

# EFEITOS DA SIBUTRAMINA SOBRE O SISTEMA REPRODUTOR MASCULINO DE RATOS

**FERNANDA FURLANETTO BELLENTANI**

Dissertação apresentada ao Instituto de Biociências, Campus de Botucatu, UNESP, para obtenção do título de Mestre no Programa de Pós-Graduação em Biologia Geral e Aplicada, Área de concentração Biologia Celular Estrutural e Funcional.

*Wilma De Grava Kempinas*

**BOTUCATU – SP  
2011**



UNIVERSIDADE ESTADUAL PAULISTA  
"JÚLIO DE MESQUITA FILHO"  
Campus de Botucatu



UNIVERSIDADE ESTADUAL PAULISTA

"Julio de Mesquita Filho"

INSTITUTO DE BIOCÊNCIAS DE BOTUCATU

EFEITOS DA SIBUTRAMINA SOBRE O SISTEMA REPRODUTOR  
MASCULINO DE RATOS

**FERNANDA FURLANETTO BELLENTANI**

**WILMA DE GRAVA KEMPINAS**

Dissertação apresentada ao Instituto de Biociências,  
Campus de Botucatu, UNESP, para obtenção do título  
de Mestre no Programa de Pós-Graduação em Biologia  
Geral e Aplicada, Área de concentração Biologia Celular  
Estrutural e Funcional.

*Wilma De Grava Kempinas*

**BOTUCATU – SP  
2011**

Ficha catalográfica elaborada pela Seção Técnica de Aquisição e Tratamento da Informação  
Divisão Técnica de Biblioteca e Documentação - Campus De Botucatu - UNESP  
Bibliotecária responsável: *Sulamita Selma Clemente Colnago* – CRB 8/4716

Bellentani, Fernanda Furlanetto.

Efeitos da sibutramina sobre o sistema reprodutor masculino de ratos /  
Fernanda Furlanetto Bellentani. - Botucatu, 2011

Dissertação (mestrado) - Instituto de Biociências de Botucatu,  
Universidade Estadual Paulista, 2011

Orientador: Wilma De Grava Kempinas

Capes: 20600003

1. Reprodução - Efeito dos produtos químicos - Estudos experimentais.

Palavras-chave: Epidídimo; Espermatozóides; Ratos Machos; Reprodução;  
Sibutramina

*Esse trabalho é dedicado a uma grande amiga, Glaura, que esteve  
presente em cada desafio e em cada sucesso.*

## **AGRADECIMENTOS**

*Aos meus pais, por apoiarem todos meus sonhos, por não me deixarem desistir e pelo amor incondicional.*

*Á minha amiga Glaura, por toda ajuda, tempo e paciência dispensados comigo. Por ser um exemplo de amizade, lealdade e capacidade.*

*À minha irmã Camila, pela presença certa nos momentos em que mais necessito e por sua franqueza.*

*Á minha querida amiga Débora, pela paciência e companhia indispensáveis na minha vida.*

*Aos meus eternos amigos, Vinícius, Danielle e Maria Fernanda, por acreditarem na força da amizade e por serem pessoas com as quais sempre poderei contar.*

*Á minha Orientadora, Prof.<sup>a</sup> Wilma, por acreditar em mim e no meu projeto.*

*Á Ana Luisa e Juliana, pelos inúmeros favores que fizeram sem hesitar.*

*À bela equipe do REPROTOX, pelo auxílio na realização do meu projeto, pelos conhecimentos que me transferiram e pela ótima convivência.*

*Aos meus amigos de Botucatu, pelo carinho e por tornarem meus dias mais leves, alegres e inesquecíveis.*

*Ao técnico José Ricardo, não só pela ajuda com o processamento dos materiais, mas também por ser sempre tão prestativo e amigável.*

*Ao amigo e Prof. Wellerson, pela oportunidade que me deu de sentir o que é ensinar e principalmente aprender.*

*A todos os alunos dos quais fui monitora, por despertar em mim a vontade de ser professora.*

*A secretária do departamento de morfologia, Luciana, por toda ajuda e atenção.*

*Aos funcionários da pós-graduação por toda ajuda.*

*Aos ratos Wistar, sem os quais a realização desse trabalho não seria possível.*

*A todos que de alguma forma estiveram envolvidos com esse trabalho e que permitiram assim, que um uma simples idéia se transformasse em um grande projeto.*

*E a Deus, por eu ser pessoa cheia de esperanças e sonhos. Por me dar uma família, amigos e mestres tão maravilhosos. Por todas as oportunidades que surgiram e surgem em minha vida!*

## ***EPÍGRAFE***

*“A mente humana é um grande teatro. Seu lugar não é na platéia, mas no palco, brilhando na sua inteligência, alegrando-se com suas vitórias, aprendendo com as suas derrotas e treinando para ser a cada dia, autor da sua história, líder se si mesmo!”*

*(Augusto Cury)*

## ***SUMÁRIO***

	Página
<b>RESUMO.....</b>	07
<b>ABSTRACT.....</b>	08
<b>INTRODUÇÃO.....</b>	09
<b>CAPÍTULO.....</b>	16
Title.....	17
Abstract.....	18
Introduction.....	19
Methods.....	20
Results.....	25
Discussion.....	27
References.....	29
Tables.....	32
Figure Legends.....	37
Figures.....	38
<b>CONCLUSÃO.....</b>	40
<b>REFÊRENCIAS BIBLIOGRÁFICAS DA INTRODUÇÃO.....</b>	41
<b>APÊNDICES.....</b>	47

## RESUMO

A sibutramina é uma droga usada mundialmente para o tratamento da obesidade. Poucos trabalhos reportam seus possíveis efeitos sobre a reprodução masculina. Dessa forma, o objetivo deste trabalho foi investigar os efeitos da sibutramina sobre a função reprodutiva de ratos machos adultos. No experimento 1, ratos da variedade Wistar foram divididos em 2 grupos: tratado com sibutramina (10 mg/Kg/dia) e controle que recebeu apenas os veículos (dimetilsulfóxido e salina). Após 28 dias de tratamento, os animais foram pesados e mortos e os pesos de seus órgãos reprodutores aferidos. Os testículos e epidídimos direitos foram utilizados para contagens espermáticas e os esquerdos para análise histopatológica. Espermatozóides foram coletados dos ductos deferentes direitos e esquerdos para análises da motilidade e da morfologia espermática, respectivamente. Foram também dosadas testosterona, hormônio folículo-estimulante (FSH) e hormônio luteinizante (LH) séricos. No experimento 2, outro lote de ratos, seguindo o mesmo delineamento, foi testado quanto ao comportamento sexual e fertilidade após acasalamentos naturais com fêmeas não tratadas. No experimento 3 ratos adultos foram utilizados para ensaios *in vitro* de reatividade farmacológica da região distal do ducto epididimário isolado, com e sem a presença de sibutramina. No final dos experimentos os pesos corpóreos dos animais não diferiram entre os grupos. Os pesos dos epidídimos e próstatas ventrais foram estatisticamente menores no grupo tratado em relação ao grupo controle, porém os pesos dos demais órgãos reprodutores foram semelhantes entre os grupos. A produção espermática diária, a morfologia e a motilidade de espermatozóides foram estatisticamente semelhantes entre os grupos experimentais. Quanto aos epidídimos, tanto o número de espermatozóides quanto o tempo de trânsito espermático foram diminuídos na região da cauda no grupo tratado. O comportamento sexual e a fertilidade mantiveram-se semelhantes entre os grupos. Os ensaios farmacológicos revelaram que a sibutramina promoveu dois efeitos distintos sobre o ducto epididimário: aumento da potência da noradrenalina e, por si só, aumento da atividade mecânica. Conclui-se que embora a sibutramina, nestas condições experimentais, não tenha interferido no processo reprodutivo de ratos, provocou aceleração do tempo de trânsito na cauda do epidídimo e diminuição das reservas espermáticas. Essa alteração provavelmente relaciona-se ao efeito simpatomimético da sibutramina, conforme demonstrado pelos ensaios *in vitro*. Na espécie humana esse fato pode representar um impacto sobre a fertilidade masculina, uma vez que as reservas espermáticas no homem são naturalmente menores do que no rato.

## ABSTRACT

Sibutramine is a drug globally used for the treatment of obesity. Few studies have reported its possible effects on male reproductive function. Therefore, the objective of this study was to investigate the effects of sibutramine on the reproductive function of adult male rats. In experiment 1, Wistar male rats were divided into two groups: treated with sibutramine (10 mg/kg/day) and control who received only the vehicles (dimethyl sulfoxide and saline). After 28 days of treatment the animals were weighed and killed and their sex organs were weighed. The right testes and epididymis were used for enumeration of sperm numbers and the left for histopathological analysis. Sperm were collected from right and left vas deferens to analyze motility and sperm morphology respectively. Serum testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were also measured. In experiment 2 another batch of rats, following the same design, was tested for sexual behavior and fertility after natural mating. In experiment 3 adult male rats were used for *in vitro* assays of pharmacological reactivity of the isolated distal epididymal duct, with and without sibutramine. At the end of experiments, the body weights of the animals did not differ between groups. The absolute weights of epididymis and prostates were significantly decreased in the treated group, but the weights of other reproductive organs were similar between groups. The daily sperm production, the sperm morphology and motility were similar in both groups. The hormone levels also were similar between groups. In the epididymis, both sperm number and epididymal transit time were decreased in the cauda area in the treated group. The sexual behavior and the fertility remained similar between groups. Pharmacological assays showed that sibutramine provoked two distinct effects in the rat distal cauda epididymis: increase in the potency of norepinephrine and, per se, increase of the mechanical activity. Thus, while sibutramine in these experimental conditions did not interfere with the reproductive process of rats, provoked acceleration of the sperm transit time in the epididymal cauda and decrease in the sperm reserves. This alteration is probably related to the sympathomimetic effect of this drug, as shown by the *in vitro* assays. In humans this fact may represent a threat for male fertility since sperm reserves in men are naturally lower compared to rats.

## **INTRODUÇÃO**

### **I. Sibutramina: função e eficácia**

A obesidade está se tornando uma epidemia mundial, contribuindo para o aumento da incidência de problemas cardiovasculares, diabetes e hipertensão (Tan et al., 2005). Frequentemente, a obesidade é definida simplesmente como uma condição de acúmulo excessivo de gordura no tecido adiposo (WHO, 2000).

Muitos pacientes têm dificuldade de perder peso e mantê-lo, especialmente quando o perdem apenas através de dietas e exercícios físicos (Wadden et al., 2005). Contudo, o tratamento médico inclui somente alguns tipos de drogas (Caterson, 1999) como, por exemplo, a sibutramina, recentemente utilizada para o tratamento da obesidade em muitos países. Nos anos 80 esse medicamento foi inicialmente desenvolvido como um antidepressivo, mas descobriu-se que ele também induzia uma importante perda de peso, afetando tanto a ingestão de alimento como o gasto de energia (Buckett et al., 1988).

Normalmente os estudos têm verificado, em humanos, perda de peso significativa com o uso de sibutramina. Cuellar et al. (2000) utilizaram 15 mg/dia de sibutramina em pacientes obesos durante seis meses e, obtiveram perda de 10,27 Kg na média do peso corporal do grupo tratado com a droga enquanto no grupo controle houve a perda de apenas 1,26 Kg. Em outro estudo, constataram a perda de mais de 5% do peso inicial em 77% dos pacientes obesos tratados durante 6 meses com 10 mg/dia de sibutramina. Além disso, a maioria dos pacientes que mantiveram o tratamento por 2 anos, apresentou sustentação da perda de peso inicial (James et al., 2000).

### **II. Mecanismo de ação da sibutramina**

Atualmente, a função esperada da sibutramina é a perda de peso, conseguida através da inibição da recaptção de noradrenalina e de serotonina (Stock, 1997; Hansen et al., 1999).

A noradrenalina é uma catecolamina liberada pelas supra-renais e pelas terminações nervosas simpáticas que tem como principais efeitos: elevação do metabolismo, aumento da frequência cardíaca e da pressão arterial. Ao inibir a recaptção da noradrenalina na fenda sináptica, a sibutramina potencializa os efeitos deste hormônio, aumentando o gasto energético pelo organismo (Hansen et al., 1998; Walsh et al., 1999).

A serotonina é produzida a partir do aminoácido triptofano em células cromafins e neurônios. Funciona tanto como um neurotransmissor como um hormônio local (no sistema vascular periférico), promovendo vasoconstrição, estimulação da musculatura lisa, regulação do sono, do apetite e do humor (Rang et al., 2007). A sibutramina, ao inibir a recaptação da serotonina, aumenta sua concentração no sistema nervoso central, diminuindo a ansiedade e aumentando a saciedade (Chapelot et al., 2000).

Portanto, a sibutramina atua através de dois mecanismos: aumento do gasto energético e diminuição do apetite. Ela também exerce suas ações farmacológicas via dois metabólitos ativos: o metabólito 1 (uma amina secundária) e o metabólito 2 (uma amina primária). Portanto, não só a sibutramina como seus metabólitos ativos inibem a recaptação de noradrenalina e serotonina. Este fato diferencia claramente a sibutramina de outras substâncias que levam a perda de peso, como a D-fenfluramina (na qual há predomínio da liberação de 5-HT) e a D-anfetamina (predominantemente um liberador de dopamina e noradrenalina) (Buckett et al., 1988; Finer, 2002).

### **III. Efeitos colaterais da sibutramina**

Os efeitos colaterais da sibutramina mais comumente encontrados nos estudos em humanos foram: secura de boca, pressão alta, fadiga, constipação, taquicardia, anorexia, dores de cabeça e insônia (Luque and Rey, 1999; Richter, 1999; Cuellar et al., 2000; Fanghanel et al., 2000; James et al., 2000).

Com relação à reprodução, recentemente foi observado que a sibutramina, administrada em ratas prenhes, causou uma diminuição no número de fetos vivos e aumentou a porcentagem de perdas pós-implantação (Francia-Farje et al., 2010).

Por outro lado, também foram relatados efeitos benéficos, como a diminuição da hiperlipidemia, auxílio no controle glicêmico em pacientes com *Diabetes Mellitus* tipo 2 (Fujioka et al., 2000; McMahon et al., 2000), diminuição da gravidade da apnéia obstrutiva do sono e redução de alguns sintomas da síndrome do ovário policístico, como sobrepeso e resistência a insulina (Tziomalos et al., 2009).

Um estudo denominado *Sibutramine Cardiovascular Outcome Trial* (SCOUT) foi desenhado para avaliar prospectivamente a eficácia/segurança da sibutramina em uma população sobrepeso/obesidade de alto risco (com doença cardiovascular, hipertensão e/ou *Diabetes Mellitus* tipo 2). Os resultados das primeiras 6 semanas, em período aberto de tratamento com sibutramina (10 mg/dia), não demonstraram efeitos colaterais alarmantes. No

entanto, os resultados finais do SCOUT mostraram que em longo prazo (5 anos) o tratamento com sibutramina (10-15 mg/dia) expôs indivíduos com doença cardiovascular anteriormente relatadas a um risco significativamente aumentado de infarto de miocárdio e de acidente vascular cerebral (AVC) não-fatais (Scheen, 2010).

Uma vez que o benefício da sibutramina, como uma ajuda para perda de peso, aparentemente não compensa os riscos cardiovasculares, a *European Medicines Agency* (EMA) recomendou a suspensão da comercialização de sibutramina em toda a União Européia (Scheen, 2010). A *Food and Drug Administration* (FDA) dos EUA afirmou que o medicamento deve levar um alerta na caixa devido a um aumento do risco de AVC e ataque cardíaco em pacientes com histórico de doença cardiovascular. No Brasil, a Agência Nacional de Vigilância Sanitária (ANVISA) publicou uma resolução (RDC 13/2010) que remaneja a substância da lista C1 (outras substâncias sujeitas a controle especial) para a lista B2 (substâncias psicotrópicas anorexígenas) dos medicamentos sujeitos a controle especial (ANVISA, 2010).

#### **IV. Morfofisiologia do sistema reprodutor masculino**

O sistema reprodutor masculino da maioria dos mamíferos é composto por testículos (gônadas), epidídimos, ductos deferentes, glândulas sexuais acessórias (como próstata e vesícula seminal), e pênis.

Esse sistema é andrógeno-dependente e qualquer tóxico que atue desordenando a interação coordenada do eixo hipotalâmico hipofisário gonadal pode levar a anormalidades reprodutivas (Sokol, 1997).

##### **IV.I - Testículo**

Cada testículo pode ser, funcionalmente e estruturalmente, dividido em duas partes: tecido intersticial e túbulos seminíferos, responsáveis pela esteroidogênese e pela espermatogênese, respectivamente (Rodrigues and Favaretto, 1999). A espermatogênese é um processo elaborado pelo qual células-tronco espermatogoniais tornam-se células haplóides altamente diferenciadas e especializadas, os espermatozóides (Clermont, 1972). O tecido intersticial está localizado entre os túbulos seminíferos e é composto por vasos sanguíneos e linfáticos, nervos e as células intersticiais ou de Leydig responsáveis pela produção de testosterona (Russell et al., 1993).

A gônada masculina é inervada pelos nervos espermáticos superiores e inferiores, do sistema nervoso autônomo. Os nervos espermáticos superiores se originam do gânglio mesentérico superior, enquanto que os nervos espermáticos inferiores originam-se do gânglio mesentérico inferior (Sosa et al., 2009). Estudos sugerem que a inervação testicular é um fator importante para a sobrevivência de células de Leydig *in vivo* (Gong et al., 2009).

Portanto, não só o eixo clássico (hipotalâmico hipofisário gonadal), mas também o sistema nervoso periférico está envolvido nas funções exercidas pelos testículos.

#### **IV.II - Epidídimo**

A função primária do epidídimo é o transporte dos espermatozóides que chegam do testículo (Orgebin-Crist, 1969; Brooks, 1983). Além do transporte, o epidídimo é responsável pela estocagem, proteção, concentração e maturação dos gametas (Cosentino and Cokett, 1986; Hermo and Robaire, 2002). Quando os espermatozóides dos mamíferos saem do testículo, eles possuem uma morfologia altamente especializada, mas são imóveis e incapazes de fertilizar um ovócito II (Brooks, 1983; Hermo and Robaire, 2002; Gatti et al., 2004). Durante a passagem pelo ducto epididimário os espermatozóides passam por um processo de maturação, conferindo aos gametas a capacidade de uma vigorosa motilidade e a habilidade para sofrer a reação acrossômica, interagir com a zona pelúcida, reconhecer e fundir-se com a membrana plasmática do ovócito II (Jones, 1999; Cuasnicú et al., 2002; Gatti et al., 2004, Sullivan et al., 2005).

No mamífero adulto, o epidídimo é formado por um ducto único e enovelado que liga os dúctulos eferentes ao ducto deferente (Cosentino and Cokett, 1986; Hermo and Robaire, 2002). Anatomicamente, é dividido em cabeça, corpo e cauda (Reid and Cleand, 1957; Cosentino and Cokett, 1986; Gatti et al., 2004; França et al., 2005; Sullivan et al., 2005). O epitélio é do tipo pseudoestratificado ciliado possui seis tipos celulares: basais, principais, estreitas, halo, claras e apicais (Hermo and Robaire, 2002). A função secretora e de reabsorção das células principais, juntamente com as junções oclusivas entre elas, garantem a manutenção do micro-ambiente intraluminal onde ocorre a maturação dos espermatozóides.

Há uma camada de músculo liso que envolve esse epitélio epididimário, apresentando-se mais fina na região da cabeça e corpo, e mais espessa na cauda. A contração dessas fibras musculares, as quais recebem inervação de fibras adrenérgicas do sistema nervoso simpático (Setchell, 2002), é importante para definir o trânsito espermático e a expulsão dos espermatozóides da região da cauda para o ducto deferente. Na cabeça e corpo do epidídimo,

a inervação é mais escassa, ao passo que a cauda apresenta-se ricamente inervada (Kaleczyc et al., 1968; Ricker, 1998).

#### **IV.III - Ducto deferente**

O ducto deferente é um órgão par que liga o epidídimo à uretra prostática. Suas funções estão relacionadas com o transporte e término do processo de maturação dos espermatozóides. Ele é caracterizado por apresentar um lúmen estreito e uma espessa camada de músculo liso, ricamente inervado por fibras nervosas adrenérgicas (Setchell, 2002), que sofre fortes contrações peristálticas e participa da expulsão do sêmen durante a ejaculação. Sua mucosa forma dobras longitudinais e a maior parte de seu trajeto é constituído por epitélio pseudo-estratificado com estereocílios (Junqueira and Carneiro, 2004).

#### **IV.IV - Vesícula seminal**

A vesícula seminal consiste de um ducto único muito dilatado e enovelado. Este ducto é revestido por um epitélio pseudo-estratificado pregueado, constituído por células epiteliais secretoras e células basais. A luz é ocupada pelo produto de secreção, de aspecto hialino. Essa secreção contribui para viabilidade espermática, induz contrações musculares do trato genital feminino e possui atividade antibacteriana. A camada muscular lisa que reveste o órgão é constituída por duas lâminas: uma interna, de fibras circulares, e outra externa, de fibras longitudinais. (Hayward et al. 1996a; 1996b). Neurônios simpáticos são previstos para controlar as contrações dessa musculatura e danos para essas vias podem resultar em incapacidade para expelir o fluído (Kepper and Keast, 1997).

#### **IV.V - Próstata**

A próstata está localizada na pelve, inferiormente à bexiga, onde circunda a uretra. Ela produz um líquido alcalino que neutraliza o conteúdo vaginal ácido, proporcionando nutrientes e transporte para os espermatozóides, e liquefazendo o sêmen (Kierszenbaum, 2008).

No homem, a próstata é um órgão compacto e internamente dividido em três zonas ou regiões distintas denominadas de zona central, zona periférica e zona de transição (McNeal, 1981). Em ratos e camundongos, essa glândula não é compacta sendo composta por um par de

lobos ventrais, um par de lobos dorsolaterais e um par de lobos anteriores ou glândula de coagulação que estão associados às vesículas seminais (Abbout et al., 2003). Em termos de homologia com as zonas da próstata humana, os lobos anteriores são considerados homólogos à zona central, enquanto os lobos dorsolaterais são considerados homólogos à zona periférica humana. Entretanto, os lobos ventrais de roedores não apresentam qualquer homologia à próstata humana, apesar de ser o lobo prostático mais estudado em modelos experimentais (Roy-Burman et al., 2004).

Histologicamente ela é constituída por glândulas túbulo alveolares ramificadas formadas por um epitélio cubóide ou pseudo-estratificado colunar. Seus ductos desembocam na porção da uretra que cruza a próstata, chamada uretra prostática. Um estroma fibromuscular cerca as glândulas (Junqueira and Carneiro, 2004). Na próstata, o hormônio dihidrotestosterona (DHT) é o andrógeno predominante (Soronen et al., 2004). Ela é inervada pelos nervos pélvico (parassimpático) e hipogástrico (simpático), ramos dos gânglios nervosos pélvicos. O sistema nervoso autônomo possui importante papel no crescimento, maturação e na função secretora desta glândula (Rodrigues et. al, 2002). A inervação age de forma sinérgica com os hormônios para a manutenção saudável da próstata (Diaz et al., 2010).

## **V. Sibutramina e o sistema reprodutor masculino**

Além dos efeitos adversos da sibutramina citados anteriormente, outro efeito reportado foi a ejaculação anormal tanto em homens (FDA, 2000) como em ratos (Nojimoto et al., 2009). Mesmo assim, os estudos destinados a compreender às possíveis ações dessa droga no sistema reprodutor masculino são muito escassos na literatura científica.

É bem estabelecido que a serotonina modula negativamente a ejaculação através da ativação de vias inibitórias eferentes (Giuliano, 2007). De fato, drogas que aumentam a neurotransmissão serotoninérgica, como antidepressivos inibidores da recaptação neuronal de monoaminas, prejudicam a ejaculação. Além disso, a sibutramina pode exercer uma ação periférica alterando a contração da musculatura lisa de órgãos envolvidos na ejaculação (Nojimoto et al., 2009).

Como já descrito, o sistema nervoso simpático inerva os órgãos que compõe o sistema reprodutor masculino e está envolvido com a contração da musculatura lisa e funcionamento adequado destes. A sibutramina, por sua vez, aumenta o tônus simpático (Hirsch et al., 2000) e pode, portanto, interferir na ação desse sistema sobre os órgãos reprodutores.

Nojimoto et al. (2009) demonstraram que a sibutramina, de acordo com a dose e na forma aguda de administração (análises realizadas cerca de 60 minutos após o tratamento), pode ter efeitos opostos na ejaculação e na contratilidade do ducto deferente e vesícula seminal. Assim, esses autores relataram que em baixas doses (5 mg/Kg), essa droga apresenta um efeito simpaticomimético indireto, esperado para um inibidor de recaptção neuronal, aumentando a contração dos músculos lisos dos ductos deferentes e das vesículas seminais e facilitando a ejaculação. Porém em altas doses (20 mg/Kg), houve uma diminuição na contratilidade desses dois órgãos e um prejuízo na ejaculação, o que pode estar relacionado à diminuição da concentração intracelular de cálcio.

É importante notar que outros órgãos do sistema reprodutor masculino também possuem inervação simpática e podem ter suas funções prejudicadas caso haja alterações na neurotransmissão por esta inervação. Kempinas et al. (1998) verificaram, em ratos, um atraso no tempo de trânsito espermático na cauda do epidídimo e disfunções ejaculatórias após serem submetidos a simpatectomia, tanto cirúrgica como química.

Assim, mesmo com a utilização crescente da sibutramina para tratamento da obesidade e com a presença de indícios de que essa droga pode afetar o sistema reprodutor masculino, ainda há falta de estudos mais detalhados sobre o tema.

Neste sentido, foi desenvolvido o presente trabalho que teve por objetivo avaliar parâmetros reprodutivos, performance reprodutiva e reatividade farmacológica da cauda do epidídimo isolada de ratos machos adultos expostos à sibutramina.

## **CAPÍTULO**

Este trabalho deu origem ao artigo “Acceleration of sperm transit time and reduction of sperm reserves in the epididymis of rats exposed to sibutramine” que foi submetido para publicação no periódico “Journal of Andrology”.

ACCELERATION OF SPERM TRANSIT TIME AND REDUCTION OF SPERM  
RESERVES IN THE EPIDIDYMIS OF RATS EXPOSED TO SIBUTRAMINE

Sibutramine and rat sperm reserves

Fernanda F. Bellentani<sup>1</sup>, Glaura S. A. Fernandes<sup>2</sup>, Juliana E. Perobelli<sup>2</sup>, Enio S. A. Pacini<sup>3</sup>,  
Luiz R. A. Kiguti<sup>3</sup>, André S. Pupo<sup>4</sup>, Wilma D. G. Kempinas<sup>5</sup>

1 – Graduate Program in General and Applied Biology, Institute of Biosciences, UNESP –  
Univ Estadual Paulista, Brazil.

2 - Graduate Program in Cellular and Structural Biology, Institute of Biology, UNICAMP -  
State University of Campinas, Brazil.

3 - Graduate Program in Biological Sciences – Pharmacology, Institute of Biosciences,  
UNESP – Univ Estadual Paulista, Brazil

4 - Department of Pharmacology, Institute of Biosciences, UNESP – Univ Estadual Paulista,  
Brazil.

5 - Department of Morphology, Institute of Biosciences, UNESP - University Estadual  
Paulista.

**Corresponding author:**

Wilma De Grava Kempinas

Department of Morphology, Institute of Biosciences, UNESP - University Estadual Paulista.

Caixa-Postal 510

CEP 18618-970, Distrito de Rubião Jr., s/n, Botucatu, SP – Brazil.

Telephone number: +55 (14) 38116264 ext. 104, Fax: +55 (14) 38116264

Email: [kempinas@ibb.unesp.br](mailto:kempinas@ibb.unesp.br)

## ABSTRACT

Sibutramine is a drug globally used for the treatment of obesity. The aim of this study was to investigate male reproductive disorders caused by sibutramine in adult rats. Wistar rats were treated for 28 consecutive days (gavage) with 10 mg/kg of sibutramine. Control animals received only vehicle (dimethyl sulfoxide and saline). Terminally the rats were sacrificed for evaluation of body and reproductive organ weights, sperm parameters, hormone levels (luteinizing hormone, follicle-stimulating hormone and testosterone), testicular and epididymal histopathology, sexual behavior, fertility and *in vitro* contractility of the epididymal duct. Sibutramine decreased ( $p < 0.05$ ) weights of epididymis and ventral prostate, but not of other reproductive organs. The sperm number and transit time in the epididymal cauda were decreased ( $p < 0.001$ ), but the daily sperm production was not altered. Moreover, the morphology and sperm motility, the histopathology of the testes and epididymis, sexual behavior, fertility and serum hormone levels were not altered by the treatment. Sibutramine increased the potency of norepinephrine and, per se, increased the mechanical activity of the epididymal duct *in vitro*. Thus, while sibutramine in these experimental conditions did not interfere with the reproductive process of rats, provoked acceleration of the sperm transit time and decrease in the sperm reserves in the epididymal cauda. This alteration is probably related to the sympathomimetic effect of this drug, as shown by the *in vitro* assays. In humans this fact may represent a threat for male fertility since sperm reserves in men are naturally lower compared to rats.

Key words: sibutramine, male rats, epididymis, sperm reserves, sperm transit time, sympathetic innervation

## INTRODUCTION

Sibutramine is a widely used anti-obesity drug (Luque and Rey, 2002). It is a centrally acting aid to weight loss that inhibits the reuptake of serotonin (5-HT) and norepinephrine involved in the control of food intake, and stimulates thermogenesis increasing energy expenditure (Ara and Brennan, 2007).

The most commonly reported side effects of sibutramine include dry mouth, high blood pressure, fatigue, constipation, tachycardia, anorexia, headaches and insomnia (Luque and Rey, 1999; Richter, 1999; Cuellar et al, 2000; Perrio et al, 2007). The European Medicines Agency (EMA) recommended the suspension of the marketing authorizations for sibutramine. This decision was taken because of a possible increased cardiovascular risk, mainly ischemic heart disease, associated with the use of sibutramine in patients with pathologies prone to develop cardiovascular complications. The conclusion was that the benefits no longer outweigh the risks (Scheen, 2010). However, this drug is still used in other parts of the world, including Brazil.

Regarding the effects of this drug on male reproduction, abnormal ejaculation was observed in rats (Nojimoto et al, 2009), but there is still a lack of studies in the scientific literature related to the topic. In fact, drugs that increase serotonergic neurotransmission, such as antidepressants inhibitors of the neuronal reuptake of monoamines, delay ejaculation. Apart from its action in the central nervous system, facilitation of ejaculation may result from augmented sensitivity of smooth muscles of vas deferens and seminal vesicle to norepinephrine while reductions of intracellular  $Ca^{2+}$  concentrations may be involved in the delay ejaculation observed with high doses of sibutramine (Nojimoto et al, 2009).

It is important to note that sibutramine increases sympathetic tone (Hirsch et al, 2000) and that other male reproductive organs, such as epididymis, may also have his functions damaged if there are changes in sympathetic innervation. Kempinas et al. (1998a) found in

rats, that chemically or surgically sympathectomy delays the transit of sperm through the cauda epididymis and produces ejaculatory dysfunction.

Based on the growing use of sibutramine for the obesity treatment and the dearth of information in the literature on the effects of this drug in male reproductive system, this study  
55 aims to evaluate reproductive parameters, reproductive performance and pharmacological reactivity of isolate distal cauda epididymis duct of adult male rats exposed to sibutramine.

## **METHODS**

### *Animals*

60 Adult male and female Wistar rats were supplied by Central Biotherium of the State University of São Paulo and were housed in polyethylene cages (43 cm×30 cm×15 cm) with laboratory-grade pine shavings as bedding. The animals were maintained in controlled environmental conditions: temperature ( $22 \pm 2^{\circ}\text{C}$ ), relative air humidity ( $55 \pm 10\%$ ), 12 hours light/dark cycle, and continuous air circulation. Rat chow and filtered tap water were provided  
65 ad libitum. The experimental protocol followed the Ethical Principles in Animal Research of the Brazilian College of Animal Experimentation and was approved by the Bioscience Institute/UNESP Botucatu Ethical Committee for Animal Experimentation (115-CEEA). In order to evaluate endpoints of general reproductive toxicity and reproductive performance the study was conducted in three steps, called Experiment 1, Experiment 2 and Experiment 3,  
70 described as follows.

### *Experiment 1: reproductive organ weights, serum hormones levels, sperm parameters and histology*

Adult male rats (120 days old, n=10) were treated with 10 mg/kg per day of sibutramine  
75 diluted in dimethyl sulfoxide (DMSO) and saline (60 mg sibutramine/1 mL DMSO/2 mL

saline), gavage (oral route) for 28 consecutive days. The respective controls (n=10) received only vehicle (1 mL DMSO/2 mL saline) at a dose of 0,17 mL DMSO/kg.

#### Body weight and weight of reproductive organs

80 On the day following the end of treatment the animals were weighed and killed by decapitation. The right testis, epididymis, vas deferens, ventral prostate and seminal vesicle (without the coagulating gland, full and empty of secretion) were removed and their weights determined.

#### 85 Hormonal Measurement

After decapitation, blood was collected (between 9:00 and 11:30 am) from the ruptured cervical vessels in a tube for the determination the serum concentrations of testosterone, luteinizing hormone (LH), and follicle-stimulating hormone (FSH). Serum was obtained after centrifugation (2400 rpm, 20 min, 4 °C) in a refrigerated apparatus and was frozen at -20 °C  
90 until the moment of hormonal determination. The analyses were determined by double-antibody radioimmunoassay at the Neuroendocrinology Laboratory, Dental School of University of São Paulo - USP campus at Ribeirão Preto, Sao Paulo state, Brazil. Testosterone was measured using the Testosterone kit (Diagnostic Systems Laboratories, USA). The LH and FSH levels were quantified by specific kits from the National Institute of Arthritis,  
95 Diabetes and Kidney Diseases (NIADDK, USA). All samples were measured in the same assay to avoid inter-assay errors. Intra- and interanalysis variations were, respectively, 4% and 10% for LH; 3% and 10% for FSH; 1.75% and 20% for testosterone.

### Sperm Counts, Daily Sperm Production, and Sperm Transit Time through the Epididymis

The spermatids resistant to the process of testicular homogenization (stage 19 of spermiogenesis) and the sperm present in the caput/corpus and cauda epididymis were enumerated as described by Robb et al. (1978), with modifications by Fernandes et al. (2007).  
105 To calculate the daily sperm production (DSP), the concentration of spermatids per testis was divided by 6.1, which is the number of days in which mature spermatids are present in the seminiferous epithelium. Then, the DSP per gram was calculated in order to determine the efficiency of the process. The sperm transit time through the epididymal caput/corpus and cauda was obtained by dividing the number of sperm present in each of these regions by the  
110 DSP.

### Histological Analysis of Testis and Epididymis

The left testis and epididymis were fixed in Alfac fixing solution (85% ethanol, 10% formaldehyde and 5% glacial acetic acid) for 24hours. The fixed organs were embedded in  
115 paraplast and subjected to histological cuts of 5  $\mu$ m thickness, subsequently stained with hematoxylin and eosin (H.E). Histological evaluation was carried out using transverse sections of the testis and longitudinal sections of the epididymis, analyzed in blind test with the aid of light microscope.

### 120 Sperm Morphology and Motility

With the aid of a syringe and a needle, sperm were removed from the left vas deferens of rats through internal rinsing with 1.0mL of saline formol. For the analysis, smears were prepared on histological slides that were left to dry for 90 minutes and observed in a phase-contrast microscope (400X magnification) (Seed et al, 1996); 200 spermatozoa were analyzed  
125 per animal. Morphological abnormalities were classified into general categories pertaining to

head morphology (without curvature, without characteristic curvature, pin head or isolated form, i.e., no tail attached) and tail morphology (broken, isolated, i.e., no head attached, or rolled into a spiral) (Filler, 1993).

Sperm motility analysis was performed as described by Perobelli et al. (2010), using  
130 sperm collected from the right vas deferens.

*Experiment 2: sexual behavior, fertility and reproductive performance after natural mating*

In this experiment another batch of 20 male rats (120 days old) and 40 females (70 days old) were used, following the same experimental design as described for Experiment 1.

135

Sexual behavior

One day after the end of sibutramine treatment male rats were placed individually in boxes of polycarbonate crystal, measuring 44 cm×31 cm×16 cm, 5 min before introduction of one adult sexually receptive female. Behavioral testing was conducted for four hours in the  
140 dark period of the cycle under dim red illumination. For the next 40 min the following measures were recorded: intromission latency, defined as the times from introduction of the female in the cage to the first intromission; intromission frequency, the number of intromissions preceding the first ejaculation; ejaculation latency, the time from introduction of the female in the cage to the first ejaculation; intromission latency post-ejaculation, the time  
145 to the first intromission after the first ejaculation; intromission frequency post-ejaculation, the number of intromissions after the first ejaculation; total number of ejaculations (Alhenius and Larsson, 1984; Ågmo, 1997). If the male did not mount or intromission in the next 10 min after the introduction of one adult female in the box, it was considered sexually inactive.

150

### Fertility and reproductive performance

Rats that ejaculated during evaluation of sexual behavior stayed with the females for an additional four hours, permitting a greater number of ejaculations. The animals that had been deemed inactive were tested again daily for the next five consecutive days during which different females in estrus were placed in their boxes during the dark period of the cycle. At the end of the afternoon males and females were separated and vaginal smears were collected, in which initial sperm detection was determined to be the day zero of gestation (GD0). On GD20 females were killed by decapitation. After collection of the uterus and ovaries, the numbers of corpora lutea, implantation sites, resorptions, live fetuses and fetal weights were determined. From these results were determined: fertility potential (efficiency of implantation):  $\text{implantation sites/corpora lutea} \times 100$ ; rate of pre-implantation loss:  $\text{number of corpora lutea} - \text{number of implantations} / \text{number of corpora lutea} \times 100$ ; rate of post-implantation loss:  $\text{number of implantations} - \text{number of live fetuses} / \text{number of implantations} \times 100$ ; sex ratio:  $\text{number of male fetuses} / \text{number of female fetuses} \times 100$ .

165

### *Experiment 3: Pharmacological reactivity of isolated distal cauda epididymis duct*

Adult male Wistar rats (90 days old, n=4) were killed by decapitation and whole epididymis was carefully excised. Strips from distal cauda epididymis (Hinton et al, 1979) were dissected and cleaned of adherent tissues and sperm. Tissues were mounted in organ baths under 9.8 mN resting tension in a nutrient solution with the following composition (mM): NaCl 138; KCl 5.7; CaCl<sub>2</sub> 1.8; NaH<sub>2</sub>PO<sub>4</sub> 0.36; NaHCO<sub>3</sub> 15, dextrose 5.5, prepared in glass-distilled, de-ionised water, maintained at 30°C, pH 7.4, and continuously bubbled with 95% O<sub>2</sub>/5% CO<sub>2</sub>. Changes of isometric tension in distal cauda epididymis were digitally recorded. After a 30 minutes stabilization period with adjustments of basal tension, tissues were challenged with 80 mM KCl until reproducible contractions were obtained. Then, a

175

concentration-response curve to norepinephrine was obtained in absence of sibutramine by adding cumulative concentrations of the agonist and was taken as a control curve. After washing and relaxation, increasing concentrations of sibutramine (1, 3 and 10  $\mu$ M) were incubated for at least 45 minutes and then a new concentration-response curve to norepinephrine was obtained. Concentration-response curves to norepinephrine were normalized by the percentage of the maximal response obtained in control curve. The potencies of norepinephrine ( $pEC_{50}$ ) in the absence and presence of sibutramine was measured by the negative logarithm concentration of norepinephrine that produces 50% of maximal response. Erratic contractile activity triggered by sibutramine was quantified by the sum of any phasic or tonic activity above baseline during 5 minutes. For normalization purpose, these measurements were taken 30 min after the addition of sibutramine.

#### *Statistical analysis*

ANOVA, Student's t-test or nonparametric Mann-Whitney test were used to compare the data, according to the experiment and characteristic of each variable. Differences were considered statistically significant when  $p < 0.05$ .

## **RESULTS**

Although the hormonal assay revealed that experimental groups did not differ significantly as to serum levels of testosterone, LH or FSH (Table 1), sibutramine-treated rats showed a significant reduction in the weights of the epididymis and prostate (Table 2).

Exposure to sibutramine did not markedly alter the number of mature spermatids in the testis, the daily sperm production and the number and sperm transit time through the caput/corpus epididymis. On the other hand there was a significant reduction in both sperm number and transit time in the epididymal cauda (Table 3).

Histopathological analysis of the testes and epididymis (data not show) did not reveal any apparent alterations that could be attributed to sibutramine exposure (mean percent values for normal tubules in the control group = 98 % and sibutramine = 97.5 %). In the same way the number of morphologically normal sperm in the sibutramine-treated group (96%) was comparable to control group (95.75%). Sibutramine also did not interfere with sperm motility (data not shown).

Tables 4 and 5 show that there were no differences among the experimental groups in sexual behavior and reproductive performance.

In the absence of sibutramine no spontaneous contractile activity was observed in the rat isolated distal cauda epididymis (Fig. 1a). The incubation with 1, 3 and 10  $\mu\text{M}$  sibutramine induced phasic contractions in the epididymal duct (Fig. 1b, 1c and 1d). The sum of the contractile activity induced by sibutramine measured during 5 minutes was increased in comparison from the contractile activity measured in the absence of sibutramine (n=7-8,  $p < 0.005$ ) (Fig. 1e).

Norepinephrine induced concentration-dependent contractions in the rat distal cauda epididymis with high potency ( $\text{pEC}_{50} = 6.53 \pm 0.06$ , n=8). Incubation with 1, 3 and 10  $\mu\text{M}$  of sibutramine increased the sensitivity of the distal cauda epididymis to norepinephrine by approximately 8- ( $\text{pEC}_{50} = 7.45 \pm 0.09$ , n=7), 16- ( $\text{pEC}_{50} = 7.72 \pm 0.10$ , n=7) and 14-fold ( $\text{pEC}_{50} = 7.66 \pm 0.19$ , n=7), respectively (n=7-8,  $p < 0.05$ ). This increase was characterized by a leftward shift in the concentration response-curve to norepinephrine (Fig. 2). In addition, the concentration of sibutramine 10  $\mu\text{M}$  reduced the maximal contractions induced by norepinephrine (n=7-8,  $p < 0.0001$ ) (Fig. 2).

## DISCUSSION

Despite the increasing utilization of drugs for treating obesity, specific detailed works on the reproductive effects of these compounds, including sibutramine, are scarce.

It is known that the autonomic nervous system plays an important role in the growth, maturation and secretory function of the prostate (Rodrigues et al, 2002). It is possible that the diminution of the weight of the prostate, in the absence of effects on testosterone levels in animals treated with sibutramine may be due to the sympathomimetic effect of this drug and is related to its capacity to inhibit noradrenaline reuptake (Haynes and Egri, 2005; Nojimoto et al, 2009). The reduction of epididymal weight caused by sibutramine may have occurred on account of the diminished number of sperm in the epididymal cauda region.

The sperm transport through the epididymis is mediated by contraction of smooth muscle surrounding the epididymal duct. The epididymis receives autonomic innervation proceeding from inferior mesenteric ganglion, major pelvic ganglion and pelvic accessory ganglion (Ricker, 1998). The sympathetic innervation controls the smooth muscle contractions of the epididymal duct in all regions of epididymis mainly by activation of  $\alpha_1$ -adrenoceptors (Pholpramool and Triphrom, 1984; Ventura and Pennefather, 1991; Ventura and Pennefather, 1994). Drugs which interfere in the sympathetic system or contractile activity of epididymis have important effects in the sperm transit time through the epididymis (Billups et al, 1990; Kempinas et al, 1998b; Fernandez et al, 2008).

Sibutramine showed two distinct effects in the rat distal cauda epididymis: increased the potency of norepinephrine and increased the mechanical activity. Similar effects were also observed in the vas deferens and seminal vesicle by Nojimoto et al. (2009). The increased potency of norepinephrine in the rat distal cauda epididymis observed in this study may result from the inhibition of neuronal reuptake by sibutramine and the effect of induction of mechanical activity may be associated with the ability of sibutramine to block voltage-gated

K<sup>+</sup> channels (Kim et al, 2007). It is known that voltage-gated K<sup>+</sup> channels have an important function in the control of smooth muscle contraction. In the bovine epididymal duct are demonstrated that Erg K<sup>+</sup> channels modulates contraction activity (Mewe et al, 2008). In addition, 10 μM sibutramine suppressed the maximal contraction obtained in the concentration-response curves to norepinephrine in the rat cauda distal epididymis. Similarly, Nojimoto et al. (2009) observed reductions in the maximal contraction in rat vas deferens and seminal vesicle with sibutramine 10, 30 and 100 μM. This effect was credited to the action of sibutramine in blocking the Ca<sup>+</sup> influx into the smooth muscle cells and could explain the same action in the rat cauda epididymis.

260 The passage of sperm through the epididymis occurs by means spontaneous contractile activity of smooth muscle in the region of caput and corpus and induced contractile activity of smooth muscle in the cauda region, which is highly innervated (Kaleczyc et al, 1993; Ricker, 1998, Kempinas and Klinefelter, 2010). In the present study the sympathomimetic effect of sibutramine in the smooth muscle of rat cauda distal epididymis could explain the decrease in the sperm number and acceleration of sperm transit time mainly by induction of contractile activity.

270 Nojimoto et al. (2009) demonstrated that sibutramine can interfere negatively in ejaculation. However, this was not observed in the present study since the animals of both experimental groups presented sexually similar behavior. This may have occurred due to differences in experimental delineation and in sibutramine doses utilized in each study. Moreover, sperm motility, fertility and reproductive performance were not altered by sibutramine exposure.

275 Thus, while sibutramine in these experimental conditions did not interfere with the reproductive process of rats, provoked acceleration of the sperm transit time and decrease in the sperm reserves in the epididymal cauda. This alteration is probably related to the

sympathomimetic effect of this drug, as shown by the *in vitro* assays. In humans this fact may represent a threat for male fertility since sperm reserves in men are naturally lower compared to rats (Johnson et al, 1997).

## 280 ACKNOWLEDGEMENTS

We are grateful to Dr. Janete Aparecida Anselmo-Franci and to Dr. 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. We are also grateful to CNPq (National Council for Scientific and  
285 Technological Development) for financial support. This paper represents part of the MS thesis presented by F. F. Bellentani to UNESP - Univ Estadual Paulista, Brazil.

## REFERENCES

- 290 Agmo A. Male rat sexual behavior. *Brain Res Protoc.* 1997; 1:203-209.
- Ahlenius S, Larsson K. Apomorphine and haloperidol-induced effects on male rat sexual behavior: no evidence for actions due to stimulation of central dopamine autoreceptors. *Pharmacol Biochem Behav.* 1984; 21:463-466.
- 295 Ara R, Brennan A. The cost-effectiveness of sibutramine in non-diabetic obese patients: evidence from four Western countries. *Obes Rev.* 2007; 8:363-371.
- Billups KL, Tillman S, Chang TS. Ablation of the inferior mesenteric plexus in the rat: alteration of sperm storage in the epididymis and vas deferens. *J Urol.* 1990;143:625-629.
- 300 Cuellar GE, Ruiz AM, Monsalve MC, Berber A. Six-month treatment of obesity with sibutramine 15 mg; a double-blind, placebo-controlled monocenter clinical trial in a Hispanic population. *Obes Res.* 2000; 8:71-82.
- 305 Fernandes GS, Arena AC, Fernandez CD, Mercadante A, Barbisan LF, Kempinas WG. Reproductive effects in male rats exposed to diuron. *Reprod Toxicol.* 2007; 23:106-112.
- Fernandez CD, Porto EM, Arena AC, Kempinas WDG. Effects of altered epididymal sperm  
310 transit time on sperm quality. *Int J Androl.* 2008; 31: 427-437.

- Filler R. Methods for evaluation of rats epididymal sperm morphology. In: Chapin RE, Heindel JH, eds. *Male reproductive toxicology*. San Diego, CA: Academic Press Inc, 1993: 334–343.
- 315 Haynes WG, Egri Z. Sibutramine and the sympathetic nervous system in obese humans. *Clin Auton Res*. 2005; 15: 189-192.
- Hinton BT, Dott HM, Setchell BP. Measurement of the motility of rat spermatozoa collected by micropuncture from the testis and from different regions along the epididymis. *J Reprod Fertil*. 1979;55:167-172.
- 320 Hirsch J, Mackintosh RM, Aronne LJ. The effects of drugs used to treat obesity on the autonomic nervous system. *Obes Res*. 2000; 8:227-233.
- 325 Johnson L, Welsh THJr, Wilker CE. Anatomy and physiology of the male reproductive system and potential targets of toxicants. In: Sipes G, Mcqueen CA, Gandolfi AJ, eds. *Comprehensive toxicology*. New York: Elsevier Science Inc, 1997; 10: 6-51.
- 330 Kaleczyc J, Majewski M, Całka J, Lakomy M. Adrenergic innervation of the epididymis, vas deferens, accessory genital glands and urethra in the boar. *Folia Histochem Cytobiol*. 1993; 31: 117-123.
- Kempinas WDG, Suarez JD, Roberts NL, Strader LF, Ferrell J, Goldman JM, Narotsky MG, Perreault SD, Evenson DP, Ricker DD, Klinefelter GR. Fertility of rat epididymal sperm after chemically and surgically induced sympathectomy. *Biol Reprod*. 1998a; 59:897-904.
- 335 Kempinas WDG, Suarez JD, Roberts NL, Strader L, Ferrell J, Goldman JM, Klinefelter GR. Rat epididymal sperm quantity, quality, and transit time after guanethidine-induced sympathectomy. *Biol Reprod*. 1998b; 59: 890-896.
- 340 Kempinas WDG, Klinefelter GR. The Epididymis as a target for toxicants. In: Charlene A, ed. *McQueen, Comprehensive Toxicology*. Oxford: Academic Press, 2010: 149-166.
- 345 Kim SE, Ahn HS, Choi BH, Jang HJ, Kim MJ, Rhie DJ, Yoon SH, Jo YH, Kim MS, Sung KW, Hahn SJ. Open channel block of A-type, kv4.3, and delayed rectifier K<sup>+</sup> channels, Kv1.3 and Kv3.1, by sibutramine. *J Pharmacol Exp Ther*. 2007;321:753-762.
- Luque CA, Rey JA. Sibutramine: a serotonin-norepinephrine reuptake-inhibitor for the treatment of obesity. *Ann Pharmacother*. 1999; 33:968-978.
- 350 Luque CA, Rey JA. The discovery and status of sibutramine as an anti-obesity drug. *Eur J Pharmacol*. 2002; 440:119-128.
- 355 Mewe M, Wulfsen I, Schuster AM, Middendorff R, Glassmeier G, Schwarz JR, Bauer CK. Erg K<sup>+</sup> channels modulate contractile activity in the bovine epididymal duct. *Am J Physiol Regul Integr Comp Physiol*. 2008;294:R895-904.
- Nojimoto FD, Piffer RC, Kiguti LR, Lameu C, de Camargo AC, Pereira OC, Pupo AS. Multiple effects of sibutramine on ejaculation and on vas deferens and seminal vesicle contractility. *Toxicol Appl Pharmacol*. 2009; 239: 233-240.
- 360

- 365 Perobelli JE, Martinez MF, Franchi CAS, Fernandez CD, Camargo JL, Kempinas WDG. Decreased sperm motility in rats orally exposed to single or mixed pesticides. *J Toxicol Environ Health A*. 2010;73:991-1002
- 370 Perrio MJ, Wilton LV, Shakir SA. The safety profiles of orlistat and sibutramine: results of prescription-event monitoring studies in England. *Obesity (Silver Spring)*. 2007; 15:2712-2722.
- 375 Pholpramool C, Triphrom N. Effects of cholinergic and adrenergic drugs on intraluminal pressures and contractility of the rat testis and epididymis in vivo. *J Reprod Fertil*. 1984;71:181-188.
- Richter WO. How safe are the new obesity drugs? Indications and contraindications of orlistat and sibutramine. *MMW Fortschr Med*. 1999; 141:32-36.
- 380 Ricker DD. The autonomic innervation of the epididymis: its effects on epididymal function and fertility. *Androl*. 1998; 19:1-4.
- Robb GW, Amann RP, Killian GJ. Daily sperm production and epididymal sperm reserves of pubertal and adult rats. *J Reprod Fertil*. 1978; 54:103-107.
- 385 Rodrigues AO, Machado MT, Wroclawski ER. Prostate innervation and local anesthesia in prostate procedures. *Rev Hosp Clin Fac Med Sao Paulo*. 2002; 57: 287-292.
- Scheen AJ. Suspension of the commercialization of sibutramine and rosiglitazone in Europe. *Rev Med Liege*. 2010; 65:574-579.
- 390 Seed J, Chapin RE, Clegg ED, Dostal LA, Foote RH, Hurtt ME, Klinefelter GR, Makris SL, Perreault SD, Schrader S, Seyler D, Sprando R, Treinen KA, Veeramachaneni DN, Wise LD. Methods for assessing sperm motility, morphology, and counts in the rat, rabbit, and dog: a consensus report. ILSI Risk Science Institute Expert Working Group on Sperm Evaluation. *Reprod Toxicol*. 1996; 10:237-244.
- 395 Ventura S, Pennefather JN. Sympathetic co-transmission to the cauda epididymis of the rat: characterization of postjunctional adrenoceptors and purinoceptors. *Br J Pharmacol*. 1991;102:540-544.
- 400 Ventura S, Pennefather JN. 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*. 1994;254:167-173.

405

410

## TABLES

Table 1. Serum hormonal levels.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 10)
FSH (ng/mL)	6.05 ± 0.46	6.10 ± 0.53
LH (ng/mL)	1.64 ± 0.32	1.50 ± 0.30
Testosterone (ng/mL)	2.23 ± 0.29	2.33 ± 0.48

Values expressed as mean ± SEM. Mann-Whitney test,  $p > 0.05$ .

Table 2. Reproductive organs and body weights.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 10)
Final body weight (g)	460.16 ± 11.52	437.34 ± 10.25
Testis (g)	1.74 ± 0.06	1.83 ± 0.06
Epididymis (mg)	715.86 ± 22.72	652.62 ± 13.37*
Ventral prostate (mg)	597.01 ± 40.33	437.17 ± 34.99*
Full seminal vesicle (g)	1.59 ± 0.13	1.37 ± 0.07
Empty Seminal vesicle (mg)	611.26 ± 38.12	563.53 ± 29.78
Vas deferens (mg)	131.51 ± 3.78	123.99 ± 5.21

Values expressed as mean ± SEM. Student's t-test, \* p <0.05.

Table 3. Sperm parameters.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 10)
Spermatid number ( $10^6$ /testis)	216.07 $\pm$ 9.45	235.22 $\pm$ 7.43
Daily sperm production ( $10^6$ /testis/day)	35.42 $\pm$ 1.55	38.56 $\pm$ 1.22
( $10^6$ /g testis /day)	24.67 $\pm$ 1.02	25.00 $\pm$ 1.02
Caput / corpus epididymis sperm number ( $10^6$ / organ)	136.18 $\pm$ 6.82	148.34 $\pm$ 7.79
Cauda epididymis sperm number ( $10^6$ /organ)	276.16 $\pm$ 12.37	180.98 $\pm$ 8.87*
Sperm transit time in the caput/corpus (days)	3.86 $\pm$ 0.16	3.86 $\pm$ 0.19
Sperm transit time in the cauda (days)	7.85 $\pm$ 0.32	4.73 $\pm$ 0.26*

Values are expressed as mean  $\pm$  SEM. Student's t- test, \* p <0.05.

Table 4. Sexual behavior endpoints.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 8)
Latency to the first intromission (s)	50.78 ± 10.09 (9)	37.25 ± 13.15 (8)
Number of intromissions until the first ejaculation	22.00 ± 1.97 (9)	17.25 ± 2.45 (8)
Latency to the first ejaculation (s)	1166.63 ± 123.72 (8)	999.29 ± 112.95 (7)
First post-ejaculatory intromission (s)	373.83 ± 40.78 (6)	461.83 ± 26.73 (6)
Number of post-ejaculatory intromissions	10.33 ± 1.41 (6)	10.17 ± 2.71 (6)
Number of ejaculations	1.62 ± 0.26 (8)	1.57 ± 0.20 (7)

Values are expressed as mean ± SEM. Latencies expressed in seconds. The number of animals that presented the behavior is indicated in parenthesis. Student's t- test, p >0.05.

Table 5. Fertility and reproductive performance.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 8)
Body weight of dams (g)	354.37 ± 8.63	373.96 ± 4.81
Number of corpora lutea	12.10 ± 0.57	13.37 ± 0.26
Number of implants	11.80 ± 0.70	12.37 ± 0.37
Number of resorptions	0.20 ± 0.20	0.50 ± 0.27
Number of live fetuses	11.60 ± 0.69	11.87 ± 0.40
Fetuses weight (g)	3.07 ± 0.05	3.07 ± 0.06
<sup>1</sup> Sex ratio (%)	100.00 (66.67 – 159.82)	78.57 (66.67 – 206.25)
<sup>1</sup> Fertility potential (%)	100.00 (100.00– 100.00)	96.43 (88.87 – 100.00)
<sup>1</sup> Pre-implantation loss (%)	0.00 (0.00 – 0.00)	3.57 (0.00 – 11.12)
<sup>1</sup> Post-implantation loss (%)	0.00 (0.00 – 0.00)	0.00 (0.00 – 7.63)

Values expressed as mean ± SEM. Student's t- test, p> 0.05.

<sup>1</sup> Values expressed as median (Q1 - Q3). Mann-Whitney test, p> 0.05

## FIGURE LEGENDS

Figure 1. Representative tracings of the contractile activity induced by vehicle (a) and in the presence of sibutramine 1 (b), 3 (c) and 10 (d)  $\mu\text{M}$  in the distal portion of rat cauda epididymis. (e) Tension developed in 5 min in the absence (vehicle, water) and presence of increasing concentration of sibutramine. Values are mean  $\pm$  SEM of 8 tissue segments taken from 4 different rats. \* Different from the tension developed in the presence of vehicle (ANOVA, followed by Newman-Keuls, \* $p < 0.0001$ , # $p < 0.005$ ).

Figure 2. Concentration-response curves to norepinephrine in the absence and presence of increasing concentrations of sibutramine. Each symbol represents the mean, and the vertical bars, the SEM of 8 tissues taken from 4 rats.

## FIGURES

Figure 1

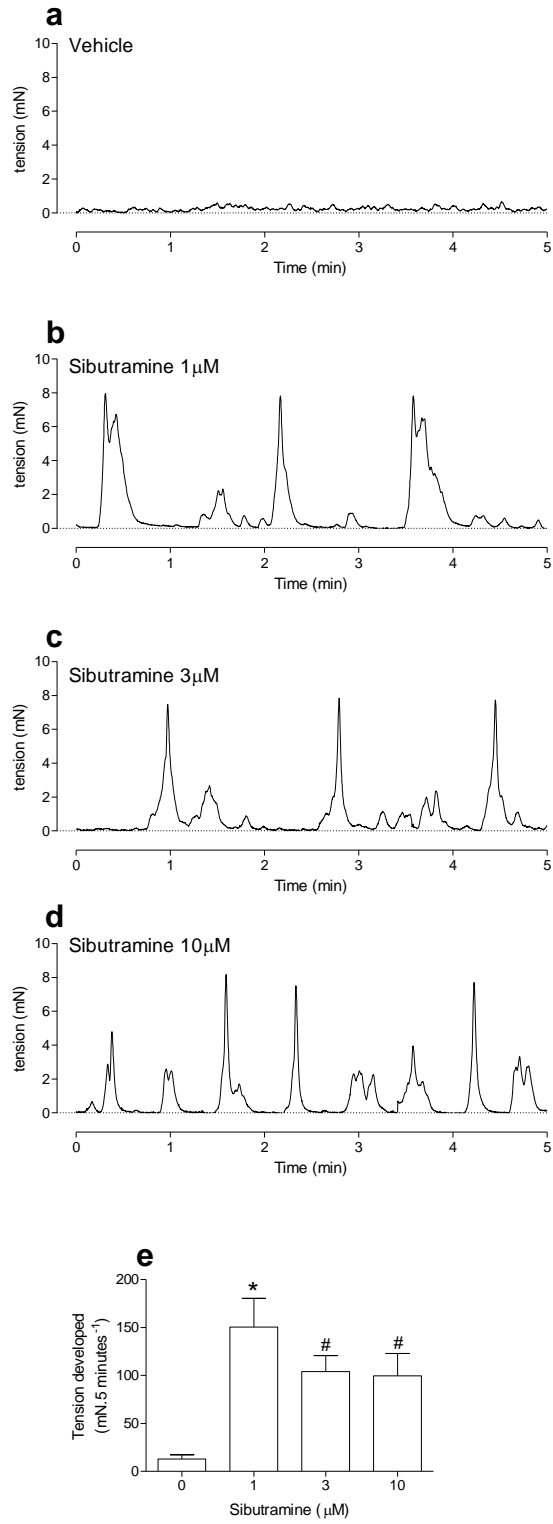
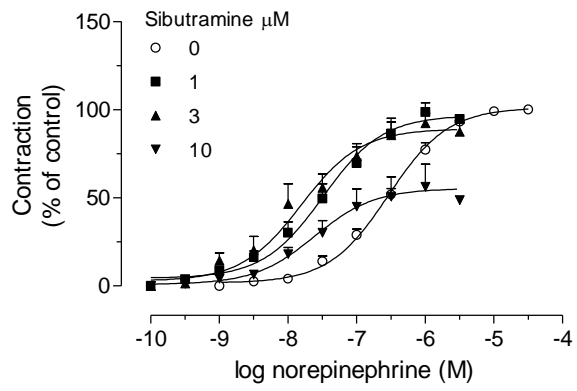


Figure 2



## CONCLUSÃO

Conclui-se que embora a sibutramina não tenha interferido no processo reprodutivo de ratos machos, provocou aceleração do tempo de trânsito e diminuiu o estoque de espermatozóides na cauda do epidídimo. Essa alteração provavelmente relaciona-se ao efeito simpatomimético da sibutramina e, na espécie humana, é possível que esse fato tenha um impacto sobre a fertilidade masculina, uma vez que as reservas espermáticas no homem são naturalmente menores do que no rato.

## REFERÊNCIAS BIBLIOGRÁFICAS DA INTRODUÇÃO

ABOUT, D.E. et al. Expressed sequence tag profiling identifies developmental and anatomic partitioning of gene expression in the mouse prostate. **Genome Biol.**, v.4, n.12, R.79.1-79.15, 2003.

AGÊNCIA NACIONAL DE VIGILÂNCIA SANITÁRIA (ANVISA). Anvisa esclarece nova resolução sobre Sibutramina. Disponível em:

<http://portal.anvisa.gov.br/wps/portal/anvisa/imprensa>. Acesso em: 6 de julho de 2010.

BROOKS, D.E. Epididymal functions and their hormonal regulation. **Aust. J. Biol. Sci.**, v.36, n.3, p. 205-221, 1983.

BUCKETT, W.R.; THOMAS, P.C.; LUSCOMBE, G.P. The pharmacology of sibutramine hydrochloride (BTS 54 524), a new antidepressant which induces rapid noradrenergic down-regulation. **Prog. Neuropsychopharmacol. Biol. Psychiatry**, v.12, n.5, p.575–584, 1988.

CATERSON, I.D. What should we do about overweight and obesity? **Med. J. Aust.**, v.171, n.11-12, p.599–600, 1999.

CHAPELOT, D. et al. Modalities of the food intake-reducing effect of sibutramine in humans. **Physiol. Behav.**, v.68, n.3, p.299-308, 2000.

CLERMONT, Y. Kinetics of spermatogenesis in mammals: seminiferous epithelium cycle and spermatogonial renewal. **Physiol. Rev.**, v.52, n.1, p.198-236, 1972.

COSENTINO, M.J.; COKETT, A.T. Structure and function of the epididymis. **Urol. Res.**, v.14, n.5, p.229-240, 1986.

CUASNICÚ, P.S. et al. Changes in specific sperm proteins during epididymal maturation. In: BOBAIRE, B.; HINTON, B. T. (Eds.). **The Epididymis: from molecules to clinical practice**. New York: Kluwer Academic/ Plenum Publisher, 2002. p.389-403.

- CUELLAR, G.E. et al. Six-month treatment of obesity with sibutramine 15 mg; a double-blind, placebo-controlled monocenter clinical trial in a Hispanic population. **Obes. Res.**, v.8, n.1, p.71-82, 2000.
- DIAZ, R. et al. Histological modifications of the rat prostate following transection of somatic and autonomic nerves. **An. Acad. Bras. Ciênc.**, v.82, n.2, p.397-404, 2010.
- FANGHANEL, G. et al. A clinical trial of the use of sibutramine for the treatment of patients suffering essential obesity. **Int. J. Obes. Relat. Metab. Disord.**, v.24, n.2, p.144-150, 2000.
- FINER, N. Sibutramine: its mode of action and efficacy. **Int. J. Obes. Relat. Metab. Disord.**, v.26, n.4, p.29-33, 2002.
- FOOD AND DRUG ADMINISTRATION (FDA). Medwatch - The FDA medical products reporting system. 2000. Summary of safety-related drug labeling changes approved by FDA. U.S. Disponível em: <http://www.fda.gov/medwatch/safety>. Acesso em: Novembro de 1999.
- FRANÇA, L.R.; AVELAR, G.F.; ALMEIDA, F.F.L. Spermatogenesis and transit through the epididymis in mammals with emphasis on pigs. **Theriogenology**, v.63, n.2, p.300-318, 2005.
- FRANCIA-FARJE, L.A.D. et al. Sibutramine effects on the reproductive performance of pregnant overweight and non-overweight rats. **J. Toxicol. Environ. Health A**, v.73, n.13, p.985-990, 2010.
- FUJIOKA, K. et al. Weight loss with sibutramine improves glycaemic control and other metabolic parameters in obese patients with type 2 diabetes mellitus. **Diabetes Obes. Metab.**, v.2, n.3, p.175-187, 2000.
- GATTI, J.L. et al. Post-testicular sperm environment and fertility. **Anim. Reprod. Sci.**, v.82-83, n.1, p.321-339, 2004.
- GIULIANO, F. 5-Hydroxytryptamine in premature ejaculation: opportunities for therapeutic intervention. **Trends Neurosci.**, v.30, n.2, p.79-84, 2007.

GONG, Y.G. et al. Deprivation of testicular innervation induces apoptosis of Leydig cells via caspase-8-dependent signaling: a novel survival pathway revealed. **Biochem. Biophys. Res. Commun.**, v.382, n.1, p.165-170, 2009.

HANSEN, D.L. et al. Thermogenic effects of sibutramine in humans. **Am. J. Clin. Nutr.**, v.68, n.6, p.1180-1186, 1998.

HANSEN, D.L. et al. The effect of sibutramine on energy expenditure and appetite during chronic treatment without dietary restriction. **Int. J. Obes. Relat. Metab. Disord.**, v.23, n.10, p.1016-1024, 1999.

HAYWARD, S.W. et al. Epithelial developmental in the rat ventral prostate, anterior prostate and seminal vesicle. **Acta Anat. (Basel)**, v.155, n.2, p.81-93, 1996a.

HAYWARD, S.W. et al. Stromal developmental in the ventral prostate, anterior prostate and seminal vesicle of the rat. **Acta Anat. (Basel)**, v.155, n.2, p.94-103, 1996b.

HERMO, L.; ROBAIRE, B. Epididymal cell types and their functions. In: Robaire, B.; Hinton, B.T. (Eds.). **The Epididymis: from molecules to clinical practice**. New York: Kluwer Academic/ Plenum Publisher, 2002. p.81-102.

HIRSCH, J.; MACKINTOSH, R.M.; ARONNE, L.J. The effects of drugs used to treated obesity on the autonomic nervous system. **Obes. Res.**, v.8, n.3, p.227-233, 2000.

JAMES, W.P. et al. Effect of sibutramine on weight maintenance after weight loss: a randomised trial. STORM Study Group. Sibutramine Trial of Obesity Reduction and Maintenance. **Lancet**, v.356, n.9248, p.2119-2125, 2000.

JONES, R.C. To store or mature spermatozoa? The primary role of the epididymis. **Int. J. Androl.**, v.22, n.2, p.57-67, 1999.

JUNQUEIRA, L.C.; CARNEIRO, J. Aparelho reprodutor masculino. In: \_\_\_\_\_. **Histologia básica**. Rio de Janeiro: Guanabara Koogan, 2004. p.415-431.

KALECZYC, J. et al. Adrenergic innervation of the epididymis, vas deferens, accessory genital glands and urethra in the boar. **Folia Histochem. Cytobiol.**, v.31, n.3, p.117-123, 1968.

KEMPINAS, W.D.G et al. Fertility of rat epididymal sperm after chemically and surgically induced sympathectomy. **Biol. Reprod.**, v.59, n.4, p.897-904, 1998.

KEPPER, M.E.; KEAST, J.R. Location, immunohistochemical features, and spinal connections of autonomic neurons innervating the rat seminal vesicles. **Biol. Reprod.**, v.57, n.5, p.1164-1174, 1997.

KIERSZENBAUM, A.L. Transporte e maturação de espermatozóides. In: \_\_\_\_\_. **Histologia e Biologia Celular**. Rio de Janeiro: Elsevier, 2008. p.601-639.

LUQUE, C.A.; REY, J.A. Sibutramine: a serotonin-norepinephrine reuptake-inhibitor for the treatment of obesity. **Ann. Pharmacother.**, v.33, n.9, p.968-978, 1999.

MCMAHON, F.G. et al. Efficacy and safety of sibutramine in obese white and African American patients with hypertension: a 1-year, double-blind, placebo-controlled, multicenter trial. **Arch. Intern. Med.**, v. 60, n.14, p.2185-2191, 2000.

MCNEAL, J.E. The zonal anatomy of the prostate. **Prostate**, v.2, n.1, p.35-49, 1981.

NOJIMOTO, F.D. et al. Multiple effects of sibutramine on ejaculation and on vas deferens and seminal vesicle contractility. **Toxicol. Appl. Pharmacol.**, v.239, n.3, p.233-240, 2009.

ORGBIN-CRIST, M.C. Studies on the function of the epididymis. **Biol. Reprod.**, v.1, suppl 1, p.S155-175, 1969.

RANG, H.P. et al. **Rang and Dale's Pharmacology**. 6ed. Nova Iorque: Churchill/Livingstone, 2006. CD-ROM.

REID, B.L.; CLEAND, K.W. The structure function of the epididymis. I-The histology of the rat epididymis. **Aust. J. Zool.**, v.5, n.3, p.223-251, 1957.

RICHTER, W.O. How safe are the new obesity drugs? Indications and contraindications of orlistat and sibutramine. **MMW Fortschr. Med**, v.141, n.49-50, p.32-36, 1999.

RICKER, D.D. The Autonomic Innervation of the epididymis: its effects on epididymal function and fertility. **J. Androl.**, v.19, n.1, p.1-4, 1998.

RODRIGUES, A.O.; MACHADO, M.T.; WROCLAWSKI, E.R. Prostate innervation and local anesthesia in prostate procedures. **Rev. Hosp. Clin. Fac. Med. São Paulo**, v.57, n.6, p.287-292, 2002.

RODRIGUES, J.A.; FAVARETTO, A.L.V. Sistema reprodutor. In: AIRES, M.M. (Ed.). **Fisiologia**. Rio de Janeiro: Guanabara Koogan, 1999. p.877-917.

ROY-BURMAN, P. et al. Genetically defined mouse models that mimic natural aspects of human prostate cancer development. **Endocr. Relat. Cancer**, v.11, n.2, p.225-324, 2004.

RUSSELL, L.D. et al. Histological and Histopathological Evaluation of the Testis. **Int. J. Androl.**, v.16, n.1, p.83, 1993.

SCHEEN, A.J. Cardiovascular risk-benefit profile of sibutramine. **Am. J. Cardiovasc. Drugs**, v.10, n.5, p.321-334, 2010.

SETCHELL, B.P. Innervation and vasculature of the excurrent duct system. In: ROBAIRE, B.; HINTON, B. T. (Eds.). **The Epididymis: from molecules to clinical practice**. New York: Kluwer Academic/ Plenum Publisher, 2002. p.35-48.

SOKOL, R.Z. The hypothalamic – pituitary – gonadal axis as a target for toxicants. In: SIPES, G.; MCQUEEN, C.A.; GANDOLFI, A.J. (Eds.). **Comprehensive toxicology**. Cambridge: Cambridge University Press, 1997. p.87-98.

SORONEN, P. et al. Sex steroid hormone metabolism and prostate cancer. **J. Steroid. Biochem. Mol. Biol.**, v.92, n.4, p.281-286, 2004.

SOSA, Z.Y. et al. Administration of noradrenaline in the autonomic ganglia modifies the testosterone release from the testis using an ex vivo system. **Int. J. Androl.**, v.32, n.4, p.391-398, 2009.

STOCK, M.J. Sibutramine: a review of the pharmacology of a novel anti-obesity agent. **Int. J. Obes. Relat. Metab. Disord.**, v.1, suppl.1, p.S25-29, 1997.

SULLIVAN, R. et al. Role of exosomes in sperm maturation during the transit along the male reproductive tract. **Blood Cells Mol. Dis.**, v.35, n.1, p.1-10, 2005.

TAN, H.M.; GUNDLACH, A.L.; MORRIS, J.M. Exaggerated feeding response to central galanin-like peptide administration in diet-induced obese rats. **Neuropeptides**, v.39, n.3, p.333-336, 2005.

TZIOMALOS, K.; KRASSAS, G.E.; TZOTZAS, T. The use of sibutramine in the management of obesity and related disorders: an update. **Vasc. Health Risk Manag.**, v.5, n.1, p.441-452, 2009.

WADDEN, T.A. et al. Randomized trial of lifestyle modification and pharmacotherapy for obesity. **N. Engl. J. Med.**, v.353, n.20, p.2111-2120, 2005.

WALSH, K.M.; LEEN, E.; LEAN, M.E. The effect of sibutramine on resting energy expenditure and adrenaline-induced thermogenesis in obese females. **Int. J. Obes. Relat. Metab. Disord.**, v.23, n.10, p.1009-1015, 1999.

WORLD HEALTH ORGANIZATION (WHO). Part I: The problem of overweight and obesity. In: **Obesity: preventing and managing the global epidemic**. Geneva: WHO Technical Report Series, 2000. 894p.

## **APÊNDICES**

## TABLES

Table 1. Serum hormonal levels.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 10)
FSH (ng/mL)	6.05 ± 0.46	6.10 ± 0.53
LH (ng/mL)	1.64 ± 0.32	1.50 ± 0.30
Testosterone (ng/mL)	2.23 ± 0.29	2.33 ± 0.48

Values expressed as mean ± SEM. Mann-Whitney test,  $p > 0.05$ .

Table 2. Reproductive organs and body weights.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 10)
Final body weight (g)	460.16 ± 11.52	437.34 ± 10.25
Testis (g)	1.74 ± 0.06	1.83 ± 0.06
Epididymis (mg)	715.86 ± 22.72	652.62 ± 13.37*
Ventral prostate (mg)	597.01 ± 40.33	437.17 ± 34.99*
Full seminal vesicle (g)	1.59 ± 0.13	1.37 ± 0.07
Empty Seminal vesicle (mg)	611.26 ± 38.12	563.53 ± 29.78
Vas deferens (mg)	131.51 ± 3.78	123.99 ± 5.21

Values expressed as mean ± SEM. Student's t-test, \* p <0.05.

Table 3. Sperm parameters.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 10)
Spermatid number ( $10^6$ /testis)	216.07 $\pm$ 9.45	235.22 $\pm$ 7.43
Daily sperm production ( $10^6$ /testis/day)	35.42 $\pm$ 1.55	38.56 $\pm$ 1.22
( $10^6$ /g testis /day)	24.67 $\pm$ 1.02	25.00 $\pm$ 1.02
Caput / corpus epididymis sperm number ( $10^6$ / organ)	136.18 $\pm$ 6.82	148.34 $\pm$ 7.79
Cauda epididymis sperm number ( $10^6$ /organ)	276.16 $\pm$ 12.37	180.98 $\pm$ 8.87*
Sperm transit time in the caput/corpus (days)	3.86 $\pm$ 0.16	3.86 $\pm$ 0.19
Sperm transit time in the cauda (days)	7.85 $\pm$ 0.32	4.73 $\pm$ 0.26*

Values are expressed as mean  $\pm$  SEM. Student's t- test, \* p <0.05.

Table 4. Sexual behavior endpoints.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 8)
Latency to the first intromission (s)	50.78 ± 10.09 (9)	37.25 ± 13.15 (8)
Number of intromissions until the first ejaculation	22.00 ± 1.97 (9)	17.25 ± 2.45 (8)
Latency to the first ejaculation (s)	1166.63 ± 123.72 (8)	999.29 ± 112.95 (7)
First post-ejaculatory intromission (s)	373.83 ± 40.78 (6)	461.83 ± 26.73 (6)
Number of post-ejaculatory intromissions	10.33 ± 1.41 (6)	10.17 ± 2.71 (6)
Number of ejaculations	1.62 ± 0.26 (8)	1.57 ± 0.20 (7)

Values are expressed as mean ± SEM. Latencies expressed in seconds. The number of animals that presented the behavior is indicated in parenthesis. Student's t- test, p >0.05.

Table 5. Fertility and reproductive performance.

	Experimental groups	
	Control (n = 10)	Sibutramine (n = 8)
Body weight of dams (g)	354.37 ± 8.63	373.96 ± 4.81
Number of corpora lutea	12.10 ± 0.57	13.37 ± 0.26
Number of implants	11.80 ± 0.70	12.37 ± 0.37
Number of resorptions	0.20 ± 0.20	0.50 ± 0.27
Number of live fetuses	11.60 ± 0.69	11.87 ± 0.40
Fetuses weight (g)	3.07 ± 0.05	3.07 ± 0.06
<sup>1</sup> Sex ratio (%)	100.00 (66.67 – 159.82)	78.57 (66.67 – 206.25)
<sup>1</sup> Fertility potential (%)	100.00 (100.00– 100.00)	96.43 (88.87 – 100.00)
<sup>1</sup> Pre-implantation loss (%)	0.00 (0.00 – 0.00)	3.57 (0.00 – 11.12)
<sup>1</sup> Post-implantation loss (%)	0.00 (0.00 – 0.00)	0.00 (0.00 – 7.63)

Values expressed as mean ± SEM. Student's t- test, p> 0.05.

<sup>1</sup> Values expressed as median (Q1 - Q3). Mann-Whitney test, p> 0.05

## FIGURE LEGENDS

Figure 1. Representative tracings of the contractile activity induced by vehicle (a) and in the presence of sibutramine 1 (b), 3 (c) and 10 (d)  $\mu\text{M}$  in the distal portion of rat cauda epididymis. (e) Tension developed in 5 min in the absence (vehicle, water) and presence of increasing concentration of sibutramine. Values are mean  $\pm$  SEM of 8 tissue segments taken from 4 different rats. \* Different from the tension developed in the presence of vehicle (ANOVA, followed by Newman-Keuls, \* $p < 0.0001$ , # $p < 0.005$ ).

Figure 2. Concentration-response curves to norepinephrine in the absence and presence of increasing concentrations of sibutramine. Each symbol represents the mean, and the vertical bars, the SEM of 8 tissues taken from 4 rats.

## FIGURES

Figure 1

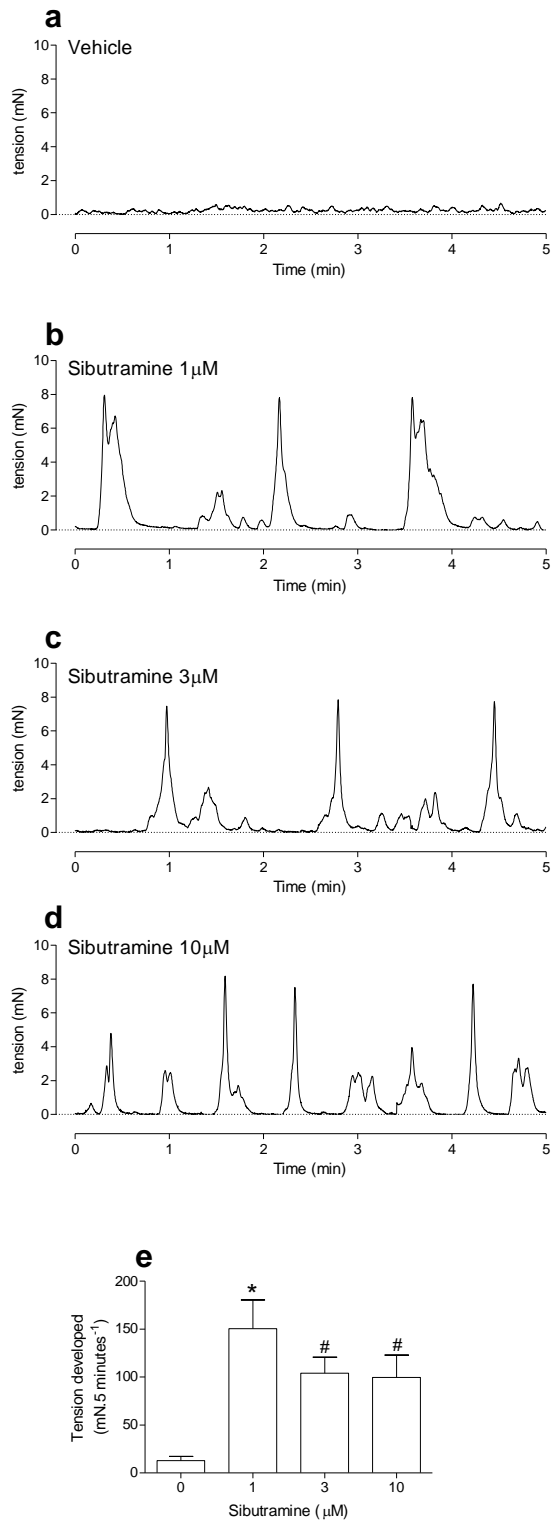
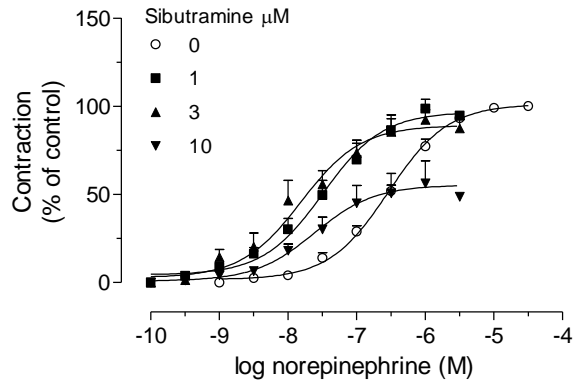


Figure 2



Parecer da Comissão de Ética na Experimentação Anicaml (CEEA) do Instituto  
de Biociências-UNESP



UNIVERSIDADE ESTADUAL PAULISTA  
"JÚLIO DE MESQUITA FILHO"  
Campus de Botucatu



## CERTIFICADO

Certificamos que o Protocolo nº **115-CEEA**, sobre "EFEITOS DA SIBUTRAMINA SOBRE O SISTEMA REPRODUTOR MASCULINO DE RATOS NORMAIS E OBESOS", sob a responsabilidade de **WILMA DE GRAVA KEMPINAS**, está de acordo com os Princípios Éticos na Experimentação Animal adotado pelo Colégio Brasileiro de Experimentação Animal (COBEA) e foi aprovado pela **COMISSÃO DE ÉTICA NA EXPERIMENTAÇÃO ANIMAL (CEEA)**, em reunião de **03/04/2009**.

Botucatu, 3 de abril de 2009.



Prof. Dra. **PATRÍCIA FERNANDA F. PINHEIRO**  
Presidente - CEEA