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# Beneficial effect of tamoxifen on sperm recovery in infertile men with nonobstructive azoospermia

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## Summary

About 10% of infertile men have azoospermia. After the introduction of microinjection [intracytoplasmic sperm injection (ICSI)], many of these men obtain the chance to be a father. But still in many cases of nonobstructive azoospermia, we are not able to find spermatozoa for ICSI. Medications may be able to increase the chance of finding spermatozoa in testis samples. So in this study, we evaluated the effect of tamoxifen citrate on the results of sperm recovery from testis tissue in infertile men with nonobstructive azoospermia. Thirty-two azoospermic infertile men with proved nonobstructive azoospermia were selected. Tamoxifen was administered for 3 months. Semen samples and in the cases of azoospermia second testis biopsy were taken, and the results were compared with the first samples. According to first testis samples, 13 patients had hypospermatogenesis, 9 had maturation arrest and 10 patients sertoli cell syndrome. After tamoxifen treatment, six patients showed spermatozoa in their ejaculates. From other patients all in hypospermatogenesis group, 75% in maturation arrest group and 20% in sertoli cell group showed spermatozoa in their second testis samples. Our study showed that treatment of patients with nonobstructive azoospermia with anti-oestrogenic drugs like tamoxifen can improve the results of sperm recovery in testis samples and also increase the chance of pregnancy by microinjection.

## Introduction

Today, infertility is a common problem all over the world. About 10–15% of couples who seek treatments are infertile, and male factor is the cause in about 50% of cases (Stephen & Chandra, 1998). Infertility and abnormal spermatogenesis can be the result of a wide range of diseases and abnormalities. The empiric use of hormones for idiopathic male infertility has been tried for many years, before the introduction of Assisted Reproductive Technology (ART) (Crottaz *et al.*, 1992; World Health Organization, 1992; Matsumiya *et al.*, 1998; Baccetti *et al.*, 2004). The term of idiopathic infertility is applied to men who have abnormal semen parameters without an identifiable cause based on history, physical examination and currently available laboratory and radiographic examinations. More than 30% of infertile men have idiopathic infertility (Oehninger & Kruger, 2007) according to above criteria (Nieschlag, 1997). Difficulties in defining this heterogeneous group of patients probably contribute to the often equivocal and disappointing results in many of the clinical trials.

Clomiphen citrate and tamoxifen citrate are two nonsteroidal selective anti-oestrogen receptor modulators commonly used for the treatment of idiopathic male infertility. Several randomised clinical trials have assessed tamoxifen performance for idiopathic infertility (Torok, 1985; AinMelk *et al.*, 1987; Krause *et al.*, 1992).

About 10% of these couples have azoospermia. Azoospermic patients can be divided into two groups of obstructive and nonobstructive azoospermia (Jarow *et al.*, 1989). Obstructive azoospermia is due to any problem that causes obstruction of sperm conduit and tube from testis to urethra. Nonobstructive azoospermia is related to any problem that causes inactivity of germinal tissues of testicles. With the advances in assisted reproductive techniques, only one spermatozoon per ovum is necessary for fertilisation with intracytoplasmic sperm injection (ICSI).



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Spermatozoa may be surgically retrieved from the testis, so treatment of these patients can be performed through macroscopic or microscopic removal of seminiferous tubules from testis and extraction of spermatozoa, if any, to use for microinjection (Bourne *et al.*, 1995; Vicari *et al.*, 2001; Tesujimora *et al.*, 2002). Some of the recent studies showed that testis sampling after medical treatment can increase chances of sperm retrieval (Hussein *et al.*, 2005). So, in this study, we selected infertile men with proven nonobstructive azoospermia and will report the rate of sperm retrieval after medical treatment with tamoxifen.

## Materials and methods

This descriptive clinical study has been carried out between 2007 and 2009 in Yazd Research and Clinical Center for Infertility, after approval by institutional review board. Forty-eight infertile men with nonobstructive azoospermia who had at least two samples of semen without spermatozoa and their previous testis biopsy had been shown nonobstructive azoospermia were selected. Physical examination and other required workup including hormone assay were carried out for all of them. Due to selecting idiopathic infertility, all patients who had varicocele or their serum FSH level was more than three times of normal range (2–10 mIU $ml^{-1}$) were excluded from the study. Thirty-two patients were selected and medical treatment with tamoxifen with dose of 20 mg orally per day started for them and continued for 3 months. Three months after treatment, semen samples were collected and semen analysis was carried out according to WHO guidelines (WHO, 1999). Morning plasma FSH assays [enzyme-linked immunoabsorbent assay (ELISA) method at Research and Clinical Center for Infertility Laboratory, Yazd, Iran] were performed for all patients. For those men who still had azoospermia, testicular sperm extraction (TESE) was carried out by open surgical method, in which after injecting 10 cc of xylocaine 2% into spermatic cord, scrotal layers incised through 1- to 2-cm skin incision. Tunica albuginea incised and at least three samples of testis tissue were taken and immediately sent to ART laboratory to check for the presence of spermatozoa. In cases that no spermatozoa found in these three initial samples, the same procedure was carried out on the other side and three other samples were taken. Tunica of the testis and other scrotal layers were sutured by chromic 4–0.

### Statistical analysis

Data were expressed in mean ± SD. For comparisons between different patterns of patients, chi-square test was assessed. A *P*-value of ≤0.05 was considered statistically significant. SPSS software version 13 (SPSS INC., Chicago, IL, USA) was used for such comparisons.

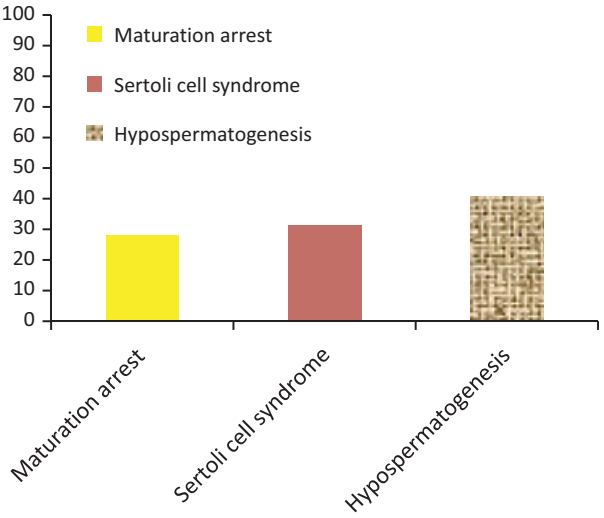


**Fig. 1** Distribution of histopathologic pattern on initial biopsy.

## Results

As shown in Fig. 1, hypospermatogenesis was seen in the initial biopsy of 13 patients (40.6%), maturation arrest was noted in 9 (28.1%) and sertoli cell syndrome was seen in 10 patients (31.2%).

After tamoxifen therapy, 6 (18.7%) patients demonstrated spermatozoa in their semen analysis with concentration ranging from 1 to 20 million spermatozoa per ml. Mean total motility, mean sperm morphology, mean age, and mean serum FSH level of these patients were listed in Table 1.

Semen parameters after tamoxifen treatment in all patients are listed in Table 2 according to initial testis biopsy results. In hypospermatogenesis group, sperm finding result was positive in 100% of cases. After tamoxifen treatment, 38.5% of patients generated spermatozoa in their ejaculate, and the others have a positive testicular

**Table 1** Age, FSH level and semen parameters of patients under investigation

| | *n* = 32 | | |
|---|---|---|---|
| Characteristics | Min | Max | Mean ± SD |
| Age (years) | 20 | 48 | 30.59 ± 7.08 |
| FSH(mIU $ml^{-1}$) | 1.50 | 20.00 | 8.33 ± 4.86 |
| Concentration (million $ml^{-1}$) | 1 | 20 | 6.50 ± 7.12 |
| Motility (%) | 1 | 20 | 11.17 ± 8.11 |
| Morphology (%) | 1 | 23 | 11.67 ± 9.37 |

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**Table 2** Sperm finding outcome by initial biopsy pattern after tamoxifen therapy

| | *n* = 32 | | | |
|---|---|---|---|---|
| Initial biopsy result | Spermatozoa in ejaculate | Positive in testicular biopsy | Negative in testicular biopsy | *P*-value |
| Hypospermatogenesis (*n* = 13) | 5 (38.5%) | 8 (61.5%) | 0 (0%) | 0.000 |
| Maturation arrest (*n* = 9) | 1 (11.1%) | 6 (66.6%) | 2 (22.2%) | |
| Sertoli cell (*n* = 10) | 0 (0%) | 2 (20%) | 8 (80%) | |
| Total | 6 (18.7%) | 16 (50%) | 10 (31.3%) | |

biopsy. In the patients with pattern of maturation arrest, the ejaculated spermatozoa were found in 11.1%, while 66.6% presented spermatozoa in testicular biopsy and only 22.2% remained azoospermic after treatment. None of the patients with sertoli cell syndrome had spermatozoa in their ejaculate after treatment. We were able to find spermatozoa in the testicular biopsy samples of 20% of patients with sertoli cell syndrome; however, 80% of patients remained azoosperm. The statistically significant difference was noted regarding sperm finding in three groups ($P = 0.000$).

## Discussion

Approximately 10% of infertile men have azoospermia, which can be divided into obstructive and nonobstructive azoospermia. Nearly two-thirds of these patients have nonobstructive azoospermia (Jarow *et al.*, 1989). After introducing ICSI, these patients have found the opportunity to be treated by several sperm acquisition techniques and IVF/ICSI (Devorey *et al.*, 1995). Spermatozoa can be retrieved by several macro- and microsurgical techniques including fine-needle aspiration, conventional TESE and recently micro-TESE (Micic & Dotlic, 1985; Gorgy *et al.*, 1998; Silber, 2000). Different histological patterns can be seen in testis biopsy of these patients like hypospermatogenesis, maturation arrest and sertoli cell syndrome. Although many studies have shown that medical treatment can improve oligoasthenospermia in some patients, but in cases of nonobstructive azoospermia, treatment is still not through medications, but sperm retrieval and microinjection (Micic & Dotlic, 1985). On the other hand, sperm retrieval is not always successful in this group of patients. Review of articles shows that TESE by macroscopic surgery is, at most, able to find spermatozoa in 16.7% of azoospermic patients that with microscopic surgery it will be reached to as high as 44.6% (Silber, 2000; Okada *et al.*, 2002). As mentioned before, administration of anti-oestrogens is a common treatment in cases of oligo-astheno-teratozoospermia. Because anti-oestrogens interfere with the normal negative feedback of sex steroids at hypothalamic and pituitary levels to increase endogenous gonadotropin-releasing hormone secretion from the hypothalamus, and FSH and LH secretion directly from the pituitary. In turn, FSH and LH stimulate Leydig cells in the testes, and this has been claimed to lead to increased local testosterone production, thereby boosting spermatogenesis with a possible improvement in fertility. There may also be a direct effect of anti-oestrogens on testicular spermatogenesis or steroidogenesis. So, in this study, infertile men with nonobstructive azoospermia were selected and treated with tamoxifen for 3 months before TESE and microinjection, and success rate of sperm retrieval in these patients was evaluated. Although clomiphen is the most common anti-oestrogenic agent for the empiric therapy of infertile men, in this study we used tamoxifen as an anti-oestrogen. It had two reasons, the first was that in another similar study, Hussein *et al.*, clomiphen was used and we changed it to tamoxifen to know that if all anti-oestrogenic drugs can produce the same results as they found. The second reason was that the daily dose of tamoxifen is more consistent in most of all previous studies, while it is not true for clomiphen, and different doses of clomiphen with or without titration have been used. So, we gave daily dose of 20 mg tamoxifen to all of our patients, and it was changed to every other day regimen in only two patients until the end of the study.

There are several other studies in literature which evaluated the effects of anti-oestrogenic medication, mostly clomiphen, on the stimulation of spermatogenesis.

In current study, only 18% of patients show spermatozoa in their ejaculate after tamoxifen therapy. One of these studies showed that clomiphen treatment in nonobstructive azoospermic patients is even able to return spermatozoa into the ejaculate in many of them (Hussein *et al.*, 2005). We also found the same results, but in our study, a few of our patients had spermatozoa in their ejaculate after treatment with tamoxifen (18%), which is much lower than that study (64.3%). It is reasonable to assume that one of the causes of this significant difference could be related to the exclusion of patients with sertoli cell syndrome in the above-mentioned study, while in our study, all of the male patients with any type of nonobstructive azoospermia including sertoli cell syndrome were included into the study. In our study, 81.3% of patients had no spermatozoa in their ejaculate after tamoxifen treatment and therefore macroscopic TESE was carried out in some of them bilaterally. In this group of patients, we found spermatozoa in 61.5% of samples. As shown before, the most common histological pattern in these


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patients who had spermatozoa in their samples was hypospermatogenesis (Table 2). On the other hand, the least successful rate of sperm retrieval was in patients with sertoli cell syndrome (20%). We had nine patients with sperm maturation arrest in our study: six of them had spermatid and three of them were in primary spermatocyte stage. Tourneye reports sperm retrieval in 84% of patients with maturation arrest in spermatid stage and 76% of patients in spermatocyte stage (Tournaye *et al.*, 1996). In this study, we found spermatozoa in seven of patients with maturation arrest (77.77%) after treatment with tamoxifen, that is, in accordance with previous studies (Okada *et al.*, 2002; Hussein *et al.*, 2005). Of these patients, one had spermatozoa in his ejaculate (14.28%), and in the other six, spermatozoa were found only in their testis samples. In these seven patients, testis histology showed spermatid stage in all except one patient. We were not able to retrieve spermatozoa in two patients with spermatocyte stage (22.22%). So, in all patients with maturation arrest in spermatid stage and in 33.33% of them in spermatocyte stage, we finally were able to retrieve spermatozoa. This difference with Tourneye's study could be related to a different stage of maturation of spermatozoa in patients who were studied, because there may be some different response rates in patients in primary and secondary spermatocyte stages, and also in early and late spermatid stages leading to this discrepancy. As we expected and other previous studies also have shown, the best response to medical treatment was seen in azoospermic patients who had hypospermatogenesis pattern in their testis histology (Tournaye *et al.*, 1996; Okada *et al.*, 2002). In our study, 100% of patients in this group produced spermatozoa in their ejaculate or testis samples after treatment with tamoxifen. Overall, we could find spermatozoa in semen or testis tissue of 22 patients after tamoxifen therapy which is statistically significant. Clinical pregnancy was achieved in nine of our patients, two by IVF and in other seven by microinjection.

## Conclusions

According to our study and the previous ones, we can conclude that medical treatment with anti-oestrogenic drugs like clomiphen and tamoxifen in male patients with nonobstructive azoospermia can improve successful sperm retrieval and improve treatment results. Therefore, we greatly recommend prior medical treatment before trying sperm retrieval and consider couples for ART cycles.

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