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Effect of Bisphenol A on Fertility of Male Mice

Shikha Chouhan[1], Jay Prakash[1], Satyndra K Yadav[1], Neeraj K Agrawal[2], Surya P Singh[1]

[1]Department of Biochemistry, Faculty of Science, Banaras Hindu University, Varanasi-221005

[2]Department of Endocrinology and Metabolism, Institute of Medical Sciences, Banaras Hindu University, Varanasi-221005.

shikhachouhan16@gmail.com, satyndra_yadav@yahoo.co.in, jaiprakash_biotech@yahoo.co.in, drnkavns@gmail.com, suryasinghbhu16@gmail.com, ssingh35@bhu.ac.in.

Abstract

Bisphenol A (BPA) is a chemical widely used for the synthesis of polycarbonate plastics. The present study was undertaken to decipher the effects of BPA exposure, on fertility of male mice. A set of eight Swiss albino mice were treated with BPA intraperitonially at a dose of 100µg/kg body weight/day, for a period of two months. Control group was treated with vehicle.

Females mated with male mice that were exposed to BPA showed a 50% decrease in litter size compared to control. There was significant reduction in sperm count as well as testosterone level in the BPA exposed mice. The histological study of testes showed immense distortion of the seminiferous tubules in treated mice along with other deformities.

The present study gives strong evidence to support that BPA is capable of impairing male fertility by distressing the steroidogenesis and spermatogenesis process in male mice.

Keywords: Bisphenol A, xenoestrogen, male fertility, testosterone level, sperm count.

Introduction

Bisphenol A (BPA) is an important industrial compound employed as a monomer of polycarbonate plastics used in food packaging, manufacturing products such as baby bottles, water bottles, epoxy resins and white dental sealants (Colborn 1993, Brotons 1995, Kim 2010). BPA molecules are bound by "ester bonds" to form the polymer and this bond is disrupted by heat and acidic or basic conditions that release BPA into food or beverages in contact with the plastics (Krishnan 1993).

BPA is known to mimic the role of estrogen once inside the body, thus acting as a xenoestrogen. Hence, BPA has been placed in the group of chemicals known as endocrine disruptors (EDs). These EDs basically mimic the role of different hormones inside our body thereby causing severe disruption of the normal functioning of the endocrine system (Sekizawa

2008).

BPA is known to have weak estrogenic activities in vivo as well as in vitro, with approximately 10,000 times less affinity for estrogen receptors compared to 17β-estradiol (Kuiper 1998).

Indeed xenoestrogen like BPA are now being implicated in human infertility, genital tract malformations, and increased cancer rates in estrogen sensitive target tissues (Sharper 1993). Bisphenol A has been tested in male fertility studies in laboratory animals. Some studies have reported no effects on the reproductive function of male offspring following maternal exposure (Ashby 1999), whilst others have reported that exposure to low doses of BPA causes reproductive toxic effects (Nagel 1997). This raises some concern about the adverse effects of BPA on human fertility.

In view of the above literature, the present study was carried out to determine whether exposure to environmentally relevant BPA levels affects testicular steroidogenesis and if so, to identify the mechanisms associated with observed effects.

Material and Methods

Animal care and maintenance

Sixteen adult male *Swiss albino* mice used in the experiment were purchased from animal house facility of the Institute of Medical Sciences, BHU, Varanasi, India. They were kept in a controlled temperature of 22 ±3˚C on a 12-h light/dark cycle. Food pellets and water were made available to these animals *ad libitum*. Proper cleaning of cages and grills were done routinely.

BPA administration

Animals were divided into two groups and labeled as treated and control. BPA was administered intraperitonially at a dose of 100µg/kg body weight/day for a period of two months. Control group was injected with normal saline alone.

Fertility assessment

Fertility was estimated in adult male mice exposed to BPA. After two months of BPA injection, each male was placed in an individual cage with two virgin untreated females of the same strain. They were left together for 10 days, during which two estrus cycles should have elapsed, BPA treated and control males were then removed and sacrificed for further evaluations. The female mice were left isolated and allowed to give birth to their litters. The number of pups/litter were counted and compared among control and BPA exposed groups.

Weight of accessory sex organs

Bisphenol A-exposed and control mice were sacrificed after completion of the exposure, by cervical dislocation. Reproductive organs including the testes and seminal vesicles were dissected out and the paired weights were recorded.

Sperm Count

Sperm count was performed according to the method of Salian (2009a). Briefly, the cauda region of epididymis was excised and collected. It was placed in 0.5 ml of phosphate buffer saline and homogenized using a manual glass homogenizer. The homogenate was mixed

using a vortex mixer, and the number of sperm was measured using a hemocytometer after a twenty fold dilution. Sperm counted were expressed as number of sperms in million per cauda epididymis.

Testosterone estimation

Radioimmunoassay (RIA) was used to quantify the levels of serum testosterone as described by D'Souza (2005) with some modifications. Briefly, blood was collected and allowed to stand at room temperature for 30 min to separate the sera. It was then centrifuged at 10,000 rpm for 10 min and, separated sera were then stored at -20°C for hormone assay.

Histological analysis

According to standard criteria given by Hess (1990), histological analysis was carried out. Briefly, the testes were dissected out and fixed in Bouin's fixative for 24 h. After primary fixation, 3 to 5 mm thick testicular slices were cut and refixed in fresh fixative for another 24 h. The paraffin embedded tissue blocks were sectioned at a thickness of 5μm, mounted on glass slide and stained with Hematoxylin and Eosin (Sigma, USA). The stained slides were reviewed under standard light microscope (Leitz orthoplan microscope- 054546).

Statistical analysis

The data were analyzed by using one way analysis of variance (ANOVA) through GraphPad prism software. All values were expressed as mean ± SEM. The significance of the data obtained was evaluated by using Student-Newman-Kules test. P-values of less than 0.05 ($p<0.05$) were considered significant.

Results

Females mated with male mice, that were exposed to BPA showed a significant reduction (***$p<0.001$) in the litter size. In control group number of pups varied from 10 to 12 whereas in treated group it varied from 5 to 6. The testis (***$p<0.001$) and seminal vesicle (** $p<0.01$) weight of BPA treated mice showed a significant reduction compared to control.

There was significant reduction (** $p<0.01$) in sperm count in the mice exposed to BPA. Sperm count was found to be in the range of 1.2 to 2.4 million in control mice whereas in BPA exposed mice it ranged from 0.54 million to 0.8 million. The testosterone level in control group was nearly 345 to 456 ng/ml and in treated group it was 156 to 262ng/ml. Thus, we saw a significant decrease (***$p<0.001$) in testosterone level after BPA exposure. The microscopic view of testes from BPA exposed mice showed immense distortion in seminiferous tubules with extensive vacuolization.

Discussion

The present study was designed to investigate any adverse effects of BPA on fertility and reproduction of adult male mice. This chemical was chosen because of its bulk production and huge consumption all over the world. Additionally, due to its estrogenic behavior there is a large possibility of its implication in male infertility.

The No Observed Adverse Effective Levels (NOAEL) of BPA has been found to be 5mg/kg body weight/day (NTP, 2001). Thus, we selected a dose much below this level in order to evaluate the effect of BPA exposure on male mice fertility.

Several reproductive parameters were adversely affected after exposure of BPA to adult male mice. First of all the reduction in number of pups/litter as a result of BPA exposure is a direct evidence of decrease in male fertility. Testis weight in adult mice is an important indicator of total germ cell number per testis. Simultaneously, the weight of seminal vesicle helps in indicating the testosterone level in adult male mice (Atanassova 1999). Thus, a decrease in both these end points due to BPA exposure gives a reliable indication of antiandrogenic effect of this chemical. Sperm count is an important parameter to analyze toxic effects of any substance on male reproduction. The result obtained here suggests that BPA causes a significant reduction in sperm production in male mice and it is in harmony with a previous study (Salian 2009b). Testosterone plays an important role in the process of steroidogenesis in male testis. An impaired histological architecture and a reduced testosterone level in BPA exposed mice gives a clear picture of impaired activity of Leydig cells.

Thus, the results obtained in the present study clearly demonstrate that long term BPA exposure to male mice at low doses is capable of adversely affecting the male fertility by causing severe damages to the spermatogenesis and steroidogenesis process.

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References

Atanassova, N., McKinnell, C., Walker, M., Turner, K.J., Fisher, J.S., Morley, M., Millar, R., Groome, N.P. and Sharpe, R.M. (1999): Permanent effects of neonatal estrogen exposure in rats on reproductive hormone levels, Sertoli cell number and the efficiency of spermatogenesis in adulthood, Endocrinology, 140: pp. 5364-5373.

Ashby, J., Tinwell, H. and Haseman, J. (1999): Lack of effects of low dose levels of Bisphenol A and Diethylstilbestrol on prostate gland of CF1 mice exposedin utero, Regul Toxicol Pharmacol, 30: pp. 156-166.

Brotons, J.A., Olea-Serrano, M.F., Villalobos, M., Pedraza, V. and Olea, N. (1995): Xenoestrogens released from lacquer coatings in food cans, Environ Health Perspect, 103: pp. 608-612.

Colborn, T., Vom Saal, F.S. and Soto, A.M. (1993): Developmental effects of endocrine disrupting chemicals in wildlife and humans, Environ Health Perspect, 101: pp. 378-384.

D'Souza, R., Gill-Sharma, M., Pathak, S., Kedia, N., Kumar, R. and Balasinor, N. (2005): Effect of high intratesticular estrogen on the seminiferous epithelium in adult male rats. Mol Cell Endocrinology, 241: pp. 41-48.

Hess, R. (1990): Quantitative and qualitative characteristics of the stages and transitions in the cycle of the rat seminiferous epithelium: light microscopic observations of perfusion-fixed and plastic embedded testes, Biol Reprod, 43: pp. 525-542.

Kim, J.Y., Hanb, E.H., Kim, H.G., Oh, K.N., Kim, S.K., Lee, K.Y. and Jeong, H.G. (2010): Bisphenol A induced aromatase activation is mediated by cyclooxygenase-2 up-regulation in rat testicular Leydig cells, Toxicol Lett, 193: pp. 200-208.

Krishnan, A.V., Stathis, P., Permuth, S.F., Tokes, L., and Feldman, D. (1993): Bisphenol A: an estrogenic substance is released from polycarbonate flasks during autoclaving,

Endocrinology, 132: pp. 2279-2286.

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Kuiper, G.G., Lemmen, J.G., Carlsson, B., Corton, J.C., Safe, S.H. and vander Saag, P.T. (1998): Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta, Endocrinology, 139: pp. 4252–6.3

NTP, (2001): National Toxicology Program's Report of the Endocrine Disruptors Low Dose Peer Review, National Toxicology Program, Research Triangle Park, NC

*Nagel, S.C., Vom Saal, F.S., Thayer, K.A., Dhar, M.G., Boechler, M. and Welshons, W. (1997): Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative in vivo activity of the xenoestrogens Bisphenol A and octylphenol, Environ Health Persp,105: pp. 70-76.

Salian, S., Doshi, T. and Vanage, G. (2009a): Perinatal exposure of rats to Bisphenol A affects the fertility of male offspring, Life Sci, 85: pp. 742-752.

Salian, S., Doshi, T. and Vanage G. (2009b): Neonatal exposure of male rats to Bisphenol A impairs fertility and expression of Sertoli cell junctional proteins in the testis, Toxicology, 265: pp. 56-67.

Sekizawa, J. (2008): Low dose effects of Bisphenol A: a serious threat to human health? J Toxicol Sci, 33: pp. 389-403.

Sharper and Skakkebaekn. (1993): Are estrogens involved in falling sperm counts and disorders of the male reproductive tract? Lancet, 1993, 341: pp.1392-1395.

Figure legends

Fig 1: Decrease in number of pups/litter in the female mated with adult mice exposed to BPA compared to females mated with control mice. Data expressed as mean±SEM, n=8, and significant changes are shown as ***p<0.001.

Fig 2: Decrease in weight of testis in BPA treated mice compared to control. Data expressed as mean±SEM, n=8 and significant changes are shown as ***p<0.001.

Fig 3: Decrease in weight of seminal vesicle in BPA treated mice compared to control. Data expressed as mean±SEM, n=8, and significant changes are shown as **p<0.01.

Fig 4: Sperm count analysis of control vs BPA treated mice. The data is expressed as mean±SEM, n=8 and significant changes are shown as ** p<0.01.

Fig 5: Testosterone level in adult male mice exposed to BPA compared to control. The data is expressed as mean±SEM, n=8 and significant changes are shown as ***p<0.001

Fig 6: Photomicrographs of testis showing seminiferous tubules (ST) of (a) control and (b) BPA treated mice. The ST of BPA treated mice show extensive vacuolization with hypospermatogenesis whereas control mice show normal histological architecture.

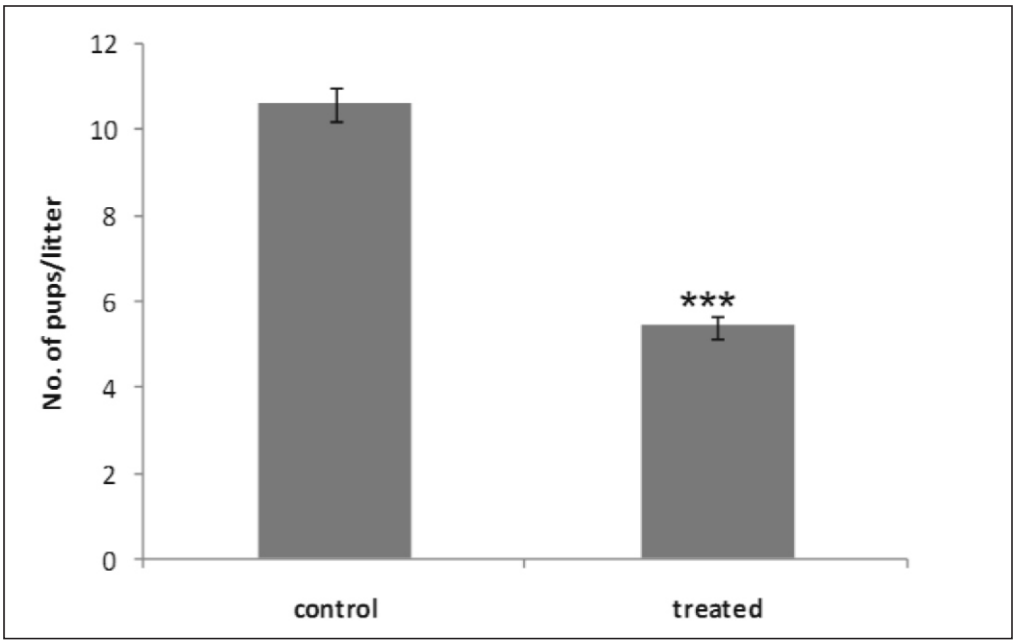

12
10
8
6
4
2
0
No. of pups/litter
***
control
treated

Fig. 1

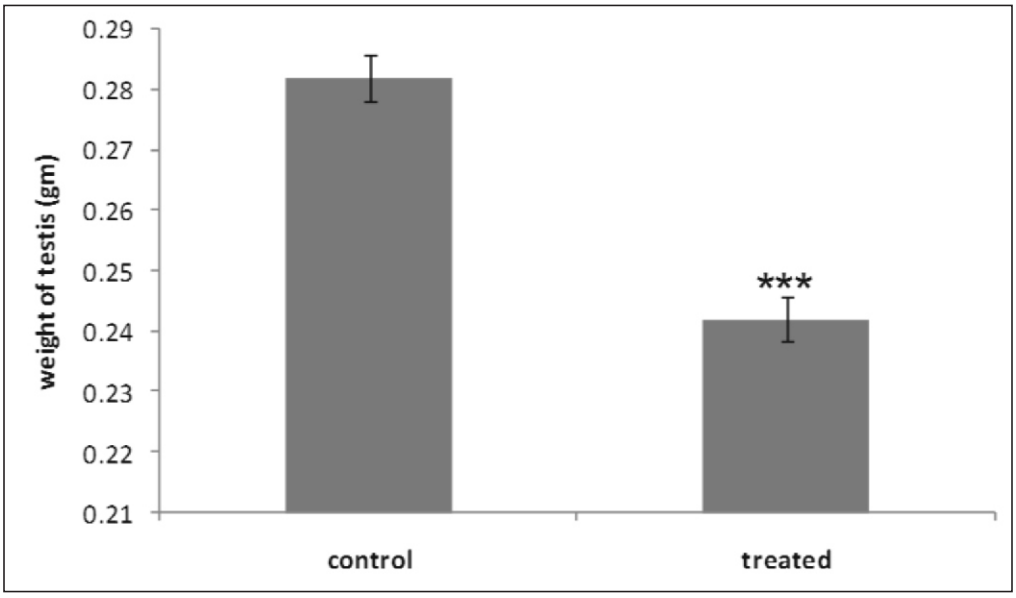

0.29
0.28
0.27
0.26
0.25
0.24
0.23
0.22
0.21
weight of testis (gm)
***
control
treated

Fig. 2

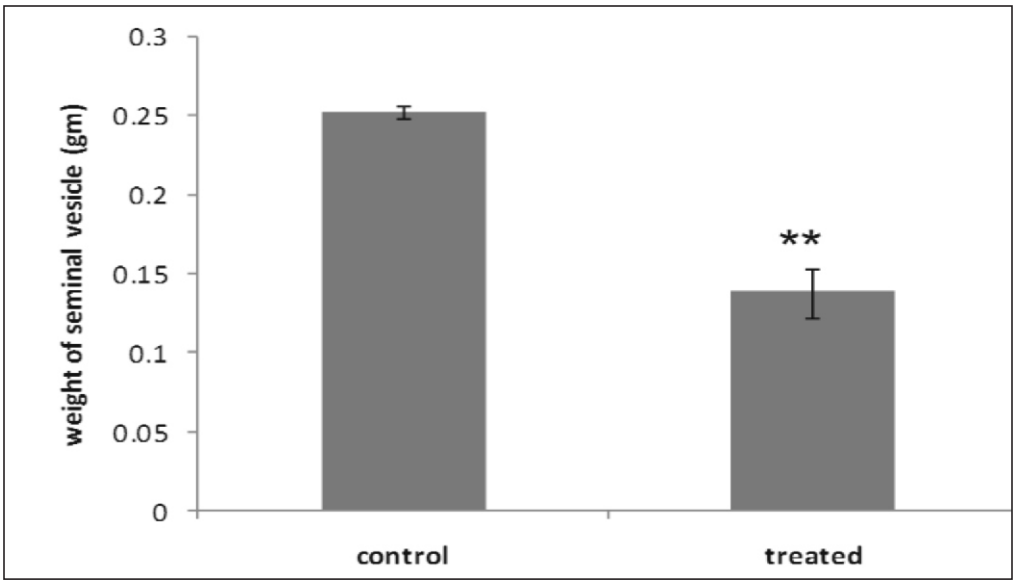

0.3
0.25
0.2
0.15
0.1
0.05
0
weight of seminal vesicle (gm)
**
control
treated

Fig. 3

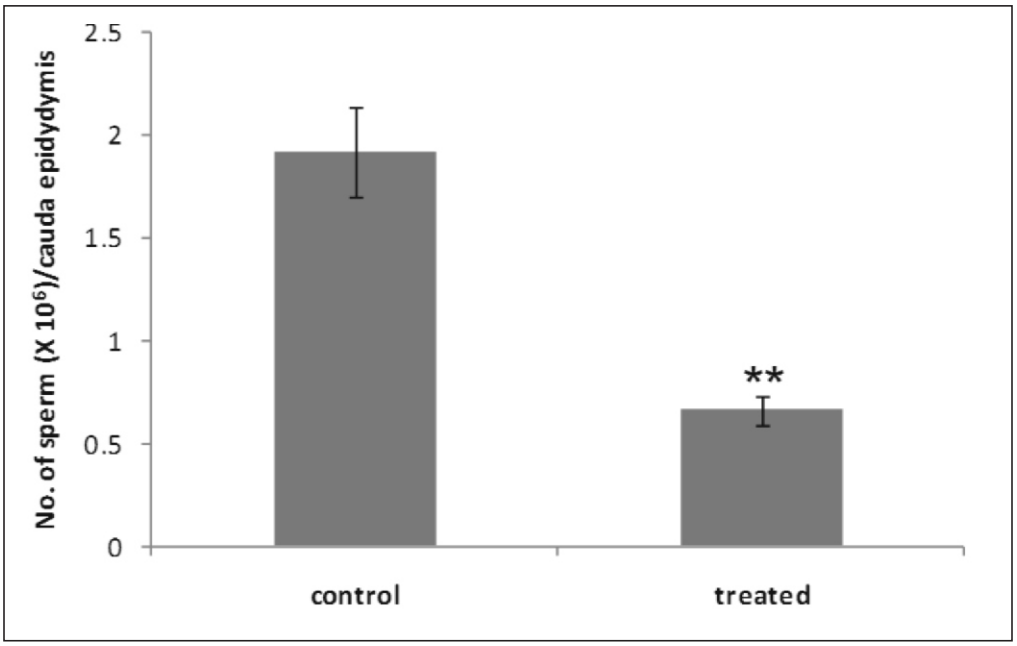

2.5
2
1.5
1
0.5
0
No. of sperm (X 10⁶)/cauda epididymis
**
control
treated

Fig. 4

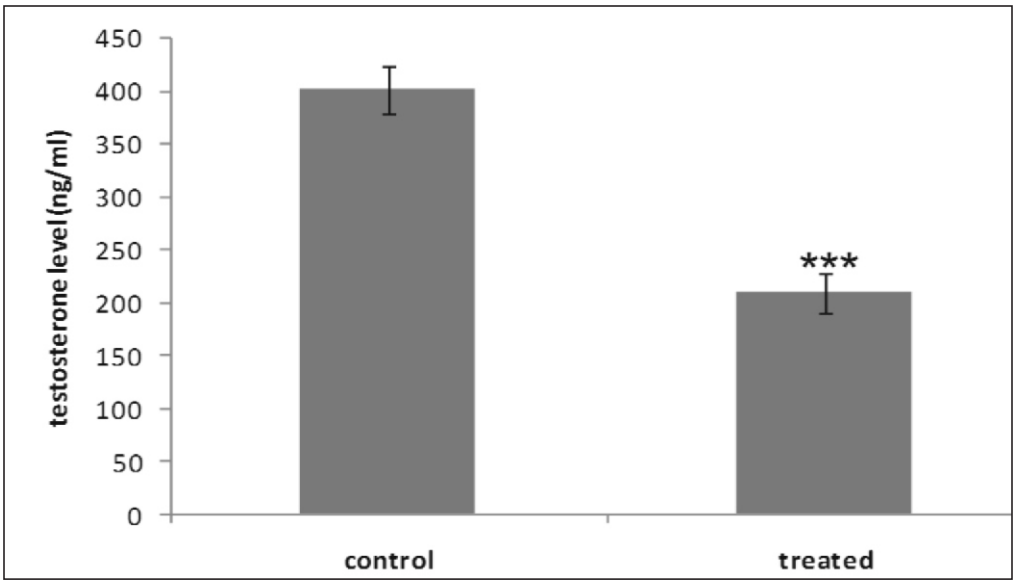
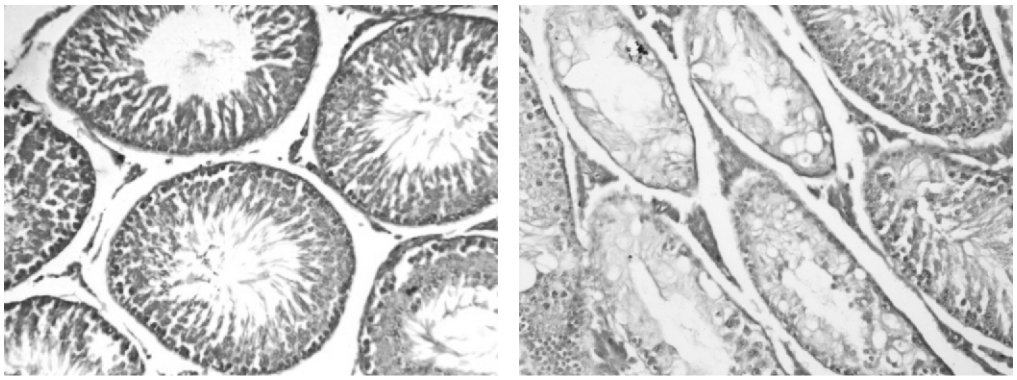

450
400
350
300
250
200
150
100
50
0
testosterone level (ng/ml)
***
control
treated

Fig. 5



(a) (b)

Fig. 6