

AZF microdeletions of the Y chromosome and in vitro fertilization outcome

Janet M. Choi, M.D.,^a Pak Chung, M.D.,^a Lucinda Veeck, M.L.T.,^a Anna Mielnik, M.S.,^a Gianpiero D. Palermo, M.D.,^a and Peter N. Schlegel, M.D.^{b,c}

Weill Medical College of Cornell University, and The Population Council, New York, New York

Objective: To determine whether the presence of a Y microdeletion confers any adverse effects on in vitro fertilization or intracytoplasmic sperm injection (IVF/ICSI) outcome.

Design: Retrospective case-control study.

Setting: Academic infertility center.

Patient(s): A total of 17 patients with Y microdeletions who attempted IVF/ICSI cycles at our center between March 1996 and March 2002 were studied. Study patients were analyzed in two groups: those who underwent testicular sperm extraction (TESE) and those for whom ejaculated sperm was used.

Intervention(s): The two patient study groups were matched to controls treated at the same time who had either nonobstructive severe oligozoospermia or azoospermia with normal Y chromosomes. Controls were matched for age of the female partner, sperm concentration, and number of embryos transferred.

Main Outcome Measure(s): Fertilization and clinical pregnancy rates.

Result(s): Sperm was only obtained from patients with azoospermic factor (AZF)^c microdeletions (and from one patient with a partial AZF^b microdeletion). A trend toward lower fertilization rates in patients with Y microdeletions was noted, which did not reach statistical significance. Clinical pregnancy rates per cycle and per transfer were similar to those for controls.

Conclusion(s): Patients with AZF^c microdeletions seem to have IVF/ICSI outcomes comparable to those of controls with normal Y chromosomes. (Fertil Steril® 2004;81:337-41. ©2004 by American Society for Reproductive Medicine.)

Key Words: Y microdeletions, IVF, ICSI, TESE

Received April 9, 2003;
revised and accepted June
30, 2003.

Reprint requests: Peter N.
Schlegel, M.D., Weill
Medical College of Cornell
University, Department of
Urology, 525 East 68th
Street, New York, New
York 10021 (FAX: 212-746-
8425; E-mail: pnschleg@
med.cornell.edu).

^a Center for Reproductive
Medicine and Infertility,
Weill Medical College of
Cornell University.

^b Department of Urology,
Weill Medical College of
Cornell University.

^c The Population Council,
Rockefeller University, New
York, New York.

0015-0282/04/\$30.00
doi:10.1016/j.fertnstert.2003.
06.030

Male infertility affects up to 50% of couples having trouble with reproduction. Among infertile men, the prevalence of Y microdeletions is approximately 7%, with a range of 1%–35% (1, 2), depending on the patients selected for study and study techniques. Most Y chromosome microdeletions occur on the long arm (q) and are subdivided into three azoospermic factor (AZF) regions: a, b, and c (see Fig. 1). Genes encoded in the AZF regions might be important in spermatogenesis. However, a small portion (up to 2%) of the fertile male population might also harbor very small microdeletions of the Y chromosome (3), likely involving noncoding regions (4).

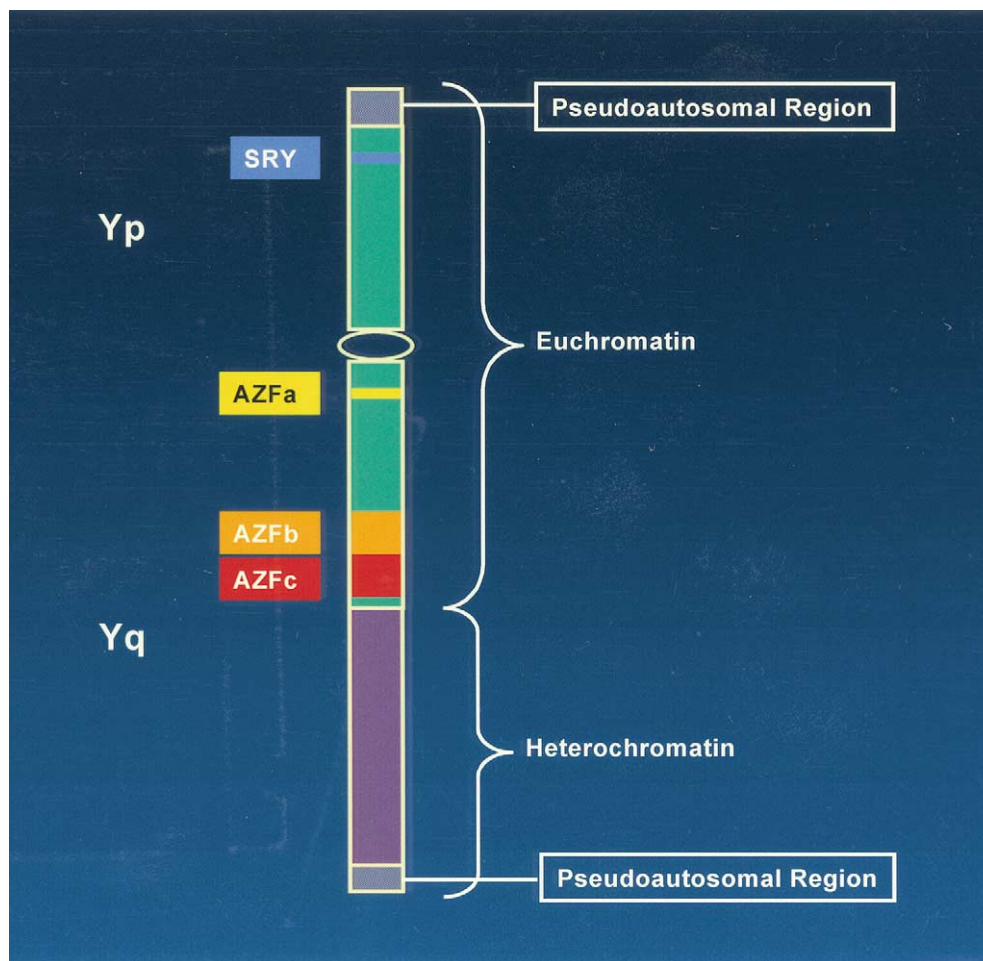
Before the advent of IVF with intracytoplasmic sperm injection (ICSI) and sperm retrieval techniques such as testicular sperm extraction (TESE), many men affected by microdeletions

of the Y chromosome could not reproduce. Recent studies (5–8) have reported successful pregnancies with the use of sperm from Y-microdeleted men and ICSI.

Mulhall et al. (5) compared 8 azoospermic men with AZF^c deletions with 28 controls with normal Y chromosomes; all patients underwent TESE with subsequent ICSI cycles. Although fertilization rates in the AZF^c-deleted group seemed to be lower than in controls (36% vs. 45%), a statistically significant difference was not observed, and pregnancy rates seemed to be similar in both groups. In a study by van Golde et al. (7), 8 AZF^c-deleted men with severe oligozoospermia who underwent ICSI cycles with ejaculated sperm were compared with controls with normal Y chromosomes; fertilization rates in this case were significantly lower in the AZF^c-deleted group (55% vs.

FIGURE 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.



Choi. Y microdeletion and IVF. *Fertil Steril* 2004.

71%, $P < .001$), with poorer embryo quality in the study group as well. Overall pregnancy and delivery rates were not statistically different.

In one of the most recent studies, Oates et al. (8) characterized IVF cycles in 26 couples in which the men were found to have an AZFc microdeletion. The overall fertilization rate was 47%, with a pregnancy rate per cycle of 27%. Little mention has been made in the literature, however, of the reproductive capabilities of men with microdeletions of the AZFa or -b regions (9, 10).

To contribute to the information available on a small but significant proportion of the infertile male population, we reviewed the ICSI outcomes in a group of patients with Y microdeletions in any of the three main regions (AZFa, -b, and -c). This group was further divided between patients with azoospermia requiring TESE and patients with severe

oligozoospermia. In addition, we matched these groups to severely oligozoospermic or azoospermic controls with normal Y chromosomes in an attempt to discern whether there were any significant differences in fertilization and pregnancy rates.

MATERIALS AND METHODS

Approval for this study was obtained from our hospital's institutional review board on human research. A retrospective chart review was then performed on a total of 17 Y-microdeleted men and their partners who attempted ICSI treatment at the Center for Reproductive Medicine and Infertility at the Weill Medical College of Cornell University from March 1996 to March 2002. This group was subdivided into those men who attempted TESE and those who used ejaculate sperm for treatment (see Table 1).

TABLE 1

ICSI outcomes of patients with Y chromosome microdeletions.

Variables	TESE patients		Ejaculate patients	
	Y-deleted	Controls	Y-deleted	Controls
No. of cycles	12	24	15	30
No. of patients	11	23	6	30
Female age (y), mean \pm SD	30.50 \pm 5.06	30.46 \pm 4.15	33.67 \pm 2.49	34.53 \pm 3.02
Sperm obtained/TESE attempts, % (n)	58.3 (7/12)	62.5 (15/24)		
Average sperm concentration ^a			2.46 \times 10 ⁶ /mL	1.98 \times 10 ⁶ /mL
2PN/injected oocytes, % (n)	41.2 (42/102)	52.8 (104/197)	53.7 (80/149)	62.4 (181/290)
No. of ET, mean \pm SD	2.57 \pm 1.05	2.62 \pm 0.62	2.93 \pm 1.03	3.36 \pm 1.0
Clinical pregnancy/cycle, % (n)	33.3 (4/12)	37.5 (9/24)	33.3 (5/15)	46.7 (14/30)
Clinical pregnancy/ET, % (n)	57.1 (4/7)	69.2 (9/13)	35.7 (5/14)	50.0 (15/30)

Note: PN = pronuclei; ET = embryo transfer.

^a Within the TESE group, average sperm numbers were less than 100 total for both case and controls.

Choi. Y microdeletion and IVF. *Fertil Steril* 2004.

A total of 11 Y-microdeleted men underwent 12 attempted TESE procedures for IVF. Of these 11 men, 1 had a microdeletion of the AZFa region, 1 had a microdeletion of the AZFb region, 1 had a partial AZFb microdeletion, 2 had complete microdeletions of both AZFb and -c, and 6 had AZFc deletions (see Table 2). These subjects were matched against 23 men who underwent 24 IVF/TESE cycles at the same time and who had known normal Y chromosomes with nonobstructive azoospermia or severe (<5 million/mL) oligozoospermia. Controls were matched for age of the female partner, average sperm concentration and motility, and number of embryos transferred. Outcomes compared included

sperm obtained per TESE attempt, fertilization rate, clinical pregnancy rate per cycle, and clinical pregnancy rate per embryo transfer.

A total of 7 Y-microdeleted men (all with AZFc deletions) attempted 16 IVF cycles using ejaculate sperm. These patients were matched against 30 controls—men cycling through IVF/ICSI at the same time with severe oligozoospermia and normal Y chromosomes. Matching parameters were the same as for the TESE group. Outcomes compared included fertilization rate, clinical pregnancy rate per cycle, and clinical pregnancy rate per embryo transfer.

TABLE 2

Testicular sperm extraction patients: location of Y microdeletions, biopsy results, and TESE and IVF outcomes.

Patient no.	Y microdeletion	Biopsy (if known)	TESE success	IVF result
1	AZFa	SCO	No sperm	N/A
2	AZFc	95% SCO	No sperm	N/A
3	AZFb + c	SCO	No sperm	N/A
4	AZFc	SCO/MA	+sperm	+pregnancy ^a
5	AZFb + c	MA	No sperm	N/A
6 (2 cycles)	AZFc	N/A	+sperm	No pregnancy
	AZFc	N/A	+sperm	+pregnancy ^b
7	AZFc	N/A	+sperm	Missed abortion
8	AZFb	MA	No sperm	N/A
9	Short AZFb	Hypospermatogenesis	+sperm	Biochemical
10	AZFc	N/A	+sperm	No pregnancy
11	AZFc	MA	+sperm ^c	+pregnancy ^d

Note: SCO = Sertoli-cell only; MA = maturation arrest.

^a Term, healthy male infant.

^b Twin male pregnancy, reduced to singleton at 13 weeks (Trisomy 18); healthy singleton male infant delivered.

^c Sperm was obtained from a frozen testicular biopsy.

^d Term, healthy male twins.

Choi. Y microdeletion and IVF. *Fertil Steril* 2004.

Testicular Sperm Extraction Technique

One surgeon (P.N.S.) performed all the TESE procedures and reviewed the diagnostic biopsy pathology. A microdissection TESE technique was used, as has been described previously (11). The procedures were performed in conjunction with programmed IVF stimulation cycles for the female partners. Spermatozoa were not cryopreserved before use with ICSI, except in one case in which frozen testicular sperm was used.

Y Chromosome Analysis

Y chromosome analysis was carried out with a multiplex polymerase chain reaction (PCR) technique on DNA extracted from peripheral leukocytes. For men who were found to have failure of amplification of a sequence-tagged site (STS) on the Y chromosome, single primer pairs were used to confirm the absence of each site in a PCR reaction on multiple occasions. Further details for the Y microdeletion screening techniques used for the men in this study have been described previously (12).

IVF Stimulation With ICSI

The IVF stimulation protocols used for the female partners have been defined in the past (13). Briefly, the patients were down-regulated with a GnRH agonist either with or without oral contraceptive pills. When adequately suppressed, the women were begun on a stimulation protocol using injectable gonadotropins. One female partner from the ejaculate group did not undergo suppression with a GnRH agonist and instead used a GnRH antagonist in conjunction with the gonadotropins once average follicle diameters reached 13 mm.

Patients were closely monitored throughout their cycles with frequent serum E_2 levels and sonograms of their ovarian follicles. Gonadotropin doses were tapered down as follicular response occurred. Human chorionic gonadotropin (5,000–10,000 IU) was used to trigger ovulation once follicle sizes were 16 mm to 17 mm in diameter. Oocyte retrievals were performed 34–36 hours after hCG triggering.

Intracytoplasmic sperm injection was performed on all metaphase II oocytes several hours after retrieval. The ICSI technique used at our center has been described elsewhere (14).

Statistical Analysis

Statistical analysis was performed with either the Student's *t* test or the χ^2 test, wherever applicable.

RESULTS

TESE Group

Of the 12 TESE attempts performed on 11 Y-microdeleteds men, 5 yielded no sperm ($P > .05$ vs. controls). No spermatozoa were found with TESE for any patient with a complete AZFa, AZFb, or AZFb+c microdeletion. In 7 of the Y-microdeleteds patients, prior testicular biopsy results

were available. There was no absolute correlation between location of the Y microdeletion and type of biopsy (see Table 2).

The trend toward lower fertilization rates (Table 1) in the Y-microdeleteds TESE group compared with the controls did not achieve statistical significance ($P = .057$). Clinical pregnancy rates (defined as the presence of a sac in utero on ultrasonography) per cycle and per transfer were not statistically different ($P > .05$). Because no spermatozoa were obtained in men with complete AZFa, AZFb, or AZFb+c microdeletions, all clinical pregnancies occurred among patients with AZFc microdeletions. Though not considered a clinical pregnancy, a biochemical pregnancy did occur with sperm obtained from the patient with a short microdeletion involving part of the AZFb region.

When comparing the STS maps detected by PCR for the patients, we found that all the AFZc-deleteds men lacked the same STS: the entire segment comprising the AZFc region. The AZFb+c-deleteds men lacked all the STS of the b and c regions, whereas the patient with the partial AZFb microdeletion (and successful TESE retrieval) was missing only the sY143 region. The AZFa-deleteds man was found to be missing the entire AZFa region.

Screening for Y microdeletions in the four resultant male offspring was not done, and karyotyping of the missed abortion was not performed.

Ejaculate Group

Within the group of AZFc-deleteds patients who used ejaculated sperm, both fertilization rates and clinical pregnancy rates per cycle and per transfer tended toward being lower. Again, the difference was not statistically significant (in all cases, $P > .05$) when compared with controls. One of the five pregnancies in the Y-microdeleteds (AZFc) ejaculate group resulted in a live birth (gender unavailable), two others resulted in the delivery of term singletons (one male, one female), and two resulted in missed abortions (the karyotype was available on one: 46, XY).

DISCUSSION

Our study demonstrates that TESE retrieval rates for the Y-microdeleteds men are not significantly different from those of controls (patients with idiopathic nonobstructive azoospermia). This finding supports prior reports (5, 7) that addressed patients with AZFc deletions. Our findings also suggest that although an AZFc microdeletion does not adversely affect a man's TESE retrieval prognosis, complete microdeletions of the AZFa, AZFb, or AZFb+c region might do so.

None of the patients with complete AZF deletions outside of the AZFc region yielded sperm on TESE. These results support our previously reported findings that complete AZFb microdeletions are a significant adverse prognostic finding for TESE (9). Interestingly, there was one patient in this

study with a deletion involving a short segment of AZFb who did yield sperm at TESE. Other than this patient, there was little variation in the STS found to be deleted among each of the subgroups of patients. Patients found to have a microdeletion of a certain AZF region generally lacked all the STS of that region.

We also found no absolute correlation between diagnostic testicular biopsy findings and type of microdeletion, nor even between biopsy findings and TESE success. Foresta et al. (1) observed no clear correlation between type of Y microdeletion and testicular pathology. Oates et al. (8) could not even discern a correlation between AZFc deletions and testicular histology. This is not surprising, because most men with nonobstructive azoospermia have a heterogeneous pattern of spermatogenesis. Previously reported results from our center and others have indicated that a single random biopsy cannot reliably predict sperm retrieval with TESE (15). Kamp et al. (10) have reported that in the case of AZFa microdeletions, all patients studied to date have been found to have Sertoli-cell-only pattern on biopsy.

What is reassuring about our findings is that, once sperm is obtained (whether by TESE or ejaculation) from men with Y microdeletions, fertilization rates and clinical pregnancy rates are not significantly different from those of the nonobstructed men with normal Y chromosomes. Although we are reporting on one of the larger case-control studies of Y microdeleted males ($n = 17$), this number is still small, and type II statistical error might have prevented detection of small differences between patient groups evaluated. Moreover, even the success rates with our controls were lower than the overall success rates at our center; this might have to do with the poor sperm quality in such severely affected patients.

Through assisted reproductive technology, men with Y microdeletions and severe oligozoospermia or azoospermia are sometimes able to reproduce. This and other studies (6, 8) have observed that male offspring can result from these IVF/ICSI attempts. Although genetic testing information of the male offspring in our study was not available, prior studies have found that all male offspring inherit their fathers' Y microdeletions (6, 8). Although the inheritance of the AZFc microdeletion does not seem to have any somatic effect on the children (8), the concern remains that the passage of microdeletions from father to son will perpetuate the adverse effects on future male fertility. Full counseling should be provided to all couples before and after genetic

testing. Rucker et al. (16) and Nap et al. (17) have both reported that up to 20% of couples will change their mind about reproductive choices once a specific genetic abnormality is defined.

References

1. Foresta C, Moro E, Ferlin A. Y chromosome microdeletions and alterations of spermatogenesis. *Endocr Rev* 2001;22:226–39.
2. Foresta C, Moro E, Ferlin A. Prognostic value of Y deletion analysis. *Hum Reprod* 2001;16:1543–7.
3. Pryor JL, Kent-First M, Muallem A, van Bergen AH, Nolten WE, Meisner L, et al. Microdeletions in the Y chromosome of infertile men. *N Engl J Med* 1997;336:534–9.
4. Repping S, Skaletsky H, Lange J, Silber S, van der Veen F, Oates RD, et al. Recombination between palindromes P5 and P1 on the human Y chromosome causes massive deletions and spermatogenic failure. *Am J Hum Genet* 2002;71:906–22.
5. Mulhall JP, Reijo R, Alagappan R, Brown L, Page D, Carson R, et al. Azoospermic men with deletion of the DAZ gene cluster are capable of completing spermatogenesis: fertilization, normal embryonic development and pregnancy occur when retrieved testicular spermatozoa are used for intracytoplasmic sperm injection. *Hum Reprod* 1997;12:503–8.
6. Page DC, Silber S, Brown LG. Men with infertility caused by AZFc deletion can produce sons by intracytoplasmic sperm injection, but are likely to transmit the deletion and infertility. *Hum Reprod* 1999;14:1722–6.
7. van Golde RJT, Wetzels AMM, de Graaf R, Tuerlings JHAM, Braat DDM, Kremer JAM. Decreased fertilization rate and embryo quality after ICSI in oligozoospermic men with microdeletions in the azoospermia factor c region of the Y chromosome. *Hum Reprod* 2001;16:289–92.
8. Oates RD, Silber S, Brown LG, Page DC. Clinical characterization of 42 oligospermic or azoospermic men with microdeletion of the AZFc region of the Y chromosome, and of 18 children conceived via ICSI. *Hum Reprod* 2002;17:2813–24.
9. Brandell RA, Mielnik A, Liotta D, Ye Z, Veeck LL, Palermo GD, et al. AZFb deletions predict the absence of spermatozoa with testicular sperm extraction: preliminary report of a prognostic genetic test. *Hum Reprod* 1998;13:2812–5.
10. Kamp C, Huellen K, Fernandes S, Sousa M, Schlegel PN, Mielnik A, et al. High deletion frequency of the complete AZFa sequence in men with Sertoli-cell-only syndrome. *Mol Hum Reprod* 2001;7:987–94.
11. Schlegel PN, Palermo GD, Goldstein M, Menendez S, Zaninovic N, Veeck LL, et al. Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia. *Urology* 1997;49:435–40.
12. Girardi SK, Mielnik A, Schlegel PN. Submicroscopic deletions in the Y-chromosome of infertile men. *Hum Reprod* 1997;12:1635–41.
13. Davis OK, Rosenwaks Z. In vitro fertilization. In: Adashi EY, Rock JA, Rosenwaks Z, eds. *Reproductive endocrinology, surgery, and technology*, Vol. 2. Philadelphia: Lippincott-Raven, 1996:2320–34.
14. Palermo GD, Cohen J, Alikani M, Adler A, Rosenwaks Z. Intracytoplasmic sperm injection: a novel treatment for all forms of male factor infertility. *Fertil Steril* 1995;63:1231–40.
15. Su LM, Palermo GD, Goldstein M, Veeck LL, Rosenwaks Z, Schlegel PN. Testicular sperm extraction with intracytoplasmic sperm injection for nonobstructive azoospermia: testicular histology can predict success of sperm retrieval. *J Urol* 1999;161:112–6.
16. Rucker GB, Mielnik A, King P, Goldstein M, Schlegel PN. Preoperative screening for genetic abnormalities in men with nonobstructive azoospermia before testicular sperm extraction. *J Urol* 1998;160:2068–71.
17. Nap AW, Van Golde RJT, Tuerlings JHAM, De Sutter P, Pieters MHEC, Giltay JC, et al. Reproductive decisions of men with microdeletions of the Y chromosome: the role of genetic counselling. *Hum Reprod* 1999;14:2166–9.