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Andrology

A Comparative Study of Y Chromosome Microdeletions in Infertile Males From Two Chinese Populations

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***Purpose*:** To compare the prevalence and type of Y-microdeletions in Hong Kong and Shanghai men with severe male-factor infertility.
***Methods*:** Seven Y-linked sequence tagged site (STS) primers and seven gene-specific primers were screened in 293 infertile males (139 from Hong Kong and 154 from Shanghai) and 161 fertile men (61 from Hong Kong and 100 from Shanghai). Serum FSH, LH, and testosterone levels were also measured in these men.
***Results*:** The incidence of Yq microdeletions in nonobstructive azoospermic men from Hong Kong (8.5%) and Shanghai (6%) was similar. Yq microdeletions were observed in severe oligospermic patients (8.5%) from Hong Kong but not from Shanghai. Among the 9 Hong Kong men with Y-microdeletions, 8 had AZFc deletion and one had AZFb deletion. In contrast, 6 of 9 men from Shanghai with Y-microdeletions had AZFb deletion. The incidence of AZFb deletion among Y-microdeleted men was statistically different between the two populations. Two of the men with AZFb deletion also had AZFa and AZFc deletions.
***Conclusions*:** Regional variations in the type of Y-microdeletion existed between Hong Kong and Shanghai infertile males.

KEY WORDS: AZF; male infertility; Y chromosome microdeletion.

INTRODUCTION

Numerous studies demonstrated that male infertility is associated with a high incidence of Y-chromosome microdeletions, ranging from 3 to 28% of oligospermic and nonobstructive azoospermic men (1,2). These microdeletions were mapped to three nonoverlapping regions, named as azoospermic factor (AZF) AZFa, AZFb, and AZFc, at Yq11.22-23 of Y chromosome (2,3).

There are a number of genes present within each AZF region, e.g. *DAZ* (Deleted in Azoospermia) (4), *RBM* (RNA binding motif) (5), *SPGY1* (spermatogenesis gene on the Y), *BPY2* (basic protein Y2), and *CDY* (chromodomain Y) (6). Some of these genes belong to multicopy gene family (2,7). The *RBM* gene has about 30 copies on both arms of the Y chromosome (7). The DAZ even has a homolog in the autosome known as *DAZLA* (DAZ-like autosomal gene) (8). It is unclear which of these genes is responsible for male infertility. Some of these gene products, e.g. *DAZ* and *RBM*, are expressed specifically in the testis and are confined to the male germ cells (9,10). Their physiological functions are yet to be determined though they are believed to play a role in male germ cell development.

Reports on the prevalence of Y chromosome microdeletions in Asian infertile populations are few

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(11–15). Three of these reports include Chinese populations. Liow *et al.* (11) studied microdeletions in two AZF regions (AZFb and AZFc) in a mixed population consisting of 64% Chinese and 36% of other undefined ethnic origins. We also recently reported our local data consisting of a mixed population of Chinese and Caucasian (15). There are only two studies on pure Chinese population from Taiwan (12,14). These two studies suggested that the microdeletions in AZFa and AZFb are relatively common in Taiwan.

Ethnic differences in genetic abnormalities are well known. Chinese population consists of people with different ethnic origins. The prevalence of Y-microdeletion in different parts of China is unknown. The objective of this study is to compare the frequency and type of Y-microdeletion between two different Chinese ethnic groups, one from Hong Kong and the other one from Shanghai.

MATERIALS AND METHODS

Patient Selection

The Ethics Committee, Faculty of Medicine, The University of Hong Kong approved this study. Semen analysis was performed according to the World Health Organization guidelines (16). One hundred and thirty-nine infertile males from Hong Kong and 154 infertile males from Shanghai participated in this study. All of them were Chinese.

In Hong Kong, the infertile males consisted of 59 (41.5%) nonobstructive azoospermic men, 47 (33.8%) severe oligospermic men (sperm count <5 M/mL), and 33 (23.2%) obstructive azoospermic men, including 22 congenital (congenital absence of the vas deferens) and 11 acquired (postsurgery or infection) obstruction of the seminal tract. Their ages ranged from 25 to 50 years (36 ± 4.8 years; mean $\pm$ SD). These men attended the assisted reproduction program at Queen Mary Hospital, Hong Kong.

In Shanghai, the infertile males consisted of 135 (87.7%) nonobstructive azoospermic men, 19 (12.3%) severe oligospermic men (sperm count <5 M/mL). Their ages ranged from 22 to 45 years (30 ± 4.5 years; mean $\pm$ SD). These patients attended the male infertility clinics in Shanghai Institute of Family Planning Technical Instruction, Shanghai.

Sixty-one normal healthy Chinese men of proven fertility from Hong Kong and 100 from Shanghai were also recruited as controls. Their ages ranged from 17 to 54 years (27.9 ± 8.3; mean $\pm$ SD). Four healthy women were used as negative controls when Y-specific STS or gene markers were used in multiplex PCR.

Hormone Assay

Serum concentrations of follicle stimulating hormone (FSH) and luteinizing hormone (LH) were measured in each subject by commercially available immunoassay using chemiluminescence (Chiron Diagnostics Corporation, East Walpole, USA). Serum concentration of testosterone was determined by radioimmunoassay using ^{125}I-labelled testosterone (Diagnostic Products Corporation, Los Angeles, USA).

Sample Preparation and PCR Amplification

All tests were performed on genomic DNA extracted from peripheral blood lymphocytes using a DNA isolation kit for mammalian blood (Roche Molecular Biochemicals, Mannheim, Germany). Table I shows the primer sequence, the order and the approximate localization of the Y-specific sequence-tagged sites (STS) primers, and the expected size of the PCR products of all Y-specific STS and the AZF-candidate genes on chromosome Y used in this study. All Y-specific STS and gene-specific primers were synthesized by a commercial company (Life Technologies, Inc., Gaithersburg, MD) using published sequence-tagged sites or gene sequence.

Each DNA sample was tested with multiplex PCR using six primer pairs of Y-specific STS that amplified in subintervals 5 and 6 of Yq11. These six primer pairs include sY84, sY86, sY127, sY132, sY254, and sY255. To prevent false negative scoring, each PCR reaction included either one of the following internal control primers: sY72 as a control marker for chromosome Y and globin as marker for quality of DNA preparation and for exclusion of possible PCR failure. In each PCR reaction, a DNA sample from a normal fertile control was included as positive control. A female genomic DNA sample and a water blank were also used as negative controls to verify that there was no cross contamination.

PCR was carried out in a 25 μL reaction volume containing 100–200 ng of human genomic DNA, 1.5–2.0 mM $MgCl_2$, dNTP (200–400 μM each of dTTP, dCTP, dGTP, dATP), primers (10–200 pmoles each), 1$\times$ PCR buffer, and 1 U *Taq polymerase*. The PCR buffer, dNTP mix, and *Taq polymerase* were all obtained from Roche Molecular Biochemicals. Thermocycling consisted of an initial denaturation of 4 min at 94°C, followed by 35 cycles of 30 s at 94°C, 30 s at 49–50°C, and 1 min at 72°C. PCR products were separated

Table I. Sequence-Tagged Sites (STS) and Gene-Specific Primer Sequences for Deletion Analysis

sY number/gene	Primer	Size (bp)
Chromosome Y		
AZFa region (interval 5A-D)		
sY84 (5C)	5′AGAAGGGTCTGAAAGCAGGT3′ 5′GCCTACTACCTGGAGGCTTC3′	326
sY86 (5C)	5′GTGACACACAGACTATGCTTC3′ 5′ACACACAGAGGGACAACCCT3′	320
AZFb region (interval 5M-6B)		
sY127 (5Q)	5′GGCTCACAAACGAAAAGAAA3′ 5′CTGCAGGCAGTAATAAGGGA3′	274
sY132 (6A)	5′GAGAGTCATAATGCCGACGT3′ 5′TGGTCTCAGGAAGTTTTTGC3′	159
RBM1 (6B)	5′CCTCTCTCCACAAAACCAACA 3′ 5′ATGCACTTCAGAGATACGG 3′	800
RBM2 (6B)	5′AGAGATGCACTTCAGAGG 3′ 5′CCTCTCTCCACAAAACCAACA 3′	800
AZFc region (interval 6C-6F)		
sY254 (6D)	5′GGGTGTTACCAGAAGGCAAA3′ 5′GAACCGTATCTACCAAAGCAGC3′	350
sY255 (6D)	5′GTTACAGGATTCGGCGTGAT3′ 5′CTCGTCATGTGCAGCCAC3′	126
DAZ (6D)	5′TAGGTTTCAGTGTTTGGATTCCG3′ 5′GGAAGCTGCTTTGGTAGATAC3′	1300
SPGY1 (6D)	5′ACAATTTTGATAGTCTGAACACAAGC 3′ 5′TTTCACATACAGCCATTAAGTTTAGC 3′	460
BPY2 (6E)	5′ CAGCGTATCATAGAAAATGT3′ 5′AGTACTTTATTTGCAGGTTCTG3′	142
CDY1 (6F)	5′GGCGAAAGCTGACAGCAA3′ 5′GGGTGAAAGTTCCAGTCA3′	75
Outside AZF region		
sY72 (4A)	5′CTTGTGTGACATTCCCTCCA3′ 5′ATGTTTGTGGGTCATTCAGG3′	100
CDY2 (5L)	5′GACCACAAGAAAACTGTGAG3′ 5′GATCTGCTGCAATAGGGTC3′	64

Note. All STS primer sequences were obtained from Genbank (http://gdbwww.gdb.org/). Gene-specific primer sequences were used as previously described (2,8,17). The order of the STS and the approximate localization of AZFa, b, and c regions have been previously defined (1,4,18,19). The numbers within the brackets represent the interval number.

on 2–3% agarose gels and visualized by staining with ethidium bromide. A sample was considered positive for the given STS markers and gene-specific primers when the PCR product of the expected size was present and it was considered negative or deleted if a product of the expected size was not amplified after three successive PCR reactions. All samples were analysed in a blinded fashion, without knowledge of the patient's clinical details. Samples from Shanghai with suspected Y-chromosome microdeletions were sent to Hong Kong. Y chromosome microdeletion analysis of these samples was repeated to confirm the diagnosis.

Statistical Analysis

SPSS for Windows was used to analyse the data. Two-tailed Fisher's exact test was used to determine (a) the observed incidences of Y chromosome microdeletions between the infertile men and the fertile controls and between the infertile men from Hong Kong and those from Shanghai; (b) the observed incidence of AZFb deletions between Hong Kong and Shanghai men with Yq microdeletions. Mann-Whitney U test was used to assess the significance of the difference in hormonal levels between the infertile group with abnormalities and the fertile control group. $p < 0.05$ was considered statistically significant.

RESULTS

Incidence of Y Chromosome Microdeletions

In a total of 59 nonobstructive azoospermic and 47 severe oligospermic patients from Hong Kong, Y microdeletions were detected in 8.5% (5/59)

Table II. Clinical Characteristics of Patients With Y Chromosome Microdeletions

Patient	Age (years)	FSH (IU/L)	LH (IU/L)	Testosterone (nmol/L)	Deletion region
Hong Kong					
Nonobstructive azoospermia					
s17	36	18.7[a]	3.9	12.1	AZFc
s45	34	28.1[a]	11.5	11.5	AZFc
s54	36	18.5[a]	4.8	13.7	AZFc
s117	33	6.3	3.8	14.0	AZFb
01M028	30	nd	nd	nd	AZFc
Severe oligospermia					
s7	36	6.7	2.4[b]	11.3	AZFc
s89	38	9.6	3.1	12.3	AZFc
s102	34	7.1	3.8	22.7	AZFc
01M021	38	nd	nd	nd	AZFc
Shanghai					
Nonobstructive azoospermia					
SH1	27	16.0[a]	19.6[a]	57.9[a]	AZFb
SH4	34	21.1[a]	5.4	15.6	AZFb&c
SH9	27	4.4	4.4	9.4	AZFc
SH12	39	39.4[a]	30.2[a]	57.9[a]	AZFb&c
SH18	25	6.8	3.4	26.0	AZFc
SH92	30	6.4	5.0	0.1[b]	AZFc
SH113	23	11.4	11.7	0.03[b]	AZFb&c
SH131	25	22.0[a]	17.8[a]	13.5	AZFa, b&c
SH151	34	35.4[a]	18.8[a]	19.8	AZFa, b&c

Note. Abbreviations: FSH = follicle stimulating hormone; LH = luteinizing hormone; nd = not determined. For Hong Kong—Normal FSH: 1.5–10 IU/L; Normal LH: 3.0–13.5 IU/L; Normal Testosterone: 10–35 nmol/L; For Shanghai—Normal FSH: 1.5–11.5 IU/L; Normal LH 1.1–8.2 IU/L; Normal Testosterone: 7–36 nmol/L.
[a] Above normal range.
[b] Below normal range.

nonobstructive azoospermic and 8.5% (4/47) severe oligospermic men. No Yq microdeletions were found in 61 fertile controls and 33 infertile patients with obstructive azoospermia from Hong Kong. The clinical data of patients with Y chromosome microdeletions are summarized in Table II. Using the two-tailed Fisher's exact test, the observed incidence of Y chromosome microdeletions in patients with severe oligospermia and nonobstructive azoospermia are significantly different ($p < 0.05$) from that in the fertile control group.

A total of 6.7% (9/135) of the nonobstructive azoospermic men from Shanghai had Y-microdeletions. No Y chromosome microdeletions were detected in the 19 severe oligospermic men and fertile controls. The incidence of Y-microdeletion in the nonobstructive azoospermic was significantly higher than that of the control ($p < 0.05$).

Using the two-tailed Fisher's exact test, the observed incidence of Y chromosome microdeletions was not significant between the two nonobstructive azoospermic populations. Unfortunately, close male relatives of the Y-deleted patients were unavailable for further Y-microdeletions analysis. Therefore, we could not determine the origin of these deletions.

Type of Y Chromosome Microdeletions

Of the nine microdeletions observed in the infertile patients from Hong Kong, two different patterns of deletion were identified. The first was localized within the AZFb region (between sY127 and sY132; subinterval 5Q–6A) in one azoospermic patient (Fig. 1(A)). Both *RBM1* and *RBM2* were deleted in this patient. The second and the most frequently deleted region was interstitial deletion localized within the AZFc region and was detected in eight infertile patients: four severe oligospermic and four nonobstructive azoospermic patients. All eight microdeletions resulted in the loss of genes localized in subinterval 6D–6F within the AZFc region (Table II).

In contrast, five different patterns of deletion were found among the nine microdeletions observed in patients from Shanghai (Fig. 2). AZFb deletions were more frequently detected in this group of patients from Shanghai (Fig. 1(B)). Gene-specific markers localized within the AZFb (*RBM1* and *RBM2*) and

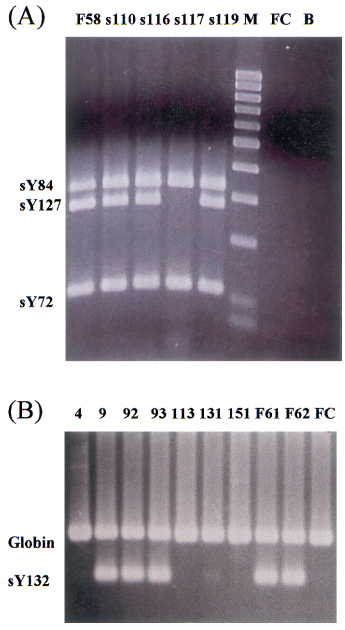

Fig. 1. (A) Polymerase chain reaction products were amplified AZFa (sY84), and AZFb (sY127) regions using oligonucleotide primers. AZFb deletion (sY127) was observed in only one patient s117 from Hong Kong. No deletions were detected in the remaining samples and the fertile control (F58). sY 72 was used as a control marker. Female genomic DNA (FC) and water (B) was used as the negative controls. Molecular weight marker (M) was shown in the third lane from right. PCR products separated by electrophorosis in 3.0% agarose gel and visualized by ethidium bromide staining. (B) Polymerase chain reaction products were amplified in the AZFb region (sY132). AZFb deletion was detected in patients 4, 113, 131, and 151 from Shanghai. No deletions were detected in the remaining samples and the two fertile controls (F61 and F62). Globin was used as a control marker. Female genomic DNA (FC) was used as the negative control for sY132. PCR products were separated by electrophorosis in 3.0% agarose gel and visualized by ethidium bromide staining.

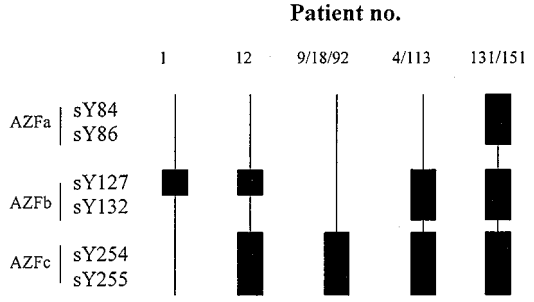

Fig. 2. Summary of the patterns of Yq microdeletions in infertile patients from Shanghai. Map locations of AZFa, AZFb, and AZFc regions have been previously defined. The position of STS markers used are indicated on the left. Solid black boxes represent the absence of STS marker(s).

AZFc (*DAZ*, *SPGY1*, *BPY2*, and *CDY1*) regions were absent in most samples with AZFb or/and AZFc deletion. However, patients SH1 and SH12 with sY127 deletion had only *RBM1* but not *RBM2* deletion. Patient SH113 with sY254 and sY255 deletions did not show CDY1 deletion in subinterval 6F.

Among the nine men from Hong Kong with Y-microdeletions, only one (11.1%) had AZFb deletion. The others had AZFc deletion. No AZFa deletions were detected. In contrast, 6 of 9 men from Shanghai with Y-microdeletion had AZFb deletion (66.7%). Two of the men (SH 131 and SH 151) with AZFb deletion also had AZFa and AZFc deletions. The difference in the incidence of AZFb deletion among Y-microdeleted males in Shanghai and Hong Kong was statistically significant ($p < 0.05$).

Serum Hormone Levels and Y Chromosome Microdeletions

Serum FSH concentration was elevated in the three nonobstructive azoospermic patients from Hong Kong (s17, s45, s54) with AZFc deletion (Table II). This increase was neither observed in the nonobstructive azoospermic man (s117) with AZFb deletion nor in the severe oligospermic patients (s7, s89, s102) with AZFc deletions. Serum FSH concentrations in majority of the infertile patients from Shanghai with AZFb or AZFb and c or AZFa and b and c deletions were increased but not in patients with AZFc deletion only. The testosterone level was normal in seven of the Hong Kong men with Y-chromosome deletion but the other two Hong Kong men with microdeletions had not been determined. The testosterone levels of the Shanghai men were variable, above the normal for two and below the normal for two.

Table III compares the hormonal parameters of infertile patients with and without Y microdeletions with that of the normal fertile controls. In the nonobstructive azoospermic group from Hong Kong, the median serum LH and testosterone concentrations were all within the normal range in both patients with and without Y microdeletions. However, the median FSH concentrations in infertile men with and without Y microdeletions were significantly higher than those in the normal fertile control group ($p < 0.05$). In the nonobstructive azoospermic group from Shanghai, the serum FSH in infertile men without deletion, but not in those with deletion, was significantly higher than in the control group. The median serum FSH, LH, and testosterone concentrations in severe oligospermic patients from Hong Kong and

Table III. Hormonal Parameters of Infertile Patients With and Without Y Microdeletion Compared With Normal Fertile Controls

	FSH	LH	Testosterone
Nonobstructive azoospermia			
Hong Kong			
Without deletion	25.4[a] (4.0–65.6)	9.6 (2.1–44.0)	11.3 (0.1–3.0)
With deletion	18.6[a] (4.6–28.1)	4.4 (3.8–11.5)	12.9 (11.5–14.0)
Shanghai			
Without deletion	11.2[a] (0.2–63.0)	7.5 (0.2–40.7)	15.9 (0.3–58.9)
With deletion	9.0 (4.3–16.0)	8.8 (3.4–18.6)	28.4 (9.4–58.2)
Severe oligospermia			
Hong Kong			
Without deletion	6.3 (2.5–36.2)	3.7 (1.5–13.5)	12.9 (3.8–33.4)
With deletion	7.1 (6.7–9.6)	3.1 (2.4–3.8)	12.3 (11.3–22.7)
Shanghai			
Without deletion	6.5 (0.2–19.3)	4.4 (0.2–11.8)	16.9 (3.1–73.2)
Control			
Hong Kong	4.3 (1.7–13.4)	3.6 (1.3–15.4)	14.6 (7.5–53.1)
Shanghai	3.4 (0.2–12.6)	3.2 (0.2–22.2)	18.7 (5.2–79.4)

Note. All values are expressed in median (range). For Hong Kong—Normal FSH: 1.5–10 IU/L; Normal LH: 3.0–13.5 IU/L; Normal Testosterone: 10–35 nmol/L; For Shanghai—Normal FSH: 1.5–11.5 IU/L; Normal LH: 1.1–8.2 IU/L; Normal Testosterone: 7–36 nmol/L; Abbreviations: FSH = follicle stimulating hormone; LH = luteinizing hormone.
[a] Significantly higher than normal fertile control group ($p < 0.05$).

Shanghai with or without Y microdeletion were not different from the control.

DISCUSSION

There have been many reports on the type and frequency of Y chromosome microdeletion in infertile patients in the Caucasian population. Similar reports on Chinese population are scarce. In this study, the prevalence of Y microdeletions examined in nonobstructive azoospermic men from Hong Kong and Shanghai was 8.5% (5/59) and 6.7% (9/135), respectively. These frequencies are in accordance with the deletion frequencies (3.5–20%) reported in other Asian populations (Table IV). The absence of Yq deletions in all 19 severe oligospermic men from Shanghai could be due to small sample size in this study. The most interesting finding in this study is the difference observed in the type of Yq microdeletion between the Hong Kong and Shanghai Chinese populations. The most prevalent type of Yq microdeletion in Hong Kong is within the AZFc region, whereas the deletion pattern in Shanghai is more variable (Fig. 2).

Table IV. Summary of the Incidence of Y Microdeletion in Asian Populations

Asian populations	Percentage	References
Singaporean		
Azoospermic	3/44 (6.8)	11
Severe oligospermic	3/86 (3.5)	11
Taiwanese		
Azoospermia/severe oligospermia	6/68 (9)	12
Azoospermia	12/134 (9)	14
oligo-asthenospermia	10/86 (11.6)	14
Korean		
Azoospermia	8/40 (20)	13

AZFc deletion is the most frequent microdeletion associated with severe male-factor in men from many populations. Vast majority of Hong Kong Chinese men with Y-microdeletion are deleted in AZFc region only. This is similar to a Singapore study on a predominately Chinese population (11). These are in contrast to the findings in Shanghai where majority of infertile men recruited in this study had AZFb deletion with or without other deletions. Coincidentally, AZFb alone and AZFb & c deletions are found in a fair proportion in Korean (13) and Taiwanese (14) populations. Shanghai, Taiwan, and Korea are in East Asia whereas Hong Kong and Singapore are in Southeast Asia. The differences in the pattern of Yq microdeletions observed in these studies suggest possible regional differences in the genetic causes of male infertility.

In our study and those of others in the Caucasian population, no Yq microdeletion was observed in any of the patients with obstructive azoospermia (20). Congenital bilateral absence of the vas deferens (CBAVD) accounts for 1–2% of obstructive azoospermic men. One recent study has demonstrated cystic fibrosis transmembrane conductance regulator (CFTR) gene mutation in CBAVD patients,

without clinical cystic fibrosis (CF), in Caucasian population (21). Although cystic fibrosis is not a common genetic disease in the Asian population, CFTR gene mutation has been reported in some Asian CF patients (22). Therefore, we cannot exclude the possibility of the presence of CF mutations in our CBAVD patients.

Candidate male infertility genes in the AZFb and AZFc regions have been proposed. Although the precise biological functions of these genes are not clear, some of these genes are testis-specific. Although most AZFb deleted infertile men from Hong Kong and Shanghai had *RBM1* and *RBM2* deletions, two nonobstructive azoospermic men (SH1 and SH12) had only *RBM1* but not *RBM2* deletion, suggesting that *RBM2* may not be the cause of azoospermic condition.

Four genes (*DAZ*, *SPGY1*, *BPY2*, and *CDY1*) have been mapped at different subintervals within the AZFc region. Most subjects from Shanghai and all subjects from Hong Kong with AZFc deletion had total loss of these AZFc candidate genes irrespective of these being azoospermia or severe oligospermia. Although the roles of these genes on spermatogenesis remain unclear, our results indicate that the total loss of these AZFc genes is not sufficient to abolish spermatogenesis completely.

In this study, we demonstrated that there is a significant difference in the pattern of Y chromosome microdeletion in infertile patients in Hong Kong and Shanghai. One of the recruited severe oligospermic subjects (s7) with AZFc deletion underwent assisted reproduction with ICSI resulting in a baby boy carrying the same AZFc deletion as the subject (23). Thus, determination of genetic abnormalities, and proper genetic counselling to infertile couples with severe male-factor before any infertility treatment is important.

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