Y chromosome deletions in azoospermic and severely oligozoospermic men undergoing intracytoplasmic sperm injection after testicular sperm extraction

S.J.Silber[1,3], R.Alagappan[2], L.G.Brown[2] and D.C.Page[2]

[1]Infertility Center of St Louis, St Luke's Hospital, St Louis, MO 63017 and [2]Howard Hughes Medical Institute, Whitehead Institute and Department of Biology, Massachusetts Institute of Technology, Cambridge, MA 02142, USA

[3]To whom correspondence should be addressed

Y chromosome deletions encompassing the AZFc region have been reported in 13% of azoospermic men and 7% of severely oligozoospermic men. We examined the impact of these Y deletions on the severity of testicular defects in 51 azoospermic men undergoing intracytoplasmic sperm injection (ICSI) after testicular sperm extraction (TESE) and 30 men with severe oligozoospermia undergoing ICSI after ejaculation of spermatozoa. In addition, five azoospermic patients shown previously to have Y chromosome deletions underwent histological evaluation of their previously obtained testis biopsy specimens. A further 27 azoospermic men underwent TESE–ICSI, but not Y chromosome DNA testing. Ten of 51 azoospermic men (20%) who underwent TESE–ICSI and Y-DNA testing were found to be deleted for portions of the Y chromosome AZFc region. Of these 10, five had spermatozoa retrievable from the testis, and in two cases the wives became pregnant. Of the 41 azoospermic men with no Y chromosome deletion, 22 (54%) had spermatozoa retrievable from the testis, and in 12 cases (29%) the wives became pregnant. Four of 30 (13%) severely oligozoospermic patients were found to be deleted for AZFc and in three (75%) of these pregnancy was achieved. The other 26 severely oligozoospermic couples who had no AZFc deletions underwent ICSI, and 12 (46%) have an ongoing or delivered pregnancy. The embryo implantation rate was not significantly different for azoospermic (22%), oligozoospermic (16%), Y-deleted (14%) or Y-intact (18%) men. Of the total of 19 infertile men who had Y chromosome deletions, 14 had deletions within Y chromosome intervals 6D–6F, in the AZFc region. Twelve of those 14 had some spermatozoa (however few in number) in the ejaculate or testis. Five of the Y-deleted men had deletions that extended more proximally on the Y chromosome, and in none of these could any spermatozoa be observed in either ejaculate or testis. These results support the concept that, in azoospermic or oligozoospermic men with Y chromosome deletions limited to intervals 6D–6F (AZFc), there are generally very small numbers of testicular or ejaculated spermatozoa. Larger Y deletions, including and extending beyond the AZFc region and encompassing more Y genes, tend to be associated with a total absence of testicular spermatozoa. In those cases where spermatozoa were retrieved, the presence of Y deletions had no obvious impact on fertilization or pregnancy rate.

Key words: AZFc/azoospermia/ICSI/TESE/Y chromosome

Introduction

Azoospermic men with germinal failure often have tiny foci of intact spermatogenesis somewhere in their testes (Silber and Rodriguez-Rigau, 1981; Silber *et al.*, 1995b). This discovery has allowed otherwise sterile men to father children by utilizing testicular sperm extraction (TESE) and intracytoplasmic sperm injection (ICSI) (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1993; Devroey *et al.*, 1995; Silber *et al.*, 1995a, 1996). In about 60% of these cases, a tiny amount of spermatozoa is produced in the testis, though this is insufficient to spill over into the ejaculate (Silber *et al.*, 1997).

Deficient spermatogenesis in men has been considered likely to have a genetic aetiology (Tiepolo and Zuffardi, 1976; Johnson *et al.*, 1989). Recently, a number of laboratories have reported submicroscopic deletions of certain portions of the Y chromosome in azoospermic and severely oligozoospermic men (Vogt *et al.*, 1996; Ma *et al.*, 1993; Najmabadi *et al.*, 1996; Nakahori *et al.*, 1996; Qureshi *et al.*, 1996; Chai *et al.*, 1997; Elliot *et al.*, 1997; Foresta *et al.*, 1997; Girardi *et al.*, 1997; Kremer *et al.*, 1997; Mulhall *et al.*, 1997; Pryor *et al.*, 1997; Simoni *et al.*, 1997; van der Ven *et al.*, 1997; Vereb *et al.*, 1997). In our laboratory, about 13% of men with non-obstructive azoospermia, and 7% of those with severe oligozoospermia, have been found to be deleted for a particular portion of the Y chromosome, the AZFc region, which contains the *DAZ* gene cluster (Reijo *et al.*, 1995, 1996; Saxena *et al.*, 1996). These deletions have been shown to be new mutations not present in fathers or brothers, nor in normal fertile controls. All of these results suggest that genes on the Y chromosome play an important role in spermatogenesis.

We wished to determine what impact the size and extent of deletions encompassing the AZFc region might have on the type and severity of spermatogenic defect in azoospermic and severely oligozoospermic patients who are undergoing TESE and ICSI, and what effect, if any, these deletions might have on the fertilization and pregnancy rate.

Materials and methods

Fifty-one men with non-obstructive azoospermia and 30 men with severe oligozoospermia ($<1\times10^6$/ml in the ejaculate) underwent

Y-chromosomal sequence tagged site (STS) mapping, and TESE–ICSI or ICSI alone in an attempt to conceive a child. None of the azoospermic men had obstruction, and all were documented to have 'Sertoli cell-only', maturation arrest, or a combination of the two. Five additional azoospermic men who were found to be Y-deleted elected not to attempt TESE–ICSI but underwent quantitative histological evaluation of a previously obtained testis biopsy. An additional 27 men with non-obstructive azoospermia were not studied for Y chromosome deletions, but nonetheless underwent TESE–ICSI.

We searched for a relationship between the presence of testicular or ejaculated spermatozoa and the presence or absence, and size and location, of a Y-chromosomal deletion. The method of quantitative study of testicular histology was previously reported (Silber *et al.*, 1996). The methodology for TESE–ICSI has also been described previously (Devroey *et al.*, 1995; Silber *et al.*, 1995a, 1996).

Using the polymerase chain reaction (PCR), we searched for Y chromosome deletions in azoospermic or severely oligozoospermic men by testing genomic DNA for the presence or absence of as many as 52 DNA landmarks (STS) distributed across the entirety of the Y chromosome (Figure 1). All tests were performed on DNA purified from blood leukocytes or lymphoblastoid cell lines. All Y-DNA markers employed had been placed previously on a physical map of the chromosome; the markers represented all known genes and gene families in the non-recombining region of the Y chromosome (Foote *et al.*, 1992; Vollrath *et al.*, 1992; Lahn and Page, 1997; Vogt *et al.*, 1997).

Results

Of the 51 azoospermic men who underwent TESE–ICSI, 10 (20%) were found to be deleted for the AZFc region, which encompasses the *DAZ* gene cluster (Table I). Of those 10, five (50%) had spermatozoa retrievable from the testis, and the wives of two (20%) became pregnant. Of the 41 azoospermic men with apparently intact Y chromosomes, 22 (54%) had spermatozoa retrievable from the testis, and the wives of 12 (29%) became pregnant.

Of the 26 severely oligozoospermic ($<1\times10^6$/ml) men with apparently intact Y chromosomes undergoing ICSI, the wives of 12 (46%) now have an ongoing or delivered pregnancy. Of the four oligozoospermic couples that were Y-deleted, the wives of three (75%) have an ongoing or delivered pregnancy (Table II). Among a total of 14 couples (azoospermic and oligozoospermic) with Y deletions undergoing ICSI, five (36%) have healthy ongoing or delivered pregnancies. The pregnancy rate per embryo transfer in cases where spermatozoa were found was not dramatically different for azoospermic (36%), oligozoospermic (46%), Y-deleted (42%) or Y-intact (50%) men.

Y chromosome testing was not done in 27 azoospermic men undergoing TESE–ICSI. In 19 of these cases, the pathology was either cryptorchidism, mumps or post-chemotherapy azoospermia, but not idiopathic Sertoli cell-only or maturation arrest. From the outset we viewed this as a distinct aetiological group in which Y deletions were very unlikely. The clinical results with this group of azoospermic patients undergoing TESE–ICSI did not differ from those of the group with idiopathic germinal failure, i.e. Sertoli cell-only or maturation arrest. Eight of these 27 TESE–ICSI cases had Sertoli cell-only or maturation arrest, but declined to undergo Y-chromosomal

testing. Of the total of 78 cases (51 Y-tested and 27 not Y-tested), neither embryo transfer rate, 2 pronuclei (2PN) fertilization rate, cleavage rate nor the delivered pregnancy rate per initiated cycle was affected by whether the pathology was Sertoli cell-only, maturation arrest, post chemotherapy, mumps or cryptorchid atrophy (Table III).

Among the 31 men (out of 78) in whom no spermatozoa were found at TESE (40%), round spermatids were also not found, either in the TESE procedure itself, or in the examination of histological sections. Round spermatids were never observed in the absence of elongated spermatids or spermatozoa.

Y deletions limited to intervals 6D–6F (the AZFc region; Figure 1) were generally associated with finding some spermatozoa at TESE, while larger Y defects (extending more proximally and/or more distally) were associated with finding no spermatozoa at TESE (Figure 1; Tables IV and V). Five Y-deleted azoospermic patients did not undergo TESE–ICSI, but underwent quantitative scrutiny of their previous testis biopsy. Ten Y-deleted azoospermic men underwent TESE–ICSI. Thus, a total of 15 Y-deleted azoospermic men were studied for the presence of testicular spermatozoa. In these non-obstructive azoospermic men, a few spermatozoa sufficient for ICSI were found in the testes of eight of the 10 with deletions limited to intervals 6D–6F, but absolutely no spermatozoa were found in the testes of any of the five azoospermic men with deletions which extended more proximally and/or more distally. Even in one of the two patients with limited Y deletions who did not have sufficient spermatozoa for ICSI, a few spermatozoa were noted on extensive histological evaluation. Thus, there was a clear trend toward larger deletions causing more severe spermatogenic defects. The four oligozoospermic men who were Y-deleted also all had small deletions limited to intervals 6D–6F.

Thus, a total of 19 Y-deleted men were scrutinized for spermatozoa either at TESE, in the testicular biopsy, or simply by the presence of some spermatozoa in the ejaculate (Table V). Fourteen (73%) had deletions limited to interval 6D–6F, and 12 of those (86%) had spermatozoa in the testis or ejaculate. Five of the 19 Y-deleted men had deletions extending beyond 6D–6F, and none of those had any spermatozoa detected in the testes or ejaculate. It is not clear whether this is caused by the terminal nature of these large deletions in four of the five cases, or because of the absence of additional spermatogenesis genes on their Y chromosome. Part or all of the AZFc region was absent in all of our Y-deleted azoospermic and severely oligozoospermic patients. When the deletion extended beyond AZFc, encompassing additional testis-specific genes, no spermatozoa could be found.

When spermatozoa were recoverable in azoospermic or oligozoospermic men, there was no significant difference in pregnancy rate with ICSI whether the man was Y-deleted or Y-intact. In azoospermic men, the pregnancy rate was 29% for Y-intact and 20% for Y-deleted cases. In oligozoospermic men, the pregnancy rate was 46% for Y-intact and 50% for Y-deleted cases.

The histological classification of the azoospermic men did not appear to relate in a straightforward manner with the extent or site of deletion. Of the 10 patients with deletions limited
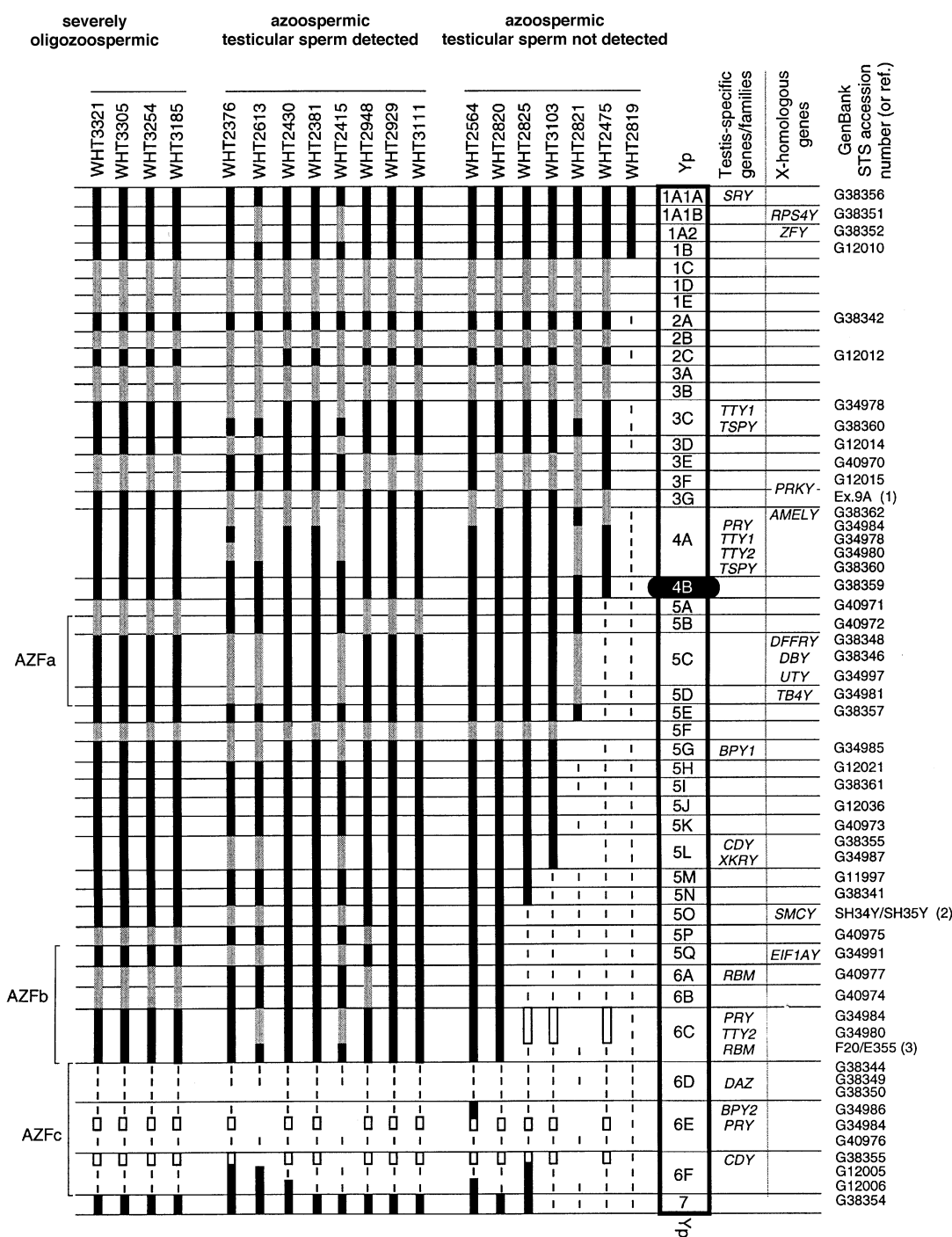
Figure 1. Y chromosome deletions in 19 azoospermic or severely oligozoospermic men. Towards the right, the Y chromosome is represented by a long rectangle; 43 deletion intervals [1A1A through 7, as defined by Vollrath *et al.* (1992)], the short arm (Yp), centromere (in black; interval 4B), and long arm (Yq) are labelled. To the right of the chromosome are indicated the names and positions of 10 X-homologous genes and 11 testis-specific genes or gene families (Lahn and Page, 1997; Vogt *et al.*, 1997). On the extreme right are listed GenBank accession numbers (or references) for the 52 STS for which we tested the patients. (GenBank accession numbers provide electronic access to PCR primer sequences and reaction conditions. Note: STS with accession numbers G40977 and G38345 appear to map near, but not necessarily within, *RBM* genes.) [References (1): Schiebel *et al.*, 1997; (2) Agulnik *et al.*, 1994; (3) Ma *et al.*, 1993.] The bars to the left of the chromosome represent the portions of the Y chromosome that were found to be present in: (i) seven azoospermic men in whom no spermatozoa were found in testis biopsies; (ii) eight azoospermic men in whom spermatozoa were found in testis biopsies; and (iii) four severely oligozoospermic men. The experimentally demonstrated presence of an STS in an individual is indicated by a black segment; the inferred presence (by interpolation) of an STS in an individual is indicated by a minus; inferred absence is indicated by the absence of any symbol. White boxes represent positive PCR results that should be interpreted in the context of the four-specific repeat nature of the sequences being considered; these positive results may simply reflect the existence of closely related, cross-amplifying sequences in other portions of the Y chromosome. Some of these results have been reported previously (Reijo *et al.*, 1995). At the extreme left of the figure, the approximate boundaries of the AZFa, AZFb and AZFc regions (as defined by Vogt *et al.*, 1996) are shown.

Table I. Y chromosome deletions and TESE–ICSI for azoospermia

	Y-intact (%)	Y-deleted (%)
No. of cycles	41	10
No. with spermatozoa	22 (54)	5 (50)
No. of MII	290	87
No. with 2 pronuclei (2PN)	123 (42)	41 (47)
No. cleaved	76 (62)	24 (58)
No. of sacs	17 (22)	3 (13)
No. pregnant (ongoing or delivered)	12 (29)	2 (20)

Table II. Y-chromosomal deletions and ICSI for oligozoospermia

	Y-intact (%)	Y-deleted (%)
No. of cycles	26	6[a]
No. with spermatozoa	26	6[a]
No. of MII	312	70
No. of 2 pronuclei (2PN)	200 (64)	48 (68)
No. cleaved	113 (56)	31 (65)
No. of sacs	17 (15)	5 (16)
No. pregnant (ongoing or delivered)	12 (46)	3 (50)

[a]Four patients. Two patients each went through two cycles.

Table III. TESE–ICSI for non-obstructive azoospermia

	All cases (%)	Sertoli cell-only (%)	Maturation arrest (%)
No. of cycles	78	33	26
No. with spermatozoa	47 (60.3)	17 (52)	16 (61.5)
No. of MII	634	247	204
No. of 2 pronuclei (2PN)	256 (40)	95 (38)	83 (41)
No. cleaved	157 (61)	49 (52)	65 (78)
No. of sacs	25 (16)	12 (24)	8 (12)
No. pregnant (ongoing or delivered)	17 (22)	7 (21)	7 (27)

Table IV. Presence or absence of spermatozoa in testes of azoospermic men in relation to the presence of absence of Y deletion

	Total cases	Spermatozoa found (%)	Spermatozoa not found
Y deletion detected	15	8 (53)	7
No Y deletion detected	41	22 (54)	19

Table V. Extent of Y deletion in all infertile men (azoospermic and severely oligozoospermic)

Deletions	Total cases	Some spermatozoa present (%)	No spermatozoa
Limited to intervals 6D–6F	14	12 (86)	2
Extending beyond intervals 6D–6F	5	0	5

to AZFc, three had maturation arrest, five had Sertoli cell-only, and two had a combination of maturation arrest and Sertoli cell-only. Of the five patients with deletions extending beyond AZFc, four were Sertoli cell-only, and one was maturation arrest.

Discussion

In this report, we undertook to summarize and analyse the results of Y chromosome deletion analysis in men with severe spermatogenic defects who were undergoing therapeutic attempts for their wives to conceive with TESE and ICSI.

Cytogenetic studies dating back to 1976 had shown, in a very small number of azoospermic men, gross defects visible in the long arm of the Y chromosome, implying the existence of an azoospermic factor somewhere in that region (Tiepolo and Zuffardi, 1976). In 1992, comprehensive Y-chromosomal maps constructed using yeast artificial chromosomes (YAC) and STS created the possibility for more detailed study of the Y chromosome in infertile men (Foote *et al.*, 1992; Vollrath *et al.*, 1992). A detailed Y-chromosomal mapping study of a large series of severely infertile men with clearly identified phenotypes revealed a common deletion in 13% of azoospermic

males. The commonly deleted region was located in the distal portion of interval 6, subsequently referred to as AZFc (Reijo *et al.*, 1995; Vogt *et al.*, 1996). The fathers of the Y-deleted, infertile men were shown to have intact Y chromosomes, demonstrating that the deletions had arisen *de novo* and providing strong evidence that the deletions were the cause of the spermatogenic failure observed in these men. The *DAZ* gene cluster was identified within the small, commonly deleted region, and has autosomal homologues (Reijo *et al.*, 1995; Saxena *et al.*, 1996; Ruggiu *et al.*, 1997). The *DAZ* genes have been shown to be transcribed specifically in spermatogonia and primary spermatocytes (Menke *et al.*, 1997).

Shortly thereafter, ICSI with testicular and epididymal sperm retrieval methods were developed for the treatment of couples with azoospermia (Schoysman *et al.*, 1993; Devroey *et al.*, 1994; Silber *et al.*, 1994, 1995c; Tournaye *et al.*, 1994). TESE and ICSI could thus be utilized to achieve pregnancy for the wives of men with azoospermia caused by deficient spermatogenesis (Devroey *et al.*, 1995; Silber *et al.*, 1995a,b, 1996). Men with the most severe spermatogenic defects causing complete azoospermia were often found, in an extensive testicular biopsy, to have a minute number of spermatozoa (or mature spermatids) very sparsely present; these spermatozoa could then be used for ICSI. This TESE–ICSI procedure was based on quantitative studies of spermatogenesis dating back to the late 1960s (Steinberger and Tjioe, 1968; Zuckerman *et al.*, 1978; Silber and Rodriguez-Rigau, 1981).

Severe oligozoospermia (which is readily treated with ICSI) is thus simply a quantitative variant of azoospermia in which there is a minute level of spermatogenesis in 60% of azoospermic men, though this is below the threshold necessary for sperm to 'spill over' into the ejaculate (Silber *et al.*, 1997).

For the purpose of comparing Y-chromosomal deletions to the degree of spermatogenic defect, azoospermic men with at least a few spermatozoa retrievable from the testes should perhaps be considered together with very severely oligozoospermic men. Azoospermic men in whom no spermatozoa at all could be retrieved from the testis may be in a different category.

In oligozoospermic men, or azoospermic men in whom

testicular spermatozoa can be retrieved, the presence or absence of Y deletions apparently has no effect on fertilization or pregnancy rates. Furthermore, in azoospermic men, the presence or absence of a Y deletion correlates poorly with whether the pathology will be maturation arrest or Sertoli cell-only. We found these deletions in azoospermic and severely oligozoospermic men with a variety of histological patterns, e.g. Sertoli cell-only or maturation arrest, either complete or partial.

In those infertile men who are Y-deleted, larger deletions (extending beyond intervals 6D–6F) appear to be associated with a total absence of testicular spermatozoa, but smaller deletions (limited to 6D–6F) are associated with the presence of small numbers of spermatozoa that are sufficient for ICSI. Also, all four Y-deleted oligozoospermic men studied were found to have deletions limited to 6D–6F. These results suggest that multiple spermatogenesis genes on the Y chromosome may contribute to and modify the severity of the spermatogenic defect in men with deletions encompassing the AZFc region. Though deletions limited to intervals 6D–6F remove the entire *DAZ* gene cluster, in most individuals such deletions are apparently not sufficient to eliminate completely all spermatogenesis.

These clinical findings in azoospermic and severely oligozoospermic Y-deleted men undergoing TESE and/or ICSI correlate well with Y chromosome studies reported recently in many different laboratories (Ma *et al.*, 1993; Najmabadi *et al.*, 1996; Nakahori *et al.*, 1996; Qureshi *et al.*, 1996; Vogt *et al.*, 1996; Elliot *et al.*, 1997; Foresta *et al.*, 1997; Girardi *et al.*, 1997; Kremer *et al.*, 1997; Pryor *et al.*, 1997; Simoni *et al.*, 1997; van der Ven *et al.*, 1997; Vereb *et al.*, 1997). The central clinical conclusion from our series is that while at least a few spermatozoa are detected in most infertile or 'sterile' men with standard AZFc deletions, spermatozoa are generally not detected in men with larger deletions encompassing AZFc plus more proximal and distal regions of the Y chromosome. Of course, this is a generalization based on a small number of clinical cases, but it does confirm the viewpoints of several other investigators.

The patients with no spermatozoa retrievable even after exhaustive TESE–ICSI attempts were found to have deletions which extend beyond AZFc (Vogt *et al.*, 1996; Elliott *et al.*, 1997). Deletions of *DAZ* and AZFc alone were not generally sufficient to prevent completely all spermatogenesis. However, when AZFb and beyond were missing, there was predictably no detectable completion of spermatogenesis. Therefore, deletion of functional members of the *RBM* gene family, or of some of the other genes found in AZFb, e.g. *EIF1AY*, *PRY* and *TTY2*, might contribute to the enhanced severity of spermatogenic defects observed in patients whose deletions extend proximal to the AZFc region (Chai *et al.*, 1997; Elliot *et al.*, 1997; Lahn and Page, 1997).

There are probably several genes on the Y chromosome that impinge on spermatogenesis (Lahn and Page, 1997; Vogt *et al.*, 1997). It may be the total effect of all these spermatogenesis genes on the Y chromosome that determines sperm production rates. Our studies suggest that it will be possible to identify subsets of Y-deleted azoospermic men who are relatively good or relatively poor candidates for TESE–ICSI.

Acknowledgements

References

Agulnik, A.I., Mitchell, M.J., Lerner, J.L. *et al.* (1994) A mouse Y chromosome gene encoded by a region essential for spermatogenesis and expression of male-specific minor histocompatibility antigens. *Hum. Mol. Genet.*, **3**, 873–878.

Chai, N.N., Salido, E.C. and Yen, P.H. (1997) multiple functional copies of the RBM gene family, a spermatogenesis candidate on the human Y chromosome. *Genomics*, **45**, 355–361.

Devroey, P., Silber, S., Nagy, Z. *et al.* (1994) Ongoing pregnancies and birth after intracytoplasmic sperm injection (ICSI) with frozen–thawed epididymal spermatozoa. *Hum. Reprod.*, **10**, 903–906.

Devroey, P., Liu, J., Nagy, Z. *et al.* (1995) Pregnancies after testicular extraction (TESE) and intracytoplasmic sperm injection (ICSI) in non-obstructive azoospermia. *Hum. Reprod.*, **10**, 1457–1460.

Elliot, D.J., Millar, M.R., Oghene, K. *et al.* (1997) Expression of RBM in the nuclei of human germ cells is dependent on a critical region of the Y chromosome long arm. *Proc. Natl. Acad. Sci. USA*, **94**, 3848–3853.

Foote, S., Vollrath, D., Hilton, A. and Page, D.C. (1992) The human Y chromosome: overlapping DNA clones spanning the euchromatic region. *Science*, **258**, 60–66.

Foresta, C., Ferlin, A., Garolla, A. *et al.* (1997) Y-chromosome deletions in idiopathic severe testiculopathies. *J. Clin. Endocrinol. Metab.*, **82**, 1075–1080.

Girardi, S.K., Mielnik, A. and Schlegel, P.N. (1997) Submicroscopic deletions in the Y chromosome of infertile men. *Hum. Reprod.*, **12**, 1635–1641.

Johnson, M.D., Tho, S.P., Behzadian, A. and McDonough, P.G. (1989) Molecular scanning of Y_q11 (interval 6) men with Sertoli-cell-only syndrome. *Am. J. Obstet. Gynecol.*, **161**, 1732–1737.

Kremer, J.A., Tuerlings, J.H., Meuleman, E.J. *et al.* (1997) Microdeletions of the Y chromosome and intracytoplasmic sperm injection: from gene to clinic. *Hum. Reprod.*, **12**, 687–691.

Lahn, B.T. and Page, D.C. (1997) Functional coherence of the human Y chromosome. *Science*, **278**, 675–680.

Ma, K., Inglis, J.D., Sharkey, A. *et al.* (1993) A Y chromosome gene family with RNA-binding protein homology: candidates for the azoospermia factor AZF controlling human spermatogenesis. *Cell*, **75**, 1287–1295.

Menke, D.B., Mutter, G.L. and Page, D.C. (1997) Expression of DAZ, an azoospermia factor candidate, in human spermatogonia. *Am. J. Hum. Genet.*, **60**, 237–241.

Mulhall, J.P., Reijo, R., Alagappan, R. *et al.* (1997) 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.*, **12**, 503–508.

Najmabadi, H., Huang, V., Yen, P. *et al.* (1996) Substantial prevalence of microdeletions of the Y-chromosome in infertile men with idiopathic azoospermia and oligozoospermia detected using a sequence-tagged site-based mapping strategy. *J. Clin. Endocrinol. Metab.*, **81**, 1347–1352.

Nakahori, Y., Kuroki, Y., Komaki, R. *et al.* (1996) The Y chromosome region essential for spermatogenesis. *Horm. Res.*, **46**(suppl. 1), 20–23.

Palermo, G., Joris, H., Devroey, P. and Van Steirteghem, A. (1992) Pregnancies after intracytoplasmic injection of single spermatozoon into an oocyte. *Lancet*, **340**, 17–18.

Pryor, J.L., Kent-First, M., Muallem, A. *et al.* (1997) Microdeletions in the Y chromosome of infertile men. *N. Engl. J. Med.*, **336**, 534–539.

Qureshi, S.J., Ross, A.R., Ma, K. *et al.* (1996) Polymerase chain reaction screening for Y chromosome microdeletions: a first step towards the diagnosis of genetically-determined spermatogenic failure in men. *Mol. Hum. Reprod.*, **2**, 775–779.

Reijo, R., Lee, T.-Y., Salo, P. *et al.* (1995) Diverse spermatogenic defects in humans caused by Y chromosome deletions encompassing a novel RNA-binding protein gene. *Nature Genet.*, **10**, 383–393.

Reijo, R., Alagappan, R.K., Patrizio, P. and Page D.C. (1996) Severe oligospermia resulting from deletions of the *Azoospermia Factor* gene on the Y chromosome. *Lancet*, **347**, 1290–1293.

Ruggiu, M., Speed, R., Taggart, M. *et al*. (1997) The mouse *Dazla* gene encodes a cytoplasmic protein essential for gametogenesis. *Nature*, **389**, 73–77.

Saxena, R., Brown, L.G., Hawkins, T. *et al*. (1996) The DAZ gene cluster on the human Y chromosome arose from an autosomal gene that was transposed, repeatedly amplified and pruned. *Nature Genet*., **14**, 292–299.

Schiebel, K., Wunkelmann, M., Mertz, A. *et al*. (1997) Abnormal XY interchange between a novel isolated protein kinase gene, PRKY, and its homologue, PRKX, accounts for one third of all (Y+)XX males and (Y-)XY females. *Hum. Mol. Genet*., **6**, 1985–1989.

Schoysman, R., Vanderzwalmen, P., Nijs, M. *et al*. (1993) Pregnancy after fertilisation with human testicular spermatozoa. *Lancet*, **342**, 1237.

Silber, S.J. and Rodriguez-Rigau, L. (1981) Quantitative analysis of testicle biopsy: determination of partial obstruction and prediction of sperm count after surgery for obstruction. *Fertil. Steril*., **36**, 480–485.

Silber, S.J., Nagy, Z.P., Liu, J. *et al*. (1994) Conventional in-vitro fertilization versus intracytoplasmic sperm injection for patients requiring microsurgical sperm aspiration. *Hum. Reprod*., **9**, 1705–1709.

Silber, S.J., Nagy, Z., Liu, J. *et al*. (1995a) The use of epididymal and testicular spermatozoa for intracytoplasmic sperm injection: the genetic implications for male infertility. *Hum. Reprod*., **10**, 2031–2043.

Silber, S.J., Van Steirteghem, A.C. and Devroey, P. (1995b) Sertoli cell only revisited. *Hum. Reprod*., **10**, 1031–1032.

Silber, S.J., Van Steirteghem, A.C., Liu, J. *et al*. (1995c) High fertilization and pregnancy rate after intracytoplasmic sperm injection with sperm obtained from testicle biopsy. *Hum. Reprod*., **10**, 148–152.

Silber, S.J., Van Steirteghem, A.C., Nagy, Z. *et al*. (1996) Normal pregnancies resulting from testicular sperm extraction and intracytoplasmic sperm injection for azoospermia due to maturation arrest. *Fertil. Steril*., **55**, 110–117.

Silber, S.J., Nagy, Z., Devroey, P. *et al*. (1997) Distribution of spermatogenesis in the testicles of azoospermic men: the presence or absence of spermatids in the testes of men with germinal failure. *Hum. Reprod*., **12**, 2422–2428.

Simoni, M., Gromoll, J., Dworniczak, B. *et al*. (1997) Screening for deletions of the Y chromosome involving the DAZ (Deleted in AZoospermia) gene in azoospermia and severe oligozoospermia. *Fertil. Steril*., **67**, 542–547.

Steinberger, E. and Tjioe, D.Y. (1968) A method for quantitative analysis of human seminiferous epithelium. *Fertil. Steril*., **19**, 960–970.

Tiepolo, L. and Zuffardi, O. (1976) Localization of factors controlling spermatogenesis in the nonfluorescent portion of the human Y chromosome long arm. *Hum. Genet*., **34**, 119–124.

Tournaye, H., Devroey, P., Liu, J. *et al*. (1994) Microsurgical epididymal sperm aspiration and intracytoplasmic sperm injection: a new effective approach to infertility as a result of congenital bilateral absence of the vas deferens. *Fertil. Steril*., **61**, 1045–1051.

van der Ven, K., Montag, M., Peschka, B. *et al*. (1997) Combined cytogenetic and Y chromosome microdeletion screening in males undergoing intracytoplasmic sperm injection. *Mol. Hum. Reprod*., **3**, 699–704.

Van Steirteghem, A.C., Nagy, Z., Joris, H. *et al*. (1993) High fertilization and implantation rates after intracytoplasmic sperm injection. *Hum. Reprod*., **8**, 1061–1066.

Vereb, M., Agulnik, A., Houston, J.T. *et al*. (1997) Absence of *DAZ* gene mutations in cases of non-obstructed azoospermia. *Mol. Hum. Reprod*., **3**, 55–59.

Vogt, P., Edelmann, A., Kirsch, S. *et al*. (1996) Human Y chromosome azoospermia factors (AZF) mapped to different subregions in Yq11. *Hum. Mol. Genet*., **5**, 933–943.

Vogt, P.H., Affara, N., Davey, P. *et al*. (1997) Report of the Third International Workshop on Y Chromosome Mapping 1997. *Cytogenet. Cell Genet*., **79**, 1–20.

Vollrath, D., Foote, S., Hilton, A. *et al*. (1992) The human Y chromosome: a 43-interval map based on naturally occurring deletions. *Science*, **258**, 52–59.

Zuckerman, Z., Rodriguez-Rigau, L., Weiss, D.B. *et al*. (1978) Quantitative analysis of the seminiferous epithelium in human testicle biopsies and the relation of spermatogenesis to sperm density. *Fertil. Steril*., **30**, 448–455.