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Male factors determining the outcome of intracytoplasmic sperm injection with epididymal and testicular spermatozoa

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Summary. During a period of 8 years, 1079 intracytoplasmic sperm injection (ICSI) procedures with aspirated epididymal or testicular spermatozoa were performed. Epididymal spermatozoa were used in 172 cycles and testicular spermatozoa or spermatids in 907 cycles. Multiple biopsies were obtained from at least two different locations in the testes. Retrieved spermatozoa were used after cryopreservation (frozen) or immediately after aspiration (fresh). Three hundred patients had obstructive azoospermia (OA) or ejaculation failure. In 414 cases, azoospermia was caused by impaired spermatogenesis resulting from maldescended testes, chemotherapy/radiotherapy, or by Sertoli-cell-only syndrome, genetic disorders or unknown aetiology. Transfer rates, pregnancy rates and birth rates per ICSI cycle showed no statistically significant differences between testicular and epididymal spermatozoa in men with OA (28% average birth rates in both cases). However, birth rates differed significantly with regard to the status of spermatogenesis. Treatment of men with nonobstructive azoospermia (NOA) resulted in a birth rate of 19% per cycle. In all patient groups, there was no difference in the birth rates achieved with fresh and cryopreserved spermatozoa. While testicular volume, follicle-stimulating hormone level and age of the male patient are no statistically significant prognostic factors, the underlying cause of azoospermia is the most important factor determining the outcome of ICSI with epididymal and testicular spermatozoa. The pregnancy rate is lower in NOA patients than in those with OA.

Introduction

Male infertility resulting from obstruction of the seminal ducts can be solved by using microsurgical refertilization techniques. Men with irreversible obstructions are candidates for microsurgical epididymal sperm aspiration (MESA) or testicular sperm extraction (TESE), both in conjunction with intracytoplasmic sperm injection (ICSI). In cases of nonobstructive azoospermia (NOA), TESE can also be used for sperm retrieval and consecutive ICSI.

Evaluating our data over a period of 8 years, we have analysed the relevant andrological factors that might influence the outcome of ICSI with epididymal and testicular spermatozoa.

Patients and methods

Operative techniques

In all cases of obstructive azoospermia (OA) with the presence of a vas deferens, an attempt was first made to reconstruct the seminal ducts with the aim of achieving patency of seminal pathways and obtaining ejaculated spermatozoa. Men with irreversible post-testicular obstruction of the seminal ducts [e.g. congenital bilateral aplasia of vas deferens (CBAVD)] underwent MESA, which is a well-defined operative procedure. In the cases of inflammatory obstruction of the epididymis with the therapeutic necessity of tubulovasostomy,

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MESA and TESE were performed additionally for the cryopreservation of epididymal and testicular sperm. If azoospermia persisted, these preserved spermatozoa were then used for the ICSI procedure.

In men with fully or focally developed spermatogenesis but no outflow of seminal fluid to the epididymis, which is necessary for MESA, the only possibility to obtain spermatozoa for ICSI is TESE. The procedure was performed by open testicular biopsy. In cases of irreversible obstructive azoospermia, testicular tissue was extracted on a unilocular basis and multiple biopsies were performed in all cases of NOA (three to 10 biopsies per testicle).

In the first year of the study period, spermatozoa or spermatids were used immediately after aspiration. Since 1994, spermatozoa have been used after cryopreservation whenever possible.

Patients

From November 1993 to November 2001, 714 azoospermic patients underwent MESA/TESE. The main indication for MESA was irreversible obstruction of the seminal ducts, either acquired or congenital (CBAVD). Indications for TESE were chiefly NOA caused by maldescended testes, chemotherapy, radiation therapy, complete fibrosis of the epididymis or genetic disorder (Fig. 1).

Despite normal spermatogenesis, a group of 42 patients had severe ejaculation failure resulting from spinal cord injury, retroperitoneal lymphadenectomy or severe psychogenic problems which were not treatable by conventional methods.

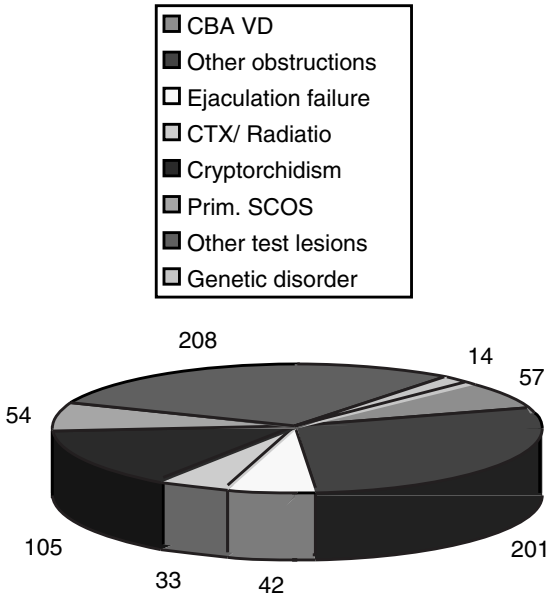

Figure 1. Underlying male pathologies as an indication for intracytoplasmic sperm injection with epididymal and testicular spermatozoa in 714 patients (*n*).

Histological examination and genetic screening

Testicular histology was available for all NOA patients. Two were shown to have testicular intraepithelial neoplasia, which was treated by radiotherapy of the involved testis.

In 1997, genetic screening was introduced of patients with primary NOA concerning the azoospermic factor (AZF = deletion of the Y chromosome) (AZF a,b,c). All patients with CBAVD and their female partners underwent screening to determine mutations of the CFTR gene (six to 32 mutations).

Statistical analysis

At the Institute of Medical Statistics, Technische Universität Munich, statistical analysis was performed by means of the chi-squared test.

Results

Between November 1993 and November 2001, 714 patients underwent 771 sperm retrieval procedures, of which 98 were combined with reconstruction of the seminal ducts. Spermatozoa were found in 247 of 414 NOA patients (60%) (Table 1). In 31 ICSI procedures, epididymal spermatozoa were used immediately after extraction (one-step procedure) in stimulated cycles. Another 141 ICSI procedures were performed with epididymal spermatozoa after cryopreservation (two-step procedure). Testicular spermatozoa were used immediately on 96 occasions and after cryopreservation on 780 occasions. In 31 ICSI procedures, testicular spermatids (fresh/frozen) were used because of lack of spermatozoa (Table 2). Of these 1079 ICSI procedures, 999 (92%) resulted in at least one oocyte fertilization and embryo transfer. Overall pregnancy occurred in 311 cases (45 twins, three triplets). However, 55 (18%) had an abortion, so the overall birth rate was 24% per cycle. Although embryo transfer was achieved in 26 (84%) of the 31 ICSI cycles using spermatids, no clinical pregnancy occurred (Table 2).

Obstructive azoospermia

Evaluating the results of ICSI in relation to the sperm origin (epididymal or testicular), there is a – statistically not significant – tendency in favour of epididymal spermatozoa (birth rate 32% versus 23%). However, the transfer rates, pregnancy rates and birth rates per ICSI cycle were not significantly different between testicular and epididymal spermatozoa, regardless of whether fresh or

Table 1. Probability of finding spermatozoa in relation to previous testicular status and definite histology in 414 patients with nonobstructive azoospermia

Cause of nonobstructive azoospermia	Number of patients (*n*)	Patients with sperm detection	Sperm detection rate (%)
Primary Sertoli-cell-only syndrome	54	10	19
Maldescended testes	105	70	67
Chemotherapy and/or radiotherapy	33	18	55
Klinefelter syndrome or other genetic disorder	14	8	57
Other causes of impaired spermatogenesis	208	141	69
Total	414	247	60

Chi-squared test, $P < 0.0001$.

Table 2. Intracytoplasmic sperm injection (ICSI; $n = 1079$) cycles with epididymal and testicular spermatozoa in relation to sperm origin (testicular/epididymal) and type of application (fresh/frozen)

Sperm origin	ICSI cycles (*n*)	Embryo transfer rate per cycle (%)	Birth rate per cycle (%)
Epididymal fresh	31	97	16
Epididymal cryopreserved	141	96	32
Testicular fresh	96	94	25
Testicular cryopreserved	780	92	23
Testicular spermatids (fresh/frozen)	31	84	0
Total	1079	92	24

The differences in birth rates between the groups are not statistically significant (chi-squared test, $P = 0.0944$).

Table 3. Intracytoplasmic sperm injection (ICSI; $n = 1079$) cycles with epididymal and testicular spermatozoa from 714 patients: results in relation to the underlying cause of azoospermia

Pathology	ICSI cycles (*n*)	Embryo transfers (*n*)	Pregnancies (*n*)	Abortions (*n*)	Birth rate (%)
Obstruction					
CBAVD	120	117	43	8	29
Refertilisation failure: post-vasectomy or post-inflammatory	359	349	120	25	26
Ejaculation failure	85	81	32	4	33
Impaired spermatogenesis					
Cryptorchidism	179	158	32	9	13
Chemotherapy/radiation	41	39	13	3	24
Sertoli-cell-only syndrome	23	18	2	0	9
Klinefelter syndrome	2	1	0	0	0
Unknown aetiology	270	236	70	6	24
Total	1079	999	312	55	24

cryopreserved (birth rate 26% versus 29% per cycle). Thus, none of the patient groups showed a significant difference between fresh and cryopreserved spermatozoa (Table 3).

Analysis of the data with regard to the underlying causes of azoospermia revealed significant differences. In all patients with OA (including those with ejaculation failure), a single biopsy yielded sufficient spermatozoa to perform ICSI.

Nonobstructive azoospermia

No spermatozoa were found in 167 of 414 nonobstructed azoospermic men (40%) (Table 1). The 60% who had spermatozoa showed a significantly lower embryo transfer rate, pregnancy rate and baby take-home rate. The birth rate in this group was 9–24% (average 19%) per ICSI cycle (Fig. 2). On the other hand, a birth rate of 28% occurred in the group with OA. The differences are statistically significant ($P = 0.0031$) (Fig. 2).

In 32 of 138 NOA patients in whom previous unilocular biopsies had been negative, spermatozoa were found after multiple TESE procedures, which corresponds to a detection rate of 23%.

Evaluation of other underlying factors that might affect the outcome of ICSI, such as testicular volume, follicle-stimulating hormone (FSH) level

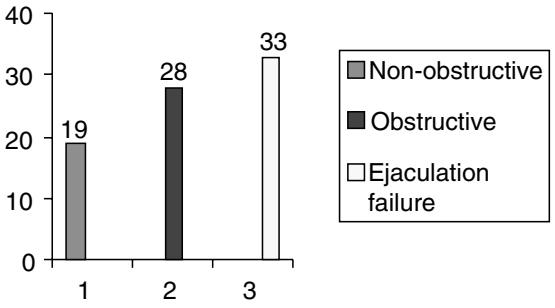

Figure 2. Intracytoplasmic sperm injection ($n = 1079$) cycles (out of 714 couples) with epididymal and testicular spermatozoa: birth rates per cycle (%) in relation to underlying aetiology of azoospermia. The difference between group 1 and 2 is statistically significant ($P = 0.0031$) while that between group 2 and 3 is not ($P = 0.27$) (chi-squared test).

and age of the male partner, did not reveal significant differences with regard to the sperm detection rate (Tables 4–7).

Genetic disorder

Pre-operative genetic screening in 226 of 381 patients with primary testicular lesions showed a numeric chromosomal disorder in 11 cases (nine men with Klinefelter syndrome, two with XO) and a deletion of the Y chromosome (AZF) in three patients. In one of the latter, testicular spermatozoa was found, but ICSI was not performed because of the couple's personal decision. Although five of the nine patients with Klinefelter syndrome had testicular spermatozoa or elongated spermatids, no pregnancy occurred. Of the 57 patients with CBAVD, 47 showed a mutation of the CFTR gene. There was one case of female CFTR mutation and because of this risk, the couple decided to refrain from ICSI.

Discussion

Since its introduction in 1992, ICSI has revolutionized the techniques of assisted reproduction and has become a popular fertilization procedure. ICSI with ejaculated spermatozoa from men with impaired semen quality achieves a delivery rate of up to 39% per cycle (Palermo *et al.*, 2000; Nygren & Anderson, 2001). Even in cases of azoospermia, ICSI can realize the couple's fertility. Azoospermia can be caused by irreversible obstruction of the seminal ducts or testicular lesions (NOA). In these patients, sperm retrieval techniques are required to achieve fertility with ICSI. While MESA and TESE are the most common retrieval procedures, percutaneous aspirating techniques are favoured by some groups (Craft *et al.*, 1997; Levine & Lisek, 1998; Turek *et al.*, 2000).

In this study, we analysed the outcome of ICSI with epididymal and testicular spermatozoa in relation to pregnancy and birth rates in 714 couples treated during 8 years. We tried to identify the most important andrological factors that might influence the result, while female aspects were not analysed.

Table 4. Testicular volume as a prognostic factor for sperm detection in 414 patients with nonobstructive azoospermia. The study is based on the volume of the larger of the two testicles (where two were present)

Testicular volume (ml)	Patients (*n*)	Patients without sperm (*n*)	Patients with sperm (*n*)	Detection rate (%)
<5	16	7	9	56
5–10	162	59	103	64
11–15	147	60	87	59
>15	89	41	48	54
Total	414	167	247	60

Detection rates show no statistically significant differences between the different groups of testicular volume (chi-squared test, $P = 0.5051$).

Table 5. Male follicle stimulating hormone (FSH) level as a prognostic factor for sperm detection in 414 patients with nonobstructive azoospermia

FSH (mIU ml^{-2})	Patients (*n*)	Patients without sperm (*n*)	Patients with sperm (*n*)	Detection rate (%)
<10	86	33	53	62
10–20	154	65	89	58
21–30	93	36	57	61
31–40	48	22	26	54
>40	33	11	22	67
Total	414	167	247	60

Detection rates show no statistically significant differences between the different group (chi-squared test, $P = 0.7836$).

Table 6. Birth rate per intracytoplasmic sperm injection (ICSI) cycle ($n = 339$) in relation to the age of patients with nonobstructive azoospermia who fathered with retrieved testicular spermatozoa

Male partner age (years)	ICSI cycles (*n*)	Birth rate per cycle (%)
<40	294	21
40–50	38	18
>50	7	29

Female age in all cases <35 years. Birth rates show no statistically significant differences between the three groups (chi-squared test, $P = 0.8205$).

Table 7. Birth rate per intracytoplasmic sperm injection (ICSI) cycle ($n = 291$) in relation to the age of patients with obstructive azoospermia, who fathered with retrieved testicular spermatozoa

Male partner age (years)	ICSI cycles (*n*)	Birth rate per cycle (%)
<40	218	34
40–50	63	25
>50	10	30

Female age in all cases <35 years. Birth rates show no statistically significant differences between the three groups (chi-squared test, $P = 0.4359$).

Obstructive azoospermia

Based on the experiences of most professional *in vitro* fertilization centres worldwide, it is generally accepted that in obstructed azoospermic men the type of retrieval technique is of secondary importance. In these cases, it is a simple matter to retrieve spermatozoa at the first attempt using any of the conventional procedures (aspiration or biopsy).

Epididymal spermatozoa

Concerning the ICSI results with MESA, we achieved delivery rates of up to 32% per cycle with epididymal spermatozoa. An unexpected and apparently contradictory result was obtained relating to the patients in whom fresh epididymal spermatozoa had been used in a one-step procedure. The birth rate of 16% per ICSI cycle was lower than that achieved in the group with cryopreserved sperm (32%). This phenomenon may be explained by the statistically nonsignificant size of the patient group with fresh spermatozoa. On the other hand, it might result from the fact that epididymal native spermatozoa were used during the initial learning curve of ICSI at our institution.

Analysing the results of ICSI with epididymal spermatozoa, no statistically significant superiority could be demonstrated. With regard to other factors, such as capacity of freezing, higher sperm density, better motility, etc., the apparent advantage of epididymal over testicular spermatozoa remains speculative.

Testicular spermatozoa

A comparison between epididymal and testicular sperm (which is only possible in the obstructive group, because NOA patients have no epididymal spermatozoa) revealed no statistically significant difference. The birth rate was 29 and 26% for epididymal and testicular sperm, respectively. This finding is supported by the fact that there is no relevant study with a representative patient number demonstrating a clear superiority of epididymal over testicular sperm in OA.

Fresh versus frozen sperm

Analogously, a comparison of the use of fresh/frozen spermatozoa showed no differences. In accordance with other authors (Tournaye *et al.*, 1999; Gil-Salom *et al.*, 2000; Janzen *et al.*, 2000; Cayan *et al.*, 2001) we found no statistically significant difference in birth rates. Cryopreserved epididymal spermatozoa tended to give the best birth rate per cycle (32%), but the difference to the other sperm groups was not statistically significant (Table 2). It is unclear why freezing should improve the sperm fertilization capacity. Data in the literature are congruent with our findings (Tournaye *et al.*, 1999, Cayan *et al.*, 2001).

Nonobstructive azoospermia

The only clear difference in birth rates showing statistical significance resulted from a comparison of men with normal spermatogenesis (obstructive azoospermia and ejaculation failure) and those with impaired spermatogenesis. In the latter group, no spermatozoa were found in 40% of patients. Nonobstructed patients with spermatozoa (60%) had a significantly lower birth rate per cycle (19%) than obstructed men in whom sperm retrieval was not the problem because the first testicular biopsy had yielded spermatozoa.

Scientific interest should be focussed on the group of NOA patients. It should be remembered that these include men with previous negative testicular biopsies (no spermatozoa), Klinefelter syndrome, Sertoli-cell-only syndrome (SCOS) and other negative preconditions relevant to the discovery of testicular spermatozoa. Therefore, the low

birth rates reported in the nonobstructive group should be seen in the light of the fact that many of the patients had a poor fertility prognosis because of inferior testicular status (Tournaye *et al.*, 1996).

Prognostic factors for the outcome of ICSI

A central question raised by this study is the evaluation of andrological factors relating to the outcome of ICSI. While it is well established that female partner age is one of the most important prognostic factors (Nygren & Andersen, 2001), the effect of male age has been little researched. So we tried to evaluate the importance of the age of male partner with regard to a couple's fertility chance. To minimize the influence of female age, only couples where the female partner was under 35 years were included in this context. The influence of impaired spermatogenesis was eliminated by including only couples with OA. Another group comprised NOA patients. In neither of the groups did the age of the male partner seem to have an influence on the birth rates.

Testicular volume

NOA patients usually have pathologically reduced testicular volumes (<15 ml). As we failed to find spermatozoa by TESE in 40% of nonobstructed men, we searched for a correlation between the degree of diminished testicular volume and sperm detection rate, but there was no statistically significant relationship. As a result, small testicular volumes should not be advanced as a reason for refusing TESE. However, volumes lower than 5 ml must indicate genetic screening for Klinefelter syndrome, other numeric chromosomal aberrations and the azoospermic factor. The sperm detection rate in cases of genetic disorder (57%) was not decreased compared with that obtained in other testicular lesions, but the fertility rate was much lower. In the rare cases of genetically caused NOA, delivery was not achieved. Despite a few reports about successful infertility management of Klinefelter patients (Silber, 1998) the use of testicular spermatozoa in these cases is not recommended.

The lowest sperm detection rate (19%) occurred in the group with primary Sertoli-cell-only syndrome (Table 1). Consequently, the birth rate (9%) was lower than that observed with other testicular lesions. In many patients with primary SCOS, the clinical status with testicular volumes of 1520 ml and moderately elevated FSH is typical. As a unilocular testicular biopsy has often been performed with negative result, we have frequently been approached about a further biopsy. In these cases, the sperm detection rate is 23% using a bilateral multiple biopsy technique. Prior to deciding on a further testicular biopsy procedure, the patient should be informed about this low detection rate.

FSH

While FSH levels can be an indicator of the degree of testicular injury, patients with SCOS usually have concentrations not higher than 20 IU ml^{-1}. So we examined the birth rates after use of testicular spermatozoa from NOA patients in relation to the FSH levels. No significant differences were found in five groups (Table 5). Consequently, highly elevated FSH levels must not be considered a negative prognostic factor for the outcome of ICSI.

The sperm nondetection rate of 40% in NOA patients may be explained by our practice of performing multiple biopsies in a mapping technique. A number of alternative procedures are available for epididymal and testicular sperm retrieval. It is not the subject of this paper to discuss the value and risks involved.

In conclusion, the underlying cause of azoospermia is the most important prognostic factor regarding the outcome of ICSI (Fig. 2). In cases of NOA, the birth rate after ICSI is the lowest in patients with SCOS and genetic disorders (Table 3). Men with normal spermatogenesis have good chances to achieve fertility, provided that there is no female sterility factor. In contrast, patients with testicular lesions should be informed that their chances of achieving fertility are lower and the probability of not detecting spermatozoa is as high as 40%.

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