# Changes in hormonal profile and seminal parameters with use of aromatase inhibitors in management of infertile men with low testosterone to estradiol ratios

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**Objective:** To compare the effects of 2.5 mg letrozole with those of 1 mg anastrazole daily on the hormonal and semen profiles of a subset of infertile men with low $T/E_2$ ratios.
**Design:** Prospective, nonrandomized study.
**Setting:** Reproductive medicine clinic.
**Patient(s):** The study group consisted of 29 infertile men with a low serum $T/E_2$ ratio ($<10$).
**Intervention(s):** Patients were divided into two groups. Group A included 15 patients treated with 2.5 mg letrozole orally once daily for 6 months, and Group B consisted of 14 patients treated with 1 mg anastrazole orally every day for 6 months.
**Main Outcome Measure(s):** Hormonal evaluation included measurement of serum FSH, LH, PRL, T, and $E_2$. In all sperm analyses pretreatment and posttreatment total motile sperm counts (ejaculate volume $\times$ concentration $\times$ motile fraction) were evaluated.
**Result(s):** The use of aromatase inhibitors (either letrozole or anastrazole) in cases of infertile men with low $T/E_2$ ratios improved both hormonal and semen parameters.
**Conclusion(s):** This study suggests that some men with severe oligospermia, low T levels, and normal gonadotropin concentration may have a treatable endocrinopathy. (Fertil Steril® 2012;98:48–51. ©2012 by American Society for Reproductive Medicine.)
**Key Words:** Aromatase inhibitors, male infertility, oligospermia, letrozole, anastrazole

Aromatase is a cytochrome p450 enzyme that is present in the ovaries, testis, adipose tissue, and brain. The enzyme is responsible for the conversion of T and androstenedione to $E_2$ and estrone, respectively. Aromatase inhibitors interact with aromatase enzyme in estrogens-secreting tissues, thus limiting estrogens production with preservation of T levels. Aromatase inhibitors are widely used for endocrine treatment of endometriosis, uterine leiomyomas, endometrial and breast cancers, impaired sperm production, and ovulation induction.

In the last 2 decades, studies have reported improved semen quality in men with normal gonadotropins and idiopathic oligospermia treated with aromatase inhibitors (1, 2).

The aim of this prospective, randomized trial was to compare the effects of 2.5 mg letrozole with those of 1 mg anastrazole daily on the hormonal and semen profiles of a subset of infertile men with low $T/E_2$ ratios. Letrozole is a nonsteroidal, selective, potent third-generation aromatase inhibitor. Anastrazole, also a nonsteroidal agent, represents the fourth generation of aromatase inhibitors. Blocking estrogen production by inhibiting aromatization will stop the conversion of androstenedione and T to estrogen. This hypoestrogenic state would release the hypothalamic–pituitary axis from estrogenic negative feedback and lead to increased FSH secretion and to the development of sperm production.

## MATERIALS AND METHODS

The study was approved by the Hospital Review Board at the Aretaieion Hospital, Athens. The study took place from March 2008 to July 2011. This prospective, nonrandomized study included 29

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infertile men with a low serum T/$E_2$ ratio (<10). These patients were divided into two groups. Group A consisted of 15 patients treated with 2.5 mg letrozole orally once daily for 6 months, and group B consisted of 14 patients treated with 1 mg anastrazole orally every day for 6 months. Patients were allocated to either group on an alternating basis by the outpatient clinic. Informed consent was received from all patients.

Liver function tests were performed every month, and serum hormones and semen parameters were assessed at the beginning and at the end of treatment. The serum hormones and semen parameters were compared before and after the treatment.

All men had a thorough history and underwent physical examination, semen analyses, serum hormonal evaluation including FSH, LH, T, $E_2$, PRL, and TSH, and karyotype analysis; Y chromosome microdeletion and patients with a total sperm count $<1 \times 10^6$ had genetic analysis for cystic fibrosis. Patients with abnormal results on karyotype analysis or Y chromosome microdeletion assay were excluded from the study.

All patients had sperm concentrations $<10 \times 10^6$ spermatozoa/mL, T/$E_2$ ratio of <10, and T levels <300 ng/dL. Testicular volume was measured with the use of ultrasound using the equation length × height × width × 0.71 (3).

Blood samples for hormonal evaluation were taken in the early morning, between 7:00 and 8:00 AM. Initial hormonal evaluation included assessment of serum FSH, LH, PRL, T, $E_2$, and TSH, and all hormones were measured using a commercially available kit (Vidas, bioMerieux).

The reference ranges of the assays used for FSH, LH, $E_2$, T, PRL, and TSH were as follows: FSH, 0.1–110 mIU/mL; LH, 0.1–100 mIU/mL; $E_2$, 9–3,000 pg/mL; T, 0.1–13 ng/mL; PRL, 0–200 ng/mL; TSH, 0–60 μIU/mL.

Idiopathic oligozoospermia was diagnosed on the basis of FSH concentrations that were within the normal range of reference values; the average value from the two most recent semen analyses being below normal according to the World Health Organization classification (4); and the absence of any abnormality that could be responsible for the impaired semen values, such as infection, trauma, autoimmunity, varicocele, or epididymal factor; the negative results of the hormonal and other investigations that the patients were submitted to have been described above.

Semen samples were collected by masturbation after 2–4 days of sexual abstinence and processed within 1 hour of ejaculation. All semen analyses were performed in the same andrology laboratory according to World Health Organization criteria (4). None of the participants had received any medication as a therapeutic regimen for at least 3 months before the study, although occasional use of analgesics (e.g., paracetamol) was acceptable.

The seminal values for the initial and 6th-month evaluations are the means of two estimations.

As an overall index of seminal quantity and quality, the total functional sperm fraction (TFSF, $\times 10^6$) was estimated. This term includes quantitative and qualitative factors of the semen and has been calculated by multiplying the sperm count ($\times 10^6$) by motility (%) and by normal morphology (%) (5, 6).

In all sperm analyses pretreatment and posttreatment TFSF were evaluated. The mean TFSF value was used for comparison from pretreatment to posttreatment. Additionally, in all cases analyzed, volume of ejaculate (in milliliters), sperm concentration (in millions per milliliter), and motility (percentage) were assessed.

Group A was treated with 2.5 mg letrozole (Femara; Novartis Pharmaceuticals), and group B was treated with 1 mg anastrazole (Arimidex; Zeneca Pharma International) orally, once daily for 6 months.

Data were statistically analyzed using Medcalc statistical software (version 12.0.4.0). Results are presented as mean ± SE. Statistical analysis was performed using Student's *t* test to compare pre- and posttreatment sperm parameters, serum hormone levels, and testicular volumes. All data are given as mean ± SE, and $P<.05$ was considered a statistically significant difference.

## RESULTS

The results in all examined parameters of the two study groups (A and B) are presented in Tables 1 and 2, respectively.

Improvement was not seen in seminal parameters in 4 of 15 patients in the letrozole group (26.6%) and in 3 of 14 patients in the anastrazole group (21.4%).

Of the patients treated with letrozole, only one presented an asymptomatic mild increase in serum liver enzymes (serum glutamic oxaloacetic transaminase [SGOT] and serum glutamic pyruvic transminase [SGPT]), but it was transient, and medication was continued. Additionally, two patients complained of transient weakness, 1 patient of nausea that lasted for 10 days, and 2 patients of mild headache. On the other hand, in two patients—from those who were treated with anastrazole—an asymptomatic increase in serum liver enzymes (alkaline phosphatase) was observed. One patient developed mild diarrhea at 1 month of use, which lasted for 3 days and subsided on its own without further sequelae; two patients developed transient nausea and one patient mild headache. No other complications were reported from the patients of both groups. In summary, both drugs were well tolerated.

## TABLE 1

**Results of semen analysis and hormonal tests before and after 6 months of treatment with letrozole 2.5 mg/d.**

| Parameter | Before treatment | After treatment | *P* value |
|---|---|---|---|
| Body mass index (kg/m$^2$) | 29.86 ± 2.53 | 30.1 ± 2.13 | >.05 |
| Testicular volume (mL) | 14.89 ± 4.32 | 15.01 ± 4.30 | .94 |
| Serum FSH (mIU/mL) | 8.35 ± 2.03 | 8.41 ± 1.95 | .93 |
| Serum LH (mIU/mL) | 9.55 ± 1.84 | 9.28 ± 1.80 | .69 |
| Serum T (ng/dL) | 275 ± 29 | 495 ± 65 | <.001 |
| Serum $E_2$ (pg/mL) | 26.7 ± 1.75 | 14.98 ± 2.58 | <.001 |
| T/$E_2$ ratio | 9 ± 0.2 | 36 ± 4.5 | <.001 |
| Ejaculate volume (ml) | 2.85 ± 0.36 | 3.35 ± 0.20 | .005 |
| Sperm count ($\times 10^6$) | 3.5 ± 1.43 | 5.19 ± 1.62 | .001 |
| Motility (%) | 11.05 ± 2.48 | 22.13 ± 4.37 | .001 |
| TFSF[a] ($\times 10^6$) | 1.71 ± 0.87 | 2.51 ± 1.09 | .013 |

*Note:* Values are mean ± SE.
[a] TFSF was estimated by multiplying total sperm count ($\times 10^6$) by motility (%) and by morphology (%).

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**TABLE 2**

**Results of semen analysis and hormonal tests before and after 6 months of treatment with anastrazole 1 mg/d.**

| Parameter | Before treatment | After treatment | *P* value |
|---|---|---|---|
| Body mass index (kg/m$^2$) | 30.14 ± 3.1 | 30.0 ± 2.75 | >.05 |
| Testicular volume (mL) | 13.65 ± 3.95 | 13.89 ± 3.42 | .86 |
| Serum FSH (mIU/mL) | 8.35 ± 1.95 | 8.45 ± 1.93 | .89 |
| Serum LH (mIU/mL) | 11.15 ± 1.58 | 11.01 ± 1.53 | .81 |
| Serum T (ng/dL) | 265 ± 25 | 513 ± 65 | <.001 |
| Serum $E_2$ (pg/mL) | 24.1 ± 2.01 | 15.15 ± 1.95 | <.001 |
| T/$E_2$ ratio | 8 ± 0.5 | 34 ± 5.9 | <.001 |
| Ejaculate volume (ml) | 2.40 ± 0.15 | 3.18 ± 0.52 | <.001 |
| Sperm count (×10$^6$) | 4.15 ± 3.38 | 8.9 ± 2.11 | <.001 |
| Motility (%) | 12.35 ± 3.89 | 22.85 ± 3.38 | <.001 |
| TFSF[a] (×10$^6$) | 1.91 ± 1.25 | 2.41 ± 1.06 | .005 |

*Note:* Values are mean ± SE.
[a] TFSF was estimated by multiplying total sperm count (×10$^6$) by motility (%) and by morphology (%).

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Statistical comparison of TFSF for the letrozole and anastrazole groups before and after treatment, using Student's *t* test for independent samples (because all samples follow normal distribution), showed that there was no statistically significant difference between TFSF of the letrozole group before treatment and TFSF of the anastrazole group before treatment ($P=.62$), as well as TFSF of the letrozole group after treatment and TFSF of the anastrazole group after treatment ($P=.81$). Therefore, it could be said that both groups are comparable with respect to TFSF before and after treatment. Additionally, the increase in average TFSF in the letrozole group after treatment compared with the pretreatment value was 31.6%, and the increase in average TFSF in the anastrazole group after treatment compared with the pretreatment value was 21.1%. To detect whether there is a statistically significant difference between the 31.6% increase of average TFSF seen in the letrozole group in comparison with the 21.1% increase of average TFSF seen in the anastrazole group, having a type I error of 0.05 and a type II error of 0.20, 273 patients are required in each group.

## DISCUSSION

Change of plasma $E_2$ levels within the male physiologic range could be associated with significant change of LH levels in plasma through an effect at the level of the pituitary gland (7). A decrease in $E_2$ levels with the administration of an aromatase inhibitor is associated with an increase in levels of LH, FSH, and T (8). Although FSH release is mainly under the control of inhibin, circulating $E_2$ has a strong effect on FSH levels in men (9).

Earlier studies using anastrozole or testolactone have shown evidence for a positive action on sperm concentration and motility (1, 2, 10). However, another trial using testolactone did not show a significant improvement of sperm quality in men with oligospermia (11). More recently, a study in which anastrozole was added to treatment with tamoxifen in men with idiopathic oligoasthenoteratozoospermia and a decreased T over $E_2$ ratio after treatment with tamoxifen alone indicated an increased pregnancy rate compared with the group without the addition of the aromatase inhibitor (12).

Saylam et al. (13) treated 27 infertile men with a low serum T/$E_2$ ratio (<10) with 2.5 mg letrozole orally once daily for >6 months. They noted that T/$E_2$ ratio, ejaculate volume, sperm motility, and total motile sperm count (TMSC) significantly increased after the letrozole treatment. Additionally, 2 of 10 oligospermic men achieved spontaneous pregnancy. In patients with azoospermia, 23.5% presented spermatozoa in the ejaculate, and 76.5% remained azoospermic after letrozole treatment.

Patry et al. (14) treated a 31-year-old man with primary infertility, normal serum FSH levels, and pattern of nonobstructive azoospermia, with use of the aromatase inhibitor letrozole orally for up to 4 months, and final testicular biopsy showed normal spermatogenesis.

Raman et al. (2) treated 140 subfertile men with abnormal T/$E_2$ ratios using either testolactone 100–200 mg or anastrazole 1 mg daily. A comparison of the efficacy of these two therapies on both hormonal and semen parameters showed similar effects. Additionally, treatment with aromatase inhibitors has been used before testicular sperm extraction in Klinefleter's syndrome patients, with favorable results (15).

Clomiphene citrate was not used in these patients because there were published data suggesting development of azoospermia after treatment with clomiphene citrate in patients having oligospermia (16).

Many infertile men with severe oligospermia can exhibit a decreased T/$E_2$ ratio, and treatment with an aromatase inhibitor can normalize values and improve semen quality.

The findings of the present study suggest that some men with severe oligospermia (<5 × 10$^6$/mL), low T levels (<300 ng/dL), a T (ng/dL) to $E_2$ (pg/mL) ratio <10, and normal gonadotropins concentration may have a treatable endocrinopathy. Accordingly, the endocrine evaluation should perhaps include an estimation of $E_2$ and calculation of the T (ng/dL) to $E_2$ (pg/mL) ratio. A ratio <10 identifies those who might benefit from treatment with an aromatase inhibitor to improve T levels and possibly the seminal parameters. The efficacy of letrozole and anastrazole in improving the seminal parameters was similar, and the nonresponse rate was 26.6% in the letrozole group and 21.4% in the anastrazole group. The T levels and the T/$E_2$ ratio were improved in all patients in both groups. This was the reason why a control arm was not used in this study. The side effects that were reported by the patients in both groups were considered well tolerated and subsided with time, and there was no significant difference in the incidence and severity of side effects between the two groups. Therefore, it seems that both anastrazole and letrozole are equally effective in the improvement of T levels and seminal parameters in patients with severe oligospermia (<5 × 10$^6$/mL), low T levels (<300 ng/dL), and a T (ng/dL) to $E_2$ (pg/mL) ratio <10, and the presented side effects are mild, well tolerated, and subside with the time. There are no available data concerning possible risks about the long-term use of aromatase inhibitors in men, but from the available data from the use of aromatase inhibitors in postmenopausal women with breast cancer it seems that the main potential concerns are about the risk of development

of osteoporosis and a possible mild increase in cholesterol levels at 5 years of use of letrozole (17). The percentage of patients taking letrozole and reporting osteoporosis was 6.9%, vs. 5.5% in the placebo group. Bisphosphonates, drugs to increase bone strength, were given to 21.1% of letrozole patients and 18.7% of placebo patients. However, there are no available data as far as we know at 6 months' follow-up concerning the risk of developing osteoporosis or increase in cholesterol levels that could have an effect on the vascular systems of the patients.

Possible limitations of this study could be the relatively small numbers of participating patients in each group and that there are no data about rates of IUI/IVF and pregnancy outcomes, which could give information about the clinical significance of the improvement seen in semen parameters in terms of pregnancy achievement rates.

A control arm was not used in the study given the previously published reports describing benefit of aromatase inhibition in men with $E_2$/T ratios $>$10:1 (18).

Further prospective, randomized, blinded, placebo-controlled studies are needed to clarify the role of aromatase inhibitors in the management of male infertility.

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