIU/ml; testosterone, 16.2 versus 15.05 ng/dl; PRL 14.27 versus 7.76 ng/ml, respectively. Testicular volume of males with Y chromosome AZFc microdeletion and those with an intact Y chromosome showed no statistically significant difference (average $\pm$ SD volumes were 12.21 ± 3.711 ml and 15.00 ± 5.00 ml, respectively) (Table 5).

Discussion

Microdeletion occurs most frequently in the AZF region located in the long arm of the Y chromosome. The frequency of microdeletion varies between 1 % [19] and 55 % [20] worldwide. AZFc, the most frequently deleted region of the Y chromosome in infertile males, is a de-novo microdeletion found in one out of 4,000 males [21]. Men with Y chromosome microdeletions and severe oligozoospermia or azoospermia are sometimes able to reproduce via assisted reproductive technology such as ICSI. The treatment outcomes of infertile men who suffered from Y chromosome microdeletion has been controversial. Some studies have demonstrated comparable treatment outcomes in patients with or without Y chromosome microdeletions [22, 23]. However, other studies showed different outcomes, particularly in fertilization rate and quality of embryos between these two groups. To date, there has been

no study on the birth defects in the offspring of Y chromosome microdeletion patients. Here, we selected the region of AZFc and STS alternatively, based on the EAA recommendations. There Success rates after ICSI are unrelated to sperm parameters, though the fertilization rate may be affected by sperm and/or oocyte factors in IVF. Despite the fact that mean sperm concentration and sperm motility are different between the Y-deleted and the control groups, there is no difference in clinical pregnancy in these two groups. Our results confirmed the results of previous studies [24, 25] in a larger cohort.

Recently, several investigators have shown that embryo characteristics following ICSI using sperm obtained from men with Y chromosome microdeletions were not adversely affected by the deletion [4, 9, 10, 26, 27]. ICSI brings new hope for severe oligozoospermic men who wish to conceive children with their own gametes [28]. High fertilization and pregnancy rates have been achieved in men with severe oligozoospermia [29]. Thus, ICSI can be applied to men with Y microdeletion, yet with the risk of passing their deleted Y chromosome to their sons [11, 12]. Studies have shown that men with Y chromosome microdeletion could obtain their offspring through ICSI with fertilization rate and pregnant rate comparable to men without microdeletion. Our study shows that Y chromosome AZFc microdeletion group has a reduced fertilization rate and an increased cleaved embryo rate compared with men without microdeletion and the difference is statistically significant. The reduced fertilization rate is consistent with the result reported by van Golde RJ et al. [9]. This result suggests that Y chromosome AZFc microdeletion would affect the fertilization rate of ICSI treatment, leading to reduced fertilizing ability of sperm, and therefore an inability to activate oocytes. The higher cleaved rate revealed in the Y chromosome AZFc microdeletion group suggests that once successful fertilization is achieved, embryo development will not be affected by the microdeletion. Our study showed that a mild decline in sperm concentration was found in two cases with AZFc deletion, and there are two similar reports available [30, 31]. Because AZFc microdeletion may be associated with a decline in sperm production over time [32], semen cryopreservation in early adulthood could be proposed. However, this differs with the report by Oates et al. [27] who concluded that sperm production appeared stable over time. Table 6 shows that

Table 5 Reproductive hormones and testicular volume in oligozoospermic men with Y chromosome AZFc microdeletions and control patients

Variables	Y-deleted	Control	<i>t/Z</i>	<i>P</i>
FSH (mIU/ml)	9.58 $\pm$ 5.812	7.65 $\pm$ 4.460	-0.655	0.514
LH (mIU/ml)	5.47 $\pm$ 3.220	5.32 $\pm$ 2.821	-0.092	0.927
T (nmol/L)	16.02 $\pm$ 21.327	15.05 $\pm$ 3.345	-0.090	0.928
PRL (ng/ml)	14.27 $\pm$ 24.186	7.76 $\pm$ 2.276	-0.536	0.593
Mean testicular volume (ml)	12.21 $\pm$ 3.711	15.00 $\pm$ 5.000	1.630	0.106

Table 6 Outcome of ICSI cycles in couples with Y chromosome microdeletion

Study	Year	ICSI cycles	Clinical pregnancy rate	Delivery rate	Boys born	Girls born
Mulhall et al.	1997	6	1 (16.6)	1 (16.6)	0	2
van Golde RJ et al.	2001	19	3 (15.8)	3 (15.8)	0	5
Oates et al.	2002	48	13 (27.1)	13 (27.1)	10	8
Choi et al.	2004	27	9 (33.3)	7 (25.9)	6	1
Kihaile et al.	2004	8	3	—	—	—
Stouffs et al.	2005	40	7 (17.5)	3 (7.5)	Unknown	
Patrat et al.	2010	42	14 (30.9)	8 (19.1)	5	9
Wu et al.	2011	56	21 (37.5)	20 (35.7)	15	9
Present study	2012	143	36 (32.7)	29 (26.4)	14	21

a have few studies precisely analyzed how Y chromosome microdeletion influences fertilization rate, embryo quality, and pregnancy rates [9, 10, 12, 26, 27, 33].

Our study showed no significant differences in embryo implantation, biochemical pregnancy, clinical pregnancy, ectopic pregnancy, delivery, early miscarriage, late miscarriage and ratio of male/female babies in men with or without microdeletion, suggesting that Y chromosome AZFc microdeletion does not affect the outcomes of ICSI treatment. The studies of Patrat et al. [33], Kihaile et al. [10] and Choi et al. [4] have shown similar results. Bellver [34] indicated that Y chromosome AZFc microdeletions do not seem to be related to recurrent spontaneous abortion (RSA) of unknown origin. There is no significant difference found in newborn body height, weight and low birth weight rate. None of the newborns in either group (35 in Y-deleted group and 37 in control group) had any congenital defects. However, a birth defect rate of 2.59 % (41 with birth defects out of 1,585 newborns after ICSI treatment) was found in our Center from 2005 to 2009. The zero birth defect in the current study is very likely due to the relatively small case number of newborns and therefore is not representative. Mau [35] reported that children born after ICSI appear to have a higher risk of urogenital malformations, especially hypospadias, which may be related to paternal subfertility. Funke implied ICSI is a specific risk factor for hypospadias [36], however, a meta-analysis demonstrates no significantly increased risks of cardiovascular defects, musculoskeletal defects, hypospadias, neural tube defects, or oral clefts after ICSI [37]. Therefore, long term follow-up on a larger cohort of couples with AZFc microdeletion in the Y chromosome is needed to explore the possible role of such deletions on the birth defect outcomes of ICSI treatment.

Prior studies have found that all male offspring inherit their fathers' Y chromosome microdeletion [27, 38]. Although the inheritance of the AZFc microdeletion does not seem to have any somatic effect on the children, the concern remains that the passage of microdeletions from father to

son will perpetuate the adverse effects on future male fertility. Aside from expected spermatogenic deficiency in male offspring, couples can be reassured that the children would be expected to be healthy. No dramatic results, especially somatic effects on children, have been shown in the follow-up of children conceived by ICSI in men with Y chromosome AZFc microdeletion. Both sons and daughters are somatically healthy. In addition, at present, no increased risk of somatic effects in the offspring of men with Y chromosome microdeletion has been described [12, 27, 33]. Presently, PGD gives these couples the possibility to select female embryos for transfer. Our main concern is how to help and fully counsel couples to make their own well-informed choice to become parents. What are the possible risks encountered by the patients: male infertility in the offspring, additional risk? Indeed, can we consider male infertility as a severe handicap, justifying sex selection [33]? Extensive counseling should be provided to all couples before and after genetic testing [23], especially for the couples that decide to pursue PGD. The PGD procedure has a high economic burden on patients and more importantly, the risks to offspring health are still debatable. Evidence from animal study indicates that PGD renders an increased risk of neurodegenerative disorders in offspring following blastomere biopsy [39].

Here, we have provided the latest evidence, with the largest sample size to date, of the possible influence of male Y chromosome AZFc microdeletion on the fertilization rate and quality of embryo as well as other clinic and laboratory parameters, particularly newborn characteristics, and have indicated no difference between patients with or without Y deletion. We have demonstrated that the embryo outcomes are comparable between patients with or without Y chromosome AZFc microdeletion once sperm is available for ICSI. This result suggests that Y chromosome AZFc microdeletion confers no adverse effects on the outcomes of ICSI. The possible negative impact and high expense of PGD may be avoided for Y chromosome AZFc microdeletion patients for ICSI in light of this result.

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