Histopathologic Effects of Anastrozole an Aromatase Enzyme Inhibitor on Rat Testicles

Aromataz Enzim İnhibitörü Anastrozolun Rat Testisleri Üzerine Etkisinin Histopatolojik Olarak İncelenmesi

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ABSTRACT Objective: To investigate effects of anastrozole that used for idiopathic male infertility on testicular histopathology. **Material and Methods:** Twenty-eight rats were divided into four groups. Group I included rats that were given saline solution (placebo). Group II included rats that received low dose of anastrozole (0.001 mg/kg). Group III included rats that received routine dose of anastrozole (0.01 mg/kg). Group IV included rats that received high dose of anastrozole (0.1 mg/kg). Anastrozole was administered to treatment groups for 21 days and at the end of the treatment period, testicles were removed for histopathologic examination. **Results:** Spermatogenetic activity was normal in 6, 5, 4 and 3 rats in group 1, group 2, group 3 and group 4, respectively. Slight testicular atrophy was detected in 1, 2, 2 and 1 rats in group1, group2, group 3 and group 4, respectively. Severe/complete testicular atrophy was detected in 1 rat in group 3 and in 3 rats in group 4. No severe/complete testicular atrophy was detected in groups 1 and 2. Leydig cells were hyperplastic in 1 rat in group 1 and in 2 rats in group 2. Leydig cells were hyperplastic in rats that had severe or complete atrophy in group 3. Leydig cells were hyperplastic in rats that had mild and complete atrophy in group 4. Basal lamina appeared normal in all groups. **Conclusion:** High dose anastrozole treatment results in a significant decrease in testicular size. Although it was not statistically significant, mild and total atrophy and hyperplasia in leydig cells secondary to atrophy were also detected.

Key Words: Infertility, male; testis; anastrozole

ÖZET Amaç: İdiyopatik erkek infertilitesinin ampirik tedavisinde kullanılan anastrozolün testis histopatolojisi üzerine olan etkilerinin incelenmesidir. **Gereç ve Yöntemler:** Yirmi sekiz rat, dört eşit gruba bölünmüştür. Kontrol grubuna (grup 1 plasebo) 1 ml serum fizyolojik, düşük doz grubuna (grup 2) 0,001 mg/kg/gün, orta doz grubuna (grup 3) 0,01 mg/kg/gün, yüksek doz grubuna (grup 4) 0,1 mg/kg/gün anastrozol verilmiştir. İşlem 21 gün boyunca devam ettirilmiş ve 21. günün sonunda testisler histopatolojik incelemeye alınmıştır. **Bulgular:** Spermatogenetik aktivite grup 1' de 6 ratta, grup 2'de 5 ratta, grup 3'de 4 ratta ve grup 4'de 3 ratta normaldi. Hafif testis atrofisi grup 1, 2, 3 ve 4'de sırasıyla 1, 2, 2 ve 1 ratta saptandı. Şiddetli ya da tam atrofi ise grup 3'de 1, grup 4'de 3 ratta tespit edildi. Grup 1 ve 2'de şiddetli ya da tam atrofi saptanmadı. Grup 1'de 1 ratta, grup 2'de iki ratta leydig hücreleri hiperplazik idi. Grup 3'de belirgin ve tam atrofinin izlendiği ratlarda leydig hücreleri hiperplazikti. Grup 4'de hafif ve tam atrofinin izlendiği ratlarda leydig hücreleri hiperplazikti. Bunun yanı sıra grupların tamamında bazal laminalar normaldi. **Sonuç:** Yüksek doz anastrozol tedavisinin testis boyutlarında azalmaya neden olduğu ortaya konmuştur. Ancak yine de bu gelişmenin tek başına testiküler volüm azalmasını aydınlatmayabileceği, buna ilaveten diğer faktörleri ve hormonal evaluasyonu da içeren ileri araştırmanın yarar sağlayacağını düşünmekteyiz.

Anahtar Kelimeler: İnfertilite, erkek; testis; anastrozol

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Male factor accounts for half of the couples with infertility.[1] Clinical findings support that there is not any semen abnormalities in most of the infertile men. Decrease in number and function of sperm can indicate a testicular problem.[2,3] The current thinking is that 30% of infertile couples have a male factor. In spite of all current advancements in diagnostic modalities, no cause can be detected in semen analysis in 25% of the men with infertility. The majority of these men are diagnosed by low sperm count. Only a small proportion has problems such as erectile dysfunction or retrograde ejaculation. Anastrozole is an aromatase inhibitor used for empiric treatment in this patient group that is named as idiopathic male infertility in which generally no pathology is found on physical examination and hormonal evaluation. Anastrozole, that inhibits conversion of both adrenal and peripheral testosterone to estrogen, has become a promise in treatment of idiopathic infertility.[4,5] In the present study, effects of anastrozole treatment on testis histology were investigated and the changes were interpreted in the light of literature.

MATERIAL AND METHODS

After obtaining ethical committee approval, the study was carried out on 28 male Wistar rats aged 80-90 days. Mean spermatogenetic cycle was 12.9 days. Male rats were kept in temperature controlled rooms (25°C) with constant humidity (40-70%) and 12 hours light/dark cycle, prior to use in the experimental protocols. All animals were treated in accordance with the Principles of Laboratory Animal Care. These 28 rats varied 200 to 300 g in weight and they were randomized into 4 equal groups, of which one was control group and 3 were treatment groups. Anastrozole was orally administered to rats. Group I included rats that were given saline solution (placebo). Group II included rats that received low dose of anastrozole (0.001 mg/kg/day). Group III included rats that received routine dose of anastrozole (0.01 mg/kg/day). Group IV included rats given high dose of anastrozole (0.1 mg/kg/day). The routine dose of anastrozole given to rats was calculated according to the dose given as a 70 kg adult human having a routine dose of 1 mg / day (approx. 0.01 mg / kg / day). Control group was given 1 ml saline, low dose group was given 0.001 mg/kg/day anastrozole, normal dose group was given 0.01 mg/kg/day, high dose group was given 0.1 mg/kg/day and this procedure was continued for 21 days. The weights of rats in control and treatment groups were chosen as similar. Rats in all groups were sedated with ether at the end of 21 day-treatment and midline incision was done in the abdomen. After anatomic layers were dissected, both inguinal canals were detected separately and bilateral orchiectomy was performed by pulling testicles toward midline. Removed testicles were placed in cups containing Bouin's solution and sent to pathology clinic for histopathologic examination. In pathology clinic, after measuring the length of the testicles, they were dissected into two at horizontal plan and fixated in Bouin's solution for a night. Samples were buried in paraffin blocks following routine procedures and sections of 4-5 μ thickness were prepared for microscopy. Sections of 4-5 μ thickness were stained with hematoxylin-eosin stain and spermatic activity, interstitial Leydig cells and basal membranes were evaluated.

STATISTICAL ANALYSIS

All statistical analyses were performed using SPSS, version 15.0. Statistical significance was considered as $p<0.05$. As supplementary statistic, frequency (percent) for the variables obtained by counting and medium± standard deviation and median (minimum and maximum) values for the variables obtained by measurement were used. Chi- square analysis was used for the variables obtained by counting. Mann-Whitney U test was used for comparing two independent groups and Kruskal-Wallis variance analysis was performed for more than two independent groups for the variables obtained by measurement.

RESULTS

As a result of macroscopic and histopathological examination, the size of the testis, spermatogenic activity, basal lamina and the status of Leydig cells were examined both in treatment groups and the control group.

TABLE 1: Testicular volume of the groups (mm3).

Groups	Mean	Standard deviation	Median
Group I	16	2	16
Group II	15	1	15
Group III	13	2	14
Group IV	11*	3	11

* Kruskall Wallis, p: 0.005, Mann Whitney U Testi p: 0.004.

In the control group, mean size of the testes was 16 mm (standard deviation 2 mm) and the median size of the testes was 16 mm. The mean testis sizes were 15 mm (standard deviation of 1 mm), 13 mm (standard deviation of 2 mm) and 11 mm (standard deviation of 3 mm) in low, medium and high dose treatment groups, respectively. The median testis size in group I, II, III and IV were 16, 15, 14 and 11 mm, respectively (Table 1). The difference in testicular size was significant in treatment groups (P <0.005). And while the difference between the control and group IV was significant (P = 0.004), there was no significantly difference between control group (Group I) and both group II and III (p>0.005).

On histopathologic examination, spermatogenic activity of the control group (Group I) of rats were normal in six rats and only one rat's spermatogenic activity showed mild atrophic changes. In the control group all the basal membranes were normal (Figure 1a).

Spermatogenic activity in the low-dose treatment group (Group II) were monitored mild atrophic changes in 2 rats while there were no problems in the 5 rats. No salient and total atrophic changes were observed. Testes showed mild atrophic changes and all the basal membranes were normal (Figure 1b) (Table 2).

In normal-dose treatment group (Group III) spermatogenic activity was normal in 4 rats. There was salient atrophy in 2 rats and total atrophy in one rat. Basal membranes were observed normal in rats that received the moderate dose therapy (Figure 1c).

In high-dose treatment group (Group IV) spermatogenic activity was normal in 3 rats. There was salient atrophy in 1 rat and total atrophy in 3 rats. In this group all the testes' basal laminaes were normal (Figure 1d).

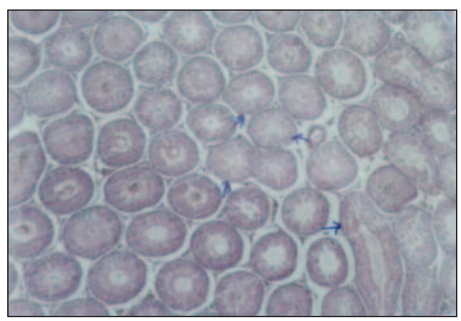

FIGURE 1a: Normal spermatogenic activity.

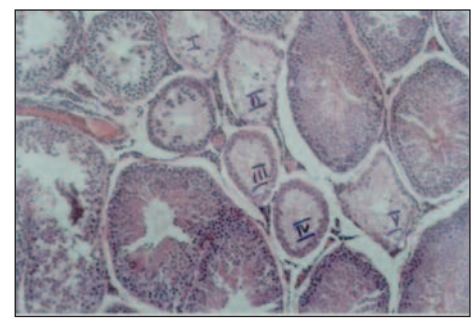

FIGURE 1b: Atrophic areas surrounding normal-appearing tubuli.

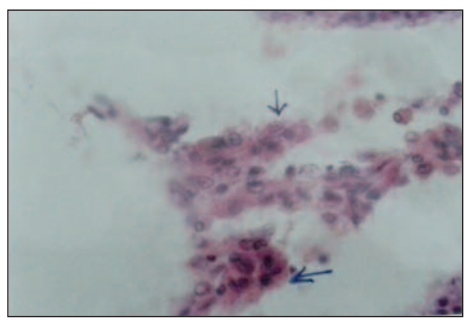

FIGURE 1c: Hyperplasic Leydig cells.

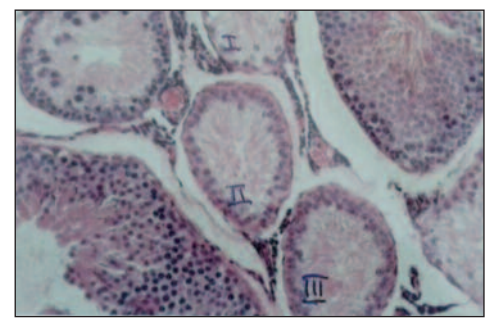

FIGURE 1d: Histologic image that displays total atrophy.

TABLE 2: Evaluation of histologic changes in the groups in terms of atrophy.

Groups	No atrphy	Mild atrophy	Market atrophy	Complete atrophy
Group I	6	1	0	0
Group II	5	2	0	0
Group III	4	0	2	1
Group IV	3	1	0	3

In terms of atrophic changes in the groups were presented in Table 3. As a result of statistical analysis of these data, there was not a significant difference between the groups.

DISCUSSION

Child bearing capacity of a couple depends on normal functioning of reproductive systems. There are several anatomic or physiologic factors that preclude pregnancy and couples can face infertility when they have one or more of these factors. However, any reasons can not be identified in 10% of infertile couples.[6] Treatment of these couples, considered as idiopathic infertility, include several empiric medications. These medications include clomiphene citrate, kallikrein, methylxanthines, antioxidants, prostaglandin synthesis inhibitors, antiestrogens, androgens and aromatase enzyme inhibitors. Although all of these agents based on a rationale, they do not always achieve success and there is no reliable treatment to boost fertility.[7-9]

Aromatase is an enzyme mostly located in testis, liver and brain, and converts irreversibly testosterone to estradiol. Testosterone is essential for spermatogenesis and decreased testosterone/estradiol ratio is mostly associated with male infertility.[9,10] Aromatase inhibitors cause an increase in testosteronee level and a decrease in estradiol level. So it is used for idiopatic male infertility especially for men with low testosterone/estradiol ratio.[4,5,9-12] One of the aromatase inhibitors, anastrozole, has presumably improving effect and has been in use for this purpose.[13,14] Itoh determined that testolactone, an aromatase inhibitor, increased the testosterone/estradiol rate and contributed to enhance semen parameters more than 20 years ago.[15] Raman and Schlegel investigated the effects of anastrozole on semen and hormone profile in infertile men with abnormal testosterone/estradiol ratio.[11] They found out that 1 mg/day anastrozole with mean 4.7 months follow-up period, significantly increased testosterone/estradiol ratio and improved semen parameters as mush as 100 to 200 mg/day testolactone. But they did not evaluate whether there were any change in the sizes of testicles during the treatment.

But, on the contrary, there are some articles that investigate the importance and effect of estrogen on spermatogenesis. It has been observed in several articles that aromatase deficiency in mice and human also causes infertility.[16-20] There are plenty of endocrine, paracrine and autocrine fac-

TABLE 3: Spermatogenic activity in groups.

		Spermatogenic activity (Group I)							
		Normal		Abnormal		All		Statistical Analysis	
		n	%	n	%	n	%	Chi-square	p
Spermatogenic activity (Group II)	Normal	5	83.33	0	0.00	5	71.43		
	Abnormal	1	16.67	1	100.00	2	28.57	Fisher'sExact	0.286
	All	6	100.00	1	100.00	7	100.00		
Spermatogenic activity (Group III)	Normal	4	66.67	0	0.00	4	57.14		
	Abnormal	2	33.33	1	100.00	3	42.86	Fisher'sExact	0.429
	All	6	100.00	1	100.00	7	100.00		
Spermatogenic activity (Group IV)	Normal	3	50.00	0	0.00	3	42.86		
	Abnormal	3	50.00	1	100.00	4	57.14	Fisher'sExact	1.000
	All	6	100.00	1	100.00	7	100.00		

tors contributing to regulation of spermatogenesis in seminiferous epithelium. Apart from androgens, estrogens are also a potential regulator of spermatogenesis.[21] In male mice with aromatase deficiency, deterioration on spermatogenesis was detected after they were born and they were all infertile after one year.[16] Destruction of estrogen receptors in mice testicles also causes the infertility just as ERαKO and ERβKO mice. It was detected in ERαKO that fluid reabsorption in efferent ductules causes an increased pressure in the testis. Because of these changes, atrophy in seminiferous tubules and infertility occurs. On the other hand, aromatase over-expression also results in infertility in mice.[22] So it is understood from the literature that there should be a balance between testosterone and estrogen.

We designed our study aiming at investigation the effects of anastrozole on testicular function and histology. When a macroscopic examination was carried out before microscopic examination of testicles, we found a gradual decrease in testicular size associated with the dose of anastrozole. It is expected that anastrozole has positive effects on testicular histology and physiology. Turner et al. reported testicular volume increases after long term anastrozole treatment in rats.[23] But although it was not statistically significantly different, there was an increased number of atrophic changes in spermatogenetic activity from Group 2 to Group 4. And increased leydig cell hyperplasia towards Group 4 were detected compared to control group associated to atrophic changes. Moreover, testicular volume decreased gradually as high dose group was approached and the difference was statistically significant. Accorcing to our knowledge, there is not an article reports testicular atrophy as a side effect of anastrozole as we presented in any of the human or animal study models. We hypothesize that, after the disruption of the testosteron/estrogene ratio, decreased estrogene caused the atrophy which was associated with the anastrozole dose as mentioned above.

We acknowledge several limitations within this study. The first limitation is that we did not evaluate the semen parameters. Unlike humans, it is not possible to follow effects of medications on spermatogenesis with semen analysis in rats, but epididymal aspiration could be performed instead. However in our study we do not have these data because we aimed at investigating macroscopic and histopathologic changes only. Another limitation of this study is that we did not investigate the effect of the aromatase on endocrinologic parameters. Therefore, our findings must be confirmed by further prospective randomized studies which are more comprehensive. Despite these shortcomings, this is an important study since there is no previous data about the potential side effects of aromatase inhibitors mentioned above in the literature.

We observed a significant decrease in testicular size in rats that received high dose anastrozole. Mild and total atrophy depending on dose, and hyperplasia in Leydig cells secondary to atrophy was also detected. We believe that our findings must be confirmed by further prospective, randomized studies.

REFERENCES

1. Sigman M, Howards SS. Male infertility. In: Walsh PC, Retik AB, Vaughan ED Jr, Wein AJ, eds. Campell's Urology. 7th ed. Philadelphia: Saunders; 1998. p.1317-8.
2. Jarow JP. ESHRE basic semen analysis courses 1995-1999: immediate beneficial effects of standardized training. J Urol 2002;168(5):2310.
3. Kruger TF, Acosta AA, Simmons KF, Swanson RJ, Matta JF, Oehninger S. Predictive value of abnormal sperm morphology in in vitro fertilization. Fertil Steril 1988;49(1):112-7.
4. Tsai-Mortis CH, Aquilano DR, Dufau ML. Cellular localization of rat testicular aromatase activity during development. Endocrinology 1985;116(1):38-46.
5. de Jong FH, Hey AH, van der Molen HJ. Effect of gonadotropins on the secretion of oestradiol-and testosterone by the rat testis. J Endocrinol 1973;57(2):277-84.
6. van der Steeg JW, Steures P, Hompes PG, Eijkemans MJ, van der Veen F, Mol BW. Investigation of the infertile couple: a basic fertility work-up performed within 12 months of trying to conceive generates costs and complications for no particular benefit. Hum Reprod 2005;20(10):2672-4.
7. Liu PY, Handelsman DJ. The present and future state of hormonal treatment for male infertility. Hum Reprod Update 2003;9(1):9-23.
8. Pasqualotto FF, Fonseca GP, Pasqualotto EB. Azoospermia after treatment with clomiphene citrate in patients with oligospermia. Fertil Steril 2008;90(5):2014.e11-2.
9. Schlegel PN. Aromatase inhibitors for male infertility. Fertil Steril 2012;98(6):1359-62.

10. Pavlovich CP, King P, Goldstein M, Schlegel PN. Evidence of a treatable endocrinopathy in infertile men. J Urol 2001;165 (3):837-41.
11. Raman JD, Schlegel PN. Aromatase inhibitors for male infertility. J Urol 2002;167(2 Pt 1):624-9.
12. Raven G, de Jong FH, Kaufman JM, de Ronde W. In men, peripheral estradiol levels directly reflect the action of estrogens at the hypothalamo-pituitary level to inhibit gonadotropin secretion. J Clin Endocrinol Metab 2006;91(9):3324-8.
13. T'sjoen GG, Giagulli VA, Delva H, Crabbe P, De Bacquer D, Kaufman JM. Comparative assessment in young and elderly men of the gonadotropin response to aromatase inhibition. J Clin Endocrinol Metab 2005;90(10):5717-22.
14. Inkster S, Yue KW, Brodie A. Human testicular aromatase: immunocytochemical and biochemical studies. J Clin Endocrinol Metab 1995;80(6):1941-7.
15. Itoh N, Kumamoto Y, Maruta H, Tsukamoto T, Takagi Y, Mikuma N, et al. [Therapeutic efficacy of testolactone (aromatase inhibitor) to oligozoospermia with high estradiol/testosterone ratio]. Nihon Hinyokika Gakkai Zassh 1991;82(2):204-9.
16. Robertson KM, O'Donnell L, Jones ME, Meachem SJ, Boon WC, Fisher CR, et al. Impairment of spermatogenesis in mice lacking a functional aromatase (cyp 19) gene. Proc Natl Acad Sci U S A 1999;96(14):7986-91.
17. Rochira V, Granata AR, Madeo B, Zirilli L, Rossi G, Carani C. Estrogens in males: what have we learned in the last 10 years? Asian J Androl 2005;7(1):3-20.
18. Antal MC, Krust A, Chambon P, Mark M. Sterility and absence of histopathological defects in nonreproductive organs of a mouse ERbeta-null mutant. Proc Natl Acad Sci U S A 2008;105(7):2433-8.
19. Rochira V, Carani C. Aromatase deficiency in men: a clinical perspective. Nat Rev Endocrinol 2009;5(10):559-68.
20. Joseph A, Shur BD, Hess RA. Estrogen, efferent ductules, and the epididymis. Biol Reprod 2011;84(2):207-17.
21. Carreau S, Bouraima-Lelong H, Delalande C. Estrogen, a female hormone involved in spermatogenesis. Adv Med Sci 2012;57(1):31-6.
22. Li X, Rahman N. Impact of androgen/estrogen ratio: lessons learned from the aromatase over-expression mice. Gen Comp Endocrinol 2008;159(1):1-9.
23. 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(2): 225-38.