The Effect of Testosterone Replacement Therapy on Bladder Functions and Histology in Orchiectomized Mature Male Rats

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OBJECTIVE To investigate the effect of testosterone replacement therapy on bladder functions and smooth muscle/collagen content in orchidectomized orchiectomized mature male rats.

METHODS The study included 25 mature male Sprague-Dawley rats divided into 3 groups. After bilateral orchiectomy, 8 rats received intramuscular saline injection, as a control group, and 8 rats received intramuscular injection of testosterone undecanoate 100 mg/kg as a treatment group. The sham group had 9 rats. Urodynamic studies were performed in all groups, before and after the study. The rats were killed after 60 days, and cystometric findings and smooth muscle/collagen ratio of the bladders were compared between the groups.

RESULTS From the beginning to the end of the experiment, mean maximal bladder capacity increased 46.61% ± 20.82 in the testosterone treatment group, while decreased 38.91% ± 17.83 in control group, revealing a significant difference ($P = .002$). Smooth muscle/collagen ratio was significantly higher in the testosterone treatment group (1.53 ± .34) than in the control group (1.05 ± .32), ($P = .01$).

CONCLUSIONS This study showed that bladder capacity and smooth muscle/collagen content improved with testosterone therapy in orchiectomized rats. Therefore, testosterone replacement therapy in late-onset hypogonadal men with urogenital dysfunction may have a positive role to improve bladder function by increasing bladder smooth muscle. UROLOGY 75: 886–890, 2010. © 2010 Elsevier Inc.

Symptomatic late-onset hypogonadism (SLOH) is defined as a clinical and biochemical syndrome associated with advancing age and characterized by typical symptoms and a deficiency in serum testosterone levels.[1] SLOH is characterized by diminished libido and erectile quality and frequency, changes in mood, sleep disturbances, decrease in lean body mass, increase in visceral fat, decrease in body hair and skin alterations, and decreased bone mineral density.[1,2] Also, androgen deficiency in older men can cause urogenital dysfunction. Testosterone replacement therapy is indicated in the presence of both clinical symptoms that are suggestive of hormone deficiency and decreased testosterone level.[3-6]

Many studies have investigated the effects of restoring testosterone levels to normal in men with complaints of low sexual desire and erectile dysfunction.[3-6] Studies also suggest that testosterone replacement therapy may promote prostate enlargement, resulting in lower urinary tract symptoms.[7,8] However, the effect of testosterone on smooth muscle of urogenital system is not well known. Our recent study has shown that in addition to improvement in sexual functions, testosterone therapy may also improve lower urinary tract symptoms/bladder functions by increasing bladder capacity and compliance and decreasing detrusor pressure at maximal flow in men with SLOH.[9]

The aim of this experimental study was to investigate the effect of testosterone replacement therapy on bladder functions and smooth muscle/collagen content in orchiectomized mature male rats.

MATERIAL AND METHODS

Animals and Study Design

The experimental protocol was approved by the Committee on Animal Research at the University of Mersin School of Medicine. The study included 25 male Sprague-Dawley 120-day-old rats, weighing 200-240 g, and the rats were divided into 3 groups. After bilateral orchiectomy, 8 rats received intramuscular (i.m.) saline injection as a control group, and 8 rats received testosterone undecanoate (Nebido, Bayer Schering Pharma, Berlin, Germany) 100 mg/kg i.m. as a treatment group. The sham group had a total of 9 rats. Urodynamic evaluation was performed to determine baseline bladder pressures, capacity, and compliance in all groups at the beginning of the study.

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After performing baseline urodynamic studies, bilateral orchiectomy was performed and 100 mg/kg i.m. testosterone undecanoate was injected into the left gluteal region in the treatment group. In the control group, after bilateral orchiectomy, i.m. saline injection was performed. In the sham group, testicles were explored but no orchiectomy was performed. Cystometric studies were repeated 2 months after the first application in all rats before they were killed. The bladders were removed and cystometric findings and smooth muscle/collagen ratio of the bladders were compared between the groups.

Orchiectomized Rat Model

The rats were anesthetized with intraperitoneal injection of ketamine (50-100 mg/kg) and received a single 20 mg/kg dose of ciprofloxacin as a prophylactic antibiotherapy. Under sterile conditions, a midline incision was made to expose the lower abdominal cavity, and the entire testicles were removed.[10,11] Sham operations were performed in a similar manner but the abdomen was closed without removing testicular tissue. The rectus fascia was closed with a running 4-0 plain absorbable catgut suture, and the skin was closed with interrupted 3-0 silk sutures.

Functional Evaluation

Urodynamic studies were performed before the treatment and just before the rats were killed, as described previously.[12-15] All rats underwent cystometry under ketamine anesthesia (50 mg/kg i.m.). A midline incision was performed in all rats and bladder was exposed. A fine incision was performed and 22-G catheter was replaced into the dome of the bladder, and residual urine was emptied. The catheter was connected by a polyethylene tube to a pressure transducer using a urodynamic equipment (Life-Tech, Inc, Houston, TX) and a computer program (Urolab Primolus, Life-Tech, Inc, Houston, TX). Each rat underwent cystometric measurements with infusion of warmed (37°C) normal saline solution at 0.1 mL/min (Abbott infusion pump). For each rat, single cystometry was done at the beginning of the experiment, and single cystometry was done at the end of the experiment. During the study, the baseline (empty bladder), opening (at first leakage), and peak pressure (maximal pressure during voiding) and the maximal bladder capacity were recorded. Bladder compliance (mL/cm H_2O) was calculated according to the following formula: Compliance = Maximal Bladder Volume/Opening Pressure-Baseline Pressure.

Histologic Evaluation

After removal of the bladders the rats were killed by pentobarbital overdose (200 mg/kg) and bilateral thoracotomy. After both ureters were ligated, the bladder was filled with 10% formalin solution through a catheter transurethrally and kept distended overnight. The specimen was split longitudinally, and the routine tissue processing for light microscopy was performed.

Bladder tissues were embedded in paraffin. Sections (3 μm) were cut by microtome and stained with Masson's trichrome for the evaluation of collagen and smooth muscle content. Slides were examined by an Olympus BX50 light microscope and photographed by an Olympus PM10SP photograph system. The slides were analyzed on a PC with Soft Imaging System (Olympus Soft Imaging Solutions, GmbH, Münster, Germany). The number of pixels within the bladder was counted and set at 100%, and the other structures were erased from the images. The smooth muscle and collagen components of the bladders were identified at 300x, and calculated as square millimeters.

Statistical Analysis

Statistical analyses were performed using the "One way ANOVA test" to compare the mean body weight and mean serum testosterone level at the beginning and end of the experiment among the 3 groups; "independent *t* test" to compare smooth muscle/collagen ratio and differences as percentage in cystometric findings at the beginning and end of each experiment between the 2 groups. Data are presented as mean ± standard error for cystometric findings and mean ± standard deviation for smooth muscle/collagen ratio. Probability values of $<.05$ were considered statistically significant.

RESULTS

Functional Study Findings

At the beginning of the experiment, the mean body weight was 218 ± 16 g in the sham group, 214 ± 14 g in the control group, and 213 ± 17 g in the treatment group, revealing no significant differences among the 3 groups ($P = .894$). At the end of the experiment, the mean body weight reached to 249 ± 21 g in the sham group, 212 ± 23 g in the control group, and 287 ± 31 g in the treatment group, revealing significant difference among the 3 groups ($P = .001$). At the beginning of the experiment, the mean serum testosterone level was 0.507 ± 0.096 ng/mL in the sham group, 0.582 ± 0.116 ng/mL in the control group, and 0.523 ± 0.127 ng/mL in the treatment group, revealing no significant differences among the 3 groups ($P = .426$). At the end of the experiment, the mean serum testosterone level reached to 0.497 ± 0.122 ng/mL in the sham group, 0.002 ± 0.0001 ng/mL in the control group, and 1.861 ± 0.376 ng/mL in the treatment group, revealing significant difference among the 3 groups ($P = .0001$).

Figures 1 and 2 show the mean maximal bladder capacity and bladder compliance at the beginning and end of the experiment in the sham, control, and testosterone treatment groups. The mean maximal bladder capacity decreased from 1.20 ± 0.15 mL to 1.17 ± 0.23 mL in the sham group, decreased from 1.29 ± 0.15 mL to 0.75 ± 0.23 mL in the control group, while it increased from 1.08 ± 0.22 mL to 1.34 ± 0.13 mL in the testosterone treatment group (Fig. 1). As percentage from the beginning to the end of the experiment, the mean maximal bladder capacity decreased 1.78% ± 1.15% in the sham group, 38.19% ± 17.83% in the control group, while increased 46.61% ± 20.82 in the testosterone treatment group. The increase of the mean maximal bladder capacity was significantly higher in the testosterone treatment group than in the control group ($P = .002$).

As shown in Fig. 2, the mean bladder compliance decreased from 0.027 ± 0.002 mL/cm H_2O to 0.026 ± 0.003 mL/cm H_2O in the sham group, decreased from 0.023 ± 0.006 mL/cm H_2O to 0.019 ± 0.005 mL/cm

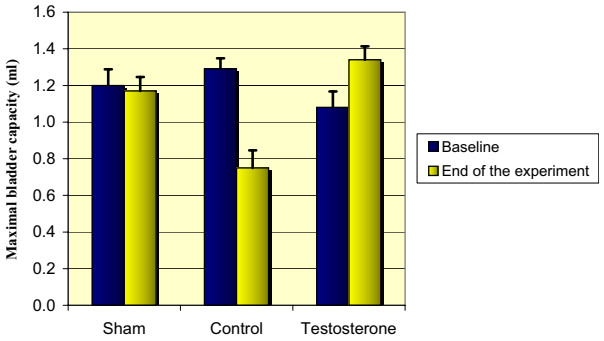


Figure 1. The mean maximal bladder volume at the beginning and end of the experiment in the sham, control, and testosterone treatment groups. As percentage from the beginning to the end of the experiment, the mean maximal bladder capacity decreased 1.78% ± 1.15 in the sham group, 38.19% ± 17.83 in the control group, while increased 46.61% ± 20.82 in the testosterone treatment group. The increase of the mean maximal bladder capacity was significantly higher in the testosterone treatment group than in the control group ($P = .002$).

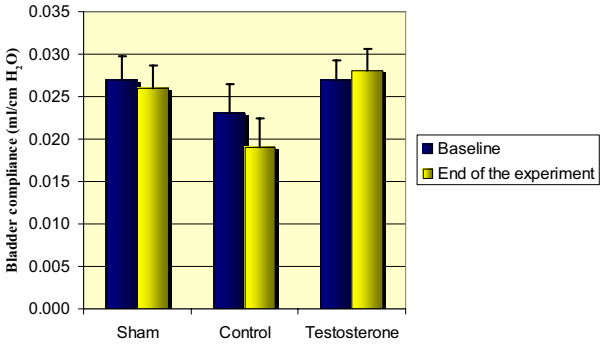


Figure 2. The mean bladder compliance at the beginning and end of the experiment in the sham, control, and testosterone treatment groups. As percentage from the beginning to the end of the experiment, the mean bladder compliance decreased 3.84% ± 1.65 in the sham group, and decreased 17.39% ± 6.97 in the control group, while increased 3.71% ± 1.52 in the testosterone treatment group, revealing no statistical significance compared with the control group ($P = .191$).

H_2O in the control group, while increased from 0.027 ± 0.008 mL/cm H_2O to 0.028 ± 0.006 mL/cm H_2O in the testosterone treatment group. As percentage from the beginning to the end of the experiment, the mean bladder compliance decreased 3.84% ± 1.65 in the sham group, and decreased 17.39% ± 6.97 in the control group, while increased 3.71% ± 1.52 in the testosterone treatment group, revealing no statistical significance compared with the control group ($P = .191$).

Histologic Findings

As shown in Fig. 3, the mean bladder smooth muscle/collagen ratio was 1.26 ± 0.34 in the sham group, 1.05 ± 0.32 in the control group, and 1.53 ± 0.34 in the testosterone treatment group. As compared with the control group, smooth muscle/collagen ratio was significantly higher in the sham group ($P = .03$) and testosterone treatment group ($P = .01$). Figure 4 shows Masson's trichrome staining of a rat bladder for each group.

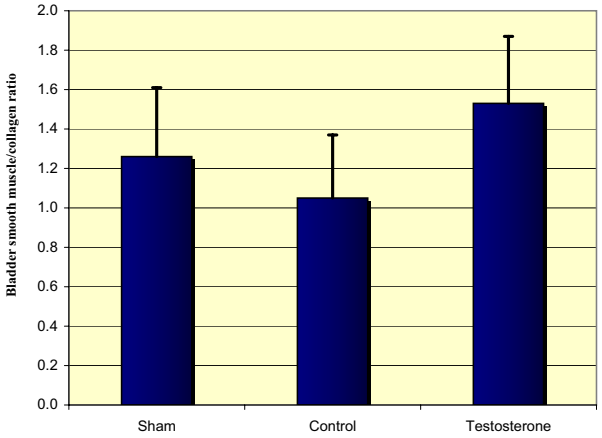
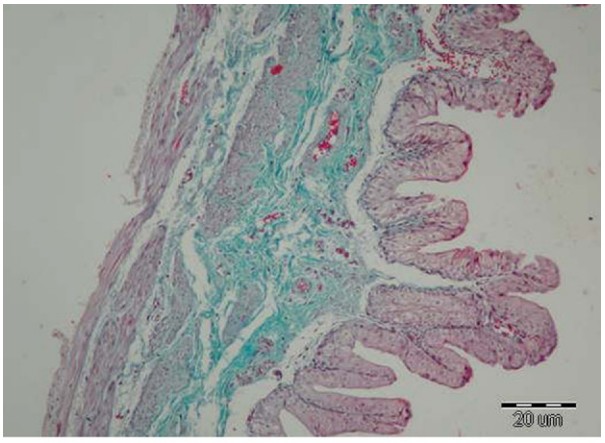


Figure 3. The mean smooth muscle/collagen ratio in the sham, control, and testosterone treatment groups. As compared with the control group, smooth muscle/collagen ratio was significantly higher in the sham group ($P = .03$) and testosterone treatment group ($P = .01$).

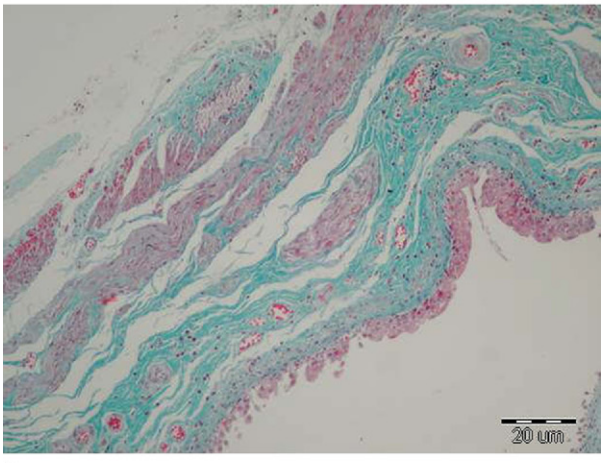
COMMENT

Orchiectomized rats have been used as a model of hypogonadism-induced testosterone deficiency in human beings.[10,11] However, late-onset hypogonadism is a condition characterized by combination of central and peripheral hypogonadism. Surgical castration is a pure model of peripheral (primary) hypogonadism, and may not be an appropriate model of late-onset hypogonadism in human beings. In the present study, we performed bilateral orchiectomy, and then we investigated the effect of testosterone replacement therapy on bladder functions and smooth muscle/collagen content in orchiectomized mature male rats. Many clinical and experimental studies focused on erectile function in testosterone deprivation. In the present study, erectile functions were not measured because the main point of the study was to investigate effect of testosterone therapy on bladder functions and histology in testosterone-deprived rats.

Androgen deprivation may inhibit the smooth muscle differentiation pathway. In the present study, we found that bladder capacity and smooth muscle content decreased after orchiectomy period. From the beginning to the end of the experiment, the increase in the mean maximal bladder capacity was significantly lower in the control group than in the sham group. In addition, orchiectomized rats showed significantly lower smooth muscle/collagen content than the sham group. Our experimental rat study showed that bladder capacity and smooth muscle/collagen content may deteriorate in testosterone deprivation period. Lin et al[16] concluded that



A



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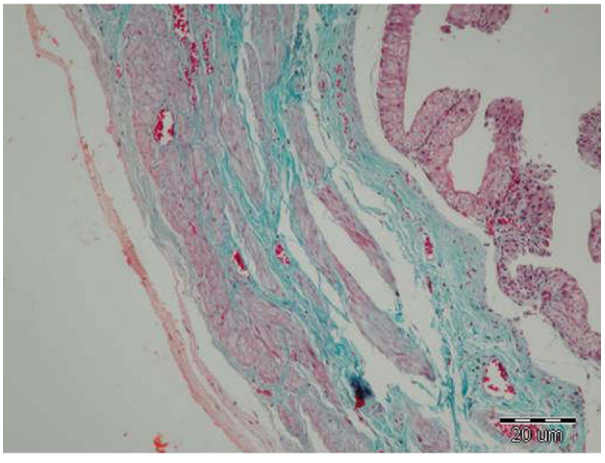

C

Figure 4. Masson trichrome staining of a rat bladder in the sham group (**A**), control group (**B**), and testosterone treatment group (**C**). The claret red color shows smooth muscle content, and the green color shows collagen content (Color version available online).

testosterone might be as important as estrogen in the bladder contractile responses. Madeiro et al[17] studied the association of androgen/estrogen on the bladder and urethra of castrated female rats, and after 28 days of medication the animals were killed for the histologic evaluation of the bladders. They reported that the bladders of the rats receiving androgen/estrogen presented a higher number of vessels, epithelial thickness, and quantity of muscular fibers than the rats receiving isolated conjugated estrogen.

Androgens, through the activation of androgen receptors, may stimulate stromal precursor cells to differentiate into smooth muscle cells.[18] Androgens also regulate growth and differentiation of vascular smooth muscle cells. Such studies have not been performed in bladder tissue. Therefore, we investigated the effect of testosterone therapy on bladder functions and smooth muscle/collagen content in the castrated mature rats. In our study, increases in maximal bladder capacity were significantly higher in the testosterone treatment group than in the control group. Also, bladder compliance was higher in the testosterone treatment group than in the control group, but the difference was not statistically significant. A recent male rabbit study showed that after injection of testosterone, bladder capacity and compliance increased with high blood testosterone levels in male rabbits undergoing bilateral orchiectomy.[19] These animal studies suggest that bladder dysfunction may be related to androgen deficiency, and testosterone treatment may improve bladder functions and smooth muscle/collagen content in castrated animals.

There are contrary opinions that testosterone has been shown to cause obstruction with or without unstable detrusor contractions and in addition, it may also induce changes in the micturition reflex. Maggi et al[20] treated adult male rats with daily testosterone injections and performed cystometric measurements. They found testosterone treatment to increase prostate weight and cause outflow obstruction seen as increased residual urine volume and detrusor instability. Less change was seen in the bladder capacity. Pandita et al[21] reported that testosterone treatment in adult male rats for 2 weeks increased prostate weight. Testosterone treatment was shown to increase micturition pressure, bladder capacity, residual volume, and micturition volume. Detrusor, urethra, and prostate were isolated and more detailed studies were performed in vitro. However, no changes were seen in the smooth muscle responsiveness. Measurement of bladder and prostate weight would give more precise and supplementary information to confirm improvement in the bladder capacity. However, in the present study, we did not measure prostate and bladder weight. We measured body weight at the beginning and end of the experiment, and we observed significantly higher body weight in the testosterone treatment group than in the orchiectomized control group, receiving no treatment.

There can be increased ρ-kinase activity, and consequently calcium sensitivity of the contractile machinery in bladder smooth muscle, and one of possible explanation for the improvement of bladder functions would be that testosterone therapy may also alleviate pelvic isch-

emia by increasing nitric oxide synthase expression and activity, as well as reducing ρ-kinase activity in bladder smooth muscle.[22,23] Muto et al[23] demonstrated that the neuronal nitric oxide synthase (nNOS) knock-out mice showed an increased urinary frequency. In addition, androgens may augment endothelial nitric oxide synthase in these nNOS knock-out animals. Therefore, they suggested that testosterone potentially has a therapeutic effect on an overactive bladder by decreasing the nNOS expression in aging males. Testosterone induces the relaxation of smooth muscle cells by modulating both the nNOS activity and cyclic guanosine monophosphate level. Testosterone is also an important maintenance factor for autonomic nerve activity.[24] Thus, the changes in cystometric parameters could be related not only to smooth muscle activity, but also to neural function.

Although several testosterone formulations are available, testosterone topical gel and long-term depot injections are the most commonly used treatment options, due to their favorable pharmacokinetic profile characterized by relatively constant plasma levels, avoiding wide fluctuations and minimal side effects.[1,4-6] We used long-acting testosterone injection preparation because of difficulty of daily or monthly administration of testosterone agents in rats. According to the studies, single injection of long-acting testosterone undecanoate provided a 3-month effects. In the present study, testosterone-treated rats had about 3-fold higher serum testosterone level than untreated controls. Therefore, the dose of testosterone in the treated group could be pharmacologic rather than physiological.

CONCLUSIONS

This study showed that bladder capacity and smooth muscle/collagen content improved with testosterone therapy in orchiectomized mature rats. Therefore, testosterone replacement therapy in late onset hypogonadal men with urogenital dysfunction may have a significant role to improve bladder function by increasing bladder smooth muscle content. However, further studies are needed to support these findings.

References

1. Nieschlag E, Swerdloff R, Behre HM, et al. Investigation, treatment and monitoring of late-onset hypogonadism in males: ISA, ISSAM, and EAU recommendations. *J Androl*. 2006;27:135-137.
2. Buvat J, Shabsigh R, Guay A, et al. Hormones, metabolism, aging, and men's health. In: Porst H, Buvat J, eds, and the standard committee of the ISSM. *Standard Practice in Sexual Medicine*. UK: Blackwell Publishing; 2006:225.
3. Lazarou S, Morgentaler A. Hypogonadism in the man with erectile dysfunction: what to look for and when to treat. *Curr Urol Rep*. 2005;6:476-481.
4. Lunenfeld B, Niesschlag E. Testosterone therapy in the aging male. *Aging Male*. 2007;10:139-153.
5. Vermeulen A. Androgen replacement therapy in the aging male-a critical evaluation. *J Clin Endocrinol Meta*. 2001;86:2380-2390.
6. Yassin AA, Saad F. Treatment of sexual dysfunction of hypogonadal patients with long acting testosterone undecanoate (Nebido). *World J Urol*. 2006;24:639-644.
7. Meikle AW, Arver S, Dobs AS, et al. Prostate size in hypogonadal men treated with a nonscrotal permeation-enhanced testosterone transdermal system. *Urology*. 1997;49:191-196.
8. Calof OM, Singh AB, Lee MC, et al. Adverse events associated with testosterone replacement in middle-aged and older men: a meta-analysis of randomized, placebo-controlled trials. *J Gerontol A Biol Sci Med Sci*. 2005;11:1451-1457.
9. Karazindiyanoğlu S, Çayan S. The effect of testosterone therapy on lower urinary tract symptoms/bladder and sexual functions in men with symptomatic late-onset hypogonadism. *Aging Male*. 2008;11:146-149.
10. Erben RG, Erberle J, Stahr K, et al. Androgen deficiency induces high turnover osteopenia in aged male rats: a sequential histomorphometric study. *J Bone Miner Res*. 2000;6:1085-1098.
11. Gunness M, Orwoll E. Early induction of alterations in cancellous and cortical bone histology after orchiectomy in mature rats. *J Bone Miner Res*. 1995;11:1735-1744.
12. Çayan S, Canpolat B, Çayan F, et al. The effect of chronic inflammatory condition of the bladder and estrogen replacement therapy on bladder functions and histology in surgically menopause and chronic cystitis induced rats. *Neurourol Urodyn*. 2006;25:194-201.
13. Çayan S, Chermansky C, Schlote N, et al. The bladder acellular matrix graft in a rat chemical cystitis model: functional and histologic evaluation. *J Urol*. 2002;168:798-804.
14. Çayan S, Coskun B, Bozlu M, et al. Botulinum toxin type A may improve bladder function in a rat chemical cystitis model. *Urol Res*. 2003;30:399-404.
15. Acar D, Çayan S, Aktaş S, et al. The effect of tamoxifene on bladder functions and histology, and the role of estrogen receptor beta in a rat chemical cystitis model. *Neurourol Urodyn*. 2007;26:309-316.
16. Lin WY, Rehfusst A, Whitbeck C, et al. Effect of letrozole on urinary bladder function in the female rabbit. *BJU Int*. 2007;100:1391-1395.
17. Madeiro A, Girao M, Sartori M, et al. Effects of the association of androgen/estrogen on the bladder and urethra of castrated rats. *Clin Exp Obstet Gynecol*. 2002;29:117-120.
18. Traish AM, Goldstein I, Kim NN. Testosterone and erectile function: from basic research to a new clinical paradigm for managing men with androgen insuffiency and erectile dysfunction. *Eur Urol*. 2007;52:54-70.
19. Celayir S. Effects of different sex hormones on male rabbit urodynamics: an experimental study. *Horm Res*. 2003;60:215-220.
20. Maggi CA, Manzini S, Giuliani S, et al. Infravesical outflow obstruction in rats: a comparison of two models. *Gen Pharmacol*. 1989;20:345-349.
21. Pandita RK, Persson K, Hedlund P, et al. Testosterone-induced prostatic growth in the rat causes bladder overactivity unrelated to detrusor hypertrophy. *Prostate*. 1998;35:102-108.
22. McVary K. Lower urinary tract symptoms and sexual dysfunction: epidemiology and pathophysiology. *BJU Int*. 2006;97(suppl 2):23-28.
23. Muto S, Yasuda M, Kamiyama Y, et al. Testosterone decreased urinary-frequency in nNOS-deficient mice. *Int J Androl*. 2008;1:67-70.
24. Keast JR. Effects of testosterone on pelvic autonomic pathways: progress and pitfalls. *J Auton Nerv Syst*. 2000;79:67-73.