TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2009; 39 (4): 519-524
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0802-10

M. Murad BAŞAR
Devrim TUĞLU

Aromatase inhibitors in infertile patients: effects on seminal parameters, serum and seminal plasma testosterone levels, and estradiol levels during short-term follow-up

Aim: To evaluate the effects of an aromatase inhibitor (anastrozole) on seminal parameters, and on serum and seminal plasma testosterone/estradiol ratios in infertile patients, as well as to clarify its place among empirical infertility treatment modalities.

Patients and Methods: The study included 32 patients with spermatozoa numbering > 5 million/mL in ejaculate and a serum testosterone/estradiol ratio < 0.14. Anastrozole, an aromatase-inhibiting agent, was given (1 mg b.i.d.) and patients were re-evaluated 2 months later based on semen analysis, and measurements of serum and seminal plasma testosterone and estradiol levels.

Results: Semen analysis parameters before and after treatment, respectively, were as follows: number of spermatozoa: 12.4 ± 4.1 million/mL and 22.3 ± 5.7 million/mL; motility: 33.4 ± 4.2% and 47.6 ± 7.4%; normal morphology: 5.4 ± 1.3% and 8.9 ± 2.7%. Differences between the values before and after treatment were statistically significant ($P < 0.05$).

Serum testosterone level was 4.1 ± 1.2 ng/mL, estradiol level was 52.1 ± 9.4 pg/mL, and testosterone/estradiol ratio was 0.13 ± 0.03 at the beginning of treatment. These values were 3.2 ± 0.6 ng/mL, 68.4 ± 7.3 pg/mL, and 0.05 ± 0.001, respectively, in seminal plasma.

Following 2 months of anastrozole treatment, testosterone and estradiol levels, and the testosterone/estradiol ratio showed statistically significant changes in serum and seminal plasma. While testosterone levels significantly increased, estradiol levels decreased (serum $p_T = 0.001$, $p_{E2} = 0.001$; seminal plasma $p_T = 0.001$, $p_{E2} = 0.001$).

Conclusion: Aromatase inhibitors are a potential treatment method for infertile male patients with increased plasma estradiol levels and decreased plasma testosterone/estradiol ratios.

Key words: Male infertility, estradiol, testosterone, aromatase inhibitors

Department of Urology,
Faculty of Medicine,
Kırıkkale University,
Kırıkkale - TURKEY

Received: February 05, 2008
Accepted: January 12, 2009

Correspondence
M. Murad BAŞAR
Birlik Mahallesi,
96. Sokak, 10/3,
Çankaya 06610
Ankara - TURKEY

mmbasar@hotmail.com

İnfertil hastalarda aromataz inhibitörleri: kısa süreli izlemde serum ve seminal plazma testosteron and östradiol düzeylerine etkileri

Amaç: Bu çalışmanın amacı, aromataz inhibitörünün infertil hastalarda seminal parametreler ve serum ve seminal plazma testosteron/östradiol oranlarına etkisi değerlendirmek ve ampirik infertilite tedavisi modalitelerinde yerini açıklamaktır.

Hastalar ve Yöntem: Ejakulat spermatozoa sayısı 5 milyon/mL'den fazla ve serum testosteron/östradiol oranı 0,14'den az olan toplam 32 hasta çalışmaya alındı. Aromotaz inhibitörü olan anastrazol günde iki defa 1 mg olarak başlandı ve hastalar iki ay sonra semen analizi ve serum ve seminal plazma testosteron ve östradiol düzeyleri ölçümü ile tekrar değerlendirildi.

Bulgular: Tedavi öncesi ve sonrası semen analiz parametreleri sırası ile spermatozoa sayısında 12,4 ± 4,1 milyon/mL ve 22,3 ± 5,7 milyon/mL, motilitede % 33,4 ± 4,2 ve % 47,6 ± 7,4 ve morfolojide % 5,4 ± 1,3 ve % 8,9 ± 2,7 idi. Bu değişiklikler istatistiksel olarak anlamlıydı ($P < 0,05$).

* This study was accepted as a poster presentation at the 101st Annual Meeting of the American Urological Association.

Tedavi öncesinde serum testosteron düzeyi 4,1 ± 1,2 ng/mL, östradiol düzeyi 52,1 ± 9,4 pg/mL, ve testosteron/östradiol oranı 0,13 ± 0,03 idi. Bu değerler seminal plazmada sırası ile 3,2 ± 0,6 ng/mL, 68,4 ± 7,3 pg/mL ve 0,05 ± 0,001 idi.

İki aylık anastrazol tedavisi sonrasında, serum ve seminal plazma testosteron ve östradiol düzeyleri istatistiksel olarak anlamlı değişim gösterdi. Testosteron düzeyi istatistiksel anlamlı olarak artarken, östradiol düzeyi azaldı (Serum $p_T = 0.001$, $p_{E2} = 0.001$; Seminal plazma $p_T = 0.001$, $p_{E2} = 0.001$).

Sonuçlar: Bu çalışmada, aromataz inhibitörlerinin artmış plazma östradiol ve azalmış plazma testosteron/östradiol oranı olan erkek infertil hastalarda tedavi seçeneklerinden biri olabileceği sonucuna vardık.

Anahtar sözcükler: Erkek infertilitesi, östradiol, testosteron, aromataz inhibitörleri

Introduction

Leydig cells in the interstitium of testes produce sex steroids, including testosterone and estrogen. While approximately 20% of estrogen in the blood is produced in the testes, the majority is converted to estradiol (E_2) in peripheral tissues via aromatase enzyme activity (1,2).

Aromatase is a cytochrome p450 enzyme active in different tissues, including testes, adipose tissue, and the brain (2-4). This enzyme converts testosterone and androstenedione into E_2 and estrone, respectively. In previous studies, it was shown that Leydig cells, germ cells, and spermatozoa also possess aromatase activity (2,5). Aromatase inhibitors interact with this enzyme, thus limiting estrogen production.

No specific cause can be found in approximately 10%-15% of infertile couples (6). These undiagnosed patients are classified as having idiopathic infertility. The hormonal profile of infertile patients changes according to etiology. Generally, increased serum follicle-stimulating hormone (FSH) levels are associated with low or normal testosterone levels in azoospermic patients. In contrast, normal serum hormone levels were observed in idiopathic infertile patients. Recently, some hormonal imbalances, such as testosterone/estradiol (T/E_2) and luteinizing hormone/testosterone (LH/T) ratios, have been described in patients previously classified as idiopathic infertile (6). Moreover, levels of these hormones in seminal plasma have also been reported to be of importance (7,8).

As empirical hormonal treatment methods have low rates of success, artificial reproductive techniques are more commonly offered to these patients (6); however, these techniques are associated with some drawbacks, such as high cost, unsuccessful results, testicular damage, and risk of ovarian hyperstimulation syndrome (9,10). Therefore, hormonal treatment is still important for some subgroups of infertile males.

The aims of the present study were to evaluate the effects of treatment with an aromatase inhibitor (anastrozole) on spermatozoa number, motility, and morphology, and serum and seminal plasma T/E_2 ratios in infertile males with oligoasthenoteratozoospermia, as well as to clarify its place among empirical infertility treatment modalities.

Materials and Methods

All patients that presented to the Andrology outpatient department were evaluated via detailed history and physical examination. Hormonal analysis, including serum FSH, LH, prolactin, total testosterone, and E_2 levels, was performed between 08^{00} and 10^{00}. Two semen analyses were performed after 3 days of sexual abstinence and were evaluated according to World Health Organization (WHO) guidelines; morphological analysis was performed after staining with Sperm Mac (Ferti Net NV, Belgium), based on Kruger's strict criteria (11). Following semen analysis, the remaining seminal plasma was centrifuged at 300 ×g for 10 min and stored at -80 °C until seminal plasma testosterone and E_2 levels were measured. Serum and seminal plasma testosterone and E_2 levels were measured using chemiluminescence methods.

After these investigations, 32 patients aged 23-42 years (mean age: 31.8 ± 4.1 years) with normal gonadotropins and prolactin levels, and a serum T/E_2 ratio < 0.14 that had participated in intrauterine insemination were enrolled in the study. Exclusion criteria included any hormonal treatment during the previous 6 months, spermatozoa numbering < 5 million per ejaculation, any scrotal pathology, such as varicocele or cryptorchidism, previous history of scrotal surgery, and abnormal serum hormone levels.

Later, anastrozole (1 mg b.i.d.) (Arimidex®, Astra Zeneca, USA), a non-steroidal aromatase-inhibiting agent, was given to all patients. The patients were re-evaluated 2 months later based on semen analysis, and serum and seminal plasma testosterone and E_2 levels were re-measured at the end of this period. Patients were queried regarding any side effects.

The second semen parameters of evaluations, and serum and seminal plasma hormone levels before and after treatment were compared using the paired t test. Statistical significance was considered as $P < 0.05$.

Results

Semen analysis results, and serum and seminal plasma hormone levels before and after treatment are given in the Table. While the increase in ejaculate volume was insignificant after treatment ($P = 0.54$), all other seminal parameters significantly increased at the end of the 2-month follow-up (Figure 1). Serum gonadotropins and prolactin levels did not change significantly in response to aromatase inhibitor treatment (Table).

Mean serum testosterone level was 4.1 ± 1.2 ng/mL, mean E_2 level was 52.1 ± 9.4 pg/mL, and mean T/E_2 ratio was 0.13 ± 0.03 before treatment,

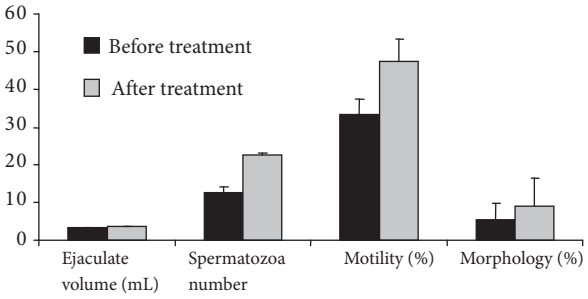

Figure 1. Seminal parameters before and after anastrozole treatment.

Table. Seminal parameters, and serum and seminal hormone levels before and after anastrozole treatment.

	Before treatment (mean ± SD)	After treatment (mean ± SD)	P*
Ejaculate volume (mL)	3.2 ± 1.6	3.4 ± 0.8	0.542
Sperm count (million/mL)	12.4 ± 4.1	22.3 ± 5.7	0.001
Motility (%)	33.4 ± 4.2	47.6 ± 7.4	0.027
Morphology (%)	5.4 ± 1.3	8.9 ± 2.7	0.040
FSH (mIU/mL)	3.3 ± 1.2	3.2 ± 1.1	0.073
LH (mIU/mL)	2.6 ± 0.5	2.5 ± 0.6	0.065
Prolactin (ng/mL)	14.9 ± 2.9	14.1 ± 2.1	0.301
Serum testosterone (ng/mL)	4.1 ± 1.2	6.9 ± 1.3	0.001*
Serum estradiol (pg/mL)	52.1 ± 9.4	31.7 ± 9.8	0.001*
Serum T/E_2	0.13 ± 0.03	0.22 ± 0.04	0.001*
Seminal plasma testosterone	3.2 ± 0.6	4.8 ± 0.2	0.001*
Seminal plasma estradiol	68.4 ± 7.3	43.6 ± 6.3	0.001*
Seminal plasma T/E_2	0.05 ± 0.001	0.11 ± 0.01	0.001*

SD: Standard deviation

*Paired t test, $P < 0.05$.

versus 3.2 ± 0.6 ng/mL, 68.4 ± 7.3 pg/mL, and 0.05 ± 0.001, respectively, in seminal plasma. At the end of 2 months of anastrozole treatment, there were significant increases in serum and seminal plasma testosterone levels (6.9 ± 1.3 ng/mL and 4.8 ± 0.2 ng/mL, respectively) ($P < 0.05$). In contrast, E_2 levels significantly decreased in serum and seminal plasma (31.7 ± 9.8 pg/mL in serum and 43.6 ± 6.3 pg/mL in seminal plasma) ($P < 0.05$). The T/E_2 ratio increased to 0.22 ± 0.04 in serum and to 0.11 ± 0.01 in seminal plasma; these changes were significant ($P < 0.05$) (Figure 2).

No side effects due to the medication were reported; however, pregnancy was not observed by the end of the 2-month treatment period.

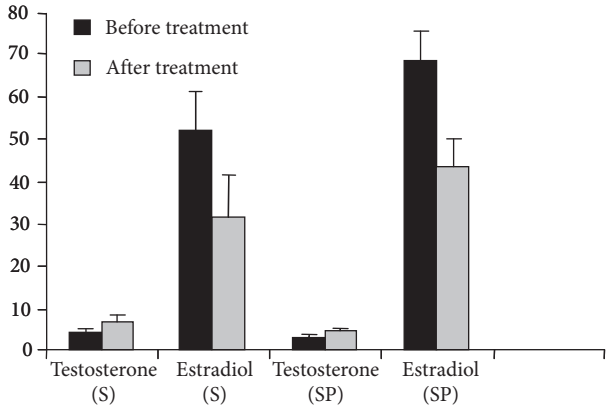

Figure 2. Serum and seminal plasma hormone levels before and after anastrozole treatment (S: serum; SP: seminal plasma).

Discussion

Results of the present uncontrolled preliminary study show that short-term aromatase inhibitor treatment had a positive effect on spermatogenesis and steroidogenesis in the testes.

Although the only role of estrogen in male reproduction is thought to be the regulation of sexual behavior and gonadotropin secretion, numerous new data have led to increasing interest in different interactions of estrogen in the male reproductive system (12,13). In studies performed in transgenic mice, 2 types of estrogen receptors, estrogen receptor α (ER α) and estrogen receptor β (ER β), were identified (14,15). These receptors are expressed widely in the testes, male reproductive tract, and accessory sex organs (12,13,16). Recently, ERs have been found in the epididymis in significant numbers (17). Hess et al. (18) observed abnormal fluid absorption in ER knock-out mice. It has thus been thought that estrogen acts on spermatogenesis during the period of epididymal maturation.

The idiopathic infertile male remains a contentious point among infertile couples. Although it is referred to as "idiopathic", different factors, including hormonal imbalance in serum/seminal plasma, autocrine factors, and genetic malformation, may be involved in this condition. Increased aromatization of testosterone to E_2 and changes in T/E_2 balance are among the etiologic factors in idiopathic infertile males. Restoration of this balance via blocking aromatization should be considered as a treatment method.

The effects of anti-estrogen treatment on male infertility were investigated in previous studies (19-21). These agents, such as tamoxifen and clomiphene, stimulate gonadotropin secretion at the hypothalamic level and, therefore, germ cell maturation and testosterone production can be achieved; however, the effects of treatment are controversial (20,21). In a meta-analysis by Vandekerckhove, it was reported that a modest improvement was observed in sperm density and motility in infertile patients treated with these drugs; however, no significant improvement in the pregnancy rate following anti-estrogen treatment has been reported in the literature (22).

In contrast, aromatase inhibitors block conversion of testosterone into estrogen in peripheral tissues. In the first animal studies it was reported that aromatase inhibitors induced dose-dependent suppression of E_2 in healthy males; however, it suppressed plasma E_2 concentration by 50% in monkeys (23). Kawakami et al. (24,25) reported that these drugs were effective in dogs with high plasma E_2 levels. In human studies, while some reported an improvement in sperm count, others observed no changes (22).

In the first human study of aromatase inhibitors, Clark et al. (26) reported no change in semen

parameters and no pregnancies resulting from their idiopathic infertile male patients; however, Pavlovich et al. (27) reported that aromatase inhibitors were effective in infertile males with decreased serum testosterone, as compared to E_2. In that study sperm count and motility increased with testolactone therapy. Recently, Raman et al. (28) reported that the aromatase inhibitors testolactone and anastrozole increased the T/E_2 ratio in serum and improved seminal parameters however, to the best of our knowledge the present study is the first to report pregnancy outcomes following treatment with these drugs.

Luboshitzky et al. (8) reported that seminal plasma hormonal balance is also important for the regulation of spermatogenesis. They observed that seminal plasma testosterone levels were lower and E_2 levels higher in their infertile group, as compared to control subjects. In addition, they observed a significant correlation between low seminal plasma T/E_2 ratios and semen analysis parameters.

Akiyama et al. reported that increased testicular aromatase activity and testicular E_2 levels can result in male infertility (29). The effect of aromatase inhibitors on seminal plasma in rats was investigated by Turner et al. (30). They observed a significant increase in testis weight, and in plasma FSH and testosterone levels in rats treated with anastrozole at the 19^{th} week of treatment. While they did not observe any suppression in plasma E_2 concentration, they detected significant reductions in testicular intestinal fluid. In the present study we observed increased T/E_2 ratios in serum and seminal plasma. Moreover, anastrozole had a positive effect on spermatogenesis by regulating the T/E_2 ratio in infertile patients.

There are major limitations to the present study, namely that it was not designed as a double-blind study and that the follow-up period was insufficient. Nonetheless, we observed significant improvement in seminal plasma parameters with short-term usage. All patients enrolled in the study underwent the first cycle of intrauterine insemination (IUI); however, no pregnancy was observed following IUI in these patients' partners. We did not follow-up the patients with respect to pregnancy rates.

We conclude that aromatase inhibitors should be used in infertile patients with increased plasma E_2 levels and/or decreased plasma T/E_2 ratios. Our results show that the drug had a positive effect on aromatization of hormones in both serum and seminal plasma. As we did not observe any pregnancy following the treatment, medical treatment with aromatase inhibitors should be used for a short period in male patients undergoing assisted reproductive techniques, particularly IUI. Further studies are needed regarding the crucial role of seminal plasma hormonal balance on spermatogenesis and, consequently, evaluation of the effects of aromatase inhibitors on seminal plasma hormonal balance. Moreover, further investigations should focus on the effects of long-term aromatase inhibitor use and in conjunction with repeated assisted reproductive technique cycles.

References

1. de Jong FH, Hey AH, van der Molen HJ. Effect of gonadotropins on the secretion of oestradiol 17β and testosterone by the rat testis. Endocrinology 1973; 57: 277-284.
2. Tsai-Mortis CH, Aquilano DR, Dufau ML. Cellular localization of rat testicular aromatase activity during development. Endocrinology 1985; 116: 38-46.
3. Inkster S, Yue KW, Brodie A. Human testicular aromatase: immunocytochemical and biochemical studies. J Clin Endocrinol Metab 1995; 80: 1941-1947.
4. Simpson ER, Zhao Y, Agarwal V, Michale MD, Bulun SE, Hinshelwood MM et al. Aromatase expression in health and disease. Recent Prog Hormone Res 1997; 52: 185-214.
5. Janulis L, Bahr JM, Hess RA, Janssen S, Osawa Y, Bunick D. Rat testicular germ cells and epididymal sperm contain active p450 aromatase. J Androl 1998; 19: 65-71.
6. Sigman M, Jarow JP. Male infertility In: Walsh PC, Retik AB, Vaughan Jr ED, Wein AJ editors: Campbell's Urology, 7th edition. Philadelphia: WB Saunders; 2002. p 1475-1531.

7. Adamopoulos D, Lawrence DM, Vassilopoulos P, Kapolla N, Kontogeorgos L, McGarrigle HH. Hormone levels in the reproductive system of normospermic men and patients with oligospermia and varicocele. J Clin Endocrinol Metab 1984; 59: 447-452.

8. Luboshitzky R, Kaplan-Zverling M, Shen-Orr Z, Nave R, Herer P. Seminal plasma androgen/oestrogen balance in infertile men. Int J Androl 2002; 25: 345-351.

9. Tassone M, Kuhn R, Talbot JM. Ovarian hyperstimulation syndrome. Aust N Z J Obstet Gynaecol 1997; 37: 95-99.

10. Abramov Y, Elchalal U, Schenker JG. Severe OHSS: An 'epidemic' of severe OHSS: a price we have to pay? Hum Reprod 1999; 14: 2181-2183.

11. Kruger TF, DuToit TC, Franken DR, Acosta AA, Oehninger SC, Menkveld R et al. A new computerized method of reading sperm morphology (strict criteria) is as efficient as technician reading. Fertil Steril 1993; 59: 202-209.

12. Carani C, Qin K, Simoni M, Faustini-Faustini M, Serpente S, Boyd J et al. Effect of testosterone and estradiol in a man with aromatase deficiency. N Engl J Med 1997; 337: 91-95.

13. O'Donnell L, Robertson KM, Jones ME, Simpson ER. Estrogen and spermatogenesis. Endocr Rev 2001; 22: 289-318.

14. Simpson ER, Mahendroo MS, Means GD, Kilgore MW, Corbin CJ, Mendelson CR. Tissue-specific promoters regulate aromatase cytochrome P450 expression. Clin Chem 1993; 39: 317-324.

15. Brewster ME, Anderson WR, Pop E. Effect of sustained estradiol release in the intact male rat: correlation of estradiol serum levels with actions on body weight, serum testosterone, and peripheral androgen-dependent tissues. Physiol Behav 1997; 61: 225-229.

16. Eddy EM, Washburn TF, Bunch DO, Goulding EH, Gladen BC, Lubahn DB et al. Targeted disruption of the estrogen receptor gene in male mice causes alteration of spermatogenesis and infertility. Endocrinology 1996; 137: 4796-4805.

17. Parlevliet JM, Pearl CA, Hess MF, Famula TR, Roser JF. Immunolocalization of estrogen and androgen receptors and steroid concentrations in the stallion epididymis. Theriogenology 2006; 66: 755-765.

18. Hess RA, Bunick D, Lee KH, Bahr J, Taylor JA, Korach KS et al. A role of oestrogen in the male reproductive system. Nature 1997; 390: 509-512.

19. Liu PY, Handelsman DJ. The present and future state of hormonal treatment for male infertility. Human Reprod Update 2003; 9: 9-23.

20. Gill-Sharma MK, Gopalkrishnan K, Balasinor N, Parte P, Jayaraman S, Juneja HS. Effects of tamoxifen on the fertility of male rats. J Reprod Fertil 1993; 99: 395-402.

21. Gill-Sharma MK, Balasinor N, Parte P, Aleem M, Juneja HS. Effects of tamoxifen metabolites on fertility of male rat. Contraception 2001; 63: 103-109.

22. Vandekerckhove P, Lilford R, Vail A, Hughes E. Clomiphene or tamoxifen for idiopathic oligo/asthenospermia. Cochrane Database Syst Rev 2000; 2: CD000151.

23. Dukes M, Edwards PN, Large M, Smith IK, Boyle T. The preclinical pharmacology of "Arimidex" (anastrozole: ZD1033)-a potent, selective aromatase inhibitor. J Steroid Biochem Mol Biol 1996; 58: 439-445.

24. Kawakami E, Taguchi N, Hirano T, Hori T, Tsutsui T. Therapeutic effect of aromatase inhibitor in two azoospermic dogs with high plasma estradiol-17β levels. J Vet Med Sci 2003; 65: 1343-1345.

25. Kawakami E, Hirano T, Hori T, Tsutsui T. Improvement in spermatogenic function after subcutaneous implantation of a capsule containing an aromatase inhibitor in four oligozoospermic dogs and one azoospermic dog with high plasma estradiol-17β concentrations. Theriogenology 2004; 62: 165-178.

26. Clark RV, Sherins RJ. Treatment of men with idiopathic oligozoospermic infertility using the aromatase inhibitor, testolactone. Results of a double-blinded, randomized, placebo-controlled trial with crossover. J Androl 1989; 10: 240-247.

27. Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. J Urol 2001; 165: 837-841.

28. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. J Urol 2002; 167: 624-629.

29. Akiyama H. A study on testicular aromatase activity-spermatogenic damage in high testicular E2 models of rat. Nippon Hinyokika Gakkai Zasshi 1997; 88: 649-657.

30. Turner KJ, Morley M, Atanassova N, Swanston ID, Sharpe RM. Effect of chronic administration of an aromatase inhibitor to adult male rats on pituitary and testicular function and fertility. J Endocrinol 2000; 164: 225-238.