Increased Incidence of Meiotic Anomalies in Oligoasthenozoospermic Males Preselected for Intracytoplasmic Sperm Injection

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Purpose: Based on data from the literature, to detect the possible presence of an increased frequency of meiotic anomalies in oligoasthenozoospermic (OA) patients preselected for intracytoplasmic sperm injection.

Methods: Meiotic studies in as many successive patients with a clinical indication for a diagnostic testicular biopsy as needed to complete at least 100 cases with a severe OA (motile sperm concentration $\leq 1.5 \times 10^6/\text{ml}$).

Results: An increased incidence of meiotic anomalies was found in 102 patients with a severe OA (17.6%) compared to the mean for 105 patients with other etiologies in the series (5.7%) or the mean for patients reviewed in the literature (6.5%).

Conclusions: Patients with a severe OA have a higher incidence of synaptic anomalies. This may result in the malsegregation of chromosomes at meiosis I, producing abnormal sperm, and could explain the high incidence of sterility and some cases of abortion (in two thirds of the couples with abortions the husband had meiotic anomalies) in this group.

KEY WORDS: Diploidy; meiotic anomalies; oligoasthenozoospermia; sterility; synaptic anomalies.

INTRODUCTION

In recent years, the widespread use of intracytoplasmic sperm injection (ICSI) in couples with a severe male factor has led to an increased frequency of chromo-

some abnormalities in children conceived using this method (1). Although occasionally one of the parents carried a balanced chromosome rearrangement, in most cases de novo anomalies were detected. Several authors have suggested that this increased frequency of chromosome abnormalities in ICSI children could be related to a generalized disruption of the meiotic process (2, 3).

The first cases of recombination deficiency resulting in a disruption of meiosis in humans were described almost 30 years ago (4, 5). The most common anomaly was described as desynapsis of individual bivalents, affected a limited number of meiotic bivalents, and was characterized by the presence of variable numbers of univalents or of oligochiasmatic or achiasmatic homologues (6). The incidence of meiotic errors in heterogeneous populations of infertile males was established at 4–8% (7, 8).

Attempts to correlate the presence of meiotic anomalies and the characteristics of the semenograms failed to disclose any significant relationships, probably because the populations studied were ill defined. However, meiotic anomalies showed a tendency to accumulate in oligoasthenozoospermic (OA) males, although at the time the term OA was used to define a number of variable situations.

To determine whether patients with extremely poor semen parameters had an increased frequency of meiotic anomalies, we carried out meiotic studies in testicular biopsies from 103 male patients with a severe oligoasthenozoospermia preselected for ICSI.

MATERIALS AND METHODS

The study was carried out in as many successive patients with a clinical indication for a diagnostic testicular biopsy as needed to complete a series of at least

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100 patients with a severe oligoasthenozoospermia (motile sperm concentration $\leq 1.5 \times 10^6$ sperm/ml in at least two successive semenograms) preselected for ICSI. Testicular biopsies are included in the protocol of study of infertile and sterile patients at the Institut Dexeus, and as such its use has been approved by the institutional ethics committee. Written informed consent was obtained from all patients.

Inclusion in the OA group was based only on seminal parameters. The other patients were selected for ICSI for heterogeneous reasons, including OA with higher sperm numbers and/or motility, different types of obstructive (variable anatomical levels and etiologies), or nonobstructive (traumatic, infectious, idiopathic, cryptorchidic) azoospermia, previous in vitro fertilization (IVF) failures, recurrent abortion, idiopathic infertility, and so forth. Somatic chromosome studies in peripheral blood also were offered, but not all patients accepted.

Unilateral testicular biopsies were obtained under local anesthesia. Meiotic preparations were made using the technique of Evans *et al.* (9). All preparations were coded, scored to locate meiotic divisions at $10\times$, and analyzed blindly by two different observers. The blind was only disclosed at the end of the study.

Statistical analyses were carried out using the χ^2 test.

RESULTS

The final series included 103 patients with OA as defined, and 106 patients of different etiologies. In the OA series 70 out of 103 patients were karyotyped, and in the heterogeneous sterile population karyotypes could be obtained from 61 of the 106 patients. Somatic chromosome studies detected an XXY/XY mosaic in the OA group and an azoospermic XXY patient and a carrier of a reciprocal $t(8;17)$ with recurrent abortions in the heterogeneous group. The mosaic patient and the translocation carrier were eliminated from the corresponding groups, because it is known that such patients are prone to meiotic anomalies, leaving a total of 102 patients in the OA group and 105 patients in the heterogeneous group.

Results were classified as: 1—normal meiosis; 2—spermatogenic arrest, when prophase I figures and occasional spermatozoa but no metaphase I or metaphase II figures were found; 3—absence of spermatogenic cells, when only Sertoli cells were observed; and 4—meiotic anomalies, when synaptic errors were present as univalents and/or oligochiasmatic or achiasmatic homologues. Meiotic errors were found in 18/

102 OA patients and in 6/105 patients in the heterogeneous group (Table I). This is a significant increase (as a result of the relatively small numbers of patients) over the mean frequency in the heterogeneous group (17.6% vs. 5.7%; $\chi^2 = 7.24$, $DF = 1$, $P < 0.01$) and a highly significant increase (17.6% vs. 6.5%; $\chi^2 = 21.38$, $DF = 1$, $P < 0.005$) over the mean of the published heterogeneous series (7). If only the karyotyped patients are taken into account, the incidence of meiotic anomalies is similar: $13/69 = 18.8\%$ in OA patients and $3/60 = 5.0\%$ in the heterogeneous group.

The anomalies observed in the OA group were two cases of complete desynapsis (synaptic anomalies affecting all bivalents), nine cases of desynapsis affecting a variable number (usually 2–6) medium-sized or large bivalents, and seven cases with the presence of a variable number of small univalents in their metaphase I figures. In the heterogeneous group, four patients had synaptic anomalies affecting two to four medium-sized or large bivalents and two patients had small univalents in their metaphase I figures.

In the OA series, 100 patients were sterile and 3 had one abortion; of these, two had meiotic anomalies. In the heterogeneous group, 91 patients were sterile and 15 had at least one abortion (mean of 1.8 abortions per couple).

DISCUSSION

Our results have shown that in patients with a severe OA, as defined by present criteria, the incidence of meiotic anomalies is much higher than that previously found in heterogeneous populations of infertile males, as had been already suspected (7). In fact, this tendency has been confirmed in relatively short series of patients by meiotic analyses (10), and several studies using fluorescence in situ hybridization (FISH) on decondensed sperm heads have shown that OA males have a higher incidence of autosomal and sex chromosome aneuploidies and of diploid sperm (2, 3, 11, 12), as

Table I. Results of Meiotic Studies in 207 Successive Patients

	OA (<i>n</i> = 102)(%)	Heterogeneous (<i>n</i> = 105)(%)
Normal	63 (61.7)	63 (60.0)
Sperm. arrest	21 (20.5)	21 (20.0)
No sperm. cells ^a	-- (----)	15 (14.2)
Meiotic anom. ^b	18 (17.6)	6 (5.7)

^a Only observed in some cases of azoospermia.

^b Univalents or oligo/achiasmatic homologues.

should be expected from the disturbance of the anaphase I checkpoint (13) by the presence of erratic chromosomes or bivalents.

Andrologic studies carried out in our patients have shown the existence of a correlation between the incidence of meiotic anomalies and decreasing numbers of motile spermatozoa, and also between the incidence of meiotic anomalies and increased levels of FSH (14); the relationship between disturbed meiosis and increased levels of FSH or other environmental anomalies also has been suggested by several authors (15, 16). Finally, preliminary results on the reproductive performance of some of these patients after ICSI indicate that patients with meiotic anomalies have a decreased pregnancy rate and an increased abortion rate when compared to infertile individuals with a normal meiotic process (17).

Since these patients produce sperm with complex chromosome abnormalities (11) and an increased rate of diploid spermatozoa (12), and their embryos produced by ICSI show slower division rates, as shown in our laboratory (unpublished), some cases of sterility resulting from a severe male factor could be related to an increased peri-implantational wastage. In fact, although it is generally accepted that about 50% of abortions from clinically recognized pregnancies have chromosome anomalies, it also has been shown that over 80% of peri-implantational losses have chromosome aberrations (18).

These observations stress the need for genetic testing (either through meiotic studies, sperm FISH, or both) and for genetic counseling in patients with a severe OA or with major sperm phenotype aberrations (14, 17, 19, 20).

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