overall increased risk for aneuploidy and any unexpected chromosome distribution in comparison to spontaneous abortions of conventional IVF pregnancies. However, in the paediatric follow-up of 130 children conceived by ICSI no difference has been observed as compared to conventional IVF (Bonduelle et al., 1995).

The range of indications for ICSI includes andrological problems which could be the result of not only chromosomal anomalies but also monogenic disorders.

**Risk for monogenic disorders**

The most studied monogenic disorder in this context is cystic fibrosis due to its association with congenital bilateral aplasia of vas deferens which most importantly mandates genetic screening of both partners, and counselling for the couple and appropriate family members (Chillon et al., 1995; Silber et al., 1995). The main problem is that more than 400 mutations in the cystic fibrosis gene are known (Dean and Santis, 1994). Thus, mutation screening can only be performed for the most common alleles, and increasing the search for rare mutations will add further costs and cannot become a routine. Counselling should incorporate this uncertainty.

Probably, screening for Y-chromosome microdeletions will be a necessary routine procedure for men with diverse spermatozoon defects in the near future (Reijo et al., 1995). This notwithstanding, using ICSI may lead to inheritable infertility in male offspring.

Successful treatment of severe male infertility of genetic origin may in the long term change the selection coefficient of these and other monogenic disorders unless preimplantation or prenatal diagnosis could be offered. Presently, only a limited number of centres can offer preimplantation diagnosis. A few IVF clinics in close collaboration with medical geneticists should specialize in treating couples at increased genetic risk.

**Ethical and societal aspects**

Determination of uniform guidelines for the genetic work-up of males with severe infertility as well as consensus on quality and safety will satisfy not only medical but also ethical concerns. The genetic screening in these patients, and the fact that some of them will prove to have an infertility of genetic origin, should not be used to exclude them from appropriate infertility treatment. Rather, the information should be used for adequate genetic counselling before deciding upon treatment. The principle of genetic integrity (Wertz et al., 1996) is of utmost importance and will certainly contribute to intricate counselling situations.

**References**


**Assisted reproductive techniques—are we avoiding the genetic issues?**

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Increased use of direct manipulation of the human gamete has led to powerful innovations in micro-assisted fertilization techniques in recent years. After subzonal insemination (SUZI) intracytoplasmatic sperm injection, ICSI, the most direct method of gamete manipulation, has revolutionized assisted
reproduction by extending the treatment population to include patients with male subfertility previously not considered viable candidates for classical in-vitro fertilization (IVF) (Palermo et al., 1992). Astounding fertilization and pregnancy rates, despite severely compromised semen parameters, have prompted some to advocate ICSI as the primary treatment for all forms of infertility. In addition, more invasive procedures of male gamete retrieval including microsurgical epididymal sperm extraction (MESE) or even testicular sperm extraction (TESE) (Devroey et al., 1995) have enabled achievement of pregnancies even in the presence of azoospermia. The increase in the number of centres using micro-assisted fertilization techniques has led to greater pressure to achieve high fertilization and pregnancy rates. It is important that this competitive environment should not reduce new techniques of micro-assisted fertilization to the mere harvesting and injection of gametes. The new patient population of males with subfertility that has now become amenable to treatment may carry particular risks pertinent to fertility treatment which may still have to be delineated.

Patients with male subfertility are a pathogenetically heterogeneous group. In our opinion it is paramount to come to an accurate final diagnosis of the cause of male subfertility before treatment is instituted. A precise diagnosis enables adequate counselling of the patient, or of a couple, regarding the nature, prognosis and effects of the condition diagnosed. Furthermore, arriving at a diagnosis is helpful in choosing the appropriate mode of treatment and follow-up. This is of particular importance where genetic defects are concerned. The imminent danger of injudicious use of assisted reproductive technologies is the propagation of genetic defects in the treatment population to the offspring. Should suspicion ever arise that these techniques have been used with insufficient prudence, it may be difficult to maintain public acceptance.

In assessing the genetic risk of assisted reproductive technologies, one has to consider the genetic risk inherent to the treatment population as well as the genetic risk inherent to the procedure performed. Experience has shown that neither the preparation of the male gamete by chemical agents nor the manipulation of gametes by ICSI seems to increase the risk of genetic defects in the offspring. However it may be necessary to reconsider this when dealing with sex chromosome aneuploidies (Libaers et al., 1995). The second risk factor which needs to be considered is the genetic risk inherent in the treatment population. It has been suggested that a wide variety of chromosomal anomalies exert an adverse effect on spermatogenesis resulting in oligo- or azoospermia (Lange, 1991). This would explain the high incidence of chromosomal anomalies, particularly translocations and deletions in the male subfertile population (Chandley, 1975). Furthermore, incidence of chromosomal anomalies and semen parameters seem to be inversely correlated (Retief, 1984). In addition new insights have been gained into the presence of gonadal sex chromosome aneuploidy mosaics in cases of non-obstructive azoospermia (Yoshida et al., 1995). The role of cryptic genetic defects is particularly well illustrated by the presence of the cystic fibrosis transmembrane regulator exon mutations in patients with congenital bilateral absence of the vas deferens (Chillon et al., 1995).

As ICSI is currently the treatment of choice in cases of male subfertility, it is used in a treatment population that carries a particularly high risk of chromosomal aberrations. It is especially desirable to know the genetic makeup of the male gamete used for ICSI for a number of reasons. Firstly, there are insufficient prospectively randomized data regarding incidences of chromosomal aberrations in the offspring of patients where ICSI was performed with a known paternal chromosomal aberration. Secondly, there is no precise information regarding fertilization and abortion rates after ICSI in the presence of paternal chromosomal aberration. Abnormal karyotypes are found more commonly in spontaneous abortions and thus may be linked to unsuccessful implantation and growth of the embryo. It is not known whether a similar preselection process prevents implantation of an embryo injected with a chromosomally abnormal spermatozoa after ICSI. Furthermore, giving Progestin supplements after embryo transfer may provide an artificially favourable environment enabling successful implantation despite an abnormal karyotype of the embryo. The 'pre-testing' of sperm karyotype in the laboratory is of academic interest only as the spermatozoan dies in the process and cannot be used for techniques of assisted reproduction. Accordingly, one can never be certain of using a male gamete with a normal karyotype. As spermatogenic arrest resulting from a chromosomal abnormality is inconsistent, the genetic makeup of the gamete is equally unpredictable. Neither sperm morphology nor morphological parameters of embryo quality prior to implantation shows any correlation with karyotype (Martin and Rademaker, 1988; Jamieson et al., 1994). Pre-implantation diagnosis is therefore the only means to determine the karyotype of the embryo prior to transfer. However, using this technique as a screening tool without clear indication of the genetic risk involved seems unwarranted. A definitive diagnosis of the embryonic/fetal karyotype after successful pregnancy can only be obtained by prenatal diagnosis.

The diagnosis of idiopathic male subfertility should be reserved for patients in whom genetic causes have been ruled out. A genetic predilection for subfertility identified in the male partner has several important implications. It may be of psychological importance for the couple to know the cause of the subfertility. Counselling about the genetic risk may actually prompt some couples to choose not to have further treatment. Further, pre-implantation diagnosis or prenatal diagnosis can be reserved for these high risk couples. In addition, deciding against unwanted treatment and avoiding unnecessary tests clearly has financial implications. Although the male could have a sex chromosome aneuploidy mosaic restricted to germ cell tissue (Held et al., 1992), standard lymphocyte culture will identify the vast majority of chromosomal aberrations. Specialized investigation for the presence of CFTR exon mutations should be carried out in males with obstructive azoospermia due to congenital absence of the vas deferens. On the basis of these results the couple should be counselled about the implications of the diagnosis regarding the risks for the offspring. In the cases of CFTR gene mutations the female
partner will need to be screened for heterozygosity of the cystic fibrosis gene.

It would seem that performing ICSI using spermatozoa from chromosomally normal males does not increase the risk of fetal chromosomal abnormalities. Therefore we do not recommend prenatal diagnosis on the basis of ICSI alone unless the woman carries an independently increased risk of chromosomal aberration in the offspring. However, in our opinion, the high incidence of abnormal karyotypes in the male subfertile population clearly warrants genetic testing of the male partner prior to ICSI. If pathology is discovered prenatal diagnosis should be performed.

References

Micro-assisted fertilization and sperm chromosome abnormalities
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Undoubtedly, the application of micro-assisted fertilization (MAF) techniques has been a major advance in the treatment of male reproductive dysfunction but it has also caused a lively debate on possibly associated genetic risks. For instance, speculations about a higher rate of sperm chromosome abnormalities in patients with oligoasthenoteratozoospermia (Ng et al., 1990) or about an elevated level of sperm chromosome breakage in infertile men (Cummins and Jequier, 1994) contributed substantially to concerns about the safety of MAF. Recent articles (De Jong and Pierce, 1995; Meschede et al., 1995; Ng et al., 1995; Patrizio, 1995) have mainly focused on the molecular basis of infertility and the transmission of gene mutations to the offspring resulting from MAF but did not summarize our current knowledge concerning the cytogenetic constitution of the haploid sperm chromosome set. However, in view of the important role in reproductive loss of numerical and structural chromosome alterations and their well-known impact on the development of the affected, surviving children it might be of interest to add some details here.

Mitotic studies based on lymphocyte culture revealed an increased frequency of chromosome abnormalities in men with impaired sperm production. As reviewed by De Braekeeleer and Dao (1991), 12% of the azoospermic and severe oligozoospermic (sperm count <10×106/ml) men have an abnormal karyotype, predominantly Klinefelter’s syndrome (47XXY) and Robertsonian or reciprocal translocations. The rate of chromosome aberrations in the oligozoospermia group lies between 5.1% (Retief et al., 1984) and 6.9% (Bourrouillou et al., 1985) and is considered to be about 10 times higher than in the normal population. Recently, Baschat et al. (1995) reported on constitutional translocations in two out of 32 men with a sperm count between 2–10×106. According to the authors, intracytoplasmic sperm injection (ICSI) was indicated in both cases due to the severe impairment of sperm quality (grade III oligoasthenoteratozoospermia). These results provide evidence that particularly the patients who benefit most from MAF have an enhanced predisposition to produce chromosomally abnormal gametes and transmit cytogenetic aberrations to the embryo. However, about 90% of the subfertile men apparently have a normal somatic karyotype. Are they at an increased risk for de novo germ cell chromosome aberrations? Does the impairment of sperm count, morphology, and function during gametogenesis extend to the cytogenetic constitution of the haploid spermatozooa? An answer to these questions should be expected from direct sperm chromosome analyses.

The male pronuclear chromosome complement can be demonstrated following penetration of human spermatozoa into zona-free hamster eggs. This technique has been used to obtain information on the kind and frequency of sperm chromosome abnormalities in normal men (Rosenbusch and Sterzik, 1994) and to study the meiotic segregation of chromosomes in carriers of constitutional chromosome rearrangements (Martin, 1991; Martin and Hulten, 1993; Cozzi et al., 1994; Martin et al., 1994). However, data on subfertile men in general and those with a normal somatic karyotype in particular have remained rather scarce. Jenderny and Rohrbom (1988) reported on 57 sperm metaphases from a 39 year old man with impaired fertility and various pathological semen parameters. The chromosomal status of somatic cells was not indicated. The total rate of cytogenetic aberrations (10.5%) was comparable to the average rate (8.6%) in spermatozoa from six healthy men with a normal somatic karyotype. Navarro et al. (1990) analysed 30 sperm chromosome complements from a ‘39-year-old, infertile 46XY alcoholic man’ with oligoasthenoteratozoospermia and abundant exfoliation of spermatogenic cells. The patient was further characterized by a partial, complete asynapsis of meiotic bivalents. The total frequency of chromosomal abnormalities (6.7%) was considered to be similar to that found in a normal control