Safety issues in assisted reproduction technology

Should ICSI patients have genetic testing before treatment? A practical proposition to help patient information

K. Aittomäki1,6, U.-B. Wennerholm2, C. Bergh2, A. Selbing3, J. Hazekamp4 and K.-G. Nygren5

1 Department of Medical Genetics, University of Helsinki and Department of Clinical Genetics, Helsinki University Central Hospital, FIN-00029 Helsinki, Finland, 2 Department of Obstetrics and Gynecology, Institute of Women’s and Children’s Health, Sahlgrenska University Hospital, SE-413 45 Göteborg, 3 Department of Obstetrics and Gynecology, Linköping University Hospital, SE-581 85 Linköping, 4 IVF Clinic at Sophiahemmet, SE-114 86 Stockholm, Sweden and 5 Department of Reproductive Medicine, Volvat Medical Center, N-0303 Oslo, Norway

6 To whom correspondence should be addressed at: Department of Clinical Genetics, Helsinki University Central Hospital, PO Box 140, FIN-00029 Helsinki, Finland. E-mail: Kristiina.Aittomaki@hus.fi

ICSI is a highly efficient treatment of male factor infertility and therefore increasingly used to treat infertile men successfully. However, when used to treat patients with a genetic cause for their infertility, there may be an increased risk for the offspring. Chromosome aberrations, Y chromosome microdeletions and CFTR (cystic fibrosis transmembrane conductance regulator) mutations alone may explain up to 25% of azoospermia and severe oligozoospermia. These genetic defects could be identified before treatment, in which case informed decisions could be made by the couple to be treated concerning the treatment, prenatal testing or preimplantation genetic diagnosis. Therefore, we propose that men with very low sperm counts (<5 × 10⁶/ml) considering ICSI should always be informed of the possibility of genetic testing. The information should include a precise statement of the implications of the results for the patient, his family and his offspring, and reassurance that a decision to test or not to test, or the subsequent test results will not be used as a reason for withholding treatment. Testing should always remain voluntary, and the couples themselves should decide whether or not they choose to be tested. If an abnormality is identified, patients should be referred to specialist genetic counselling.

Introduction

Male infertility caused by azoospermia or severe oligozoospermia is known to be due to several genetic factors (Chandley 1998; Johnson, 1998; Maduro and Lamb, 2002). Of these, chromosome abnormalities, such as Klinefelter syndrome due to 47, XXY or certain chromosome translocations, are well known. More recently, submicroscopic deletions of the Y chromosome and specific mutations of known genes such as the CFTR (cystic fibrosis transmembrane conductance regulator) gene have been shown to underlie male infertility. When testing for chromosome aberrations, Y chromosome microdeletions and CFTR mutations, 24% of males with azoospermia or severe oligozoospermia recently were shown to carry a genetic abnormality (Dohle et al., 2002). At present, ICSI is increasingly used for the treatment of male factor infertility in these patients, thereby creating the possibility of causing an unbalanced chromosome aberration, inherited infertility or cystic fibrosis (CF) in the offspring. While genetic testing for these conditions is both recommended (Johnson, 1998; Kurinczuk, 2003; Land and Evers, 2003) and mostly available, it may not be as widely used as could be assumed from the literature. The practice of genetic testing varies between and within countries. In Norway, testing for chromosomal aberrations before ICSI is offered to both males and females, but only to males with non-obstructive oligo- and azoospermia. In Finland, chromosome analysis is usually offered to males with non-obstructive oligo- and azoospermia and their female partners. Positive family history is an important indicator of the presence of a genetic condition underlying infertility (Meschede et al., 2000) and therefore should be routinely collected. However, it is unable to reveal de novo aberrations, such as Y chromosome microdeletions, and is often unreliable in questions of infertility and subfertility (Van Der Avoort et al., 2003). Identifying a genetic cause for infertility has several implications. First, identification of the specific cause of
infertility is in itself important both to the treating professional and to the patient by providing an explanation as to why the patient is infertile. Secondly, the identification of a specific cause may have clinical value relevant to the choice of treatment for an individual couple (Giltay et al., 1999; Nap et al., 1999). More importantly, the possible genetic risk for the offspring can only be assessed individually if genetic testing has been performed. Therefore, we suggest, at this point in time, that at least all ICSI candidates with azoospermia or with a sperm count \( \leq 5 \times 10^5/\text{ml} \) be thoroughly informed about currently available genetic testing procedures, their testing powers and consequences, as explained below. The pre-testing information needs to be structured and concise to enable decision making. Thereafter, the patients should decide whether they would like to go through one or all of these tests before treatment.

Chromosome analysis

Chromosome aberrations are perhaps the best known genetic cause of male infertility. The frequency of chromosome aberrations varies from 1.9 to 12% in infertile males (de Braekeleer and Dao, 1991; Montag et al., 1997; Meschede et al., 1998; Tuerlings et al., 1998a). The most common chromosome aberrations associated with male infertility include numerical sex chromosome aberrations and translocations (Johnson, 1998). Of translocations, the most common Robertsonian translocation between chromosomes 13 and 14 [rob(13;14)] is also most frequently associated with male infertility, although it has not been proven unequivocally that it is the inherent cause of infertility in carrier males. Apart from infertility, the carriers of balanced translocations are healthy; however, for the offspring, there is a varying risk of an unbalanced translocation with a severe phenotype including congenital malformations and mental retardation. Although the risk of an unbalanced form in babies born to translocation carriers (mostly ranging from 0 to 20% depending on the translocation) may be low for some translocations [for rob(13;14), \(<1\%\), recent studies on PGD (preimplantation genetic diagnosis) embryos (Conn et al., 1998; Escudero et al., 2000) and spermatozoa from translocation carriers (Frydman et al., 2001) have shown a much higher risk of aneuploidy. As many of these may not lead to clinical pregnancies or would result in early miscarriage, the possible translocation also affects the results of IVF treatment. Since prenatal diagnosis or preimplantation genetic testing and often both are available, the identification of a translocation is of importance for the couple to be treated.

The value of chromosome analysis of female partners of ICSI patients in general remains unclear, particularly as a proportion of chromosome aberrations identified in studies so far, such as low level sex chromosome mosaicism, are of uncertain importance (Gekas et al., 2001). However, in cases where the indication for ICSI is a poor reproductive outcome, chromosome analysis for both partners should be considered.

The implications of identifying a chromosome aberration

- Certain chromosome aberrations are a highly probable explanation for low sperm counts, although it is not always possible to conclude unequivocally whether a translocation is causal or not.
- Numerical sex chromosome aberrations (such as 47,XXY) are not inherited from the patient’s parents and there is no risk for the same condition for the siblings. Translocations can either be de novo rearrangements or inherited, in which case the information is also relevant to other family members, particularly siblings of the patient.
- Carriers of balanced translocations are healthy, but may be infertile.
- In most cases, there are three possibilities for the offspring of a translocation carrier: the translocation may not be inherited; it may be inherited in a balanced form (which the carrier parent has); or it can be inherited in an unbalanced form. Unbalanced chromosome translocations usually cause congenital malformations and mental retardation, and there is a high rate of spontaneous abortions in these pregnancies.
- When a person is a carrier of a chromosome abnormality, prenatal testing and PGD are an option to study the chromosomes of the fetus or the embryo.

Even after normal results of parental karyotyping, there may be a higher risk of chromosomal rearrangement in a child born after ICSI. Based on fluorescence in situ hybridization (FISH) studies, it has been suggested that some infertile men with a normal lymphocyte karyotype have a high frequency of chromosomally abnormal spermatoocytes (Rubio et al., 2001). In addition, sperm retrieved with testicular sperm extraction (TESE) and used for ICSI seem to result in a higher percentage of chromosomally abnormal embryos compared with ejaculated sperm (Silber et al., 2003). The present studies also suggest that sperm aneuploidy is associated with implantation failure and early fetal loss, thus lowering the success rate of treatment (Bernardini et al., 1998; Pang et al., 1999; Burrello et al., 2003).

Y chromosome microdeletions

In a proportion of subfertile men, loss of genetic material (deletion) from the long arm of the Y chromosome can be demonstrated. The existence of a spermatogenesis-controlling factor(s), called azoospermia factor (AZF), in this chromosomal area was first based on identification of large deletions in azoospermic and oligozoospermic men (Tiepolo and Zuffardi, 1976). Subsequently, it has been shown in many studies that smaller submicroscopic deletions, unidentifiable in normal karyotyping, called microdeletions are also found in infertile men. These microdeletions are identified with a special test (Simoni et al., 1999; Simoni, 2001). The overall prevalence of Y chromosome microdeletions in this group of patients is 8.2%, but the frequency is largely dependent on the type of patients being studied and is higher in azoospermic or severely oligozoospermic males (Foresta et al., 2001). In men with a sperm count \( >5 \times 10^5/\text{ml} \), the frequency is very low. There are three specific regions of deletions on the Y chromosome, namely AZFa, AZFb and AZFc, which have been defined by
the deletions found in infertile men (Vogt et al., 1996). Microdeletions of the Y chromosome are due to recombination of nearly identical DNA sequences flanking the deleted region (Kuroda-Kawaguchi et al., 2001; Repping et al., 2002). The deletions usually arise de novo in the sperm and have not been inherited from a fertile father in normally conceived pregnancies (Page et al., 1999). However, once a deletion has occurred, it will be inherited by all male offspring if the infertility is treated with ICSI. The microdeletions seem to be stable when inherited, as no change in the size of the AZFc deletion was identified in the study by Oates et al. (2002). No sex chromosome aneuploidies were found in the offspring of the AZFc-deleted men in this study, although it has been suggested that Y chromosome microdeletions may predispose to sex chromosome aneuploidies and therefore also to phenotypes associated with sex chromosome mosaicism (Siffroi et al., 2000; Patsalis et al., 2002).

The implications of identifying a Y chromosome microdeletion

- A highly probable explanation for the low number of sperm.
- The deletions are almost always the result of a de novo deletion in the patient and are not inherited from the father of the patient.
- The deletion is always inherited by all male offspring of a man who carries the deletion as sons always inherit the Y chromosome from their father. Daughters will not inherit the deletion.
- The male offspring who inherit the microdeletion are likely to be infertile/subfertile as adults.

The microdeletions cause spermatogenetic failure due to loss of important genes residing in the AZF regions. It is possible that some infertile men do not have microdeletions but carry mutations in the actual genes within the AZF regions, although only one case has been demonstrated to date (Sun et al., 1999). These mutations would also cause inherited infertility in male offspring, although such mutations cannot be tested for. Based on genetic studies on 621 infertile couples treated with ICSI, Meschede et al. (2000) state that male factor infertility should be considered a potentially heritable condition. Therefore, it is not possible to exclude entirely inheritance of infertility by male offspring through microdeletion or other present testing, but it is possible to identify families in whom this would be inevitable. For these patients, the inheritance of a microdeletion could be avoided by using donor sperm, if this option is more acceptable to the patient (Nap et al., 1999). More recently, preimplantation genetic testing and sex selection have also been suggested, as these patients would have to be treated with ICSI anyway.

Genetic testing for the CFTR gene

CF is an autosomal recessively inherited multisystem disorder causing pulmonary disease, which is the major cause of both morbidity and mortality of CF. The disease also affects the exocrine pancreas, intestine, hepatobiliary system, sweat glands and male genital tract. The majority of males with CF have obstructive azoospermia due to congenital bilateral absence of the vas deferens (CBAVD) (Kaplan et al., 1968). More recently, it was found that CBAVD may also occur as the only manifestation of CF without pulmonary or gastrointestinal signs (Rigot et al., 1991; Anguiano et al., 1992).

CF is caused by mutations in the CFTR gene located on chromosome 7. Over 900 mutations have been described in the gene. CBAVD usually results from a combination of a severe mutation with a mild CF mutation or with a specific intronic variant (5T). Unfortunately, routinely used clinical testing identifies far fewer mutations (45–80%) than are found in research studies. This, however, varies in different populations due to recurrent mutations, which are more prevalent in some populations (Mak et al., 1999; Claustres et al., 2000). As genetic testing is not able to identify all causative mutations, it then follows that a negative test does not exclude the existence of an unknown mutation. However, when a patient tests positive, i.e. has CBAVD due to CFTR mutations, there is a risk for both male and female offspring to have CF and for male offspring to have CBAVD. The risk for the offspring depends on whether or not the spouse is a carrier, since one mutated allele will always be inherited from the affected male. As the carrier frequency of CFTR mutations in many Caucasian populations is in the order of 1:22–28, it is recommended that genetic testing for CFTR mutations be offered to the partners of men with CBAVD prior to treatment. In performing such testing, it is important to note that the 5T variant frequently found in patients with CBAVD is often not routinely tested for in clinical testing panels.

The implications of identifying CFTR mutations

- CFTR mutations would be an explanation for obstructive azoospermia.
- One mutated allele will always be inherited from the affected male by the offspring.
- Genetic testing for CFTR mutations should be offered to the partner as CFTR mutations have a high carrier frequency (1:22–28) in many populations. If the partner of a male with CBAVD is a healthy carrier of a CFTR mutation, there usually is a 25% risk of CF to all children and a 25% risk of CBAVD in male offspring.
- If a partner is a carrier, prenatal testing or preimplantation genetic testing is offered.

Different mutations of the CFTR gene cause varying phenotypes, and therefore it is sometimes difficult to predict the phenotype in the offspring. Although previously suggested, mutation testing is not useful in men with idiopathic oligozoospermia or azoospermia (Tuerlings et al., 1998b).

Discussion and conclusions

There are two important principles for genetic testing: the right of every person to decide whether or not they want to be genetically tested and the right to informed decision making. How do these principals apply to genetic testing associated with infertility treatment? Although, judging from the available medical data, there may be a high risk for a person to carry a certain genetic condition, the decision about whether or not genetic testing is performed is always a decision to be made by
the person to be tested and this must also remain the practice when treating infertility. Furthermore, this also means that treatment must not be withheld if the patient decides not to be tested, although there is a high risk for affected offspring. To enable decision making, the patients need to be informed about the tests, which means that the treating professionals at the IVF clinics need to have knowledge on the genetics of infertility and genetic testing.

If testing is performed and an abnormality is identified, professional genetic counselling should be offered. In a genetic counselling session, patients are provided with an explanation of the cause of the genetic defect they have been identified with by an expert in genetics. More importantly, the consequences for the person tested, his future children and his family members are discussed and, if necessary, further counselling and testing for other family members is organized. The options for treatment of infertility considering the genetic aspects, prenatal diagnosis or PGD are also discussed. At all times, it must be clear to all parties that infertility treatment will not be withheld on genetic grounds if the patient choses to go ahead with ICSI treatment despite positive genetic testing. Indeed, Giltay et al. (1999) found that out of 75 patients that tested positive for chromosomal aberrations, 44 decided to proceed with ICSI treatment, while most patients (79%) with microdeletions chose ICSI treatment in the study by Nap et al. (1999).

It is clear that men with non-obstructive azoospermia should have both karyotyping and Y chromosome microdeletion screening. However, it is not as easy to set criteria on testing when the patient is oligozoospermic. The recent report of the ESHRE consensus meeting suggests chromosome analysis for oligozoospermia with $<5 \times 10^6$ sperm/ml, but Y chromosome microdeletion testing with $\leq 1 \times 10^6$ sperm/ml (Land and Evers, 2003). In a comprehensive review of the literature on Y chromosome microdeletions, Foresta et al. (2001) state that most studies have been performed on oligozoospermic men with $<5 \times 10^6$ sperm/ml and the overall prevalence of microdeletions in these studies is 10.5% while prevalence in patients with a higher sperm count is very low. Based on this and on the practicalities of testing, as Y microdeletion testing is much easier than chromosome analysis, we suggest using the same criteria ($<5 \times 10^6$ sperm/ml) for both although the proportion of positive results is lower when less stringent criteria are used. This could be debated further, as Cruger et al. (2003) recently studied 392 men referred for ICSI and found that Y chromosome microdeletions were present in only 2% of men with extreme oligozoospermia and 6.5% of those with azoospermia. They suggest that all couples referred for ICSI should be offered chromosome analysis but only males with $<1 \times 10^6$ sperm/ml should have microdeletion testing.

Although genetic causes of infertility can now be identified in a proportion of patients, it is clear that in the future, genetic causes will be identified in a much larger number of patients than today and we may need to consider testing options other than those available at present. It is also likely that interpreting the results and communicating their significance to the patients will become more difficult as alleles bearing a lower risk are identified. At the same time, new methods of treating infertility are increasingly applied to help families with known genetic diseases. This all means that in the future there will be a much greater overlap between reproductive medicine and genetics and that a close collaboration between professionals working in these two fields is imperative in treating patients with infertility in the best possible way.

Acknowledgements

We thank Organon Nordic for making the writing of this article possible.

References


Kurinczuk JJ (2003) From theory to reality—just what are the data telling us about ICSI offspring health and future fertility and should we be concerned? Hum Reprod 18,925–931.

The AZFc region of the Y chromosome features massive palindromes and uniform recurrent deletions in infertile men. Nature Genet 29,279–86.


