

UNIVERSIDADE ESTADUAL DE CAMPINAS

Sérgio Pereira

**Proliferação Celular da Próstata Ventral de Ratos Castrados e
Vasectomizados**

**Tese apresentada ao Instituto de Biologia
para obtenção do Título de Mestre em
Biologia Celular e Estrutural na área de
Anatomia.**

Orientador: Prof. Dr. Wílson de Mello Júnior

**FICHA CATALOGRÁFICA ELABORADA PELA
BIBLIOTECA DO INSTITUTO DE BIOLOGIA - UNICAMP**

P414p

Pereira, Sérgio

Proliferação celular da próstata ventral de ratos castrados e
vasectomizados / Sérgio Pereira . --
Campinas, SP:[s.n.], 2003.

Orientador: Wilson de Mello Júnior

Dissertação (mestrado) – Universidade Estadual de Campinas.
Instituto de Biologia.

1. Proliferação celular. 2. Próstata. 3. Castração. 4. Vasectomia. 5. Rato.
I. Mello Júnior, Wilson. II. Universidade Estadual de Campinas. Instituto de
Biologia. III. Título.

Campinas, 29 de janeiro de 2004.

Banca Examinadora

Prof. Dr. Wilson de Mello Júnior (Orientador)

Assinatura

Profa. Dra. Máira Aparecida Stefanini

Assinatura

Prof. Dr. Sebastião Roberto Taboga

Assinatura

Prof. Dr. Francisco Eduardo Martinez

Assinatura

DEDICATÓRIA

Dedico,

A *DEUS*, porque sem Ele nada seria possível.

Aos *MEUS PAIS*, *Joaquim Geraldo Pereira e Aparecida do Prado Pereira*, que muitas vezes renunciaram aos seus sonhos para que pudesse realizar o meu, pela dedicação e apoio incondicionais.

À *ANA LÚCIA BANHARA*, pelo companheirismo, incentivo e amor.

A toda *MINHA FAMÍLIA*, pelo amparo material e espiritual que me ofereceu até aqui.

AGRADECIMENTOS

Em especial ao Prof. Dr. *WÍLSON DE MELLO JÚNIOR*, antes orientador agora amigo, que me orientou sempre com muita dedicação e diligência, incentivando-me a prosseguir no caminho da ciência.

Em especial, ainda, ao Prof. Dr. *FRANCISCO EDUARDO MARTINEZ*, que me orientou no início do mestrado com dedicação, sem medir esforços para que pudesse atingir meus objetivos.

Aos Professores Dr. *FRANCISCO EDUARDO MARTINEZ*, Dr. *SEBASTIÃO ROBERTO TABOGA* e Dr. *HERNANDES FAUSTINO DE CARVALHO*, pelas valiosas sugestões, durante a análise prévia deste trabalho, que colaboraram e muito com a redação final da tese.

Aos *PROFESSORES* do Programa de Pós-Graduação em Biologia Celular e Estrutural do Instituto de Biologia da Universidade Estadual de Campinas (UNICAMP) com quem pude aprender muito durante as disciplinas cursadas.

À *LÍLIAM ALVES SENNE PANAGIO*, secretária do Departamento de Biologia Celular da Universidade Estadual de Campinas (UNICAMP), pelos serviços prestados e, ainda, pela atenção, compreensão e paciência.

Aos *PROFESSORES* do Departamento de Anatomia do Instituto de Biociências da Universidade Estadual Paulista (UNESP), *Campus* Botucatu, por terem disponibilizado os laboratórios para o desenvolvimento deste trabalho.

Aos *FUNCIONÁRIOS* do Departamento de Anatomia do Instituto de Biociências da Universidade Estadual Paulista (UNESP) *Campus* Botucatu pelos valiosos serviços prestados.

Aos *AMIGOS DE LABORATÓRIO*, Patrícia Fernanda Felipe Pinheiro, Raquel Fantin Domeniconi, Camila Contin Diniz de Almeida, Luiz Alberto Domingo Francia Farje, Kátia Aparecida da Silva Viegas, Karina Simões, Gelson Rodrigues e Rodrigo Vieira Rodrigues, pelo auxílio, pelas dicas e pela amizade.

Aos *AMIGOS DE CURSO* e aos *AMIGOS DE REPÚBLICA*, pela amizade e agradável convívio.

Ao *CONSELHO NACIONAL DE PESQUISA CIENTÍFICA E TECNOLÓGICA* (CNPq) e a *COORDENADORIA DE APERFEIÇOAMENTO DE PESSOAL DE NÍVEL SUPERIOR* (CAPES) pelo suporte financeiro.

Enfim, a *TODOS* que de forma direta ou indireta, colaboraram para a realização deste trabalho ou, ainda, para a minha formação pessoal e profissional: *FAMILIARES, AMIGOS, COLEGAS, PROFESSORES E FUNCIONÁRIOS*.

“Há homens que lutam um dia e são bons,
Há homens que lutam um ano e são melhores,
Há os que lutam muitos anos e são muito bons,
Mas há os que lutam toda a vida e esses são imprescindíveis.”

Bertold Brecht

“Toda a nossa ciência, comparada com a realidade, é primitiva e infantil...
E, ainda assim, é a coisa mais preciosa que nós temos.”

Albert Einstein

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RESUMO

Doenças como o câncer e a hiperplasia benigna de próstata estão relacionadas à falha no mecanismo de regulação do equilíbrio funcional entre os processos de proliferação celular e apoptose nas células prostáticas. Esse equilíbrio é controlado por níveis séricos de andrógenos, KFG (keratinocyte growth factor), EGF (epidermal growth factor), IGFs (insulinlike growth factors) e TGFs (transforming growth factors). A castração provoca quebra abrupta dos níveis circulantes de andrógenos, alterando esse equilíbrio. A vasectomia pode interferir na ação de fatores de crescimento tumorais e, também, na produção local de fatores de crescimento, alterando tal equilíbrio. Assim, o presente trabalho teve como objetivo avaliar a proliferação celular, a apoptose e a altura do epitélio secretor, nas regiões distal e intermediária do sistema ductal, da próstata ventral de ratos, 48h e 7 dias após castração e vasectomia. Foram realizados estudos de imuno-histoquímica com marcadores de proliferação celular (PCNA e Ki 67), estudo de citoquímica para detecção de células apoptóticas e, também, estudo morfométrico do epitélio secretor do sistema ductal prostático. Observou-se que os índices de proliferação celular diminuíram significativamente, em ambas regiões do sistema ductal prostático, 48h e 7 dias pós-cirurgia, comparado ao grupo controle. O índice de apoptose, entretanto, aumentou drasticamente 48h após castração em ambas as regiões, declinando aos 7 dias após castração. Os índices de proliferação não diferiram significativamente 48h após vasectomia, porém aumentaram 7 dias após vasectomia, em ambas as regiões do sistema ductal. No entanto, o índice de apoptose não diferiu significativamente em ambos os momentos estudados após vasectomia. Assim, a castração causa desequilíbrio entre os processos de

proliferação celular e de apoptose em favor da apoptose, enquanto que a vasectomia causa desequilíbrio em favor da proliferação celular.

ABSTRACT

Proliferative disorders such as cancer and benign prostatic hyperplasia are related to the disruption in the mechanism of regulating the balance between both processes of cell proliferation and apoptosis in the prostatic cells. That balance is controlled by androgens, keratinocyte growth factor (KGF), epidermal growth factor (EGF), insulinlike growth factors (IGFs) and transforming growth factors (TGFs). After castration, the blood levels of androgens decrease abruptly, altering that balance. Vasectomy might interfere in action of TGF and in local production of growth factor, altering that balance. Thus this study evaluates the cell proliferation, apoptosis and height of the secretory epithelium in both distal and intermediate regions of the ductal system of rat ventral prostate 48h and 7 days after castration and vasectomy. The histological sections were immunohistochemical stained using the antibodies anti-PCNA and anti-Ki 67 for detection of cell proliferation and cytochemically stained using *Fuelgen* reaction for detection of apoptosis. The height of the secretory epithelium was measured through the Image Analysis System KS-300 (ZEISS). It was observed that the indices of cell proliferation decreased significantly in both regions of the ductal system 48h and 7 days after castration compared to the sham operated group. The index of apoptosis increased significantly after 48h, declining 7 days after castration. The indices of cell proliferation did not differ significantly after 48h however they increased 7 days after vasectomy in both regions of the ductal prostatic system. The index of apoptosis did not differ significantly in either study moment after vasectomy. Thus castration caused imbalance between both processes of cell proliferation

and apoptosis in favor of apoptosis, whereas vasectomy caused imbalance in favor of cell proliferation.

INTRODUÇÃO GERAL

Morfofisiologia da próstata

A próstata, glândula sexual acessória de fundamental importância no processo reprodutivo, secreta líquido fino de aspecto leitoso, essencial para a motilidade e fertilidade dos espermatozóides, contribuindo para que a fertilização tenha êxito (Aumüller, 1989). A próstata de roedores é uma glândula complexa formada por três pares de lobos: ventral, dorsolateral e anterior (Jesik *et al.*, 1982). A próstata ventral, glândula túbulo-alveolar, é revestida por epitélio simples. Cada alvéolo consiste de células colunares secretoras estreameadas por células basais assentadas em nítida membrana basal (Carvalho & Line, 1996). O estroma é formado por fibras colágenas e elásticas, células musculares lisas, fibroblastos e vasos sangüíneos (Brandes & Portela, 1960; Carvalho *et al.*, 1997ab).

A próstata ventral de ratos é constituída por oito sistemas ductais. Cada sistema ductal prostático é a unidade funcional que compartilha um único ducto de drenagem da secreção na uretra. Cada um dos sistemas ductais pode ser dividido em três regiões – proximal, intermediária e distal – com características morfológicas diferentes (Janulis & Lee, 1998).

Na região distal, a mais distante da uretra, as células epiteliais são colunares altas e seu núcleo está freqüentemente localizado na parte apical. Figuras mitóticas podem ser identificadas ocasionalmente nas células dessa região. As células epiteliais colunares da região intermediária são mais baixas que as da distal, seu núcleo está localizado

basalmente. Células em apoptose não são evidentes nem na região distal, nem na intermediária. Na região proximal, imediatamente à uretra, as células são colunares baixas ou cúbicas e estão em processo de morte celular ou apoptose (Janulis & Lee, 1998).

O crescimento normal, a diferenciação e a manutenção da integridade funcional (secretora) e estrutural da próstata são dependentes de níveis constantes de andrógenos circulantes e ocorrem através de interações recíprocas entre o estroma e o epitélio (Price, 1963; Pour *et al.*, 1989; Kyprianou *et al.*, 1996; Cunha *et al.*, 1996).

Alguns estudos sugerem que os andrógenos possuem três funções principais nas células prostáticas que contribuem para o desenvolvimento, crescimento e manutenção da próstata: (1) podem estimular a proliferação, especialmente nas células epiteliais; (2) estimulam a diferenciação das células epiteliais secretoras; (3) inibem a apoptose de células prostáticas (Buttayan *et al.*, 1999; Maffini *et al.*, 2002; Lissbrant *et al.*, 2003).

Além disso, os andrógenos auxiliam na regulação do equilíbrio funcional entre os processos de proliferação celular e de apoptose (morte celular). Doenças como o adenocarcinoma e a hiperplasia benigna de próstata, que acometem o homem com frequência, estão relacionadas à falha nos mecanismos de controle desse equilíbrio entre os processos de proliferação e de apoptose das células prostáticas (Xie *et al.*, 2000).

Castração

A castração provoca queda abrupta e significativa nos níveis séricos de andrógenos, bem como no fluxo sanguíneo para a próstata, que é controlado pelos níveis de andrógenos. Essa redução induz apoptose nas células endoteliais dos vasos prostáticos e nas células secretoras (Kyprianou & Issacs, 1988). Em estudo realizado por Shabsigh *et al.* (1998), constatou-se que o fluxo sanguíneo para a próstata ventral de rato está reduzido em 38% durante as primeiras 18h e 48% durante as primeiras 24h, após a castração. Shabsigh *et al.* (1999) realizaram a quantificação de células apoptóticas após castração e demonstraram que a apoptose inicia após 24h, aumenta drasticamente 48h e 72h após castração e declina-se em seguida. Segundo os mesmos autores, a redução do fluxo sanguíneo precede o aparecimento de células epiteliais apoptóticas, caracterizando a relação potencial causal entre os efeitos dos andrógenos sobre o fluxo sanguíneo da próstata e a regressão da próstata ventral de rato.

Após a castração, o epitélio regride e as células epiteliais remanescentes da próstata continuam sensíveis aos andrógenos. A reposição de testosterona regenera o epitélio prostático, restaurando a função e o número de células normais (Risbridger *et al.*, 2001). As células da próstata ventral de rato respondem aos andrógenos pela alteração de uma vasta cadeia de processos bioquímicos, envolvidos na secreção e/ou proliferação celular, dependendo do estado funcional da glândula. A testosterona é bem conhecida por induzir alterações na síntese de DNA, RNA e proteínas na próstata ventral de rato (Okuda *et al.*, 1991).

Embora muitos trabalhos utilizem a castração como ferramenta para a compreensão da fisiologia dos órgãos dependentes de andrógenos (Shabisgh *et al.*, 1999; Ílio *et al.*, 2000; Vilamaior *et al.*, 2000; Acosta *et al.*, 2001), poucos enfatizam a análise quantitativa da proliferação celular.

Vasectomia

A vasectomia é uma cirurgia simples, econômica e sem muitas complicações pós-operatórias, utilizada como método anticoncepcional masculino desde a primeira metade do século passado. Na década de sessenta, já havia no mundo cerca de 50 milhões de homens vasectomizados. Nessa cirurgia, o ducto deferente é seccionado, impedindo que os espermatozóides sejam expelidos juntamente com o líquido seminal durante a ejaculação (Linnet, 1983).

Há alguns anos surgiu controvérsia sobre a correlação entre a vasectomia e o câncer de próstata. Embora alguns estudos epidemiológicos tenham apontado aumento do risco de câncer de próstata em homens vasectomizados (Giovanucci *et al.*, 1993ab; Emard *et al.*, 2001), outro relaciona a vasectomia à redução do risco de câncer de próstata (Ross *et al.*, 1983) e outros, ainda, inferem que não há correlação (Bernal-Delgado *et al.*, 1998; Lesko *et al.*, 1999; Stanford *et al.*, 1999, Cox *et al.*, 2002). Fazendo a análise dos trabalhos publicados, observa-se que a controvérsia persiste até hoje, principalmente, quando a vasectomia é realizada precocemente.

Howards (1993) levantou quatro hipóteses para tentar relacionar vasectomia com o risco de câncer de próstata. São elas: (1) endocrinológica, a vasectomia poderia alterar os parâmetros hormonais favorecendo o desenvolvimento do câncer de próstata; (2) imunológica, o aumento na produção de anticorpos antiespermático após vasectomia poderia, através de via desconhecida, favorecer o desenvolvimento do câncer, (3) do inibidor do fator de crescimento, a vasectomia, através de via indefinida, impediria a ação dos inibidores dos fatores de crescimento, ou ainda, aumentaria a produção local de fatores

de crescimento, favorecendo o desenvolvimento e/ou a progressão do câncer; (4) do inibidor direto do câncer e/ou fator de crescimento prostático, alterações no fluido seminal provocadas pela vasectomia poderiam favorecer o desenvolvimento do câncer de próstata. Porém essas hipóteses foram pouco discutidas e não apresentaram estudos conclusivos.

Embora os trabalhos epidemiológicos discorram sobre a correlação entre a vasectomia e o câncer de próstata, informações sobre a morfologia e a fisiologia da próstata após vasectomia são escassas e insuficientes para o entendimento dessa possível relação, ou mesmo para a compreensão plena da morfofisiologia desse órgão.

Proliferação celular e apoptose

Os processos de proliferação celular e de apoptose são importantes no desenvolvimento normal e neoplásico. Ambos processos são atividades biológicas fundamentais tanto para processos fisiológicos como patológicos. A regulação do ciclo celular é dependente de proteínas quinases (Wang *et al.*, 2003) e outras proteínas como a p16, p27 e p53 (Fernandez *et al.*, 2002). Assim, o ciclo celular pode ser estudado em diferentes fases que podem ser relacionadas a eventos ou a alterações moleculares. Essas fases e eventos moleculares são de grande interesse para histopatologistas e biólogos moleculares, sendo um enfoque atual (Polek *et al.*, 2003; Davis & Day, 2002; Jiang *et al.*, 2002; Venkatesweram *et al.*, 2002; Maffini *et al.*, 2002).

O PCNA (Proliferation Cell Nuclear Antigen) é a proteína acessória da DNA polimerase, que é sintetizada nas fases G1 e S do ciclo celular. O PCNA, entretanto, correlaciona-se com o estado proliferativo da célula. A detecção imuno-histoquímica do PCNA indica que a célula está em divisão e não na fase G0 do ciclo. Já o Ki 67, outro marcador imuno-histoquímico para proliferação celular, é encontrado no exterior dos nucléolos, especialmente nos componentes granulares durante as fases G1, G2 e M (Leite *et al.*, 1999; Marker *et al.*, 2003).

A apoptose, morte celular induzida ou antecipada por ação de estímulos fisiológicos, está associada a uma série de alterações histológicas e bioquímicas bem definidas. Tais alterações incluem uma redução drástica na síntese de proteínas totais, RNA e DNA, que é, entretanto, acompanhada de um aumento concomitante na síntese de uma subclasse específica de RNA e proteínas, embebição de água e aparecimento de corpos

apoptóticos e vacúolos autofágicos responsáveis pela degradação do material celular (Kyprianou & Isaacs, 1988). Essas alterações histológicas e bioquímicas podem ser detectadas por métodos citoquímicos, histoquímicos e imuno-histoquímicos.

O monitoramento da proliferação celular e da apoptose pode ser utilizado para observar os efeitos de tratamento hormonal em cânceres prostáticos (Pour *et al.*, 1989; Lissbrant *et al.*, 2003), ou ainda, para a compreensão dos mecanismos do câncer e da hiperplasia benigna de próstata (Kyprianou *et al.*, 1996; Xie *et al.*, 2000). No entanto, pesquisas, como as realizadas por Sulik (2002), fazem apenas uma abordagem qualitativa da proliferação celular prostática e não quantitativa com marcadores específicos das células em proliferação.

OBJETIVO

Estudar a proliferação celular, a apoptose e a altura do epitélio secretor nas regiões distal e intermediária do sistema ductal da próstata ventral de ratos castrados e vasectomizados.

“Repercussions of Castration and Vasectomy on the Ductal System of the Rat Ventral Prostate”

Sérgio Pereira^{1,2}, Wilson de Mello Júnior².

¹Departament of Cell Biology, Biology Institute, UNICAMP, Campinas, São Paulo, Brazil.

²Departament of Anatomy, Bioscience Institute, UNESP, Botucatu, São Paulo, Brazil.

Address for correspondence:

Prof. Dr. Wilson de Mello Júnior

Departament of Anatomy – Bioscience Institute – UNESP

Distrito de Rubião Júnior, Botucatu, São Paulo, Brasil CEP: 18.618-000

Telefone: +55 14 38116040

E-mail: mellojr@ibb.unesp.br, s019631@dac.unicamp.br

Abstract

BACKGROUND: Diseases such as cancer and benign prostatic hyperplasia are related to disruption in the mechanism regulating the balance between both processes of cell proliferation and apoptosis in prostatic cells. As castration and vasectomy might alter that balance, this study evaluates the cell proliferation, the apoptosis and the height of the secretory epithelium in both distal and intermediate regions of the ductal system of the rat ventral prostate 48h and 7 days after castration and vasectomy.

METHODS: The histological sections were immunohistochemically stained using the antibodies anti-PCNA and anti-Ki 67 for detection of cell proliferation and cytochemically stained using *Fuelgen* reaction for detection of apoptosis. The height of the secretory epithelium was measured.

RESULTS: It was observed that the cell-proliferation indices decreased significantly in both regions of the ductal system 48h and 7 days after castration compared to the sham-operated group. The apoptotic-cell index increased significantly after 48h, declining 7 days after castration. The cell-proliferation indices did not differ after 48h significantly however they increased 7 days after vasectomy in both regions of the ductal system. The apoptotic-cell index did not differ significantly in either study moment after vasectomy.

CONCLUSIONS: Castration caused imbalance between the processes of cell proliferation and apoptosis in favor of apoptosis, whereas vasectomy caused imbalance in favor of cell proliferation.

KEYWORDS: cell proliferation, apoptosis, ventral prostate, vasectomy, castration, rat.

Introduction

The epidemiological studies that associate vasectomy with prostate cancer are contradictory and not conclusive. Although some studies have indicated increased risk of prostate cancer in vasectomized men [1, 2, 3], another study reports that vasectomy decreases of that relative risk [4] and others infer that there is no association between vasectomy and prostate cancer [5, 6, 7].

Prostate cancer is one of the most frequent carcinomas found in mankind. Its incidence is increasing greatly as life quality expands longevity. Benign prostatic hyperplasia is also a common pathology in men. Both diseases are related to disruption in the molecular mechanism that regulates the delicate balance between cell proliferation and cell death in the prostate [8].

The balance between these processes is controlled by androgens, keratinocyte growth factor (KGF), epidermal growth factor (EGF) and insulinlike growth factors (IGFs) that stimulate cell proliferation and inhibit apoptosis. That balance is also controlled by transforming growth factor- β (TGF- β) which possesses antagonistic functions; in other words, TGF- β stimulates apoptosis and inhibits cell proliferation [9]. To execute such functions, the androgens act in a great chain of biochemical processes – for example, synthesis of DNA, RNA and proteins – involved in molecular mechanisms of cell proliferation and apoptosis [10].

After castration, the blood levels of androgens decrease abruptly, as well as the blood flow in the prostate, which is controlled by androgen levels. That reduction induces apoptosis in both endothelial and secretory epithelial cells [11].

According to Howards [12], it is possible that vasectomy prevents the inhibitors of transforming growth factors from reaching the prostate, or, in an undefined way, vasectomy increases the local production of the growth factors which favor the development and/or progression of prostate cancer.

Thus, this study evaluated the cell proliferation, apoptosis and height of the secretory epithelium in both distal and intermediate regions of the ventral-prostate ductal system of castrated and vazectomized rats.

Materials and methods

Forty-eight adult male Wistar rats about a hundred days old were divided into three experimental groups: castrated, vasectomized and sham-operated (control). Each group was studied at two moments: 48h and 7 days after surgery.

1. Surgeries:

The rats were weighted and afterwards anaesthetized with ketamin and xylasin intraperitoneally. The surgeries were performed under aseptic conditions through median sagittal abdominal incision.

1.1. Castrated group:

The ductus deferens and the testis vessels were fastened bilaterally and afterwards the testis and epididymis were removed.

1.2. Vasectomized group:

The deferent ducts were fastened at two points. The first point was located about 1.5 cm from the duct end in the urinary bladder base whereas the second point was 0.5 cm from the first. Afterwards the small fragment of the ductus deferens between fastenings was removed. The deferens vessels were fastened and removed also.

1.3. Sham-operated group (control):

The genital organs were manipulated and the abdominal incision subsequently sutured.

In the vasectomized and sham-operated groups, the rats were observed post-surgery, to look for cryptorchidism.

2. Morphometric study:

The rats were weighted after euthanasia with excess of anesthetic. The ventral prostate was collected 48h and 7 days after surgeries and afterwards weighted. Fragments of the ventral prostate were fixed in Bouin's solution and histologically processed. The histological sections with 5µm of thickness were stained with hematoxylin-eosyn, examined and photographed with an AXIOPHOT 2 photomicroscope (ZEISS). Four different sections of each animal were used to measure the height of the secretory epithelium in both distal and intermediate regions of the ductal system of the rat ventral prostate. These sixty measures were made through Image Analysis System KS-300 (ZEISS).

3. Detection of cell proliferation:

The histological sections were immunohistochemically stained using the antibodies anti-PCNA and anti-Ki 67 (Novocastra Laboratories Ltd) according to the manufacturer's instructions.

4. Detection of apoptosis:

The occurrence of apoptosis was determined by evaluation of the morphological alterations after the Feulgen reaction.

5. Quantitative analysis of immunohistochemical and cytochemical staining

The counting of cells was done with an AXIOPLAN 2 microscope, using 40x objective. In total, about 1000 cells were counted in each region of the ductal prostatic system. The indices of PCNA and that of Ki 67-positive cells and that of apoptotic cells were expressed as a percentage from the ratio of positive cells to the total number of cells counted.

6. Statistical Analysis:

The values of the rat body weight before surgery (g), rat body weight after surgery (g), absolute ventral prostate weight (g) and relative ventral prostate weight (%), as well as the secretory epithelium height (μm), indices of PCNA and Ki 67-positive cells (%) and index of apoptotic cells (%) in both regions of the ductal prostatic system at both moments of the study were presented as the mean plus or minus the standard deviation. These data were statistically analyzed through variance analysis complemented by the Tukey test for multiple comparisons.

Results

The relative ventral prostate weight, in the groups castrated and vasectomized, did not present alteration 48h after surgery. They also stay unaffected 7 days after vasectomy, however the same decreased in the castrated group, compared to the group control.

Table 1. The corporal weight before surgery (in g), corporal weight after surgery (in g), absolute ventral prostate weight (in g) and relative ventral prostate weight (in %), presented as the mean plus or minus the standard deviation.

Parameters	Group	Study moment	
		48h	7 days
Corporal weight before surgery	Control	433.75 ± 17.97	415.00 ± 11.18
	Castrated	410.00 ± 16.43	412.50 ± 33.58
	Vasectomized	420.00 ± 44.32	418.75 ± 19.31
Corporal weight after surgery	Control	417.50 ± 15.55	395.00 ± 23.02
	Castrated	382.50 ± 22.75	387.50 ± 27.70
	Vasectomized	395.00 ± 40.53	411.25 ± 20.15
Absolute ventral prostate weight	Control	0.6532 ± 0.0806 ^{Aa*}	0.6113 ± 0.1231 ^{Ba}
	Castrated	0.4707 ± 0.0668 ^{Ab}	0.1173 ± 0.0156 ^{Aa}
	Vasectomized	0.6311 ± 0.0916 ^{Aa}	0.6085 ± 0.0404 ^b
Relative ventral prostate weight	Control	0.133 ± 0.029 ^{Aa}	0.109 ± 0.026 ^{Ba}
	Castrated	0.125 ± 0.021 ^{Ab}	0.033 ± 0.009 ^{Aa}
	Vasectomized	0.137 ± 0.028 ^{Aa}	0.120 ± 0.014 ^{Ba}

* Different upper-case letters indicate difference between groups; different lower-case letters signify difference between the moments studied; P ≤ 0.05.

The height of the secretory epithelium in the vasectomized rats was not different statistically from that of the sham-operated rats in both regions of the ductal prostatic system and at both moments of the study. However the secretory epithelium height in the castrated rats decreased significantly in both regions of the ductal prostatic system. The reduction was highest 7 days after castration (Fig. 1).

The immunoreactivity for PCNA in the secretory epithelial cells was equally distributed by nucleus. The index of PCNA-positive cells decreased significantly in the castrated group in both regions of the ductal prostatic system and at both moments of study compared with the sham-operated group. For the vasectomized group this index was not different 48h post-vasectomy, although it increased 7 days after surgery in both regions (Fig. 2).

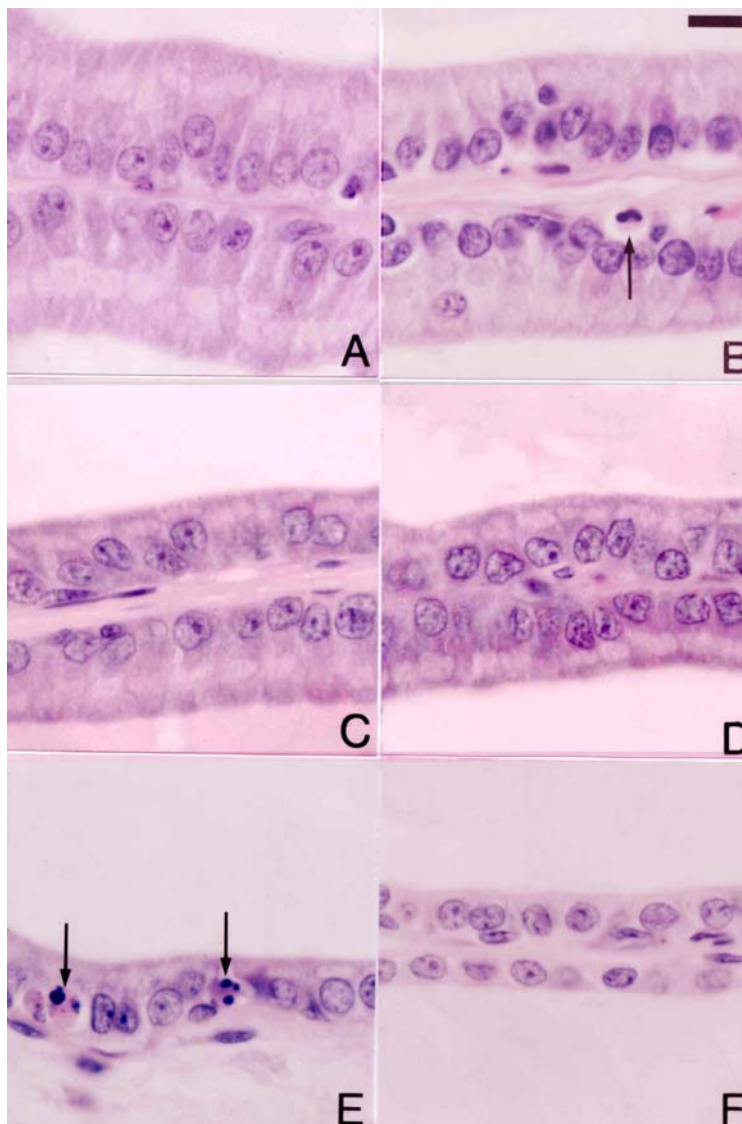
The immunoreactivity for Ki 67 in the secretory epithelial cells was located predominantly in the nucleoli, although diffuse staining throughout the nucleus was usually also seen. The index of Ki 67-positive cells in both regions of the ductal prostatic system and at both moments of the study presented a pattern similar to that of the PCNA index but with smaller absolute values. However, there were no Ki 67-positive cells in the intermediate region of the ductal prostatic system in the castrated group 7 days after castration (Fig. 2).

The apoptotic-cell index did not differ significantly 48h and 7 days after vasectomy in either region of the ductal system compared to the sham-operated group. However, that index increased significantly after 48h and it declined 7 days after castration in both regions.

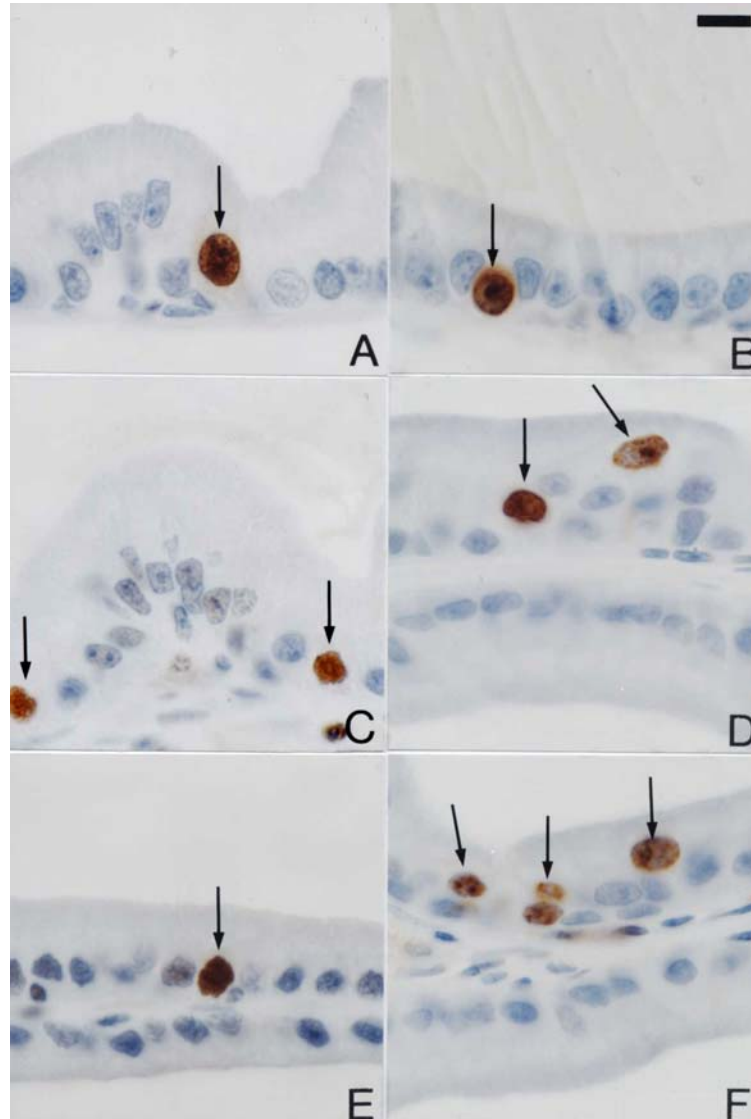
Table 2. Height (in μm), PCNA-positive cells indices (in %), Ki67-positive cells indices (in %) and apoptotic-cell indices (in %) in the secretory epithelium in the regions of the ductal system, presented as the mean plus or minus the standard deviation.

Parameter	Region	Group	Study moment	
			48h	7 days
Epithelium height	Distal	Control	64,40 \pm 5,58 ^{Ba*}	65,48 \pm 6,26 ^{Ba}
		Castrated	53,91 \pm 4,69 ^{Ab}	40,32 \pm 3,94 ^{Aa}
		Vasectomized	63,50 \pm 6,87 ^{Ba}	64,48 \pm 5,70 ^{Ba}
	Intermediate	Control	37,92 \pm 3,71 ^{Ba}	38,13 \pm 4,33 ^{Ba}
		Castrated	28,29 \pm 2,71 ^{Ab}	18,90 \pm 2,83 ^{Aa}
		Vasectomized	38,23 \pm 3,82 ^{Ba}	38,70 \pm 4,28 ^{Ba}
PCNA-positive cells indices	Distal	Control	1.13 \pm 0.14 ^{Ba}	1.07 \pm 0.17 ^{Ba}
		Castrated	0.58 \pm 0.07 ^{Ab}	0.23 \pm 0.05 ^{Aa}
		Vasectomized	1.22 \pm 0.21 ^{Ba}	3.40 \pm 0.80 ^{Cb}
	Intermediate	Control	0.65 \pm 0.09 ^{Ba}	0.63 \pm 0.11 ^{Ba}
		Castrated	0.27 \pm 0.07 ^{Ab}	0.05 \pm 0.05 ^{Aa}
		Vasectomized	0.65 \pm 0.19 ^{Ba}	1.52 \pm 0.28 ^{Cb}
Ki67-positive cells indices	Distal	Control	0.82 \pm 0.11 ^{Ba}	0.83 \pm 0.14 ^{Ba}
		Castrated	0.35 \pm 0.09 ^{Ab}	0.03 \pm 0.05 ^{Aa}
		Control	0.92 \pm 0.11 ^{Ba}	2.37 \pm 0.60 ^{Cb}
	Intermediate	Castrated	0.45 \pm 0.09 ^{Ba}	0.47 \pm 0.07 ^{Aa}
		Vasectomized	0.12 \pm 0.07 ^A	-----
		Vasectomized	0.47 \pm 0.11 ^{Ba}	1.15 \pm 0.17 ^{Bb}
Apoptotic-cell indices	Distal	Control	0.24 \pm 0.05 ^{Aa}	0.24 \pm 0.09 ^{Aa}
		Castrated	4.96 \pm 0.39 ^{Bb}	1.46 \pm 0.21 ^{Ba}
		Vasectomized	0.24 \pm 0.11 ^{Aa}	0.22 \pm 0.08 ^{Aa}
	Intermediate	Control	0.76 \pm 0.11 ^{Aa}	0.74 \pm 0.11 ^{Aa}
		Castrated	3.62 \pm 0.33 ^{Bb}	1.20 \pm 0.21 ^{Aa}
		Vasectomized	0.70 \pm 0.16 ^{Aa}	0.88 \pm 0.13 ^{Aa}

* Different upper-case letters indicate difference between groups within each region; different lower-case letters signify difference between the moments studied; $P \leq 0.05$.

Figure 1

Legend: Photomicrographs of the secretory epithelium of ventral prostate stained with hematoxylin-eosyn. **A.** Distal region, group control 7 days post-surgery, anti-PCNA; **B.** Distal region, castrated group 48h post-surgery; **C.** Intermediate region, control group 48h post-surgery; **D.** Intermediate region, vasectomized group 48h post-surgery; **E.** Intermediate region; castrated group 48h post-surgery; **F.** Intermediate region, castrated group 7 days post-surgery; **Arrow:** apoptotic cells; **Bar** = 0.2μm.

Figure 2

Legend: Photomicrographs of Immunohistochemistry (anti-PCNA and anti-Ki 67) of the secretory epithelium of ventral prostate, counterstained with hematoxylin. **A.** Distal region, group control 7 days post-surgery, anti-PCNA; **B.** Intermediate region, control group, 48h post-surgery, anti-Ki 67; **C.** Distal region, vasectomized group 48h post-surgery, anti-PCNA; **D.** Distal region, vasectomized group 48h post-surgery, anti-Ki 67; **E.** Intermediate region; castrated group 48h post-surgery, anti-PCNA; **F.** Distal region, vasectomized group 7 days post-surgery, anti-Ki 67; **Arrow:** PCNA or Ki 67-positive cells; **Bar** = 0.2µm.

Discussion

The relative ventral prostate weight, in the groups castrated and vasectomized, did not present alteration 48h after surgery. They also stay unaffected 7 days after vasectomy, however the same decreased in the castrated group, compared to the group control. Those results are in accordance with Shabisgh *et al.* [13] and Acosta *et al.* [14] which also told reduction of the weight of the ventral prostate after castration.

The morphology of the rat ventral prostate observed by light microscope is similar to descriptions from the specialized literature [15, 16]. The morphometric analysis showed that the height of secretory epithelium was higher in distal region than in the intermediate region of the ductal system of ventral prostate. That demonstrated the different functional states of the epithelium as observed also by Nemeth & Lee [17] which made possible the distinction between both regions of the ductal prostatic system in that study.

Comparing the indices of PCNA and that of Ki 67-positive cells in all the groups and the study moments verified the largest specificity of Ki 67, although the results and interpretations were the same for both proliferation markers, however with different absolute values. Those results are in accordance with the literature that reported PCNA immunoreactivity in all cell-cycle phases except in G0 as well as the presence of Ki 67 immunoreactivity in the G1, G2 and M phases of the cell cycle. Therefore, Ki 67 is more restricted than PCNA [18].

The proliferative index in the distal region of the sham-operated group was higher than that of the intermediate region of the ductal prostatic system, and the apoptotic index in the intermediate region was higher than that of the distal region. This fact corroborates

the literature data in which the researchers found that the distal region presents more proliferative activity while the cells in the intermediate region show augmented secretory activity [17, 19].

As the prostatic cells are androgen-dependent, the mean height of the secretory epithelium decreased in both regions of the ductal prostatic system after castration because castration promoted an abrupt fall in androgen blood levels [20]. Such reduction of circulating androgen levels can be the cause of the decrease in the proliferative indices in both regions of the ductal prostatic system in the castrated group because the androgens stimulate cell proliferation and inhibit apoptosis.

The apoptotic-cell index increased significantly after 48h and declined 7 days after castration in both regions of the ductal system. These results are in accordance with the literature which reported that apoptosis starts after 24h and increases dramatically at 48h and 72h after castration, declining thereafter [21].

As there were no morphologic or morphometric alterations in the secretory epithelium in the vasectomized group compared with the sham-operated group, we suggest that vasectomy does not alter the circulating androgen levels as Whibty *et al.* [22] and Miller *et al.* [23] observed when they measured hormonal levels in their studies.

Kyprianou *et al.* [24] related the benign prostatic hyperplasia in men to the imbalance between cell proliferation and apoptosis. Xie *et al.* [8] posited that the disruption in the balance between cell proliferation and apoptosis might result in transformation and progression of the premalignant lesions in the Noble rat. Cell proliferation increased while apoptosis did not alter in either region 7 days after vasectomy, indicating an imbalance between cell proliferation and apoptosis.

As suggested by Howards [12] the vasectomy might prevent the inhibitors of transforming growth factors or increase the local production of growth factors which favor the development and/or progression of prostate cancer. This might promote an imbalance between cell proliferation and apoptosis that would the development of hyperplasia or cancer [9, 25]. Perhaps such alterations promoted by vasectomy can explain the results obtained.

Thus, we conclude that vasectomy caused an imbalance between cell proliferation and apoptosis in favor of cell proliferation in the secretory epithelium in both the distal and intermediate regions of the ductal system 7 days after surgery, whereas castration caused an imbalance in favor of apoptosis. That genomic instability generated by the vasectomy allied to an environmental or genetic factor can cause hyperplasia or prostate cancer.

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CONCLUSÃO GERAL

A vasectomia causa desequilíbrio entre os processos de proliferação celular e de apoptose em favor da proliferação celular, no epitélio secretor nas regiões distal e intermediária do sistema ductal da próstata ventral de ratos aos 7 dias pós-cirurgia; porém não altera a morfologia e a altura do mesmo. A castração causa desequilíbrio em favor da apoptose e reduz a altura do epitélio secretor do sistema ductal prostático em ambos os momentos de estudo, promovendo atrofia da próstata ventral e reduzindo seu metabolismo. Essa instabilidade genômica gerada pela vasectomia, aliada a um fator ambiental ou genético, pode causar hiperplasia ou câncer de próstata.

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