unesp

CAMPUS DE SÃO JOSÉ DO RIO PRETO

A influência da testosterona nas glândulas reprodutivas acessórias de *Artibeus planirostris* (Chiroptera: Phyllostomidae)

Cintia Cristina Isicawa Puga

DOUTORADO

PÓS GRADUAÇÃO
EM BIOLOGIA ANIMAL

UNIVERSIDADE ESTADUAL PAULISTA
"JÚLIO DE MESQUITA FILHO"
Câmpus de São José do Rio Preto

Cintia Cristina Isicawa Puga

A influência da testosterona nas glândulas reprodutivas acessórias de *Artibeus planirostris* (Chiroptera: Phyllostomidae)

São José do Rio Preto
2015

Cintia Cristina Isicawa Puga

A influência da testosterona nas glândulas reprodutivas acessórias de *Artibeus planirostris* (Chiroptera: Phyllostomidae)

Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto.

Orientador: Prof. Dr. Sebastião Roberto Taboga
Co-orientadora: Profa. Dra. Patrícia Simone Leite Vilamaior

São José do Rio Preto
2015

Puga, Cintia Cristina Isicawa.
A influência da testosterona nas glândulas reprodutivas acessórias de Artibeus planirostris (Chiroptera: Phyllostomidae) / Cintia Cristina Isicawa Puga. -- São José do Rio Preto, 2015
100 f. : il., tab.

Orientador: Sebastião Roberto Taboga
Tese (doutorado) – Universidade Estadual Paulista "Júlio de Mesquita Filho", Instituto de Biociências, Letras e Ciências Exatas

1. Biologia. 2. Morcego - São José do Rio Preto. 3. Castração. 4. Testosterona. 5. Próstata. I. Taboga, Sebastião Roberto. II. Universidade Estadual Paulista "Júlio de Mesquita Filho". Instituto de Biociências, Letras e Ciências Exatas. III. Título.

CDU – 599.4

Ficha catalográfica elaborada pela Biblioteca do IBILCE
UNESP - Câmpus de São José do Rio Preto

Cintia Cristina Isicawa Puga

A influência da testosterona nas glândulas reprodutivas acessórias de *Artibeus planirostris* (Chiroptera: Phyllostomidae)

Tese apresentada como parte dos requisitos para obtenção do título de Doutor em Biologia Animal, junto ao Programa de Pós-Graduação em Biologia Animal, do Instituto de Biociências, Letras e Ciências Exatas da Universidade Estadual Paulista "Júlio de Mesquita Filho", Campus de São José do Rio Preto.

Comissão Examinadora

Prof. Dr. Sebastião Roberto Taboga
UNESP – São José do Rio Preto
Orientador

Profª Drª Rejane Maira Góes
UNESP – São José do Rio Preto

Profª Drª Sandra Regina de Carvalho Marchesin
Universidade Paulista – UNIP

Prof. Dr. Sérgio Luis Pinto da Matta
Universidade Federal de Viçosa – UFV

Profª Drª Valéria Helena Alves Cagnon Quitete
Universidade Estadual de Campinas – UNICAMP

São José do Rio Preto
27 de fevereiro de 2015

AGRADECIMENTOS

Meus sinceros agradecimentos...

Ao meu orientador **Prof. Dr. Sebastião Roberto Taboga** por todos esses anos de dedicação para com a minha formação profissional. Sebaka obrigada pela confiança que depositou em mim quando aceitou ser meu orientador no mestrado permanecendo no doutorado. Obrigada por acreditar no meu trabalho, pela orientação, incentivo e bom convívio.

À **Profª Drª Patrícia Simone Leite Vilamaior**, por esses seis anos de co-orientação, incentivo, auxílio e pela ótima convivência, obrigada por toda contribuição.

Às professoras do meu exame de qualificação**, Profa. Dra. Rejane Maira Góes** e **Profa. Dra. Sandra Regina de Carvalho Marchesin** pelas valiosas sugestões, os quais contribuíram para que o primeiro manuscrito ficasse mais coeso.

Aos professores titulares e suplentes da **banca examinadora** desse trabalho, por toda a futura contribuição, a qual tem certeza que enriquecerão ainda mais o meu trabalho.

Ao **Mateus**, não tenho palavras para agradecer, pela amizade, pelo auxílio nas coletas, por todo o apoio e ajuda durante todo o desenvolver deste trabalho, principalmente por me auxiliar nas discussões dos resultados e na correção de grande parte deste manuscrito.

Aos amigos do Laboratório de Microscopia e Microanálise, por todas as experiências vividas, pelos bons momentos de descontração, por compartilharem os conhecimentos teórico-práticos específicos, pelo auxílio em várias atividades, e, sobretudo pela amizade que levaremos para vida toda....**Ana Paula, Bianca, Bruno Sanches, Bruno Zani, Camila, Carol Christante, Carol Frandsen, Carol Negrin, Cássia, Douglas, Ellen, Eloísa, Guilherme, Júlia, Larissa, Luiz Henrique, Maê, Mari Marcielo, Mari Zanatelli, Marina, Mateus, Patrícia, Ricardo, Simone, Silvana, Vanessa** e **Viviane.**

Aos **docentes do Programa de Pós-Graduação em Biologia Animal** que contribuíram no aperfeiçoamento dos meus conhecimentos e, consequentemente para minha formação profissional.

À **Profª Drª Eliana Morielle-Versute** por disponibilizar seu laboratório quando precisei, por esclarecer dúvidas tanto teóricas quanto de identificação de animais e também por me receber em sua sala de aula, e compartilhar seus conhecimentos e vivência no meu estágio docência.

Ao técnico **Luiz Roberto Falleiros Jr** por toda a ajuda desprendida tanto no trabalho quanto na vida, pela sua preocupação e conselhos pessoais e profissionais, e principalmente por toda sua animação a qual deixa nosso ambiente de trabalho muito mais alegre.

À técnica **Rosana Silistino de Souza**, por toda a ajuda relacionada ao meu material de eletrônica, obrigada pela disposição em cortar meus milhões de bloquinhos.

Aos professores da Anatomia da Unesp-Botucatu, **Luiz Gustavo de Almeida Chuffa, Raquel Fantin Domeniconi** e **Renato Ferretti** por abrirem as portas de suas aulas e laboratórios, por compartilharem conhecimentos anatômicos e a experiência docente de vocês. Muito obrigada por aumentar ainda mais a minha paixão pela anatomia.

À **CAPES** e ao **CNPq** pelos primeiros meses de bolsa de estudo concedida.

À **FAPESP** pela bolsa de estudos concedida (Processo: 2011/01323-2).

Em especial, agradeço...

À **Deus** por me amparar nos momentos difíceis, me dar força interior para superar as dificuldades, mostrar os caminhos nas horas incertas e me suprir em todas as minhas necessidades.

Aos meus pais **José**, **Sonia** e **Claudinei** que não me deram apenas a vida, mas também a educação, condições de estudo, e me ensinaram fazer tudo da melhor forma possível com dignidade, confiando em mim e apoiando para que conseguisse realizar os meus sonhos, obrigada por sempre estarem presentes.

Ao meu irmão **Tiago**, que apesar de todas as nossas discussões, me ajuda em tudo que preciso. Obrigada por me entender, pelo companheirismo, amizade e alegrias.

A toda a **minha família** e **amigos**, por todo o apoio e amor desprendido, por compreender a minha ausência, e por todas as orações feitas ao meu favor.

Aos meus queridos amigos do quinteto fantástico (**Caio, Camila, Tais e Verônica**) pela amizade que perdura há anos, por todas as mensagens e e-mails trocados, os quais nos aproximaram e proporcionaram que compartilhássemos momentos mesmo estando longe.

Às minhas amigas de república **Amanda, Carol Frandsen, Fernanda, Marina e Milena,** pela amizade e risadas, por todas as gordices divididas, pelos bons momentos de lazer, por escutarem as minhas lamentações nas dificuldades que surgiram durante este trabalho, mesmo que na maioria das vezes não faziam ideia do que eu estava falando, e principalmente por ser a minha família neste ultimo ano de doutorado.

E por fim ao **Rapha**! Acho que jamais conseguiria listar o quanto você, mesmo sendo de outra área, me ajudou e fez parte deste trabalho: agradeço por torcer que minhas coletas dessem certo, por ter entendido que não voltaria em alguns finais de semana para adiantar meu trabalho, por ter escutado meus desabafos quando algo dava errado e compartilhado minha felicidade quando algo dava certo... Agradeço todos os conselhos que você passou para mim durante estes anos e também pelo apoio nas minhas (in)decisões profissionais. Obrigada por sua infinita paciência e compreensão com a minha personalidade explosiva. Te amo muito!

“O que prevemos raramente ocorre; o que menos esperamos geralmente acontece.”

Benjamin Disraeli

RESUMO

Chiroptera é a segunda maior ordem de mamíferos. Esta apresenta diferentes estratégias e características reprodutivas únicas. Entretanto, são raros os relatos relacionados com as glândulas reprodutivas acessórias (GRAs) masculinas de morcegos brasileiros. *Artibeus planirostris* (Phyllostomidae: Chiroptera) apresenta um complexo prostático compacto composto por duas regiões (ventral e dorsal) e um par de glândulas bulbouretrais. O complexo prostático desta espécie apresenta variações durante o ano: no peso, na altura de epitélio, na porcentagem dos componentes glamdulares (lúmen, epitélio e estroma), na quantidade de células receptor de andrógeno positiva e células em processo de proliferação e morte, bem como variações hormonais. Indicando assim que o nível de testosterona influencia nessas variações. A castração (supressão de testosterona) e a subsequente suplementação deste hormônio podem elucidar a relação das GRAs com a testosterona. Assim, o objetivo deste estudo foi avaliar o efeito da castração e suplementação de testosterona nas GRAs de *A. planirostris*. Os animais foram coletados na cidade de São José do Rio Preto, no noroeste do Estado de São Paulo, Brasil. As capturas foram realizadas no verão (21 de dezembro – 21 de março), a fim de minimizar as variações decorrentes da sazonalidade. Foram utilizados apenas machos adultos, com a determinação da idade dos animais baseada no peso corporal, ossificação completa das epífises metacarpo-falange, desgaste dos dentes, o posicionamento dos testículos e na presença de espermatozoides no testículo, cauda do epidídimo e/ou na uretra. Neste trabalho foram analisados quarenta e seis espécimes machos sexualmente maduros, os quais foram divididos em oito grupos: I) 3 dias castrado (CA3d, n = 7); II) controle três dias (CO3d, n = 4); III) 15 dias castrado (CA15d, n = 7); IV) 15 dias controle (CO15d, n = 5); V) 15 dias castrado com suplementação de cipionato de testosterona (1 ug / kg / dia) durante 3 dias (T3d, n = 7); VI) 15 dias castrado com suplementação de cipionato de testosterona (1 ug / kg / dia) durante 7 dias (T7d, n = 7); VII) controle da suplementação durante 3 dias (O3d, n = 4); e VIII) controle da suplementação durante 7 dias (O7d, n = 5). Os últimos dois grupos receberam injeção de óleo mineral no lugar do hormônio. Os resultados indicaram que ambas as regiões prostáticas são afetadas pela ablação de testosterona, apresentando queda na proliferação celular e aumento na apoptose. Já a glândula bulbouretral apresentou uma pequena redução na altura do epitélio e no número de células AR + e

um aumento no número de células basais no grupo CA15d. Nos grupos castrados por mais tempo (O3d; O7d), essa regressão fica acentuada, tendo também um aumento no número de células apoptóticas. A suplementação hormonal ocasionou um retorno da morfologia normal em ambas as glândulas. Os dados demonstraram que a região ventral foi mais sensível a alterações na testosterona do que a dorsal, apresentando uma maior renovação celular. Observou-se que a glândula bulbouretral leva mais tempo para responder a ablação de testosterona do que o complexo prostático, já que apresentou regressão evidente apenas no grupo com 22 dias de castração.

Palavras-chave: morcego, castração, suplementação hormonal, testosterona, glândula bulbouretral, próstata.

ABSTRACT

Chiroptera is the second largest order of mammals. This presents different reproductive strategies with some unique characteristics. However, there are very few reports related to the accessory reproductive glands (GRAs) of Brazilian bats. Artibeus planirostris (Phyllostomidae: Chiroptera) has a prostatic complex comprised of two regions (ventral and dorsal) and a pair of bulbourethral glands. The prostatic complex of this species shows variations during the year in: weight, epithelial height, percentage of components glands (lumen, epithelium and stroma), the amount of cells: positive androgen receptor and proliferation and death, as well as variations hormonal. Indicating that testosterone levels influence these variations. Castration (testosterone suppression) and subsequent supplementation of this hormone may elucidate the relationship of the GRAs with testosterone. Thus, the aim of this study was to evaluate the effect of castration and testosterone supplementation in GRA of A. planirostris. The animals were collected in the city of São José do Rio Preto, in the northwest of São Paulo State, Brazil. The captures were concentrated in the summer (21st December to 21st March) in order to minimize variations arising from seasonality. The bats were aged as adults based on body weight, complete ossification of the metacarpal-phalangeal epiphyses, wear of the teeth, positioning of the testes and the presence of sperm inside the testes, cauda epididymis and/or urethra. Thus, forty-six sexually male mature specimens were analyzed. Eight groups were formed: I) 3 days castrated (CA3d, n=7); II) 3 days control (CO3d, n=4); III) 15 days castrated (CA15d, n=7); IV) 15 days control (CO15d, n=5); V) 15 days castrated with testosterone cypionate (1µg/Kg/day) repositioned for 3 days (T3d, n=7); VI) 15 days castrated with testosterone cypionate (1µg/Kg/day) repositioned for 7 days (T7d, n=7); VII) Control of the reposition for 3 days (O3d, n=4); and VIII) Control of the reposition for 7 days (O7d, n=5). The last two groups received mineral oil instead of testosterone.The results indicated that both prostatic regions were affected by the ablation of testosterone, presenting a decrease in cell proliferation and an increase in apoptosis. Already the bulbourethral glands showed a small decrease in epithelium height and amount of AR + cells and an increase in the amount of basal cells in CA15d group. In groups castrated fora longer period (O3d; O7d), this regression is accentuated, and there is also an increase in amount of apoptotic cells. Hormone supplementation caused a return to normal morphology in

both glands. The data have shown that the ventral region was more sensitive to changes in testosterone than the dorsal, presenting greater cell renewal. And the BG takes longer to respond to ablation of testosterone than other reproductive glands, since it showed evident aspects of regression only in the animals castrated for 22 days.

Keywords: *bat, bulbourethral gland, castration, hormone supplementation, prostate, testosterone.*

SUMÁRIO

I- INTRODUÇÃO

A Ordem Chiroptera

Os morcegos compõem uma das ordens mais diversa e distinta no grupo dos mamíferos, sendo os únicos que possuem estruturas que permitem voo verdadeiro. O nome Chiroptera deriva do grego “cheir” e “pteron” os quais significam mão e asa, respectivamente, o que indica que a asa do morcego é uma mão altamente modificada (PERACCHI et al., 2006).

Chiroptera é a segunda maior ordem de mamíferos, com 18 famílias, 202 gêneros e aproximadamente 1.116 espécies vivendo amplamente distribuídas pelo planeta (Fig. 1), ocorrendo em regiões tropicais e temperadas, com exceção de algumas ilhas oceânicas remotas e a Antártica (SIMMONS, 2005).

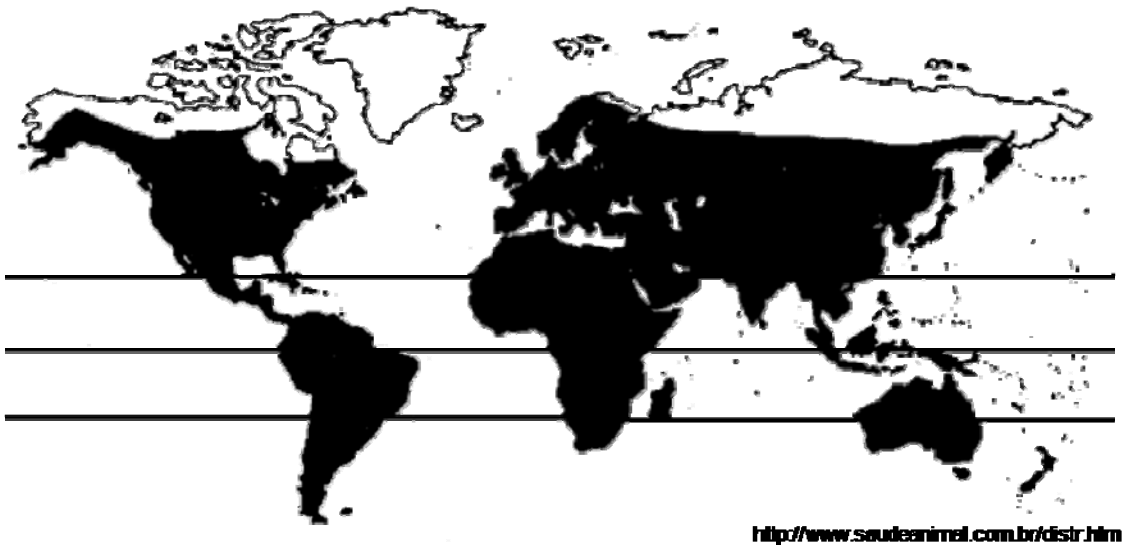


Figura 1. Mapa da distribuição geográfica da Ordem Chiroptera no mundo; as áreas em negro representam a distribuição das espécies.

No Brasil, a quiropterofauna está representada por aproximadamente 164 espécies de morcegos, pertencentes a nove famílias: Emballonuridae, Furipteridae Molossidae, Mormoopidae, Phyllostomidae, Natalidae, Noctilionidae, Thyropteridae e Vespertilionidae (PERACCHI et al., 2011), as quais habitam todo o território brasileiro, ocorrendo desde a Amazônia, o Cerrado, a Mata Atlântica, o Pantanal, o nordeste, até os pampas gaúchos, em regiões rurais e também em áreas urbanas (REIS et al., 2007).

Dentre as famílias de Chiroptera, Phyllostomidae é a mais representativa, com 89 espécies de 40 gêneros registrados em território brasileiro (PERACCHI et al.,

2011), a qual é subdividida em 5 subfamílias: Carollinae, Desmodontinae, Glossophaginae, Phyllostominae e Stenodermatinae (REIS et al., 2007). Phyllostomidae é um clado endêmico do Novo Mundo, com registros que se estendem do sudoeste dos Estados Unidos da América até o norte da Argentina (PERACCHI et al., 2006). Este grupo é caracterizado por apresentar um apêndice nasal em forma de folha ou ponta de lança, exceto em Desmodontinae, que apresenta esse apêndice nasal reduzido e em forma de ferradura (Fig. 2).

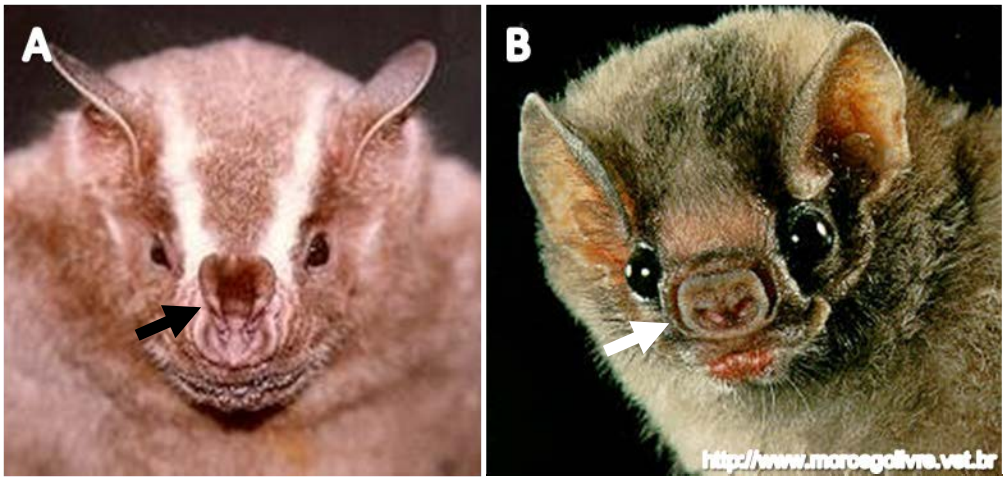


Figura 2. Representantes da família Phyllostomidae. Destaque para a característica que define a família, o apêndice nasal. A. *Artibeus lituratus*, subfamília Stenodermatinae, apresenta o apêndice nasal em forma de folha (seta negra). B. *Diphylla ecaudata*, subfamília Desmodontinae a qual difere das outras subfamílias e apresenta o apêndice nasal em forma de ferradura (seta branca).

Stenodermatinae é um grupo monofilético e compõe a subfamília mais abundante com 68 espécies, 42,2% das espécies descritas para Phyllostomidae (SIMMONS, 2005). Nesta subfamília, Simmons (2005) reconhece duas tribos: Sturniri e Stenodermatini; a primeira representa os morcegos do gênero *Sturnira*, e a segunda as demais espécies. De todos os 17 gêneros conhecidos dentro da subfamília, 12 ocorrem no Brasil, num total de 33 espécies.

Os estenodermatíneos são predominantemente frugívoros, mas algumas espécies complementam a dieta com outros itens, tais como: recursos florais, insetos e folhas. Essa preferência por frutos, juntamente com o comportamento de retirá-los da planta mãe para consumi-los em abrigos, faz destes animais excelentes dispersores de sementes de plantas da região Neotropical.

Apesar dos poucos estudos encontrados na literatura, este grupo apresenta padrões reprodutivos diversificados, com espécies monoéstricas, poliéstricas bimodais e espécies que se reproduzem ao longo de todo o ano (REIS et al., 2007).

Artibeus planirostris [Stenodermatinae: Phyllostomidae (Figura 3)] é uma espécie abundante e endêmica da região Neotropical, apresenta massa corporal entre 40 e 69 g (BÁRQUEZ et al., 1993) e comprimento do antebraço de 62-73 mm (HOLLIS, 2005). Esta espécie ocorre em praticamente todas as regiões de florestas tropicais e subtropicais (EISENBERG, 1989; HOLLIS, 2005), no Cerrado e Cerradão, e também nas Caatingas (MARES et al., 1981; WILLIG; MARES, 1989), mas é mais abundante em florestas de transição (BÁRQUEZ et al., 1991).

Figura 3. *Artibeus planirostris*, espécie da família Phyllostomidae, Sub-família: Stenodermatinae. (Fonte:www.morcegosdobrasil.blogspot.com.br)

Essa espécie se abriga em árvores, e muitas vezes é capturada perto de árvores frutíferas ou na proximidade de rios (BÁRQUEZ et al., 1991; BÁRQUEZ et al., 1993). Apresenta uma dieta predominantemente frugívora, mas com plasticidade, também, por vezes, consumindo pólen, néctar e/ou insetos (REIS et al., 2007). Os espécimes são solitários ou formam pequenas colônias de 5-16 indivíduos. O período gestacional é de aproximadamente 3,5 meses, sendo normalmente um único jovem produzido em cada gestação (TADDEI, 1976). De acordo com Willig (1985), esta espécie apresenta poliestria bimodal, com picos de reprodução em fevereiro-março e outubro-novembro. No entanto, Taddei (1976) e Graham (1987) postulam que essa espécie pode ser capaz de produzir jovens a qualquer momento ao longo o ano, principalmente pela observação durante todo o ano de fêmeas simultaneamente prenhes e lactantes, e machos escrotados com espermatozoides na cauda do epidídimo.

Reprodução de Chiroptera

A ampla distribuição geográfica da ordem Chiroptera permite que os morcegos sofram considerável influência de fatores abióticos sobre suas estratégias reprodutivas, as quais são detectadas através do comportamento ou da avaliação funcional das gônadas e das glândulas anexas (SWAMI; LALL, 1982). Morcegos hibernantes de zonas temperadas são reprodutores sazonais, enquanto que aqueles de zonas tropicais apresentam padrões reprodutivos diversos, influenciados diretamente por variações da temperatura, pluviosidade e disponibilidade de alimento (FLEMING et al., 1972; TADDEI, 1976).

Um grande número de estudos morfofuncionais tem mostrado, em algumas espécies de morcegos, características interessantes relacionadas às estratégias reprodutivas, tais como o armazenamento de espermatozoides por longos períodos em prolongamentos da cauda do epidídimo nos machos e na região do corno uterino nas fêmeas, além do atraso na implantação embrionária no trato reprodutor feminino (RACEY, 1972, 1973; KRUTZSCH, 1975; KRUTZSCH et al., 1976; RACEY, 1979; KRUTZSCH; CRICHTON, 1987; HAPPOLD; HAPPOLD, 1989; HOOD; SMITH, 1989; VAN DER MERWE; RAUTENBACH, 1990; HEIDEMAN et al., 1992; ENCARNAÇÃO et al., 2004; SHARIF et al., 2004). A avaliação da espermatogênese em nível morfológico, especialmente de espécies neotropicais, também tem sido abordada (SINGWI; LALL, 1983; McGUCKIN; BLACKSHAW, 1987; SAIDAPUR; PATIL, 1992; MORIGAKI et al., 2001; KUROHMARU et al., 2002; LEE, 2003; BEGUELINI et al., 2009). Alguns autores observaram uma assincronia no ciclo do epitélio seminífero de espécies de Vespertilionidae (WILSON; FINDLEY, 1971; LEE, 2003; BEGUELINI et al., 2009).

As glândulas reprodutivas acessórias (GRA) masculinas são órgãos que compõem o sistema reprodutor masculino, e são de suma importância por secretarem produtos que garantem a sobrevivência, viabilidade e a motilidade dos espermatozoides (ROCHEL et al., 2007), fato este que tem grande influência no sucesso reprodutivo dos mamíferos. Crichton e Krutzsch (2000), ao compilarem dados sobre reprodução de morcegos, afirmam que a estrutura, tanto anatômica quanto microscópica, dos órgãos reprodutivos, testículos e glândulas acessórias, seguem o padrão de mamíferos, ou seja, apresentam um par de testículos e glândulas acessórias, tais como: próstata, vesículas seminais, glândulas ampulares e glândulas bulbouretrais, as quais não estão presentes

juntamente em todas as famílias, ou seja, a ausência ou presença dos tipos glandulares estão relacionados à nível de família.

Puga et al. (2013, 2014) observaram que, macroscopicamente, as GRA de *Artibeus planirostris* (Fig. 4) apresentam um complexo glandular prostático (CGP) compacto, o qual apresenta duas regiões distintas: uma ventral e outra dorsal, as quais circundam a uretra.

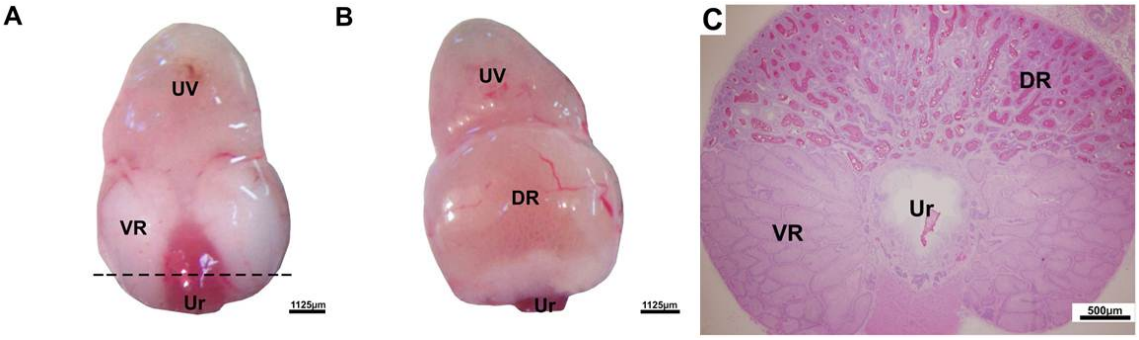


Figura 4. Anatomia e histologia do complexo glandular prostático de *Artibeus planirostris.* **A.** Vista ventral do complexo prostático, observe a relação da região ventral com a uretra. **B.** Vista dorsal do complexo prostático. **C.** Secção do complexo glandular no plano indicado em **A** (Coloração: Hematoxilina-eosina), note a relação entre a região ventral e dorsal, bem como destas com a uretra. DR, Região Dorsal; Ur, uretra; UV, vesícula urinária; VR, região ventral.

Puga et al. (2014) observaram que o CGP de *A. planirostris* apresenta variações ao longo do ano. Os dados hormonais corroboraram os dados biométricos (peso do CGP e peso testicular), já que ambos apresentaram aumento do outono até o verão, sendo no verão o maior pico que ocorre na estação de maior reprodução, indicando assim que o nível de testosterona influencia tanto na variação de peso do CGP quanto testicular. A maioria dos dados existentes referentes às dosagens hormonais está relacionada a espécies hibernantes (GUSTAFSON; SHEMESH, 1976; GUSTAFSON; DAMASSA, 1985) e relatam de um a três picos de testosterona, os quais variam conforme a espécie e o ambiente ao qual a mesma está inserida.

Em análises dos receptores de andrógeno, Puga et al. (2014) mostraram que ambas as regiões expressam esses receptores, porém na região ventral não há variação significativa ao longo do ano. Já a região dorsal apresenta a menor porcentagem de células receptor de andrógeno (AR) positivas no verão. Quando estes dados são correlacionados com a dosagem hormonal observaram que são inversamente proporcionais, ou seja, quanto menor número de células AR-positivas observa-se maior nível de testosterona no sangue. Puga et al. (2014) inferiram que isto tem relação com o

fato de que no momento que o CGP está se preparando para a secreção, os níveis hormonais estão baixos. Este nível baixo estimula, por *feedback* positivo, a síntese de receptores de andrógeno para que as células prostáticas captem a pouca testosterona circulante. Na estação do verão o organismo desta espécie necessita de um alto nível de testosterona para as atividades reprodutivas, porém o CGP não precisa captar toda essa testosterona. Então a testosterona estimula por *feedback* negativo, a redução de síntese desses receptores. Esses autores afirmam que a região dorsal sofre influência direta da testosterona, porém ainda não concluíram se as mudanças da região ventral são mediadas por este hormônio. Assim, os níveis hormonais podem estar intimamente relacionados com diferentes respostas destas glândulas ao longo do ano.

A próstata e sua dependência hormonal.

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 níveis constantes de andrógenos circulantes produzidos pelos testículos e pelas adrenais (PRICE, 1963; ORTIZ, 1976; CUNHA et al., 1987; DOUNJACOUR; CUNHA, 1988; AUMULLER; SEITZ, 1990; COOKE et al., 1991; PRICE; ROSAI, 1996; HAYWARD et al., 1997; THOMSON et al., 1997).

Desses andrógenos, 95% correspondem à testosterona sintetizada pelas células de Leydig dos testículos sob estimulação do hormônio luteinizante (LH) da hipófise, que por sua vez, é regulado pelo hormônio liberador de gonadotrofina (GnRH) do hipotálamo. Os outros 5% de andrógenos são sintetizados nas adrenais como resposta a ação do hormônio adrenocorticotrófico (ACTH) da hipófise e liberados principalmente na forma de androestenediona, que é perifericamente convertida nos tecidos prostáticos em testosterona (TAPLIN; HO, 2001).

A estimulação das células epiteliais prostáticas é dada pela transformação da testosterona em di-hidrotestosterona (DHT), que é catalisada pela enzima 5α-redutase presente principalmente no citoplasma das células epiteliais (SÁNCHEZ et al., 2013). Esta conversão é importante porque DHT tem maior atividade excitatória por ter grande afinidade ao AR (CUNHA et al., 1987). A ligação do DHT ao AR das células epiteliais desencadeia diferentes cascatas de reações e sinalizações que regulam principalmente a produção e secreção prostáticas. Do mesmo modo, a estimulação através de células estromais é fornecida pela conversão da testosterona em estradiol (E2), a qual é

catalisada pela aromatase, presente principalmente no citoplasma das células estromais (ELLEM et al., 2004; ELLEM; RISBRIDGER, 2010; BEGUELINI et al., 2015).

O estradiol é essencial para a homeostase do tecido normal dentro da próstata, e tem papel duplo no tecido prostático: ativação do receptor de estrógeno tipo alfa (ER-α) levando à proliferação, e ativação do receptor de estrógeno tipo beta (ER-β) mediando efeitos antiproliferativos (apoptose), que equilibra a ação proliferativa de andrógenos no epitélio (HELDRING et al., 2007; ELLEM; RISBRIDGER, 2009).

A prolactina e o hormônio de crescimento (GH) também estimulam a produção de andrógenos, tanto nos testículos como nas adrenais, tendo a primeira um efeito mitogênico direto adicional sobre as células epiteliais da próstata (GALBRAITH; DUCHESNE, 1997).

A castração e a morfofisiologia prostática.

A remoção ou a redução de andrógenos de origem gonadal pode ser obtida por castração cirúrgica, administração de estrógeno ou inibição da síntese e liberação de LH (Hormônio Luteinizante) e FSH (Hormônio Folículo-Estimulante) por bloqueio na liberação de gonadotrofina.

Na castração cirúrgica ocorre uma queda abrupta e significativa dos níveis séricos de andrógenos, e também a redução do fluxo sanguíneo para a próstata, já que este é controlado pelo nível de andrógeno. Esta redução induz apoptose nas células endoteliais e nas células secretoras prostáticas (KYPRIANOU; ISSACS, 1988). Em estudo realizado por Shabsigh et al. (1999) constatou-se que a apoptose inicia 24h após a castração, aumenta significativamente 48 e 72h após a castração e declina-se em seguida.

Após a castração o epitélio regride e as células epiteliais restantes da próstata continuam sensíveis aos andrógenos. A reposição de testosterona regenera o epitélio prostático, restaurando a função e o número de células normais (RISBRINDGER 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).

A glândula bulbouretral: morfologia e função.

A glândula bulbouretral (GB) é uma glândula par do sistema reprodutor masculino, sendo muito importante na função reprodutora de diferentes espécies de mamíferos. A secreção dessas glândulas normalmente limpa a uretra e, posteriormente, ajuda na lubrificação da vagina, já que é secretada antes da ejaculação (SAMUELSON, 2007).

A GB apresenta algumas diferenças entre espécies de mamíferos: ela está completamente ausente em toupeiras (RACEY, 1978), cães e mamíferos aquáticos (DYCE et al., 2002), é pouco desenvolvida no homem (JEQUIER, 1995), mas bem desenvolvida no porco (BADIA et al., 2006). A morfologia da GB tem sido estudada em vários mamíferos, especialmente roedores, tais como ratos, hamster, chinchilas, coelhos e ratazana-do-capim (JESIK et al., 1982; TOMA; BUZZELL, 1988; ADARO et al., 2001; VASQUEZ; DEL SOL, 2002; CAKIR; KARATAS, 2004; ADEBAYO et al., 2015).

Em estudos anteriores, observou-se que, macroscopicamente, a GB de *Artibeus planirostris* (PUGA et al., 2013), *Platyrrhinus lineatus* (PUGA et al., 2011; MARTINS et al., 2015) e *Molossus molossus* (CHRISTANTE et al., 2014) é uma glândula acinar em forma oval, sendo parcialmente revestida por tecido muscular. As células epiteliais colunares são preenchidas com muitas gotículas de secreção de densidade eletrônica variável. Essas vesículas são liberadas no ápice do epitélio, onde existem pequenas projeções da membrana celular.

Os estudos relacionados a andrógenos e as glândulas reprodutivas acessórias, na maioria das vezes citam a influência desse hormônio apenas na próstata (KYPRIANOU; ISSACS, 1988; SHABSIGH et al., 1999; RISBRINDGER et al., 2001). Há na literatura, poucos estudos encontrados relacionando o efeito da castração na GB, e estes mostram diferentes respostas entre os mamíferos. Aykroyd e Zuckerman (1938) mostraram que havia regressão apenas na parte periférica da GB de machos adultos de macaco Rhesus castrados por cinco meses, o que está de acordo com Barrington (1913 apud AYKROYD; ZUCKERMAN, 1938) que verificou pequena regressão na GB de cobaias com um ano de castração. Estes resultados são muito diferentes daqueles encontrados para ratos (HELLER, 1932), que mostram rápidas e acentuadas mudanças após castração.

Dessa forma, a castração (consequente ablação do nível de testosterona) e posterior reposição hormonal poderá elucidar a relação desses dois tipos glandulares prostáticos e da glândula bulbouretral com a testosterona.

II- OBJETIVOS

- Geral

O presente trabalho objetivou avaliar o efeito da castração e suplementação de testosterona nas glândulas reprodutivas acessórias de *Artibeus planirostris* (Chiroptera: Phyllostomidae).

- Específicos

1. Avaliar o padrão histológico, morfométrico, estereológico, histoquímico e imunohistoquímico da próstata na espécie *A. planirostris*, objetivando a avaliação das possíveis influências da depleção e posterior suplementação hormonal.

2. Avaliar o padrão histológico, morfométrico, estereológico, histoquímico, ultraestrutural e imunohistoquímico da glândula bulbouretral na espécie *A. planirostris*, objetivando a avaliação das possíveis influências da depleção e posterior suplementação de testosterona.

Esta tese foi escrita na forma de dois artigos científicos, os quais foram preparados nas normas e serão submetidos às revistas internacionais.

Artigo 1: **The effects of castration followed testosterone supplementation in prostatic complex of *Artibeus planirostris* (Chiroptera: Phyllostomidae).**

Artigo 2: **The effects of bilateral orchiectomy followed testosterone supplementation on the bulbourethral gland of *Artibeus planirostris* (Chiroptera: Phyllostomidae).**

III- Artigo 1: The effects of castration followed testosterone supplementation in prostatic complex of *Artibeus planirostris* (Chiroptera: Phyllostomidae).

Short title: Effects of castration in prostate of bats

Cíntia C. I. Puga[1], Mateus R. Beguelini[2], Eliana Morielle-Versute[3], Patricia S. L. Vilamaior[1], Sebastião R. Taboga[1*]

[1] Departamento de Biologia, Instituto de Biociências, Letras e Ciências Exatas, IBILCE; Universidade Estadual Paulista, UNESP, São José do Rio Preto, São Paulo, Brasil.

[2] Campus Reitor Edgard Santos, Centro das Ciências Biológicas e da Saúde, Universidade Federal do Oeste da Bahia, UFOB, Barreiras, Bahia, Brasil.

[3] Departamento de Zoologia e Botânica, Instituto de Biociências, Letras e Ciências Exatas, IBILCE; Universidade Estadual Paulista, UNESP, São José do Rio Preto, São Paulo, Brasil.

*Corresponding author: Sebastião Roberto Taboga – Rua Cristóvão Colombo nº 2265, Jardim Nazareth, 15054-000, São José do Rio Preto, São Paulo, Brazil. Tel.: +55 17 32212380. FAX: + 55 17 3221-2390. E-mail address: taboga@ibilce.unesp.br

Abstract

The prostatic complex (ventral and dorsal regions) of *Artibeus planirostris* exhibits seasonal variations throughout the year. Circulating testosterone was correlated with prostate weight, showing an increase from autumn to summer, with the highest peak in summer corresponding to the largest breeding season. This indicates that the level of serum testosterone influences variations in both testicular and prostatic weights. Serum testosterone levels seem to be closely related to the different responses of these glands throughout the year. The castration (consequent suppression of testosterone) and subsequent hormone supplementation may elucidate the relationship of these two glandular types with testosterone. Thus, the aim of this study was to evaluate the effect of castration and the testosterone supplementation in the male prostatic complex of *A. planirostris*. The results indicated that both prostatic regions were affected by the ablation of testosterone, presenting a decrease in cell proliferation and an increase in apoptosis. Similarly, the prostate was responsive to hormonal supplementation, having a recovery of the active morphophysiological pattern with testosterone supplementation. However, data have shown that the ventral region was more sensitive to changes in testosterone than the dorsal, presenting greater cell renewal.

Keywords: Bat, Castration, Hormone supplementation, Testosterone, Prostate.

1. Introduction

The male reproductive accessory glands (RAGs) are organs that compose the male reproductive system and are of great importance for secreting products that ensure the survival, viability and motility of spermatozoa (Setchell and Breed 2006), a fact that has a great influence on the reproductive success of mammals. Crichton and Krutzsch (2000) compiled the existing data on reproduction in bats and stated that both the anatomy and histology of their reproductive organs (testes and accessory glands) followed the mammalian pattern, with a pair of testis and epididymis and accessory glands, such as: prostate, seminal vesicles, ampullary glands and the bulbourethral glands, which may or may not be present in the reproductive system.

Studies demonstrated that the RAGs of the phyllostomid bats *Artibeus planirostris* (Puga et al. 2013) and *Platyrrhinus lineatus* (Puga 2011; Martins et al. 2014) were composed of a compact prostatic complex (PC), which had two distinct regions (ventral and dorsal) with associated paraurethral glands, and a pair of extra-abdominal bulbourethral glands. The ventral region completely surrounded the urethra in *P. lineatus*; however, in *A. planirostris*, the urethra was partially exposed. On the other hand, in both species, the bulbourethral glands had similar locations and morphology.

The PC of *A. planirostris* exhibited seasonal variations throughout the year. Circulating testosterone was correlated with prostate weight, as they showed an increase from autumn to summer, with the highest peak in summer corresponding to the largest breeding season. This indicates that the level of serum testosterone influences variations in both testicular and prostatic weights (Puga et al. 2014).

Analyses of androgen receptors (AR) indicated that both regions expressed these receptors; however, the ventral region had no significant variation throughout the year, while the dorsal had the highest expression in the autumn and lowest in the summer. Data showed that the expression pattern of AR in the dorsal region was inversely correlated to the testosterone dosage, presenting a small number of AR-positive cells when the level of serum testosterone was high in summer and the reverse was seen in the autumn (Puga et al. 2014). In summer, the body requires a high level of testosterone to directly stimulate the reproductive activities, but in both region of PC, already fully developed, did not need to capture all of this testosterone, resulting in a negative feedback that reduced the synthesis of these receptors. On the other hand, in

autumn, the PC, undeveloped, needs to prepare itself for the subsequent secretion of substances for the breading season; as the testosterone level was low in this period, the PC needs to increase the synthesis of AR to capture the low circulating testosterone and appropriately induce production and secretion in the prostatic epithelium (Puga et al. 2014). This study showed that the dorsal region suffered the direct influence of testosterone; however, the impact of this hormone in the ventral region was not fully elucidated. Thus, testosterone levels may be related to the different responses of these glands throughout the year.

Experiments of castration (consequent suppression of testosterone) and subsequent supplementation were a general process to evaluate the impact of testosterone in different organs and types of treatments (Huttunen et al. 1981; Kiplesund et al. 1988; Carvalho et. al. 1997; Shabisgh et al. 1999; Nishino et al. 2004; Góes et al. 2007). Thus, castration and subsequent testosterone supplementation may elucidate the relationship between this hormone and variation in these two prostatic regions. Therefore, the aim of this study was to evaluate the effect of castration and posterior testosterone supplementation in the two prostatic regions of *A. planirostris*.

2. Materials and methods

2.1. Species, ageing and experimental design

The species analyzed was *Artibeus planirostris* (Spix 1823), which is an exclusively Neotropical species of phyllostomid bat that is not listed as endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species. The bats were aged as adults based on body weight, complete ossification of the metacarpal-phalangeal epiphyses, wear of the teeth (Knegt et al. 2005), positioning of the testes and the presence of sperm inside the testes, cauda epididymis and/or urethra (Puga et al. 2013; Puga et al. 2014; Beguelini et al. 2013a; Beguelini et al. 2013b; Beguelini et al. 2014a; Beguelini et al. 2014b).

Artibeus planirostris is a wild species that is protected by federal laws in Brazil (IBAMA). Adding to these, its harem structure (with a single male mated to multiple females) means that the collection of male specimens directly influences the structure and dynamics of the populations. Thus, the number of specimens used in this study was the maximum number obtained from a balance between the minimum number required

for relevant statistical analyses and the maximum number of individuals that could be collected without destroying or disrupting the colonies of bats.

Thus, forty-six sexually male mature specimens were analyzed. Eight groups were formed (Figure 1): I) Uncastrated animals kept in captivity for 3 days (CO3d, n=4); II) Castrated animals kept in captivity for 3 days (CA3d, n=7); III) Uncastrated animals kept in captivity for 15 days (CO15d, n=5); IV) Castrated animals kept in captivity for 15 days (CA15d, n=7); V) Castrated animals kept in captivity for 15 days, thereafter treated with 0,1 µl mineral oil (on alternate days) for 3 days (O3d, n=4); VI) Castrated animals kept in captivity for 15 days, thereafter treated with 0,1 µl testosterone cypionate (dose: 1µg/Kg on alternate days) for 3 days (T3d, n=7); VII) Castrated animals kept in captivity for 15 days, thereafter treated with 0,1 µl mineral oil (on alternate days) for 7 days (O7d, n=5) and VIII) Castrated animals kept in captivity for 15 days, thereafter treated with 0,1 µl testosterone cypionate (dose: 1µg/Kg on alternate days) for 7 days (T7d, n=7).

2.2. Study area, capture and ethics statement

The animals were collected in the city of São José do Rio Preto, in the northwest of São Paulo State, Brazil, (49W22'45", 20S49'11"), which is located 500 meters above sea level. This is a semi-flat region, set in a degraded Cerrado biome, with meso-thermal climate with rainy summers and dry winters.

The captures were concentrated in the summer (21st December to 21st March) in order to minimize variations arising from seasonality (Puga et al. 2014) and to focus on changes caused by treatments. The captures were performed at night using mist nets (3m x 6m), which were positioned to intercept bats flying 1–3 m above the ground. The nets were precisely placed on possible flight routes or exits from shelters.

The experimental procedures were approved by the Ethics Committee of IBILCE-UNESP (Process: 036/2010 – CEUA), and the capture of animals was authorized by the Brazilian Institute of the Environment, IBAMA (Process: 21707-1). The animals were treated according to the recommendations of the Committee on Care and Use of Laboratory Animals from the Institute of Laboratory Animal Resources, National Research Council, “Guide for the Care and Use of Laboratory Animals” (Committee on Care and Use of Laboratory Animals, 1980).

147 **2.3. Castration and maintenance of the animals**

148 To standardize the study, after capture, the animals were kept in cages (40 cm x
149 20 cm x 20 cm) with water and food (pieces of papaya, banana or other fruit) *ad libitum*,
150 in a specific room in darkness at 25–30^{o}C. These conditions were only used in the
151 period between the end of the capture and the next morning, at which point the bats
152 were anesthetized with a mixture of ketamine (Dopalen-Vertebrands, Paulínia, SP,
153 Brazil; 370 mg/kg) and xylazine (Rompun – Bayer S.A., São Paulo, SP, Brazil; 16
154 mg/kg), which was applied at an amount of 0.1 ml per 50g of body weight (0.08 ml
155 ketamine plus 0.02 ml of xylazine). After anesthesia, bilateral orchidectomy was
156 performed, with a small incision in the inguinal region, in which the testicle and
157 epididymis were removed after cutting the deferent duct. Finally the animals were
158 sutured. After castration, the animals were maintained in the Laboratory of Microscopy
159 and Microanalysis of the Department of Biology (IBILCE/UNESP) in a specific room
160 in darkness at 25–30^{o}C. They were accommodated in cages (100cm length x 50cm
161 width x 30cm height) dotted with grids at the superior portion to facilitate aeration,
162 proper positioning of the animal (upside down) and feeding. Only 2 or 3 animals were
163 placed per cage to avoid the stress of overpopulation. The animals received water *ad*
164 *libitum* and the feeding corresponded to fresh and tender fruits (papaya, banana, etc.),
165 which were changed every day, since it is a frugivorous species. The cages were cleaned
166 every day, with the water exchange and the supplementation of the feed being
167 performed simultaneously in order to cause the lowest possible stress to the animal,
168 with these processes occurring without the direct manipulation of the animals. All
169 animals were manipulated only for the performance of the treatments (injections of
170 testosterone).
171
172 **2.4. Processing of the animals**

173 To standardize, all specimens were euthanized by deep anesthesia (mixture of
174 ketamine and xylazine), administered subcutaneously, and positioned in dorsal
175 decubitus on the dissection board. After the body weight was measured, blood samples
176 were collected by endocardial puncturing. A longitudinal incision in the skin and
177 subcutaneous tissues was performed, from the xiphoid process to the pubic symphysis,
178 with the PC and adrenal glands removed, weighed and fixed for analysis.

All specimens are archived in the Chiroptera Collection of the Laboratory of Chiroptera at the Univ. Estadual Paulista, UNESP; Instituto de Biociências, Letras and Ciências Exatas, IBILCE.

2.5. Serum hormone dosage

The blood, collected by endocardial puncture, was immediately centrifuged at 1,200 g, to separate the serum, and frozen at -80°C for subsequent analysis of testosterone levels. The testosterone dosages were performed by ELISA Capture/Sandwich (antibody-antigen-antibody), using specific commercial kits (Cayman Chemical Company, Ann Arbor, Michigan, USA - Item # No. 582701) of high sensitivity (6.0 pg/ml), with coefficients of variation for an inter-assay of 32.0 pg/ml. Readings were taken using an Epoch™ Multi-Volume Spectrophotometer System (BioTekInstruments, VT, USA) reader.

2.6. Histology

The PC was fixed by immersion in Metacarn fixative solution (methanol: chloroform: acetic acid – 6:3:1) for 3 h at 4^{o}C, dehydrated in ethanol, diaphanized in xylene and embedded in paraffin (Histosec – MERK, Darmstadt, Germany). Sections of 4μm were stained with hematoxylin-eosin (HE [Ribeiro and Lima 2000]), periodic acid-Schiff (PAS [Behmer et al. 1976]) and Picrosirius (Junqueira et al. 1979) or submitted to immunostainings. Microscopic analyses were performed using an Olympus BX60 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) with coupled image analyzer (Image Pro Plus version 6.1 for Windows - Copyright © 1993-2006 Media Cybernetics, Inc.).

2.7. Stereological analysis and morphometry

The following measurements were performed on cross sections of the PC (ventral and dorsal regions) using the Image Pro-Plus-Media Cybernetics, version 6.1 for Windows computer software for image analysis: the relative percentage of glandular compartments (epithelium, lumen and stroma), the epithelium height and the cell population census.

Stereology: for this analysis, 50 fields of the slides stained by HE (dorsal region) and Picrosirius (Ventral region) were randomly selected from each experimental

group. The relative frequency of glandular components (epithelium, lumen and stroma) was calculated by applying an M130 multipoint test system (Weibel 1963).

Morphometry: to measure the epithelium height, random fields were captured from each animal, with three measurements performed in each field, accumulating a total of 300 measurements for each experimental group.

The cell population census was conducted on histological sections submitted to p63 immunohistochemistry (protocol described below). For this analysis, 50 fields of the slides submitted to p63 immunohistochemistry were selected from each experimental group. In each field, the amounts of basal and other cells were measured by direct counting of nuclei.

2.8. Immunohistochemistry and TUNEL analysis

After microwaving for antigen retrieval, non-specific binding was blocked prior to incubation with primary antibodies against: androgen receptor (AR) – rabbit polyclonal IgG (sc-816/Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1:75); p63 – mouse monoclonal IgG2a (sc-25268/Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1:100); proliferating cell nuclear antigen (PCNA) – mouse monoclonal IgG2a (sc-56/Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1:100); and apoptosis (TUNEL – TdT-Fragel-Calbiochem, second manufacturer's instructions, Oncogene Research Products, Boston). The sections were submitted to a reaction with diaminobenzidine (DAB) and counterstained with Harris hematoxylin. Negative controls were used, omitting the incubation step with the primary antibody or enzyme (in the case of TUNEL).

The relative percentage of positive and negative cells in the acinar epithelium was determined using measurement fields of the entire length of the slides. The ratio between the proliferation and cellular apoptosis of the acinar epithelium was also calculated by dividing the percentage of PCNA-positive by that of TUNEL-positive cells (% cell PCNA-positive/% cell TUNEL-positive).

2.9. Statistical analysis

All numerical data were expressed as means and standard error, and analyzed initially by analysis of variance (One-way ANOVA) and, subsequently, by a Tukey test for multiple comparisons ($p \leq 0.05$ = significant), using the Statistica 7.0 program (Statsoft Inc., Tulsa, USA).

3. Results

3.1. Body, adrenal and PC weights and testosterone hormone levels

Biometric data (Table 1) showed no significant differences between the initial and final values of body and adrenal weights in all experimental groups, despite the increased weight of all animals held in captivity. The PC weights of the CO3d, CA3d and CO15d groups also did not differ statistically; however, the CA15d group had a significant reduction. The PC weights of the O3d and O7d groups had similar values to that of the CA15d group. Similarly, the T3d group did not show any significant difference when compared to the CA15d group. On the other hand, the T7d group presented an increase in PC weight, which displayed similar values to the normal pattern (control animals). These data indicated that the short-term treatments (3 days: CA3d and T3d) had weak effects on PC; however, the long-term treatments had a direct impact on PC. In the same way, they demonstrated the importance of testosterone for normal PC physiology, where its absence triggered a regression of the glandular complex and its supplementation promoted a recovery in function, observed in the increased weight.

The serum hormone dosage (Table 1) showed a decrease in the level of testosterone in the CA3d and CA15d groups when compared with the CO3d and CO15d groups, but this decrease was not statistically significant. The O3d and O7d groups showed even lower values and the groups with hormone supplementation (T3d and T7d) had high values.

3.2. Histology, stereological and morphometric analyses

3.2.1. Castration effect

The ventral region of the CO3d and CO15d groups showed normal morphology (epithelium with basal nuclei, lumen filled with globular secretion and a little of stroma - Figs. 2A-B, G-H) and the CA3d group (Figs. 2D-E) had no changes in morphology compared to these controls (Figs. 2A-B, 2G-H). However, the CA15d group presented a pronounced glandular regression with a large cell concentration (Figs. 2J-K), regions with cellular debris (Fig. 2J) and changes in the glandular secretion (Fig. 2K). Similarly, the dorsal region had no morphological differences between the CO3d (Fig. 2C), CA3d (Fig. 2F) and CO15d (Fig. 2I) groups; however, the CA15d group also

presented glandular regression, with increases in the stromal areas and in the epithelium height at the more peripheral regions (Fig. 2L).

Stereologically, the ventral region presented no significant difference in the amount of epithelium between groups; however, the lumen and stroma varied inversely (Fig. 4A). The lumen presented higher values in the CO3d, CA3d and CO15d groups and a significant reduction in the CA15d group, while the stroma had lower values in the CO3d, CA3d and CO15d groups and a significant increase in the CA15d group (Figs. 2J-K). Similarly, the dorsal region showed similar behavior in relation to the lumen and stromal components (Fig. 3C).

The morphometry of the dorsal region showed a small decline in the epithelium height in castrated groups (CA3d and CA15d - Fig. 4E).

3.2.2. The effect of hormone supplementation

The ventral region of the O3d (Figs. 3A-B), T3d (Figs. 3D-E) and O7d (Figs. 3G-H) groups showed a similar morphology to the CA15d group (Figs. 2J-K), presenting pronounced glandular regression with regions indicative of a higher cell concentration, cellular debris, a change in the pattern of glandular secretion and also an increase in stromal areas. However, the long-term treatment with testosterone (T7d) showed a return to normal glandular morphology (Figs. 3J-K). Similarly, the dorsal region had no morphological differences between the O3d (Fig. 3C), T3d (Fig. 3F) and O7d (Fig. 3I) groups and the CA15d group (Fig. 2L); however, the long-term treatment with testosterone (T7d) showed a return to normal glandular morphology, with a decline in stromal areas (Fig. 3L).

Stereologically, the ventral region presented no significant difference in the amount of epithelium between groups; however, the lumen and stroma varied inversely (Fig. 4B). The stroma was higher than the lumen in the O3d, T3d and O7d groups; however, the T7d group had a significant increase in the lumen and a consequent significant decrease in the stroma (Fig. 4B). Unlike the ventral region, the dorsal region showed a significant increase in the amount of epithelium in the groups treated with testosterone (T3d and T7d - Fig. 4D). However, it had a similar behavior in relation to the lumen and stromal components (Fig. 4D).

The morphometry of the dorsal region showed an increase in the epithelium height in groups treated with testosterone (T3d and T7d - Fig. 4F).

3.3. Cell population census

3.3.1. Ventral region

The cell population census revealed a significant increase in the number of basal and the other cells in the CA15d group (Figs. 5A, D), in relation to the CO3d, CA3d and CO15d groups (Figs. 5A, C). This number remained high in the O3d, O7d and T3d groups (Figs. 5B, E), and returned to normal in the T7d group (Figs. 5B, F).

3.3.2. Dorsal region

The dorsal region presented a significant increase in the number of basal and other cells in the CA15d group (Figs. 5G, J) in relation to the CO3d, CA3d and CO15d groups (Figs. 5G, I). This number remained high in the O3d, O7d and T3d groups (Figs. 5H, K), and was normal in the T7d group (Figs. 5H, L).

3.3.3. Index p63-positive cell/p63-negative cell

Dividing the number of basal cells by the other cells enabled the development of an index, which indicates that the CA15d group has a large number of basal cells, which was not found in the other groups (CA3d, CO3d and CO15d, Table in Figure 5).

The groups with hormonal supplementation had similar patterns between the groups. The dorsal region of the T7d group showed a similar index to the control group on 3 and 15 days; however, the ventral region, even with supplementation, continued to have a higher index than the CA15d or control groups (Table in Figure 5).

3.4. Immunohistochemistry

3.4.1. Androgen receptor (AR)

The AR immunohistochemistry showed, in both regions, a small decrease in AR-positive cells in the CA3d and CA15d groups in relation to the control groups (CO3d and CO15d); however, this decrease was not statistically significant (Ventral: Figs. 6A-E; Dorsal: Figs. 6G-K). On the other hand, in the hormone supplementation groups, there was a significant decrease in AR-positive cells in the O7d group (Figs. 6F, L).

3.4.2. Cell proliferation (PCNA)

PCNA showed a significant decrease of cell proliferation in the ventral region of the CA15d group (Figs. 7A, D) when compared with the CO3d (Fig. 7C), CO15d and CA3d groups.

Groups submitted to hormone supplementation [T3d (Figs. 7B and 7G-H) and T7d] showed a recovery in cell proliferation, especially in the first days of the application of the hormone. This result indicates that the glandular remodeling is mediated by testosterone and this remodeling has a peak in the first application of the hormone (T3d, Figs. 7G-H).

The dorsal region showed no significant differences in the number of PCNA-positive cells in any of the studied groups (Figs. 7I-M).

3.4.3. Apoptosis (TUNEL)

The ventral region showed a significant increase in apoptosis in the CA15d group (Figs. 8A, D) when compared with the CO3d (Figs. 8A, C), CA3d and CO15d groups (Fig. 8A). O3d and O7d (Figs. 8B, E) had high rates of cell death, which were slightly lower than that of the CA15d group. The testosterone supplementation (T3d and T7d groups) caused a decline in cellular apoptosis (Figs. 8B, F).

Similarly, the dorsal region also showed a significant increase in apoptosis in the CA15d group (Figs. 8G, J) when compared with the CO3d, CA3d (Figs. 8G, I) and CO15d groups (Fig. 8G). O3d (Figs. 8H, K) and O7d (Figs. 8H and 8L) had high rates of cell death, but they were lower than that of the CA15d group, and the T3d and T7d groups had a decline in cellular apoptosis (Fig. 8H); however, the values did not differ significantly.

3.4.4. Rate of Cell Proliferation (PCNA/TUNEL)

The PCNA/TUNEL ratio showed similar results for both regions (Figs. 8): a decline in castrated groups (CA15D, O3D and O7d) compared to control groups (CO3d and CO15d) and an increase in the groups treated with testosterone (T3d and T7d) compared to castrated groups (O3d and O7d).

4. Discussion

Aumuller and Seitz (1990) confirmed that the depletion of androgens in castration caused atrophy of the androgen-responsive organs, with a remarkable reduction in size and weight. In our work, the reduction in hormone levels caused changes in glandular morphology, such as increases in the cellular debris, percentage of stroma and modifications in their secretory pattern. The authors, together with Risbridger et al. (2001), postulated that this morphology returned to the normal pattern when testosterone was injected, since this hormone regenerated the prostatic epithelium, restoring the function and number of normal cells. Despite the fact that the decrease in the levels of serum testosterone in castrated groups (CA3d and CA15d) was not statistically significant, several similar morphological and physiological changes in the prostatic complex were related to castration, as was a return to the normal pattern in groups with hormone supplementation (T3d and T7d). Thus, the reduction in circulating testosterone (castration) caused a decrease in prostatic weight (Ca15d), which returned to the initial state with hormone supplementation (T7d), showing that the testosterone actively regulated the prostatic morphophysiology in *A. planirostris*, a pattern also observed in seasonal analyzes (Puga et al. 2014).

Stereologically, we observed that the CA3d group maintained the morphology of the control groups (CO3d and CO15d), which indicates that both prostatic regions take longer than three days to respond to decreased levels of testosterone. Rochel-Maia et al. (2011) observed a similar pattern in gerbils, indicating significant differences in these parameters only in the 21 day castrated group; this pattern was also observed in the present study, where an increase in the stroma and the lumen regression occurred after 15 days of castration, with this morphology remaining altered in the groups treated with oil (O3D and O7d). The data also indicated that short-term treatments (injection of testosterone) have little effect on both prostatic regions, with the gland presenting patterns of reactivation only after a longer period (T7d) of testosterone administration.

The cell population census indicated an increase in the density of basal and other cells in groups with reduced testosterone (castrated and oil injected groups) as expected due glandular regression and acini atrophy. Despite this, the Index p63-positive/p63-negative, which is not affected by a decrease in the glandular area, furthermore showed the increase in the index in long-term castrated groups (CA15D, O3d and O7d), indicated the marked cell death and loss of secretory cells.

411 The data showed that the AR expression was directly related to the levels of
412 serum testosterone, where it decline in castrated and increased in treated groups, as
413 described in the literature (Cordeiro et al. 2008). However, Puga et al. (2014) identified
414 that the dorsal region of *A. planirostris* had fewer AR-positive cells in the season with a
415 higher level of testosterone in the blood. This divergence can be explained by the
416 situation to which each study was subjected. In the first case, the AR-positive cells
417 increased with testosterone level, because this gland was atrophied and needed to return
418 to the normal activity pattern due to testosterone stimulation. In the second case, the
419 gland was already morphologically active, so it did not need to increase the expression
420 of AR.

421 Similarly, there was a decline in cell proliferation in the CA15d group and a
422 tendency to increase in the hormone supplementation groups, which indicated that
423 testosterone was related to the rate of cell proliferation. However, when there was
424 supplementation, the proliferation remained low, indicating that the testosterone was not
425 the principal factor causing cell proliferation and the consequent glandular
426 reestablishment. The data showed that the factor which most influenced the glandular
427 restructuring was cell death, as it significantly increased in the castrated group (CA15d)
428 along with a decline of cell proliferation, causing glandular regression. With hormone
429 supplementation, cell death declined, which allowed a return of glandular activity, even
430 in a low proliferation state.

431 Cunha et al. (1996) affirmed that the morphology and function of the adult
432 prostate tissue were maintained by the stimulation of the androgenic hormones, which
433 regulated a balance between a very low proliferation rate and cell death. Pereira et al.
434 (2006), studying the rat ventral prostate, also observed a decline in the rate of cell
435 proliferation in castrated animals. In addition, Puga et al. (2014) had observed that the
436 decrease in cellular proliferation in animals collected in the fall was related to the
437 decline in testosterone levels. Therefore, we can conclude that, in *A. planirostris*,
438 testosterone levels were more closely related to maintaining a low mortality rate than to
439 promoting cellular proliferation.

440 Kyprianou and Issacs (1988) claimed that surgical castration caused an abrupt
441 and significant decrease in serum androgen levels, also causing a reduction in the
442 prostate, as this is controlled by the level of androgens. This reduction induces apoptosis
443 in prostatic secretory cells. Although our hormone data did not show significant

differences, we still observed a decline in testosterone, which directly influenced the prostatic morphology of the CA15d group.

In the castrated group observed a higher rate of cellular death, which decreases in the castrated groups for prolonged periods (O3D and O7d), a fact that indicate that the bats have a response to the lack of testosterone slower than in rodents, in which the apoptosis process began after 24 hours of castration, increased significantly between 48 and 72 hours and declined thereafter (Shabisgh et al. 1999).

The decline in cellular apoptosis, observed in the ventral region of the hormone supplementation groups, was in agreement with that postulated by Puga et al. (2014), who showed that there was a decline in cellular apoptosis in the summer, which was the season with the highest hormone level. On the other hand, the dorsal region of the CA15d group showed an increase in apoptotic cells when compared to the control groups (CO3d and CO15), which was clearly explained by the surgical castration that caused an abrupt and significant decrease in serum androgen levels and also a reduction in blood flow to the prostate, thus inducing apoptosis in prostatic secretory cells (Kyprianou and Issacs 1988).

Our data indicated that both prostatic regions were responsive to the ablation and supplementation of testosterone; however, the ventral region was more sensitive to changes in testosterone than the dorsal, having a higher cell renewal, which may be explained by its holocrine pattern. The prostatic regulation by testosterone seemed to be more closely related to maintaining a low mortality rate than to the promotion of cellular proliferation, with an accentuated decrease in proliferation and an increase in apoptosis in the castrated groups and inhibition of apoptosis and only a small increase in proliferation in the groups treated with testosterone.

Acknowledgements

Technical help from Luiz Roberto Falleiros Junior is highly appreciated. Also greatly appreciate the collaboration of the Carolina Frandsen Pereira da Costa in the scheme of the experimental design.

References

Aumuller G and Seitz J 1990 Protein secretion and secretory process in male accessory sex glands. *Int. Ver. Cytol.* **121** 127-231

Beguelini MR, Bueno LM, Caun DL, Taboga SR and Morielle-Versute E 2014a Ultrastructure of spermatogenesis in the short-tailed fruit bat, *Carollia perspicillata* (Chiroptera: Phyllostomidae: Carollinae). *J. Morphol.* **275**(1) 111-123

Beguelini MR, Falleiros LR, Góes RM, Rahal P, Morielle-Versute E and Taboga SR (2014b) Differential expression of aromatase, estrogen receptor alpha and 17β-HSD associated with the processes of total testicular regression and recrudescence in the bat *Myotis nigricans* (Chiroptera: Vespertilionidae). *Gen. Comp. Endocrinol.* **201** 53-64

Beguelini MR, Puga CC, Martins FF, Betoli AH, Taboga SR and Morielle-Versute E 2013a Morphological variation of primary reproductive structures in males of five families of neotropical bats. *Anat. Rec. (Hoboken)* **296**(1) 156-167

Beguelini MR, Puga CC, Taboga SR and Morielle-Versute E 2013b Annual reproductive cycle of males of the flat-faced fruit-eating bat, *Artibeus planirostris* (Chiroptera: Phyllostomidae). *Gen. Comp. Endocrinol.* **185** 80–89

Behmer OA, Tolosa EMC and Freitas-Neto AG 1976 *Manual de técnicas para histologia normal e patológica.* São Paulo: EDART, Editora da Universidade de São Paulo, 241p.

Carvalho HF, Vilamaior PSL and Taboga SR 1997 Elastic system of the rat ventral prostate and its modifications following orchiectomy. *Prostate* **32** 27-34

Cordeiro RS, Scarano WR, Campos SGP, Santos FCA, Vilamaior PSL, Góes RM and Taboga SR 2008 Androgen receptor in the Mongolian gerbil ventral prostate: Evaluation during different phases of postnatal development and following androgen blockage. *Micron* **39** 1312–1324

Crichton EG and Krutzsch PH 2000 *Reproductive Biology of Bats*. London: Academic Press.

504 Cunha GR, Donjacour AA, Cooke PS, Mee S, Bigsy RM, Higgins SJ and Sugimura Y
505 1996 The endocrinology and developmental biology of the prostate. *Endocr. Rev.*
506 **8** 338-362

507 Góes RM, Zanetoni C, Tomiosso TC, Ribeiro DL and Taboga SR 2007 Surgical and
508 chemical castration induce differential histological response in prostate lobes of
509 Mongolian gerbil. *Micron* **38**(3) 231-236

510 Huttunen E, Romppanen T and Helminen HJ 1981 A histoquantitative study on the
511 effects of castration on the rat ventral prostate lobe. *J. Anat.* **3** 357– 370

512 Junqueira LCU, Bignolas G and Brentani R 1979 Picrosirius staining plus polarization
513 microscopy, specific method of collagen detection in tissue section. *J. Histochem.*
514 **11** 447-455

515 Kiplesund KM, Halgunset J, Fjosne HE and Sunde A 1988 Light microscopic
516 morphometric analysis of castration effects in the different lobes of the rat
517 prostate. *Prostate* **13** 221–232

518 Knegt LV, Silva JA, Moreira EC and Sales GL 2005 Bats found in the city of Belo
519 Horizonte, MG, 1999–2003. *Arq. Bras. Med. Vet. Zootec.* **57** 576–583

520 Kyprianou N and Issacs JT 1988 Activation of programmed cell death in the rat ventral
521 prostate after castration. *Endocrinology* **122** 552-562

522 Martins FF, Puga CCI, Beguelini MR, Morielle-Versute E, Vilamaior PSL and Taboga
523 SR 2014 Comparative Analysis of the Male Reproductive Accessory Glands of
524 Bat Species from the Five Brazilian Subfamilies of the Family Phyllostomidae
525 (Chiroptera). *Reprod. Fertil. Dev.* – submitted.

526 Nishino T, Wedel T, Schmitt O, Bühlmeyer K, Schönfelder M, Hirtreiter C, Schulz T,
527 Kühnel W and Michna H. 2004 Androgen-dependent morphology of prostates and
528 seminal vesicles in the Hershberger assay: evaluation of immunohistochemical
529 and morphometric parameters. *Annals Anatomy* **186** 247–253

530 Pereira S, Martinez M, Martinez FE and Júnior WM 2006 Repercussions of castration
531 and vasectomy on the ductal system of the rat ventral prostate. *Cell Biol. Int.*
532 **30**(2) 169-174
533

Puga CCI 2011 Morfofisiologia das glândulas reprodutivas acessórias masculinas de morcegos: estudo nas espécies *Artibeus planirostris* e *Platyrrhinus lineatus* (Chiroptera: Phyllostomidae). *Dissertação (Mestrado)* - Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas – São José do Rio Preto, 129 f.

Puga CCI, Beguelini MR, Martins FF, Morielle-Versute E, Faleiros-Jr LR, Morielle-Versute E, Vilamaior PS and Taboga SR 2014 Seasonal changes in the prostatic complex of *Artibeus planirostris* (Chiroptera: Phyllostomidae). *Gen. Comp. Endocrinol.* **197** 33-42

Puga CCI, Beguelini MR, Negrin AC, Christante CM, Morielle-Versute E, Vilamaior PS and Taboga SR 2013 Structure, histochemistry and ultrastructure of the male reproductive accessory glands in the Neotropical flat-faced fruit-eating bat, *Artibeus planirostris* (Chiroptera: Phyllostomidae). *Reprod. Fertil. Dev.* **25** 558–569

Ribeiro MG and Lima SR 2000 *Iniciação às técnicas de preparação de material para o estudo e pesquisa em morfologia*. Belo Horizonte: SEGRAC – Editora e Gráfica Limitada.

Risbridger GP, Wang H, Frydenberg M and Cunha G 2001 The metaplastic effects of estrogen on mouse prostate epithelium: proliferation of cells with basal cell phenotype. *Endocrinology* **142** 2443-50

Rochel-Maia SS, Santos FC, Vilamaior PS, Justulin LA and Felisbino SL 2011 MMP-2 and TIMP-2 in the prostates of male and female Mongolian gerbils: effects of hormonal manipulation. *Histol. Histopathol.* **26**(11) 1423-1434

Setchell BP and Breed WG 2006 Anatomy, vasculature, and innervation of the male reproductive tract In ‘Knobil and Neill’s Physiology of Reproduction’. 3rd edn. (Ed. J. D. Neill.) pp. 771–808. (Elsevier: New York.)

Shabisgh A, Tanji N, D'Agati V, Burchardt M, Rubin M, Goluboff ET, Heitjan D, Kiss A and Buttyan R 1999 Early effects of castration on the vascular system of the rat ventral prostate gland. *Endocrinology* **140** 1920-1932

564 Spix JB 1823 *Simiarum et Vespertilionum Brasiliensium Species Novae: ou histoire*
565 *naturelle des espèces nouvelles de singes et de chauve-souris observées et*
566 *recueillies pendant le voyage dans l'intérieur du Brésil execute par ordre de S.M.*
567 *le Roi de Bavière dans les années 1817, 1818, 1819, 1820*. Monaco: Francisci
568 Seraphici Hubschmanni.

569 Weibel ER 1963 Principles and methods for the morphometric study of the lung and
570 other organs. *Laboratory Investigation* **12** 131–155

Figure 1. Schematic representation of the experimental design used in this study.

FIGURE 1.

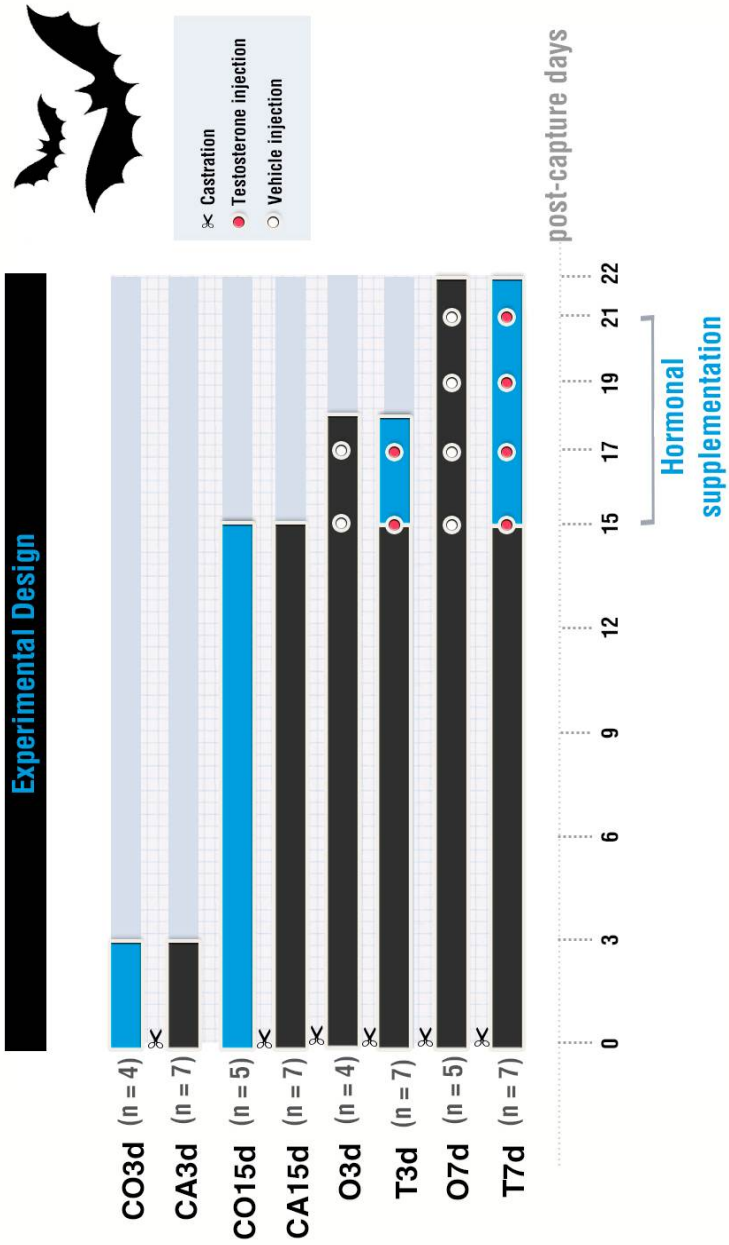

Experimental Design
Castration
Testosterone injection
Vehicle injection
post-capture days
0
3
6
9
12
15
17
19
21
22
Hormonal supplementation
CO3d (n = 4)
CA3d (n = 7)
CO15d (n = 5)
CA15d (n = 7)
O3d (n = 4)
T3d (n = 7)
O7d (n = 5)
T7d (n = 7)

Figure 2. Histological general pattern of castration effects in the prostatic complex of *Artibeus planirostris*. *Ventral region:* **A-B.** Three days control group (CO3d); **D-E.** Three days castrated group (CA3d); **G-H.** Fifteen days control group (CO15d); **J-K.** Fifteen days castrated group (CA15d): observe the larger number of cells in the glandular epithelium, the occurrence of cellular debris (**J**, arrow) and the modification of the glandular secretion (**K**, asterisk). *Dorsal region:* **C.** Three days control group (CO3d); **F.** Three days castrated group (CA3d); **I.** Fifteen days control group (CO15d); **L.** Fifteen days castrated group (CA15d): note the increase in stromal areas (#) and in the epithelium height (insert). (Ep, epithelium; St, stroma; L, lumen). Stained: **A-D-G-J:** Picrosirius, details HE; **B-E-H-K:** PAS; **C-F-I-L:** Picrosirius, details HE. **Scale bar**: All figures= 50 µm; all details = 10 µm.

FIGURE 2.

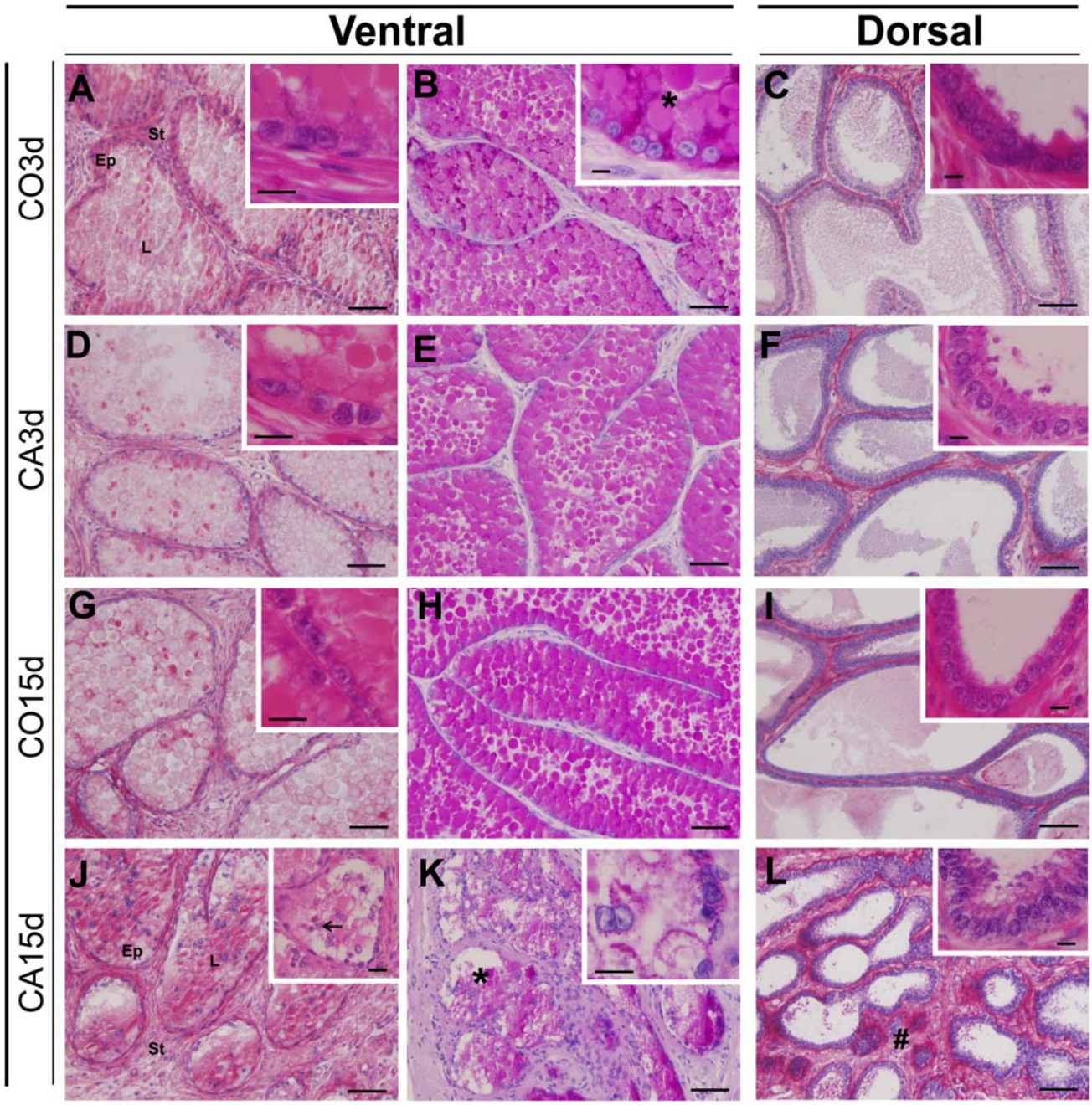


Figure 3. Histological general pattern of the hormonal supplementation effects in the prostatic complex of *Artibeus planirostris*. *Ventral region:* **A-B.** Castrated - three days oil injected group (O3d); **D-E.** Castrated - three days testosterone injected group (T3d); **G-H.** Castrated - seven days oil injected group (O7d), the arrows indicated cellular debris (inserts); **J-K.** Castrated - seven days testosterone injected group (T7d): note the decline in stromal areas (**J**) and the restoration of the full secretory activity (**K**, asterisk). *Dorsal region:* **C.** Castrated - three days oil injected group (O3d); **F.** Castrated - three days testosterone injected group (T3d); **I.** Castrated - seven days oil injected group (O7d). **L.** Castrated - seven days testosterone injected group (T7d). (Ep, epithelium; St, stroma; L, lumen). Stained: **A-D-G-J:** Picrosirius, details HE; **B-E-H-K:** PAS; **C-F-I-L:** Picrosirius, details HE. **Scale bar**: All figures= 50 µm; all details = 10 µm.

FIGURE 3.

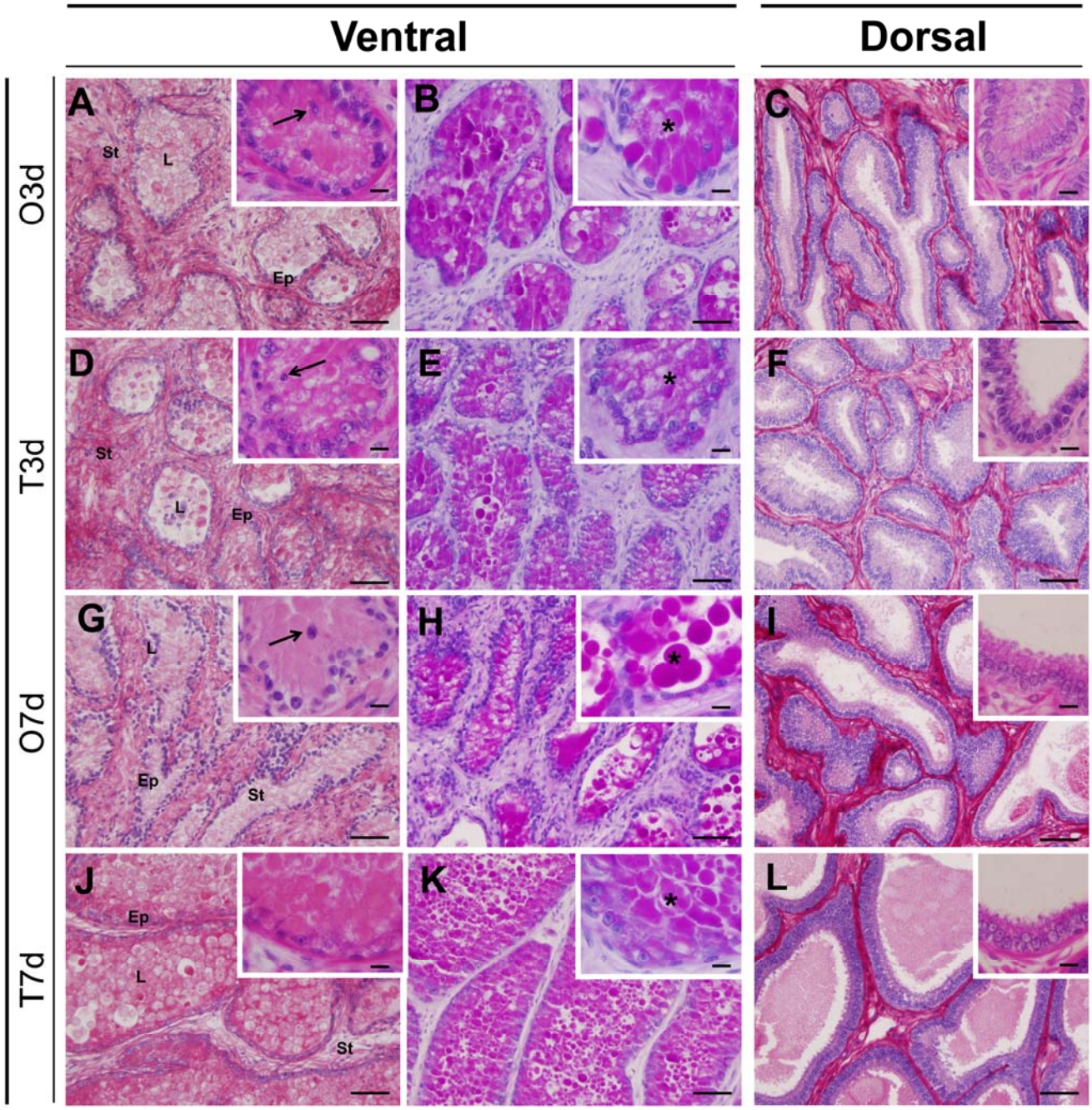


Figure 4. Stereology and morphometry of the prostatic complex of the eight experimental groups of *Artibeus planirostris*: three days control group (CO3d); three days castrated group (CA3d); fifteen days control group (CO15d); fifteen days castrated group (CA15d); castrated - three days oil injected group (O3d); castrated - three days testosterone injected group (T3d); castrated - seven days oil injected group (O7d); and castrated - seven days testosterone injected group (T7d). (**A-B.** Ventral region; **C-F.** Dorsal region; **A-C-E.** Castrated groups; **B-D-F.** Hormonal supplementation groups). ^{a,b,c} Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$).

FIGURE 4.

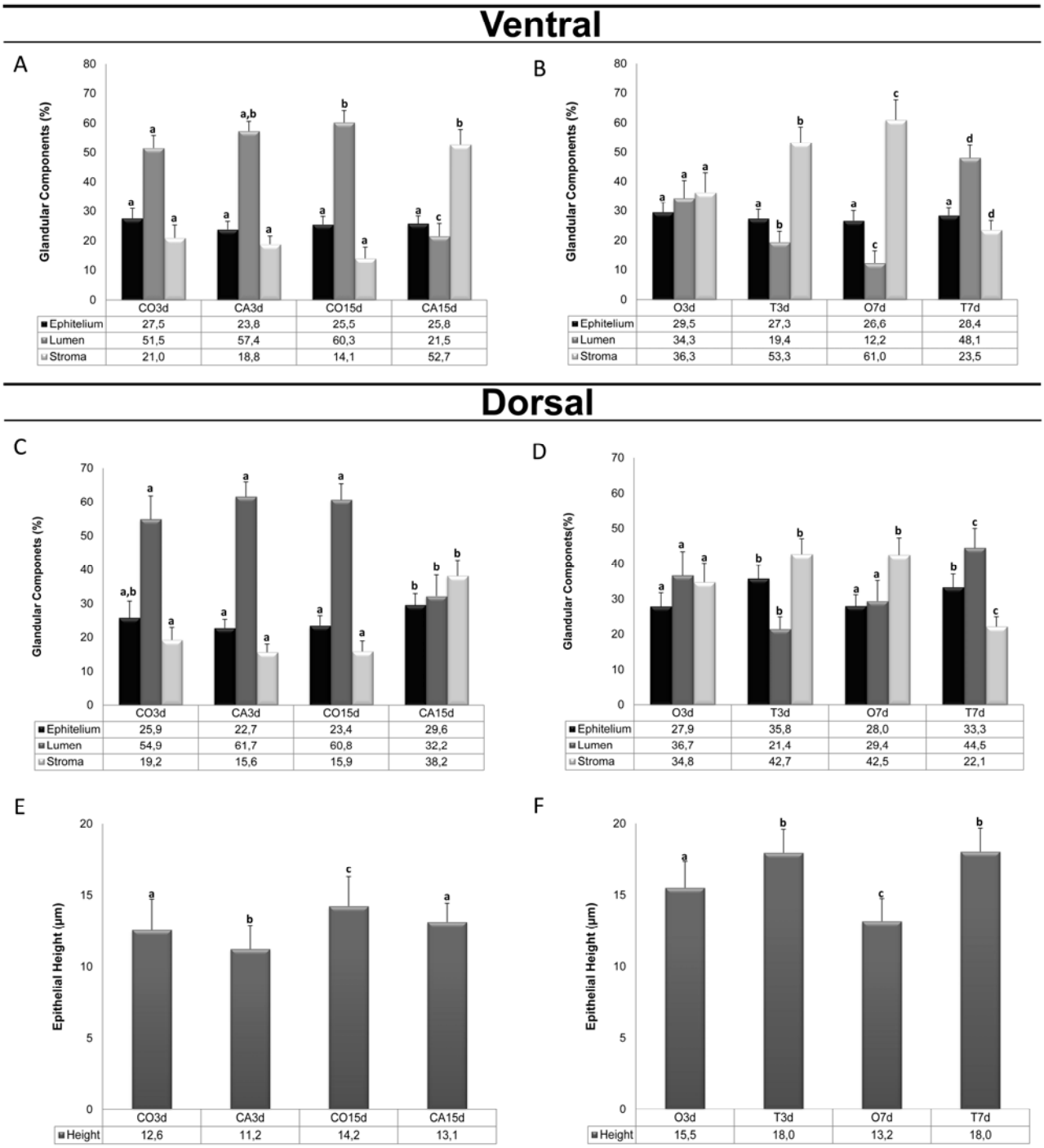
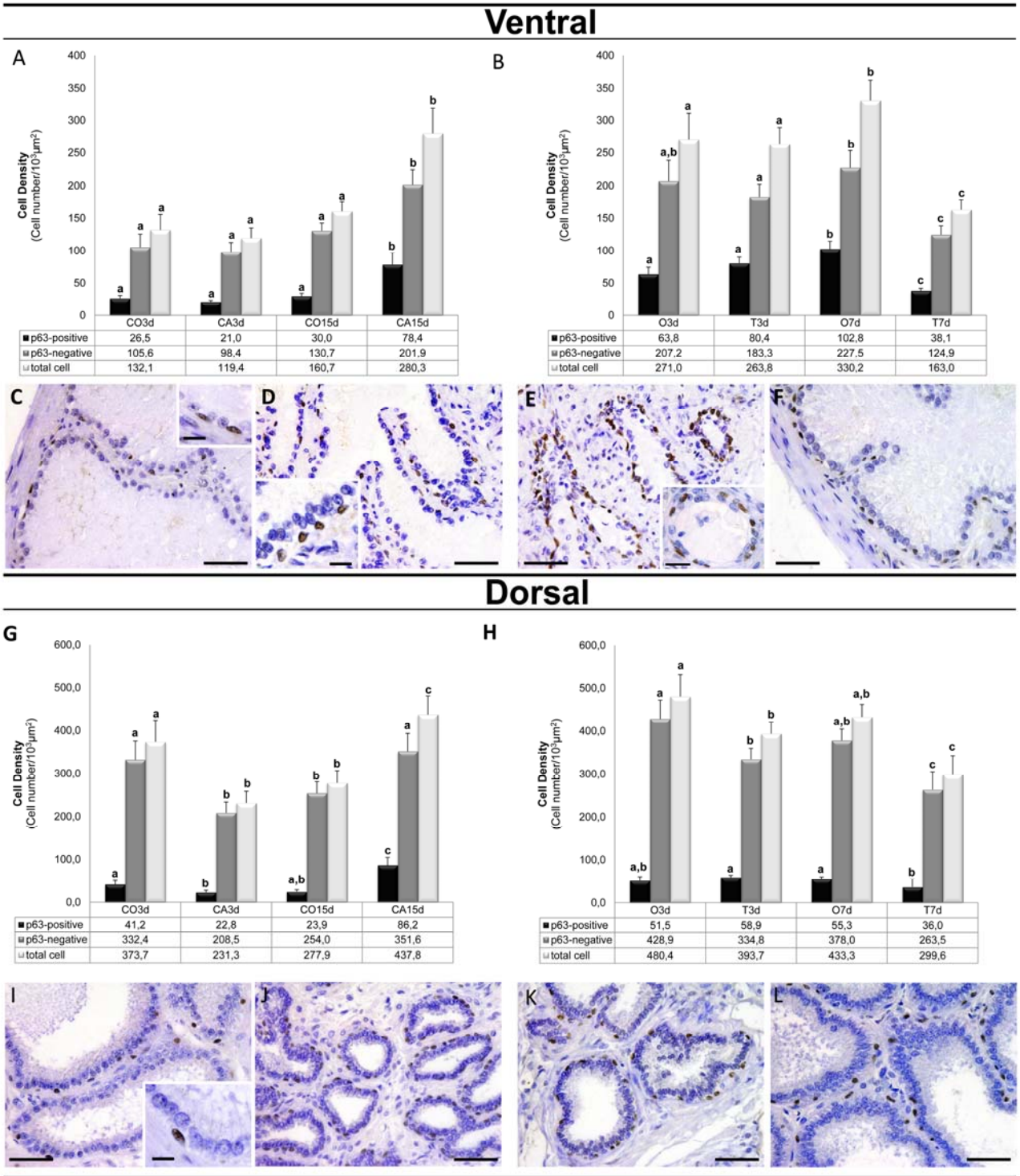

Ventral
A
Glandular Components (%)
CO3d CA3d CO15d CA15d
Ephitelium 27,5 23,8 25,5 25,8
Lumen 51,5 57,4 60,3 21,5
Stroma 21,0 18,8 14,1 52,7
B
Glandular Components (%)
O3d T3d O7d T7d
Ephitelium 29,5 27,3 26,6 28,4
Lumen 34,3 19,4 12,2 48,1
Stroma 36,3 53,3 61,0 23,5
Dorsal
C
Glandular Componets (%)
CO3d CA3d CO15d CA15d
Ephitelium 25,9 22,7 23,4 29,6
Lumen 54,9 61,7 60,8 32,2
Stroma 19,2 15,6 15,9 38,2
D
Glandular Componets(%)
O3d T3d O7d T7d
Ephitelium 27,9 35,8 28,0 33,3
Lumen 36,7 21,4 29,4 44,5
Stroma 34,8 42,7 42,5 22,1
E
Epithelial Height (µm)
CO3d CA3d CO15d CA15d
Height 12,6 11,2 14,2 13,1
F
Epithelial Height (µm)
O3d T3d O7d T7d
Height 15,5 18,0 13,2 18,0

Figure 5. Cell population census analyses of the prostatic complex of *Artibeus planirostris* submitted to p63 immunohistochemistry. A-F. Ventral region. **G-L.** Dorsal region. **A-B.** Graphics corresponding to the quantification of the basal (p63 positive) and secretory cells of the ventral region. **C.** CO3d. **D.** CA15d. **E.** O7d. **F.** T7d. **G-H.** Graphics corresponding to the quantification of the basal (p63 positive) and secretory cells of the dorsal region. **I.** CO3d. **J.** CA15d. **K.** O7d. **L.** T7d. **TABLE.** Index p63-positive/p-63-negative. ^{a,b,c} Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$). **Scale bar**: All figures= 50 µm; details Figs, C, D and I= 10 µm; detail Fig. E= 20 µm.

FIGURE 5.



Index p63-positive/p63-negative

Groups	CO3d	CA3d	CO15d	CA15d	O3d	T3d	O7d	T7d
Ventral	$0.27\pm0.04^{a,e}$	0.23 ± 0.03^{a}	0.23 ± 0.03^{a}	$0.37\pm0.06^{b,c,f}$	$0.31\pm0.04^{a,c,h}$	$0.45\pm0.05^{b,g}$	$0.47\pm0.06^{d,g}$	$0.32\pm0.04^{e,f,h}$
Dorsal	0.12 ± 0.02^{a}	0.11 ± 0.02^{a}	0.10 ± 0.02^{a}	0.30 ± 0.10^{b}	0.12 ± 0.02^{a}	0.18 ± 0.02^{a}	0.15 ± 0.02^{a}	0.13 ± 0.02^{a}

Figure 6. Immunostaining of the androgen receptor (AR) in all experimental groups of *Artibeus planirostris*. A-F. Ventral region. **G-L.** Dorsal region. **A.** CO15d; **B.** T3d; **C.** T7d; **D.** Negative control of ventral region; **E-F.** Graphics corresponding to the quantification of AR-positive cells of the ventral region; **G.** CO15d; **H.** CA15d; **I.** T3d; **J.** Negative control of dorsal region; **K-L.** Graphics corresponding to the quantification of AR-positive cells of the dorsal region. Counter-staining: Hematoxylin. ^{a,b,c} Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$). **Scale bar**: All figures= 10 µm

FIGURE 6.

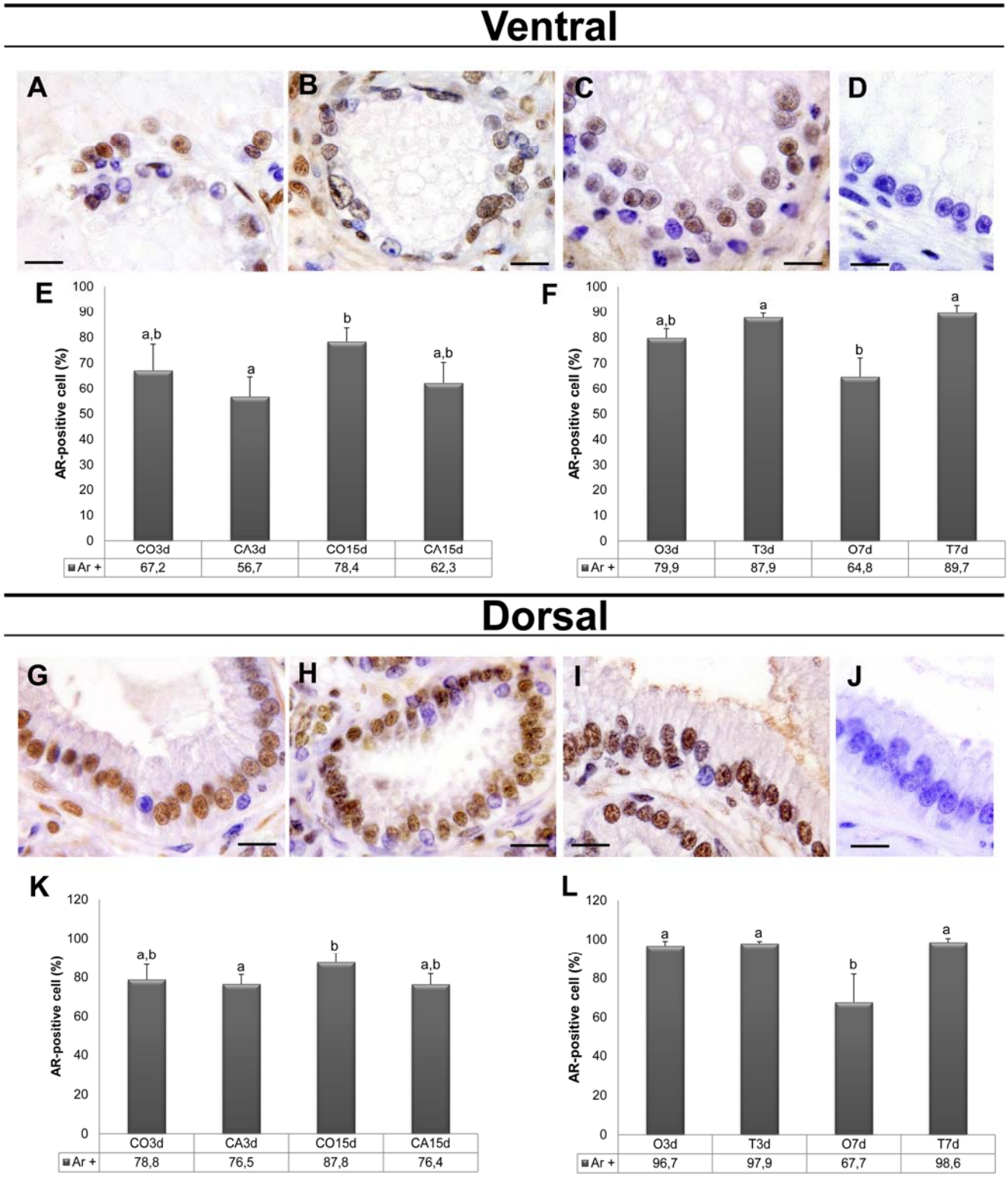

Ventral
A
B
C
D
E
AR-positive cell (%)
a,b
a
b
a,b
CO3d
CA3d
CO15d
CA15d
Ar +
67,2
56,7
78,4
62,3
F
AR-positive cell (%)
a,b
a
b
a
O3d
T3d
O7d
T7d
Ar +
79,9
87,9
64,8
89,7
Dorsal
G
H
I
J
K
AR-positive cell (%)
a,b
a
b
a,b
CO3d
CA3d
CO15d
CA15d
Ar +
78,8
76,5
87,8
76,4
L
AR-positive cell (%)
a
a
b
a
O3d
T3d
O7d
T7d
Ar +
96,7
97,9
67,7
98,6

Figure 7. Expression of the PCNA in all experimental groups of both glandular regions of *Artibeus planirostris*. A-B. Graphics corresponding to the quantification of PCNA-positive cells of the ventral region. **C-H.** Ventral region: **C.** CO3d. **D.** CA15d. **E-F.** O3d. **G-H.** T3d. **I-J.** Graphics corresponding to the quantification of PCNA-positive cells of the dorsal region. **K-M.** Dorsal region: **K-L.** CO3d. **M.** T7d. (Arrows, PCNA-positive cells; Ep, epithelium; St, stroma; L, Lumen). Counter-staining: Hematoxylin. ^{a,b,c} Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$). **Scale bar**: Figs. G, K and M= 50 µm; Figs. C,D, E and L=20 µm; Figs. F, H and detail M= 10 µm.

FIGURE 7.

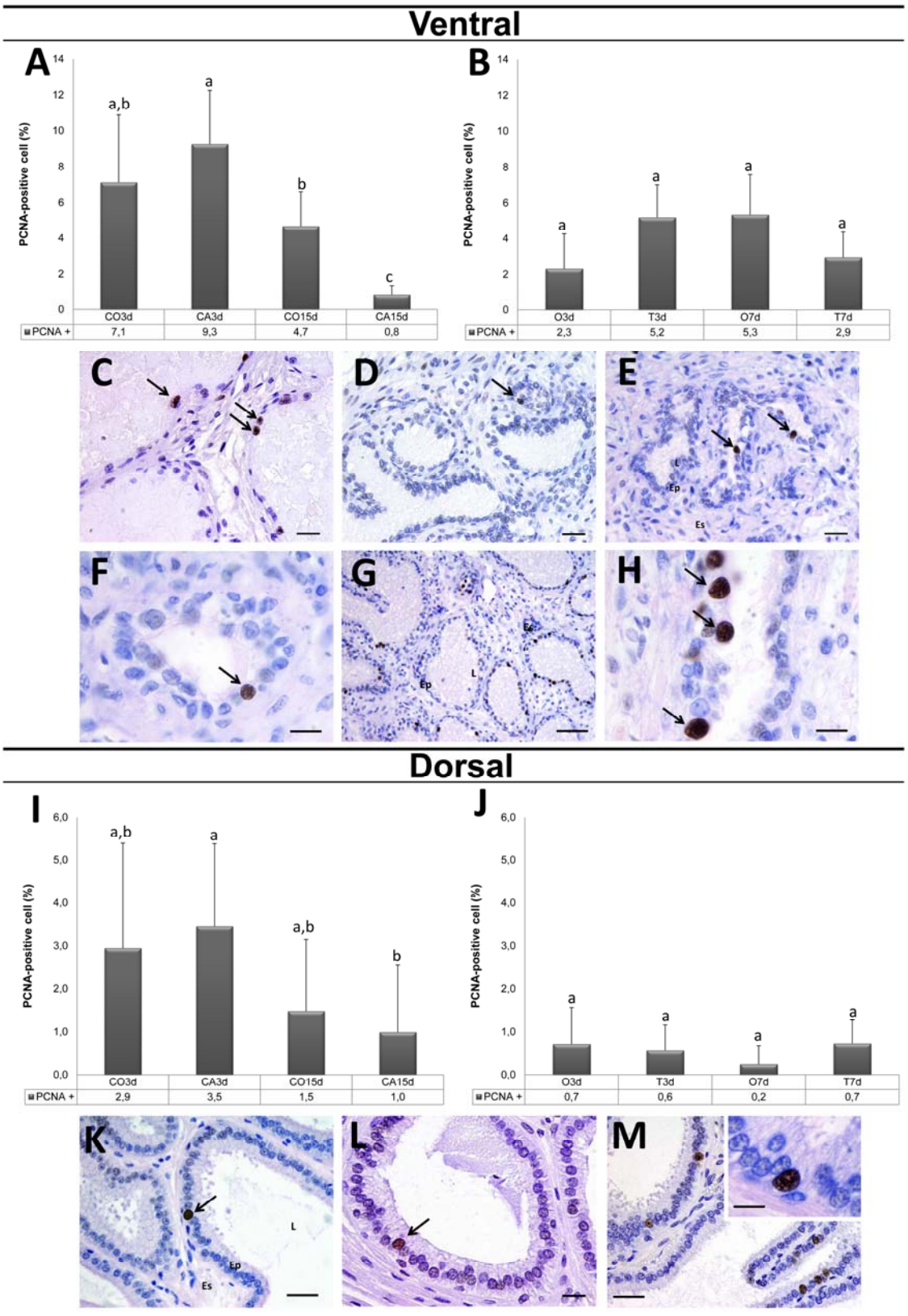
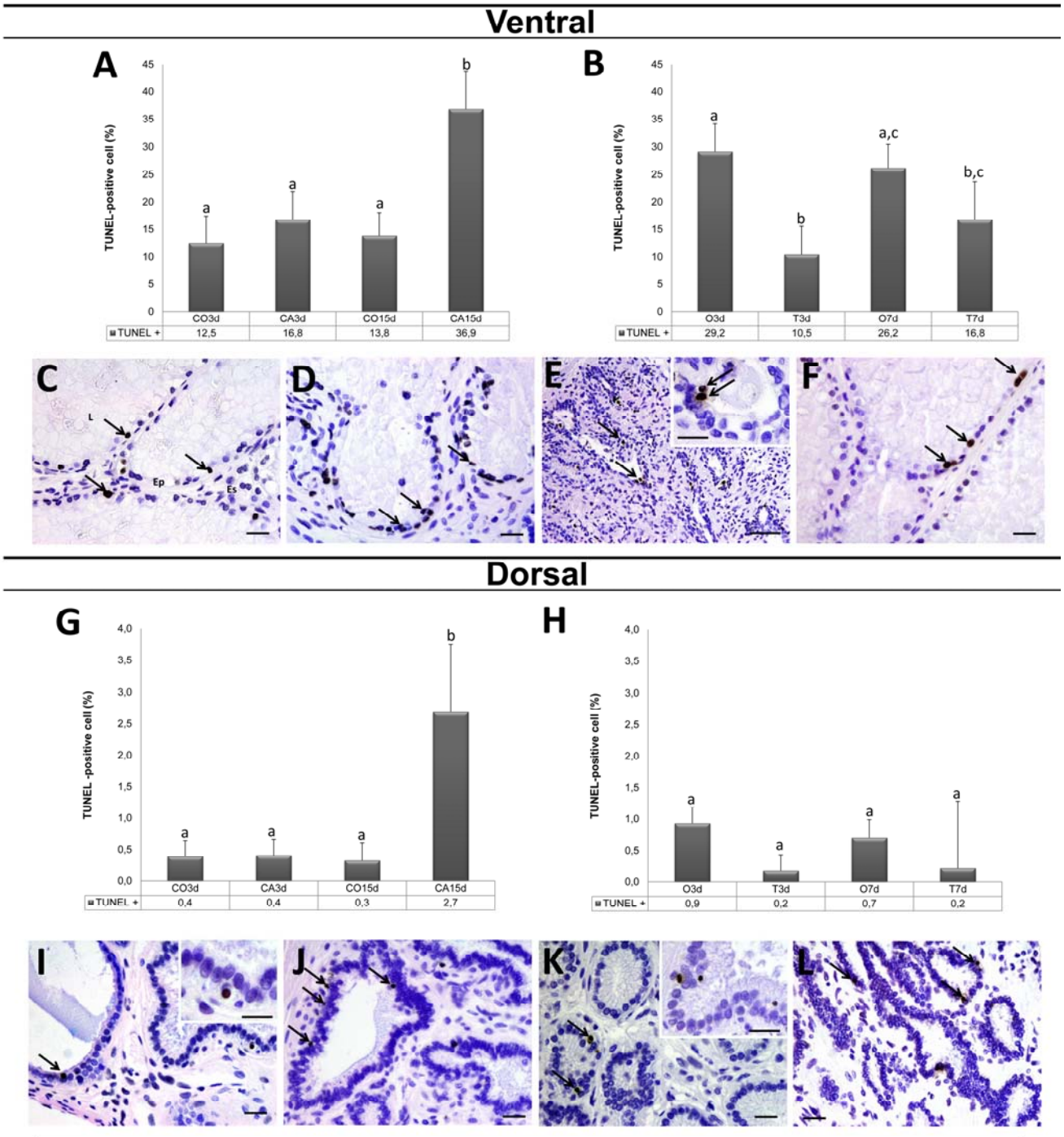

Ventral
A
PCNA-positive cell (%)
a,b
a
b
c
CO3d
CA3d
CO15d
CA15d
PCNA +
7,1
9,3
4,7
0,8
B
PCNA-positive cell (%)
a
a
a
a
O3d
T3d
O7d
T7d
PCNA +
2,3
5,2
5,3
2,9
C
D
E
L
Ep
Es
F
G
Es
Ep
L
H
Dorsal
I
PCNA-positive cell (%)
a,b
a
a,b
b
CO3d
CA3d
CO15d
CA15d
PCNA +
2,9
3,5
1,5
1,0
J
PCNA-positive cell (%)
a
a
a
a
O3d
T3d
O7d
T7d
PCNA +
0,7
0,6
0,2
0,7
K
L
Ep
Es
L
M
Es

Figure 8. A**poptotic cell (TUNEL) in all experimental groups of both glandular regions of *Artibeus planirostris*. A-B.** Graphics corresponding to the quantification of TUNEL-positive cells of the ventral region. **C-F.** Ventral region. **C.** CO3d. **D.** CA15d. **E.** O7d. **F.** T7d. **G-H.** Graphics corresponding to the quantification of TUNEL-positive cells of the dorsal region. **I-L.** Dorsal region. **I.** CA3d. **J.** CA15d. **K.** O3d. **L.** O7d. (Arrows, TUNEL-positive cells; Ep, epithelium; St, stroma; L, lumen). Counter-staining: Hematoxylin. **TABLE.** Index PCNA/TUNEL. ^{a,b,c} Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$). **Scale bar**: Fig. E= 50 µm; Figs. C, D, F, I, J, K and L=20 µm; detail Figs. E and K= 10 µm.

FIGURE 8.



Index PCNA/TUNEL

Groups	CO3d	CA3d	CO15d	CA15d	O3d	T3d	O7d	T7d
Ventral	0.57	0.55	0.34	0.02	0.08	0.50	0.20	0.18
Dorsal	7.59	8.49	4.60	0.37	0.77	3.26	0.35	3.38

Table 1. Biometric and hormonal data in the eight experimental groups.

	BIOMETRIC AND HORMONAL DATA					
	Group	**Initial Weight (g)**	**Final Weight (g)**	**Adrenal (mg)**	**Prostatic Complex (mg)**	**Testosterone (ng/dL)**
Castration Effect	CO3d	47.7±1.0	51.2±1.7	8.6±0.9	64.5±19.2^{a,b}	68.8±29.9^{a}
	CA3d	47.1±1.5	48.7±1.5	8.9±0.6	70.6±8.7^{a,c}	29.9±2.1^{a}
	CO15d	45.7±1.5	50.2±2.2	6.7±0.6	77.6±14.8^{a}	63.5±5.0^{a}
	CA15d	47.8±1.1	52.2±2.6	6.6±0.6	26.9±2.8^{b}	21.7±0.9^{a}
Supplementation Effect	O3d	47.0±2.0	52.0±1.7	8.2±0.6	32.2±9.3^{b,c,d}	7.2±3.5^{a}
	T3d	47.3±1.5	51.0±1.6	7.4±0.6	35.9±3.2^{b}	463.7±103.6^{b}
	O7d	48.5±1.5	50.4±1.5	7.0±0.4	32.6±2.7^{b}	8.3±3.9^{a}
	T7d	47.6±1.2	48.3±1.1	8.1±0.9	70.9±5.8^{a,d}	489.5±76.5^{b}

^{a,b,c} Different superscript letters indicate significant differences between experimental groups (ANOVA at $p < 0.05$).

IV- Artigo 2: The effects of bilateral orchiectomy followed testosterone supplementation on the bulbourethral gland of *Artibeus planirostris* (Chiroptera: Phyllostomidae).

Les effets de la castration suivies supplémentation en testostérone sur la glande bulbo du *Artibeus planirostris* (Chiroptera: Phyllostomidae).

Cíntia C. I. Puga[1], Mateus R. Beguelini[2], Eliana Morielle-Versute[3], Patricia S. L. Vilamaior[1], Sebastião R. Taboga[1*]

[1] Univ. Estadual Paulista, UNESP; Instituto de Biociências, Letras e Ciências Exatas, IBILCE; Departamento de Biologia, São José do Rio Preto, São Paulo, Brasil.
[2] Univ. Federal do Oeste da Bahia, UFOB; Centro Das Ciências Biológicas e da Saúde, Campus Reitor Edgard Santos, Barreiras, Bahia, Brasil.
[3] Univ. Estadual Paulista, UNESP; Instituto de Biociências, Letras e Ciências Exatas, IBILCE; Departamento de Zoologia e Botânica, São José do Rio Preto, São Paulo, Brasil.

*Corresponding author: Sebastião Roberto Taboga – Rua Cristóvão Colombo nº 2265, Jardim Nazareth, 15054-000, São José do Rio Preto, São Paulo, Brasil. Tel.: +55 17 32212380. FAX: + 55 17 3221-2390. E-mail address: taboga@ibilce.unesp.br

ABSTRACT

This study evaluated the effects of testosterone in the bulbourethral glands (BG) of the bat, *Artibeus planirostris*, performing the castration and posterior hormonal supplementation of the animals. The results showed a decrease in testosterone levels in animals 15-days castrated (CA15d), which induced a small reduction in epithelium height and in AR+ cells percentage and an increase in amount of basal cells. This regression became more severe in longer castrated groups (O3D; O7d), where there was also an increase in apoptotic cells. Moreover, the hormonal supplementation increased testosterone levels (T3; T7), causing a glandular reactivation that increased the epithelium height and AR expression. In conclusion, observed that the BG takes longer to respond to ablation of testosterone than other reproductive glands, since it showed evident aspects of regression only in the animals castrated for 22 days.

KEYWORDS: Bat, Castration, Hormone supplementation, Testosterone, bulbourethral

MOTS-CLÉS: chauve-souris, castration, suppléments hormonaux, testosterone, glande bulbo

INTRODUCTION

The bulbourethral gland (BG), one of the reproductive accessory glands, is very important in the male reproductive function of different mammalian species. The secretion of these glands usually cleans the urine from the urethra, and subsequently helps in the lubrication of the vagina, as this is released before ejaculation [1].

The BG structurally differs in different species of mammals. It is completely absent in moles [2], in dogs and in aquatic mammals [3], is poorly developed in man [4], but well developed in the pig [5]. The morphology of the BG has been studied in various mammals, especially rodents such as hamster, rat, chinchillas, rabbits and the cane rat [6,7,8,9,10,11].

Despite the few studies about the reproductive glands of bats, Puga et al. [12,13,14] and Christante et al. [15] observed that, macroscopically, the BG of *Artibeus planirostris*, *Platyrrhinus lineatus* and *Molossus molossus*, respectivelly, is an oval-shaped acinar gland, which was partially surrounded by muscular tissue and contained a columnar epithelium with basal nuclei. The columnar epithelial cells were filled with many secretory droplets of variable electron density, which are released at the apex of the epithelium, where there are small projections of the cell membrane.

The literature related to the influence of androgens in the reproductive glands is always related to the prostate gland. These studies show that surgical castration cause an abrupt and significant decrease in serum androgen levels and a reduced blood flow to the prostate, which induces apoptosis in endothelial and secretory cells [16,17]. After castration, the epithelium regresses, but the epithelial cells remain sensitive to androgens. Thus, testosterone replacement regenerates the prostatic epithelium by restoring the function and number of normal cells [18].

There are few studies related to the effect of castration on BG, with these showing different responses among mammals. Aykroyd e Zuckerman [19] showed that there was regression only in the peripheral part of the BG of mature male Rhesus monkey, which had been castrated for five months. Similarly, Barrington (1913 apud [19]) noticed little regression in the first year after castration in guinea pigs. On the other hand, these results are very different from those found in rats [20], which show quick and marked changes post-castration.

Thus, based on the conflicted relationship of the BG with testosterone in mammals and the wide variation in reproductive patterns of bats, the aim of this study

was to evaluate the testosterone effect in BG of *A. planirostris*, through castration and posterior hormone supplementation.

MATERIALS AND METHODS

Species, ageing and experimental design

The species analyzed was *Artibeus planirostris* [21], which is an exclusively Neotropical species of phyllostomid bat that is not listed as endangered on the International Union for Conservation of Nature (IUCN) Red List of Threatened Species.

The bats were aged as adults based on body weight, complete ossification of the metacarpal-phalangeal epiphyses, wear of the teeth [22], positioning of the testes and the presence of sperm inside the testes, cauda epididymis and/or urethra [12,23,24,25,26,27].

Artibeus planirostris is a wild species that is protected by federal laws in Brazil (IBAMA). Adding to these, its harem structure (with a single male mated to multiple females) means that the collection of male specimens directly influences the structure and dynamics of the populations. Thus, the number of specimens used in this study was the maximum number obtained from a balance between the minimum number required for relevant statistical analyses and the maximum number of individuals that could be collected without destroying or disrupting the colonies of bats.

Thus, forty-six sexually male mature specimens were analyzed. Eight groups were formed (Fig. 1): I) 3 days control (CO3d, n=4); II)) 3 days castrated (CA3d, n=7); III) 15 days control (CO15d, n=5); IV) 15 days castrated (CA15d, n=7); V) 15 days castrated with injection of mineral oil for 3 days (O3d, n=4); VI) 15 days castrated with testosterone cypionate (1µg/Kg/day) supplementation for 3 days (T3d, n=7); VII) 15 days castrated with injection of mineral oil for 7 days (O7d, n=5) and VIII) 15 days castrated with testosterone cypionate (1µg/Kg/day) supplementation for 7 days (T7d, n=7).

Study area, capture and licenses

The animals were collected in the city of São José do Rio Preto, in the northwest of São Paulo State, Brazil, (49W22'45", 20S49'11"), which is located 500 meters above sea level. This is a semi-flat region, set in a degraded Cerrado biome, with meso-thermal climate (rainy summers and dry winters).

The captures were concentrated in the summer (21st December to 21st March) in order to minimize variations arising from seasonality [23] and to focus on changes caused by treatments. The captures were performed at night using mist nets (3m x 6m), which were positioned to intercept bats flying 1–3 m above the ground. The nets were precisely placed on possible flight routes or exits from shelters.

The experimental procedures were approved by the Ethics Committee of IBILCE-UNESP (Process: 036/2010 – CEUA), and the capture of animals was authorized by the Brazilian Institute of the Environment, IBAMA (Process: 21707-1). The animals were treated according to the recommendations of the Committee on Care and Use of Laboratory Animals from the Institute of Laboratory Animal Resources, National Research Council, "Guide for the Care and Use of Laboratory Animals" (Committee on Care and Use of Laboratory Animals, 1980).

Castration and maintenance of the animals

To standardize the study, after capture, the animals were kept in cages (40 cm x 20 cm x 20 cm) with water and food (pieces of papaya, banana or other fruit) *ad libitum*, in a specific room in darkness at 25–30°C. These conditions were only used in the period between the end of the capture and the next morning, at which the bats were anesthetized with a mixture of ketamine (Dopalen-Vertebrands, Paulínia, SP, Brazil; 370 mg/kg) and xylazine (Rompun – Bayer S.A., São Paulo, SP, Brazil; 16 mg/kg), which was applied at an amount of 0.1 ml per 50g of body weight (0.08 ml ketamine plus 0.02 ml of xylazine). After anesthesia, bilateral orchiectomy was performed, with a small incision in the inguinal region, in which the testicle and epididymis were removed after cutting the deferent duct.

After castration, the animals were maintained in the Laboratory of Microscopy and Microanalysis of the Department of Biology (IBILCE/UNESP) in a specific room in darkness at 25–30°C. They were accommodated in cages (100cm length x 50cm width x 30cm height) dotted with grids at the superior portion to facilitate aeration, proper positioning of the animal (upside down) and feeding. Only 2 or 3 animals were placed per cage to avoid the stress of overpopulation. The animals received water *ad libitum* and, since it is a frugivorous species, the feeding corresponded to fresh and tender fruits (papaya, banana, etc.), which were changed every day. The cages were cleaned every day, with the water exchange and the supplementation of the feed being performed simultaneously in order to cause the lowest possible stress to the animal,

with these processes occurring without the direct manipulation of the animals. All animals were manipulated only for the performance of the treatments (injections of testosterone).

Processing of the animals

To standardize, all specimens were euthanized in the morning by deep anesthesia (mixture of ketamine and xylazine), administered subcutaneously, and positioned in dorsal decubitus on the dissection board. After the body weight was measured, blood samples were collected by endocardial puncturing. A longitudinal incision in the skin was performed, from the anus to the penis, thereby exposing the penile root, allowing the BG removed and fixed for analysis.

All specimens are archived in the Chiroptera Collection of the Laboratory of Chiroptera at the Univ. Estadual Paulista, UNESP; Instituto de Biociências, Letras e Ciências Exatas, IBILCE.

Serum hormone dosage

The blood, collected by endocardial puncture, was immediately centrifuged at 1,200g to separate the serum, and frozen at -80°C for subsequent analysis of testosterone levels. The testosterone dosages were performed by ELISA Capture/Sandwich (antibody-antigen-antibody), using specific commercial kits (Cayman Chemical Company, Ann Arbor, Michigan, USA - Item # No. 582701) of high sensitivity (6.0 pg/ml), with coefficients of variation for an inter-assay of 32.0 pg/ml. Readings were taken using an Epoch™ Multi-Volume Spectrophotometer System (BioTekInstruments, VT, USA) reader.

Histology

The BG was fixed by immersion in Metacarn fixative solution (methanol: chloroform: acetic acid – 6:3:1) for 3 h at 4^{o}C, dehydrated in ethanol, clarified in xylene and embedded in paraffin (Histosec – MERK, Darmstadt, Germany). Sections of 4µm were stained with hematoxylin-eosin (HE [28]), periodic acid-Schiff (PAS [29]) and Picrosirius [30]) or submitted to immunostainings. Microscopic analyses were performed using an Olympus BX60 microscope (Olympus Optical Co., Ltd., Tokyo, Japan) with coupled image analyzer (Image Pro Plus version 6.1 for Windows - Copyright © 1993-2006 Media Cybernetics, Inc.).

Stereological analysis and morphometry

The following measurements were performed on cross sections of the BG using the Image Pro-Plus-Media Cybernetics, version 6.1 for Windows computer software for image analysis: the relative percentage of glandular compartments (epithelium, lumen and stroma), the epithelium height and the cell population census.

Stereology: for this analysis, 50 fields of the slides stained by HE (dorsal region) and Picrosirius (Ventral region) were randomly selected from each experimental group. The relative frequency of glandular components (epithelium, lumen and stroma) was calculated applying an M130 multipoint test system [31].

Morphometry: to measure the epithelium height, 20 random fields were captured from each animal, with three measurements performed in each field, accumulating a total of 300 measurements for each experimental group.

The cell population census was conducted on blades submitted to P63 immunohistochemistry (protocol described below). For this analysis, 50 fields of the slides submitted to P63 immunohistochemistry were selected from each experimental group. In each field, the amounts of basal and secretory cells were measured by direct counting of nuclei.

Transmission electron microscopy

The BG were processed for transmission electron microscopy (TEM) as follows. First, the BG was fixed 24 h by immersion in a solution of 3% glutaraldehyde plus 0.25% tannic acid in Millonig buffer (pH 7.3) containing 0.54% glucose [32]. After washing with same buffer, the samples were post-fixed with 1% osmium tetroxide for 2 h, dehydrated in a graded series of acetone and embedded in Araldite 502 Resin (Electron Microscopy Sciences, Hatfield, PA, USA). Ultrathin sections (50 nm) were made using diamond knives, stained with 2% uranyl acetate for 30 min [33] followed by 2% lead citrate in sodium hydroxide for 10 min [34] and then analysed using TEM (906; Leo-Zeiss, Cambridge, UK) at the Prof. Dr Celso Abbade Mourão Center for Microscopy (IBILCE-UNESP).

Immunohistochemistry and TUNEL analysis

After microwaving for antigen retrieval, non-specific binding was blocked prior to incubation with primary antibodies against: androgen receptor (AR) – rabbit polyclonal IgG (sc-816/Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution

1:75); p63 – mouse monoclonal IgG2a (sc-25268/Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1:100); proliferating cell nuclear antigen (PCNA) – mouse monoclonal IgG2a (sc-56/Santa Cruz Biotechnology, Santa Cruz, CA, USA, dilution 1:100); and apoptosis (TUNEL – TdT-Fragel-Calbiochem, second manufacturer's instructions, Oncogene Research Products, Boston). The sections were submitted to a reaction with diaminobenzidine (DAB) and counterstained with Harris hematoxylin. Negative controls were used, omitting the incubation step with the primary antibody or enzyme (in the case of TUNEL). To confirm the results, immunostainings were performed in triplicate sets.

The relative percentage of positive and negative cells in the acinar epithelium was determined using measurement fields of the entire length of the slides. The ratio between the proliferation and cellular apoptosis of the acinar epithelium was also calculated by dividing the percentage of PCNA-positive by that of TUNEL-positive cells (% cell PCNA-positive/% cell TUNEL-positive).

Statistical analysis

All numerical data were expressed as means and standard error, and analyzed initially by analysis of variance (One-way ANOVA) and, subsequently, by a Tukey´s test for multiple comparisons ($p \leq 0.05$ = significant), using the Statistica 7.0 program (Statsoft Inc., Tulsa, USA).

RESULTS

Biometrics and hormonal data

Although there has been an increase in body weight of all animals held in captivity, no significant differences were observed between the initial and final values of body (Fig. 2A) in all experimental groups. The serum testosterone dosage (Fig. 2B) showed the decrease in the level of testosterone in CA3d (29.9±2.1ng/dL) and CA15d (21.7±0.9ng/dL) groups, when compared with the CO3d (68.8±29.9ng/dL) and CO15d (63.5±5.0ng/dL) groups. The O3d (7.2±3.5ng/dL) and O7d (8.3±3.9ng/dL) groups showed even lower values (Fig. 2B) and the groups with hormone supplementation [T3d (463.7±103.6ng/dL) and T7d (489.5±76.5ng/dL)] had high values (Fig. 2B).

Histology, stereological and morphometric analyses

The BG of the CO3d and CO15d groups showed normal morphology, (Figs. 3A-B, E-F) contained a columnar epithelium with basal nuclei and secretion granular and PAS positive. The CA3d group (Figs. 3C-D) had no changes in morphology compared to these controls (Figs. 3A-B, E-F). However, the CA15d group presented a small glandular regression with an acinar reduction (Fig. 3G-H). The groups O3d (Figs. 4A-B) and T3d (Figs. 4C-D) showed a similar morphology to the CA15d group (Figs. 3C-D), presenting a small glandular regression.

The long-term treatment without testosterone (O7d) showed a great glandular regression (Figs. 4E-F), with a change in the pattern of glandular secretion and also an increase in stromal areas. However, the long-term treatment with testosterone (T7d) showed a return to normal glandular morphology, with a decline in stromal areas (Fig. 4G-H).

Stereologically, the BG did not show significant changes in groups CO3d, CA3d, CO15d and CA15d. Observes a tendency to a small reduction in the epithelium and increased stromal in CA15d group (Fig. 5A). In the supplementation of testosterone the epithelium decreased significantly in the group O7d; the lumen increased in groups treated with testosterone (T3d and T7d) and stroma decreased in the T7d (Fig. 5B).

The morphometry showed a small significant decline in the epithelium height in the CA15d group (Fig. 5C). Oil-treated groups (O3d and O7d) showed a decrease in epithelium height. The groups treated with testosterone (T3d and T7d - Fig. 5D) showed an increase in the epithelium height, being more pronounced in group treated with testosterone for a longer time (T7d).

Ultrastructural data

The BG did not present differences in the ultrastructure in the groups CO3d, CA3d, CO15d and CA15d (Fig. 6A-L). The BG had a columnar epithelium, with basal nuclei (Fig. 6A-D-G-J). The columnar epithelial cells were filled with many secretion droplets of variable electron density (Fig. 6). These vesicles are released at the apex of the epithelium, where there are small projections of the cell membrane (Fig. 6C-F-L and detail in the Fig. 6I). In all groups, the cells presented a cytoplasm with large volume of endoplasmic reticulum and Golgi complexes (Fig. 6B-E-H-K).

The groups O3d (Fig. 7A-B-C) and T3d (Fig. 7D-E-F) showed ultrastructure similar to the groups already described. Moreover, O7d group (Fig. 7G-HI) showed a

decrease in secretory vesicles, which were concentrated at the apex of the cell and also of cellular components, such as reticulum and Golgi complex. And the group treated with testosterone for 7 days (T7d) showed a return of the glandular activity, with many secretory vesicles, with large volume of endoplasmic reticulum and Golgi complexes (Fig. 7J-K-L)

Cell population census

The cell population census revealed a significant increase in the number of basal and the other cells in the CA15d group (Figs. 8A, D), in relation to the CO3d, CA3d and CO15d groups (Figs. 8A, C). In the groups treated with testosterone for long period (T7d) has a decreased in the number of other cells in relation to the O3d, T3d and O7d groups (Figs. 8B, E), but the basal cell remains constant (Figs. 8B, E, F).

Index p63-positive cell/p63-negative cell

Dividing the number of basal cells by the other cells enabled the development of an index, which indicates that the BG did not show significant changes in groups CO3d, CA3d, CO15d and CA15d. Observes a tendency for an increase in the index in the group CA15d (Table in Figure 8). In the supplementation of testosterone observed that the O3d showed a decline in the index, and a return to the initial index in T7d group (Table in Figure 8).

Immunohistochemistry

Androgen receptor (AR)

The AR immunohistochemistry showed an increase in AR-positive cells in the CO15d group in relation to CO3d, CA3d and Ca15d groups (Figs. 9A, C and D). On the other hand, in the hormone supplementation groups, there was a significant increase in AR-positive cells in the groups treated with testosterone (T3d and T7d), and a decrease in the groups private of testosterone to long periods (O3d and O7d) (Figs. 9B, E and F).

Cell proliferation (PCNA)

PCNA did not show significant differences in the castrated groups (CO3d, CA3d, CO15d and CA15d, Figs. 10A, C, D). The groups O3d and O7d showed a decrease of PCNA-positive cells in comparison with CA3d and CA15d groups (Fig. 10A-E). The groups submitted to hormone supplementation for 3 and 7 days [T3d (Figs. 10B and 10E-H) and T7d] had an increase of the proliferation.

Apoptosis (TUNEL)

The BG showed significant increase in the number of apoptotic cells only in O7d group (Figs. 10H). In the other groups the values did not differ significantly.

Rate of Cell Proliferation (PCNA/TUNEL)

The PCNA/TUNEL ratio showed an increase in CA15d group compared to control groups (CO3d, CA3d and CO15d) and a decline in the group castrated for long period (O7d).

DISCUSSION

This study represents the first evaluation of the relation of testosterone and the BG in bats. The BG is a gland very important of the reproductive system of the different mammalian species. Its secretion is released before ejaculation, its function is to clear the urethra and help the vagina lubrication [1].

There are few studies related to the effect of castration on BG, with these showing different responses among mammals [19,20]. The literature related to the influence of androgens in the reproductive glands is always related to the prostate gland. Thus, based on the importance of this gland, in the absence of work related to the subject, conflicted relationship of the BG with testosterone in mammals and the wide variation in reproductive patterns of bats. Emphasize the importance of this study, in which to evaluate the effect of testosterone in BG of *A. planirostris*, through castration and posterior hormone supplementation.

In this study did not observe significant differences between the initial and final values of body parameters in all experimental groups, although the increased body weight of all animals held in captivity. Thus showing that castration and subsequent hormone supplementation does not influence significantly the weight of the animals.

The testosterone dosage showed a tendency of decrease in CA3d and CA15d groups, when compared with the CO3d and CO15d groups, but the BG presented only a small regression in the CA15d. Only in the O7d there was a great glandular regression, indicating that the BG of the *A. planirostris* is less dependent on testosterone, as its regression happened after 22 days of castration, when testosterone levels is very low. However, the long-term treatment with testosterone (T7d) showed a return to normal glandular morphology, with a decline in stromal areas, thus showing that there is a fast return of the gland when injected testosterone.

The cell population census indicated an increase in the number of basal and others cells in CA15d group; however, this increase is closely associated with the initial glandular regression itself, as there was an accentuated decrease in the acini and decrease of epithelial height.

The androgen receptor showed little marking of epithelial cells in the control groups. This may be related to a delay in response of this gland to testosterone. When the testosterone level decreases, the number of AR-positive cells also decreases, causing the glandular regression confirmed in morphology. Cordeiro et al. [35] observed that, in prostate, the AR expression was directly related to the levels of serum testosterone, where it decline in castrated and increase in groups treated with testosterone, as described here for the GB.

The cell proliferative and apoptotic rate changed significantly only in long-term castrated group (O7d), showing that the BG has a smaller dependency to the lack of testosterone than other male reproductive glands The prostate for example presents a significant regression in animals castrated for at least 15 days [36].

There are few studies related to the effect of castration on BG, with these showing different responses among mammals. In these studies the effect of castration was observed over long periods. Aykroyd e Zuckerman [19] showed that there was regression only in the peripheral part of the BG of mature male Rhesus monkey that had been castrated for five months and Barrington (1913 apud [19]) noticed little regression in the first year after castration in guinea pigs. These results are very different from those found for rats [20], which show quick and marked changes post-castration.

In conclusion, the present study provides the first information on the histological and ultrastructural features of BG in the bat (*Artibeus planirostris*) subjected to castration and hormone supplementation. The study showed that the BG

takes longer to respond to ablation of testosterone than other reproductive glands, as evident regression was observed only in the group with 22 days of castration (O7d).

ACKNOWLEDGEMENTS

We thank the technical help from Luiz Roberto Falleiros Junior and Rosana Silistino de Souza.

FUNDING

This work was supported by the São Paulo State Research Foundation - FAPESP [2011/01323-2 to C.C.I.P.; 2012/09194-0 to M.R.B.]; and the Brazilian National Research and Development Council (CNPq) [300163/2008-8 and 301596/2011-5 to SRT.

REFERENCES

[1] D.A. Samuelson (ed.), Male reproductive system, 1st edn, in: Textbook of Veterinary Histology. St. Louis, MI: Saunders Elsevier, 63146, 2007.

[2] P.A. Racey, Seasonal changes in testosterone levels and androgen dependent organs in male moles (*Talpa europaea*). J. Reprod. Fertil. 52 (1978) 195–200.

[3] K.M. Dyce, W.O. Sack, C.J.G. Wensing, Text Book of Veterinary Anatomy. 3rd edn. Philadelphia, PA: Saunders. 2002.

[4] A.M. Jequier, Clinical disorders affecting semen quality, in: Gametes—The Spermatozoon (J. L. Yovich, ed), Cambridge: University Press, 175–191, 1995.

[5] E. Badia, M.D. Briz, E. Pinart, S. Sancho, N. Garcia, J. Bassols, A. Pruneda, E. Bussalleu, M. Yeste, I. Casas, S. Bonet, Structural and ultrastructural features of boar bulbourethral glands, Tissue Cell, 38 (2006) 7–18.

[6] L. Adaro, A. Mendoza, R. Cepeda, C. Orostegui, Anatomo-radiographic study of the seminal vesicles of chinchilla (*Chinchilla laniger*) in captivity, Rev. Chil. Anat. 19 (2001) 297–300.

[7] A.O. Adebayo, A.K. Akinloye, S.G. Olukole, M.O. Oyeyemi, V.O. Taiwo, A.O. Ihunwo, B.O. Oke, Gross, histological and ultrastructural features of the bulbourethral gland in the greater cane rat (*Thryonomys swinderianus*), Anat. Histol. Embryol. 44(1) (2015) 59–65.

[8] M. Cakir, A. Karatas, Histo-anatomical studies on the accessory reproductive glands of the anatolian souslik (*Spermophilus xanthoprymnus*) (Mammalia: Sciuridae), Anat. Histol. Embryol. 33 (2004) 146–150.

[9] C. Jesik, J. Holland, C. Lee, An anatomic and histologic study of the rat prostate, Prostate, 3 (1982) 81–97.

[10] J. Toma, G. Buzzell, Fine estructure of the ventral and dorsal lobes of the prostate in the young adult Syrian hamster, *Mesocricetus auratus*, Am. J. Anat. 2 (1988) 132–140.

[11] B. Vasquez, M. Del Sol, Prostatic complex in the rabbit (*Oryctolagus cuniculus*). Rev. Chil. Anat. 20 (2002) 175–180.

[12] C.C.I. Puga, M.R. Beguelini, A.C. Negrin, C.M. Christante, E. Morielle-Versute, P.S.L. Vilamaior, S.R. Taboga, Structure, histochemistry and ultrastructure of the male reproductive accessory glands in the Neotropical flat-faced fruit-eating bat, *Artibeus planirostris* (Chiroptera: Phyllostomidae). Reprod. Fertil. Dev. 25 (2013) 558–569.

[13] C.C.I. Puga, Morfofisiologia das glândulas reprodutivas acessórias masculinas de morcegos: estudo nas espécies *Artibeus planirostris* e *Platyrrhinus lineatus* (Chiroptera: Phyllostomidae). Dissertação (Mestrado) - Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas – São José do Rio Preto, 2011.

[14] F.F. Martins, C.C. Puga, M.R. Beguelini, E. Morielle-Versute, P.S. Vilamaior, S.R. Taboga, Comparative analysis of the male reproductive accessory glands of bat species from the five Brazilian Subfamilies of the family Phyllostomidae (Chiroptera). J Morphol., 2015 (In press).

[15] C.M. Christante, M.R. Beguelini, C.C.I. Puga, A.C. Negrin, E. Morielle-Versute, P.S.L. Vilamaior, S.R. Taboga, Structure, histochemistry and seasonal variations of the male reproductive accessory glands in the Pallas's mastiff bat, *Molossus molossus* (Chiroptera: Molossidae), Reprod Fertil Develop. 2014 (In press).

[16] N. Kyprianou, J.T. Issacs, Activation of programmed cell death in the rat ventral prostate after castration, Endocrinology, 122 (1988) 552-562.

[17] A. Shabisgh, N. Tanji, V. D'Agati, M. Burchardt, M. Rubin, E.T. Goluboff, D. Heitjan, A. Kiss, R. Buttyan, Early effects of castration on the vascular system of the rat ventral prostate gland. Endocrinology 140 (1999) 1920-1932.

[18] G.P. Risbridger, H. Wang, M. Frydenberg, G. Cunha, The metaplastic effects of estrogen on mouse prostate epithelium: proliferation of cells with basal cell phenotype. Endocrinology 142 (2001) 2443-50.

[19] O.E. Aykroyd, S. Zuckerman, The effect of sex-hormones on the bulbourethral glands of *Rhesus monkeys*, J. Anat. Oct. 73(1) (1938) 135–144.

[20] R.E. Heller, Cowper's gland and its reaction to castration and to different sex hormone conditions. Am. J. Anat. 50 (1932) 73-95.

[21] J.B. Spix, Simiarum et Vespertilionum Brasiliensium Species Novae: ou histoire naturelle des espèces nouvelles de singes et de chauve-souris observées et recueillies pendant le voyage dans l'intérieur du Brésil execute par ordre de S.M. le Roi de Bavière dans les années 1817, 1818, 1819, 1820. Monaco: Francisci Seraphici Hubschmanni, (1823).

[22] L.V. Knegt, J.A. Silva, E.C. Moreira, G.L. Sales, Bats found in the city of Belo Horizonte, MG, 1999–2003, Arq. Bras. Med. Vet. Zootec, 57 (2005) 576–583.

[23] C.C.I., Puga, M.R. Beguelini, F.F. Martins, E. Morielle-Versute, L.R. Faleiros-Jr, P.S.L. Vilamaior, S.R. Taboga, Seasonal changes in the prostatic complex of *Artibeus planirostris* (Chiroptera: Phyllostomidae), Gen. Comp. Endocrinol, 197 (2014) 33-42.

[24] M.R. Beguelini, C.C. Puga, F.F. Martins, A.H. Betoli, S.R. Taboga, E. Morielle-Versute, Morphological variation of primary reproductive structures in males of five families of neotropical bats, Anat. Rec. (Hoboken) 296(1) (2013) 156-167.

[25] M.R. Beguelini, C.C. Puga, S.R. Taboga, E. Morielle-Versute, Annual reproductive cycle of males of the flat-faced fruit-eating bat, *Artibeus planirostris* (Chiroptera: Phyllostomidae), Gen. Comp. Endocrinol. 185 (2013) 80–89.

[26] M.R. Beguelini, L.M. Bueno, D.L. Caun, S.R. Taboga, E. Morielle-Versute, Ultrastructure of spermatogenesis in the short-tailed fruit bat, *Carollia perspicillata* (Chiroptera: Phyllostomidae: Carollinae), J. Morphol. 275(1) (2014) 111-123.

[27] M.R. Beguelini, L.R. Falleiros, R.M. Góes, P. Rahal, E. Morielle-Versute, S.R. Taboga, Differential expression of aromatase, estrogen receptor alpha and 17β-HSD associated with the processes of total testicular regression and recrudescence in the bat *Myotis nigricans* (Chiroptera: Vespertilionidae), Gen. Comp. Endocrinol. 201 (2014) 53-64.

[28] M.G. Ribeiro, S.R. Lima, Iniciação às técnicas de preparação de material para o estudo e pesquisa em morfologia. Belo Horizonte: SEGRAC – Editora e Gráfica Limitada, 2000.

[29] O.A. Behmer, E.M.C. Tolosa, A.G. Freitas-Neto, Manual de técnicas para histologia normal e patológica, São Paulo: EDART, Editora da Universidade de São Paulo, 1976.

[30] L.M. Junqueira, J. Carneiro, Histologia Básica, (Guanabara Koogan: Rio de Janeiro, 2008.

[31] E.R. Weibel, Principles and methods for the morphometric study of the lung and other organs. Laboratory Investigation 12 (1963) 131–155.

[32] G. Cotta-Pereira, F.G. Rodrigo, J.F. David-Ferreira, The use of tannic acid–glutaraldehyde in the study of elastic related fibers. Stain. Technol. 51 (1976) 7–11.

[33] M.L. Watson, Staining tissue section of electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. 4 (1958) 475–478.

489 [34] J.H. Venable, R.A. Coggeshall, A simplified lead citrate stain for use in electron
490 microscopy. J. Cell Biol. 25 (1965) 407–408.

491 [35] R.S. Cordeiro, W.R. Scarano, S.G.P. Campos, F.C.A Santos, P.S.L Vilamaior,
492 R.M. Goes, S.R. Taboga, Androgen receptor in the Mongolian gerbil ventral prostate:
493 Evaluation during different phases of postnatal development and following androgen
494 blockage, Micron 39 (2008) 1312–1324.

495 [36] C.C.I. Puga, M.R. Beguelini, E. Morielle-Versute, P.S.L. Vilamaior, S.R. Taboga,
496 The effects of testosterone in prostatic complex of *Artibeus planirostris* (Chiroptera:
497 Phyllostomidae), Data not published, 2015.

Figure 1. Schematic representation of the experimental design used in this study.

FIGURE 1.

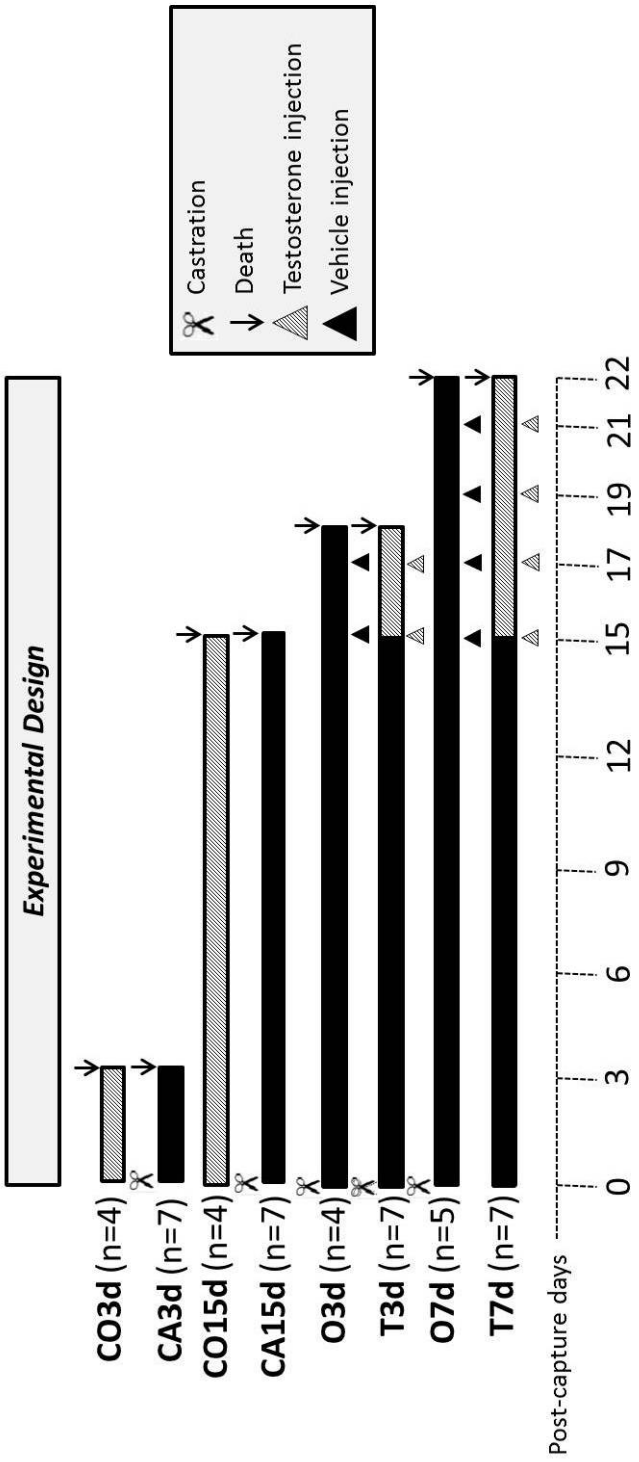

Experimental Design
Castration
Death
Testosterone injection
Vehicle injection
CO3d (n=4)
CA3d (n=7)
CO15d (n=4)
CA15d (n=7)
O3d (n=4)
T3d (n=7)
O7d (n=5)
T7d (n=7)
0
3
6
9
12
15
17
19
21
22
Post-capture days

Figure 2. Biometric and hormonal data. A. Graphs of the initial and final corporal weight of eight experimental groups of *Artibeus planirostris*. B. Testosterone levels of eight experimental groups of *Artibeus planirostris*.

FIGURE 2.

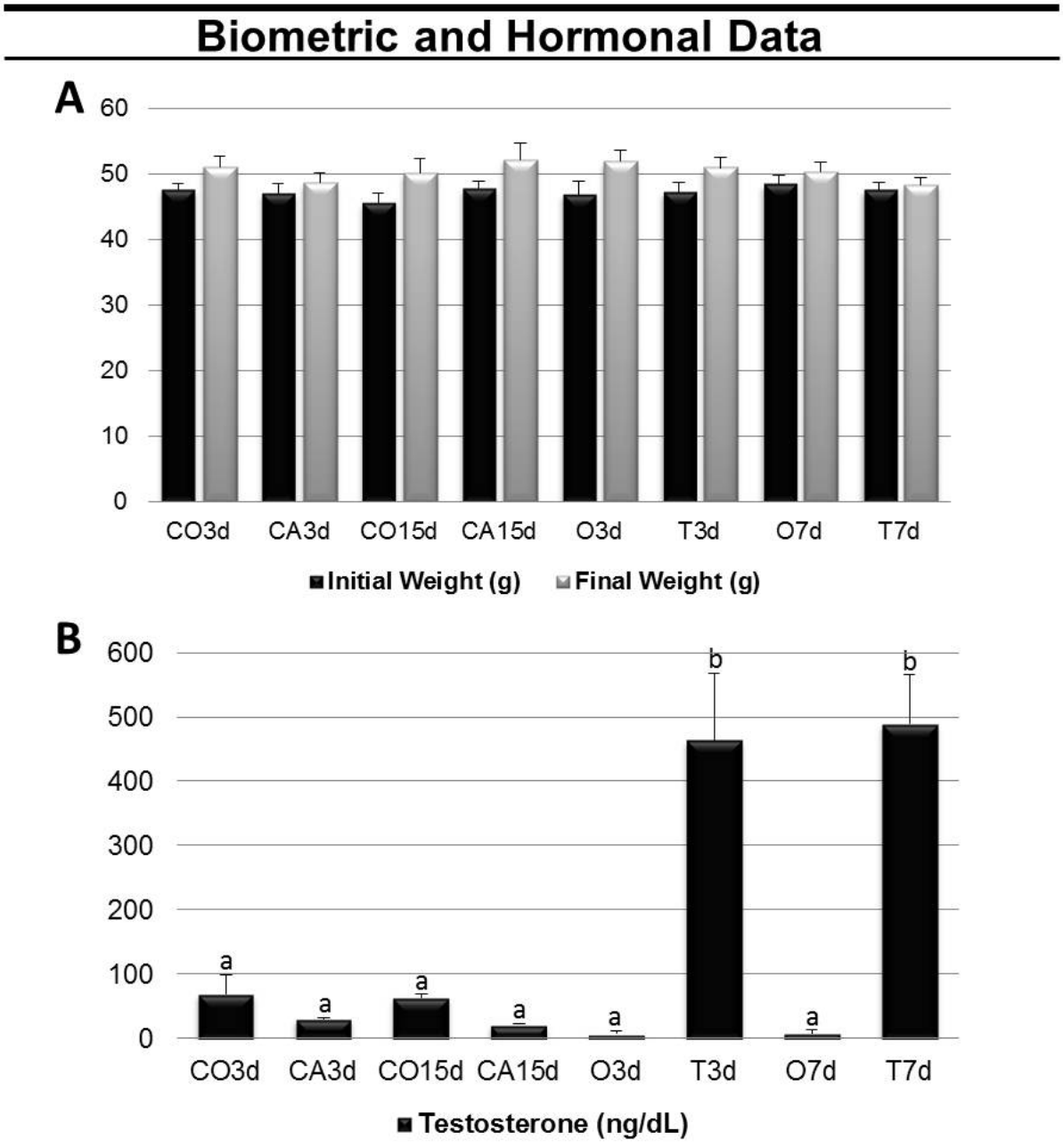

Biometric and Hormonal Data
A
60
50
40
30
20
10
0
CO3d
CA3d
CO15d
CA15d
O3d
T3d
O7d
T7d
Initial Weight (g)
Final Weight (g)
B
600
500
400
300
200
100
0
a
a
a
a
a
b
a
b
CO3d
CA3d
CO15d
CA15d
O3d
T3d
O7d
T7d
Testosterone (ng/dL)

Figure 3. Histological general pattern of the bulbourethral gland of four experimental groups of *Artibeus planirostris*. A-B. Three days control group (CO3d); **C-D.** Three days castrated group (CA3d); **E-F.** Fifteen days control group (CO15d); **G-H.** Fifteen days castrated group (CA15d). (Ep, epithelium; St, stroma; L, lumen). Stained: **A-C-E-G:** HE, details Picrosirius; **B-D-F-H:** PAS. **Scale bar: A-C-E-G=** 50 µm; all details = 20 µm; **B-D-F-H=** 10 µm.

FIGURE 3.

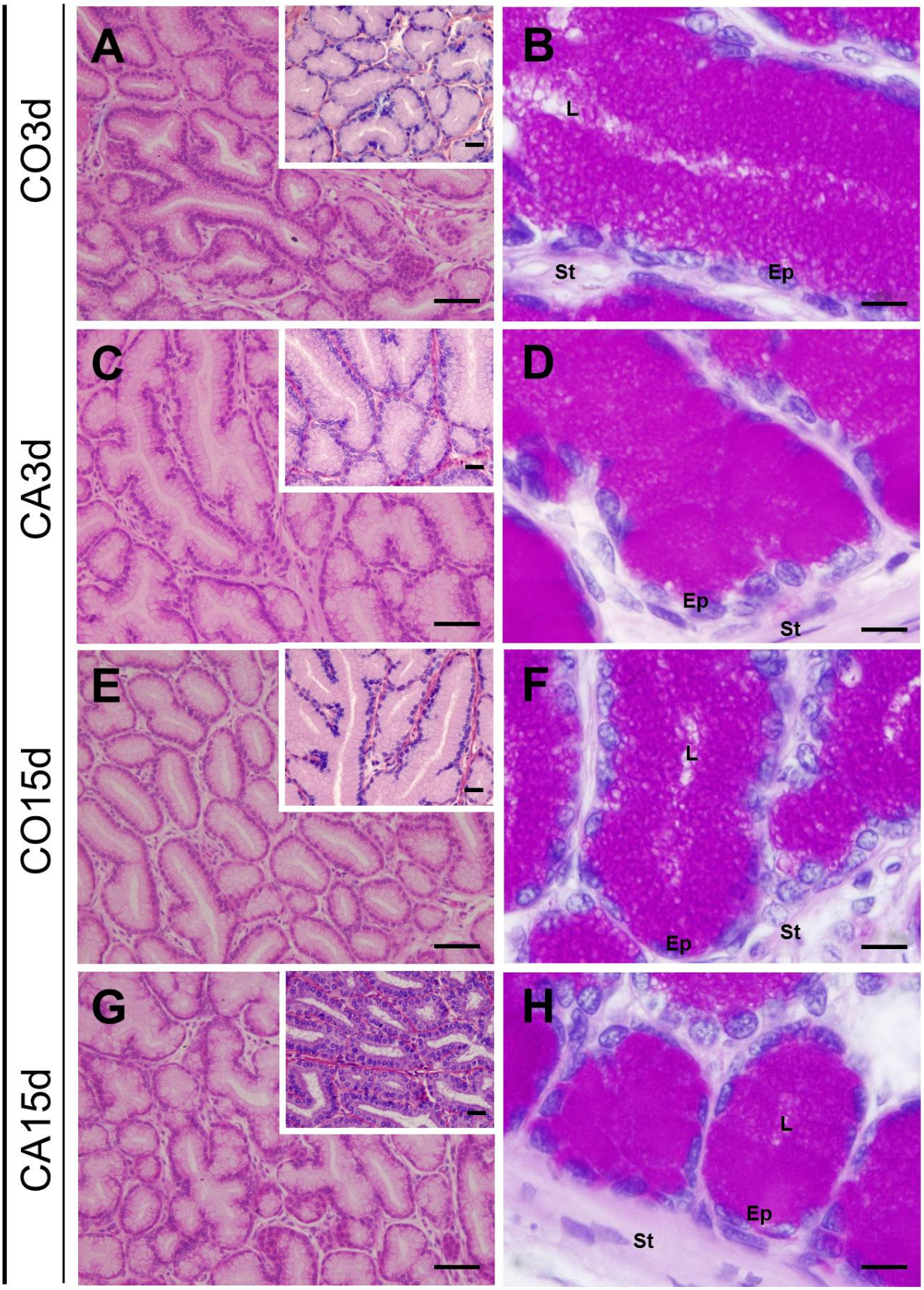


Figure 4. Histological general pattern of the bulbourethral gland of four experimental groups of *Artibeus planirostris*. A-B. Castrated - three days oil injected group (O3d); **C-D.** Castrated - three days testosterone injected group (T3d); **E-F.** Castrated - seven days oil injected group (O7d); **G-H.** Castrated - seven days testosterone injected group (T7d). (Ep, epithelium; St, stroma; L, lumen). Stained: **A-C-E-G:** HE, details Picrosirius; **B-D-F-H:** PAS. **Scale bar: A-C-E-G=** 50 µm; all details = 20 µm; **B-D-F-H=** 10 µm.

FIGURE 4.

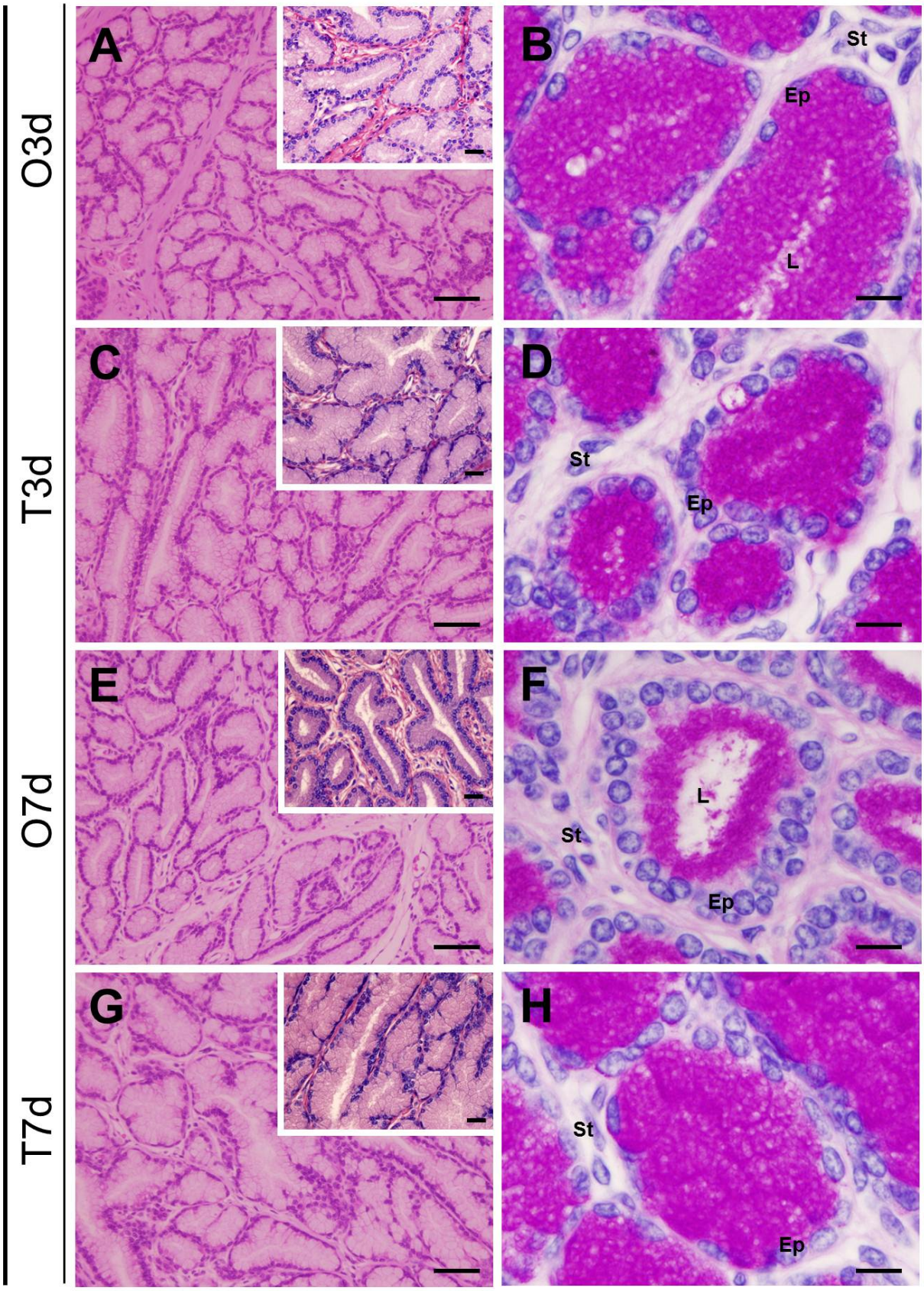


Figure 5. Stereology (A and B) and morphometry (C and D) of the bulbourethral gland of the eight experimental groups of *Artibeus planirostris*: three days control group (CO3d); three days castrated group (CA3d); fifteen days control group (CO15d); fifteen days castrated group (CA15d); castrated - three days oil injected group (O3d); castrated - three days testosterone injected group (T3d); castrated - seven days oil injected group (O7d); and castrated - seven days testosterone injected group (T7d). A-B. Stereology: percentage of the glandular components (epithelium, lumen and stroma). C-D. Morphometry: epithelial height (μm). ^{a,b,c} Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$).

FIGURE 5.

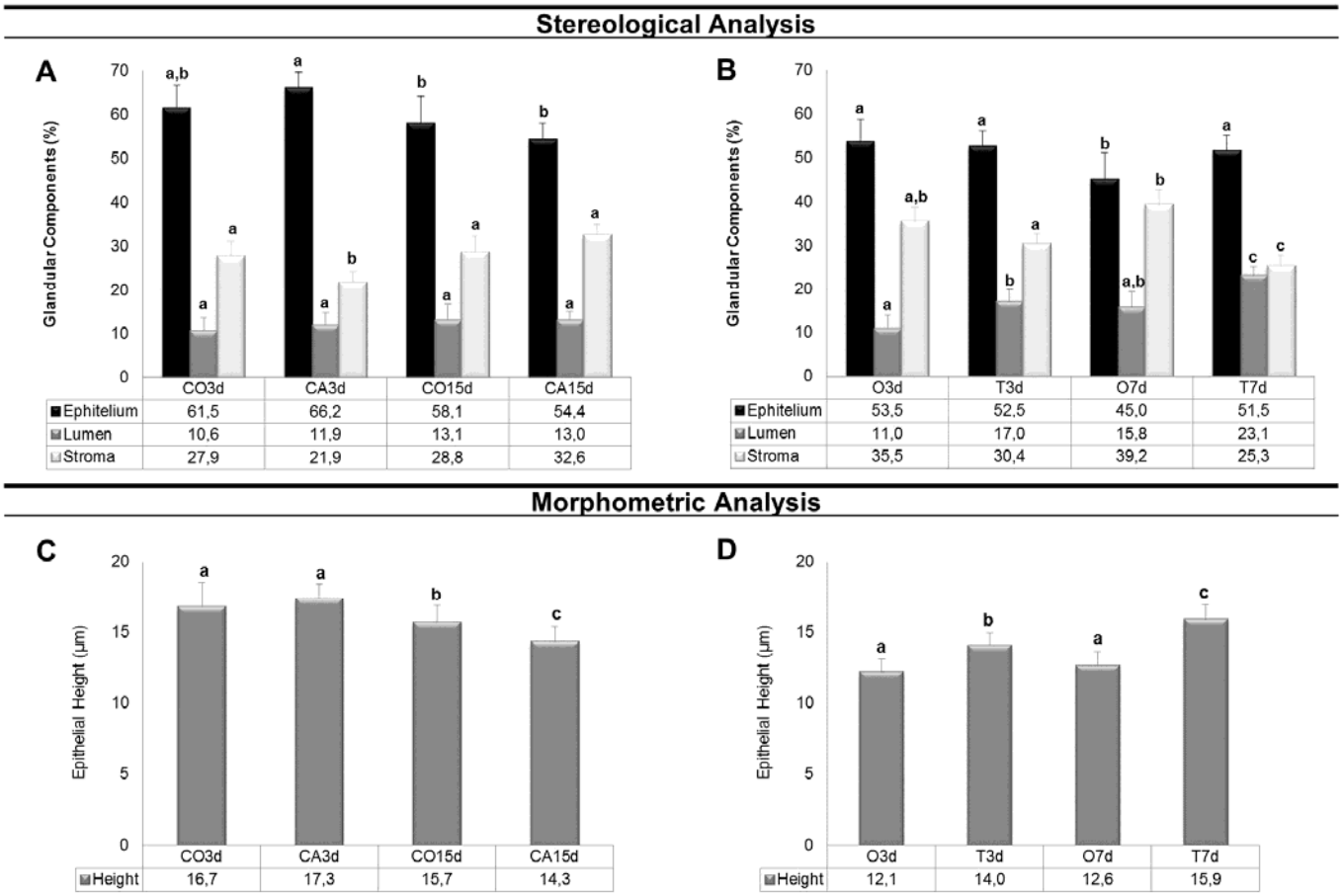

Stereological Analysis
A
Glandular Components (%)
70
60
50
40
30
20
10
0
a,b
a
a
a
b
a
b
a
b
a
a
a
CO3d
CA3d
CO15d
CA15d
Ephitelium 61,5 66,2 58,1 54,4
Lumen 10,6 11,9 13,1 13,0
Stroma 27,9 21,9 28,8 32,6
B
Glandular Components (%)
a
a
a,b
a
b
a
b
a,b
b
a
c
c
O3d
T3d
O7d
T7d
Ephitelium 53,5 52,5 45,0 51,5
Lumen 11,0 17,0 15,8 23,1
Stroma 35,5 30,4 39,2 25,3
Morphometric Analysis
C
Epithelial Height (µm)
20
15
10
5
0
a
a
b
c
CO3d
CA3d
CO15d
CA15d
Height 16,7 17,3 15,7 14,3
D
Epithelial Height (µm)
a
b
a
c
O3d
T3d
O7d
T7d
Height 12,1 14,0 12,6 15,9

Figure 6. Ultrastructural data of the bulbourethral gland of four experimental groups of *Artibeus planirostris*. **A-B-C.** Three days control group (CO3d); **D-E-F.** Three days castrated group (CA3d); **G-H-I.** Fifteen days control group (CO15d); **J-K-L.** Fifteen days castrated group (CA15d). Note the nuclei basal and the vesicle secretion in the apex (**A-D-G-J)** and the large amount of endoplasmic reticulum in the cellular cytoplasm (**B-E-H-I-K** and detail of **F**), in **C-F-L** and detail of **I**, note the apex of the cell with secretory surface (arrows). (CG, Golgi complex; m, mitocrondria; N, nucleo; RER, endoplasmic reticulum; V, vesicle). **Scale bar**: 2000nm (D-G-J); 1000nm (A-B-E-H-I-K-L); 250nm (C-F).

FIGURE 6.

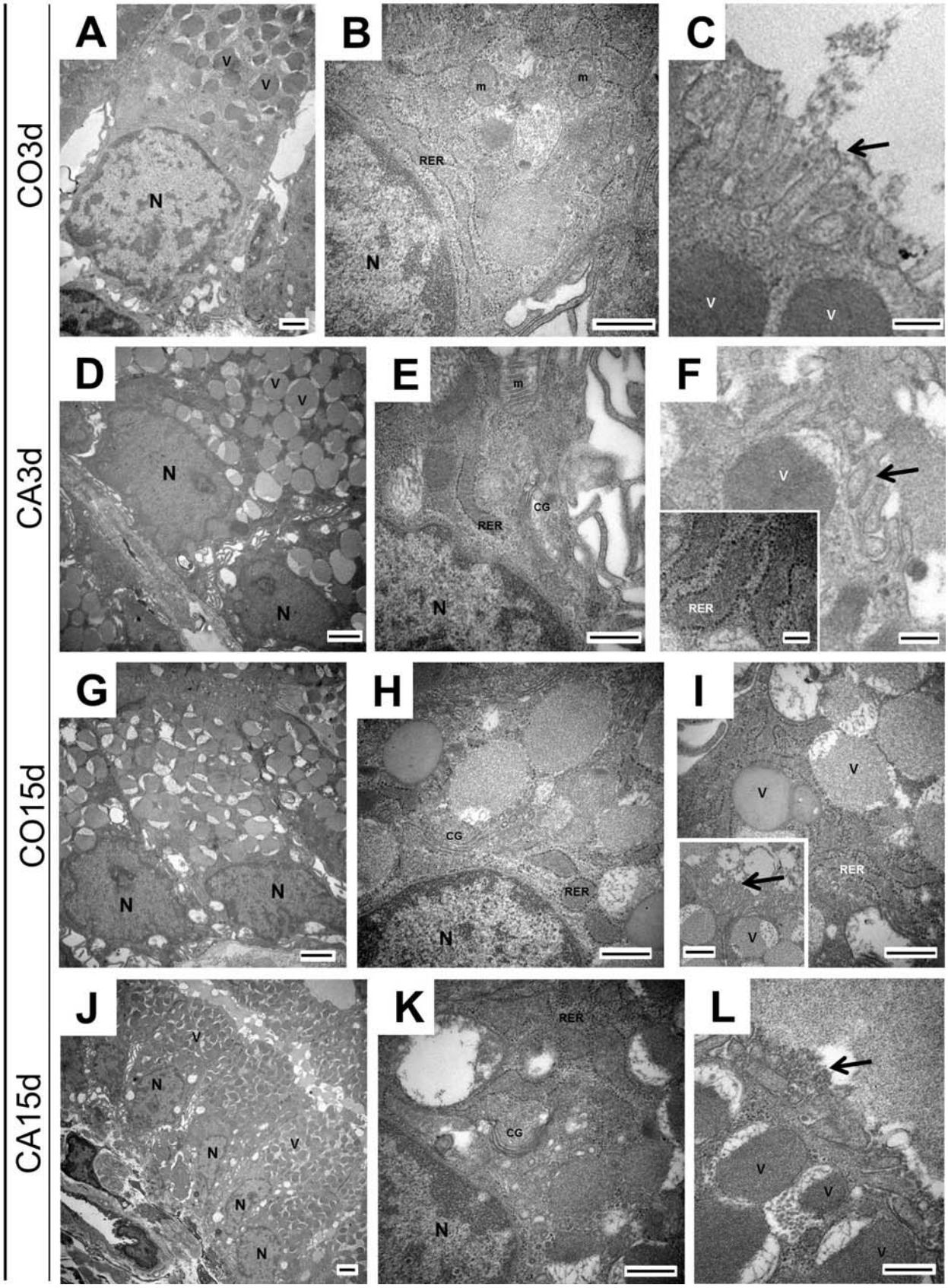


Figure 7. Ultrastructural data of the bulbourethral gland of four experimental groups of *Artibeus planirostris.* A-B-C. Castrated - three days oil injected group (O3d). **D-E-F.** Castrated - three days testosterone injected group (T3d), note that both groups are similar to the groups already described. **G-H-I.** Castrated - seven days oil injected group (O7d), note decreased secretory vesicles, which were concentrated at the apex cell and also of cellular components. **J-K-L.** Castrated - seven days testosterone injected group (T7d), note the return of the gland activity: with many secretory vesicles, with large volume of endoplasmic reticulum and Golgi complex apparent. (Arrow, Apex secretion; Ep, epithelium; CG, Golgi complex; L, lumen; m, mitocrondria; N, nucleo; RER, reticulum endoplasmatico; St, stroma; V, vesicle). **Scale bar**: 5µm (G); 4000nm (J); 2000nm (A-B-D-F-H-I); 1000nm (C-K); 500nm (detail of C-L).

FIGURE 7.

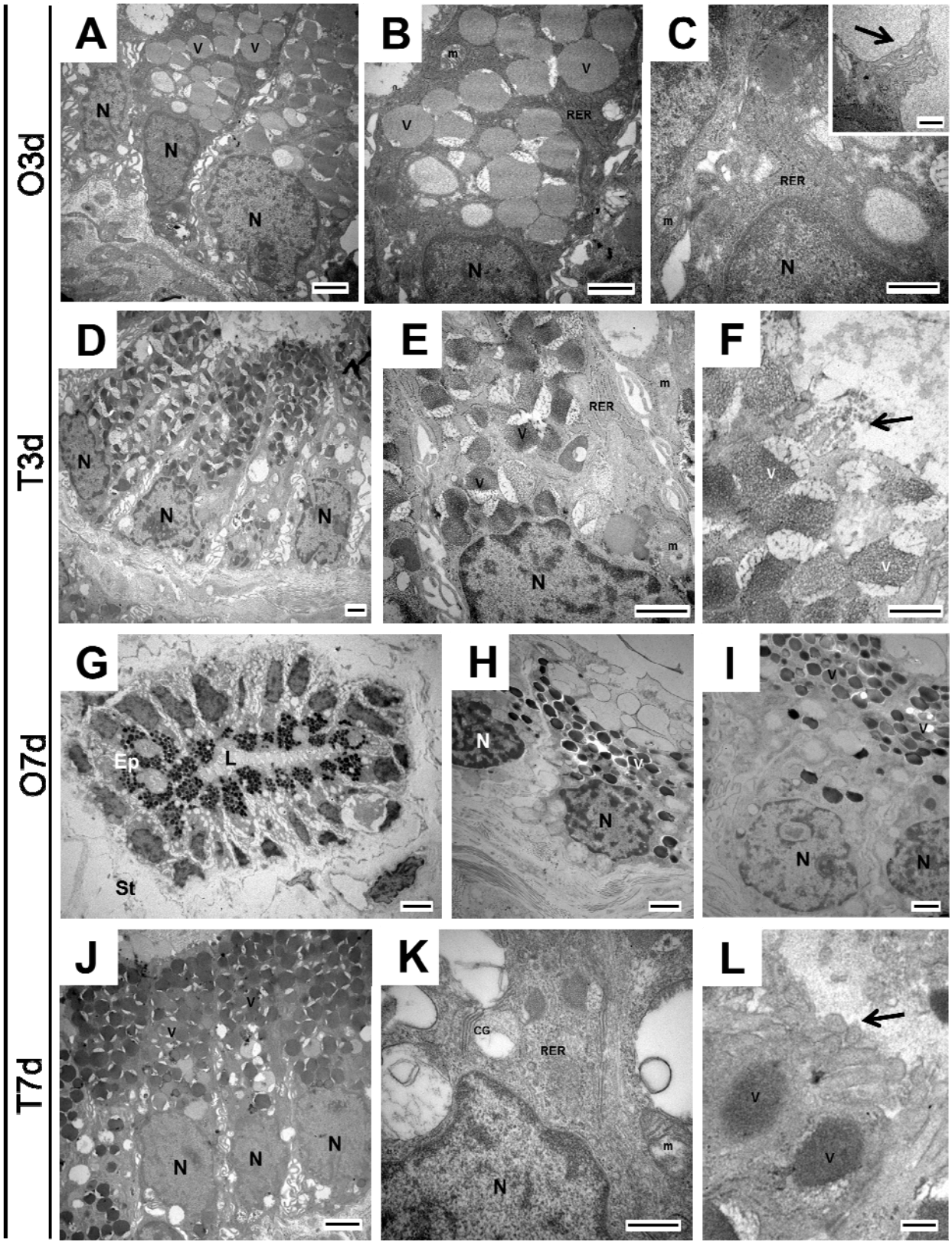
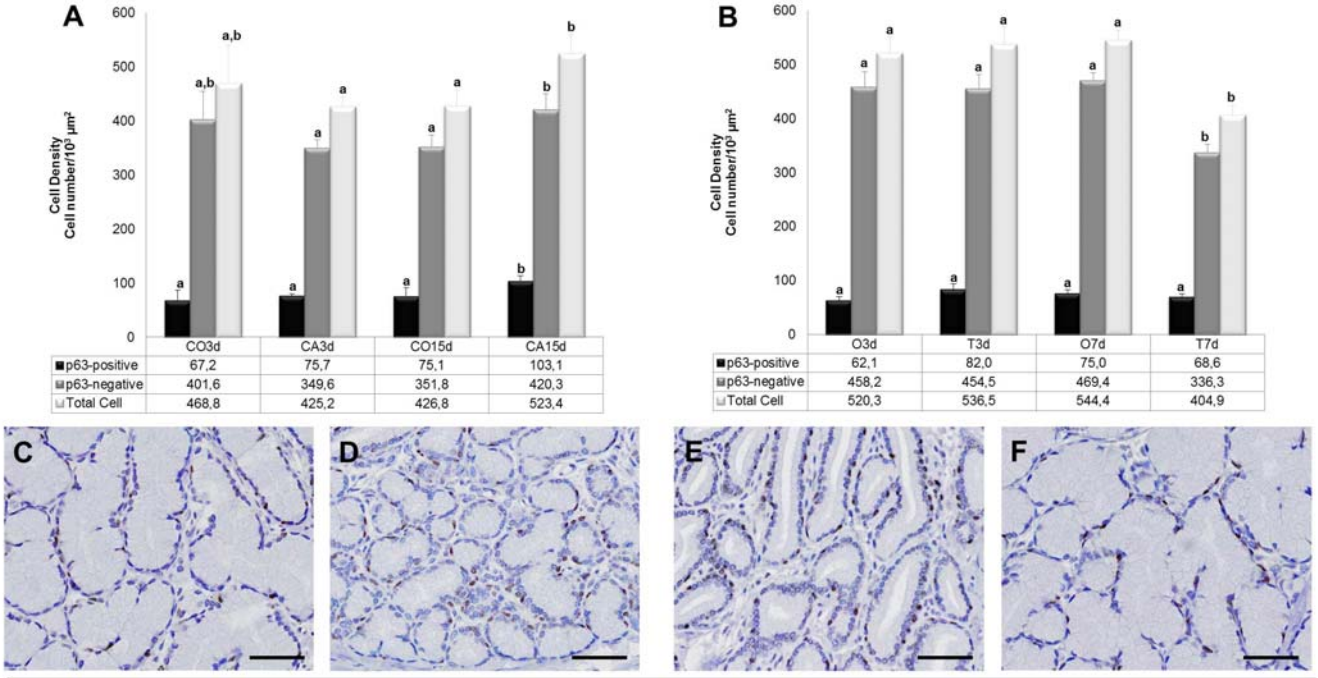


Figure 8. Cell population census analyses of the bulbourethral gland of *Artibeus planirostris* submitted to p63 immunohistochemistry. A-B. Graphics corresponding to quantification of the basal (p63 positive) and others cells. **C.** CO15d. **D.** CA15d. **E.** O7d. **F.** T7d. **Table:** Index p63-possitive/p63-negative cell. Counter-staining: Hematoxylin. [a,b,c] Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$). **Scale bar**: 50 µm.

FIGURE 8.

Cell Population Census



	CO3d	CA3d	CO15d	CA15d
p63-positive	67,2	75,7	75,1	103,1
p63-negative	401,6	349,6	351,8	420,3
Total Cell	468,8	425,2	426,8	523,4

	O3d	T3d	O7d	T7d
p63-positive	62,1	82,0	75,0	68,6
p63-negative	458,2	454,5	469,4	336,3
Total Cell	520,3	536,5	544,4	404,9

Index p63-positive/p63-negative

Groups	CO3d	CA3d	CO15d	CA15d	O3d	T3d	O7d	T7d
Bulbourethral Gland	$0.16\pm0.03^{a,c}$	$0.22\pm0.01^{b,d}$	$0.21\pm0.04^{a,b}$	0.25 ± 0.02^{b}	0.14 ± 0.02^{c}	$0.18\pm0.03^{a,c,d}$	$0.16\pm0.02^{a,c,d}$	$0.20\pm0.02^{a,b}$

Figure 9. Immunostaining of the androgen receptor (AR) in all experimental groups of bulbourethral gland of *Artibeus planirostris*. A-B. Graphics corresponding to the quantification of AR-positive cells. **C.** CO3d. **D.** CA3d. **E.** O3d. **F.** T3d. Counter-staining: Hematoxylin. [a,b,c] Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$). **Scale bar**: 10 µm.

FIGURE 9.

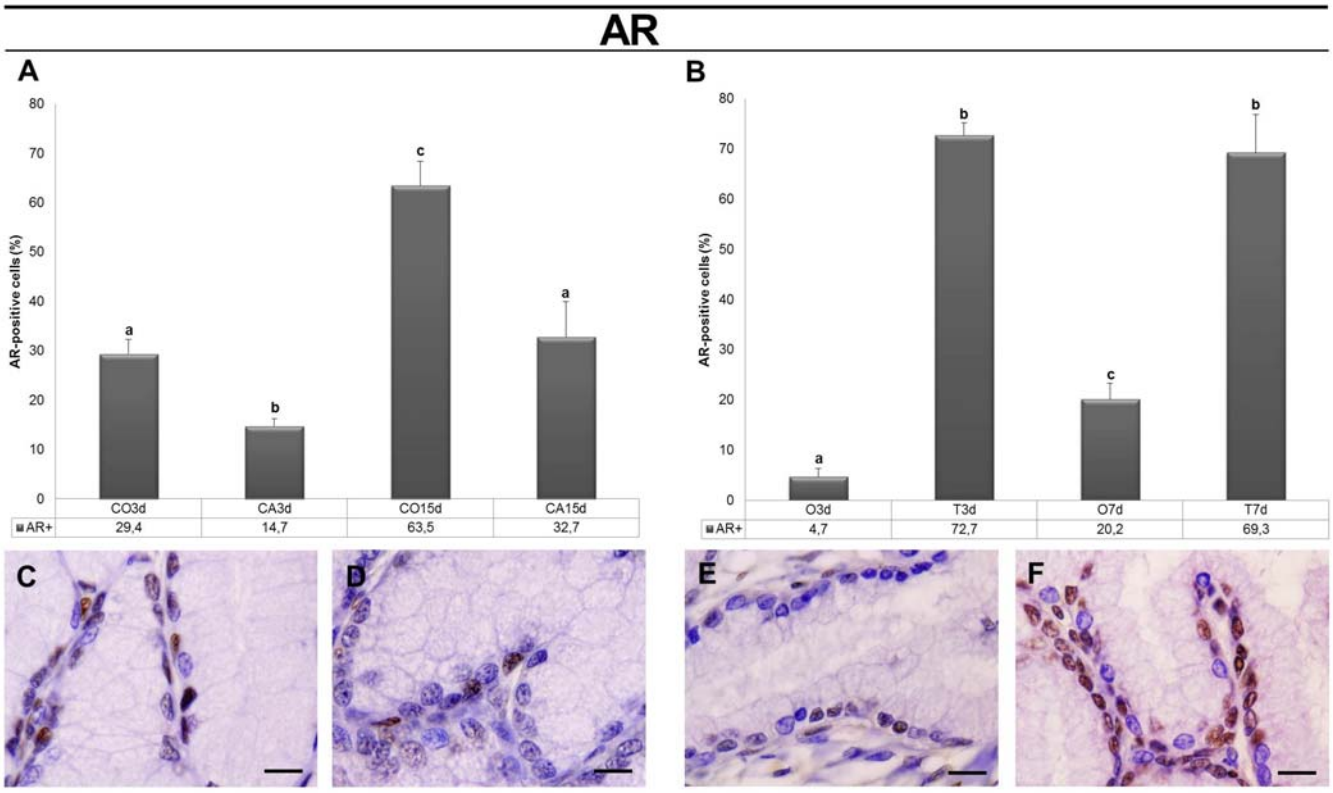
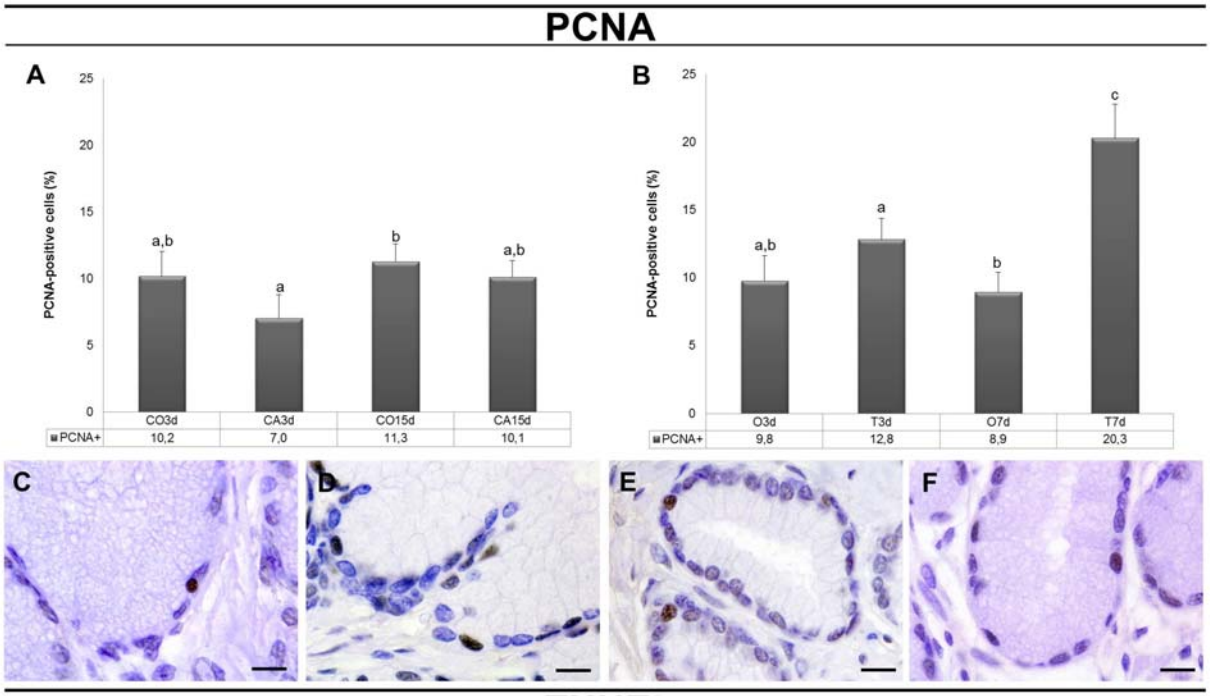
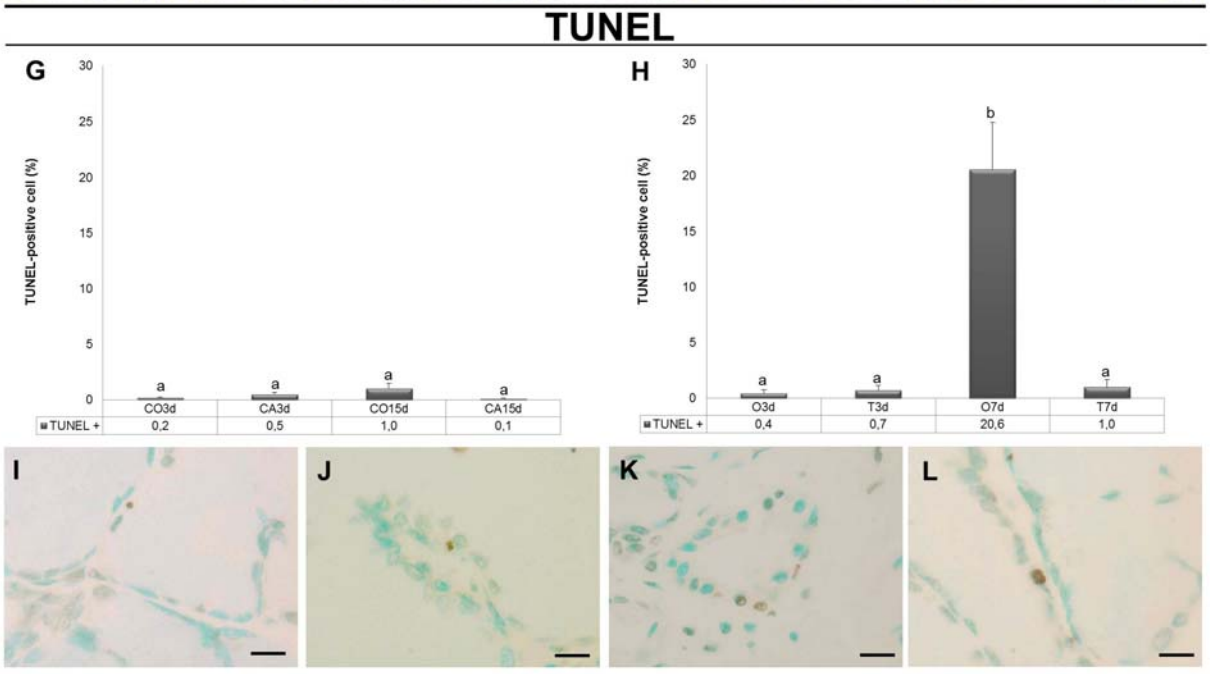

AR
A
B
AR-positive cells (%)
80
70
60
50
40
30
20
10
0
a
b
c
a
CO3d
CA3d
CO15d
CA15d
AR+
29,4
14,7
63,5
32,7
a
b
c
b
O3d
T3d
O7d
T7d
AR+
4,7
72,7
20,2
69,3
C
D
E
F

Figure 10. Amount of proliferation (PCNA) and apoptotic cell (TUNEL) in all experimental groups of bulbourethral gland of *Artibeus planirostris*. A-F. PCNA analysis. **G-L.** TUNEL analysis. **A-B.** Graphics corresponding to the quantification of PCNA-positive cells. **C.** CA3d. **D.** CO15d. **E.** O7d. **F.** T7d. **G-H.** Graphics corresponding to the quantification of TUNEL-positive cells. **I.** CO3d. **J.** CA3d. **K.** O7d. **L.** T3d. **Table:** Index PCNA/TUNEL positive cell. Counter-staining: PCNA=Hematoxylin; TUNEL=metil green. ^{a,b,c} Different superscript letters indicate significant differences between groups (ANOVA at $p < 0.05$). **Scale bar:**10 µm.

FIGURE 10.





Index PCNA/TUNEL

Groups	CO3d	CA3d	CO15d	CA15d	O3d	T3d	O7d	T7d
Bulbourethral Gland	52.9	14.0	10.7	84.8	21.7	17.6	0.4	19.9

VI- CONSIDERAÇÕES FINAIS

Quanto aos animais no cativeiro...

Este trabalho objetivou avaliar a dependência das glândulas reprodutivas acessórias de machos de *Artibeus planirostris* (Chiroptera: Phyllostomidae) pelo hormônio testosterona. E para que esse objetivo fosse alcançado havia a necessidade de fazer a cirurgia (castração) e manter os morcegos em cativeiro por um maior período. Este foi o primeiro desafio deste trabalho, como manter esse tipo de animal de forma que o experimento não fosse afetado?

No intuito de minimizar o efeito do cativeiro nos animais, esse trabalho começou com um projeto piloto, no qual se verificou como os animais seriam tratados e como eles reagiriam ao cativeiro. E com as adequações esses animais supreendentemente não apresentaram sinais de estresse, se alimentaram durante todo o período de cativeiro. Assim, pode-se afirmar que o cativeiro não teve influência nos resultados mostrados neste trabalho.

Quanto à relação das glândulas reprodutivas acessórias com a testosterona...

Os resultados indicaram que ambas as regiões prostáticas são afetadas pela ablação de testosterona, apresentando uma queda na proliferação celular e um aumento na apoptose. Já a glândula bulbouretral apresentou uma pequena redução na altura do epitélio e na quantidade de células AR + e um aumento no número de células basais no grupo castrado por 15 dias. No entanto, nos grupos castrados por tempo maior (18 e 22 dias), essa regressão foi mais acentuada com um aumento significativo do número de células apoptóticas. A suplementação hormonal ocasionou um retorno da morfologia normal em ambas as glândulas. Os dados demonstraram que a região ventral foi mais sensível a alterações na testosterona do que a dorsal, apresentando uma maior renovação das células. E observou-se que a BG leva mais tempo para responder a ablação de testosterona do que o complexo prostático, já que apresentou regressão evidente apenas no grupo com 22 dias de castração.

REFERÊNCIAS

ADARO, L.; MENDOZA, A.; CEPEDA, R.; OROSTEGUI, C. Anatomo-radiographic study of the seminal vesicles of chinchilla (*Chinchilla laniger*) in captivity. **Rev. Chil. Anat.** 19: 297–300, 2001.

ADEBAYO, A.O.; AKINLOYE, A.K.; OLUKOLE, S.G.; OYEYEMI, M.O.; TAIWO, V.O.; IHUNWO, A.O.; OKE, B.O. Gross, histological and ultrastructural features of the bulbourethral gland in the greater cane rat (*Thryonomys swinderianus*). **Anat. Histol. Embryol.** 44(1): 59–65, 2015.

AUMULLER, G.; SEITZ, J. Protein secretion and secretory process in male accessory sex glands. **Int. Ver. Cytol.**, 121: 127-231, 1990.

AYKROYD, O.E.; ZUCKERMAN, S. The effect of sex-hormones on the bulbourethral glands of Rhesus monkeys. **J. Anat. Oct.** 73(1): 135–144, 1938.

BADIA, E., BRIZ, M.D.; PINART, E.; SANCHO, S.; GARCIA, N.; BASSOLS, J.; PRUNEDA, A.; BUSSALLEU, E.; YESTE, M.; CASAS, I.; BONET, S. Structural and ultrastructural features of boar bulbourethral glands. **Tissue Cell**, 38:7–18, 2006.

BÁRQUEZ, R.M.; MARES, M.A.; OJEDA, R.A.; GIANNINI, N.P. **Mammals of Tucuman (Mamiferos de Tucuman).** Oklahoma Museum of Natural History, Norman, 1991.

BÁRQUEZ, R.M.; GIANNINI, N.P.; MARES, M.A. **Guide to the Bats of Argentina.** Oklahoma Museum of Natural History, Norman, 1993.

BEGUELINI, M.R.; MOREIRA, P.R.L.; FARIA, K.C.; MARCHESIN, S.R.C.; MORIELLE-VERSUTE, E. Morphological characterization of the testicular cells and seminiferous epithelium cycle in six species of neotropical bats. **J Morphol.** 270: 943-953, 2009.

BEGUELINI, M.R.; GÓES, R.M.; RAHAL, P.; MORIELLE-VERSUTE, E; TABOGA, S.R. Impact of the processes of testicular regression and recrudescence in the prostatic complex of the bat *Myotis nigricans* (Chiroptera: Vespertilionidae). **J Morphol.** 2015 (In press).

CAKIR, M.; KARATAS, A. Histo-anatomical studies on the accessory reproductive glands of the anatolian souslik (*Spermophilus xanthoprymnus*) (Mammalia: Sciuridae). **Anat. Histol. Embryol.** 33: 146–150, 2004.

CHRISTANTE, C.M.; BEGUELINI, M.R.; PUGA, C.C.I; NEGRIN, A.C.; MORIELLE-VERSUTE, E.; VILAMAIOR, P.S.L.; TABOGA, S.R. Structure, histochemistry and seasonal variations of the male reproductive accessory glands in the Pallas's mastiff bat, *Molossus molossus* (Chiroptera: Molossidae). **Reprod Fertil Develop.** 2014 (In press).

COOKE, O.S.; YOUNG, P.; CUNHA, G.R. Androgen receptor expression in developing male reproductive organs. **Endocrinology.** 128: 2867-2873, 1991.

CRICHTON, E.G.; KRUTZSCH, P.H. **Reproductive biology of bats.** London: Academic Press, 2000.

CUNHA, G.; DONJACOUR, A.A.; COOKE, P.S.; MEE, S.; BIGSBY, R.M.; HIGGINS, S.J.; SUGIMURA, Y. The endocrinology and developmental biology of the prostate. **Endocrinol Rev.** 8: 338-362, 1987.

DOUNJACOUR, A.A.; CUNHA, G.R. The effect of androgen deprivation on branching morphogenesis in the mouse prostate. **Dev. Biol.** 128: 1-14, 1988.

DYCE, K.M.; SACK, W.O.; WENSING, C.J.G. **Text Book of Veterinary Anatomy.** 3rd edn. Philadelphia, PA: Saunders. 2002.

EISENBERG, J.F. **Mammals of the Neotropics:** Panama, Columbia, Venezuela, Guyana, Suriname, French Guiana. University of Chicago Press, Illinois, 1989.

ELLEM, S.J.; RISBRIDGER, G.P. The dual, opposing roles of estrogen in the prostate. steroid. Enzym. **Cancer: Ann NY Acad Sci.** 1155: 174–186, 2009.

ELLEM, S.J.; RISBRIDGER, G.P. Aromatase and regulating the estrogen: androgen ratio in the prostate gland. **J Steroid Biochem Mol Biol.** 118: 246–251, 2010.

ELLEM, S.J.; SCHMITT, J.F.; PEDERSEN, J.S.; FRYDENBERG, M.; RISBRIDGER, G.P. Local aromatase expression in human prostate is altered in malignancy. **J Clinic Endocrinol Metabol.** 89: 2434–2441, 2004.

ENCARNAÇÃO, J.A.; DIETZ, M.; KIERDORF, U. Reproductive condition and activity pattern of male Daubenton's bats (*Myotis daubentonii*) in the summer habitat. **Mamm. Biol.** 69: 163-172, 2004.

FLEMING, T.H.; HOOPER, E.T.; WILSON, D.E. Three Central American bat communities: structure, reproductive cycles and movement patterns. **Ecology.** 53: 555-569, 1972.

GALBRAITH, S.M.; DUCHESNE, G.M. Androgens and prostate cancer: Biology, pathology and hormonal therapy. **Eur J Cancer.** 33 (4): 545-554, 1997.

GRAHAM, G.L. Seasonality of reproduction in Peruvian bats. In: B.D. Patterson, R.M. Timm (Eds.), **Studies in Neotropical Mammalogy, Essays in Honor of Philip Hershkovitz, Fieldianna, Zoology (New Series), Field Museum of Natural History,** Chicago, 173–181, 1987.

GUSTAFSON, A.W.; SHEMESH, M. Changes in plasma testosterone levels during the annual reproductive cycle of the hibernating bat, *Myotis lucifugus lucifugus* with a survey of plasma testosterone levels in adult male vertebrates. **Biol. Reprod.** 15: 9–24, 1976.

GUSTAFSON, A.W.; DAMASSA, D.A. Annual variations in plasma sex steroid-binding protein and testosterone concentrations in the adult male little brown bat: Relation to the asynchronous recrudescence of the testis and accessory reproductive organs. **Biol. Reprod.** 33: 1126-1137, 1985.

HAPPOLD, D.C.; HAPPOLD, M. Reproduction of Angola free-tailed bats (*Tadarida condylura*) and little free-tailed bats (*Tadarida pumila*) in Malawi (Central Africa) and elsewhere in Africa. **J. Reprod. Fertil.** 85: 133-149, 1989.

HAYWARD, S.W.; ROSEN, M.A.; CUNHA, G.R. Stromal-epithelial interations in the normal and neoplasic prostate. **Br. J. Urol.** 79 (Suppl. 2): 18-26, 1997.

HEIDEMAN, P.D.; DEORAJ, P.; BRONSON, F.H. Seasonal reproduction of a tropical bat, *Anoura geoffroyi*, in relation to photoperiod. **J. Reprod. Fertil.** 96: 765-773, 1992.

HELDRING,N.; PIKE, A.; ANDERSSON, S.; MATTHEWS, J.; CHENG, G.; HARTMAN, J.; TUJAGUE, M.; STRÖM, A.; TREUTER, E.; WARNER, M.; GUSTAFSSON, J.A. Estrogen receptors: how do they signal and what are their targets. **Physiol Rev.** 87: 905–931, 2007.

HELLER, R.E. Cowper's gland and its reaction to castration and to different sex hormone conditions. **Am. J. Anat.** 50: 73-95, 1932.

HOLLIS, L. *Artibeus planirostris*. **Mammal. Spec.** 775: 1-6, 2005.

HOOD, C.S.; SMITH, J.D. Sperm storage in a tropical nectar-feeding bat, *Macroglossus minimus* (Pteropodidae). **J. Mammal.** 70: 404-406, 1989.

JEQUIER, A.M. Clinical disorders affecting semen quality. In: **Gametes—The Spermatozoon.** (J. L. Yovich, ed). Cambridge: University Press. 175–191. 1995.

JESIK, C.; HOLLAND, J.; LEE, C. An anatomic and histologic study of the rat prostate. **Prostate.** 3: 81–97, 1982.

KRUTZSCH, P.H. Reproduction of the canyon bat, *Pipistrellus hesperus*, in Southwestern United States. **Amer. J. Anat.** 143: 163-200, 1975.

KRUTZSCH, P.H.; CRICHTON, E.G. Reproductive biology of the male little mastiff bat, *Mormopterus planiceps* (Chiroptera: Molossidae), in Southeast Australia. **Amer. J. Anat.** 178: 352-368, 1987.

KRUTZSCH, P.H.; WATSON, R.H.; LOX, C.D. Reproductive biology of the male leaf-nosed bat, *Macrotus waterhousii* in Southwestern United States. **Anat. Rec.** 184: 611-635, 1976.

KUROHMARU, M.; SARUWATARI, T.; KIMURA, J.; MUKOHYAMA, M.; WATANABE, G.; TAYA, K.; HAYASHI, Y. Seasonal changes in spermatogenesis of Japanese Lesser Horseshoe Bat, Rhinolophus cornutus from a morphological viewpoint. **Okajimas Folia Anat. Jpn.** 79: 93-100, 2002.

KYPRIANOU, N.; ISSACS, J.T. Activation of programmed cell death in the rat ventral prostate after castration. **Endocrinology.** 122: 552-562, 1988.

LEE, J.H. Cell differentiation and ultrastructure of the seminiferous epithelium in *Myotis macrodactylus*. **Kor. J. Elec. Micro.** 33(1): 25-39, 2003.
MARES, M.A.; WILLIG, M.R.; STREILEIN, K.E.; LACHER, T.E.J.R. The mammals of northeastern Brazil: a preliminary assessment. **Ann. Carnegie Mus.** 50: 81–137, 1981.

MARTINS, F.F.; PUGA, C.C.; BEGUELINI, M.R.; MORIELLE-VERSUTE, E.; VILAMAIOR, P.S.; TABOGA, S.R. Comparative analysis of the male reproductive accessory glands of bat species from the five Brazilian Subfamilies of the family Phyllostomidae (Chiroptera). **J Morphol.,** 2015 (In press).

MCGUCKIN, M.A.; BLACKSHAW, A.W. Cycle of the seminiferous epithelium in the grey-headed fruit bat, *Pteropus poliocephalus.* **Aust. J. Biol. Sci.** 40: 203-210, 1987.

MORIGAKI, T.; KUROHMARU, M.; KANAI, Y.; MUKOHYAMA, M.; HONDO, E.; YAMADA, J.; AGUNGPRIYONO, S.; HAYASHI, Y. Cycle of the seminiferous epithelium in the Java Fruit Bat (*Pteropus vampyrus*) and the Japanese Lesser Horseshoe Bat (*Rhinolophus cornutus*). **J. Vet. Med. Sci.** 63(7): 773- 779, 2001.

OKUDA,Y.; FUJISAWA, M.; MATSUMOTO, O.; KAMIDONO, S. Testosterone dependent regulation of the enzymes involved in DNA synthesis in the rat ventral prostate. **J. Urol.** 145: 188-91, 1991.

PERACCHI, A.L.; LIMA, I.P.; REIS, N.R.; NOGUEIRA, M.R.; ORTÊNCIO-FILHO, H. Ordem Chiroptera. In: REIS, N.R.; PERACCHI, A.L.; PEDRO, W.A.; LIMA, I.P. **Mamíferos do Brasil.** Londrina, 2ª ed., p. 155-234, 2011.

PERACCHI, A.L.; LIMA, I.P.; REIS, N.R.; NOGUEIRA, M.R.; HORTÊNCIO-FILHO, H. Ordem Chiroptera. In: REIS, N.R.; PERACCHI, A.L.; PEDRO, W.A.; LIMA, I.P. **Mamíferos do Brasil.** Londrina: Nélio R. dos Reis. p. 153-230, 2006.

PRICE, D. Comparative aspects of development and structure in the prostate. **Natl Cancer lnst Monogr.** 12: 1-27, 1963.

PRICE, D.; ORTIZ, E. **Organogenesis.** R. L. Dehaan and H. Ursprung, eds. Holt, New York. pp. 629-652, 1965.

CUNHA, G.R. Epithelial-stromal interactions in developrnent of the urogenital tract. **lnt Rev Cytol.** 47: 137-194, 1976.

PUGA, C.C.I. **Morfofisiologia das glândulas reprodutivas acessórias masculinas de morcegos:** estudo nas espécies *Artibeus planirostris* e *Platyrrhinus lineatus* (Chiroptera: Phyllostomidae). 129 f. Dissertação (Mestrado) - Universidade Estadual Paulista, Instituto de Biociências, Letras e Ciências Exatas – São José do Rio Preto, 2011.

PUGA, C.C.I.; BEGUELINI, M.R.; MARTINS, F.F.; MORIELLE-VERSUTE, E.; FALEIROS-JR, L.R.; VILAMAIOR, P.S.L.; TABOGA, S.R. Seasonal changes in the prostatic complex of *Artibeus planirostris* (Chiroptera: Phyllostomidae). **Gen Comp Endocrinol.** 197: 33-42, 2014.

PUGA, C.C.I.; BEGUELINI, M.R.; NEGRIN, A.C.; CHRISTANTE, C.M.; MORIELLE-VERSUTE, E.; VILAMAIOR, P.S.L.; TABOGA, S.R. Structure, histochemistry and ultrastructure of the male reproductive accessory glands in the Neotropical flat-faced fruit-eating bat, *Artibeus planirostris* (Chiroptera: Phyllostomidae). **Reprod Fertil Dev.** 25: 558–569, 2013.

RACEY, P. A. Seasonal changes in testosterone levels and androgen dependent organs in male moles (*Talpa europaea*). **J. Reprod. Fertil.** 52: 195–200, 1978.

RACEY, P.A. The prolonged storage and survival of spermatozoa in Chiroptera. **J. Reprod. Fertil.** 56: 391-402, 1979.

RACEY, P.A. The viability of spermatozoa after prolonged storage by male and female European bats. **Period. Biol.** 75: 201-205, 1973.

RACEY, P.A. Viability of bat spermatozoa after prolonged storage in the epididymis. **J. Reprod. Fertil.** 28: 309-311, 1972.

REIS, N.R.; PERACCHI, A.L.; PEDRO, W.A.; LIMA, I.P. **Morcegos do Brasil.** Londrina: REIS, N.R.; PERACCHI, A.L.; PEDRO, W.A.; LIMA, I.P. 2007.

RISBRIDGER, G.P.; WANG,H.; FRYDENBERG, M.; Cunha, G. The metaplastic effects of estrogen on mouse prostate epithelium: proliferation of cells with basal cell phenotype. **Endocrinology.** 142: 2443-2450, 2001.

ROCHEL, S. S.; BRUNI-CARDOSO, A.; TABOGA, S. R.; VILAMAIOR, P. S. L.; GÓES, R. M. Lobe Identity in the Mongolian Gerbil Prostatic Complex: A New Rodent Model for Prostate Study. **Anat Rec (Hoboken).** 290: 1233-1247, 2007.

ROSAI, J. **Male reproductive system.** In: Ackerman's Surgical Pathology. 8ed, ROSAI, J. eds. Mosby-Year, St. Louis-USA, VoI. I, p.1221-1256, 1996.

SAIDAPUR, S.K.; PATIL, S.B. Kinetics of spermatogenesis in megachiropteran bat, *Rousettus leschenaulti* (Desmarset): seminiferous epithelial cycle, frequency of stages, spermatogonial renewal and germ cell degeneration. **Indian J. Exp. Biol.** 30: 1037-1044, 1992.

SAMUELSON, D.A. (ed.), Male reproductive system, 1st edn. In: **Textbook of Veterinary Histology.** St. Louis, MI: Saunders Elsevier, 63146. pp. 418–441, 2007.

SÁNCHEZ, P.; TORRES, J.M.; CASTRO, B.; OLMO, A.; DEL MORAL, R.G.; ORTEGA, E. Expression of steroid 5α-reductase isozymes in prostate of adult rats after environmental stress. **FEBS Journal** 280: 93–101, 2013.

SHABSIGH, A.; TANJI, N.; D'AGATI, V.; BURCHARDT, M.; RUBIN, M.; GOLUBOFF, E.T.; HEITJAN, D.; KISS, A.; BUTTYAN, R. Early effects of castration on the vascular system of the rat ventral prostate gland. **Endocrinology.** 140: 1920-32. 1999.

SHARIFI, M.; GHORBANI, R.; AKMALI, V. Reproductive cycle in *Pipistrellus kuhlii* (Chiroptera, Vespertilionidae) in western Iran. **Mammalia.** 68(4): 323-327, 2004.

SIMMONS, N.B. Order Chiroptera. In: WILSON, D.E.; REEDER, D.M. (Eds). **Mammal Species of the World:** a taxonomic and geographic reference. 3 ed. v. 1. Baltimore: Johns Hopkins University Press, p. 312-529, 2005.

SINGWI, M.S.; LALL, S.B. Spermatogenesis in the non-scrotal bat – *Rhinopoma kinneari wroughton* (Microchiroptera: Mammalia). **Acta Anat.** 116: 136-145, 1983.

SWAMI, D.R.; LALL, S.B. Histoenzymological comparison of the prostate gland of sexually 'quiescent' and 'active' *Taphozous melanopogon melanopogon Temmnick* (Microchiroptera: Mammalia). **Acta Anat (Basel).** 113(2): 128-34, 1982.

TADDEI, V.A. The reproduction of some Phyllostomidae (Chiroptera) from the northwestern region of the State of São Paulo. **Biol. Zool. Univ. São Paulo.** 1: 313-330, 1976.

TAPLIN, M.R.; HO, S.M. The endocrinology of prostate cancer. **J Clin. Endo. Metab.** 86(8): 3476-77, 2001.

THOMSON, A.A.; FOSTER, B.A.; CUNHA, G.R. Analyses of growth factor and receptor rnRNA levels during development of the rat seminal vesicle and prostate. **Development.** 124: 2431-2439, 1997.

TOMA, J.; BUZZELL, G. Fine estructure of the ventral and dorsal lobes of the prostate in the young adult Syrian hamster, *Mesocricetus auratus*. **Am. J. Anat.** 2, 132–140, 1988.

VAN DER MERWE, M.; RAUTENBACH, I.L. Reproduction in the rusty bat, *Pipistrellus rusticus*, in the northern Transvaal bushveld, South Africa. **J. Reprod. Fertil.** 89: 537-542, 1990.

VASQUEZ, B.; DEL SOL, M. Prostatic complex in the rabbit (*Oryctolagus cuniculus*). **Rev. Chil. Anat.** 20, 175–180, 2002.

WILLIG, M.R., MARES, M.A. Mammals from the Caatinga: an updated list and summary of recent research. **Braz. J. Biol.** 49: 361–367, 1989.

WILLIG, M.R. Reproductive patterns of bats from Caatingas and Cerrado biomes in northeast Brazil. **J. Mammal.** 66: 668–681, 1985.

WILSON, D.E.; FINDLEY, J.S. Spermatogenesis in some Neotropical species of *Myotis*. **J. Mammal.** 52(2): 420-426, 1971.

Autorizo a reprodução xerográfica para fins de pesquisa.

São José do Rio Preto, 31/03/2015

_____

Assinatura
Cintia Cristina Isicawa Puga