ACTA REVIEW II

Severe male infertility. Impact of genetic factors on diagnosis and counselling

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Background

Until few years ago, men with severely compromised sperm production (azoo-, oligo-, astheno-, or teratospermia) were infertile, and no effective treatment was available.

With the development of percutaneous epididymal/microsurgical sperm aspiration (PESA/MESA), testicular sperm extraction (TESE), and intracytoplasmic sperm injection (ICSI), the possibility of treating severe male infertility has improved significantly. During the last few years, however, our knowledge about the causes of male infertility has increased, elucidating that different types of genetic abnormalities may account for a substantial part of the etiological background of these cases.

The objective of this survey has been to summarize our knowledge on genetic causes of different types of male infertility, to draw relevant clinical consequences of this knowledge, and finally to suggest guidelines on genetic counselling of men (couples) with these conditions.

Chromosomal abnormalities

It has been recognized for at least 30 years that the frequency of chromosome abnormalities increases with decreasing sperm count (1). The frequency and type of chromosome aberrations is also associated with the cause of the low sperm count. Thus, men with obstructive azoospermia have less chromosomal defects than men with non-obstructive azoospermia (2).

In azoospermic men, the frequency of chromosome abnormalities is 10–15%.

Of these, 92% are sex chromosome anomalies, and eight per cent are autosomal anomalies (Table I) (2). The majority of sex chromosome anomalies are Klinefelter syndrome (47,XXY), which occurs about 50 times more frequently in men with azoospermia than among men in general. Not all Klinefelter patients, however, have azoospermia. A minority, primarily men with mosaicism, may have sperm production (3). Reciprocal and Robertsonian translocations account for the majority of the autosomal abnormalities.

Yoshida et al. (4) detected 65 (6.5%) with chromosome aberrations among 1,007 males presenting with infertility, of which 41 (63%) were sex chromosome aneuploidies. Klinefelter syndrome accounted for 31 (73%) of these sex chromosome abnormal patients, of which seven were mosaic.

Veld et al. analyzed 80 men with azoospermia ($n=11$) or sperm count below 1 mio/ml ($n=69$) for an abnormal karyotype. Seven (9%) had an abnormal karyotype (5).

In oligospermic men (differently defined, typic-

Table I. The presence of genetic abnormalities in men with different types of infertility

UGS=urogenital system, CF=cystic fibrosis, US=ultrasound, CBAVD=congenital bilateral absence of the vas deferens, CFTR=cystic fibrosis transmembrane conductance regulator gene

Clinical condition Abnormalities	Azoospermia Obstructive with CBAVD	Azoospermia Non-obstructive	Sperm count <1 mio/ml	Sperm count <20mio/ml	Men with normal sperm count
Chromosomal	~0.4%	10–20%	8–10%	3–8%	0.4%
Sex-chromosomes	~0.2%	~13%	?	1–2%	0.2%
Autosomes	~0.2%	~1%	?	2–4%	0.2%
Y-deletions[1]	<1%	7–35%	5–23%	1–8%	<1%
CFTR mutations	>70%	few	few	few	<0.01
Normal US of UGS	~80%	–	–	–	–
+ UGS malformations	few	–	–	–	–
Genetic abn. total	>70%	25–50%	10–30%	4–10%[2]	~1%

[1] Deletions in the azoospermic factor (AZF) region of the long arm of Y-chromosome.

[2] Increasing per cent by decreasing sperm count.

ally <10–20mio/ml), the frequency of chromosome abnormalities is 3–8% (2, 6). Here the autosomal rearrangements account for about two thirds and sex chromosome anomalies for one third (Table I). The most frequent autosomal anomaly is 13;14 Robertsonian translocations.

Thus generally, with decreasing sperm count, the frequency of chromosomal abnormalities increases, and the proportion of autosomal anomalies of these abnormalities decreases.

In normal men, about 10% of the spermatozoa have chromosome abnormalities (2). In men with oligoasthenoteratozoospermia, the frequency of specific abnormalities (chromosome 7, 11, 12 and 18) analyzed during recent years by fluorescence-in-situ hybridization (FISH) were increased up to ten times (7). In the survey of Assche et al. (2) it was concluded that some infertile men with normal karyotype may have specific problems with meiosis, resulting in spermatogenic disruption. The quantitative impact of this phenomenon, however, still has to be assessed.

In the past, a majority of men with these disorders did not reproduce themselves. With the ICSI techniques, the risk of transmitting these chromosomal abnormalities is obviously increased. Therefore a relevant genetic screening should precede invasive infertility treatments (see later).

Y chromosome deletions

On the long arm of the Y chromosome, a gene or gene-complex called azoospermia factor (AZF) has been localized, which is anticipated to be responsible for the production of one or more hitherto unidentified factor(s) necessary for normal spermatogenesis. The AZF region has been subdivided in AZF-a, AZF-b, and AZF-c (8). The damage of each of the two latter regions seems to result in azoospermia or severe oligospermia. The AZF-c region includes the so called 'deleted-in azoospermia' gene or DAZ. These deletions are too small to be visible cytogenetically.

In 89 men with azoospermia 12 (13%) and in 35 men with severe oligozoospermia (<1 mio/ml) Reijo et al. (9) found two (6%) with AZF deletions (10). They suggested that 'severe oligozoospermia, complete testicular maturation arrest, and Sertoli-cell-only syndrome are not etiologically distinct... but represent clinically diverse manifestations of the same underlying genetic anomaly'.

Veld et al. found three (5%) who had deletions in the AZF-c region in 58 men with a sperm count of less than 1 mio/ml (5).

Girardi et al. (11) recently published an analysis of 160 men referred with male-factor infertility, none of whom had any objective sign of obstruction. The frequency of Y chromosome deletions was 7% among men with azoospermia, and 10%, 8% and 0% among men with sperm count of >0–1 mio/ml, >1–5 mio/ml, and >5 mio/ml, respectively. All the deletions were detected in the AZF regions of the Y chromosome.

Foresta et al. (12), on the other hand, found in 16 men with Sertoli-cell-only-syndrome six men (38%) and in 22 with oligospermia (<5 mio/ml) five (23%) with deletions of the AZF region. These figures are several fold higher than previously reported. The patients were, however, more selected than in the other studies, by including also a specific histological picture as inclusion criterion.

Not all men with the described deletions of the Y chromosome are infertile. Pryor et al. (13) studied 200 consecutive men with infertility, of whom 26 had azoospermia, 30 severe oligospermia (<5 mio/ml), 42 oligospermia (5–20 mio/ml), and 102 normospermia. Fourteen of these men (7%) were found to have Y chromosome microdeletions including six with azoospermia, three with severe oligospermia, four with oligospermia, and one of

normospermia. Thus the relationship between genotype and phenotype with our present knowledge is neither specific nor entirely clear. Germ line mosaicism may further complicate the diagnostics, but is probably of little quantitative significance.

ICSI may obviously increase the risk of transmitting these abnormalities to the male offspring. In fact Kent et al. found that 9% of sons born after ICSI had Y chromosome deletions (14). Therefore, diagnostic tools for assessing these Y chromosome deletions should be established, and routine screening generally be performed before ICSI.

Cystic fibrosis

Cystic fibrosis (CF) is an autosomal recessive disease affecting about one in 4,000 children (in Denmark), corresponding to a carrier frequency of three per cent. The disease is caused by mutations in the cystic fibrosis transmembrane conductance regulator (CFTR) gene, affecting the corresponding CFTR protein. CF may be caused by several different mutations. The most frequent (ΔF508) accounts for 90% in Denmark, however, less ($\sim$70%) in other countries (15).

More than 95% of men with cystic fibrosis are also infertile, primarily due to congenital bilateral absence of the vas deferens (CBAVD), which in turn account for about two per cent of all male infertility (15).

Veld et al. tested 13 common mutations in CFTR in 58 men with oligo- or azoospermia, and found eight (14%) with a single mutation (5), or four-fold more than the estimated population frequency of CFTR mutations of 3.1%. Three of the eight men had congenital bilateral absence of the vas deferens, while in five patients the vas was present.

Not all men with CBAVD have cystic fibrosis. It may alternatively be a part of a more general maldevelopment of the urogenital system. These maldevelopments are usually not associated with cystic fibrosis, but the risk of CBAVD in the male offspring is unknown. CBAVD without a detectable CFTR mutation may be due to mutations in other so far unidentified genes (16).

In general, men with CBAVD should be screened for mutations in the CFTR gene. This screening is, however, rather complex as more than 500 different mutations have been detected in patients with CF. By screening for the three most frequent mutations, 95% of CF (if present) will be detected. Those men, in which the only clinical manifestation is CBAVD, may have a different picture of mutations, compared with patients with the classical symptoms of CF. Therefore the guidelines for screening infertile men with CBAVD may differ

from the routine screening of patients with CF in general. Infertile men with no sign of CF could preferably be examined by ultrasound in order to detect anomalies of the urogenital system, although the risk of similar disease in the offspring of men with these conditions is unknown. Another possibility is to restrict the CF screening to those men, with a normal ultrasound examination of the urogenital system.

Genetic counselling of patients with CF should be restricted to few highly specialized centers in collaboration with the involved fertility clinics.

Diagnosis

In principle, there could be one or more of three reasons for screening infertile men with potential genetic abnormalities:

- Some genetic disorders with spermatogenic arrest are incompatible with fertilization even when using PESA/MESA/TESE and ICSI. These couples could be saved from hopeless treatments.
- Some genetic disorders imply a high risk of passing on a genetic constitution to the (male) offspring, with male infertility and/or chromosomal abnormalities as consequence. This risk should be evaluated before fertilization attempts are done, either because donor fertilization could be a more attractive alternative for many couples or by selecting couples among which preimplantation diagnosis or prenatal diagnosis should be offered.
- If we had a possibility of improving the treatment having knowledge about the specific abnormality.

An operationalization of the diagnosis in couples with severe male infertility taking these three considerations into account is suggested in Table II. In men with an autosomal structural aberration, the success rate of ICSI is lowered, and an increased risk of miscarriages exists (2). In case of sex chromosome aneuploidy, the success rate of ICSI is variable, but if successful the risk of aneuploidy in the offspring is probably low. We would suggest all men with pregnancy wish and non-obstructive azoospermia or who are referred to ICSI should be karyotyped.

Diagnostic tests for Y-chromosome deletions are not as yet established in a majority of fertility clinics. Attempts should be undertaken in order to be able to offer such diagnosis in all men referred for ICSI. Until such facilities are available, the counselling of the couple should include information about the risk of transmitting these deletions (if present) to the male offspring who, in

Table II. Clinical conditions and diagnostic examinations in men in treatment for severe male infertility
CF=cystic fibrosis, US=ultrasound, CBAVD=congenital bilateral absence of the vas deferens

Clinical condition Diagnostics	Azoospermia Obstructive[1]	Azoospermia Non-obst. or normal VAS	Sperm count <1 mio/ml	Sperm count 1–10 mio/l
Objective investigation	yes	yes	yes	yes
Ultrasound examination	yes	yes	yes	if prev ret[2].
Testis biopsy[3]	no	yes	if suspect US	if suspect US
Genetic Y-screening	no	before ICSI	before ICSI	no
Chromosomal screening	no	yes	yes	before ICSI
Cystic fibrosis screen	if CBAVD[4]	no	no	no
US of urogenital syst.	if no CF[5]	no	no	no

[1] Known obstruction, e.g. CBAVD, previous sterilization, other surgery.

[2] If previous retention.

[3] Biopsy in fertility clinic.

[4] Also wife should be examined.

[5] Only in men with obstructive azoospermia and negative screening for cystic fibrosis.

these instances, we expect will suffer from the same infertility problem as the infertile father. Thereby couples who don't wish to take this risk could be donor-fertilized.

Several initiatives have been taken during recent years in order to ensure the quality of clinical fertility work, including obligate registration of all IVF cycles. If society is to remain confident with the management of fertility treatment in the fertility clinic (public and private), one precondition is that these clinics behave responsibly in regard to proper information and counselling of couples in which an increased risk of transmitting genetic disorders is present. A suggestion for a continued high quality and responsibility may include:

- A systematic quality control of the offspring resulting from the new technologies, including test of the at present known genetic abnormalities.
- An adequate information of couples with a potential risk of transmitting genetic abnormalities to their offspring, and the consequences of these abnormalities.
- Establishment of relevant diagnostic tools in order to be able to select those couples in which the application of the new technologies does not seem to confer any significant genetic risk, and on the other hand to identify couples in which such risk is increased and alternative offers considered.
- Establishment of a professional network catalyzing improvement in quality assurance in public as well as private clinics.
- Standardized professional Nordic recommendations is an attractive option as a frame for these initiatives.
- Intensified research.

Conclusion

The proportion of infertile men with genetic abnormalities increases with decreasing sperm count. Among men with non-obstructive azoospermia, 25–50% have genetic abnormalities. Half of these are chromosomal aneuploidies, primarily affecting the sex-chromosomes, the other half Y chromosome deletions. Among men with low sperm count (<10–20 mio/ml), 4–10% have genetic abnormalities, the majority being autosomal chromosomal changes. Men with obstructive azoospermia with congenital bilateral absence of the vas deferens, and whose urogenital system otherwise appears normal at ultrasound examination, have more than 95% probability of having CFTR-gene mutations.

Men with severe oligo-, or azoospermia without evidence of obstructive disease should be screened in order to detect chromosomal aneuploidies. Diagnostic tools based on polymerase chain reaction (PCR) should be established and offered routinely to the same group of men in order to disclose Y chromosome deletions. The clinical consequences of confirmed genetic disorders should be discussed with the couple. If no screening is conducted, the couple should be informed about the potential genetic consequences for the male offspring.

Men with obstructive azoospermia and congenital bilateral absence of the vas deferens as well as their wives should be screened for mutations in the CFTR gene in order to provide knowledge for genetic counselling.

Men with low sperm count or non-obstructive azoospermia may have an increased risk of carcinoma *in situ* of testes, and should therefore also be screened primarily by ultrasound examination, secondarily if this is suspect by testis biopsy.

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