OPINION

Are there genetic risks associated with microassisted reproduction?

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Of all the techniques available for microassisted reproduction, the direct injection of individual sperm cells or spermatids into the cytoplasm of the oocyte (ICSI) is the most invasive, through which any possible selection against sperm cells with genomic defects would be excluded. It has, however, been shown that such a possible selection is present neither in the female genital tract nor at the zona pellucida. Selection against genetic-based defects occurs after the fertilization of the oocyte, during both embryonic and fetal development. Based on the data to date, it can be assumed that ICSI would not result in either a significant increase in genetic-based diseases, or in an increase in the number of infertile males. If, however, mutations of X-chromosomal or Y-chromosomal genes should play a major role in male fertility disorders, one could expect, over generations, an increase, though probably very slight, in the number of males with such disorders.

Key words: fertility disturbances/genetic risk/intracytoplasmic sperm injection/malformations/sperm selection

Introduction

The various techniques of microassisted reproduction can be used to help fulfill the desire for children of couples when less invasive techniques such as in-vitro fertilization (IVF), gamete intra-Fallopian transfer (GIFT) etc., fail. However, the former techniques should be reserved for couples in whom the cause of infertility or subfertility is andrological or where fertilization of the egg is not possible despite morphologically and functionally normal sperm cells and one can assume that the disturbance lies at the level of the zona pellucida. It must be noted that the fundamental causes of male infertility, as well as defects of the zona pellucida, remain unclear. The reason for this lies in our limited understanding of the molecular mechanisms involved in male germ cell differentiation, sperm–egg interaction and the process of fertilization.

The most invasive technique for microassisted reproduction is intracytoplasmic sperm injection (ICSI), i.e. the direct injection of a single spermatozoon into the oocyte. This technique, first described for use in human pregnancy by Palermo \textit{et al.} (1992), is now used worldwide. In Germany alone, there are more than 30 clinics employing ICSI. As opposed to IVF, ICSI has proved to be a considerable success. ICSI can be used successfully in couples for whom pregnancy cannot, or can very rarely, be achieved through IVF. For example, in the case of prominent oligozoospermia or teratozoospermia; absence of an acrosome in the sperm cells; immotile sperm cells or congenital bilateral absence of the vas deferens (CBAVD). Nagy \textit{et al.} (1995) showed that the only criterion for successful implementation of ICSI is the presence, in the ejaculate sediment, of one live sperm cell per oocyte.

It has since been shown that not only can sperm cells in the ejaculate be used but also those found in the epididymis (micro-epididymal sperm aspiration, MESA) or in the testis itself (testicular sperm aspiration, TESA). Even in the case of the Sertoli cell-only syndrome, sperm cells have been found in the testis of 50% of the patients (Silber \textit{et al.}, 1995b). Also, sperm cells have been isolated from the testes of a patient with Klinefelter syndrome (47XXY) and successfully used in ICSI (Staessen \textit{et al.}, 1995). Presently, methods are being developed in which haploid spermatids can be used in cases where the testis contains no sperm cells (Edwards \textit{et al.}, 1995; Vanderzwalmen \textit{et al.}, 1995).

Controversy surrounds the use of ICSI, especially in Germany, with regard to genetic risks which may result from the use of this technique (Betendorf, 1994; Diedrich \textit{et al.}, 1995; Engel and Schmid, 1995). The medical service for health insurance (Medizinische Dienst der Krankenkassen, MDK) has adopted a policy of rejection with regard to ICSI, as they assume that ICSI will result in a rise in the level of genetic disorders and chromosomal aberrations among the resulting children. There is also an international debate on the use of ICSI (Bhasin \textit{et al.}, 1994; Bourne \textit{et al.}, 1995; Butler, 1995; Ng \textit{et al.}, 1995; Patrizio, 1995; Silber \textit{et al.}, 1995a; Yanagimachi, 1995). However, as has been pointed out, too few children resulting from the use of ICSI have been examined, to draw any final conclusions with regard to the question of genetic risks. This is reminiscent of a similar situation involving the introduction of IVF which also proved controversial. Statistically, as in the case of IVF, at least 2000 children from ICSI-aided pregnancies are required in order to detect a possible rise in the level of genetic disorders and chromosomal aberrations resulting from ICSI. The views of the MDK as well as those found in the literature are based on the assumption that, during normal reproduction, selection...
against sperm cells with genetic alterations (mutations) or chromosomal disturbances takes place in the male/female genital tract and at the zona pellucida. As such a selection would be eliminated with ICSI, the risk of disability among the resulting children would be expected to rise.

We appreciate the moral and ethical doubts concerning modern reproductive techniques and, in particular, ICSI. It is indeed a decisive question whether or not all techniques which are possible as a result of advances in modern reproductive medicine should actually be employed. This area is similar to other areas of medical advancement, such as gene technology and preimplantation diagnostics. For the sake of our patients, we should permit our scientific knowledge to guide any decisions made in this regard. Here we present relevant biological observations which support the following statement: 'It is justified to assume that ICSI, the most invasive micro-assisted reproduction technique available, will not lead to a relevant increase in genetically based diseases or genetically based infertility/subfertility'. The question of whether or not ICSI-assisted pregnancies will lead to lower birth weights, as is the case with IVF, cannot yet be answered (Tarin and Cano, 1995). However, it should be noted that assisted pregnancies result in a higher frequency of multiple pregnancies.

Factors necessary for the development of the human embryo

We shall now deal with the question of which presently understood biological factors are required for normal development of the human embryo. The male haploid genome and the male germ cell proximal centriole constitute, together with the mature oocyte, the major factors necessary. After the completion of meiosis the spermatids contain only a haploid genome. It has been well documented that embryonic development requires this paternal haploid genome, without which functional extraembryonic tissue (e.g. placenta) will not develop. It has also been shown that in human embryonic development, as opposed to that of the mouse, the male proximal centriole plays a crucial role (Sathananthan et al., 1991; Palermo et al., 1994; Simerly et al., 1995), being responsible for the building of the first zygotic spindle and thus for the creation of the 2-cell stage. The proximal centriole is first detectable in middle spermatid stages, thus permitting the successful use of such cells in ICSI. It remains unclear if other elements of the male germ cell are required for normal embryonic development (Ménézo and Dale, 1995), but it is quite probable, especially when one considers the relatively low level of success of ICSI using immotile sperm cells (Silber, 1995). There will, of course, be couples who lack genetically determined factors which may be crucial for, for example, oocyte activation, completion of the second meiotic division of the oocyte or fusion of the maternal and paternal genomes. Even the use of ICSI cannot presently help such couples. However, important scientific work is already being carried out directed towards these problems (Dozortsev et al., 1994, 1995; Janny and Ménézo, 1994; Sousa and Tesarik, 1994; Asch et al., 1995; Gearon et al., 1995; Simerly et al., 1995). Overall, the assumption, that the morphological and functional differentiation of sperm cells and their large number present in the ejaculate, serves, from an evolutionary stand-

Two aspects of a possible rise in the rate of genetically-determined defects due to ICSI

The possible risk of a rise in the level of genetically determined defects following ICSI consists of two aspects: firstly, the aspect of genetically determined diseases and disabilities, and, secondly, the aspect of genetically determined infertility/subfertility. The above statement is valid for both aspects.

Could ICSI lead to a rise in the rate of genetically determined disorders after ICSI?

The rate of severe disorders among newborns is estimated to be 1–2%. An examination of the rate of disorders among newborns, born after IVF, GIFT or zygote intra-Fallopian transfer (ZIFT), reveals no significant deviation from this figure. The Society for Assisted Reproductive Technology (SART), the American Fertility Society (AFS, 1993), reports the rates of disorders after IVF, GIFT and ZIFT to be 1.5, 1.1 and 2.1% respectively. What do these results tell us?

Without doubt morphologically and motility defective sperm cells, as well as normal sperm cells, are eliminated in the female genital tract. Despite the absence of this selection there is no difference in the frequency of disorders between normally born children and those born where the sperm cell does not travel through the female genital tract.

The crucial difference between IVF and ICSI, with respect to selection against sperm cells with genetic defects, consists of the involvement of the zona pellucida. It is well documented that morphologically and/or functionally defective sperm cells are selected against at the zona pellucida. There is, however, no evidence that morphologically and functionally normal sperm cells carrying a mutation in one gene are prevented from entering the zona pellucida. If sperm cells carrying mutations in, for example, the genes for Huntington's chorea, cystic fibrosis, chondrodystrophy or epidermolysis bullosa penetrated the zona pellucida at a lower frequency than sperm cells without such mutations, one would not expect to observe Mendelian inheritance in such cases. However, Mendelian inheritance is observed in all cases of single gene inherited diseases known (numbering 4500 to date). In the case, for example, of Huntington's chorea, an autosomal dominant disease, the probability of an affected man passing on the defective gene to an offspring is 50%. The Mendelian laws of inheritance clearly show that sperm cells carrying single gene defects in their genome (DNA) are not selected against in the testis, male or female genital tract or at the zona pellucida.

Such a selection against morphologically and/or functionally defective sperm cells at the zona pellucida would be expected to affect the frequency of disorders among children born after ICSI if such sperm cells are more likely than normal sperm cells to carry genetic defects, such as chromosomal aberrations. While in the case of normal fertilization and IVF this would not play a role, it would lead to an increase in the number of disorders among children after ICSI.
Figure 1. Hybridization of lymphocyte metaphases (a, b, c) and sperm cell nuclei (a'-c'') with α-satellite DNA probes specific for chromosome 1 (a-a''), chromosome 7 (b-b'') and chromosome 17 (c-c''). Normal sperm cell nuclei are shown in a', b' and c', each with one distinct hybridization signal. Sperm cell nuclei with numerical chromosomal aberration are shown in a'', b'' and c'', each with two hybridization signals, representing a disomy for the respective chromosomes. The arrows (a'-c'') mark the darkly stained base of the flagella. For details on the method of in-situ hybridization see Guttenbach et al. (1994a,b).

There are extensive details in the literature dealing with the frequency with which chromosomal aberrations are present in the sperm cells of fertile men. Among the results, those obtained using hamster oocytes lacking a zona pellucida are of lesser relevance. Using this system, the number of sperm cells on which chromosomal analysis can be carried out is limited and, due to the use of a heterologous system, these results must be treated carefully. This view is supported by the frequent observation of chromosomal fragments during sperm cell chromosomal analysis (Banduff et al., 1988; Genesca et al., 1990). Even if such chromosomal fragments do occur in sperm cells, they would, generally, be expected to disappear after a few rounds of mitotic division of the fertilized oocyte due to the lack of centromeric structures. Over the last few years there have been major advances in the field of molecular biological techniques, especially in the area of non-radioactive in-situ hybridization (NISH), which permit the analysis of sperm cell chromosomes without the need for a heterologous system. For every human chromosome, specific probes exist which can be used for hybridization of the sperm cell nucleus. Whether a particular chromosome is present once (as is the expected case in a haploid sperm cell) or more than once, can be precisely determined from the number of hybridization signals observed. An example of such a NISH analysis of sperm cells from a fertile man is shown in Figure 1. The most extensive work carried out in this area has been presented by Guttenbach and Schmid (1990, 1991) and Guttenbach et al. (1994a,b). This group examined >110 000 sperm cells from 20 fertile men, aged 23–57 years, using fluorescent in-situ hybridization (FISH) using chromosome-specific, centromeric (α-like) DNA probes. They observed that the frequency of meiotic non-disjunction for each autosome and sex chromosome is approximately equal. The rate of disomy for a particular chromosome (e.g. there are two rather than one chromosome 18 in the sperm cell nucleus) was estimated to lie between 0.31 and 0.41%. No significant difference between the rate of disomy for the individual chromosomes, nor correlation with the age of the man was observed, although differences between individuals were observed. That would imply that ~10% of the sperm cells from a fertile man carry an additional chromosome. However, these men have successfully reproduced and their children are quite healthy.
Where, then, are the newborns with chromosomal aberrations which would be expected to have been born? The rate of chromosomal aberrations among newborns is estimated to be 0.4–0.6%.

Selection against chromosomally unbalanced sperm cells, of these fertile men, at the zona pellucida could play a role, if such sperm cells were more often morphologically or functionally defective. They would be expected to be stopped at the zona pellucida. However, one can conclude from the results of NISH carried out on morphologically normal sperm cells presented above that chromosomal aberrations per se do not lead to morphological and/or functional defects in mature sperm cells.

Perhaps then, chromosomal aberrations are more common in the sperm cells of subfertile men who may consider ICSI. There has, as yet, been no extensive examination of such men using NISH for this reason. However, some data on the sperm chromosomal constitution of subfertile men show that the rate of chromosomal aberrations does not differ from that of fertile men. Martin and Rademaker (1988) have shown that there is no correlation between sperm cell morphology and chromosomal aberrations, Rosenbusch et al. (1993) present data which show no positive correlation between the proportion of morphologically abnormal sperm cells and the proportion of sperm cells with chromosomal aberrations and Miharu et al. (1994) observed no difference in the frequency of numerical chromosomal aberrations in the sperm cells of fertile and infertile/subfertile men. We have carried out FISH analysis of the sperm nuclei of 40 infertile men with various sperm cell defects (M.Guttenbach et al., unpublished). As in the case of fertile men, we observed differences in the frequency of chromosomal aberrations between individuals, but no evidence that such aberrations are more frequent among infertile men than among fertile men.

Thus it can be concluded that morphologically defective sperm cells are not more likely to carry chromosomal aberrations. The zona pellucida selects against sperm cells with morphological defects but not against sperm cells carrying chromosomal aberrations. Strong evidence supporting this view is provided by the observation of penetration of the zona pellucida and successful fertilization by human sperm cells with a disomy for chromosome 1 (Watt et al., 1987) and the presence of an additional chromosome of paternal origin in children with trisomies: 100% of patients with 47XYY; ~50% of patients with 47XXY; 5% of patients with either 47XXX, trisomy 21 or trisomy 13 (reviewed in Fisher et al., 1993; Williams et al., 1993). In these cases a sperm cell with an additional chromosome was responsible for fertilization. Similarly, successful fertilization has been observed in mice and other species involving morphologically intact sperm cells carrying chromosomal aberrations (Stolla, 1984).

Chromosomal analyses or testis biopsies reveal chromosomal aberrations in ~5–7% of all men with subfertility. Such aberrations primarily affect the diploid stage of spermatogenesis and result, in general, in azoosperma or prominent oligozoospermia. Among these men, cases of familial chromosomal translocations are observed. However, it is often the case that the fertility of other male members of the family, who also carry the translocation, remains unaffected (Figure 2). These men can thus pass the chromosome containing the translocation onto the next generation, which implies that sperm cells, of the male relatives, which carry the translocation, are capable of normal fertilization. These observations support the statement that sperm cells carrying chromosomal aberrations are not selected against in either the male genital tract, female genital tract or at the zona pellucida.

On average, 50% of the sperm cells of men with chromosomal translocations are chromosomally unbalanced. The frequency is 19–77% depending on which chromosomes are involved (Martin and Spriggs, 1995). For such a man, the probability of a child being born with the unbalanced translocation and a severe disability is 5–20%. This reduction in the probability of a child with such a disorder being born cannot be due to selection against chromosomally unbalanced sperm cells in the genital tract or at the zona pellucida. The frequency of chromosomally unbalanced fetuses for which the father carries the translocation is no different to that for which the mother carries the translocation (Boué and Gallano, 1984). The reduction is far more likely to be due to a prenatal selection against embryos with the translocation (i.e. an increase in the level of spontaneous abortions). In this regard, we advise that a lymphocyte chromosomal analysis should be carried out for every patient with either a greatly reduced sperm cell count (<10X10⁶/ml) and/or severe morphological defects of the sperm cells, especially before ICSI. Baschat et al. (1995) examined the karyotypes of 32 men with a sperm cell count of between 2X10⁶ and 1X10⁶ before carrying out ICSI. They found reciprocally balanced translocations in two men with oligozoospermia (OAT) III.

Subfertile or infertile men are, generally, perfectly healthy. There are only a few examples of infertile/subfertile men who also have physical disabilities. One example is the Kartagener...
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Oocytes 32%
Sperm cells 8%
Fertilized oocytes 37%
Preimplantation embryos 20.6%
1st trimester embryos 8-10%
Newborns 0.6%

Figure 3. Frequency of chromosomal aberrations during human prenatal development. Plachot et al. (1987) estimated the frequency of chromosomal aberrations in unfertilized oocytes, sperm cells, fertilized oocytes and in various stages of prenatal development. The rate of chromosomal aberrations decreases during embryonic development, from 37% in the fertilized oocyte to 0.6% in the newborn.

The Kartagener syndrome is autosomal recessive, i.e. the individual is affected only when the defect is present on both homologous chromosomes. Heterozygotes are perfectly healthy. If the sperm cells from a man with Kartagener syndrome are used in ICSI, the resulting child is usually heterozygous for the defective dynein gene. Such children are healthy and the sperm cells of the male children are motile. The same is true for other autosomal recessive diseases associated with male infertility. Such autosomal recessive diseases are so rare that there is, practically, no relevant genetic probability of homozygosity for this genetic defect, apart from the case of intra-familial marriages. There are no autosomal dominant, X-linked recessive or Y-linked diseases known for which both physical disorders and male infertility have been reported. There are, however, a large number of mice mutants which, in the homozygous state, are both infertile and have severe disorders. But all heterozygous animals are fertile and physically normal (Cebra-Thomas and Silver, 1991; Chubb, 1993).

Selection against DNA mutations in the sperm cell genome occurs post-conceptionally. A total of 98% of all embryos with chromosomal aberrations will be spontaneously aborted (e.g. 100% of trisomy 16 embryos; 80% of all trisomy 21 embryos; 99% of all Turner syndrome, 45X0 embryos; chromosomal aberrations are found in 60% of all spontaneous abortions) (Hansmann, 1993). The observations of Plachot et al. (1987) show just how successful prenatal selection against chromosomally unbalanced embryos is. These authors estimated the frequency of chromosomal aberrations in oocytes at 32%, in sperm cells at 8%, in fertilized oocytes at 37%, in preimplantation embryos at 20.6%, in 1st trimester embryos at 8-10% and in newborns at 0.6% (Figure 3). Bourgoyne et al. (1991) found chromosomal aberrations in 5% of induced abortions carried out in the seventh week of pregnancy. Such strong selection against chromosomally unbalanced embryos and fetuses has also been reported in other mammals. This has been very clearly demonstrated in the mouse. Gropp et al. (1983) developed a mouse model with which it is possible to generate trisomies for each of the 19 autosomes. As shown in Figure 4, embryos and fetuses with trisomies for the various chromosomes are eliminated at different times during development, only trisomy 19 fetuses survive till birth.

In the case of humans, such stringent prenatal selection is not only applicable to chromosomally determined but also to non-chromosomally determined birth defects. A summary of the results of a study by Nishimura (1970) is presented in Table I. This group compared the frequency of various defects among embryos in the second month of pregnancy with those of newborns. The embryos from the second month of pregnancy were from abortions carried out for non-medical reasons. Table I shows that the rate of defects among embryos in the second month of pregnancy is clearly higher than in the newborns. Thus, there is prenatal selection against defects even in cases where such defects either do not affect prenatal development (e.g. cleft lip) or are not even pathological (e.g. polydactyly) (see review in Witschi, 1970).
Table 1. Frequency of various malformations found in embryos and newborns

<table>
<thead>
<tr>
<th>Malformations per thousand</th>
<th>Second month of pregnancy</th>
<th>Newborns</th>
<th>Reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anencephaly</td>
<td>2.8</td>
<td>0.64</td>
<td>4.4X</td>
</tr>
<tr>
<td>Myeloschisis</td>
<td>3.0</td>
<td>0.21</td>
<td>14.3X</td>
</tr>
<tr>
<td>Hydrocephalus</td>
<td>7.3</td>
<td>0.33</td>
<td>22.2X</td>
</tr>
<tr>
<td>Cyclopia</td>
<td>6.2</td>
<td>0.06</td>
<td>103.3X</td>
</tr>
<tr>
<td>Cleft lip</td>
<td>9.0</td>
<td>2.3</td>
<td>3.7X</td>
</tr>
<tr>
<td>Cleft lip and palate</td>
<td>21.4</td>
<td>2.3</td>
<td>6.7X</td>
</tr>
<tr>
<td>Polydactyly</td>
<td>9.1</td>
<td>1.0</td>
<td>90X</td>
</tr>
</tbody>
</table>

*Data taken from Nishimura (1970). We can conclude from these results that during embryonic development there is a strong selection against not only severe defects (e.g. anencephaly) but also against minor, non-pathological disorders (e.g. polydactyly).

Such examples demonstrate just how strong the selection against embryonic defects is. One can, thus, conclude that even if ICSI should increase the number of defective embryos, an increase in the level of children born with disorders, as compared to unaided pregnancies, cannot be expected. The Brussels ICSI group recently reported a total of 669 ICSI-assisted births, of which 18 children (2.7%) had serious birth defects (Bonduelle et al., 1995). This rate of birth defects does not represent a significant difference to the normal rate, especially when one takes into account the number of twins and triplets born. The critics of ICSI should always take into consideration the well documented evidence that the rate of defects is increased by two to three times (up to 5%) in multiple births. The group also reported no significant increase in the rate of chromosomal aberrations (1.2%), and the rate of spontaneous abortions was not higher than that in IVF pregnancies. However, despite these biologically orientated observations and their obvious results, we are of the opinion that chromosomal analyses should be carried out on all couples who decide on ICSI, until the data are statistically significant (i.e. >2000 ICSI-aided births). Within the framework of ICSI-related genetic counselling, detailed pedigrees should be made to identify familial genetic risks. This should be followed by prenatal chromosomal diagnostics (chorionic villus sampling or amniocentesis) and, in order to identify physical defects, a high-resolution ultrasound examination in weeks 18–22 of pregnancy.

ICSI, CBAVD and CF

ICSI is of ever increasing importance for couples where the male partner suffers from an azoospermia due to either a congenital bilateral aplasia of the vas deferens (CBAVD), or an exogenously determined obstruction of the epididymis or ductus deferens. The rates of pregnancies and births resulting from ICSI, using sperm cells isolated from the epididymis of such patients, do not differ from the rates observed after conventional IVF (Silber et al., 1994). It is now well known that there is a connection between cystic fibrosis (CF) and CBAVD (CBAVD is found in 97–98% of all men with CF). CF is an autosomal recessive disease with a frequency of 1:2500 among newborns, every 25th person being heterozygous for a mutation in the CF gene. If ICSI is carried out using sperm cells from a man with CF, and assuming the partners are not related and that no case of CF has been reported for relatives of the female partner, the risk of the couple having a child with CF is 2%. Over 500 different mutations have been reported in the CF gene to date (CF Genetic Analysis Consortium, see Mickle et al., 1995). The most common mutation is the so-called DF508 mutation, which accounts for ~70% of CF alleles. Through the analysis of the most common mutations, it is possible to detect >80–90% of all mutations. Therefore, every couple, in which the male partner suffers from CF, should attend genetic counselling and both partners should be tested for the presence of mutations in the CF gene before ICSI. With the aid of direct DNA diagnostics, it is possible, in the case of an increased risk of the couple having a child with CF, to carry out predictive prenatal diagnostics.

CBAVD is found in 1:5000 of all men, but in ~1–2% of infertile, though otherwise healthy, men and in 5% of men with an obstructive azoospermia (Holsclaw et al., 1971; Jequier et al., 1985). A mutation in the CF gene is found in about 60–66% of all cases of CBAVD, although often a defective CF allele was only detected on one chromosome 7. It is assumed that these men do in fact carry a mutation on the other chromosome 7 which could not be identified (Patrizio et al., 1993; Oates and Amos, 1994; Casals et al., 1995; Mercier et al., 1995). Such men would be expected to be compound heterozygotes. Oates and Amos (1994) examined 49 patients with CBAVD and found the second mutation in 18% of the cases. Casals et al. (1994) found the second mutation in 10% of 30 patients examined, Mercier et al. (1995) report a figure of 24% and Zielenski et al. (1995) of 13%. The most common mutations found among the CBAVD patients are: DF508, R117H, R1070W (Le Lannou et al., 1995) and the 5T variant (Zielenski et al., 1995). However, no CBAVD-specific mutation has been reported to date (Patrizio et al., 1993).

In the cases in which the second mutation can be identified, one can assume that these mutations are the cause of CBAVD, i.e. the genital manifestations found in CF patients. Such an assumption cannot, as yet, be made in the case of men with CBAVD where only one defective allele is found, particularly if the sweat test is perfectly normal. Pairs of brothers have been described who both carry the same chromosomes 7, shown by molecular analysis, but only one brother suffers from CBAVD (Mercier et al., 1995; Rave-Hariel et al., 1995). Because both brothers must have the same CF mutations on both chromosomes, it would be expected that both suffer from CBAVD. Such observations demonstrate the genetic heterogeneity of CBAVD and that at least one other gene must be involved which, when mutated, can lead to a CBAVD. As a result of this an examination of the family should definitely be carried out if the patient has a brother without a CBAVD. With the aid of indirect DNA diagnostics, it is possible to identify both chromosomes 7 in the patient and his brother. If the same chromosomes 7 are found in the brother and patient then it is justified to assume that the CBAVD is not the result of mutations in the CF gene.

Every couple, in which the man suffers from a CBAVD
without the clinical symptoms of CF, should undergo genetic counselling prior to ICSI. Due to the above mentioned reasons, the counselling of such patients is problematic. The various possibilities can be divided up into a number of categories (i) both CF mutations can be identified in the CBAVD patient. If the partner of such a patient is tested for the presence of the most frequent CF mutations and none is found then the risk of a child with CF, or a male child with CBAVD, being born is <1%. If the partner is found to carry a CF mutation, then the risk of a child being born with CF or possibly, in the case of a male child, a CBAVD without clinical CF symptoms rises to 50%. Such couples can avail themselves of prenatal diagnostics for CF; (ii) only one CF mutation can be identified in the CBAVD patient. He has either no brother or none of his brothers suffers from a CBAVD and there is no concordance between the brothers and patient with respect to the chromosomes 7. In this case it cannot be excluded that the patient does carry a second, undetectable CF mutation. A risk analysis would follow category 1 and would depend on the results of an examination of the female partner; (iii) only one mutation can be identified in the CBAVD patient. One of his unaffected brothers has the same chromosomes 7 as the affected man and no mutation could be found in the partner. In this case no relevant increase in the risk of CF among the children, or CBAVD among male children, can be assumed. If a mutation is found in the female partner then the risk increases to 25%. Because of this risk of CF, such couples can avail themselves of prenatal diagnostics.

Genetic counselling of these couples remains complicated because the CF phenotype can depend on the combination of the mutations on both chromosomes 7. Therefore, the counselling of each couple, risk analysis and the informativeness of the results of a possible prenatal diagnosis will vary according to the mutations found by the molecular biological analysis. Until now we have assumed that CBAVD is inherited in an autosomal recessive fashion and, despite the results from a number of families (reviewed in Martin et al., 1992) which support this view, there appear to be other possible patterns of inheritance. Kleczkowska et al. (1989) describe a family in which, apart from the patient, an uncle on the mother's side of the family is also affected with a CBAVD. Martin et al. (1992) describe two brothers, one of which has a CBAVD and the other a congenital unilateral aplasia of the vas deferens (CUAVD). These cases would appear to represent an X-chromosomal recessive inheritance and a sex-linked autosomal dominant inheritance, respectively. In the case of X-chromosomal recessive inheritance, a man with an isolated CBAVD would be expected to have, after ICSI, sons without CBAVD and daughters with a 100% chance of being carriers for CBAVD. In the case of a sex-linked autosomal dominant inheritance of CBAVD, a man with an isolated case of CBAVD would be expected to have, after ICSI, a risk of 50% of having a son with CBAVD and a risk of 50% of a daughter being a carrier for CBAVD.

The situation for CBAVD becomes even more complicated in cases in which no, or only very rare, mutations of the CF gene are found together with kidney defects (e.g. agenesis of a kidney). This was observed in 10 out of 47 (21%) CBAVD patients (Augarten et al., 1994). Such a constellation has also been observed in CUAVD patients (Casals et al., 1995; Mickle et al., 1995). According to Rubin (1975), 10% of CBAVD patients suffer from kidney disorders. Augarten et al. (1994) suggest that a separate form of CBAVD is involved, independent of the form of CBAVD caused by two CF mutations. Precisely how this form of CBAVD is inherited remains unknown. There are presently no results of ultrasound examinations of the parents, in particular the mother, available in the literature. Theoretically, autosomal recessive inheritance, X-chromosomal recessive inheritance or sex-linked autosomal dominant inheritance could be involved in these cases. Thus, a risk of up to 50% of having a son with CBAVD cannot be ruled out in the genetic counselling.

The genetic heterogeneity of CBAVD should prove, over the next few years, to be one of the most important areas of research, particularly with respect to the molecular genetics of the disease. The progeny of men with CBAVD, as a result of ICSI, should undergo very detailed clinical and genetic examinations in order to separate the various forms of CBAVD from one another and, thus, clarify the precise patterns of inheritance which will lead to the identification of the genes and the mutations involved. Only then will it be possible to offer couples, in which the man has a CBAVD, a reliable risk analysis and prenatal diagnosis.

We suggest the following in the cases of couples, in which the man suffers from a CBAVD, before ICSI is carried out (i) genetic counselling should be carried out including a detailed pedigree analysis. The couple should be questioned about possible relatives with CF and/or childless male relatives; (ii) an ultrasound examination of the kidneys should be carried out on each patient with CBAVD. If a kidney disorder is found then the parents and other relatives, in particular childless male relatives, should also be examined; (iii) molecular diagnostics for mutations in the CF gene should be carried out on every couple. If only one mutation is found in the man then a sweat test should also be performed (65% of all CBAVD patients have a conspicuous sweat test result) (Augarten et al., 1994; Casals et al., 1995). This should be followed by indirect DNA diagnostics of the family to characterize the chromosomes 7 of the patient and his non-CBAVD brother(s); (iv) as Klinefelter's syndrome has been diagnosed in some cases of patients with a CBAVD (Martin et al., 1992), each patient should undergo a chromosomal analysis.

During such genetic counselling a prenatal diagnostic is available for couples with a risk of CF. However, this is no reason to refuse ICSI to such couples with a risk of having a son with CBAVD as even a son born with CBAVD will otherwise be perfectly healthy. Could ICSI lead to a relevant rise in the risk of genetically determined infertility/subfertility?

We estimate the frequency of men with a fertility disorder to be, at most, 1.8%, although it must remain clear that it is not known what proportion of these fertility disorders is genetically determined. Taking chromosomal aberrations and possible defects in the ~1000 genes involved in sperm cell differentiation, maturation and function into account one arrives at a
Globozoospermla, Spermatogonia, a heterozygous for the mutation

Figure 5. Syncytial junctions between male germ cells. The germ cells developing from a single spermatogonia remain cytoplasmically connected to one another during spermatogenesis. Therefore, there is no effect on germ cell differentiation if the spermatogonia contains a mutation in one allele of a gene important for sperm cell maturation, as the germ cells are capable of exchanging mRNAs and proteins. All sperm cells are morphologically and functionally normal and fertile.

Haploid spermatids

Figure 6. Pedigree of a family with globozoospermia. Globozoospermia is an autosomal recessive disease. The parents of a man with globozoospermia are both heterozygous (□ [ ]), the father produces sperm cells with the gene defect and sperm cells without. Both types of sperm cell are morphologically and functionally normal and are capable of fertilizing the oocyte. The male offspring can be homozygous (□) for the gene defect and, therefore, infertile (IF), heterozygous (□) and fertile (F) or homozygous normal (□) and fertile. All female offspring are fertile.

This phenomenon can be explained by an ancient evolutionary mechanism. All germ cells produced from a single spermatogonia (e.g. 64 preleptotene spermatocytes or 400-500 haploid spermatids) are connected with one another, until the conclusion of sperm cell differentiation, by means of cytoplasmic ‘bridges’ (reviewed in Chubb, 1993) (Figure 5). That implies that all spermatocytes and spermatids, stemming from one spermatogonia, communicate with one another, i.e. exchange of proteins, ribosomal RNA, messenger RNA. Thus, defective spermatocytes and spermatids would be rescued by the neighbouring intact cells. In the case of the NO mutant (loss of ribosomal genes on one chromosome) in Xenopus laevis, the heterozygote males (i.e. one of the homologous chromosomes has the genes, the other does not) produce sperm cells (sperm cells with and without the ribosomal genes) normally (Brown and Gurdon, 1964). However, if the cytoplasmic junctions are absent then only sperm cells with the ribosomal genes will mature (own unpublished results). Furthermore, Braun et al. (1989) have shown in transgenic mice carrying the human growth factor gene under the control of the spermatid-specific promoter for protamine 1 that transcripts are found in all spermatids of heterozygous animals. This can only be explained if an exchange of mRNA and proteins between such cells is possible.

In the case of humans this would imply that, if haploid germ cells are used for ICSI from a man whose germ cell...
The gene for the androgen receptor is located at band q12 on the X chromosome. Mutations in the various regions of the gene result in different sexual phenotypes. If a mutation in the androgen receptor gene leads to a very slight androgen insensitivity, resulting in an oligozoospermia, then intracytoplasmic sperm injection (ICSI) using such sperm cells would result in only fertile offspring. All the female offspring would carry a X chromosome with a copy of the mutation in the androgen receptor gene, the sons of whom would have a 50% risk of having an oligozoospermia and the daughters would have a 50% risk of being carriers of the gene defect.

Until now, we have been dealing with the relevance of autosomal genes with respect to fertility. There is, as yet, no known gene on the X chromosome important for male germ cell differentiation and which, in a mutated form, could lead to male infertility. However, one gene on the X chromosome which may play a role in male germ cell differentiation is the androgen receptor gene (located at q12). Mutations in this gene are known to lead to androgen resistance/insensitivity resulting in a wide spectrum of phenotypes with infertility or subfertility (testicular feminization, Reifenstein syndrome, Lubs syndrome, Gilbert–Dreyfuss syndrome, Rosewater syndrome) (Fauser and Hsueh, 1995). There have been cases reported of azoospermia or prominent oligozoospermia in men with reduced androgen sensitivity (Aiman et al., 1979; Aiman and Griffin, 1982; Smallridge et al., 1984; Rouchard et al., 1986). Unfortunately, molecular genetic analysis of the androgen receptor gene of these patients was not carried out. One short report describes a patient with azoospermia whose blood concentrations of testosterone and luteinizing hormone (LH) were normal and the cause of azoospermia was put down to a very slight androgen insensitivity due to a mutation in the androgen receptor gene (loss of exon 4 of the gene) (Akin et al., 1991). Recently, Young et al. (1994) reported a patient who had a point mutation in exon 5 coding for part of the hormone binding domain of the androgen receptor, with consequent oligozoospermia. However, it seems to us rather unlikely that this point mutation is the underlying cause of the sperm disturbance. The patient responded to mesterolone therapy and pregnancy could be achieved. Assuming that mutations of the androgen receptor gene or other X chromosomal genes can result in disturbances of male fertility, how would this affect the risk of infertility among the male offspring of such men after ICSI? As shown in Figure 7, such a mutation would first have an effect in the second generation. The male offspring would all be healthy and not carry the mutation, whereas the female offspring would all be carriers for the mutation and 50% of their sons would be expected to suffer from X chromosomally determined infertility. Therefore, one could only expect, over generations, a very slight rise in the frequency of male infertility.

There are a large number of Y chromosomal genes known...
to play a role in male germ cell differentiation. They are found in the region Yq11, the so-called azoospermia locus (AZL). Patients with azoospermia and prominent oligozoospermia have been described with microdeletions in this region. Such deletions can be observed under a microscope. There is the possibility that up to 10% of men with azoospermia have microdeletions, i.e. not observable under the microscope, in the Yq11 region (Ma et al., 1992; Vogt et al., 1992; Nagafuchi et al., 1993; Kobayashi et al., 1994; Reijo et al., 1995). Just how frequent such deletions are among patients with oligozoospermia is not known only isolated cases have, as yet, been described. If the microdeletions in the Yq11 region lead to a complete lack of sperm cells or spermatids then the affected men cannot participate in ICSI. If, however, these patients have an oligozoospermia or it is possible to isolate sperm cells from the testes of these patients ICSI can be performed. In this case all the sons would be expected to have either an oligozoospermia or azoospermia (Figure 8). Therefore, the importance of deletions in Yq11 for male infertility, with respect to ICSI, depends on how often such deletions are found in patients with either severe oligozoospermia or azoospermia. Furthermore, the precise role played by microdeletions in Yq11 in male infertility is still not absolutely clear. However, apart from this, it is possible, due to molecular techniques available for the analysis of such deletions, to inform potential ICSI couples about possible infertility of a male child. It is important to note that men with deletions in the Yq11 region are, apart from fertility disorders, perfectly healthy. Why then should such couples be denied ICSI? Their healthy sons can themselves fulfill a possible desire for children through the use of ICSI. The daughters, and their children, would be expected to be fully fertile.

Comments

The data and arguments presented here permit us to draw the conclusion that the microassisted reproduction will lead to an almost negligible increase in the risk of genetic disorders and infertility among the offspring of couples, who may consider this technique. We hope that this article will contribute towards an objective discussion concerning possible risks involved in microassisted reproduction, including ICSI. We are, however, also of the opinion that it is time for all involved (doctors and patients) to take part in discussions with regard to the ethical aspects of the new reproductive technology.

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