Clinical application of new micromanipulative technologies to treat the male

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Traditional treatments of the male have produced no improvement in sperm parameters. Since the rate of normal fertilization rate is significantly lower in these cases after classic in-vitro fertilization (IVF), intracytoplasmic sperm injection (ICSI) has become the preferred method of treatment. It has been successfully applied in cases of extreme oligoasthenoteratozoospermia, and obstructive and non-obstructive azoospermia. Cryopreservation of testicular tissue will replace the repeated use of fresh testicular tissue. Full information has to be provided to patients about the frequency (~3%) of sex chromosome and autosomal aberrations in men with extreme oligoasthenoteratospermia. The need for screening for Yq deletions is still under study, and any therapeutic consequences for the newborn child will have to be analysed. The incidence of major malformations in newborn children is ~2.5%, i.e. comparable with that of the general population. Screening for de-novo sex chromosome aberrations may be particularly useful for men with sperm counts of $<5\times10^6$ and those with non-obstructive azoospermia.

Key words: intracytoplasmic sperm injection/in-vitro fertilization/ oligoasthenoteratozoospermia/sperm parameters

Introduction

For decades, many publications have attempted to demonstrate improvements in sperm counts after vacuum treatment in men. Unfortunately, these attempts have not been successful. Neither medical treatment nor surgery, except for obstructive azoospermia, have proved to be efficient (O'Donovan *et al.*, 1993; ESHRE Capri Workshop, 1996; Devroey *et al.*, 1997). The fertility potential of a male is closely related to his sperm count; if he repeatedly produces $<1\times10^6$ spermatozoa/ml, it can be assumed that he is less fertile than if he had a normal semen sample (Barratt *et al.*, 1995).

It is still debatable whether we have the correct scientific tools to judge the cut-off number of spermatozoa required for fertilization. It seems that the evaluation of semen samples as 'fertile' is an impossible task. A grey area has to be accepted since fertility is a complex system. The fertilizing capacity of

human spermatozoa is closely linked to female fecundity and especially to maternal age (Devroey *et al.*, 1996). It is therefore almost impossible to set up criteria. The criteria of the World Health Organization (WHO) are authority-based, and these criteria which include number, morphology and motility do not reflect a predictive figure of any value (WHO, 1992). More prospective research is needed to distinguish between a fertile and infertile population and to correlate semen parameters to this distinction. Attempts to do this have been made in a prospective manner, and the results do not correlate with the WHO criteria. Obviously, mean semen parameters do differ between the fertile and infertile population. Needless to say, the ranges overlap, indicating that for an individual patient no precise answer can be given (Barratt *et al.*, 1995). In specific clinical conditions, such as in extreme oligoasthenoteratozoospermia and azoospermia, it is obvious that in-vivo conception is unlikely to occur, so that assisted reproductive techniques in these conditions are mandatory. In oligoasthenoteratospermia, in-vitro fertilization (IVF) may be a solution for moderate cases. In a retrospective analysis comparing the outcome of IVF with normal semen versus impaired semen, it became apparent that incidence of eggs fertilized *in vitro*, and producing two pronuclei is significantly reduced in cases of impaired semen (Tournaye *et al.*, 1992).

Conditions alleviated by intracytoplasmic sperm injection (ICSI)

Microinjection has opened new perspectives, since it has now become feasible to inject one spermatozoon into one metaphase II oocyte. Treatment of male infertility is thus possible at the gamete level using ICSI (Palermo *et al.*, 1992; Van Steirteghem *et al.*, 1993a,b). Three conditions in the human are exemplify this:

'Azoospermia' or cryptozoospermia

Naturally, in this clinical condition, conception *in vivo* and conception after IVF are excluded. If a few spermatozoa are found after thorough centrifugation of the semen sample, an acceptable fertilization rate can be attained using ICSI (Nagy *et al.*, 1995). The ability to achieve fertilization with so few spermatozoa implies that only cases of azoospermic men can be offered no help at all to achieve their own fertilization.

Absence of morphologically normal spermatozoa

In the past, it was thought that ~4% of the spermatozoa in an ejaculate had to be normal in order to obtain normal fertilization after classic IVF (Kruger *et al.*, 1986). However, even in the presence of no morphologically normal spermatozoa at all, an acceptable normal rate of fertilization is obtained. This observation demonstrates that there is no limiting threshold as far as morphology is concerned (Nagy *et al.*, 1995).

Table I. Results of intracytoplasmic sperm injection (ICSI) in the first and subsequent cycles in cases of extreme asthenozoospermia (Vandervorst *et al.*, 1997)

	First cycles (n = 11)	Subsequent cycles (n = 12)
No. oocytes/cumulus complex	192	166
No. metaphase II oocytes (%)	162 (84)	137 (84)
No. intact oocytes (%)	145 (89)	121 (88)
No. fertilized oocytes (%)	18 (12)	74 (64)
Deliveries	0	4

Absence of motile spermatozoa

A clear distinction has to be made between asthenozoospermia where all spermatozoa are immotile and where a few have some motility. If a few spermatozoa with some motility are found, an acceptable normal fertilization rate is obtained using ICSI. If all spermatozoa are immotile, the fertilization rate is significantly reduced.

The question which had to be answered here is whether all spermatozoa remain totally immotile in repetitive trials. Motile spermatozoa were found in 12 out of 16 cases during subsequent cycles (Table I). Overall, 137 metaphase II oocytes were injected. A normal fertilization rate of 64% was obtained, 11 embryo transfers were performed and four deliveries ensued (36%). The ongoing implantation rate per embryo was 13%, i.e. four out of 30 replaced embryos implanted. These observations indicate that total asthenozoospermia is not a permanent condition, except in the presence of structural defects (Vandervorst *et al.*, 1997).

Azoospermia

More attention has to be paid to azoospermia, for which two different conditions have to be taken into account. The conditon can result from obstructive or non-obstructive origins. The biological status of each condition is totally different, indicating that spermatogenesis is normal in obstructive azoospermia and abnormal in non-obstructive azoospermia.

In obstructive azoospermia, the obstruction can be located at different points, such as the vas deferens, the epididymis and the rete testis. A special category involves bilateral absence of the vas deferens (CBAVD). Since it is known that motile spermatozoa are present in testicular tissue and that they have undergone full spermatogenesis, it seems rational to perform ICSI with testicular spermatozoa (Jow *et al.*, 1993).

If the obstruction is present in the vas deferens, spermatozoa can be retrieved from the vas deferens, the epididymis and the testis. If the obstruction is present in the epididymis, spermatozoa can be retrieved from the epididymis

Table II. Sensitivity and specificity of a prior biopsy in germ-cell aplasia and maturation arrest (Tournaye *et al.*, 1997)

	Sensitivity (%)	Specificity (%)
Germ-cell aplasia	78	88
Maturation arrest	26	87

(microsurgical epididymal sperm aspiration or MESA) (Tournaye *et al.*, 1994). Supernumerary epididymal spermatozoa can be cryopreserved and thawed for subsequent cycles (Devroey *et al.*, 1995a). It goes without saying that if more vital spermatozoa are used, the better are the fertilization ratios expected. For the above reasons, aspiration of spermatozoa from the head of the epididymis is preferential.

In the absence of spermatozoa from the epididymis, the only source of spermatozoa is the testicle. The association of testicular sperm extraction (TESE) and ICSI is a standard treatment nowadays (Devroey *et al.*, 1994). By further simplification of the procedure, aspiration of testicular spermatozoa can be performed by a fine needle aspiration (FNA) (Bourne *et al.*, 1995). Nowadays, there is no evidence that epididymal spermatozoa are superior to testicular spermatozoa or *vice versa*. It seems logical therefore to choose the simplest technique. In a case-controlled study it was clearly demonstrated that comparable fertilization and implantation rates were obtained after fine-needle aspiration (FNA) and TESE (Tournaye, personal communication). In this respect the association of FNA and ICSI is the preferred method in cases of obstructive azoospermia.

In non-obstructive azoospermia, spermatogenesis is defective. Different defects are recognizable, e.g. germ-cell aplasia (Sertoli cell-only syndrome and maturation arrest). If both conditions are absolute, no therapy is foreseeable. However, on many occasions the condition is partial, as in partial germ-cell aplasia and incomplete maturation arrest. Pregnancies have been described in cases of non-obstructive azoospermia (Devroey *et al.*, 1995b). It is still not clear as to which patients will provide spermatozoa and which will not. It is accepted that spermatozoa are recovered in all cases in obstructive azoospermia, wheras they are recovered in only one-half of patients with germ aplasia and maturation arrest.

An important question has to be answered with respect to the role of a prior biopsy. If spermatozoa are distributed equally throughout the testis, a prior biopsy will be predictive. Experience so far does not confirm the hypothesis of an equal distribution of spermatozoa in all tubules. It has been demonstrated that in non-obstructive azoospermia, the sensitivity and specificity of a prior testicular biopsy are low, as shown in Table II. These observations preclude a homogeneous distribution of spermatozoa in the testis in cases of germ-cell aplasia, and especially in cases of maturation arrest (Tournaye *et al.*, 1997). These results indicate that a prior biopsy does not have a statistically significant predictive value.

Table III. Clinical results of freezing of testicular tissue

Author	No. of cycles transferred	No. of metaphase II oocytes injected	Fertilization rate (%)	No. of embryos transferred	Ongoing pregnancies
Romero *et al.* (1996)	2	22	59	9	0
Podsiadly *et al.* (1996)	1	17	59	2	1
Gil-Salom *et al.* (1996)	12	131	51	41	6
Fischer *et al.* (1996)	1	8	37	3	1
Hovatta *et al.* (1996)	1	10	10	1	1
Khalifeh *et al.* (1997)	1	9	?	3	1
Oates *et al.* (1997)	19	149	48	50	2
Total	37	346	–	109	12 (32 %)

Implications of ICSI

Cryopreservation of testicular tissue

If spermatozoa of testicular origin are to be used, the cryopreservation of testicular tissue has to be considered since it has many advantages. Testicular tissue can be frozen if spermatozoa are found. This avoids unnecessary ovarian stimulation as well as repeated testicular biopsies. Freezing of testicular tissue, especially in obstructive azoospermia, allows many specimens to be frozen, so that repeated surgery is avoided. Different authors have reported the results of freezing of testicular tissue, and ongoing pregnancy rates of 32% have been reported (Table III). If these results are confirmed, the use of testicular spermatozoa will be closely linked to a cryopreservation policy. On the other hand, it can be argued that in obstructive azoospermia fine needle aspiration is a simpler approach.

Spermatid injections into oocytes

The injection of round spermatids has also to be assessed. If no spermatozoa are present in cases of non-obstructive azoospermia, the use of round spermatids may be considered. The round spermatid is the youngest haploid stage during the formation of male germ cells. Human pregnancies have been described after the use of round spermatids of ejaculatory origin (Tesarik *et al.*, 1995, 1996). A fundamental question yet to be answered relates to the conversion from round spermatids to elongated spermatids and mature spermatozoa, i.e. the process of spermiogenesis. Classically, it is widely accepted that a maturation arrest does not take place at the level of spermiogenesis. This conclusion implies that if round spermatids are present in a testis preparation then elongated spermatids

Table IV. Number of sex chromosomal and autosomal abnormalities in oligozoospermic males

Reference	No. of cases	Sperm count (10^6/ml)	No. (%) of sex chromosome abnormalities	No. (%) of autosomal abnormalities	Total (%)
Hendry *et al.* (1976)	108	<20	1 (0.9)	1 (0.9)	
Micic *et al.* (1984)	464	<20	0 (0.0)	8 (1.7)	
Retif *et al.* (1984)	390	<10	14 (3.6)	10 (2.6)	
Bourrouillou *et al.* (1985)	569	<10	11 (1.9)	28 (4.9)	
Matsuda *et al.* (1989)	170	<20	2 (1.2)	4 (2.4)	
	1701		28 (1.6)	51 (3.0)	4.6

will also be found. If this argument is correct, there can be no need to use round spermatids for injection. At present, it should be assumed that arrest does occasionally take place at the level of round spermatids.

The miscroscopic recognition of round spermatids is still under investigation, as is their haploidy. Clinical results published so far are disappointing. An implantation rate of 3.6% per embryo has been reported (Antinori *et al.*, 1997). Furthermore, the terminology is still confusing. Some authors report results after the microinjection of late spermatids, i.e. before spermiation. Spermiation is a late stage of spermiogenesis. The results with this stage are also disappointing, i.e. a 8 % pregnancy rate (Araki *et al.*, 1997). More research is needed to evaluate arrest at the level of round spermatids, to develop criteria of recognition, investigate their haploid status and to demonstrate acceptable rates of normal fertilization after microinjection. So far, the clinical experience with round spermatids has been extremely disappointing (Amer *et al.*, 1997).

Genetic screening and counselling

The need to screen couples requiring ICSI is still under investigation. It is obvious that the male partner must also be investigated where screening is performed. The intention to evaluate the health of the offspring. Several questions must be raised, and the first of these relates to the ICSI procedure. Does this procedure carry a risk for the offspring or is the possible risk related to the types of spermatozoa used? Moreover, the level of screening has to be defined, e.g. karyotyping the husband, carrying out tests for the presence of Yq deletions, analysing the karyotype of the fetus and evaluating the health of the newborn child. The prevalence of these conditions has to be analysed and the costs and benefits have to be compared. There is a higher risk of sex chromosome and autosomal abnormalities in oligospermic men which reaches an overall percentage of 4.6% (Table IV). These studies demonstrate that approximately 1 in 25 men with oligozoospermia carries a chromosomal abnormality.

In our own population of infertile men with teratozoospermia, we found an incidence of chromosomal aberrations in men of 2.9% (12/415), with anomalies of all three parameters (i.e. number, motility and morphology; Van Assche *et al.*, 1996). A second group consisted of patients suffering from non-obstructive

Table V. Number of chromosomal abnormalities in azoospermic males

Reference	No. of cases	No. (%) Klinefelter cases (XYY)	No. (%) of sex chromosome abnormalities	No. (%) of autosomal aberrations
Hendry *et al.* (1976)	54	3 (5.6)	–	2 (3.0)
Micic *et al.* (1984)	356	26 (7.3)	2 (0.6)	2 (0.6)
Retif *et al.* (1984)	106	12 (11.3)	6 (0.6)	–
Bourrouillou *et al.* (1985)	383	49 (12.8)	5 (1.5)	5 (1.3)
Rivas *et al.* (1987)	163	31 (19.0)	5 (3.0)	2 (1.2)
Matsuda *et al.* (1989)	89	3 (3.41)	2 (2.2)	2 (2.2)
Total	1151	124 (10.8)	20 (1.7)	13 (1.1)

azoospermia. As shown in Table V, except for Klinefelter syndrome, their proportion of sex and autosomal chromosomal abnormalities was 2.8%. Similar results in oligozoospermic and azoospermic males have been observed. The percentage is especially high (11%) in patients with Klinefelter syndrome, indicating an important bias. Klinefelter patients represent a special clinical entity, and microinjection is not routine practice, so they have to be assessed separately (Table V).

Another important group includes men with autosomal structural aberrations, where multiple congenital abnormalities and/or mental retardation in their offspring can be expected. The risk of autosomal structural anomalies is 3% in oligozoospermia and 1% in non-obstructive azoospermia. This information has to be provided to the patient, who must be given proper counselling. It is advisable to perform a karyotype of peripheral blood in cases of extreme oligoasthenoteratozoospermia and of non-obstructive azoospermia.

The discovery of Y chromosome deletions and their relation to an impaired semen production has opened a new field of concern (Tiepolo and Zuffardi, 1976; Vogt *et al.*, 1992). The deletion overlaps a region which probably contains genes related to spermatogenesis (the azoospermia factor *AZF*), and deletions in the AZF region are related to various testicular defects such as germ-cell aplasia and maturation arrest. These regions contain a single-copy gene (deleted in azoospermia, or *DAZ*), which is transcribed in the testis (Reijo *et al.*, 1995). Men with non-obstructive azoospermia and extreme oligospermia have an increased risk of carrying the Yq deletions (Silber *et al.*, 1997). It is also well known that in cases of extreme oligoasthenoteratozoospermia, the histology of random testicular biopsies reveals cases of partial maturation arrest and of partial germ-cell aplasia.

Scientifically, it is important to calculate the prevalence of the Yq deletions in extreme oligo/azoospermia. There is ample evidence that male offspring will carry the same deletions, an observation that introduces several new questions. After the couple has been fully informed, will they accept ICSI? If they do not accept, will they abandon the procedure or will they ask for the replacement of only female embryos, in order to avoid the same anomalies arising in their male children? There are important ethical consequences in making decision in this

Table VI. Semen parameters, maternal age and de-novo sex chromosome aberrations found in eight out of 977 cases (0.8%)

Maternal age (years)	De-novo sex chromosome aberrations	Sperm concentration per ml	Normal sperm morphology (%)	Sperm motility (%)
26	47,XXY	3.6×10^6	15	0
28	47,XXY	2.7×10^6	12	0
28	47,XXY	0.2×10^6	2	2
32	47,XXY	0.1×10^6	1	0
44	46,XX/47,XXX	1.6×10^6	0	9
37	47,XXX	0.4×10^6	16	0
32	47,XXX	0.03×10^6	not done	not done
25	47,XYY	2.8×10^6	40	18

complex area, and so far no studies have been performed, either at the social or the clinical level. A debate is needed between different opinions to clarify the position and to assess any economic consequences.

Follow-up of children

Two different forms of screening can be performed to assess the health of concepti. Screening of the newborn child is extremely important, and our group has reported that the frequency of major malformations is ~2.5% (23/877) (Bonduelle *et al.*, 1996). Up to March 1997, 1672 children were born in our centre, of whom 39 had major malformations (2.3%). These updated findings confirm previously published data, indicating that the expected rate of major congenital malformation is ~2.5%. Compared with registers of the general population, this rate is not abnormally high.

It also remains an open question as to whether all pregnancies ensuing after ICSI have to undergo prenatal testing by chorionic villus sampling (CVS) or amniocentesis. Special attention has to be paid to the occurrence of de-novo sex chromosome aberrations. An increased percentage of de-novo sex chromosome aberrations can result from the ICSI procedure itself or be linked to a defined subgroup of males with impaired semen samples. If it is related to sperm parameters, it could be correlated with severely impaired semen parameters. In 977 cases of prenatal diagnoses, eight de-novo sex chromosome aberrations were found (0.8%), and sperm parameters for these eight patients are shown in Table VI. It is interesting to observe that each of the eight de-novo sex chromosome aberrations were found after using spermatozoa from males with extreme oligoasthenoteratozoospermia. From these preliminary data, it is justifiable to suggest that the group at risk includes men with sperm concentrations of $<5\times10^6$/ml.

Conclusions

Despite careful diagnosis of the infertile male, therapeutic modalities are almost non-existent. Most cases of oligoasthenoteratozoospermia and non-obstructive azoospermia are idiopathic or unexplained. Even if diagnoses of men with impaired semen quality are explicable, e.g. as in men suffering from cryptorchidism, no treatment can be offered. Research laboratories must find modalities of diagnosis and therapy, in the hope that efficient treatments for oligospermic males will be found. Such a development could dramatically decrease the need for ICSI. However, if male infertility has a genetic origin, then few or no forms of medical treatment will be helpful.

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