

## Natural Course of Severe Oligozoospermia in Infertile Male: Influence on Future Fertility Potential

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**ABSTRACT:** Most couples with severe male factor infertility are treated with assisted reproduction technology and little has been known about the prognosis of severe male factor infertility itself. We investigated the prognosis of infertile male patients with severe oligozoospermia. Thirty-nine patients with severe nonobstructive oligozoospermia were followed more than 6 months without any medical or surgical intervention. Retrospective analyses of the natural sequence of the condition and influences on the future fertility potential of the study participants were conducted. Sperm concentration, motility, and morphology between first semen analysis and last semen analysis were not significantly different. However, during the follow-up period, 5 (12.8%) patients became azoospermic. In 7 (17.9%) patients, the sperm count declined to a

severe level that could be detected only after centrifugation. Three patients underwent microdissection testicular sperm extraction (TESE) for sperm retrieval after confirmation of azoospermia. The sperm retrieval was successful only in 1 of the 3 patients. Therefore, male patients diagnosed with severe oligozoospermia should be informed about possible aggravation of their residual spermatogenesis function and the necessity of intermittent follow-up semen analyses. If follow-up semen tests show a declining tendency, sperm cryopreservation may be recommended for these patients. If azoospermia develops during the follow-up period, early TESE procedure should be considered to improve the chance of sperm retrieval.

Key words: Male infertility, prognosis.

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Infertility affects about 15% of couples attempting pregnancy, with male factor infertility identified in approximately 50% of the cases (Bhasin et al, 1994). However, many aspects of male infertility are poorly understood, and many cases of male infertility are diagnosed as idiopathic. Although researchers believe that many cases of male infertility have a contributing genetic background, our ability to diagnose these defects remains limited (Lilford et al, 1994; Short, 1994; Silber and Repping, 2002). Detailed genetic studies such as chromosomal abnormality and Y chromosome micro-deletion have been conducted for less than 2 decades (Kleiman et al, 1999). On the other hand, over the past 30 years, the treatment of infertility has seen the development of revolutionary new assisted reproductive

technologies such in vitro fertilization (IVF) and intracytoplasmic sperm injection (ICSI).

Recent advances in IVF techniques, such as conventional IVF and, more importantly, ICSI, require only a small number of viable sperm and, hence, have revolutionized the treatment of severe male factor infertility by allowing infertile men with severely compromised semen parameters to achieve fatherhood (Palermo et al, 1992; Devroey et al, 1995; Nagy et al, 1998; Silber, 2000). Therefore, the couples with severe male factor infertility are treated mostly with assisted reproduction technology (ART). However, male patients have not drawn much clinical attention, and furthermore, most male patients are not regularly followed up after the IVF/ICSI procedure; thus, little has been known about the prognosis of male factor infertility disease itself. For the infertile couples with severe male factor etiology, there may be a successful pregnancy with the aid of advanced ART procedures. However, the couple might want another baby several years later. Thus, the prognosis and future fertility potential of these severe male factor patients are very important. We therefore investigated the natural course of patients with severe oligozoospermia and discuss the future fertility potential in these patients.

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Table 1. Patient characteristics at initial evaluation

Characteristic	Mean ± SD
No. of patients	39
Age, years	32.8 ± 3.0
Follow-up, months	34.8 ± 26.1
Semen volume, mL	2.9 ± 1.4
Sperm concentration, $\times 10^6/\text{mL}$	1.0 ± 1.3
Sperm motility, %	24.7 ± 15.4
Sperm morphology, %	15.6 ± 11.4
FSH, IU/mL	9.2 ± 6.2
Testis volume, mL	11.9 ± 4.2

## Materials and Methods

We retrospectively reviewed the natural history of patients diagnosed with severe male factor infertility at our andrology clinic. The study was approved by the institutional review board. Evaluation of men consisted of a thorough personal and family history, physical examination, semen analyses, and laboratory tests, including hormonal profile and genetic tests. From January 2002 to December 2007, a total of 425 patients were initially diagnosed with severe male factor infertility. Of them, 39 patients met the inclusion criteria of this study. Inclusion criteria were severe, nonobstructive-type oligozoospermia at initial evaluation and more than 6 months follow-up. Severe oligozoospermia was defined as a concentration of less than  $5 \times 10^6$  sperm/mL. Patients who underwent any medical or surgical treatment during the follow-up period were excluded. Other exclusion criteria were a previous history of genital infection, varicocele, cryptorchidism, exposure to gonadotoxin, hypogonadism, or an insufficient sexual abstinence period. The patients with suggestive obstructive-type oligozoospermia based on semen volume, testis size, serum FSH level, or obstructive lesion as revealed by an image study such as transrectal ultrasonography were also excluded. All patients underwent multiple semen analyses ( $\geq 3$  times) during the follow-up period. Sperm concentration, motility, and morphology were assessed according to the World Health Organization (1999) manual. All semen samples were obtained by masturbation into a wide-mouthed plastic container from patients in a separate room after 3 days or more of sexual abstinence; the samples were allowed to liquefy for at least 20 minutes at 37°C before analysis. During follow-up, the patients repeated semen analyses, if sperm was not detected under conventional microscopic Makler chamber evaluation, centrifugation at  $1500 \times g$  for 10 minutes was performed to detect any viable motile sperm. Azoospermia was determined with centrifuged specimen on 2 different occasions. The changes of fertility status and semen analysis data were analyzed and compared. All statistical analyses were performed with the use of a commercially available software program (SPSS 11.5, Chicago, Illinois). The Kaplan-Meier method was used to generate the time to event (azoospermia) curve, and the Wilcoxon signed rank test was used to compare the difference between semen parameters. Statistical significance was defined as  $P < .05$ , and all statistical tests were 2-sided.

Table 2. Changes in semen variables during the follow-up period

	Initial Semen Analysis	Last Semen Analysis	<i>P</i>
Semen volume, mL	2.9 ± 1.4	2.9 ± 1.7	.228
Sperm concentration, $\times 10^6/\text{mL}$	1.0 ± 1.3	0.8 ± 1.2	.499
Sperm motility, %	24.7 ± 15.4	23.0 ± 13.1	.498
Sperm morphology, %	15.6 ± 11.4	12.9 ± 11.1	.105

## Results

Thirty-nine patients with severe nonobstructive oligozoospermia met the inclusion criteria, including a follow-up period of more than 6 months with multiple repeated semen analysis and no medical or surgical intervention for male factor infertility during the follow-up period at our andrology clinic between January 2002 and December 2007 (Table 1). The median follow-up period was 30.0 months (range, 6.0–134.0 months). Sperm concentration, motility, or morphology between first and last semen analysis was not statistically significantly different (Table 2). However, 7 of 39 (17.9%) patients exhibited a sperm count reduced to the extremely severe level—that is, detectable only after centrifugation. Median concentration of initial semen analysis in these patients was  $0.1 \times 10^6$  sperm/mL (range,  $0.05$ – $5.0 \times 10^6$  sperm/mL). Five patients (12.8%) became azoospermic during the follow-up period. Azoospermia was confirmed with multiple ( $\geq 2$  times per patient) centrifuged semen specimens. Median concentration of initial semen analysis in these azoospermic patients was  $1.3 \times 10^6$  sperm/mL (range,  $0.05$ – $5.0 \times 10^6$  sperm/mL). The median duration to become azoospermic was 42.0 months (range, 6.0–62.0 months). With Kaplan-Meier survival analysis, the mean survival time of the study population during the follow-up period was 105.7 months (95% CI, 83.53–127.87 months; Figure). After azoospermia was confirmed with a centrifuged specimen, 3 patients underwent microdissection testicular sperm extraction (micro-TESE) procedure for sperm retrieval; this procedure was successful only in one of them (33.3%). Sperm was not found in the other 2 patients, and their testes biopsies showed Sertoli cell-only syndrome.

## Discussion

Male factor infertility has increasingly become a central topic in treating infertile couples. However, many aspects of male infertility are still poorly understood, and medical therapy for these patients is largely

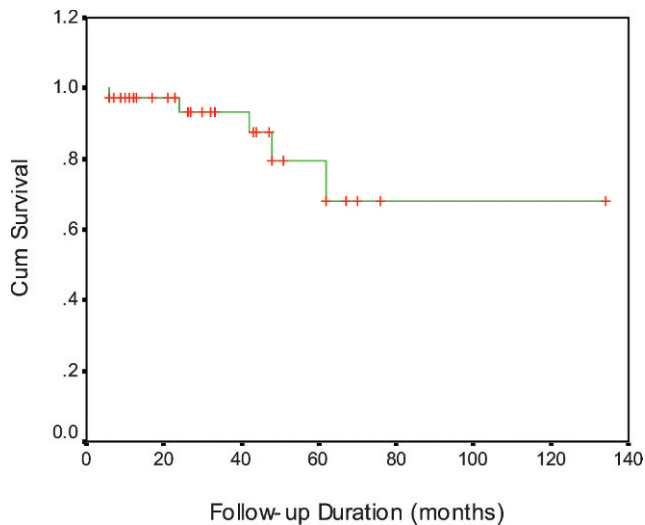


Figure. Survival curve of residual spermatogenesis function.

unsuccessful. More than 90% of male factor infertility is characterized either by low number of sperm in semen or by production of poor-quality sperm. Therefore, a management plan for severe male infertility often does not consider a pharmacologic treatment, and couples are treated directly with ART. Clinicians are interested mainly in a successful pregnancy using an advanced ART procedure, and little has been known about the natural history of male factor infertility itself. However, the prognosis and future fertility potential are very important not only for patients themselves but also for the couples. Previously, there were a few case reports about the course of patients with severe male factor infertility, and the decline in sperm count over time was reported in patients with Y chromosome microdeletion (Girardi et al, 1997; Simoni et al, 1997; Calogero et al, 2001). In one study, a 25-year-old male patient with a Y chromosome microdeletion was diagnosed as severely oligozoospermic at initial evaluation. However, he became azoospermic over time, and a testis biopsy revealed spontaneous germ cell regression. The authors reported that spermatogenic defects with Y chromosomal microdeletions could worsen with age (Calogero et al, 2001). Five patients (12.8%) in our study group also became azoospermic during the follow-up period. This finding is consistent with previous case reports and suggests that, in some of these severe oligozoospermic patients, the underlying spermatogenic defects are ongoing, and, furthermore, future fertility potential might be lost over time.

Recent advances in IVF techniques, such as conventional IVF and, more importantly, ICSI, require only a small number of viable sperm. It is widely known that severely oligozoospermic semen may be reported as

azoospermia unless semen samples are examined carefully. Although routine microscopic semen examination might find no sperm in severe oligozoospermic patients, after centrifugation, several viable sperm can be found to use for IVF/ICSI procedure (Ron-El et al, 1997; Swanton et al, 2007). Five patients (12.8%) in our study group became azoospermic and no sperm were found even after a thorough search following centrifugation of semen specimens. If no viable motile sperm for IVF/ICSI procedure were found in the ejaculate after centrifugation, the next step would be TESE procedure, preferably under microdissection. However, this sperm retrieval method requires an invasive surgical procedure with possible complications, and the chance of successful sperm retrieval is usually 40%–60% in patients with nonobstructive-type azoospermia (Schlegel and Li, 1998; Okada et al, 2002; Tsujimura et al, 2002; Ramasamy et al, 2009). Three out of the 5 azoospermic patients in our study underwent microdissection TESE for sperm retrieval. After the surgical procedure, sperm were retrieved in only 1 patient, and in the other 2 patients, no sperm at all was retrieved. The testis biopsy during the TESE procedure showed Sertoli-only syndrome. These findings were consistent with the previous report that there was pathologic germinal cell regression over time. We suggest that early sperm cryopreservation should have been done in these patients.

Another important consideration is that a window of time might exist for the surgical retrieval of sperm between the onset of azoospermia development and complete loss of residual testicular spermatogenesis. To achieve higher sperm retrieval rates in these newly azoospermic patients during the follow-up period, TESE should be performed as early as possible before complete loss of residual spermatogenesis function. Sometimes clinicians dealing with infertile couples might start the IVF cycle without reevaluation of severe male factor infertility for long periods of time. In such cases, sperm collection from the male partner is requested on the same day of ovum pick-up for IVF. If the residual spermatogenesis function was completely depleted and azoospermia had developed in these patients, the sperm for the IVF procedure would not be acquired at all. We suggest that severe oligozoospermic patients should be reevaluated before a new IVF cycle if a long period of time has elapsed since the prior IVF cycle. The sperm concentration of 7 (17.9%) patients became so reduced that sperm were detected only after centrifugation. Although, these people maintained very few spermatogenesis functions during the follow-up period, these patients might also become azoospermic over a longer period, as time goes by. Therefore, the patients should be considered as potential candidates for sperm cryopreservation.

From several classifications of severe oligozoospermia, we adopted the less than 5 million sperm/mL inclusion criterion for our study group. Although still debatable, the general consensus is that, at this level of severe oligozoospermia, patients possess an underlying spermatogenesis defect (Calogero et al, 2001).

In our study, 5 patients had definitive identifiable genetic defects. One patient had chromosomal abnormality with translocation, and 4 patients had Y chromosome microdeletion. One patient with Y chromosome microdeletion became azoospermic during the follow-up period, and the remaining 4 patients maintained a severe oligozoospermic state. It is suggested that genetic causes are responsible for many cases of male factor infertility. However, the precise function and contribution of genetic defects to infertility phenotype are not yet known. We analyzed oligozoospermic patients with known genetic defects together with idiopathic patients because we think there are underlying unknown genetic factors in many cases of idiopathic oligozoospermia and they might have a similar natural course (Quilter et al, 2003).

Despite several limitations of our study, including the nature of its retrospective study design, lack of a control group, and relatively small study population, the present study clearly showed the diminishing tendency of spermatogenesis function over time in patients with severe nonobstructive oligozoospermia. This is the first report about the natural history of patients with severe oligozoospermia. Our study shows that patients with nonobstructive oligozoospermia at initial evaluation should be informed about the possible decline of residual spermatogenic function; hence, intermittent follow-up semen analyses should be recommended. If a decreasing tendency is noticed, early sperm freezing should be strongly considered. If azoospermia develops during the follow-up period and sperm had not been cryopreserved previously, early TESE might be considered to improve the chance of sperm retrieval. Medical personnel dealing with fertility problems should be more familiar with the natural pattern for men with severe male factor infertility. Further prospective studies about the prognosis of severe male factor infertility are warranted.

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