Universidade Estadual Paulista "Júlio de Mesquita Filho"

Gabriela Missassi

EFFECTS OF SUBCHRONIC EXPOSURE TO SIBUTRAMINE ON REPRODUCTIVE PARAMETERS AND FERTILITY OF WISTAR ADULT MALE RATS

EFEITOS DA EXPOSIÇÃO SUBCRÔNICA À SIBUTRAMINA SOBRE PARÂMETROS REPRODUTIVOS E FERTILIDADE DE RATOS MACHOS WISTAR ADULTOS

Botucatu - SP
2016

Gabriela Missassi

EFFECTS OF SUBCHRONIC EXPOSURE TO SIBUTRAMINE ON REPRODUCTIVE PARAMETERS AND FERTILITY OF WISTAR ADULT MALE RATS

EFEITOS DA EXPOSIÇÃO SUBCRÔNICA À SIBUTRAMINA SOBRE PARÂMETROS REPRODUTIVOS E FERTILIDADE DE RATOS MACHOS WISTAR ADULTOS

Relatório final, apresentado a Universidade Estadual Paulista ”Júlio de Mesquita Filho”, como parte das exigências para a obtenção do título de Bacharel em Ciências Biológicas.

Orientadora: Profª. Drª. Wilma De Grava Kempinas

Universidade Estadual Paulista “Júlio de Mesquita Filho”, Botucatu
2016

FICHA CATALOGRÁFICA ELABORADA PELA SEÇÃO TÉC. AQUIS. TRATAMENTO DA INFORM.
DIVISÃO TÉCNICA DE BIBLIOTECA E DOCUMENTAÇÃO - CÂMPUS DE BOTUCATU - UNESP
BIBLIOTECÁRIA RESPONSÁVEL: ROSEMEIRE APARECIDA VICENTE-CRB 8/5651

Missassi, Gabriela.
Efeitos da exposição subcrônica á sibutramina sobre parametros reprodutivos e fertilidade de ratos machos Wistar adultos / Gabriela Missassi. - Botucatu, 2016

Trabalho de conclusão de curso (bacharelado - Ciências Biológicas) - Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências de Botucatu
Orientador: Wilma De Grava Kempinas
Capes: 20600003

1. Testes de toxicidade subcrônica. 2. Epidídimo. 3. Sibutramina. 4. Drogas - Efeito fisiológico. 5. Sêmen. 6. Rato como animal de laboratório.

Palavras-chave: Epidídimo; Qualidade espermática; Sibutramina.

EFFECTS OF SUBCHRONIC EXPOSURE TO SIBUTRAMINE ON REPRODUCTIVE PARAMETERS AND FERTILITY OF WISTAR ADULT MALE RATS

Gabriela Missassi[1*], Cibele S. Borges[1], Raquel F. Silva[1], Janete Anselmo-Franci[2], Wilma G. Kempinas[1].

[1]Laboratory of Biology and Toxicology of Reproduction and Development, Department of Morphology, Institute of Biosciences, UNESP–Universidade Estadual Paulista, Botucatu, São Paulo, Brazil

[2]Department of Morphology, Stomatology and Physiology, School of Dentistry, USP–Universidade de São Paulo, Ribeirão Preto, São Paulo, Brazil

Running Title: Sibutramine administration in the dark phase impairs rat sperm quality

*Corresponding author:

Gabriela Missassi

Laboratory of Reproductive and Developmental Biology and Toxicology

Department of Morphology, Institute of Biosciences of Botucatu

UNESP – Univ Estadual Paulista, 18618-970, Botucatu, SP, Brazil

Phone: +55 14 98167-4847, e-mail: gabby_missassi@hotmail.com.

ABSTRACT

Sibutramine is a non-selective serotonin-norepinephrine reuptake inhibitor largely used for weight loss. In a previous work, we showed the pharmacological mechanisms involved in the reduction on sperm quality and fertility of rats exposed to this anorexigen in the light phase of light-dark (l/d) cycle. Considering that rodents are nightlife animals, with higher metabolic activity during the dark phase, the present work aimed to further investigate whether the alterations found in rat sperm quality would be maintained after treatment in this period. For this, adult male *Wistar* rats were treated with sibutramine (10 mg/kg/day) or vehicle for 15 days in the dark phase of the light-dark cycle. Sibutramine decreased final body and reproductive organ weights, as well as the testosterone levels and number of sperm with progressive motility. These animals also had an acceleration in the sperm transit time in the epididymis and a decrease in sperm concentrations throughout this organ (histopathological analysis). In conclusion, the impairment of reproductive parameters were enhanced when the exposure occurred during the dark phase of the l/d cycle, which corresponds to more active period of these animals.

INTRODUCTION

Sibutramine is an anorexigen that acts as a non-selective inhibitor of norepinephrine reuptake (Nisoli and Carruba 2000; Choi et al. 2011; Binsaleh 2012). Its efficacy as a weight loss agent has been proven, since it reduced the incidence of mortality and morbidity related to obesity (Yen and Ewald 2012; Suplicy et al. 2014). However, because of severe side effects, such as increased blood pressure, several countries prohibited its use (James et al. 2010; Barétic 2013; Seimon et al. 2014; Krentz et al. 2016).

Currently, some studies in these countries reported the presence of sibutramine in mixed teas and natural compounds to accelerate weight loss (Zhang et al. 2013; Kim et al. 2014; Mathona et al. 2014). Hachem et al (2016) analyzed the composition of 100% natural food supplements used for weight loss and observed that almost 50% had an active pharmaceutical ingredient, among them, sibutramine.

After sibutramine intake, the drug is metabolized in the liver and forms two metabolites: primary (M1) and secondary amines (M2). These compounds mediate sibutramine activity and reach peak plasma concentrations 3-4 hours, respectively (Bae et al. 2009; Bae et al. 2011). Studies have shown that between 2-8 hours after administration of sibutramine, the animals showed a marked reduction in caloric intake due to the action that amines exert on α_1-adrenoceptor, which is important to increase energy expenditure and thermogenesis (Jackson et al. 1997).

Moreover, because male genital system receives innervation from sympathetic (adrenergic fibers) and parasympathetic fibers (cholinergic fibers), which exert distinct control functions mediated by neurotransmitters, such as norepinephrine (Purves et al. 2001), some studies also investigate the effects of this compound in the reproductive system. In a previous work, it was demonstrated that the increased availability of norepinephrine caused by

sibutramine administration impaired sperm quality and fertility in adult male rats (Bellentani et al. 2011; Borges et al. 2013).

Considering these informations and the fact that rodents, unlike humans, are nocturnal animals (Sthefan 1972; Bravo et al. 2014) and have higher metabolic activity during this period, the administration of drugs at night possibly exerts more effect in their system. Thus, the present study aimed to evaluate the effects of sibutramine on sperm quality of adult male rats treated for 15 days in the dark phase of light/dark (l/d) cycle.

MATERIAL AND METHODS

Ethics Statement

The experimental procedures were approved by the local Ethics Committee for the Use of Experimental Animals of the University of São Paulo State (protocol number 248-CEEA) and were in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health). The euthanasia was performed by decapitation, all efforts were made to minimize suffering.

Animals

Male (110 days old/ 490-540 g) *Wistar* rats were obtained from the Central Biotherium of UNESP – Univ Estadual Paulista, and maintained under controlled conditions (25°C, 30% air humidity, 12/12-h light/dark cycle) with food and water available *ad libitum*.

The male rats were randomly allocated into two groups: control, in which rats were treated by gavage with the vehicle solution (33.3% dimethylsulfoxide and 66.7% saline) in 0.5 ml/kg, and treated, in which rats were trated with sibutramine (10 mg/kg/day) in vehicle solution by gavage for 15 days.

Drugs and Solutions

Drugs were obtained from the following sources: sibutramine hydrochloride monohydrate from Deg (Jiangyin Eas, China) and dimethylsulphoxide (DMSO) from Sigma (St. Louis, MO, USA).

Sibutramine was diluted in 33.3% dimethylsulfoxide and 66.7% saline and administered once a day in 0.5ml/kg. This dose was chosen as the minimum anorectic dose in this experimental model.

Body and reproductive organ weights

The animals were weighed during the treatment and euthanized by decapitation at the end of it. The right testis, epididymis, ventral prostate, and seminal vesicle (without the coagulating gland) were removed and their weights recorded.

Hormonal measurements

Blood samples were allowed to coagulated and the serum obtained by centrifugation (2400 rpm) for 20 min at 4°C and stored at -20°C. Serum testosterone, FSH and LH levels were determined by radioimmunoassay. Testosterone levels were measured using the Coat-A-Count® assay (Diagnostics Products Corporation, Los Angeles, USA) while LH and FSH were measured using specific kits supplied by the National Institute of Arthritis, Diabetes and Kidney Diseases (NIADDK). All samples were measured within the same assay to avoid the inter-assay errors. Intra-assay variabilities were 3.4% for LH, 2.8% for FSH, and 4% for testosterone.

Sperm counts, daily sperm production, and sperm transit time through the epididymis

Homogenization-resistant testicular spermatids (stage 19 of spermiogenesis) in the testis were counted as described previously, with adaptations adopted by Fernandez et al. (2007). Briefly, the testis, was decapsulated, weighed, and soon after the collection, was homogenized in 5 ml of NaCl 0.9% containing Triton X 100 0.5%, followed by sonication for 30 s. After a 10-fold dilution, one sample was transferred to Neubauer chambers (4 fields per animal), and mature spermatids were counted. To calculate the daily sperm production (DSP), the number of spermatids at stage 19 was divided by 6.1, which is the number of days of the seminiferous cycle during which these spermatids are present in the seminiferous epithelium. In the same manner, caput/corpus and cauda epididymidis were cut into small fragments with

scissors and homogenized, and sperm counted as described for the testis. The sperm transit time through the epididymis was determined by dividing the number of sperm in each portion by DSP.

Sperm motility

The male rats were used for sperm isolation as described elsewhere. Briefly, the sperm were released from the left proximal epididymal cauda, first site where fertile sperm is encountered in the rat, by pinching the duct and collecting the sperm in 2 mL of modified human tubular fluid (HTF) medium (Irvine Scientific). Sperm motility was evaluated using an aliquot of 10μL of sperm suspensions was immediately transferred to a Makler chamber maintained at 34ºC. Using a phase-contrast microscope (400 x magnification), 100 sperm were counted and classified as Type A (mobile with progressive movement) Type B (mobile without progressive movement) and Type C (immobile).

Histological Analysis of Testis and Epididymis

The left testis and epididymis were fixed in Bouin solution for 24 h. The pieces were dehydrated in a graded ethanol series and routinely processed for embedding in paraffin, sectioned at 5 m, and subsequently stained with hematoxylin and eosin (H&E). Testis and epididymis sections were examined by light microscopy following specific guidelines for toxicological studies (Foley, 2001).

Statistical analysis

Data are presented as mean ± standard error of mean (SEM) or median and interquartil range. Student's t-test or Mann-Whitney test were used for comparison of parametric or

nonparametric variables respectively. Differences were considered significant when $p < 0.05$. The statistical analyses were performed by GraphPad InStat (version 5).

RESULTS

During treatment, the weight of the animals was monitored and from the twelfth day, until the last day of treatment the sibutramine group had a significant reduction ($p < 0.05$) in weight gain compared with the control group (Figure 1).

At the end of the experiment, the animals were weighed and killed. The absolute weight of all reproductive organs and accessory glands collected from animals exposed to sibutramine were reduced (Table 1). In addition, the hormone measurements demonstrated a significant reduction in serum testosterone levels of these animals (Figure 2), although the levels of FSH and LH were not altered.

Daily sperm production was similar in both groups (Figure 3A), however, despite the short period of treatment, sibutramine group presented lower sperm reserves that was shown in the histopathological analysis (Figure 4C - 4F) and sperm count (Figure 3B). Besides that, this groups also had an acceleration in sperm transit time through epididymal caput/corpus and cauda (Figure 3C). Regardless of these alterations, the histopathological analysis showed no changes on the structure and dynamics of spermatogenesis in the testis (Figure 4A and 4B).

The analysis of sperm motility showed an increase in the percentage of immotile sperm as well as a reduction of motile sperm in sibutramine-treated group (Figure 3D).

DISCUSSION

Pharmacotherapy designed to treat obese patients besides helping them to lose weight, significantly reduces the risk of associated morbidities like type 2 diabetes and cardiovascular disease. Despite the adverse effects associated with these medications, and the fact that many countries prohibit its use (Krentz et al. 2016), it is common to find traces of these compounds in natural food supplements and teas (Zhang et al. 2013; Kim et al. 2014; Mathona et al. 2014; Hachem et al. 2016).

Sibutramine is considered the method of choice for weight loss due to suppression of appetite through inhibition of monoaminergic neurotransmitters reuptake in the central nervous system (Araujo and Martel 2012). Thus, from the twelfth day until the last, the group treated with this drug had a significant reduction in weight gain compared to control, and this confirms its efficacy as an anoxerigen.

The assessment of absolute and relative organ weights is an important parameter to evaluate the risk of toxicity on male reproductive system (Clegg 2001). In a previous work, we showed that sibutramine reduced the weight of epididymis, ventral prostate and seminal vesicle (Borges et al. 2013). In the present study, the same results were observed but additionally, this group also had a decrease in the weight of testis. It is known that testicular development and the formation of secondary sexual characters are mediated by testosterone and for that reason, its concentration is essential to spermatogenesis and sperm production. Despite the fact that LH and FSH levels were unchanged, there was a significantly reduction in testosterone concentrations that did not alter the morphology of the testis but is possibly the cause for low testicular weight.

In the same manner, the reduced weights of prostate and seminal vesicle may have resulted from low levels of testosterone combined with the sympathomimetic effect of the drug. Studies demonstrated that sibutramine increased the contractile activity of ventral prostate

(Borges et al. 2013) and seminal vesicle (Nojimoto et al. 2009) leading to a more prominent fluid elimination and consequently decreasing their weight.

Regarding the epididymis, the low sperm reserves resulted from the acceleration in sperm transit time was possibly the cause for its weight reduction. This alteration was also confirmed once the histopathological analysis showed less sperm present in this organ. Considering the accelerated sperm transit time caused by sibutramine exposure, it might be resulted from increased contractility of this organ in the presence of this drug (Borges et al. 2013) and from low levels of testosterone (Fernandez et al. 2007).

The smooth muscle present in the epididymis cauda receives sympathetic innervation that is manly mediated by the activation of α1-adrenoreceptors (Ventura and Pennefather 1991; Ventura and Pennefather 1994; Ricker 1998; Gerendai et al. 2001). Considering that, drugs that act in the autonomic nervous system exerts influence in sperm transit time (Pholpramool et al. 1984; Billups et al. 1990; Kempinas et al. 1998). Once the passage through the epididymis has a fundamental role in the post-testicular sperm maturation and current alterations in this parameter may impair this process (Klinefelter 2002; Fernandez et al. 2007; Bellentani et al. 2011; Borges et al. 2013), the acceleration observed in the sibutramine group could be the cause for the increased percentage of immotile sperm.

In conclusion, sibutramine administered in the dark phase of circadian rhythms of rats promoted enhanced deleterious reproductive effects, as shown by reduced reproductive organ weights, lower sperm quality and quantity and decreased serum testosterone levels. More studies are needed to investigate the outcomes of longer periods of sibutramine exposure. Considering that this drug is widely used as an anorexigen, the results of the present work may have implications for human fertility.

CONFLICT OF INTEREST STATEMENT

The authors declare that there are no conflicts of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Ruither de Oliveira Gomes Carolino of the Department of Morphology, Stomatology and Physiology, Dental School of Ribeirão Preto, University of São Paulo – USP, for the collaboration with the hormonal assays, to Dr. Adriana Lúcia Mendes endocrinologist of the Department of Clinical Medicine, Medicine School of Botucatu, São Paulo State University – UNESP, for the collaboration with the sibutramine prescription, and to José Eduardo Bozano, assisting academic support, of the Department of Morphology, Institute of Biosciences, São Paulo State University – UNESP, for the collaboration and assistance throughout the project.

REFERENCES

Araujo, J.R. and Martel, F. 2012. Sibutramine effects on central mechanisms regulating energy homeostasis. *Curr Neuropharmacol.* 10: 49-52.

Bae, J.W., Choi, C.I., Jang, C.G. and Lee, S.Y. 2011. Simultaneous determination of sibutramine and its active metabolites in human plasma by LC-MS/MS and its application to a pharmacokinetic study. *Biomed Chromatogr.* 25: 1181–1188.

Bae, K., Noh, K., Jang, K., Kim, S., Yong, C.S., Choi, H.G., Kang, J.S., Chen, J., Ma, E., Lee, M., Shin, B.S., Kwon, K. and Kang, W. 2009. Analysis of enantiomers of sibutramine and its metabolites in rat plasma by liquid chromatography–mass spectrometry using a chiral stationary-phase column. *J Pharm Biomed Anal.* 50: 267–270.

Barétic, M. 2013. Obesity drug therapy. *Minerva Endocrinol.* 38(3): 245-254.

Bellentani, F.F., Fernandes, G.S., Perobelli, J.E., Pacini, E.S., Kiguti, L.R., Pupo, A.S. and Kempinas, W.D. 2011. Acceleration of sperm transit time and reduction of sperm reserves in the epididymis of rats exposed to sibutramine. *J Androl.* 32: 718-724.

Billups, K.L., Tillman, S. and Chang, T.S. 1990. Ablation of the inferior mesenteric plexus in the rat: alteration of sperm storage in the epididymis and vas deferens. *J Urol.* 143: 625-629.

Binsaleh, S. 2012. Sibutramine for the treatment of recurrent high-flow priapism. *J Androl.* 33: 566-569.

Borges, C.S., Missassi, G., Pacini, E.S.A., Kiguti, L.R.A., Sanabria, M., Silva, R.F., Banzato, T.P., Perobelli, J.E., Pupo, A.S. and Kempinas, W.G. 2013. Slimmer or Fertile? Pharmacological Mechanisms Involved in Reduced Sperm Quality and Fertility in Rats Exposed to the Anorexigen Sibutramine. *Plos One* 8.

Bravo, R., Cubero, J., Franco, L., Mesa, M., Gala´n, C., Rodriguez, A.B., Jarne, C. and Barriga, C. 2014. Body weight gain in rats by a high-fat diet produces chronodisruption in activity/inactivity circadian rhythm. *Chronobiol Int.* 31(3): 363–370.

Choi, J.W., Joo, J.I., Kim, D.H., Wang, X., Oh, T.S., Choi, D.K. and Yun, J.W. 2011. Proteome changes in rat plasma in response to sibutramine. *Proteomics*. 11: 1300-1312.

Clegg, E.D., Perreault, D., and Klinefelter, G. R. 2001. Assessment of male reproductive toxicity. In *Principles and Methods of Toxicology*, 4th ed., ed. A. W. Hayes, 263-300. Philadelphia, PA: Taylor & Francis.

Fernandez, C.D., Porto, E.M., Arena, A.C. and Kempinas Wde, G. 2008. Effects of altered epididymal sperm transit time on sperm quality. *Int J Androl.* 31: 427-437.

Foley, G.L. 2001. Overview of male reproductive pathology. *Toxicol Pathol.* 29: 49-63.

Gerendai, I., Toth, I.E., Kocsis, K., Boldogkoi, Z., Rusvai, M. and Halasz, B. 2001. Identification of CNS neurons involved in the innervation of the epididymis: a viral transneuronal tracing study. *Auton Neurosci.* 92: 1-10.

Gerendai, I., Toth, I.E., Kocsis, K., Boldogkoi, Z., Rusvai, M. and Halasz, B. 2001. Identification of CNS neurons involved in the innervation of the epididymis: a viral transneuronal tracing study. *Auton Neurosci* 92: 1-10.

Hachem, R., Assemat, G., Martins, N., Balayssac, S., Gilard, V., Martino, R. and Malet-Martino, M. 2016. Proton NMR for detection, identification and quantification of adulterants in 160 herbal food supplements marketed for weight loss. *J Pharm Biomed Anal.* 124: 34-37.

Jackson, H.C., Bearham, M.C., Hutchins, L.J., Mazurkiewicz, S.E., Needham, A.M. and Heal. D.J. 1997. Investigation of the mechanisms underlying the hypophagic effects of the 5-HT and noradrenaline reuptake inhibitor, sibutramine, in the rat. *Br J Pharmacol*. 121: 1613-1618.

James, W.P., Caterson, I.D., Coutinho, W., Finer, N., Van Gaal. L.F., Maggioni, A.P., Torp-Pedersen, C., Sharma, A.M., Shepherd, G.M., Rode, R.A. and Renz, C.L. 2010. Effect of sibutramine on cardiovascular outcomes in overweight and obese subjects. *N Engl J Med.* 363: 905-917.

Kempinas, W.D., Suarez, J.D., Roberts, N.L., Strader, L., Ferrell, J., Goldman, J.M. and Klinefelter, G.R. 1998. Rat epididymal sperm quantity, quality, and transit time after guanethidine-induced sympathectomy. *Biol Reprod.* 59: 890-896.

Kim, H.J., Lee, J.H., Park, H.J., Cho, S.H., Cho, S. and Kim, W.S. 2014. Monitoring of 29 weight loss compounds in foods and dietary supplements by LC-MS/MS. *Food Additives & Contaminants: Part A.*

Klinefelter, G. R. 2002. Actions of toxicants on the structure and function of the epididymis. In The epididymis—From molecules to clinical practice, ed. B. Robaire and B. T. Hinton, 353–69. New York, NY: Kluwer Academic.

Krentz, A.J., Fujioka, K. and Hompesch, M. 2016. Evolution of Pharmacological Obesity Treatments: Focus on Adverse Side-Effects Profiles. *Diabetes Obes Metab*.

Mathona, C., Ankli, A., Reich, E., Bieri, S. and Christen, P. 2014. Screening and determination of sibutramine in adulterated herbal slimming supplements by HPTLC-UV densitometry. *Food Additives & Contaminants: Part A*. 31(1): 15-20.

Nisoli, E. and Carruba, M.O. 2000. An assessment of the safety and efficacy of sibutramine, an anti-obesity drug with a novel mechanism of action. *Obes Ver*. 1: 127-139.

Nojimoto, F.D., Piffer, R.C., Kiguti, L.R., Lameu, C., de Camargo, A.C., Pereira, O.C. and Pupo, A.S. 2009. Multiple effects of sibutramine on ejaculation and on vas deferens and seminal vesicle contractility. *Toxicol Appl Pharmacol.* 239: 233-240.

Pholpramool, C. and Triphrom, N. 1984. Effects of cholinergic and adrenergic drugs on intraluminal pressures and contractility of the rat testis and epididymis in vivo. *J Reprod Fertil.* 71: 181-188.

Purves, D.A., G.J.; Fitzpatrick, D. et al.., editors. 2001. Autonomic Regulation of Sexual Function. In: D.A. Purves, G.J.; Fitzpatrick, D. et al.., editors. (Ed), Neuroscience, Sunderland (MA).

Ricker, D.D. 1998. The autonomic innervation of the epididymis: its effects on epididymal function and fertility. *J Androl.* 19: 1-4.

Seimon, R.V., Espinoza, D., Ivers, L., Gebski, V., Finer, N., Legler, U.F. Sharma, A.M., James, W.P.T., Coutinho, W. and Caterson, I.D. 2014. Changes in body weight and blood pressure: paradoxical outcome events in overweight and obese subjects with cardiovascular disease. *Int J Obesity*. 1–7.

Stephan, F.K. 1972. Circadian Rhythms in the Rat: Constant Darkness Entrainment to T Cycles and to Skeleton Photoperiod. *Physiol Behav.* 30: 451- 462.

Suplicy, H., Boguszewski, C.L., Santos, C.M.C., Desterro de Figueiredo, M., Cunha, D.R. and Radominski, R. 2014. A comparative study of five centrally acting drugs on the pharmacological treatment of obesity. *Int J Obesity*.1–7.

Ventura, S. and Pennefather, J.N. 1991. Sympathetic co-transmission to the cauda epididymis of the rat: characterization of postjunctional adrenoceptors and purinoceptors. *Br J Pharmacol.* 102: 540-544.

Ventura, S. and Pennefather, J.N. 1994. Alpha 2-adrenoceptor binding sites vary along the length of the male reproductive tract: a possible basis for the regional variation in response to field stimulation. *Eur J Pharmacol.* 254: 167-173.

Yen, M. and Ewald, M.B. 2012. Toxicity of Weight Loss Agents. *J Med Toxicol.* 8: 145-152.

Zhang, Y., Huang, X., Liu, W., Cheng, Z., Chen, C. and Yin, L. 2013. Analyses of drug Illegally added into Chinese traditional patent medicine using surface-enhanced raman scattering. *Anal Sci.* 29: 985-990.

FIGURE LEGENDS

Figure 1. Evolution of body weight gain during the treatment with sibutramine or vehicle. The data are expressed as the mean ± SEM. * $p < 0.05$ (Student's t-test).

Figure 2. FSH (folicle-stimulating hormone), LH (Luteinizing hormone) and T (testosterone). The data are expressed as the mean ± SEM. * $p < 0.05$ (Student's t- test).

Figure 3. A: Sperm number in the testis of the control and sibutramine-treated groups. **B:** Sperm reserves in caput/corpus and cauda of the control and sibutramine-treated groups. **C:** Sperm transit time in caput/corpus and cauda of the control and sibutramine-treated groups. **A-C:** The data are expressed as the mean ± SEM. * $p < 0.05$, ** $p < 0.01$ (Student's t- test). **D:** Sperm motility with Type A (mobile with progression; $p < 0.05$) Type B (mobile without progression) and Type C (immobile; $p < 0.05$). The data are expressed as median. * $p < 0.05$ (Mann- Whitney test).

Figure 4. Histological aspect of testis from control (a), and sibutramine group (b), the epididymal caput and cauda from animals of control (c,e) and sibutramine (d,f) group (n=5/ group). GC: germ cells; L: lumen; In: interstitial tissue; Spz: spermatozoa; Ep: epithelium. Final magnification: 200x.

Figure 1.

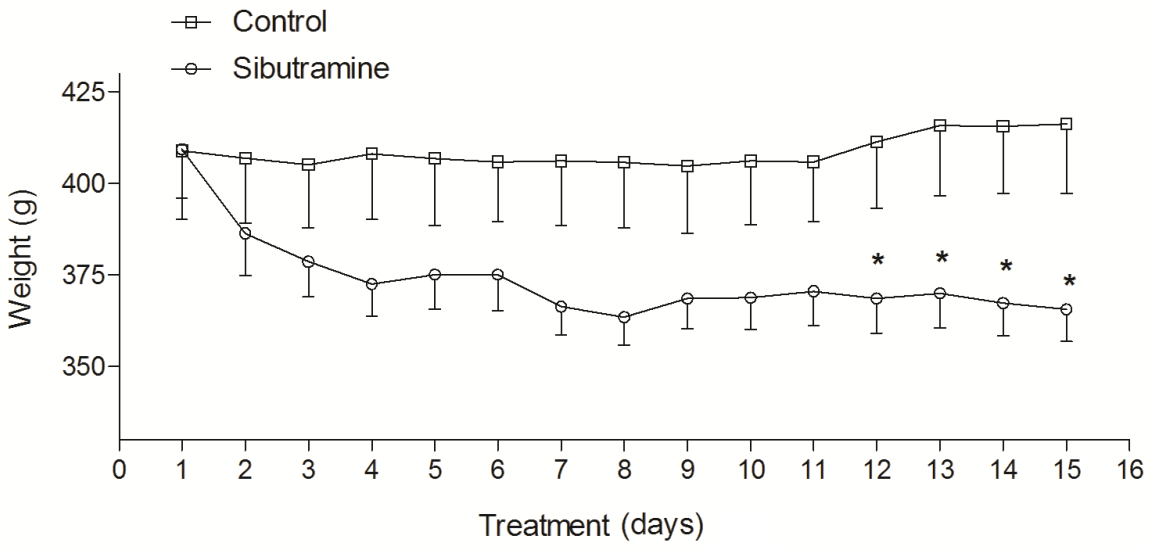
Control
Sibutramine
Weight (g)
425
400
375
350
0
1
2
3
4
5
6
7
8
9
10
11
12
13
14
15
16
Treatment (days)
*
*
*
*

Figure 2.

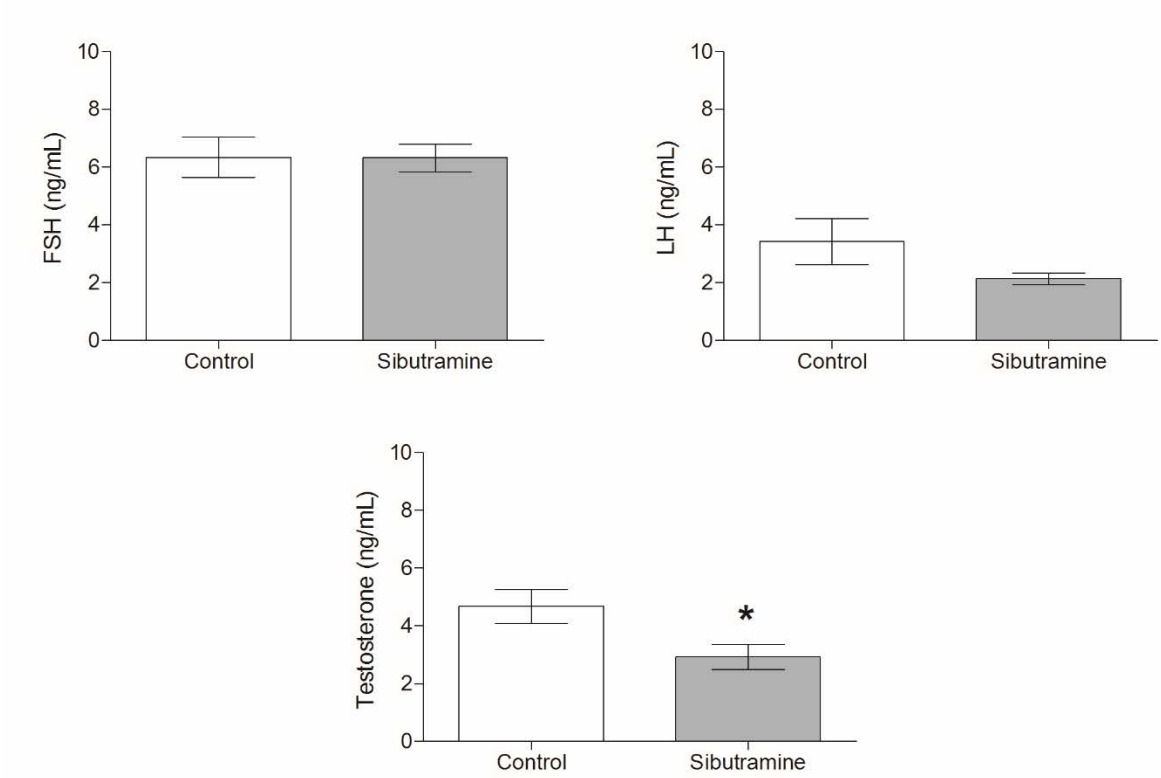
FSH (ng/mL)
10
8
6
4
2
0
Control
Sibutramine
LH (ng/mL)
10
8
6
4
2
0
Control
Sibutramine
Testosterone (ng/mL)
10
8
6
4
2
0
*
Control
Sibutramine

Figure 3.

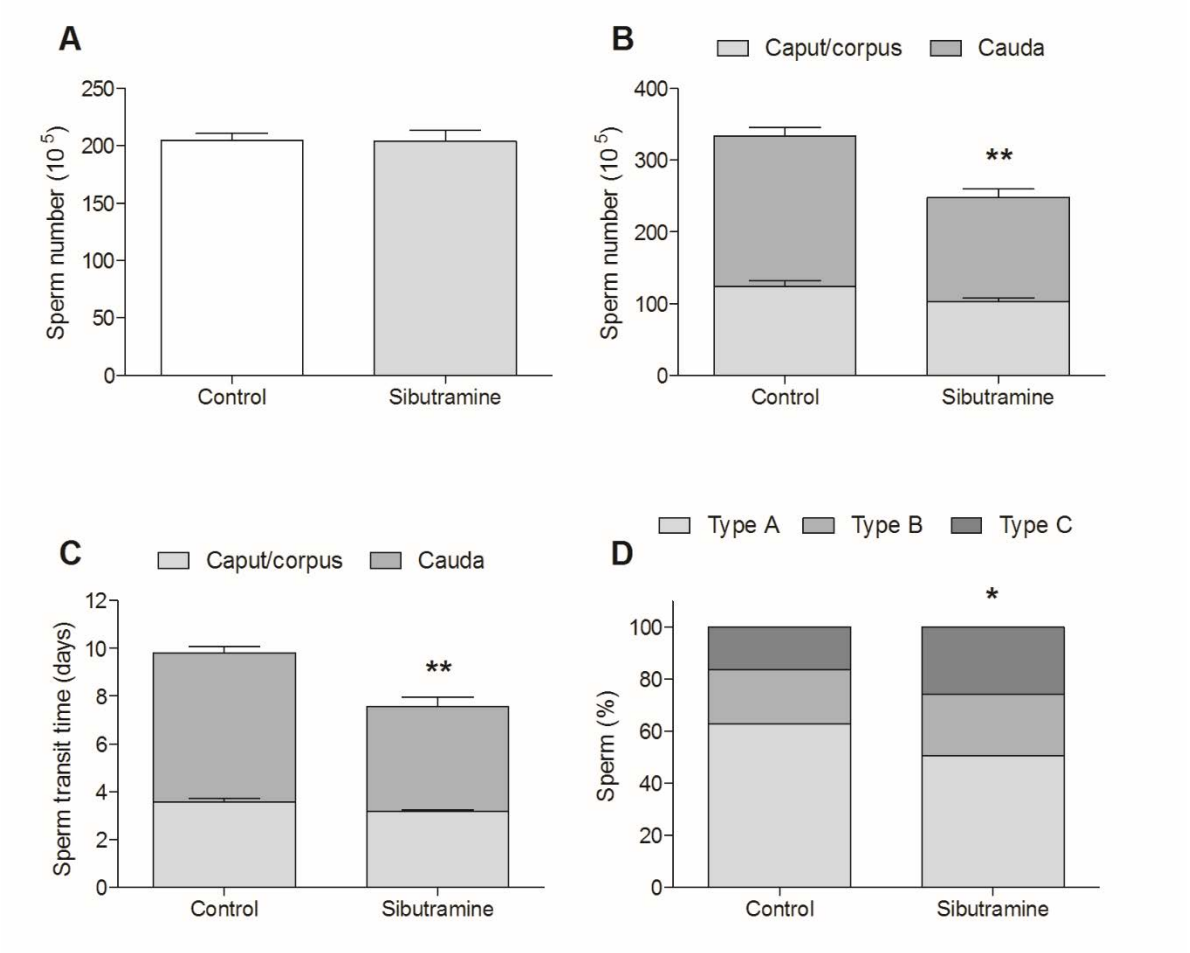
A
Sperm number (10^5)
250
200
150
100
50
0
Control
Sibutramine
B
Caput/corpus
Cauda
Sperm number (10^5)
400
300
200
100
0
**
Control
Sibutramine
C
Caput/corpus
Cauda
Sperm transit time (days)
12
10
8
6
4
2
0
**
Control
Sibutramine
D
Type A
Type B
Type C
Sperm (%)
100
80
60
40
20
0
*
Control
Sibutramine

Figure 4.

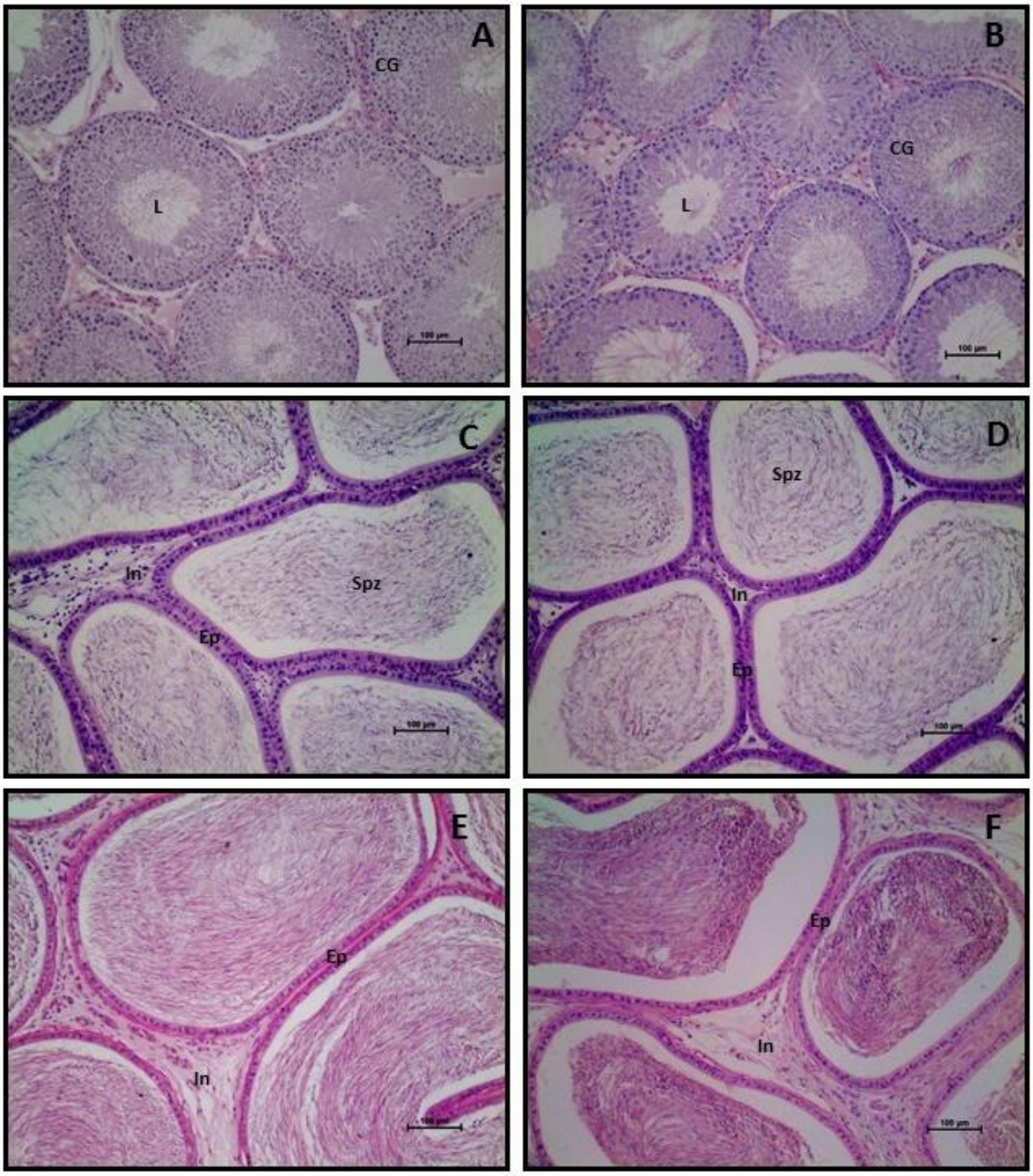

Table 1.

Table 1 - Body and reproductive organ weights.

Parameters	Control (n=8)	Sibutramine (n=8)
Final body weight (g)	416.30 ± 19.06	365.60 ± 8.78*
Testis (g)	1.72 ± 0.05	1.56 ± 0.03*
Testis (g/100g)	0.42 ± 0.01	0.42 ± 0.01
Epididymis (mg)	683.20 ± 30.31	585.10 ± 14.49*
Epididymis (mg/100g)	165.6 ± 7.12	159.1 ± 5.43
Ventral prostate (mg)	527.40 ± 37.37	333.50 ± 16.27*
Ventral prostate (mg/100g)	128.4 ± 10.63	90.89 ± 5.63*
Seminal vesicle (g)	1.45 ± 0.09	0.97 ± 0.07*
Seminal vesicle (g/100g)	0.36 ± 0.28	0.26 ± 0.02*

Values expressed as mean ± S.E.M. * $p<0.05$ (Student's t-test).