10 The treatment of azoospermia with surgery and ICSI

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ICSI for oligoasthenospermia

Before 1992, male infertility had been considered in most cases to be untreatable. Then came a surprisingly neat solution: ICSI, i.e the injection of a single spermatozoon into a single egg. Since the publication of the first papers on ICSI for oligozoospermia in 1992 and 1993, an intense flurry of scientific effort has been dedicated to extending its application to virtually every type of male infertility (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1993; Silber, 1995; Silber *et al.*, 1995*a*). First it was confirmed that the most severe cases of oligoasthenoteratospermia enjoyed the same success rates as mild cases of male factor infertility and that these pregnancy rates were no different from those of couples where men with normal spermatozoa were undertaking conventional IVF (Nagy *et al.*, 1995*a*). Next, it was discovered that it does not really matter how the spermatozoon is pretreated prior to ICSI, and that any method for aspirating the spermatozoon into an injection pipette and transferring it into the oocyte is adequate (Liu *et al.*, 1994*a*). In fact, no sperm defect precluded success with the ICSI technique. Any fertilization failure was always related either to poor egg quality or to sperm non-viability (Liu *et al.*, 1995). It appeared that there was no negative effect on the pregnancy rate with ICSI even with the most severe morphological sperm defects, the most severe reduction in motility, or with the tiniest number of spermatozoa in the ejaculate ('pseudoazoospermia') (see Tables 10.1 and 10.2). Only absolute immotility of ejaculated or epididymal spermatozoa lowered the fertilization rate, and this was found to be due not to the immotility of spermatozoa but rather to their non-viability. Completely non-motile spermatozoa, if viable, were capable of normal fertilization and pregnancy rates (see Fig. 10.1), however few of them there might be in an ejaculate.

Sperm retrieval and ICSI for obstructive azoospermia

Long-term studies of vasoepididymostomy using the single-tubule microsurgical technique has been remarkably successful for obstructive

Table 10.1. Result of ICSI with ejaculated sperm not related to sperm quality

	No. of cycles	*2PN (%)*	*Transfer (%)*	*Clinical pregnancies (%)*
Sperm Count (Total)				
'0'	57	58	86	25
up to 1×10^6	97	64	96	26
>1 to 5×10^6	128	70	96	22
$>5 \times 10^6$	684	71	93	30
Motility				
0[a]	12	10	42	0
0	54	60	87	13
>0 to 5%	19	68	100	32
>5 to 50%	479	70	88	31
>50%	337	74	95	26
Morphology				
0	48	68	88	31
>1 to 3	125	70	96	33
>4 to 13	307	71	94	26
>14	203	75	95	29

2PN, 2 pronuclei.

[a] Nagy *et al.*, 1995a.

Table 10.2. Fertilization failure, October 1992 to December 1994

No. of cycles	2PN (%)	Clinical pregnancies
2732	71%	964 (36%)

Failed fertilization in 29 (1%) cycles (28 couples)

of 26 couples with failed fertilization, who underwent repeat ICSI,
22 (85%) achieved fertilization in subsequent cycles

2PN, 2 pronuclei.

From Liu et al., 1995.

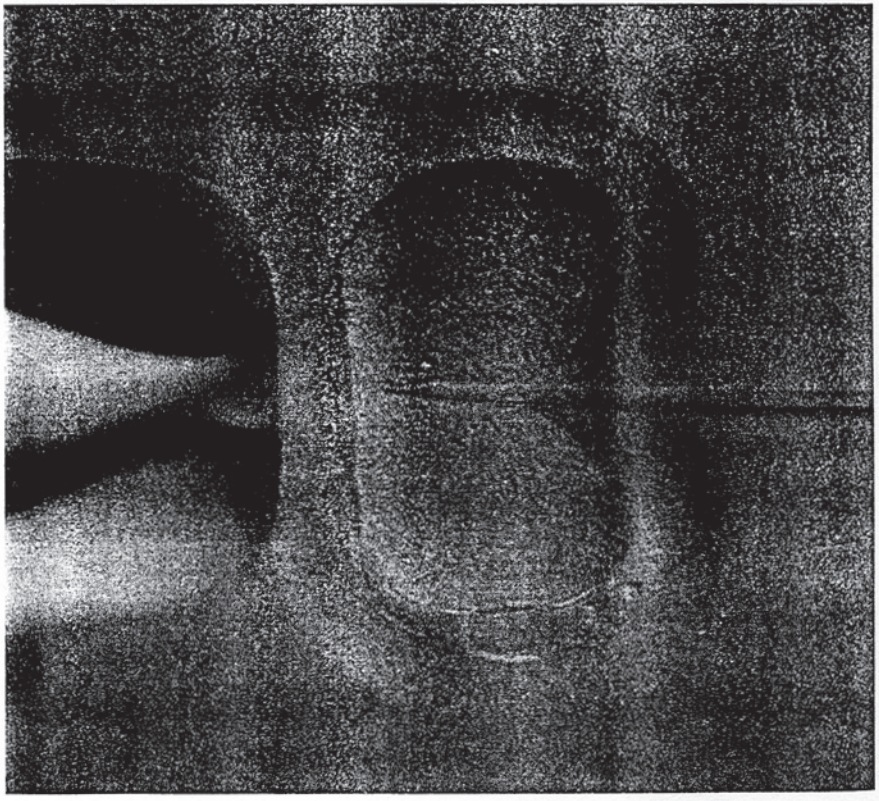
azoospermia and vasectomy reversal. However, very few centres had sufficient experience to obtain such good results. Furthermore, many patients with obstructive azoospermia, e.g. congenital absence of the vas deferens (CAVD) were not reconstructable (Silber, 1980, 1988, 1989*a*,*b*; Silber & Rodriguez-Rigau, 1981; Silber *et al.*, 1988)

With sperm aspiration and extraction techniques, even if the male were absolutely azoospermic, pregnancy rates could be achieved that were no

different from those of a man with a normal sperm concentration (Nagy *et al.*, 1995*b*; Silber *et al.*, 1995*b*). The first successful attempts at sperm aspiration combined with ICSI were reported by Silber and Tournaye in 1994 (Silber *et al.*, 1994; Tournaye *et al.*, 1994). We coined the term 'MESA' for this procedure (microsurgical epididymal sperm aspiration). Conventional *in vitro* fertilization (IVF) with aspirated epididymal spermatozoa, yielded a pregnancy rate of only 9% and a delivery rate of only 4.5%, whereas ICSI with aspirated epididymal spermatozoa in men with CAVD yielded a pregnancy rate of 47% and a delivery rate of 33%. Furthermore, there was no difference in pregnancy rate with retrived epididymal spermatozoa whatever the cause of the obstruction, be it failed vasoepididymostomy, CAVD, or simply irreparable obstruction (Silber *et al.*, 1995*c*).

It was soon realized that testicular spermatozoa could fertilize as well as epididymal spermatozoa, and also give rise to normal pregnancies (Schoysman *et al.*, 1993; Devroey *et al.*, 1995; Silber *et al.*, 1995*d*). This procedure was called TESE (testicular sperm extraction). TESE truly revolutionized the treatment of infertile couples with azoospermia. Even patients with zero sperm motility in the ejaculate or in the epididymis (or even in men with no epididymis) could still have their own biological child, just so long as

Fig. 10.1. ICSI (intracytoplasmic sperm injection).



there was normal spermatogenesis. It also meant that surgeons who had no microsurgical skill would be able easily to perform a testicular biopsy, and thus retrieve spermatozoa which could be used for ICSI without the need for microsurgical expertise in performing a conventional MESA procedure.

Next, it was shown that epididymal spermatozoa, despite fairly weak motility, could be frozen and, on thawing, give pregnancy rates that which were no different from those obtained using active (freshly retrieved) epididymal spermatozoa (Devroey *et al.*, 1994). This meant that a man with obstructive azoospermia could undergo a microsurgical reconstruction, without the need to have his partner prepared for simultaneous IVF. Moreover, spermatozoa could be retrieved from the epididymis at the time of the vasoepididymostomy and could be frozen and stored. Thus, frozen stored epididymal spermatozoa could serve as a back-up for future ICSI procedures without the man having to undergo anymore invasive surgeries or aspirations (Silber, 1989*a*,*b*). This also meant that couples did not have to time their MESA cycle exactly with the woman's egg retrieval, and also that men about to undergo chemotherapy and/or radiation for cancer could have just a single ejaculate frozen and thus have no delay in undergoing cancer treatment. This is because one ejaculate would be sufficient for an almost infinite number of IVF-ICSI cycles.

Sperm retrieval for testicular failure

In the majority of cases of patients with testicular failure (caused by maturation arrest, Sertoli cell only syndrome, cryptorchid testicular atrophy, postchemotherapy azoospermia, or even Klinefelter's syndrome), there is usually a very tiny number of spermatozoa or spermatids which can be extracted and utilized for ICSI (Devroey *et al.*, 1995; Silber *et al.*, 1995*a*,*d*, 1996; Silber, 1995). Men who appear to be making no spermatozoa whatsoever could now have their own biological child. This surprising development came out of basic studies in quantitative analysis of testicular biopsy begun by Steinberger and Zuckerman, and finally continued by Silber and Rodriguez-Rigau in the late 1960s and 1970s (Steinberger & Tjioe, 1968; Zuckerman *et al.*, 1978; Silber & Rodriguez-Rigau, 1981). These early studies of the kinetics of spermatogenesis in the testicle demonstrated that there was often a small degree of spermatogenesis present if one looked quantitatively and carefully at a testicular biopsy of men who were azoospermic from non-obstructive testicular failure. However, the significance of this 'threshold' phenomenon was not appreciated until the era of ICSI, when it was realized that these spermatids could be harvested and normal pregnancy rates achieved in the approximately 60% of such patients who had this miniscule degree of spermatogenesis present in otherwise completely deficient testicles (Silber *et al.*, 1995*c*).

The current ability of most men now to father a child no matter how poor

the sperm count, even if there appears to be no spermatozoon at all, is dramatic. It appeared that the results of ICSI were not related either to the source of the spermatozoa (whether ejaculated, testicular, or epididymal), or to the quality of the spermatozoa (morphology or motility), whether the spermatozoa have been frozen or obtained fresh, whether they were retrieved with ease (as in the cases of normal spermatogenesis or with ejaculated spermatozoa), or whether they have to be extracted in very small numbers directly from the Sertoli cell after hours of painstaking searching of a testis specimen (see Table 10.3). Interestingly, recent research has indicated that there are significantly higher pregnancy rates in couples with testicular aspiration of spermatozoa from men with normal spermatogenesis compared to men with hypospermatogeneis and germ cell aplasia (see Table 8.2, p. 000). However, the age of the female partner is also a significant factor in determining the success of ICSI (Silber *et al.*, 1995*c*; and Table 10.4).

The genetics of infertile men about to undergo ICSI

Genetics of oligozoospermia and germinal failure

From 1975 to 1997, in various previous published reports, 7876 infertile men have undergone karyotyping. Of these, 3.8% were found to have sex chromosomal abnormalities and 1.3% were found to have autosomal chromosomal abnormalities, giving a total of 5.1% chromosomal abnormalities in over 7000 men studied and reported. This compares with the incidence in newborn infants of sex chromosomal abnormalities of 0.14%, autosomal chromosomal abnormalities of 0.25%, and of total chromosomal abnormalities in the newborn population of 0.38% (Koulischer & Schoysman, 1974; Chandley, 1979; Abramsson *et al.*, 1982; Zuffardi & Tiepolo, 1982; Gardelle *et al.*, 1983; Matsuda *et al.*, 1989; Dumur *et al.*, 1990; Yoshida *et al.*, 1995; Tables 10.5 and 10.6).

On average, 2% of men with severe oligozoospermia or oligoasthenoteratospermia, exhibited chromosomal defects, five- to six-fold that of a normal population. These chromosomal defects resulted in a higher rate of miscarriage and transmission of paternal chromosomal defects to the offspring. Therefore, in a very small percentage of infertile men (2%), chromosomal abnormalities appear to be able to interfere with spermatogenesis at the meiotic level. However, there are much more subtle genetic defects that appear to be responsible for male factor infertility and will not show up in routine chromosomal analysis.

In a study initiated by David Page and myself in 1992, 89 men with non-obstructive azoospermia caused either by maturation arrest, Sertoli cell only syndrome, or a combination of these two histological defects, underwent detailed sequence-tagged sites mapping of the Y chromosome (Reijo *et al.*, 1995). This study demonstrated microdeletions in 13% of such azoospermic

Table 10.3. ICSI with ejaculated versus epididymal (fresh and frozen) and testicular sperm

	No. of cycles	*2PN (%)*	*Transfer (%)*	*Clinical pregnancies (%)*
Ejaculated	965	70	93	30
Fresh epididymal	43	56	93	30
Frozen epididymal	9	56	100	33
Testicular	17	48	77	39

2PN, 2pronuclei

From Nagy et al., 1995b.

Table 10.4. Obstructive azoospermia – effect of age of wife

Age of wife	*No. of cycles*	*No. of eggs M-II*	*2PN (%)*	*Normal cleaved embryos*	*Ongoing and delivered per cycle*
<30	48 (26%)	907	382 (42%)	292 (76%)	22 (46%)
30–36	87 (48%)	1111	610 (55%)	413 (68%)	30 (34%)
37–39	22 (12%)	195	104 (53%)	84 (81%)	3 (14%)
40+	25 (14%)	281	147 (52%)	101 (69%)	1 (4%)
Totals	182 (100%)	2494	1243 (54%)	890 (70%)	56 (31%)

2PN, 2 pronuclei; M-II, meiosis-II.

patients. These microdeletions are located at the distal portion of the euchromatic region of the Y chromosome which is positioned approximately in the middle of the long arm of the Y. These microdeletions are being detected with mapping signposts that presently have a sensitivity of only 20 000 base-pairs. Thus, it is possible that many more, much smaller mutations in the Y chromosome, perhaps in this region (*DAZ*), could be responsible for varying degrees of azoospermia and oligozoospermia (Reijo *et al.*, 1995; Silber, 1995; Silber *et al.*, 1995*a*, 1995*c*). This is a particularly difficult region of the human genome to sequence accurately because of the presence of so many confusing 'Y-specific repeats'. That is why this region is so easily prone to spontaneous mutations and perhaps why male infertility is very common in human beings.

Even patients with Klinefelter's syndrome (XXY), who were thought never to be able to father children, often have a minute amount of sperm production that can be discovered in the testis. These patients can undergo the TESE-ICSI procedure, develop normal embryos, and live births have been reported (Tournaye *et al.*, 1996). The question arises of whether spermatozoa from these Klinefelter's patients (presumably like those spermatozoa from other severely infertile men, who seem to yield offspring with a higher

Table 10.5. Autosomal abnormalities observed in infertile men (including azoospermic and oligozoospermic males)

Reference	*Number*	*Robertsonian translocation*	*Reciprocal translocation*	*Inversion*	*Extra marker*	*Total*
Total	7876	45 (0.6%)	36 (0.5%)	8 (0.1%)	8 (0.1%)	104 (1.3%)
Newborn studies	94465	76 (0.08%)	98 (0.10%)	23 (0.02%)	35 (0.04%)	363 (0.38%)

From Van Assche et al., 1996

Table 10.6. Chromosomal abnormalities observed in seven series of infertile men compared to normal newborn population

References	*Number*	*Sex chromosome*	*Autosomes*	*Total*
Koulischer & Schoysman, 1974	1000	27 (2.7%)	6 (0.6%)	33 (3.3%)
Chandley, 1979	2372	33 (1.4%)	18 (0.7%)	51 (2.1%)
Zuffardi & Tiepolo, 1982	2542	175 (6.9%)	40 (1.6%)	215 (8.6%)
Abramsson *et al.*, 1982	342	6 (1.8%)	4 (1.2%)	10 (2.9%)
de Gardelle *et al.*, 1983	318	13 (4.1%)	7 (2.2%)	20 (6.3%)
Matsuda *et al.*, 1989	295	0 (0)	5 (1.7%)	5 (1.7%)
Yoshida *et al.*, 1995	1007	41 (4.1%)	24 (2.4%)	65 (6.5%)
Total	7876	295 (3.8%)	104 (1.3%)	399 (5.1%)
Newborn infants	94465	131 (0.14%)	232 (0.25%)	366 (0.38%)

From Van Assche et al., 1996

incidence of sex chromosomal abnormalities) may be a heterogeneous variety (mosaic) consisting of some that are disomic for XX, and some that are disomic for XY, as well as some that are normal haploid Y and normal haploid X. Thus far, the replaceable embryos for the most part do not have sex chromosomal abnormalities, except for one which was a mosaic of XXY and XY (Handyside, 1993; Liu *et al.*, 1994*b*; Staessen *et al.*, 1996). This matter, therefore, still remains under debate.

Thus, because the ICSI procedure allows us to fertilize the eggs of partners from almost any man, no matter how apparently sterile, we have been able to study and understand better the genetic causes of male infertility, and the possible transference of this male infertility to male offspring generated by the ICSI procedure.

Cystic fibrosis

The genetics of cystic fibrosis and congenital male obstructive sterility have been worked out in great detail thanks to the introduction of ICSI (Silber *et al.*, 1991; Anguiano *et al.*, 1992; Patrizio *et al.*, 1993; and Chillon *et al.*,

1995). In the past, we never dreamed that congenital absence of the vas might be a genetic condition transmitted via the cystic fibrosis gene. The only clue was the clinical observation that all men with cystic fibrosis also have congenital absence of the vas deferens. However, almost all men appearing in fertility clinics because of azoospermia caused by congenital absence of the vas deferens had normal sweat chloride tests and no clinical signs of cystic fibrosis. Yet when these men were studied genetically, we discovered in 1991 and 1992, that 70% had common cystic fibrosis mutations on one allele, and 10% had common cystic fibrosis mutations on both alleles. In those cases where both alleles were affected, one of the mutations was always an extremely mild one. Now we also see men with frank cystic fibrosis, with both alleles having strong mutations, who are also presenting to our fertility clinic to attempt to get their wives pregnant using MESA-ICSI.

It has now been demonstrated that a splicing error in intron 8 called the T_5 allele was found in patients with congenital bilateral absence of vas deferens (CBAVD) who had either no coding mutations, or none on the opposite allele. Thus, CBAVD patients, who are heterozygous for cystic fibrosis, or in both alleles of those who showed no mutations, each have an intron defect in the cystic fibrosis gene that diminishes cystic fibrosis transmembrane regulator gene (CFTR) protein synthesis. This results in a defective production of CFTR protein which was adequate to prevent cystic fibrosis but inadequate to prevent the development of congenital absence of the vas (Dumur *et al.*, 1990).

Thus, the development of ICSI for treatment of the most severe cases of male factor infertility has led to major molecular genetic discoveries that would never have been anticipated in the era of classical andrology.

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Male **Fertility** & **Infertility**

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