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RENATO SIMÕES CORDEIRO

"Expressão de Receptores Androgênicos no Lóbulo Ventral da Próstata do Gerbilo da Mongólia"

Este exemplar corresponde à redação final da tese defendida pelo(a) candidato (a) Renato Simões Cordeiro.
e aprovada pela Comissão Julgadora.

Tese apresentada ao Instituto de Biologia para obtenção do Título de Doutor em Biologia Celular e Estrutural, na área de Biologia Celular.

Orientador: Prof. Dr. Sebastião Roberto Taboga

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"As coisas que o olho não viu, e o ouvido não ouviu,
e não subiram ao coração do homem
são as que Deus preparou para os que o amam"

SUMÁRIO

I. RESUMO

O crescimento normal, a diferenciação e a manutenção da integridade morfofuncional da glândula prostática são dependentes das interações de concentrações constantes de andrógenos com seus receptores. A necessidade de se estudar esta glândula em resposta aos hormônios e o efeito do bloqueio destes, deve-se ao fato da próstata humana ser o sítio de um grande número de doenças relacionadas à idade, sendo que as de maior importância clínica são o câncer prostático e a hiperplasia prostática benigna, as quais podem ser tratadas por estratégias de remoção de andrógenos. Este estudo teve por objetivo a análise imuno-histoquímica do grau de expressão do receptor androgênico (RA) no lóbulo ventral prostático do gerbilo após terapias de bloqueios androgênicos. Setenta e cinco gerbilos machos foram distribuídos, aleatoriamente, em 3 grupos de 25 animais, cada grupo representando uma fase do desenvolvimento pós-natal: jovem, adulto e senil. Em cada fase foi realizada uma análise morfológica e estereológica dos compartimentos prostáticos, bem como a análise imuno-histoquímica da expressão do RA. Além disso, estabeleceu-se a dosagem hormonal das concentrações séricas de testosterona, como método para verificar a relação da quantidade desse andrógeno com a expressão dos RA. Os resultados demonstraram haver um padrão heterogêneo de distribuição dos RA no lóbulo ventral ao longo do desenvolvimento pós-natal, em que quanto mais jovem for o animal maior a interação de andrógenos estimulando a expressão de RA nos compartimentos prostáticos. As terapias de bloqueios androgênicos diminuíram a expressão de RA no lóbulo ventral e a reposição androgênica após esses bloqueios não apresentou o mesmo grau de intensidade de expressão de RA próximo às condições fisiológicas normais. A regulação e a distribuição do RA nos tecidos prostáticos do gerbilo são mecanismos complexos e que, provavelmente, são geneticamente regulados por andrógenos antes do nascimento ou por outros fatores ainda desconhecidos. O gerbilo parece ser um modelo valioso na tentativa de melhorar o conhecimento do comportamento morfofisiológico e patológico dessa importante glândula em humanos ao longo do envelhecimento e da formulação de novas idéias de terapias de combate ao câncer de próstata.

II. ABSTRACT

The normal growth, differentiation and maintenance of the morphofunctional integrity of the prostate gland are dependent on the interaction of constant levels of androgens with their receptors. The need to study the responses to hormones under several conditions and the effect of their blockage is due to the fact that the human prostate is the site of a great number of age-related diseases, and the ones with a major medical importance are prostate cancer and benign prostatic hyperplasia, which can both be treated with androgen suppression. The aim of this study was to analyze immunohistochemical degree of expression of androgen receptor (AR) of the ventral lobe of the gerbil prostate during different phases of the postnatal development employing differents treatments for androgen blocking. Seventy-five male gerbils were distributed, randomly, into 3 groups of 25 animals each, where each group corresponded to one phase of postnatal development: young, adult and aged phase. In each phase, it was possible to morphologically and stereologically analyze the compartments of prostatic ventral lobe, as well as to immunohistochemically analyze the degree of expression of androgen receptor. In addition, it was possible to establish the hormonal dosage of serum testosterone concentrations given the comparative approach of the expression of androgen receptors. There is a heterogeneous pattern of AR distribution in the prostatic ventral lobe throughout postnatal development, in which the younger animal is the higher, the interaction of circulating androgens that stimulate the AR expression in the compartments prostatics. The androgen blockage therapies decreased AR expression in the ventral lobe, but the androgen reposition after these blockages was not showed the same the degree of expression of androgen receptor near normal physiological conditions. The regulation and distribution of AR along the gerbil prostatic tissues are complex mechanisms that are likely to be genetically regulated by androgens prenatally or by other factors that are still unknown. The gerbil seems to be a valuable model in the attempt to improve the understanding of the morphophysiological and pathological behavior of this important gland in humans throughout aging and to stimulate new therapeutic ideas to fight prostate cancer.

III. INTRODUÇÃO

Aspectos gerais do desenvolvimento e da fisiologia prostática

A próstata é uma glândula acessória do trato reprodutor masculino responsável pela produção de nutrientes, gradientes iônicos e pH ótimo para os espermatozóides no fluido seminal (Untergasser *et al*., 2005).

A próstata origina-se a partir do epitélio do seio urogenital, servindo como glândula sexual e uretral (Dounjacour e Cunha, 1988, Cooke *et al*., 1991, Marker *et al*., 2003, Untergasser *et al*., 2005). Ela é uma glândula túbulo-alveolar composta com atividade secretora principalmente ligada à sua parte alveolar (Reese *et al*., 1986). O epitélio prostático apresenta ao menos três tipos celulares distintos, os quais podem ser diferenciados por suas características morfológicas e funcionais em células: luminais secretoras, basais e neuroendócrinas (Fig.1) (Abate-Shen e Shen, 2000; Garraway *et al.*, 2003).

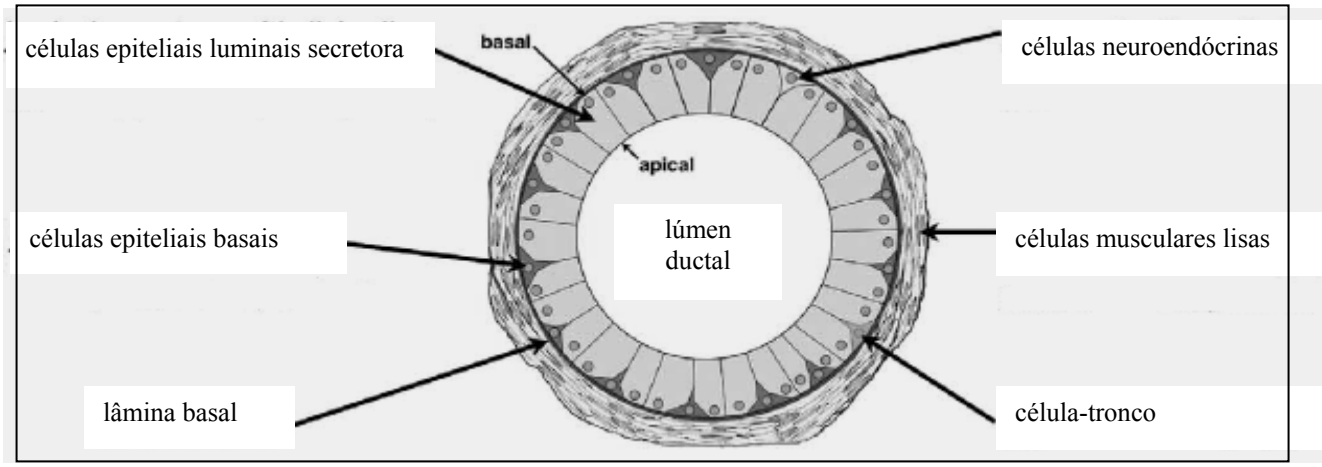

Fig. 1 – Esquema de um ducto prostático em corte transversal, indicando os diferentes tipos celulares (Marker *et al.*, 2003).

Entremeando as partes glandulares existe um estroma ricamente vascularizado, com esparsas fibras conjuntivas, células musculares lisas que têm papel contráctil durante a ejaculação, fibroblastos, macrófagos fixos, além de nervos e terminações nervosas, cada

qual com seu papel importante e específico na viabilidade e função secretora do tecido como um todo. Envolvendo este órgão tem-se uma fina cápsula fibromuscular, que confere diferentes formas ao órgão nos diversos mamíferos já estudados (Carvalho e Line, 1996 e Tuxhorn *et al*., 2001).

Segundo Slayter e colaboradores (1994), no que se refere à homologia das partes da próstata humana com os lóbulos prostáticos de ratos, o lóbulo ventral em rato corresponde à zona mediana da próstata humana enquanto o lóbulo dorsal corresponde à zona posterior. Partindo dessas homologias morfo-funcionais pré-estabelecidas pode-se, então, buscar condições experimentais, em que a análise das respostas do epitélio e dos componentes do estroma são avaliadas. Assim, existem na literatura trabalhos relacionados à ação de agentes químicos (carcinógenos) na próstata, como os trabalhos de Pollard e Luckert (1987) e ação de agentes endógenos (hormonais) como os trabalhos desenvolvidos por Carvalho *et al.,* (1996, 1997a,b) e Scarano *et al.,* 2006, em que os resultados sugerem similaridade com a espécie humana.

O crescimento normal, a diferenciação e a manutenção da integridade funcional (secretora) e estrutural da próstata e dos demais órgãos acessórios do sistema reprodutor masculino são dependentes de concentrações constantes de andrógenos circulantes e ocorrem através de interações recíprocas entre o mesênquima e o epitélio (Cunha, 1976, Cunha *et al*., 1996, Hayward *et al*., 1997, Thomson *et al*., 1997, Banerjee *et al*., 2001; Taplin e Ho, 2001; Debes e Tindall, 2002).

Durante a vida fetal, existe um período em que a dependência de andrógenos é absoluta, sendo que sua remoção neste período suprime a formação da próstata (Wells, 1954, Sugimura *et al*., 1986, Dounjacour e Cunha, 1988).

Nemeth e Lee (1996) e Sugimura *et al* (1986) sugerem que o estroma seja o primeiro alvo da ação androgênica, sendo a reação do epitélio mediada por fatores estromais. Certos efeitos do estroma sobre o epitélio podem ser simplesmente uma função da matriz extracelular produzida em grande parte pelas células estromais.

A necessidade de se estudar as respostas aos hormônios, sob várias condições e o efeito do bloqueio destes, deve-se ao fato de ser a glândula prostática em humanos, o sítio de um grande número de doenças relacionadas à idade, sendo que as de maior importância

clínica são o câncer prostático (CaP) e a hiperplasia prostática benigna (HPB) que se instalam em resposta as descompensações hormonais. Os hormônios androgênicos e seus receptores, entre outros fatores, exercem papel na etiologia dessas lesões, as quais podem ser tratadas por estratégias de remoção de andrógenos (Droller, 1997, Rauch *et al*., 1997, Cordeiro *et al*., 2004, Corradi *et al*., 2004; Oliveira, 2005).

Ação dos andrógenos e anti-androgênicos

Os andrógenos regulam o crescimento, a diferenciação e a morte celular programada em células alvo via interação com o receptor androgênico (RA), os quais são fatores de transcrição que regulam a expressão de genes-alvo por mecanismo dependente do ligante (Cunha *et al*., 1986; Nevalainen, 1997; Brum *et al*., 2005). A produção de andrógenos é regulada pelo eixo hipotalâmico-hipofisário-gonadal, sendo que, o principal andrógeno é a testosterona, com as células de Leydig dos testículos produzindo mais de 95% e a glândula adrenal menos de 5% desse (Debes e Tindall, 2002; Hsing *et al*., 2002).

Na próstata, a testosterona é convertida pela ação da enzima 5-α-redutase tipo 2, em 5-α-di-hidrotestosterona (DHT), a forma androgênica mais ativa, tendo uma afinidade maior pelo RA do que pela testosterona (Galbraith e Duchesne, 1997; Hsing *et al*., 2002; Dehm e Tindall, 2006). Caso haja um bloqueio na conversão de testosterona para DHT durante o desenvolvimento prostático há uma acentuada redução da morfogênese e crescimento do órgão ou até mesmo importantes modificações no estroma dos indivíduos adultos quando tratados com o inibidor desta enzima (Marker *et al*., 2003, Corradi *et al*., 2004).

Segundo Dehm e Tindall (2006), existem três formas de ação da testosterona nos tecidos alvos (Fig. 2):

1°- Pode difundir através da membrana plasmática das células-alvo e se ligar diretamente ao RA ativando-os;

2°- Se converter em DHT pela enzima 5α-redutase antes de se ligar ao RA;

3°- Ou ser aromatizada para estrógeno e atuar através dos receptores de estrógeno.

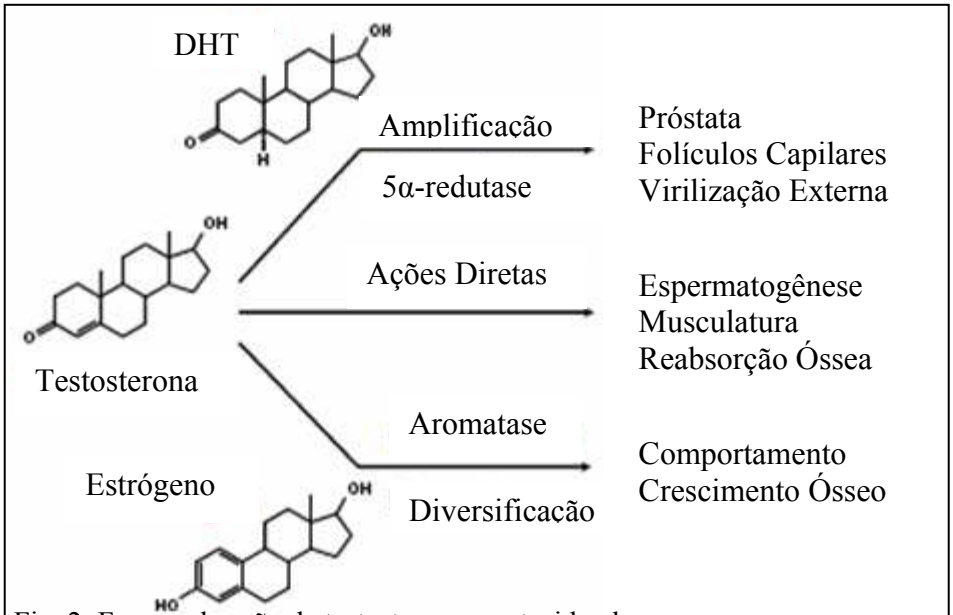

Fig. 2: Formas de ação da testosterona nos tecido alvos

As células alvos para andrógenos estão localizadas em muitas partes do corpo, com altas concentrações de RA nos órgãos acessórios masculinos, como por exemplo, folículo capilar, pele genital, epidídimo, glândula seminal, próstata, bem como no hipotálamo (Brinkmann *et al*., 1999, Santos *et al*., 2004, Farla *et al*., 2005, Dehm e Tindall, 2006).

O RA é uma fosfoproteína solúvel de 110 kDa, pertencente a uma superfamília de receptores esteróides que funciona como fator de transcrição intracelular. Estruturalmente o RA contém três domínios funcionais (Fig. 3): Domínio N-Terminal (DNT), Domínio Central de Ligação ao DNA (DLD) e Domínio C-Terminal de ligação ao ligante (DLL) (Galbraith e Duchesne, 1997, Farla *et al*., 2005, Gao *et al*., 2006).

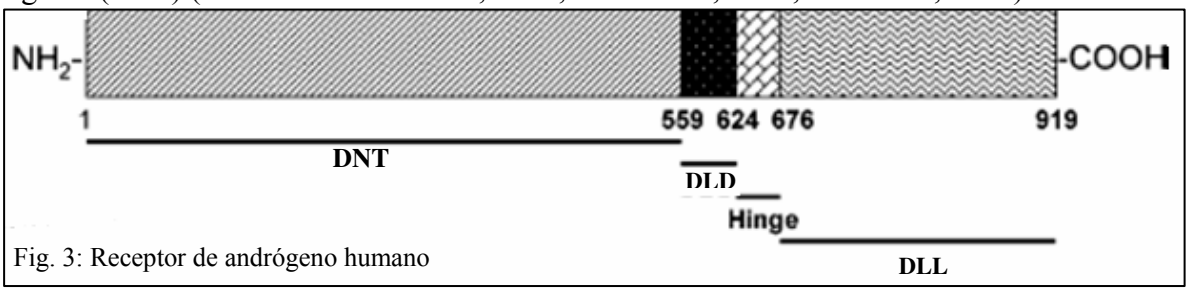

Fig. 3: Receptor de andrógeno humano

Em estágio desligado ou inativo, o RA existe no citoplasma formando um complexo que inclui chaperonas e co-chaperonas (família de proteínas *heat shock*, Hsp 90, Hsp70 e Hsp 56). Após a ligação com andrógeno ocorrem alterações na conformação e

composição deste complexo, o receptor então sofre dissociação das chaperonas, dimerização e fosforilação. Em seguida, o RA é translocado para núcleo dentro de 15-60 minutos da entrada do andrógeno na célula, ligando-se aos elementos de resposta androgênica do DNA, promovendo ou estimulando a expressão de genes alvos (Fig. 4) (Hughes *et al*., 2001; Heinlein e Chang, 2004; Farla *et al*., 2005). A entrada dessa proteína-RA no núcleo pode ocorrer através de difusão simples pelos poros nucleares ou pode ligar-se a sinais de localização nuclear que resultará na ativação do transporte (Jenster *et al*., 1993).

A expressão gênica do RA pode ser modulada em múltiplos níveis por mecanismos de transcrição, pós-transcrição e pós-tradução. Além disso, complexos formados por co-ativadores ou co-repressores, podem atuar como moléculas adaptadoras, ligando-se diretamente aos receptores nucleares, recrutando proteínas adicionais e interagindo com a maquinaria transcricional basal para aumentar a transcrição de genes-alvo (Brum *et al*., 2005).

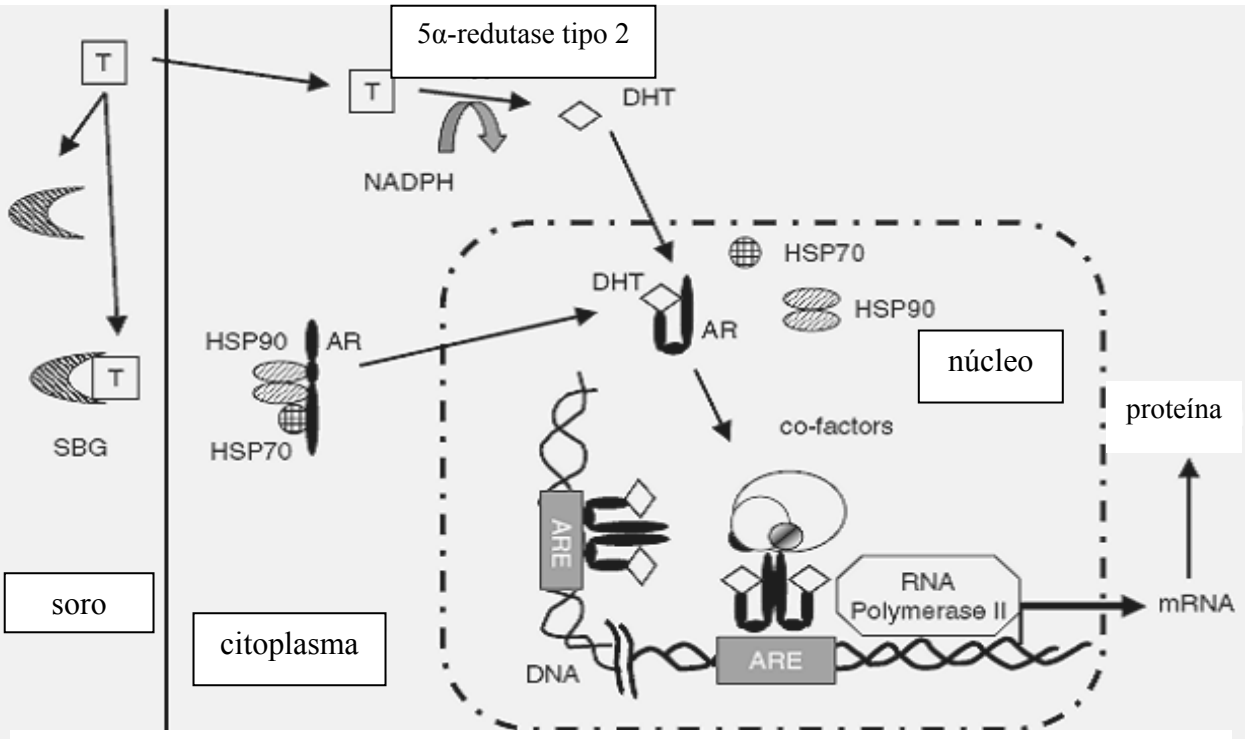

Fig. 4. Mecanismo de ligação do RA. ARE: elemento de resposta androgênica; DHT: di-hidrotestosterona; HSP: protein *heat shock*; SBG: globulina de ligação a hormônios sexuais; T: testosterona.

Embora a função dos andrógenos seja importante, esta é insuficiente para manter a homeostase prostática. Esse processo também requer interações entre fatores de crescimento peptídicos que são secretados por células epiteliais e estromais de maneira autócrina ou parácrina, atuando no controle de proliferação e morte celular, em ambos os compartimentos celulares (Galbraith e Duchesne, 1997; Untergasser *et al*., 2001; Lee e Chang, 2003).

Em células epiteliais da próstata humana há uma baixa, porém balanceada, taxa de proliferação e morte celular (<0,20% dia), o que resulta em um estado estável do órgão sem crescimento líquido, com as células sendo renovadas continuamente (Berges *et al*., 1995). Há um grande interesse em compreender melhor a biologia prostática devido à sua alta propensão em desenvolver tumores malignos, estando entre as mais comuns, as neoplasias que acometem o homem (Bonkhoff e Remberger, 1996; Abate-Shen e Shen, 2000).

A próstata humana é dividida em três discretas regiões, definidas pela sua localização em relação à uretra. As células de cada região variam significativamente no desenvolvimento de HPB e CaP. O CaP é considerado uma doença com grande heterogeneidade, englobando a existência simultânea de entidades patológicas múltiplas como neoplasia intra-epitelial (PIN), adenocarcinomas dependentes e independentes de andrógenos e lesões metastáticas em um mesmo tecido (Droller, 1997; Tang e Porter, 1997; Lucia *et al*., 1998; Bostwick *et al*., 2000). Enquanto muitos carcinomas prostáticos retêm um padrão de crescimento baixo, aproximadamente um terço torna-se invasivo localmente ou produz metástases, expandindo-se para os linfonodos locais e órgãos distantes como ossos, fígado e pulmão (Shulz *et al*., 2003).

No início, o crescimento e a sobrevivência das células alteradas dependem da ação androgênica, em que a castração e/ou a administração de uma droga anti-androgênica seria a melhor alternativa como terapia. Inicialmente o bloqueio androgênico resulta da inibição dos RA, indicado pela redução da expressão de determinados genes alvos e consequentemente redução do tumor (Feldman e Feldman, 2001; Dehm e Tindall, 2006).

Contudo, o CaP pode reincidir em uma forma que é resistente às manipulações hormonais de tratamentos, sendo referido como uma doença andrógeno-independente.

Embora o CaP seja resistente à tentativa de ação do bloqueio androgênico, o RA permanece um fator crítico para crescimento e sobrevida da maioria dos tumores e que, portanto o eixo hormonal de expressão do RA permanece um valioso alvo à terapia (Buchanan *et al.,* 2001).

A HPB é a doença mais prevalente da próstata, com aproximadamente 50% dos homens apresentando evidências histológicas aos 50 anos e 90% aos 80 anos de idade. É considerada uma doença progressiva, definida como o crescimento contínuo da próstata, caracterizando-se por uma predominante proliferação estromal e, embora um aumento substancial do epitélio também ocorra, a integridade regional da glândula é mantida. Está doença leva à intensificação de sintomas e ao aumento do risco de complicações ao longo do tempo, como a retenção urinária aguda e necessidade de procedimentos cirúrgicos.

As ações androgênicas têm sido amplamente demonstradas na próstata humana, seja por sua ação na morfogênese, diferenciação, proliferação celular e secreções da glândula, ou pela resposta ao tratamento hormonal do CaP e HPB. Contudo, os mecanismos moleculares de transformação neoplásica e carcinogênese ainda não estão bem estabelecidos (Krieg *et al*., 1993; Bonkhoff e Remberger, 1996; Raghow *et al*., 2000; Banerjee *et al*., 2001; Leav *et al*., 2001; Brum *et al*., 2005).

Alterações nas concentrações endógenas de hormônios esteróides relacionados ao envelhecimento, também promovem alterações prostáticas em outras espécies de mamíferos. Em ratos *Brown Norway* senis o crescimento espontâneo da próstata é confinado aos lóbulos laterais e dorsais, sendo, portanto, lóbulo-específico (Banerjee *et al*., 2001). Em cães também há uma propensão correlacionada com o envelhecimento ao aparecimento de lesões proliferativas espontâneas na próstata (Leav *et al*., 2001), bem como em primatas (McEntee *et al*., 1996).

Os andrógenos têm papel fundamental na carcinogênese prostática. Partindo desse princípio, a inibição de vias hormonais permanece como a primeira medida para a prevenção dessa lesão (Lee e Chang, 2003). A supressão das concentrações séricas de testosterona e DHT pode ser realizada por orquiectomia (castração cirúrgica) e/ou administração de drogas antiandrogênicas (Ruijter *et al*., 1999; Corradi *et al*., 2004; Cordeiro, *et al*., 2004).

Após a orquiectomia bilateral, as concentrações séricas de testosterona diminuem de 90 a 95% (Heinlein e Chang, 2004). Em ratos, o lóbulo ventral prostático e a glândula seminal involuem, perdendo aproximadamente 90% de suas células após 21 dias de castração (Meeker *et al*., 1996; Carvalho *et al*., 1997a,b). A regressão da glândula ocorre devido à ativação do programa de morte celular apoptótica, a qual afeta, principalmente, células epiteliais glandulares. As células epiteliais basais e estromais são menos sensíveis a supressão androgênica e são mantidas (Bruyninx *et al.*, 1999; Balk, 2002).

Sugimura e colaboradores (1986) demonstraram que a administração de andrógenos pós-castração promove a restauração da estrutura ductal, semelhante à condição normal. Em ratos *Brown Norway*, a apoptose induzida pela castração é lóbulo-específica e uma diminuição nesse processo celular foi observada com o envelhecimento, sugerindo uma evolução para independência a andrógeno relacionada com a senescência nos quatro lóbulos prostáticos (Banerjee *et al*., 2001). Esses eventos indicam que há uma variação quanto à dependência androgênica nas regiões prostáticas, bem como, entre as células epiteliais, podendo haver outros fatores que permitam a sobrevivência de grupos de células independentes de hormônio.

Para Scher e colaboradores (2004), o sucesso do tratamento anti-androgênico está vinculado à dependência das células à DHT, embora as concentrações hormonais sejam baixas no sangue de pacientes com tumores em progressão pós-castração, a concentração androgênica intratumoral pode ser suficiente para manutenção da lesão. Desta maneira, tumores prostáticos, aparentemente, não se encontram em um ambiente completamente independente de andrógenos após castração (Mohler *et al*., 2004).

Embora, as concentrações plasmáticas de testosterona declinem após a castração, vários estudos indicam que a produção intraprostática de DHT continua a ocorrer, por meio da conversão de andrógenos adrenais. Desta forma, a DHT está presente mesmo após supressão androgênica, sendo necessários procedimentos terapêuticos adicionais para que o bloqueio hormonal seja efetivo (Nishiyama *et al*., 2004).

A utilização de medicamentos chamados anti-androgênicos esteroidais e não-esteroidais (castração química) no tratamento de lesões prostáticas têm sido muito indicada,

principalmente nas lesões proliferativas adenocarcinomatosas que dependem desses hormônios, e, além disso, parece ser mais eficiente que a orquiectomia, uma vez que mesmo após a depleção hormonal, a glândula adrenal ainda produz cerca de 5% de hormônios androgênicos (Brooks *et al*., 1991).

As drogas anti-androgênicas inibem ou diminuem a síntese e/ou a liberação de hormônios hipotalâmicos e de seus fatores de liberação e de hormônios da hipófise anterior (gonadotropinas), inibindo assim a biossíntese ou a secreção de andrógenos e, consequentemente, suas ações sobre os tecidos alvos e, além disso, essas drogas atuam na inibição da união dos andrógenos aos respectivos receptores celulares (Galbraith e Duchesne, 1997; Taplin e Ho, 2001; Heinlein e Chang, 2004).

Uma característica particular da farmacocinética dos anti-androgênicos não-esteroidais, quando comparado com os esteroidais, é que eles proporcionam uma melhor eficiência no bloqueio androgênico para tratamento de CaP, além disso, estimulam a liberação de hormônio luteinizante (LH) e conseqüentemente há um aumento das concentrações de testosterona testiculares e por esta razão, muitos pacientes permanecem sexualmente potentes durante os tratamentos terapêuticos com anti-androgênicos não-esteroidais (Pompeu *et al*., 1998; Singh *et al*., 2000;Goto *et al*., 2004; Foster e Harris, 2005).

A flutamida é um dos mais conhecidos anti-androgênicos não-esteroidais e atua como inibidor competitivo de testosterona e DHT na ligação com RA no tecido prostático (Fig. 5). Essa droga quando absorvida pelo trato gastrointestinal sofre alterações conformacionais, formando a 2-hidroxyflutamida, metabólito mais potente antiandrogênico *in vivo* e com alta afinidade de ligação para RA do que a flutamida. Esse metabólito inibe a ação da testosterona endógena e exógena, responsáveis pelo crescimento da próstata, atuando principalmente em nível citoplasmático por inibição competitiva da ligação da DHT em seu receptor, bem como possível bloqueio de translocação do complexo receptor-DHT para interior do núcleo inibindo assim, a síntese de DNA prostático estimulada pela testosterona resultando na involução da glândula (Metzger *et al*., 1993; Singh *et al* 2000; Miyata *et al*., 2003; Singh *et al*., 2006).

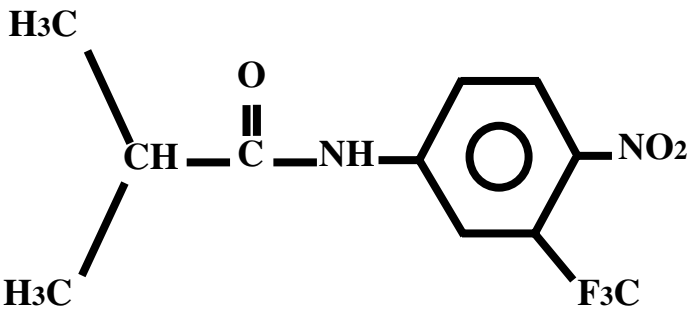

Figura 5: Estrutura química da Flutamida (Eulexin): 3α,-trifluoro-2-methyl1-4'-nitro-m-propionotoluidide (Sufrin e Coffey, 1975).

Estudos mais recentes descrevem que o metabólito ativo da flutamida, ao se ligar ao RA provoca mudanças conformacionais, agindo como medida terapêuctica no combate ao câncer de próstata. Esse anti-androgênico além de bloquear completamente a translocação do complexo receptor-DHT para interior do núcleo, também pode agir na translocação de RA, porém de forma mais lenta e incompleta, diminuindo assim a expressão de genes alvos (Farla *et al*., 2005; Gao *et al*., 2006).

A distribuição da flutamida nos tecidos foi examinada em ratos machos após a administração de 5 mg/kg de ^{14}C-flutamida. Enquanto a concentração da flutamida estava geralmente baixa em todos os tecidos examinados, o seu metabólito apresentava um aumento de 70 vezes após 6 horas da dosagem. O metabólito foi relativamente concentrado no lóbulo ventral prostático e na glândula seminal de ratos, demonstrando serem os órgãos alvos da atividade farmacológica da flutamida (Neri, 1989).

Vários trabalhos e ensaios experimentais demonstram que a administração diária de flutamida, em doses variando entre 1 a 50 mg/kg de peso corporal, reduz consideravelmente o peso e volume da próstata, e das glândulas seminais de ratos sem alterar a potência sexual (Neri, 1989; Sogani *et al*., 1984; Srougi 1992). Narayan e colaboradores (1996), estudando 372 pacientes durante dois anos, demonstraram que 250mg de flutamida diária foi suficiente para reduzir de 14% a 29% o volume prostático.

A administração de 10 mg/kg/animal de flutamida durante 10 dias em cobaias macho ao longo do desenvolvimento pós-natal, promoveu alterações substanciais estruturais e ultra-estruturais nos componentes epiteliais e no padrão de distribuição e na

concentração dos componentes fibrilares, levando em conta a fase do desenvolvimento pós-natal (Cordeiro *et al*., 2004).

Em camundongos transgênicos com adenocarcinoma (TRAMP), este anti-andrógeno em altas doses diminuiu a incidência de tumor e aumentou o período de latência, sugerindo ser uma medida preventiva viável (Raghow *et al*., 2000). Porém, a terapia prolongada com flutamida pode levar a seleção de células tumorais, as quais não sofrem a ação antiandrogênica e assim, contribuir para a progressão da lesão (Lee e Chang, 2003 e Foster e Harris, 2005). Isso poderia ocorrer provavelmente devido ao efeito estimulatório da Flutamida sobre a liberação de LH e conseqüente aumento dos níveis de andrógeno testicular, podendo haver dessa forma, um maior estímulo proliferativo destas células tumorais.

A depleção de andrógenos, seja por castração cirúrgica e/ou química, após a próstata ter atingido o seu tamanho final, causa à atrofia do órgão, com notável redução do seu tamanho e peso, inicialmente caracterizados por uma parada na síntese e uma acelerada liberação da secreção luminal, seguida pela diminuição do tamanho das células epiteliais por processos de morte e degeneração celular, resultando em lóbulos reduzidos e células epiteliais menores (Aumüller e Seitz, 1990). São observados ainda um declínio na síntese de DNA e de proteínas no conteúdo e na complexidade do RNA e uma diminuição do receptor de andrógeno. A castração na idade adulta leva à morte, predominantemente, as células epiteliais luminais (Hayward *et al*., 1996; Carvalho *et al*., 1997a,b; Vilamaior, 2000).

Uma diminuição de 48% no tamanho da glândula foi notada em homens nos seis primeiros meses de terapia com anti-andrógeno flutamida, enquanto que a combinação de castração cirúrgica com esta droga (bloqueio androgênico completo-BAC) promoveram uma diminuição prostática de 56% (Noldus *et al*., 1996; Cordeiro *et al*., 2004). Em ratos, a regressão do lóbulo ventral também foi mais acentuada após o tratamento combinado (Montalvo *et al*., 2002). Assim, há uma redução de estimulação androgênica nas células anômalas uma vez que, estas apresentam dependência hormonal inicial (Raghow *et al*., 2000). Porém, essas terapias podem conduzir ao desenvolvimento de células insensíveis a andrógenos, as quais são resistentes aos sinais apoptóticos e resultam em doença

metastática (O´Neill, 2001). Tais eventos patológicos dificultam o encontro de uma solução terapêutica para a lesão e, equivalem a um ponto crucial na compreensão do potencial maligno do câncer de próstata (Shulz *et al*., 2003).

Além disso, há ressaltar-se que a sinalização de RA é mantida ou regulada positivamente em tumores que retomam crescimento após falha de terapia androgênica e a ativação de genes reguladores de andrógenos é suficiente para facilitar a sobrevivência do tumor. Há evidências sugerindo que a seleção de mecanismos que facilitem a permanência da sinalização por RA, ou que ative novas vias de proliferação celular, podem depender da terapia utilizada durante o tratamento (Scher *et al*., 2004).

Enquanto os eventos associados às modificações alcançadas pelas células epiteliais da parte glandular e no estroma foram intensamente investigados após a castração cirúrgica e química, as modificações morfológicas, bioquímicas e imuno-histoquímicas que ocorrem no epitélio e no estroma prostático, ainda necessitam de maiores investigações após efeito de aplicação de anti-androgênicos, principalmente frente à tentativa de melhor compreensão das relações andrógenos-receptores e epitélio-estroma.

Características do modelo experimental

Os estudos sobre a fisiologia da próstata têm despertado grande interesse para as Ciências Biomédicas, uma vez que este órgão ser alvo do desenvolvimento de graves lesões no homem, tanto na fase adulta quanto na senescência. Assim, é de fundamental importância que se tente estabelecer padrões comparativos com outros animais, para melhor compreender os mecanismos envolvidos em condições malignas nos homens.

Os gerbilos, também conhecidos com esquilos da Mongólia, são roedores da família Muridae, subfamília Gerbillinae (McKenna e Bell, 1997), provenientes das regiões áridas da China e Mongólia (Schwentker, 1963). Introduzidos por Vitor Schwentker, nas Américas em 1954 como nova proposta de animal experimental, ficaram durante muito tempo nas limitações dos Estados Unidos como animais de excelência para a pesquisa biomédica (Williams, 1974). Nas últimas décadas, esse animal vem sendo gradativamente introduzido nos biotérios das universidades brasileiras e têm assumido importante papel

nos experimentos biológicos e biomédicos, juntamente com outras espécies clássicas como *Rattus novergicus* (rato) e *Mus muscullus* (camundongo).

Na pesquisa científica é cada vez maior a utilização dos gerbilos na experimentação biomédica, principalmente nas áreas da imunologia (Jeffers *et al*., 1984; Nawa *et al*., 1994) e fisiologia (Muller e Nielsen, 1979) e também em estudos sobre o sistema genital (Santos *et al*., 2003; Corradi *et al*., 2004; Pinheiro *et al*., 2004; Segatelli *et al*., 2004). Os estudos a respeito do sistema genital masculino dessa espécie têm descrito as relações dos ductos das glândulas acessórias com a uretra (Williams, 1974; Pinheiro *et al*., 2004) e os efeitos de bloqueios ou manipulações hormonais sobre a organização estrutural do lóbulo prostático ventral (Corradi *et al*., 2004; Oliveira, 2005).

Nosso grupo de pesquisa adotou como modelo experimental para o estudo da próstata o gerbilo *Meriones unguiculatus*. De anatomia similar à do rato e camundongo, os gerbilos adultos de ambos os sexos variam entre 11,5 e 14,5 cm de comprimento corpóreo. Os machos adultos pesam em torno de 90-100g enquanto as fêmeas adultas pesam valores próximos a 85g (Kramer, 1964). Esses animais têm sido amplamente utilizados para estudos de natureza didático-científica, principalmente pelo fato de terem comportamento dócil em cativeiro. Outra característica importante a ser considerada sobre esses roedores é a facilidade de manutenção no biotério, pois, por serem de origem desértica, apresentam micção infreqüente, agilizando muito a limpeza das gaiolas no processo de manutenção, promovendo uma maior facilidade e plasticidade no manuseio e manutenção dos animais nos biotérios.

A glândula prostática dos gerbilos tem se mostrado semelhante à humana, no referente à compacidade e fusão de lóbulos. Entre as partes glandulares, encontra-se um estroma conjuntivo vascularizado, com poucas fibras conjuntivas e elásticas, além de abundantes células musculares lisas dispostas ao redor dos ductos prostáticos. Ultra-estruturalmente, o epitélio prostático apresenta heterogeneidade entre os seus tipos celulares e o estroma glandular mostra abundantes células musculares lisas entremeadas às camadas de colágeno (Williams, 1974; Zanetoni *et al*., 2001). Além disso, o modelo vem apresentando respostas significativas em estudos sobre tratamentos hormonais (Santos *et al*., 2003; Scarano *et al*., 2004), drogas contra hiperplasia prostática humana (Corradi *et al*.,

2004; Cordeiro *et al*., 2005), bem como, desenvolvimento de neoplasias espontâneas associadas ao envelhecimento (Zanetoni *et al*., 2001). O surgimento dessas últimas está correlacionado com declínio nas concentrações séricas de testosterona nos animais senis (Zanetoni *et al*., 2001). Assim, parece interessante a tentativa de se estabelecer novos modelos para estudos da próstata. E nestes, o gerbilo pode ser incluído como uma proposta valiosa na melhoria do conhecimento do comportamento morfofisiológico e patológico dessa importante glândula masculina.

Atualmente sabe-se que vários fatores hormonais são responsáveis pela manutenção da estrutura histofisiológica da próstata masculina (Reiter, 1999). No entanto, o conhecimento sobre o grau de expressão do RA na próstata normal e anormal, bem como após terapias de bloqueios androgênicos ainda é limitado. Desta forma, investigar as diferentes interações dos andrógenos com seus receptores nos compartimentos prostáticos ao longo do desenvolvimento pós-natal e o comportamento desses receptores em diferentes ambientes de ablação androgênica utilizados em tratamento de lesões prostáticas, permitirá um melhor entendimento da biologia prostática e possibilitará condutas terapêuticas no tratamento do CaP.

IV. OBJETIVOS

O presente estudo teve como objetivo avaliar o grau de expressão do receptor androgênico no lóbulo ventral da próstata do gerbilo *Meriones unguiculatus* (Muridae, Gerbillinae) nas diferentes idades do desenvolvimento pós-natal (jovens, adultos e senis) mediante diferentes terapias de bloqueios androgênicos. Além disso, foi realizado a dosagem hormonal da concentração sérica de testosterona e avaliação estereológica dos volumes ocupados pelo compartimento epitelial, luminal e estromal visto sua abordagem comparativa em relação à expressão do receptor de andrógeno.

V. ARTIGO

Título: "Androgen receptor expression in the Mongolian Gerbil ventral prostate: Evaluation during different phases of post-natal development and following androgen blockage"

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Androgen receptors expression in the Mongolian Gerbil ventral prostate: Evaluation during different phases of post-natal development and following androgen blockage

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Abstract

Background: The normal growth, differentiation and maintenance of the morphofunctional integrity of the prostate gland are dependent on the interaction of constant levels of androgens with their receptors. The need to study the responses to hormones under several conditions and the effect of their blockage is due to the fact that the human prostate is the site of a great number of age-related diseases, and the ones with a major medical importance are prostate cancer and benign prostatic hyperplasia, which can both be treated with androgen suppression. The aim of this study was to analyze immunohistochemical degree of expression of androgen receptor (AR) of the ventral lobe of the gerbil prostate during different phases of the postnatal development employing differents treatments for androgen blocking.

Methods: Seventy-five male gerbils were divided, randomly, into 3 groups of 25 animals, where each group corresponded to one phase of postnatal development: young, adult and old. In each phase, it was possible to morphologically and stereologically analyze the compartments prostatics, as well as to immunohistochemically analyze the degree of expression of AR after the androgen blockage therapies. In addition, it was possible to establish the hormonal dosage of serum testosterone concentration given the comparative approach of the expression of AR.

Results: There is a heterogenic pattern of AR distribution in the ventral lobe throughout postnatal development, in which the younger animal was the higher the interaction of circulating androgens that stimulate the AR expression in the compartments prostatics. The androgen blockage therapies decreased AR expression in the prostatic compartments, but the androgen reposition after these blockages was not sufficient to recover the glandular structure or stimulate the AR expression up to normal physiological conditions.

Conclusions: Both the regulation and distribution of androgen receptors along the gerbil prostatic tissues are complex mechanisms that are likely to be genetically regulated by androgens prenatally or by other factors that are still unknown. This rodent species seems to be a valuable model in the attempt to improve the understanding of the morphophysiological and pathological behavior of this important gland in humans throughout aging and to stimulate new therapeutic ideas to fight prostate cancer.

Background

Androgens regulate the growth, differentiation and programmed death of cells in the prostate gland and other male reproductive organs via interaction with the androgen receptor (AR). The main androgen is testosterone, which, inside the prostate, is converted by the action of type 2 5α -reductase enzyme to 5α-dihydrotestosterone (DHT), which is a more active androgen form that presents a higher affinity for AR than testosterone [1-6]. If there is a blockage in the conversion of testosterone to DHT during the prostatic development, an intense reduction in the morphogenesis and growth of the organ occurs or there are even important changes in the stroma of adult individuals when they are treated with the inhibitor of this enzyme [7, 8].

The AR, a member of the steroid receptor family that is activated by androgens, is the major regulatory transcription factor in normal development of the prostate and in the growth of androgen-dependent prostate cancer. Thus, AR is assumed to contribute to prostate cancer development during its recurrence in the androgen-deprived patient, but the function of AR in the development of androgen-independent and dependent prostate cancers is still unknown [5, 9, 10-12].

The need to study the responses to hormones under several conditions and the effect of their blockage is due to the fact that the human prostate is the site of a great number of age-related diseases, and the ones with a major medical importance are prostate cancer (PC) and Benign Prostatic Hyperplasia (BPH). Although PC is resistant to attempts at androgen blockage, AR remains a critical factor for the growth and survival of most of the tumors and, therefore, the hormonal axis of AR expression constitutes a valuable target for antiandrogenic therapy [4, 13-15]. These lesions, whether malignant or not, can be treated with androgen removal strategies [8, 16, 17]. The suppression of testosterone and DHT concentrations in the serum can be obtained through orchiectomy (surgical castration) and/or treatment with antiandrogenic drugs [8, 17, 18].

Flutamide is one of the most well-known non-steroidal antiandrogens that act as competitive inhibitors of testosterone and DHT in the binding of AR with the prostatic tissue. This drug, once absorbed by the gastrointestinal tract, undergoes conformational

changes, forming the most active metabolite, 2-hydroxyflutamide, which is the most potent antiandrogenic agent *in vivo*, with higher AR binding affinity than flutamide [19, 20-23].

Androgen blockers cause a reduction of androgenic stimulation in the anomalous cells since they initially present hormonal dependency [24]. However, these therapies can cause the development of androgen-insensitive cells that are resistant to apoptotic signaling, resulting in metastatic disease [25]. Such pathological events make it difficult to find a therapeutic solution to the lesion and they constitute a key factor in understanding the malignant potential of prostate cancer [26].

Although the events associated with the changes undergone by epithelial cells in the glandular portion and in the stroma have been intensely investigated after either surgical or chemical castration, the morphological, biochemical and immunohistochemical changes that occur in both the epithelium and the prostatic stroma still require further investigation after the effects of antiandrogenic treatment, mainly in terms of trying to better understand the relationships between androgen and receptors as well as between epithelium and stroma.

Thus, the objective of this work is to evaluate, based on immunohistochemical and stereologic studies, the expression of androgen receptors in both epithelial and stromal compartments of the prostatic ventral lobe of the gerbil *Meriones unguiculatus* (Muridae, Gerbillinae) during different ages of postnatal development (young, adult, and old) after the androgen blockage therapies.

Methods

Animals

Seventy-five male gerbils (*Meriones unguiculatus,* Gerbilinae: Muridae), supplied from the Universidade de São Paulo State UNESP (Botucatu, SP, Brazil) were housed under adequate conditions of luminosity (12-12 cicle) and temperature (24°C) and were fed rodent ration (Labina®, Purina, Agribrands do Brasil Ltda.) and water *ad libitum*.

Experimental protocol

The animals were distributed, randomly, into 3 groups of 25 animals each, where each group corresponded to one of the following postnatal development phases: young (with mean age of 48 ± 15.9 days), adults (112 ± 27.7 days), and old (18 ± 5.4 months). In each postnatal development phase 5 groups were formed of 5 animals each, according to the following experimental protocol of androgen blockade: **Group I - Control**: Received the pharmacological vehicle (0.9% saline solution) for 7 days; **Group II - Surgical Castration**: The animals were submitted to bilateral orchiectomy by abdominal surgical incision after anesthesia with intra-muscular injections of ketamine-chlorohydrate (50 mg/kg body weight). After surgery the animals of this group were placed in individual boxes and submitted to the experiments for 7 days; **Group III - Chemical Castration**: Daily subcutaneous injections of 0.3 ml of antiandrogenic flutamide 10 mg/Kg/body weight (Sigma Chemical Co, St. Louis, Missouri-EUA) for 7 days [17]; **Group IV - Blockade Androgen Completed (BAC)**: Surgical Castration associated with Chemical Castration for 7 days; **Group V** - BAC for 7 days, following received intradermic testosterone injections (1 mg/kg testosterone cypionate [Novaquimica/Sigma Pharma, Hortolândia, São Paulo, Brazil] in 0.25 ml corn oil) for 7 additional days [27].

Morphological and Stereological Analysis

After the respective treatment periods, the five groups of animals at each age were anesthetized lightly by CO_2 inhalation and weighed. After this procedure, the animals

were sacrificed by decapitated and blood collected for serological analysis. For analysis under light microscopy, the entire prostatic complex was excised, weighed and cut into fragments. The samples were fixed for 24h in formaldehyde 4% just prepared in phosphate buffer, pH 7.2. After fixation, the material was dehydrated in graded ethanol series and the embedding was performed in Paraplast (Histosec-Merck). The 4μm histological sections were submitted to hematoxylin-eosin (HE) staining for general morphological and stereological analysis of the tissue compartments. The microscopy analyses were performed with an Olympus photomicroscope. The Image-Pro Plus computer software, version 4.5 (©Media Cybernetics) for Windows®, was used to digitize the histological images the each phase of the postnatal development.

The stereological analyses of the tissue compartments was determined according to the procedure of Weibel using a 168-point grid test system [28], as applied to the rat male prostate [29]. The data were obtained from 20 random microscopic fields per experimental group in the ages studied at 100x objective. The relative volume (%) was calculated after counting the number of points that coincided with each of the tissue compartments (epithelium, lumen of ducts, stroma and non-muscle stroma). The absolute volume (mm^3) of each of these compartments was determined by multiplying the volume relative by the mean prostatic weight based on the determination that 1 mg of fresh rat ventral tissue had a volume of approximately 1 mm^3 [30].

Animal handling and experiments were done according to the ethical guidelines of the State University of Campinas, following the Guide for Care and Use of Laboratory Animals (process Nr. 1214-1). The large sample size used in this work was justified by the minute size of the organ and the large number of analytical procedures employed.

Immunohistochemistry (IHC)

Tissue sections (4μm) of formalin-fixed, paraffin-embedded gerbil prostatic ventral lobe were immunostained as described previously. Antigen retrieval was performed in citrate buffer (pH 6.0) by steamer for 45 min. and endogenous peroxidase activity was quenched by incubation in 3% hydrogen peroxide in methanol for 10 min. Thereafter, the

sections were incubated overnight at 4°C in a humidified chamber in rabbit polyclonal AR (N-20) primary antibody (Santa Cruz Biotechnology, CA, USA), diluted 1:100 in PBS containing 3% goat serum. The reaction sites were visualized by incubating the tissue sections with biotinylated second antibody, avindin-biotin-peroxidase complex, and diaminobenzidine reagent. In the negative control sections, PBS containing 3% goat serum replaced the primary antibody and sections were stained with Hematoxylin as a blue nuclear counterstain for 8 sec.

Immunostaining for AR was assessed by visual estimation under light microscopy at x 1000 magnification (oil immersion). Immunostaining for AR was additionally assessed by visual counting of immunopositive and immunonegative nuclei and expressed as a percentage of the total of 30 acini and 30 area stromal nuclei counted [31-33].

Hormonal serum concentrations

After blood collection the serum was separated by centrifugation and stored at -20ºC for subsequent hormone assay. The determination of serum concentration of testosterone was performed by luminescence-immunoassay (mouse antibodies anti-testosterone – Johnson & Johnson ®, USA) in an automatic analyzer. The sensitivity was 0.1–150 ng/ml for testosterone. The intra-assay and inter-assay variations were 4.6 and 4.3%, respectively.

Statistical Analysis

All statistical analyses were performed with Statistica 6.0 software (StatSoft). The ANOVA and Tukey honest significant difference (HSD) tests were applied and $p \leq 0.05$ was considered statistically significant.

Results

Morphological and stereologic aspects of the prostatic ventral lobe

The analysis of histological sections shows that the ventral prostate lobe of the gerbil is formed by tubular alveolar glands, lined with a prismatic epithelium which is simple and active in terms of production of secretion (depending on the phase of postnatal development), and with the nuclei of these cells chiefly disposed in a polarized way in the basal region (Fig. 1).

The prostatic ducts are surrounded throughout their extent by layers of smooth muscle fibers (muscular stroma – MS) which connect themselves by closely forming a concentric cluster and by means of a loose connective tissue, non-muscular stroma (NMS), detectable between the epithelium and the muscular stroma. The NMS is also present between the ducts, in the form of loose connective fibrovascular tissues containing fibroblasts and low-caliber blood vessels, each with its important and specific role in the viability and secretory function of the tissue (Fig. 1).

According to the age of the animal, it has been observed that the prostatic ducts have three different types of architectural differentiation, conferring a higher or lower diameter and luminal structure. In the glandular lumen, there is a secretion of an array of proteins since it is stained intensely by eosin (Fig. 1).

Young phase

The control group animals reached an average body weight of 38g and their prostatic complex weighed an average of 0.05g. The ventral lobe is composed of small (or developing) secretory ducts constituted by a simple epithelium with folds in their extension and with mainly oval nuclei. The ducts are lined with a thick layer of concentric smooth muscle fibers and with a large NMS between the epithelium and the smooth muscle layer (Fig. 1). The analysis of the absolute volume of the prostatic compartments have shown that the epithelium occupies 0.83 mm^3 of the organ, the lumen 0.45 mm^3, the MS 1.1 mm^3 , and the NMS 2.6 mm^3 (Table 1).

The biometrical results show that the group treated with testosterone after BAC presented a significant 221% increase in relative prostatic weight in comparison with the control group, and they have also demonstrated that this growth was reflected in the prostatic compartments after the stereologic analysis, presenting highly significant values. The other treatments, despite decreasing the values of the biometrical parameters, did not present a statistically significant reduction.

Adult phase

In this phase the animals reached an average body weight of 70.4g, and their prostatic complex weighed an average of 0.73g. The morphological analysis associated with stereological data show that the prostatic ducts appeared wider, with a luminal volume of 32.0 mm^3, resulting in an epithelium of 11.0 mm^3 that displays small epithelial cells, without any type of folding, probably due to its distention during the secretory process. Besides, the ducts are surrounded by only one layer of smooth muscle fiber, in which this muscular stroma occupies a volume of 8.5 mm^3, compared with a volume of 22.0 mm^3 for non-muscular stroma (Fig. 1 and Table 2).

The biometric data for descriptive statistics presented significant changes only the BAC group, showed a significant decrease in relation to the prostatic complex weight, resulting in a decrease of 39% in relative weight. The animals treated with flutamide and BAC displayed significant reduction in absolute volumes of the epithelium, lumen and non-muscular stroma, when compared to the other groups (Table 2).

Old phase

After completing one year of life, the animals were sacrificed. By then, their average body weight had reached 93.0g and their prostatic complex weighed an average of 0.92g. The ventral prostate region of these animals is composed of prostatic ducts with a luminal volume of 46.0 mm^3 and it contains an epithelium of 11.0 mm^3 with small folds whose cells can be columnar or short. An interesting feature was the presence of ducts lined with a thick smooth muscle layer with a volume of 11.6 mm^3 and a large region of

non-muscular stroma measuring 23.5 mm^3, which differs, mainly, the young phase (Fig. 1 and Table 3).

Among the treatments, the castrated group presented a statistically significant reduction in prostatic complex weight, associated with a reduction of the absolute volume of the epithelium, lumen and the non-muscular stroma. In addition, the group treated with testosterone after the androgen blockage showed a significant growth of 22% in prostate relative weight (Table 3).

The morphological variations presented in the muscular and non-muscular stroma of the control animals along the three ages analyzed appear to be related to the extensive proliferation of the secretory compartment of the epithelium during sexual maturity (Fig. 1). After the analyses of the relative volumes (%) of the prostatic compartments, it was possible to notice that the proportion of MS and NMS had apparently decreased from the young age to adulthood and it had also shown a luminal increase (Tables 1, 2 and 3).

Hormonal profile

The hormonal dosage of serum testosterone presented a particular characteristic for each phase of postnatal development. The young control group presented 3.2 ng/ml of serum testosterone, 3.8 ng/ml in the adult group, and 2.7 ng/ml in the old group. Among the different treatments to which the animals were subjected, the groups treated only with antiandrogenic flutamide and those treated with testosterone after BAC were the ones that showed statistically significant results. The treatment with flutamide for 7 days was sufficient to increase the testosterone concentrations in the 3 ages researched. Hence, the treatment with testosterone after BAC for 7 days caused the levels to exceed 300 ng/ml at all ages (Tables 1, 2, and 3).

Immunohistochemical analysis

The immunohistochemical analyses allowed the standardization of different staining intensity degrees related to AR in the ventral lobe of the gerbil prostate as degrees of intensity ranging from Strong and Moderate to Negative (no stain).

The immunohistochemical evaluation associated with stereology demonstrates that in the control group there is a heterogeneous pattern of positive responses for AR in the stromal region and mainly in the epithelial region of each phase of gerbil postnatal development. The AR expression was detected in the nuclei of basal and luminal epithelial cells and of some stromal cells (nuclei of smooth muscle cells and fibroblasts) and the epithelial region has presented a greater intensity of expression to AR than the stromal region (Fig. 2).

The epithelial basal cells, discontinuously located along the basal membrane and in lesser frequency than the secretory luminal cells, showed apparently the same degree of stain intensity for each phase and, therefore, they can also be direct targets of androgenic action (Fig.2). In the negative control of immunohistochemical reaction, no AR expression was detected in the nuclei of either stromal or epithelial cells during the 3 ages evaluated (Fig. 2A).

In addition, it could be noted that all the types of androgen blockage therapies used in the 3 phases of postnatal development significantly reduced the intensity degree of AR expression compared to the control group. In the each group experimental was observed in increase in the AR-positive stromal cells when compared to the epithelium after the androgen blockage therapies (Fig. 3 and 4).

Young phase

In the control group 49% of the epithelial cells (luminal + basal) presented strong staining intensity, 32% stained moderately and 22% were AR negative. The same analysis of the stromal region demonstrated a balance between strong staining intensity corresponding to 33%, whereas moderate and negative staining corresponded to 36% and 31%, respectively (Fig. 2, 3 and 4). Thus the morphostereological characteristics

associated with the hormonal profile during the young age apparently contributed to stimulating a major expression of AR in the stromal region (69% AR-positive cells and 31% AR-negative cells) and even more so in the epithelial region (78% AR-positive cells and 22% AR-negative cells), in comparison with the other phases of postnatal development (Fig. 2, 3 and 4).

The stereological results of AR expression in the prostate ventral lobe show a significant decrease, mainly the high degree of staining, in all the experimental groups compared to the control group. The nuclei of both the epithelial and stromal cells responded positively to AR expression, but with very heterogeneous degrees of staining intensity (Fig. 2, 3 e 4). Moreover, after the androgen blockage therapy was possible to observe an increase in the stromal region AR-positive cells associated an increase the AR-negative epithelial cells.

When one compares the possible efficiency of androgen blockage during this developmental phase, it is possible to observe that orchiectomy and antiandrogenic action, as well as both associated can considerably decrease the AR expression in the prostatic ventral region (Fig. 3 and 4). Among the 3 ages researched, the young phase presented the highest AR stain intensity, both in the epithelium and in the stroma, even after the androgen blockages.

Adult phase

Although the intensity of AR expression in comparison with the young phase was lower, it was greater than that of the old phase. A highly heterogeneous pattern of AR-positive cells can be observed both in the epithelial and stromal compartments (Fig. 2).

Within the control group 42.5% of cells in the epithelial region presented strong staining intensity, 27% moderate staining, and 30.5% negative; but in the stroma, 23.0% of cells presented strong stain, 29% moderate, and 48% AR-negative. These data demonstrate that, although the adult phase presents the highest concentration of serum testosterone, there was a sharp decrease of AR expression mainly in the stromal region in comparison with the young phase (Figs. 3 and 4).

In addition, it was possible to observe that within all the experimental groups, mainly in groups II and IV which had been surgically castrated, there was a marked and significant decrease of AR expression in the prostatic compartments. In the animals that had been surgically castrated 7 days before, 92% of the epithelial cells and 84% of the stromal cells had been AR-negative (Fig. 3 and 4).

Hormonal reposition with testosterone after BAC resulted in a high concentrations of this androgen in the serum, but in contrast to the young phase, it did not cause a significant increase in the absolute and relative volumes of the different prostatic compartments analyzed and, in addition, a significant increase could be noted in the AR-negative cells (80% in the epithelium and 71.5% in the stroma) (Fig. 3 and 4).

Old Phase

The immunohistochemical analyses associated with AR stereology in the control group revealed that 40% of the epithelial cells were AR-negative, while 35% and 25% presented moderate and strong staining, respectively. The highly significant results were revealed in the stroma with strong staining intensity corresponding to only 17% of the cells, 19% moderate staining, and 64% of AR-negative cells. Among the 3 phases of postnatal development studied, the old phase presented the lowest degree of expression of androgen receptors in the ventral lobe of the gerbil prostate (Figs. 2, 3 and 4).

As for the 4 experimental groups, it was possible to notice highly significant differences of reduction of AR expression in comparison with the control group. Within the castrated group, 91.5% of the epithelial cells and 89.0% of the stromal cells were AR-negative while in the BAC group, 91% of epithelial cells and 87% of the stromal cells were AR-negative (Figs. 3 and 4).

Since the prostate of senile animals is an androgen-dependent gland, it was possible to observe a statistically significant increase of AR expression in the group V in comparison with the other androgen-blocked groups, that is, in the epithelial compartment, 32% of the cells were AR-positive and 68% were AR-negative, and in the stroma, the breakdown between AR-positive and AR-negative was 46% to 54%, respectively. Therefore, the gerbil old age provides the best evidence that the prostate is an androgen-

dependent organ and that the 7-day androgen treatment after androgen blockage resulted in the immunostained need normal conditions in comparison with both the adult and the young phases of this species (Fig. 2, 3 and 4).

Thus, the immunohistochemical analyses associated with the stereology of androgen receptor expression in the ventral lobe of the gerbil prostate have contributed to elucidating the fact that in this rodent species there is a heterogeneous pattern of positive responses to AR in each prostatic compartment and to each phase of postnatal development in the treated groups, compared to the control groups. Besides, the analysis of variance (ANOVA) shows that the different staining degrees related to androgen receptors at each age were highly significant, with $p \leq 0.05$.

Discussion

The immunohistochemical analyses of the ventral lobe of the gerbil prostate have shown that androgen receptor expression has been detected in the nuclei of luminal and basal epithelial cells as well as in some stromal cells.

The basal cells of the rat adult prostate and the prostatic ventral region of mice were AR negative and, for that matter, they do not seem to be direct targets of androgenic action [31, 34-37]. This absence of AR in the basal cells can be a common characteristic in other species of rodents [46].

In the gerbil, the basal cells express the androgen receptor protein in the 3 phases of postnatal development, but the greatest concentrations are apparently in the young animals. The AR staining intensity corresponded to that of luminal cells in all the experimental groups. Besides, this cluster of cells showed strong staining after hormonal reposition of testosterone in animals that had been subjected to BAC, probably by responding to the androgenic action with an increase in proliferation.

These data can be best explained based on studies of castrated rats, in which it is possible to observe that, after castration, the luminal cells undergo drastic atrophy while the basal cells remain unchanged and they also increase their proliferation rate in order to

reconstruct the glandular epithelium when there is androgen reposition [44, 45, 47]. The functional meaning of basal cells in rat prostates is not quite established, although it has been suggested that they can act as either reserves or stem cells of the luminal epithelium as well as phagocytic cells that regulate the number of secretory cells or as potential mediators between the activities of luminal cells related to regulation of the transportation of materials between the stromal and epithelial compartments [31, 48].

More recent immunohistochemical analyses have shown that these cells express the cytokeratins CK5 and CK14, as seen in undifferentiated epithelial-cell staining (in proliferation activity) [49]. Based on these findings, this cellular type has been assigned a role in the regeneration of the glandular epithelium and it has also been suggested that at least a part of this cell population has stem cell properties [45, 47]. This could explain the AR expression in the basal cells of the gerbil ventral prostate, even after the androgen blockages.

Other important components of the glandular epithelium include the luminal secretory epithelial cells since they are the most abundant and are responsible for the secretion of prostatic fluid. These cells, often cylindrical and differentiated, not only express activity specific to enzymes such as prostatic acid phosphatase [51] and prostate specific antigen–PSA, but also can be identified by their selective expression of cytokeratins CK8 and CK18 [49, 52].

In the gerbil, these cells have presented a high intensity of AR expression located in the nucleus, which indicates that they are direct targets of androgenic action. The androgen blockage caused a decrease in AR expression, which thus provoked the emergence of a heterogeneous pattern of AR positive and AR negative cells at all the ages evaluated. The intense AR staining observed in luminal epithelial cells contrasts with the abundance of 5α-reductase enzyme present in the stroma, which converts testosterone into dihydrotestosterone (DHT) in the prostate and can also directly act on the epithelial region [38-41, 53, 54].

The populations of luminal and basal cells have been apparently resistant to the androgenic blockages used for a period of 7 days, that is, these cells activated during the gland recovery process after the exogenic androgen reposition and presented a strong

staining intensity for AR, which has also been observed in adult mice that had been castrated 3 weeks earlier and subjected to hormonal reposition [31]. It is important to observe that hormonal reposition with testosterone resulted an increase in AR expression in the ventral lobe of the gerbil prostate, though; this expression was not the same as the one observed in the control groups. Therefore, the androgenic reposition after some period of androgen blockage not showed the same comportament in AR expression for motives that are still unknown.

The according by [55], described the regional morphofunctional differences along the system of ducts of the rat prostate ventral lobe. These authors suggested that the epithelium responds in different ways to the same concentration of circulating androgen, with responses ranging from cellular proliferation to cellular death, and that the activity of epithelial cells is determined by the stromal component.

At first, the epithelium was regarded as the main target of the action of androgens in the prostate. However, in recent decades, several findings indicate that the androgens act primarily on the prostatic stromal cells and the epithelial activity is, for the most part, mediated by stromal factors [38, 43, 55-57].

Although there are some variations among the species, the prostatic stroma in mammals is composed of smooth muscle cells and fibroblasts spread among the elements of the extracellular matrix. The stromal cells produce a complex gamut of components of the extracellular matrix including types I and III fibrillar collagen as well as type VI micro-fibrillar collagens [58, 59]. A series of studies have attempted to clarify the participation of these macromolecules in controlling the prostatic function based on the phenomenon of prostatic regression caused by castration [58-62].

In the gerbil, it was possible to observe that the stromal cells are AR-positive, mainly the smooth muscle cells around the ducts, but they present a significantly lesser staining intensity in relation to the epithelial layer, which suggests that the epithelial cells present a greater amount of stroma-related androgen receptor protein. However, after hormonal treatments, it was possible to notice an increase in stromal AR expression in relation to the epithelium in the all phase of gerbil postnatal development. Therefore, in this species of rodents, there is a homeostasis regulated by both the direct and the indirect

action of androgen receptors and that both the epithelial and stromal compartments seems to be the mains targets of the androgenic and antiandrogenic action and the emergence of prostatic age-related lesions [62].

Among the 3 ages studied, the young phase has been the one that presented the highest staining intensity for AR, even after the androgen blockers. The according by [63], observed that the presence of AR in the prostate after castration and in the absence of endogenous ligands cannot prevent receptor transcriptional activity by the cells, and they have also stated that several molecular signaling pathways of the growth factors can influence the transcriptional activity of androgen receptors.

The concentrations of serum testosterone produced by the testicles in the young phase apparently contributed to stimulating a major expression of AR in the prostatic compartments, which is necessary for the development and functionality of this organ during this phase of postnatal development until it reaches adulthood [3, 42]. Besides, the effective response to the concentrations of circulating androgens caused the prostate of the surgically and / or chemically castrated animals not to present significant decrease in its relative prostate weight as well as in the expression of androgen receptors in comparison with the other ages of postnatal development.

These data are based on the works of [64] and [7], about the rat prostate, where it was possible to observe that the serum testosterone concentrations are very low during the first postnatal weeks and that, during puberty (young phase), there is a significant increase in serum testosterone, which induces prostatic growth. Moreover, it is important to observe that the total cellular content for AR is influenced by the presence or absence of androgens, a process called autoregulation [10, 11, 65].

Statistically, the androgenic blockage reduces AR expression much more in the epithelial region than in the stromal region, and all of this is associated with a decrease in absolute and relative volumes of the epithelial compartment and with an increase in the stromal compartment during the young phase. Exogenous hormonal reposition after BAC resulted in a high concentration of serum testosterone, which stimulated a significant increase in the absolute volume of different prostatic compartments, resulting in an increase in the relative weight of the prostate. But this hormonal reposition was not

sufficient to stimulate a recovery or an AR major expression after the androgenic blockage, that is, it was possible to notice a significant increase of AR-negative cells (78% in the epithelium and 74% in the stroma). Therefore, it was not possible to observe a close relationship between the size of the prostatic complex and the intensity of AR expression during the young phase.

In the adult phase, once the prostatic gland had reached its normal size, an increase was observed in the luminal diameter associated with an apparent decrease in epithelial height and a reduction in the stromal area in comparison with the young phase. In addition, the concentrations of circulating androgens in this period are responsible for the morphofunctional maintenance of the organ, which indicates a heterogeneous pattern of AR expression in the epithelial and stromal cells probably due to the balance between the proliferation and the death of cells in the prostatic compartments. Statistical analyses after the androgenic blockage therapies showed a significant reduction in the AR expression degree.

Moreover, in the adult phase, AR expression does not seem to be directly related to the weight of the prostatic complex and even less to the detected concentration of circulating androgen. Therefore, there must be an intrinsic androgen-dependent autoregulatory mechanism in AR expression in the gerbil prostate, as shown in experiments carried out by [3], with Brown Norway rats subjected to orchiectomy. A series of partial studies developed by [42, 46, 66, 67], with young and adult Sprague Dawley rat prostates reported that there is a specific autoregulation in each prostatic lobe for the androgen receptor.

When the animals reached old age, a highly significant decrease in the serum testosterone concentrations associated with the high rate of AR-negative cells was observed not only in the control group but also in the experimental groups. Therefore, this phase of development, in comparison with the others, seems to be much more susceptible to variations and hormonal treatments. One of the hypotheses for explaining this phenomenon is the fact that there are changes, whether molecular or not, in the nuclear AR expressions due to the decrease of testicular androgen production with age, causing the epithelial cells

to contribute to the evolution of cellular androgen-dependent hyperplasia and, in more serious cases, they can become androgen-independent [3, 7, 68, 69].

After the moment when the prostate has reached its highest level of normal growth, cellular hyperplasia or prostatic carcinoma appears in some species, including humans, dogs and some species of rats. These changes are related to the decrease of testicular androgen production and to peripheral concentrations of androgens acting on the prostate. Besides, AR mutations can occur with a certain frequency, especially in advanced prostatic tumors, which develop resistance to hormonal therapy. But the molecular mechanisms involved in the processes of proliferation, differentiation and apoptosis have not yet been completely established, not to mention the mechanisms of neoplasic transformation and carcinogenesis [40, 41, 70-72].

The androgens, whether from testicular or adrenal origins, are not necessary for the development of epithelial receptors in the postnatal rat prostate and that the AR expression in the epithelial region is genetically determined by androgens prenatally or by other factors that remain unknown [73]. This could explain the heterogeneous pattern of AR expression in the prostatic compartments in each phase of gerbil postnatal development in the control groups and especially in the experimental groups.

The influence of the androgens on both the structural organization and physiology of the prostate is a well-known fact. It is known that androgen suppression leads to a process of involution known as prostatic regression, mainly characterized by its epithelial atrophy and progressive decrease in glandular volume [16]. Thus the androgen blockage therapies dispensed during the 3 phases of gerbil postnatal development significantly influenced the decrease of AR expression in the prostatic compartments.

According to [74], described the effect of castration on the adult gerbil prostatic lobes. The results demonstrate that the decrease of androgens caused a progressive reduction of the wet weight and the relative weight of the set of prostatic lobes. Three weeks after castration, the lobes had lost more than a half of their weight. This process of involution resembles the one described for the rat [57, 61, 75] and it is characterized by reduction of acinar size and by epithelial atrophy, which in the species studied had become clearer two weeks after castration. In addition, the comparison of the

involution process between the lobes of the Mongolian gerbil shows that the ventral lobe decreased in epithelial height and volume during the first week after castration, which was not detected in the dorsal lobe. Such an observation may suggest a greater androgenic dependence on the epithelial cells of the ventral lobe in comparison with the dorsal lobe for the maintenance of secretory activity, as observed in other rodents [3].

According to [75], androgen depletion through chemical or surgical castration causes an important prostatic involution and, although about 5% of residual circulating testosterone remains after orchiectomy, peripherally produced by the adrenal gland, this testosterone is not enough to maintain the organ homeostasis, which could explain the significant changes in the prostatic compartments.

Flutamide is a potent non-steroidal antiandrogenic agent functionally specific for the sexual androgen-dependent accessory structures, which is the case in the prostate gland. Although this drug is efficient in treating BPH by inhibiting the union of androgens to their respective cellular receptors, it also stimulates the release of LH and, consequently, the increase of testosterone concentrations and, for that reason, many patients remain potent during the treatment [19, 21, 22, 76]. These physiological results have also been observed in the gerbil, in which the testosterone concentrations significantly increased in association with the decrease of AR expression in comparison with the control group.

In the lateral prostate of male guinea pigs, androgen blockage with flutamide has provoked distinct morphological and structural changes in some tissue components taking into account the phase of postnatal development. This differentiated action of flutamide in several phases of the postnatal development seems to be directly connected to hormonal influence that is sometimes absolute and sometimes relative or non-existent in rodents [17, 64]. It could explain the heterogeneous pattern of AR expression in the gerbil prostate ventral lobe.

Several studies have demonstrated that the combination of flutamide with surgical castration in the treatment of prostate cancer increased the degree of responses to the treatment and, most importantly, it increased survival by an average of 7 months compared to the utilization of only LHRH agonist and orchiectomy. Besides, these studies

state that, for best efficiency, the anti-androgen must be combined with surgical castration during the initial phases of the treatment [19, 23, 64, 76].

The combination of flutamide with surgical castration for 7 days resulted in the decrease of the serum testosterone concentrations associated with a significant decrease of AR expression, mainly in the old phase where the concentration of endogenous androgen is lower in comparison with the other ages. Therefore, this therapeutic combination dispensed to this rodent species seems to be the most effective means of blocking circulating androgen, which in turn will be able to be used for treatment of age-related prostatic lesions.

The vast scope of research in the area of steroidal receptors in the last 3 decades has provided considerable information on the physiological and pathologic functions of steroidal receptors. The androgen receptor is the most recently cloned steroidal receptor and it has been extensively researched due to its involvement in the male reproductive system and in prostate cancer [16, 42, 77-79]. The analyses of each modulation stage of AR activity constitute a prerequisite for better understanding the of androgen receptor expression in the target tissues. Molecular and clinical studies have provided important information on how the androgen-receptor signaling pathways can act on these diseases. However, the evaluation of other molecular mechanisms is necessary to understand the physiological and pathological functions of these androgen-receptor signaling pathways, as well as the development of therapeutic drugs to treat the patients.

Conclusions

In the Mongolian gerbil there is a distribution pattern of AR in the prostatic ventral lobe through postnatal development in which the younger animal the greater the interaction of circulating androgens that stimulate the AR expression in the epithelial and stromal compartments. Besides, in this species of rodent, the basal cells express AR and it is possible to assign this cell population property of stem cells that play an important role in the regeneration of the glandular epithelium.

The androgen blockage therapies reduced AR expression in the prostatic compartments but the androgenic reposition after these blockages was not showed the same the degree of expression of androgen receptor near normal physiological conditions. In general, the complete androgen blockage therapy seems to be the most efficient means of androgen depletion in the gerbil, which, in turn, can be used to treat prostatic age-related lesions.

It is possible to conclude that the regulation and distribution of androgen receptors in the gerbil prostatic compartments are complex mechanisms that probably are genetically regulated by prenatal androgens or other factors that are still unknown. The gerbil appears to be a valuable experimental model in the attempt to improve the knowledge of the morphophysiological and pathological behavior of the human prostate throughout aging and the formulation of new therapeutic ideas to prevention prostate cancer.

Authors' contributions

RSC carried out the sampling, tissue processing, developed the methods and wrote the manuscript. SGPC helped immunohistochemical assays, supervised analysis and participated in discussing the results. WRS carried out the hormonal immunoassay and participated in discussing the results, and helped write the manuscript. FCAS made the statistical analysis, participated in the discussion of the results, and helped write the manuscript. SRT and PSLV participated in the planning of the experiments and, the discussion of the results, and helped write the manuscript.

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Figures Legends

Figure 1: Histological sections stained with HE of young, adult and old gerbil lobe ventral prostate. Legends: **ep** - epithelium; **L** - lumen; **SMC**– smooth muscle cell; *** -** non-muscular stroma. Bars: 50 μm.

Figure 2: Histologic sections submitted to AR IHC. Brown stain means positive demarcation. Legends: **arrow-stron** – stained stronger; **arrow-mod –** stained moderate; **arrow-neg –** stained negative; **arrowheads –** basal cell. Bars: 20 μm. **Figure 2A**: Negative Control for IHC.

Figure 3. Graphic representation of epithelial cells counting with different staining degrees of intensity for the AR expression in the different phases of the postnatal development. *Statistically significant differences between groups ($p \leq 0.05$).

Figure 4. Graphic representation stromal cells counting with different staining degrees of intensity for the AR expression in the different postnatal development phases. *Statistically significant differences between groups ($p \leq 0,05$).

TABLE 1: Quantitative analysis of experimental groups in the young phase (mean ± SEM).

Quantitative analysis (g)		Control	Castrated	Flutamide	BAC	BAC+Testosterone
Body weight *		$38.0^{a} \pm 0.73$	$42.5^{a} \pm 0.9$	$43.3^{a} \pm 2.0$	$38.0^{a} \pm 2.0$	$58.4^{b} \pm 1.5$
Prostatic complex weight *		$0.05^{a} \pm 0.005$	$0.05^{a} \pm 0.003$	$0.04^{a} \pm 0.003$	$0.04^{a} \pm 0.003$	$0.23^{b} \pm 0.02$
Relative prostate weight † *		$0.001^{a} \pm 0.00012$	$0.0009^{a} \pm 0.0002$	$0.0009^{a} \pm 0.00008$	$0.001^{a} \pm 0.00007$	$0.004^{b} \pm 0.003$
Relative weight variation ‡ (%)		___	- 7.7	- 23.5	- 14	+ 221
Testosterone concentrations ng/ml *		$3.2^{a} \pm 0.38$	$1.2^{a} \pm 0.12$	$13.0^{b} \pm 2.5$	$3.6^{a} \pm 0.8$	$318^{c} \pm 11.6$
Stereological Data						
Relative Volume (%)	Epithelium *	$17.0^{a} \pm 0.56$	$11.0^{b} \pm 0.8$	$20.0^{c} \pm 0.6$	$11.0^{b} \pm 0.56$	$20.0^{c} \pm 0.9$
	Lumen *	$9.0^{a} \pm 1.4$	$5.0^{a} \pm 0.5$	$8.0^{a} \pm 0.5$	$3.5^{b} \pm 0.5$	$45.0^{c} \pm 1.75$
	Muscular Stroma *	$23.0^{a} \pm 1.4$	$32.0^{b} \pm 2.3$	$21.0^{a} \pm 1.0$	$30.0^{b} \pm 1.9$	$16.0^{c} \pm 0.8$
	Non-Muscular Stroma *	$51.0^{a} \pm 1.82$	$52.0^{a} \pm 2.4$	$51.0^{a} \pm 0.9$	$55.5^{a} \pm 1.95$	$19.0^{b} \pm 1.0$
Absolute Volume (mm^3)	Epithelium *	$0.83^{a} \pm 0.02$	$0.6^{a} \pm 0.04$	$0.8^{a} \pm 0.025$	$0.45^{b} \pm 0.02$	$4.6^{c} \pm 0.21$
	Lumen *	$0.45^{a} \pm 0.7$	$0.24^{a} \pm 0.025$	$0.34^{a} \pm 0.02$	$0.14^{a} \pm 0.02$	$10.3^{b} \pm 0.4$
	Muscular Stroma *	$1.1^{a} \pm 0.07$	$1.6^{b} \pm 0.12$	$0.8^{a} \pm 0.04$	$1.2^{a} \pm 0.07$	$3.6^{c} \pm 0.2$
	Non-Muscular Stroma *	$2.6^{a} \pm 0.09$	$2.6^{a} \pm 0.12$	$2.0^{b} \pm 0.04$	$2.2^{a} \pm 0.08$	$4.5^{c} \pm 0.24$

* Statistically significant differences between control and treatments; superscript letters (a, b, c) represent statistically significant differences between the experimental groups, $*p \leq 0.05$.

† Relative weight corresponds to the ratio between the weight of the prostate and that of the whole body.

‡ Relative weight variation is shown with respect to the control, which was taken as 100%.

TABLE 2: Quantitative analysis from experimental groups in the adult phase (mean ±SEM).

Quantitative analysis (g)		Control	Castrated	Flutamide	BAC	BAC+Testosterone
Body weight		70.4 ± 1.3	68.0 ± 4.3	65.2 ± 4.0	61.0 ± 2.6	70.0 ± 3.2
Prostatic complex weight *		0.73^{a} ± 0.025	0.6^{a} ± 0.1	0.52^{a} ± 0.1	0.4^{b} ± 0.1	0.7^{a} ± 0.05
Relative prostate weight †		0.01 ± 0.0002	0.008 ± 0.0013	0.008 ± 0.0009	0.006 ± 0.0015	0.009 ± 0.0004
Relative weight variation ‡ (%)		—	- 17	- 16	- 39	- 4
Testosterone concentrations ng/ml *		3.8^{a} ± 0.15	9.7^{a} ± 0.66	258.4^{b} ± 47.0	8.0^{a} ± 0.96	328^{c} ± 5.2
Stereological Data						
Relative Volume (%)	Epithelium *	15.0^{a} ± 0.75	15.0^{a} ± 0.9	12.4^{a} ± 0.9	11.0^{b} ± 0.8	18.0^{a} ± 0.9
	Lumen	43.0 ± 2.4	41.5 ± 2.8	44.5 ± 2.8	46.0 ± 2.5	42.0 ± 1.7
	Muscular Stroma *	11.5^{a} ± 0.9	12.0^{a} ± 0.8	14.5^{a} ± 1.2	17.5^{b} ± 1.0	15.0^{a} ± 1.3
	Non-Muscular Stroma	30.5^{a} ± 1.9	31.5 ± 1.8	28.6 ± 2.3	25.5 ± 1.8	25.0 ± 1.7
Absolute Volume (mm^3)	Epithelium *	11.0^{a} ± 0.54	9.0^{a} ± 0.55	6.4^{b} ± 0.45	4.5^{c} ± 0.32	12.5^{a} ± 0.64
	Lumen *	32.0^{a} ± 1.75	25.0^{b} ± 1.7	23.0^{b} ± 1.5	18.4^{b} ± 1.0	29.3^{a} ± 1.2
	Muscular Stroma *	8.5^{a} ± 0.7	7.5^{a} ± 0.5	7.5^{a} ± 0.62	7.0^{a} ± 0.4	11.0^{b} ± 0.93
	Non-Muscular Stroma *	22.0^{a} ± 1.4	19.0^{a} ± 1.1	15.0^{b} ± 1.2	10.2^{c} ± 0.7	17.3^{a} ± 1.2

* Statistically significant differences between control and treatments; superscript letters (a, b, c) represent statistically significant differences between the experimental groups, *$p \leq 0.05$.

† Relative weight corresponds to the ratio between the weight of the prostate and that of the whole body.

‡ Relative weight variation is shown with respect to the control, which was taken as 100%.

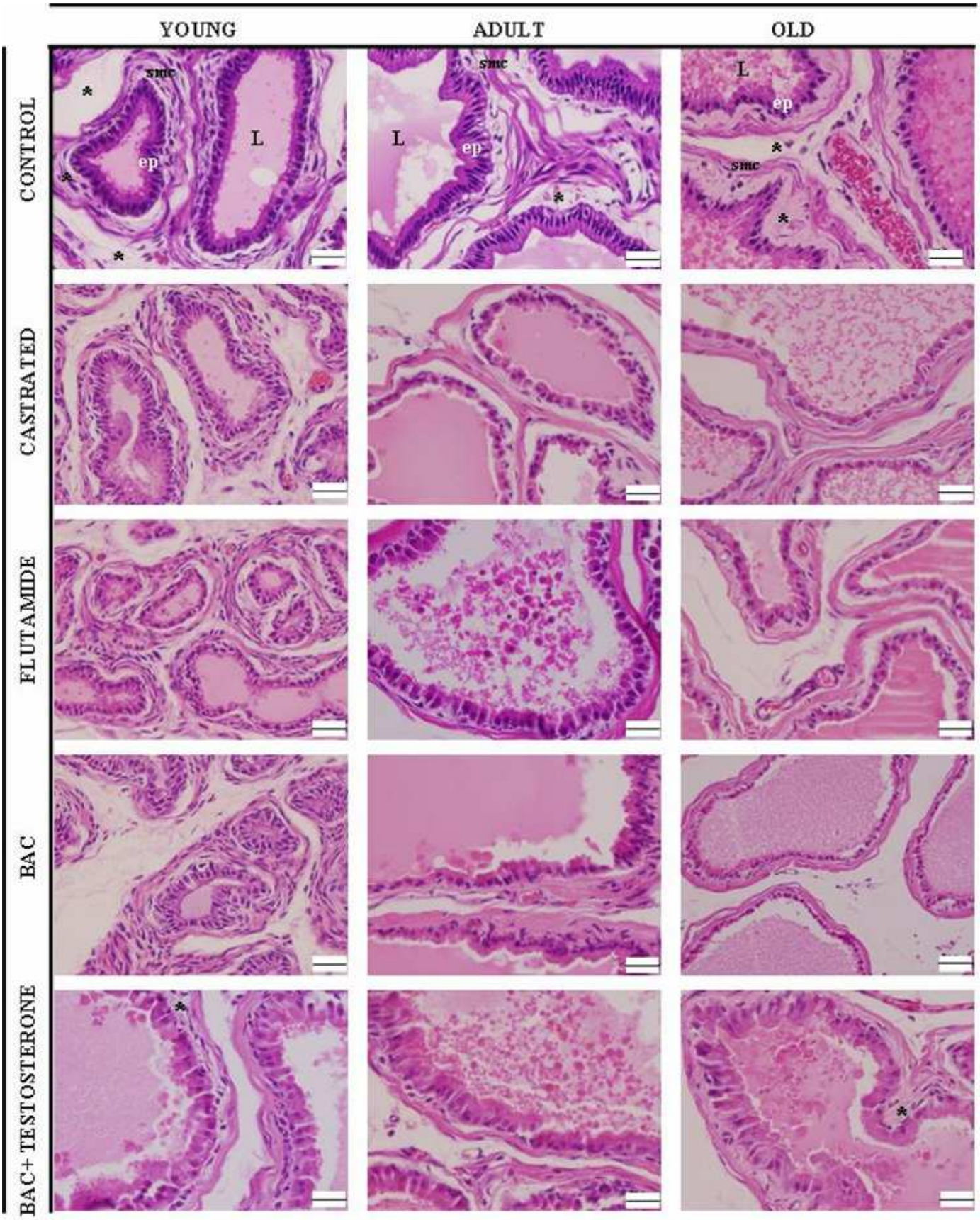
TABLE 3: Quantitative analysis from experimental groups in the old phase (mean ± SEM).

Quantitative analysis (g)		Control	Castrated	Flutamide	BAC	BAC+Testosterone
Body weight		93.0 ± 2.6	96.0 ± 12.3	100.5 ± 3.5	80.0 ± 2..3	72.2 ± 4.3
Prostatic complex weight *		0.92^{a} ± 0.06	0.7^{b} ± 0.05	0.94^{ac} ± 0.04	0.8^{a} ± 0.06	0.85^{a} ± 0.03
Relative prostate weight † *		0.01^{a} ± 0.0003	0.007^{a} ± 0.0006	0.009^{a} ± 0.0005	0.009^{a} ± 0.0008	0.013^{b} ± 0.0012
Relative weight variation ‡ (%)		—	- 25	- 2.6	+ 2	+ 22
Testosterone concentrations ng/ml *		2.7^{a} ± 0.25	1.3^{a} ± 0.2	55.2^{b} ± 11	2.5^{a} ± 0.65	347^{c} ± 9.0
Stereological Data						
Relative Volume (%)	Epithelium *	12.0^{a} ± 0.8	11.0^{a} ± 0.5	13.5^{a} ± 0.9	10.5^{a} ± 0.5	15.5^{b} ± 0.9
	Lumen *	50.0^{a} ± 1.6	51.5^{a} ± 2.0	48.0^{a} ± 1.5	45.0^{a} ± 2.0	40.5^{b} ± 2.65
	Muscular Stroma	13.0 ± 0.7	13.0 ± 1.5	13.5 ± 0.85	14.0 ± 0.9	12.0 ± 1.0
	Non-Muscular Stroma	25.0 ± 1.8	24.5 ± 1.7	25.0 ± 1.2	30.5 ± 1.6	32.0 ± 2.3
Absolute Volume (mm^3)	Epithelium *	11.0^{a} ± 0.74	7.6^{b} ± 0.33	12.6^{a} ± 0.86	8.3^{a} ± 0.44	13.0^{a} ± 0.8
	Lumen *	46.0^{a} ± 1.5	36.0^{b} ± 1.5	45.3^{a} ± 1.4	36.3^{b} ± 1.6	34.4^{b} ± 2.3
	Muscular Stroma	11.6^{a} ± 0.62	9.2 ± 1.1	12.6 ± 0.8	11.0 ± 0.75	10.3 ± 0.85
	Non-Muscular Stroma *	23.5^{a} ± 1.6	17.0^{b} ± 1.2	23.5^{a} ± 1.1	24.5^{a} ± 1.3	27.2^{a} ± 1.9

* Statistically significant differences between control and treatments; superscript letters (a, b, c) represent statistically significant differences between the experimental groups, *$p \leq 0.05$.

† Relative weight corresponds to the ratio between the weight of the prostate and that of the whole body.

‡ Relative weight variation is shown with respect to the control, which was taken as 100%.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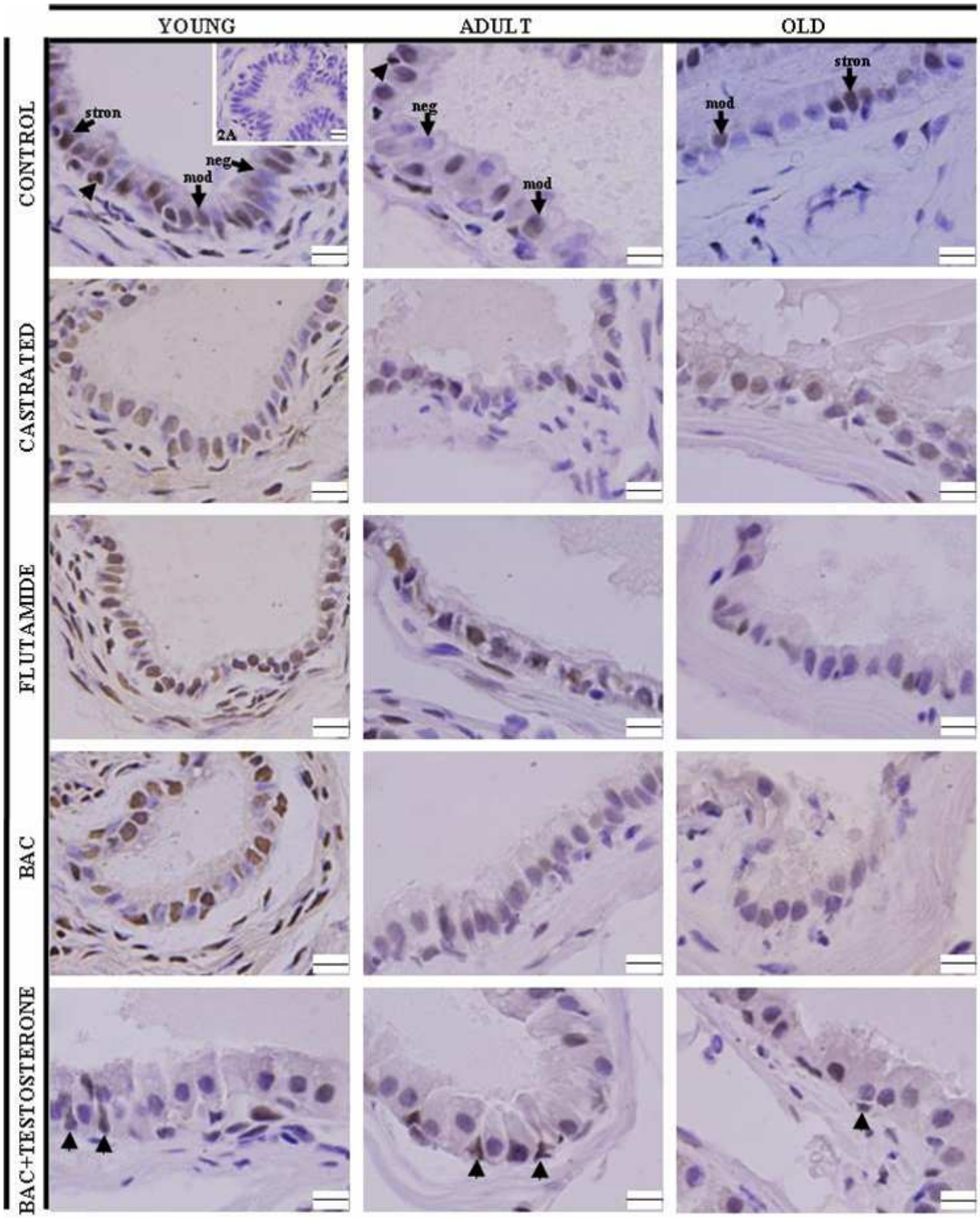
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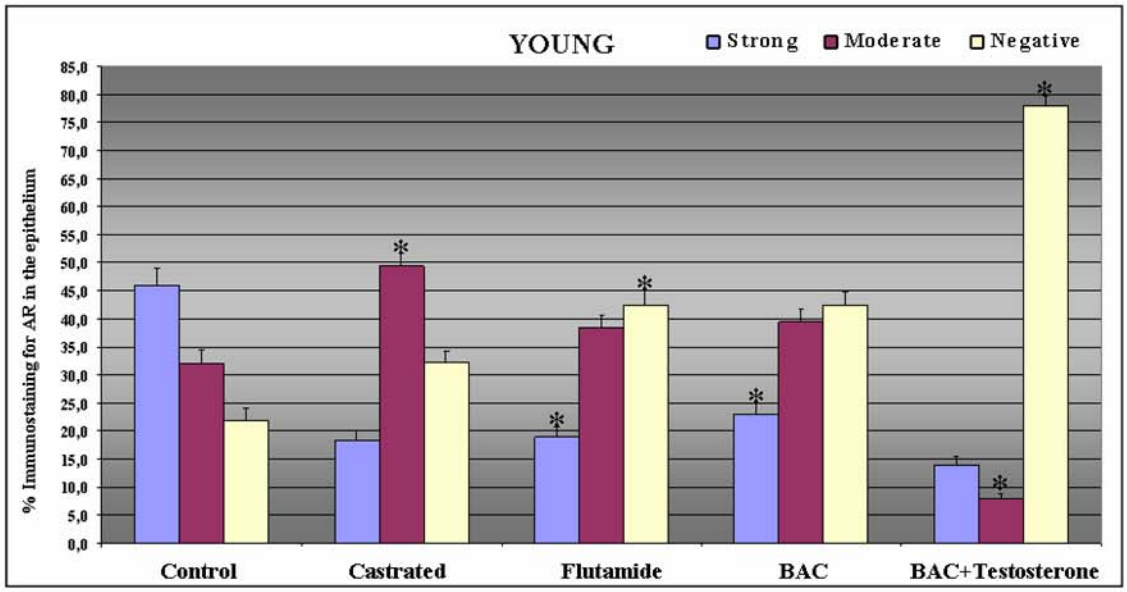
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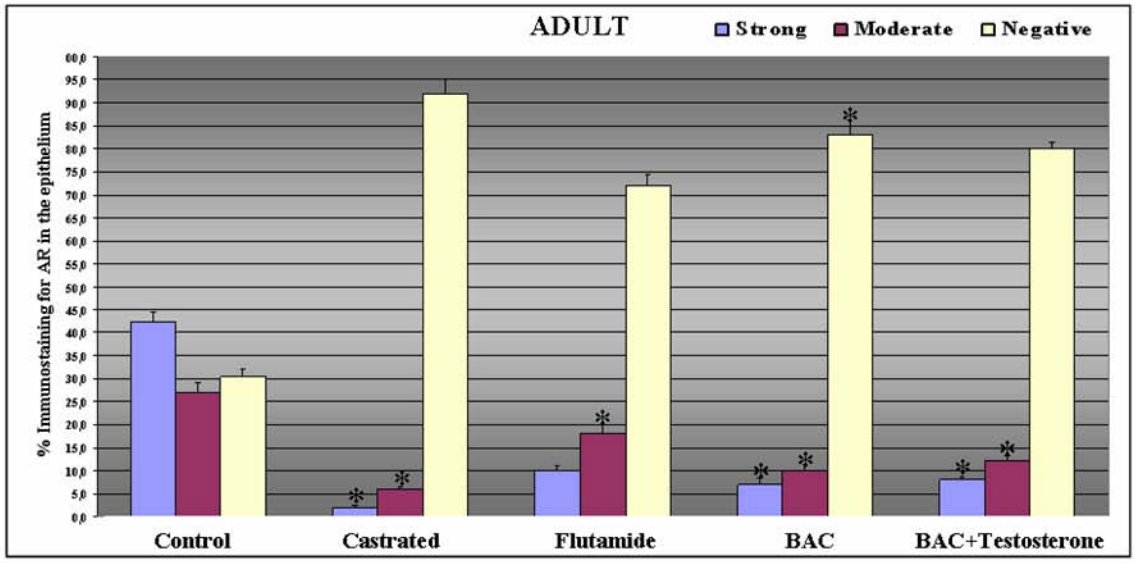
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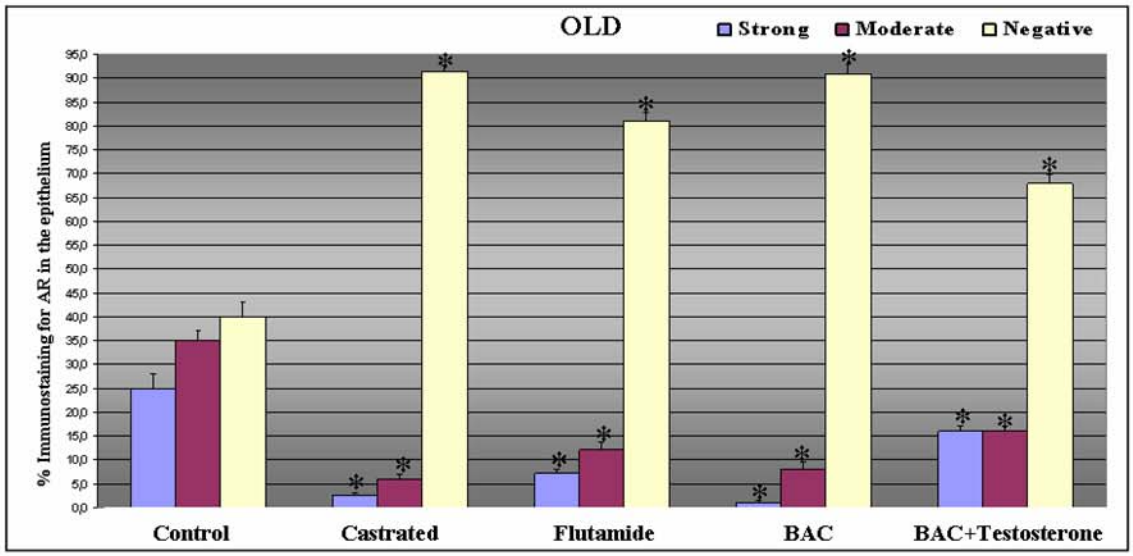
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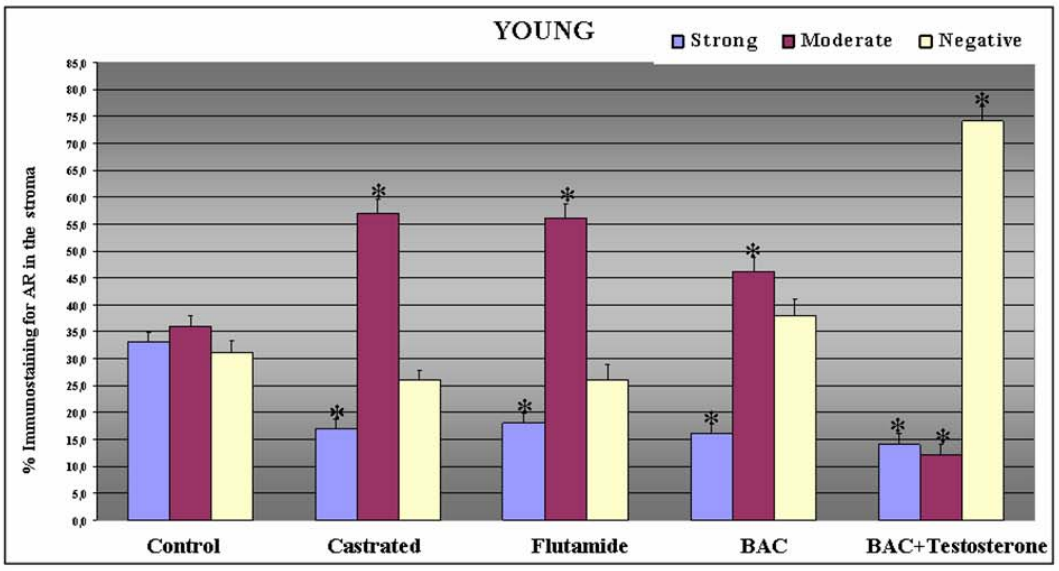
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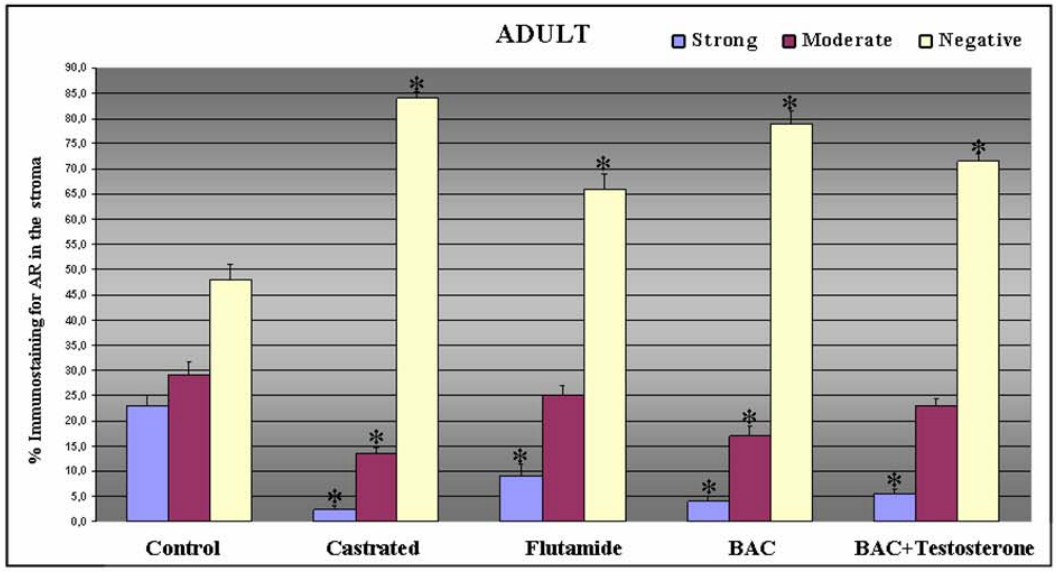
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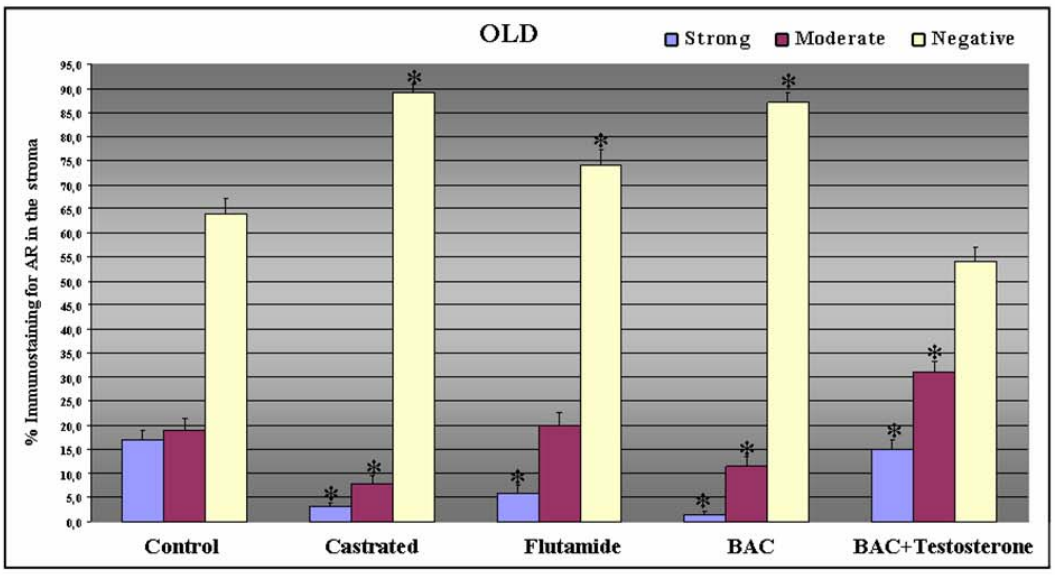
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VI. CONCLUSÕES GERAIS

No gerbilo da Mongólia há um padrão de distribuição do RA no lóbulo ventral prostático ao longo do desenvolvimento pós-natal, sendo que quanto mais jovem for o animal maior a interação de andrógenos circulantes estimulando a expressão de RA nos compartimentos epitelial e estromal. Além disso, nessa espécie de roedor as células basais expressaram RA, podendo atribuir a essa população de célula propriedades de células-tronco exercendo um papel importante na regeneração do epitélio glandular.

As terapias de bloqueios androgênicos diminuíram a expressão de RA nos compartimentos prostáticos e a reposição androgênica após esses bloqueios não apresentou o mesmo grau de intensidade de expressão de RA próximo às condições fisiológicas normais. De maneira geral, a terapia de bloqueio androgênico completo parece ser a maneira eficaz de depleção androgênica no gerbilo, e que por sua vez, poderá ser utilizado no tratamento de lesões prostáticas relacionadas à idade.

Conclui-se que a regulação e a distribuição do RA nos compartimentos prostáticos do gerbilo são mecanismos complexos que, provavelmente, são geneticamente regulados por andrógenos antes do nascimento ou por outros fatores ainda desconhecidos. O gerbilo parece ser um modelo experimental valioso na tentativa de melhorar o conhecimento do comportamento morfofisiológico e patológico da próstata humana ao longo do envelhecimento e da formulação de novas idéias de terapias de combate ao câncer de próstata

VII. REFERÊNCIAS BIBLIOGRÁFICAS

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DECLARAÇÃO

Declaro para os devidos fins que o conteúdo de minha dissertação/tese de mestrado/doutorado intitulada "Expressão de Receptores Androgênicos no Lóbulo Ventral da Próstata do Gerbilo da Mongólia"

( ) não se enquadra no Artigo 1°, § 3° da Informação CCPG 002/06, referente a bioética e biossegurança.

( ) está inserido no Projeto CIBio (Protocolo n°_____), intitulado _____

(X) tem autorização da Comissão de Ética em Experimentação Animal (Protocolo n° 1214 – 1).

( ) tem autorização do Comitê de Ética para Pesquisa com Seres Humanos (?) (Protocolo n°_____).

Aluno(a)

Orientador(a)

Para uso da Comissão ou Comitê pertinente:

(X) Deferido ( ) Indeferido

Nome: Profa. Dra. ANA MARIA A. GUARALDO
Função: Presidente
Comissão de Ética na Experimentação Animal
CEEA/IB - UNICAMP