Antônio Carlos Tonelli de Toledo

# EFEITO DA TADALAFILA NA PREVENÇÃO DE ALTERAÇÕES DO CORPO CAVERNOSO APÓS LESÃO VASCULO-NERVOSA DO FEIXE PERI PROSTÁTICO. ESTUDO EXPERIMENTAL EM RATOS.

Tese de Doutorado apresentada ao Programa de Pós-graduação em Bases Gerais da Cirurgia da Faculdade de Medicina de Botucatu-UNESP, para a obtenção do título de Doutor.

Orientador: Prof. Titular João Luiz Amaro

Botucatu- SP
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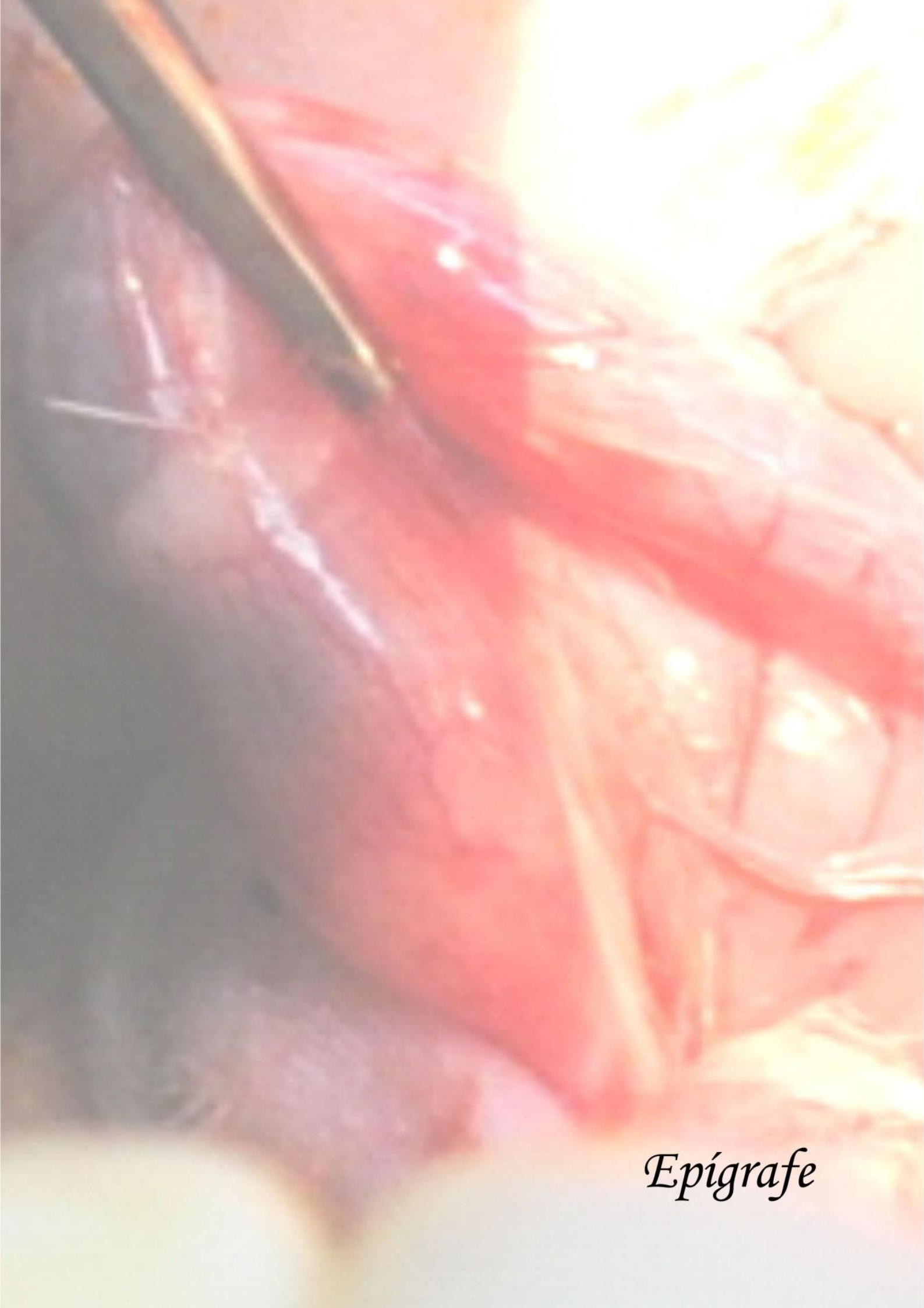
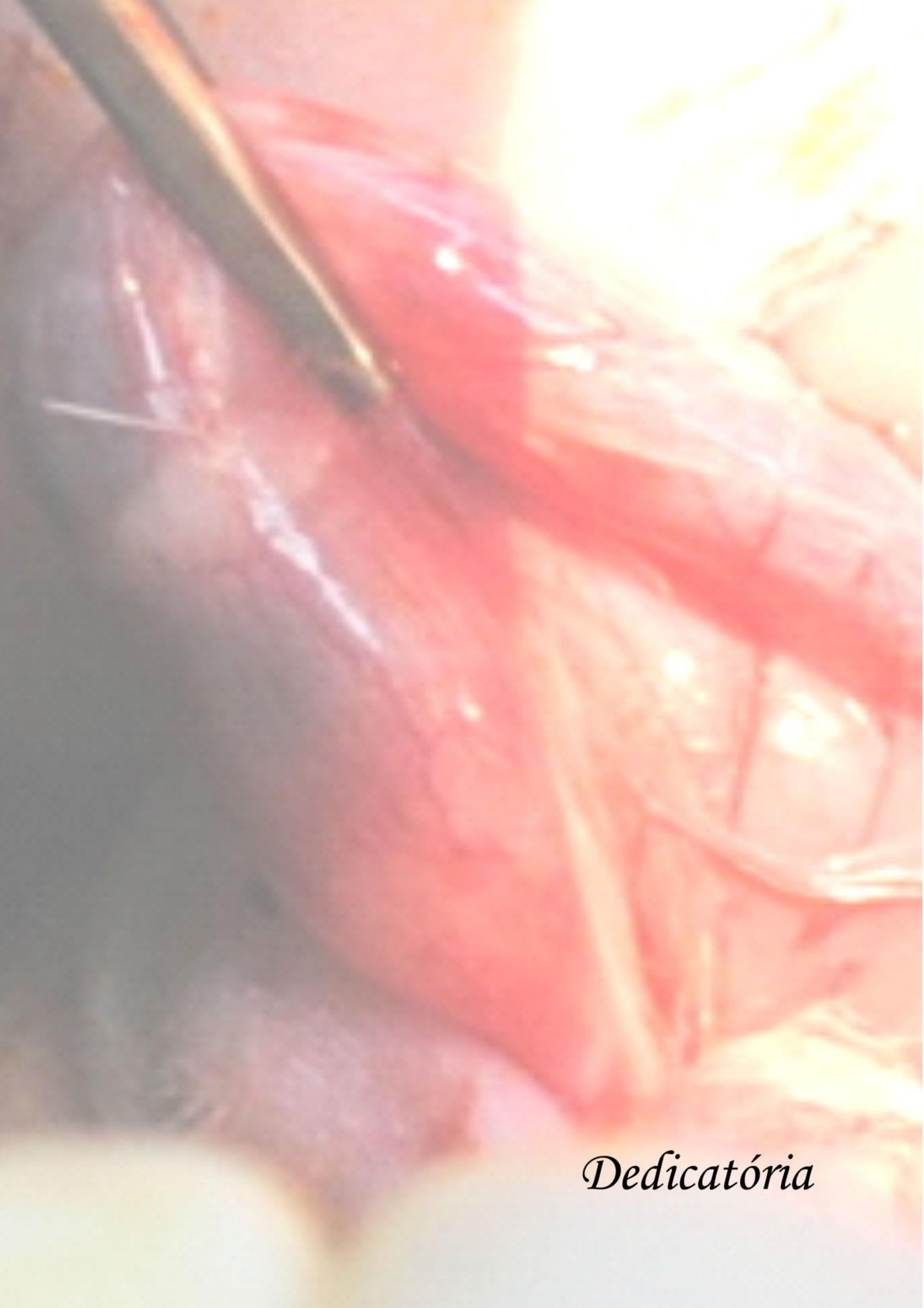


# *Epígrafe*

*"Não confunda derrotas com fracasso nem vitórias com sucesso. Na vida de um campeão sempre haverá algumas derrotas, assim como na vida de um perdedor sempre haverá vitórias. A diferença é que, enquanto os campeões crescem nas derrotas, os perdedores se acomodam nas vitórias."*

*Roberto Shinyashiki*



# *Dedicatória*

*A Deus, que com sabedoria e bondade iluminou o meu caminho sempre na linha da verdade. Aperfeiçoando a arte real e suprema do pensamento*

*Aos meus queridos pais, José Antonio e Rosa, pelo exemplo de vida, honestidade e carinho. Pelas inúmeras idas ao aeroporto e torcida constante. Agradeço pela família construída em todos esses anos.*

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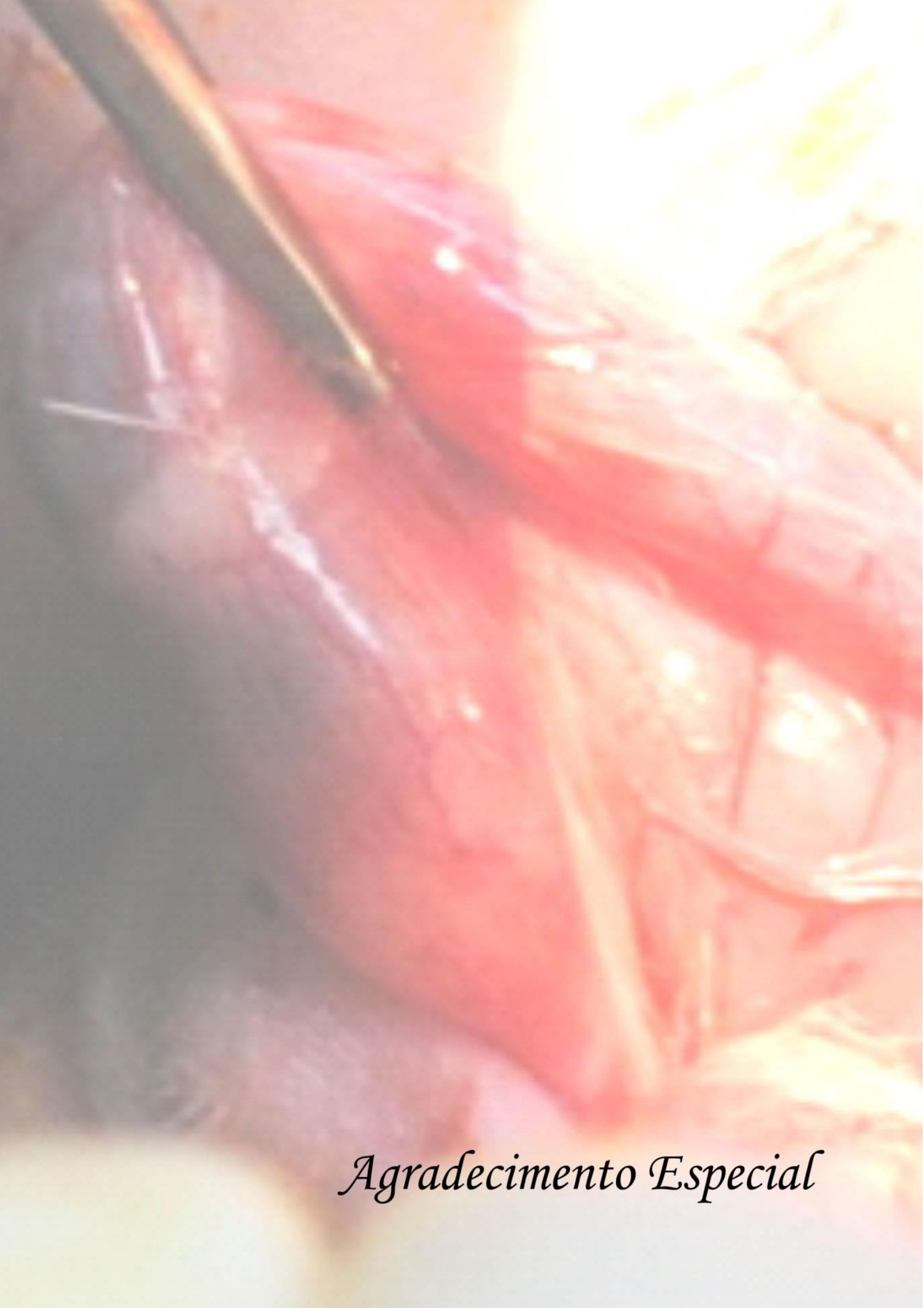

*Agradecimento Especial*

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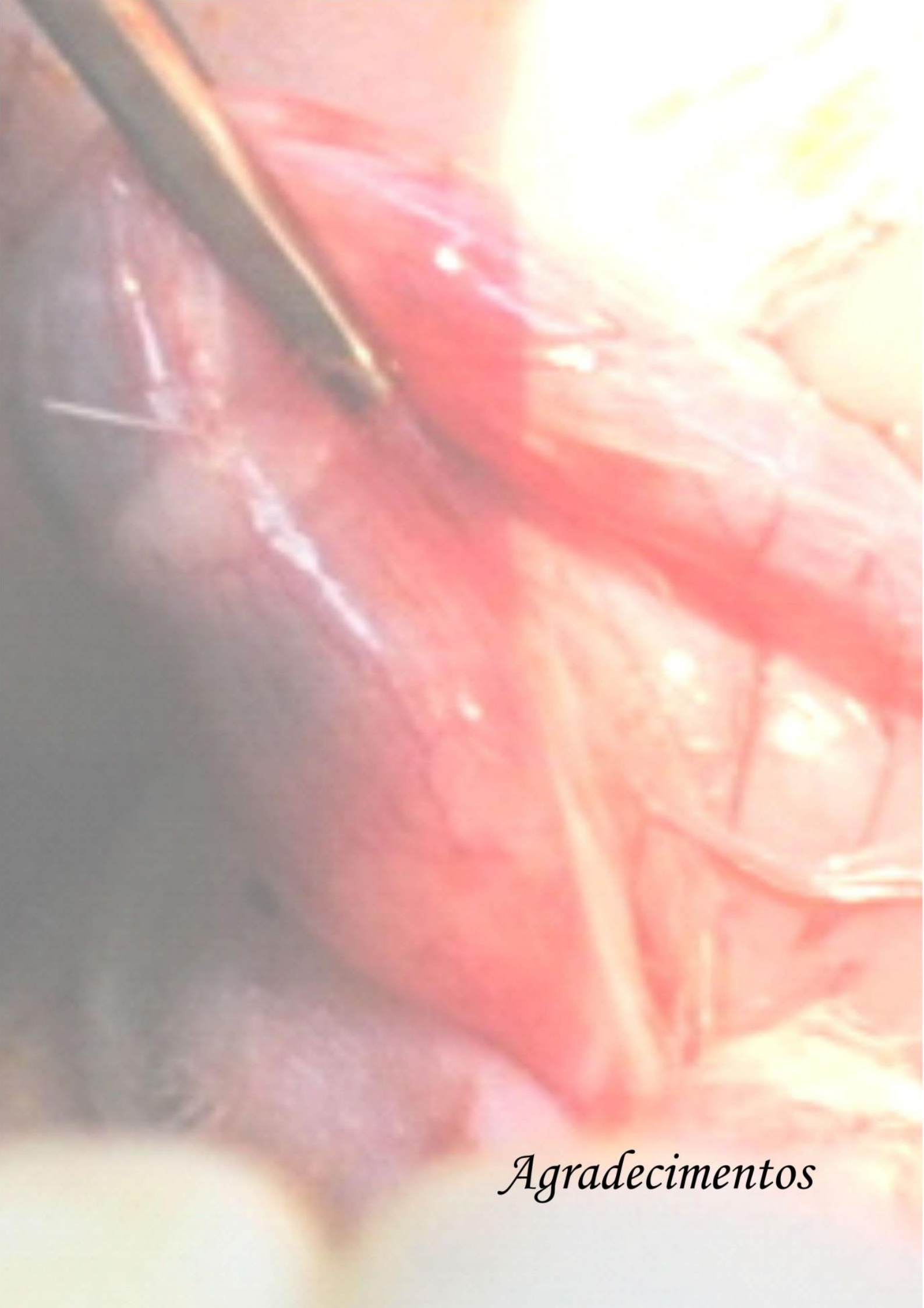

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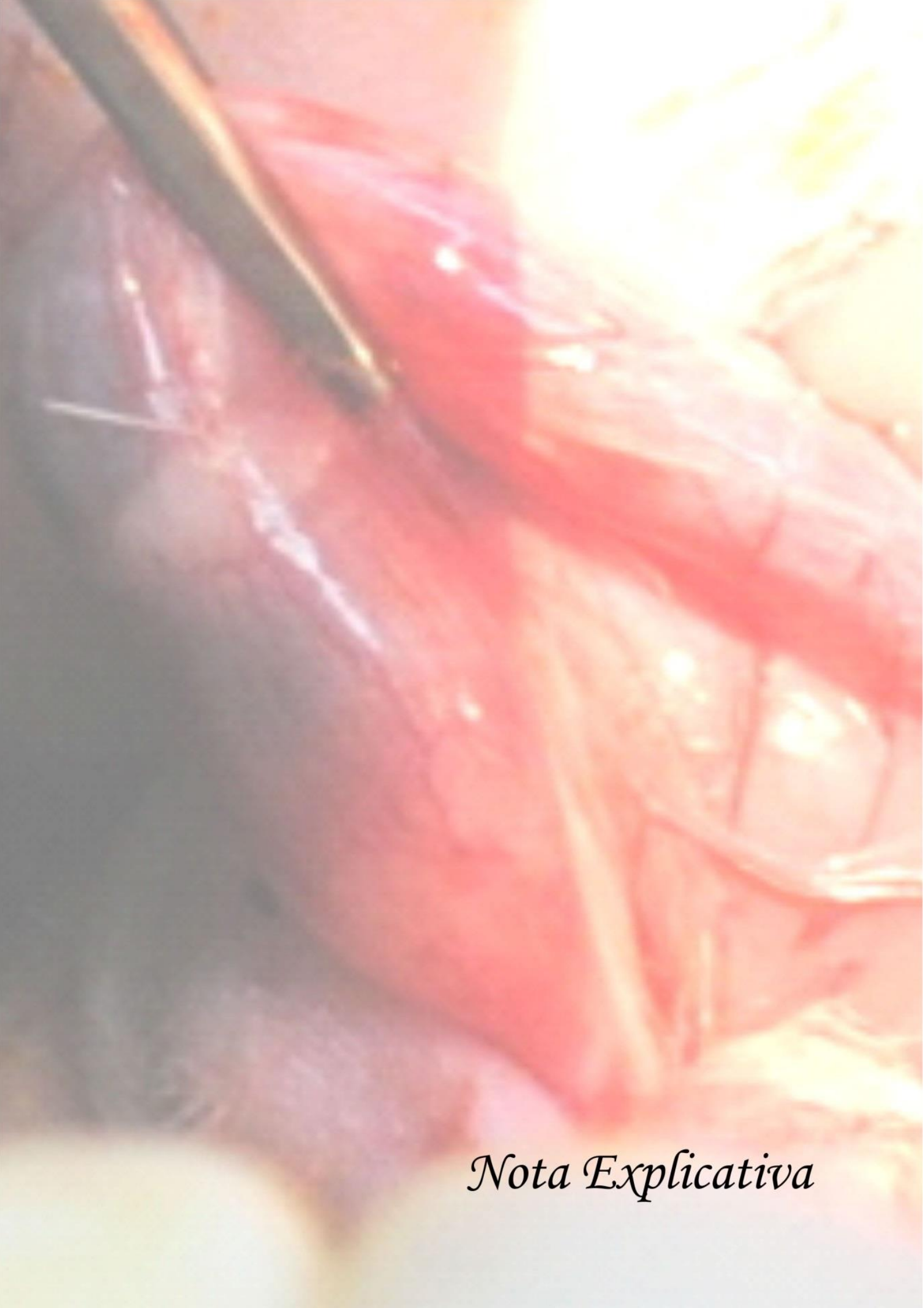


# *Nota Explicativa*

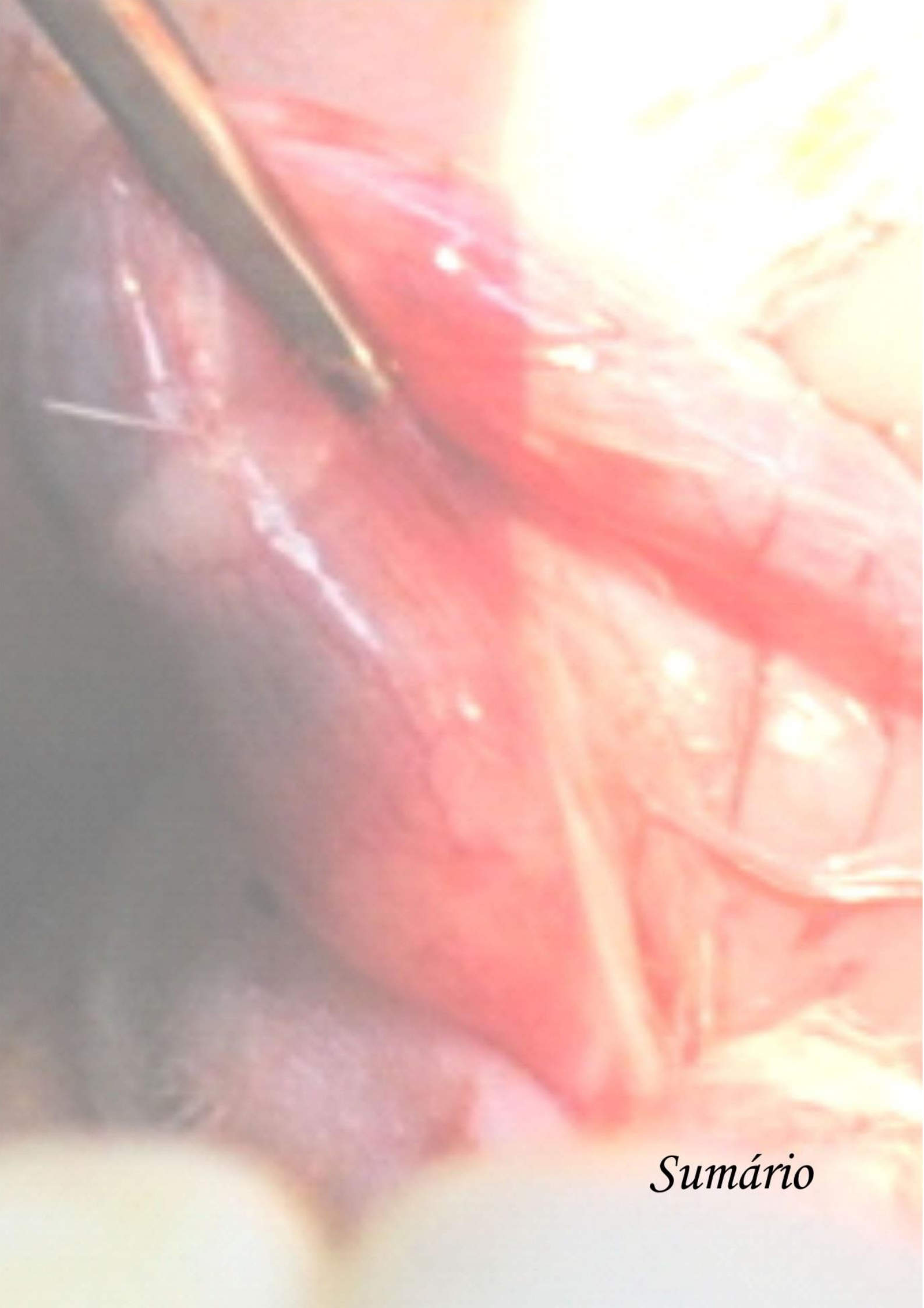
*A forma de apresentação desta tese de doutorado segue uma nova orientação do Programa de Pós-Graduação em Bases Gerais da Cirurgia da Faculdade de Medicina de Botucatu - UNESP, com o objetivo de facilitar sua publicação em revista científica. Está dividido em dois capítulos e um anexo. O primeiro capítulo versa sobre a revisão da literatura, e o segundo foi escrito de acordo com as normas internacionais de publicação em periódicos.*

*O capítulo 1 corresponde a revisão da literatura sobre adenocarcinoma da próstata, disfunção erétil pós prostatectomia radical, função erétil normal e modelos experimentais para estudo do corpo cavernoso.*

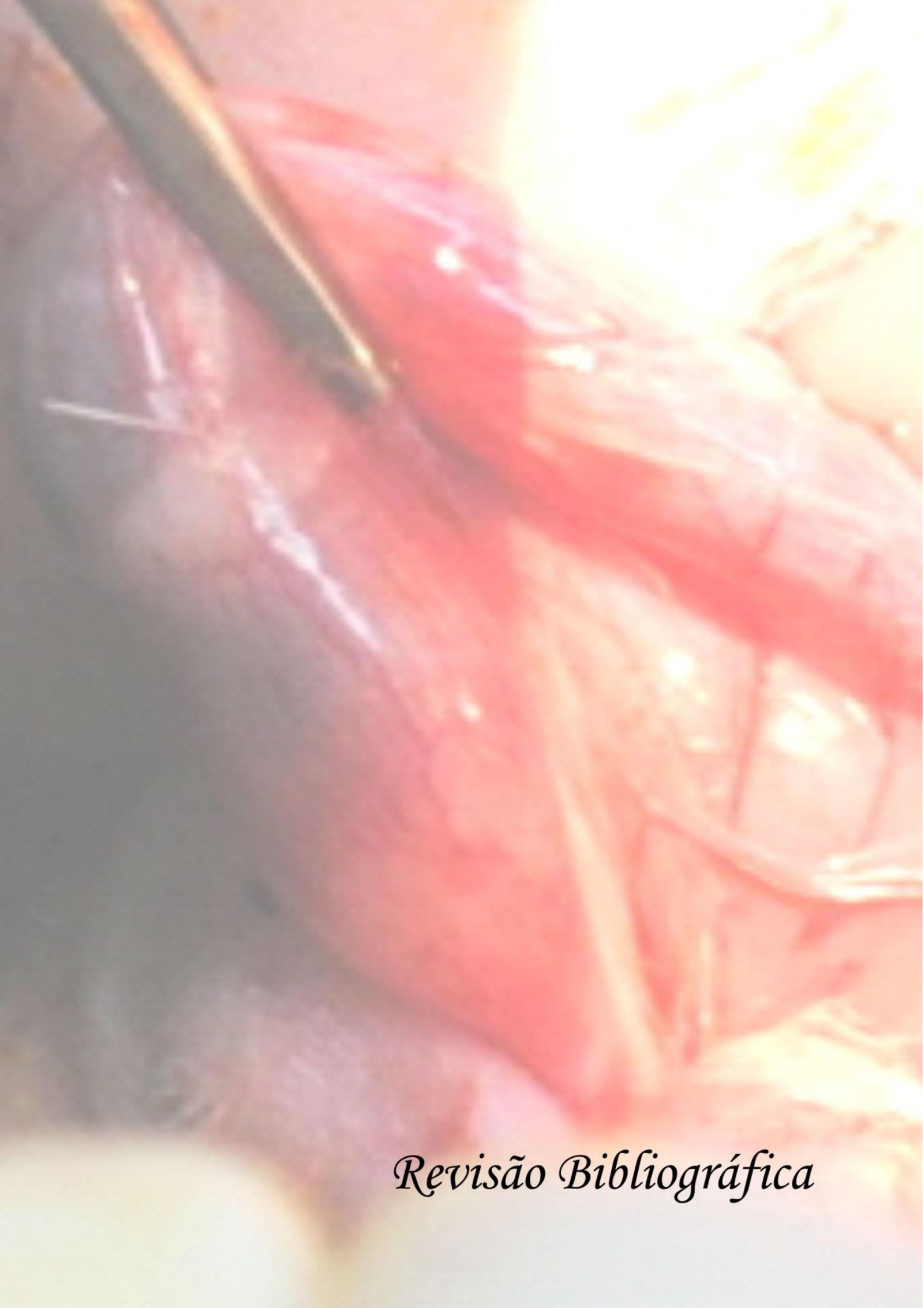
*O capítulo 2 refere-se ao trabalho para publicação, com o título: "Efeito da tadalafila na prevenção de alterações do corpo cavernoso após lesão vásculo-nervosa do feixe peri prostático. Estudo experimental em ratos.*

*No anexo é mostrado as informações para autores da revista a ser publicada.*

*Tal proposta visa o aprendizado do pós-graduando em redação de artigos científicos e divulgação do conhecimento, contemplando, assim, os objetivos finais do programa de pós graduação.*



# *Sumário*



# Revisão Bibliográfica

# 1- INTRODUÇÃO

O câncer de próstata (CaP) é o tumor sólido não cutâneo de maior incidência em homens acima dos 50 anos, correspondendo por aproximadamente 40% dos tumores do sexo masculino. Representa a segunda maior causa de mortalidade câncer específica nos Estados Unidos[1]. Em 2012, no Brasil, o câncer de próstata teve uma incidência de 62 novos casos por 100.000 habitantes.[2,3]. São bem estabelecidos como fatores de risco a idade, a etnia e antecedente familiar, podendo ainda ser influenciado pelo ambiente, além da dieta, presença prévia de hiperplasia prostática benigna (HPB) e fatores hormonais[4, 5,6].

O rastreamento do câncer de próstata é realizado por meio do exame digital retal e dosagem sérica do antígeno prostático específico (APS), sua realização anual pode diminuir a mortalidade câncer específica [7,8] .

O diagnóstico definitivo é confirmado pelo estudo histopatológico após biópsia prostática. Em 2011 foram realizadas aproximadamente um milhão de biópsias prostáticas, sendo que em 25 % dos casos foi confirmado o diagnóstico de CaP [9, 10].

O tratamento do câncer da próstata depende do seu estadiamento clínico, e em casos selecionados, pode-se realizar a vigilância ativa[11, 12]. Na doença localizada com maior chance de evolução desfavorável, o tratamento pode ser realizado por prostatectomia radical (PR) ou radioterapia[13, 14]. O tratamento cirúrgico é o mais utilizado (51,6%), sendo que 86,3% dos pacientes submetidos a este procedimento

tem menos de 60 anos[15]. Nos casos de doença localmente avançada, tanto a radioterapia como a cirurgia, em combinação com tratamento hormonal, podem ser oferecidos, seguindo critérios clínicos já estabelecidos [16, 17]. Nos casos de doença metastática, o melhor tratamento é hormonioterapia[18, 19, 20].

Em 1904, Young [21] realizou a primeira PR, utilizando a via perineal, Millen em 1947 introduziu o acesso retropúbico [22]. Entretanto, o índice de complicações cirúrgicas imediatas como sangramento excessivo, e tardias como elevadas taxas de disfunção erétil (DE) e incontinência urinária fizeram com que a PR fosse menos utilizada, ocasionando aumento da indicação de radioterapia [21, 22]. A falta de conhecimento adequado da anatomia da região pélvica pode ter sido responsável por este alto índice de efeitos adversos[23]. A partir do melhor entendimento da drenagem vascular prostática o tratamento cirúrgico tornou-se mais seguro, e após a identificação dos nervos cavernosos foi possível a realização da cirurgia com preservação da função erétil[23, 24, 25]. Estudos das fácias prostáticas permitiram cirurgias com margens oncológicas mais adequadas e menores danos ao esfíncter uretral, com conseqüente diminuição da incontinência urinária pós operatória [26, 27]. O refinamento técnico na realização deste procedimento permitiu a realização da PR com preservação de nervo (“nerve sparing”), que tornou esta cirurgia a mais utilizada para o tratamento do câncer de próstata localizado. Revisões recentes mostram taxas de sobrevida câncer específica superior a 85% em pacientes acompanhados por mais de 15 anos após PR[24].

O controle oncológico e a execução segura da cirurgia foram bem estabelecidos após este período, entretanto, a proximidade dos planos de dissecção

em relação ao feixe vásculo nervoso (Figuras 1 e 2) mantém uma taxa de disfunção erétil variando entre 30 e 50% [28, 29, 30].

A DE causa grande impacto na qualidade de vida dos pacientes submetidos a PR [31]. A recuperação da função erétil é lenta, podendo levar vários anos.

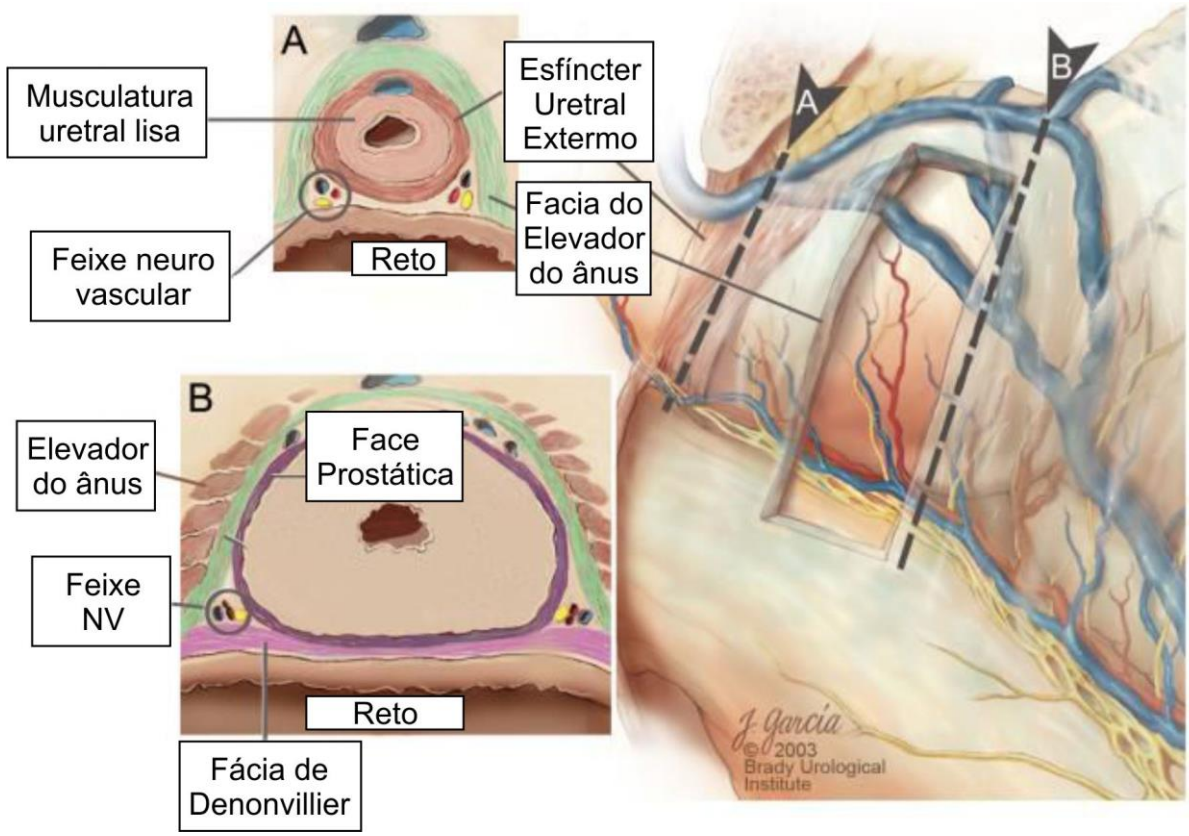



**Figura 1 –** A) Secção transversal do ápice prostático, demonstrando a relação entre o esfíncter uretral estriado, o músculo liso uretral e o feixe neuro vascular, que localiza-se póstero lateralmente a uretra

B) Secção transversa da porção medial da próstata, mostrando a relação entre as fácias prostáticas, do elevador do ânus e de Denonvillier. O feixe neuro vascular localiza-se entre as fácias do elevador do ânus e prostática

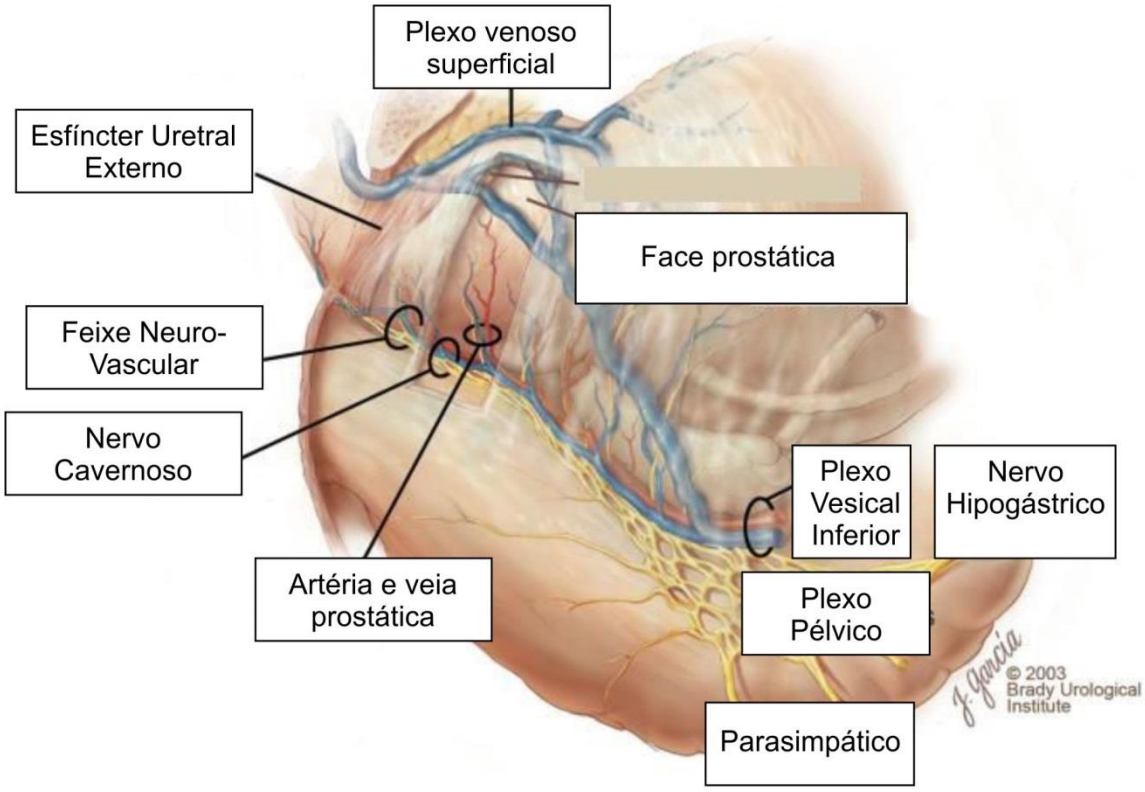



**Figura 2 -** Visão lateral mostrando a passagem de plexo nervoso e vascular prostático entre as fácias prostática e do elevador do ânus

## 2. FUNÇÃO ERÉTIL NORMAL

### 2.1- BASES MORFOLÓGICAS DA EREÇÃO

O termo ereção é derivado do latim (“erectio”) e designa estado de elevação, enrijecimento ou endurecimento. Este fenômeno é decorrente do aumento da irrigação arterial nos corpos cavernosos. Após sua drenagem o pênis irá se encontrar em estado de flacidez [33, 34]. Durante este estado, existe uma contração tônica da musculatura lisa peniana e dessa forma a irrigação peniana é reduzida pelos capilares com propósito nutricional, sem passar pelo interior dos espaços

sinusoidais. No momento da ereção, ocorre vasodilatação das artérias heliciformes desviando o sangue para os espaços sinusoidais, e assim comprimido a drenagem venosa contra a túnica albugínea, conferindo rigidez ao pênis[35].

Os corpos cavernosos constituem as câmaras de pressão responsáveis pela rigidez do pênis durante a ereção. Seu tecido é constituído de fibras musculares lisas, fibras colágenas e elásticas. Essas estrututras se dispõem em múltiplas direções gerando lacunas revestidas por endotélio vascular, chamadas de espaços sinusoidais (Figura 3).

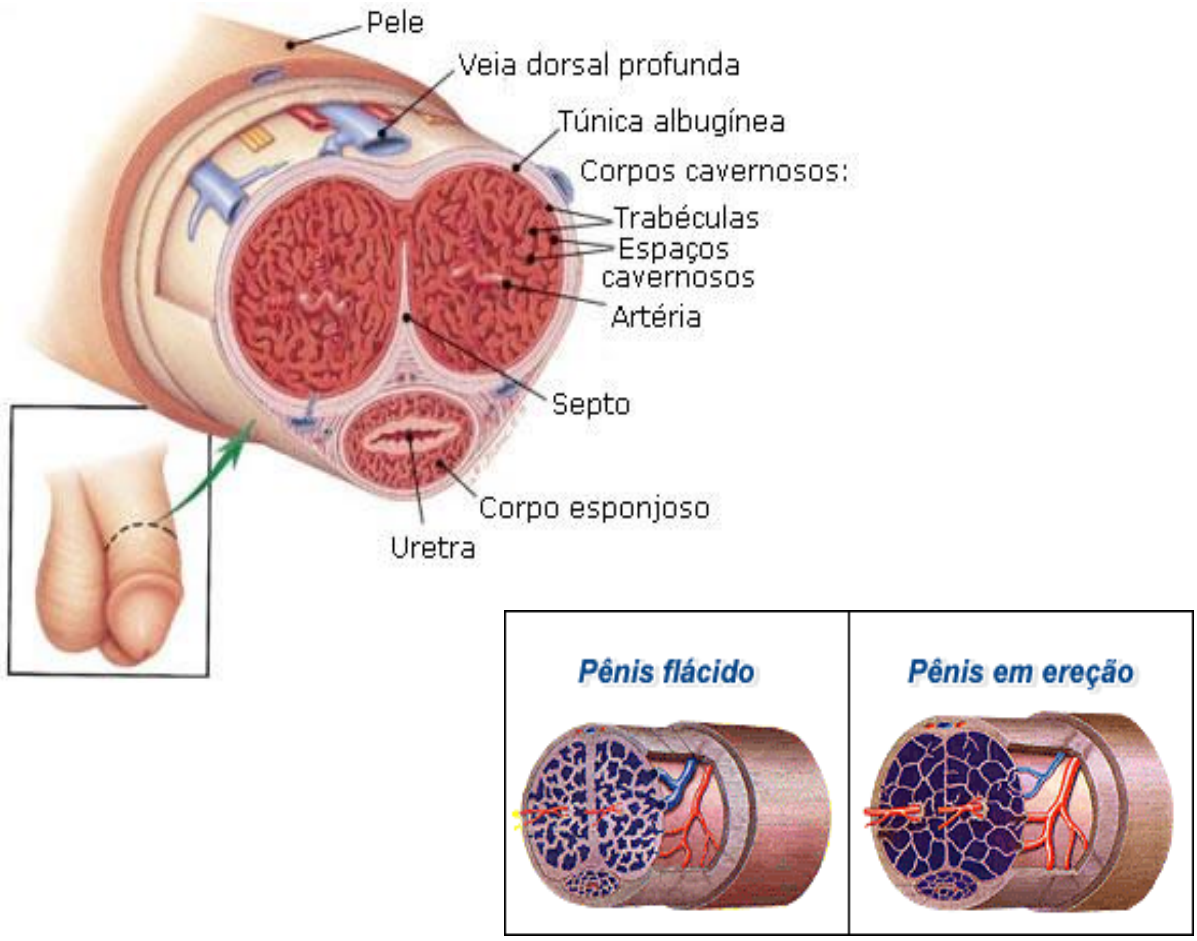



**Figura 3 -** Ilustração do corte transversal do pênis, demonstrando as micro estruturas do corpo cavernoso durante o estado de flacidez e ereção.

Os corpos cavernosos são revestidos por uma túnica albugínea espessa e fibrosa que dentro de certos limites é bastante expansível. Estas túnicas fundem-se, na linha mediana, formando o septo do pênis, que na extremidade distal permite a comunicação entre os corpos cavernosos.

Os vasos que irrigam o pênis originam-se da artéria pudenda interna, que é ramo terminal da artéria ilíaca interna. Esta artéria emite quatro ramos para o pênis, que são: dorsal do pênis, bulbar, uretral e cavernosa[36]. A mais importante durante a ereção é a cavernosa que ocupa posição central no corpo cavernoso, e se divide em dois ramos. O primeiro situa-se no interior das trabéculas e termina em rede capilar, nutrindo o tecido cavernoso. O segundo assume a forma espiralada (artérias helicinais) e desemboca diretamente nos espaços sinusoidais, sendo responsável pelo seu enchimento e, consequentemente, pela ereção [37]. Esse padrão clássico de irrigação peniana apresenta algumas anastomoses, que permitem aos vasos ipsilaterais suprirem o lado oposto ou ainda, os ramos das artérias dorsais suprirem as trabéculas cavernosas [38].

A inervação do pênis é complexa, com participação de fibras simpáticas e parassimpáticas. O parassimpático exerce função miorrelaxante e vasodilatadora, que intervem na ereção[39]. O simpático tem ação inotrópica positiva e vasoconstritora, promovendo detumescência, mas também auxiliando na ereção [40]. Essas fibras reunem-se no plexo ganglionar pélvico de onde partem os nervos cavernosos [41,42]. Após deixar o plexo pélvico, o nervo cavernoso segue entre o reto e a próstata, perfurando o diafragma urogenital acompanhando inicialmente a porção terminal da artéria pudenda interna e, posteriormente, a artéria cavernosa, até

penetrar no corpo cavernoso[43]. Seus ramos inervam os vasos penianos e o tecido cavernoso, atuando sobre a musculatura lisa destas estruturas, promovendo sua constrição e relaxamento.

A inervação somática do pênis é feita pelo nervo pudendo, que contém fibras aferentes (sensoriais) e eferentes (motores). O nervo dorsal do pênis é um ramo sensorial do nervo pudendo. As fibras sensoriais são responsáveis pela condução de impulsos sensitivos táteis, de pressão, vibratórios, térmicos e dolorosos[44]. As fibras motoras inervam os músculos estriados do pênis, o bulbocavernoso e o ísquiocavernoso[45.]

A drenagem venosa peniana pode ser dividida em planos superficial, intermediário e profundo. No superficial, o sangue é drenado da pele até a fácia de Buck seguindo para a veia dorsal superficial, safena e epigástrica inferior. O sistema intermediário drena a glande e a porção distal dos corpos cavernosos e esponjoso. Neste as veias circunflexas drenam para a  dorsal profunda e desemboca no plexo periprostático de Santorini. No profundo o sangue é drenado das porções proximais dos corpos cavernosos e esponjoso. Estes sistemas se comunicam formando o plexo venoso retropúbico que drena para a veia pudenda interna

## 2.2 - FISIOLOGIA DA EREÇÃO

A musculatura lisa do corpo cavernoso desempenha importante papel na ereção, detumescência e flacidez peniana[46,47,48,49,50]. A ereção necessita do relaxamento da musculatura lisa peniana, tanto no corpo cavernoso como na irrigação vascular. Distúrbios nestes mecanismos têm sido apontados como fatores causais de disfunção erétil[51].

A musculatura lisa cavernosa e a musculatura das artérias e arteríolas cavernosas encontram-se contraídos com o pênis em flacidez, permitindo apenas a passagem de sangue para nutrição tecidual. Durante o processo excitatório, mediado por neurotransmissores, há o relaxamento desta musculatura, e consequente vasodilatação arterial[48,49]. Este processo irá comprimir a túnica venosa entre a albugínea e os sinusóides, diminuindo o retorno venoso, e aumentando a pressão intra cavernosa. Como conseqüência temos a rigidez e ereção do pênis. Para o funcionamento adequado deste processo é fundamental a integridade da musculatura lisa, espaço sinusoidal e organização de fibras elásticas no CC, que podem ser afetadas pós prostatectomia radical [50, 51].

## 2.3- DISFUNÇÃO ERÉTIL PÓS PROSTATECTOMIA RADICAL

A prostatectomia radical pode causar lesão do feixe vasculo-nervoso peri porstático[52,53], este fato pode ocasionar DE, mesmo nos casos com preservação deste feixe entre 30-50% dos casos. Esta complicação tem grande impacto na qualidade de vida sexual destes pacientes[55,56, 57].

A etiopatogenia da DE pós prostatectomia é multifatorial, no pós operatório imediato ocorre uma neuropraxia do nervo cavernoso. Esta é ocasionada pela tração excessiva, lesão térmica ou processo inflamatório decorrente da cauterização e dissecção tecidual durante a remoção da próstata[61]. Esta lesão nervosa pode permanecer por até 24 meses, o que desencadeia uma reação em cascata, causada principalmente pela hipóxia do corpo cavernoso, que leva a uma desorganização micro-estrutural do tecido erétil, com aumento da produção de colágeno, remodelação venosa e consequente diminuição de músculo liso [58,59,60].

A apoptose parece ter papel significante na DE, uma vez que trabalhos experimentais tem demonstrado que a denervação peniana acarretou apoptose de músculo liso no tecido cavernoso de ratos [62]. Esse processo foi mais significativo nas lesões bilaterais do feixe vásculo nervoso, quando comparadas as lesões unilaterais. Nestas o dano neural ocasionou diminuição dos fatores de crescimento, aumento dos radicais livres e ocitocina, com consequente aumento da apoptose [63].

A influência da lesão vascular isolada do feixe peri prostático no desenvolvimento de DE ainda não é bem estabelecida. Entretanto a diminuição do fluxo arterial nos corpos cavernosos ocasiona queda da oxigenação e possivelmente alterações ultra estruturais no CC[64,65]

## 3- O PAPEL DOS INIBIDORES DA FOSFODIESTERASE E-5 (IPDE-5)

Na flacidez peniana a musculatura lisa do corpo cavernoso encontra-se contraída. Esta contração depende de dois fatores: alta concentração intracelular de cálcio e de fosforilação da miosina. Estes fenômenos são controlados pelo adenosina mono fosfato cíclica (AMPc) e guanosina monofosfato cíclico (GMPc), produzidos no citoplasma a partir de estímulos e receptores da parede celular [66,67,68]

A fosfodiesterase é uma enzima que degrada por hidrólise as ligações que existem na AMPc e na GMPc. Desta forma, quando inibidas, aumentam a concentração intracelular dos nucleotídeos cíclicos e, em consequência ocorre o relaxamento do músculo liso cavernoso. São conhecidas 11 tipos de fosfodiesterases, todas com efeitos similares, porém, com concentrações diferentes nos diversos tecidos [69]. No pênis encontramos a fosfodiesterase tipo 5, que também está presente na musculatura lisa dos vasos pulmonares [70].

O primeiro IPDE-5 aprovado pelo FDA (Food and Drug Administration - EUA), foi o citrato de sildenafila, em Março de 1998 [71], seguidos pela vardenafila e pela tadalafila.

## 3.1- MECANISMO DE AÇÃO DA TADALAFILA

A tadalafila apresenta seletividade 700 vezes maior pela fosfodiesterase 5 em relação a tipo 6 quando comparada com o sildenafila e o vardenafila, o que diminui o potencial de efeitos visuais adversos.

A tadalafila tem tempo de concentração máxima (tmax) de 2 horas e uma meia vida de 17 horas. Em 60 % dos pacientes que ingeriram 20 mg de tadalafila foi possível ter relações sexuais satisfatórias por até 36 horas da ingestão, demonstrando uma janela de oportunidade bastante ampla [72].

O consumo de alimentos ou bebida alcoólica associados a esta droga não alteraram sua absorção e excreção, mesmo nos pacientes mais idosos [73].

Os efeitos adversos mais comuns foram cefaléia, dor lombar e dispepsia, o uso da droga mostrou-se seguro em pacientes com hipertensão arterial.

A tadalafila não pode ser utilizada em pacientes que fazem uso de nitratos, pelo risco de hipotensão grave.

Atualmente existem estudos demonstrando a ação protetora dos IPDE-5 na hipóxia do corpo cavernoso pós PR, pois estas drogas auxiliam na preservação das estruturas celulares, o que poderia levar a uma recuperação mais precoce da função erétil [74, 75, 76, 77, 78].

# 4- O USO DE MODELOS ANIMAIS PARA ESTUDO DA DISFUNÇÃO ERÉTIL PÓS PR

Em 1863, Eckhard et al iniciaram estudos com modelos animais para avaliação da ereção peniana em cães [79]. Este fato permitiu a reprodução de uma ereção peniana utilizando estímulos elétricos com medida da pressão intra cavernosa. Semans e Langworthy descreveram a neurofisiologia da função sexual em felinos [80], entretanto, em 1965, Langworthy descreveu pela primeira vez a inervação pélvica do rato[81]. Pelo baixo custo, fácil manuseio, e semelhança estrututral com o corpo cavernoso humano, o modelo utilizando ratos tornou-se um dos principais para estudo de DE após a realização de PR.

Quinlan et al foi o primeiro a identificar os nervos cavernosos em ratos, que localizam-se na região dos gânglios pélvicos maiores, lateralmente a próstata [82]. Foi demonstrado que a estimulação elétrica destes nervos ocasionava ereção peniana. Permitindo assim se testar o efeito da lesão deste plexo no corpo cavernoso, simulando condições semelhantes as causadas pós prostatectomia radical [83, 84, 85].

Vários tipos de lesão nervosa foram descritos em ratos, porém a mais eficaz é a transsecção e ressecção de um segmento nervoso[86, 87]. Estudos foram realizados avaliando apenas a lesão do nervo cavernoso[63,64], entretanto, está bem estabelecido que a DE pós PR é multifatorial, não se sabe ao certo qual o papel da lesão isolada da porção vascular do feixe peri prostático na alteração estrutural do corpo cavernoso.

Desenvolvemos um modelo experimental em ratos, que permitiu a identificação da artéria e do nervo cavernoso no feixe peri prostático (figura 4). Possibilitando testar os efeitos da secção isolada da artéria e nervo cavernoso na estrutura do CC, assim como o efeito protetor da tadalafila

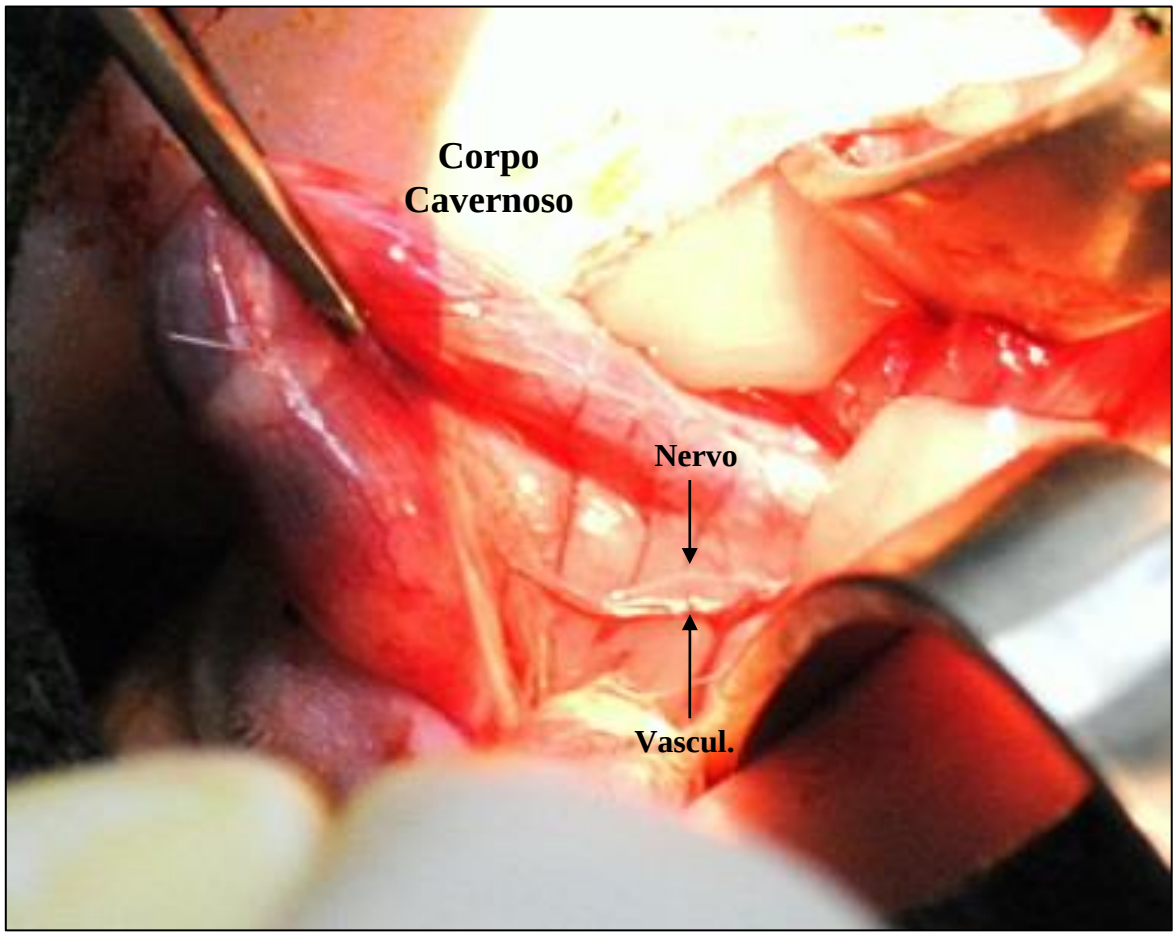



**Figura 4 -** Demonstração do componente vascular e nervoso no feixe peri prostático em modelo experimental de ratos.

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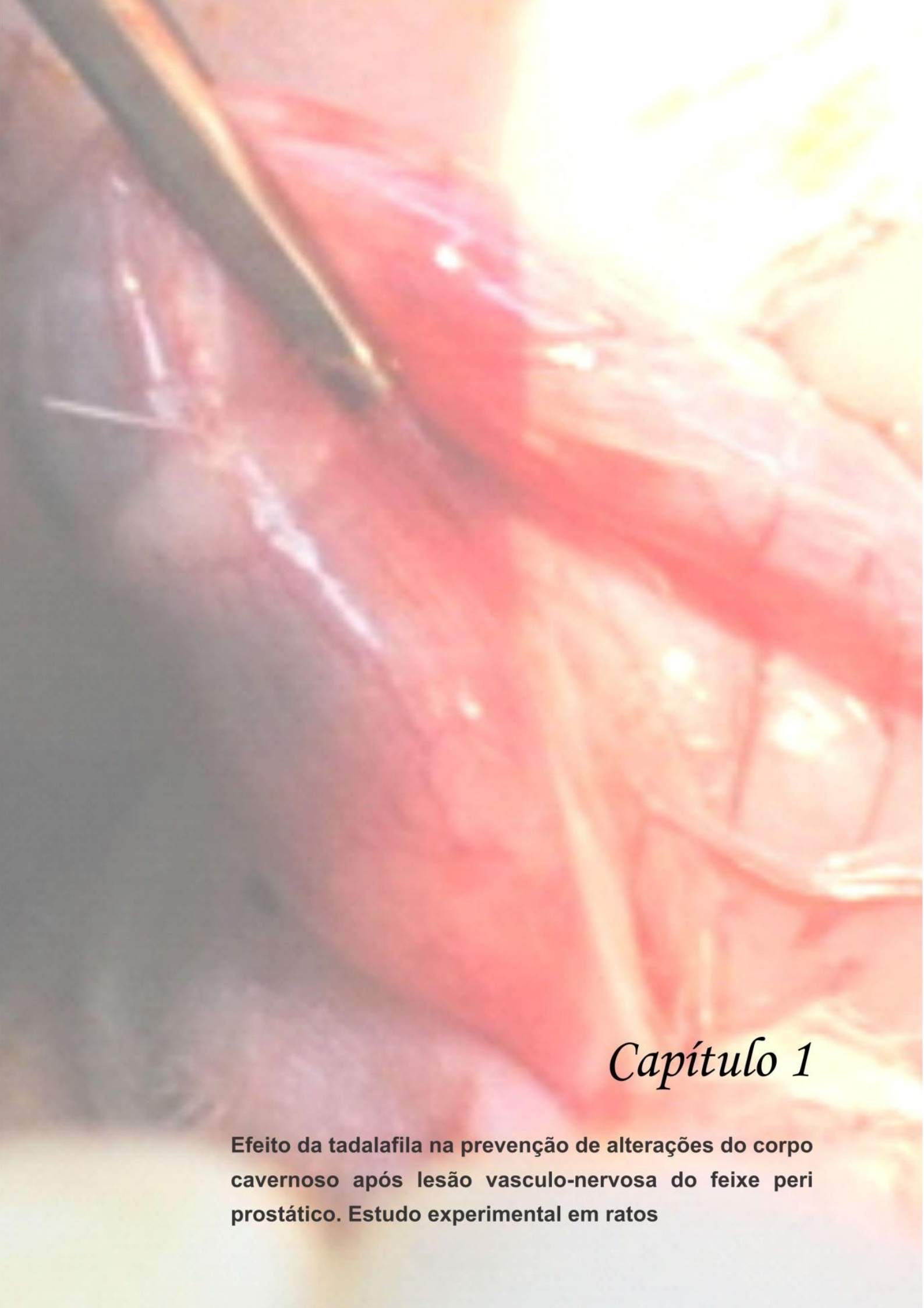

# *Capítulo 1*

**Efeito da tadalafila na prevenção de alterações do corpo cavernoso após lesão vasculo-nervosa do feixe peri prostático. Estudo experimental em ratos**

# Efeito da tadalafila na prevenção de alterações do corpo cavernoso após lesão vasculo-nervosa do feixe peri prostático. Estudo experimental em ratos

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## RESUMO

OBJETIVO: O presente estudo tem como objetivo avaliar o impacto no corpo cavernoso causado pela lesão isolada do nervo e artéria cavernosa, assim como determinar o efeito da tadalafila (IPDE-5) na remodelação tecidual, utilizando um modelo experimental de ratos. Material e Métodos: 50 ratos machos, da raça Whistar, pesando entre 250 e 350 gramas, foram aleatoriamente divididos, de forma randomizada em cinco grupos. Grupo 1 (G1): controle cirúrgico - CCir (n=10), submetidos apenas a exposição do feixe vásculo nervoso peri prostático. Grupo 2 (G2): Lesão Nervosa Isolada - LNI (n=10), os animais foram submetidos a lesão seletiva do nervo cavernoso bilateralmente. Grupo 3 (G3): Lesão Vascular Isolada - LVI (n=10), os animais foram submetidos a lesão seletiva da artéria cavernosa bilateralmente. Grupo 4 (G4): Lesão Nervosa Isolada + Tadalafila - LNI+T (n=10), os animais foram submetidos a lesão do nervo cavernoso bilateralmente e receberam tadalafila na dose de 5mg/Kg/dia. Grupo 5 (G5): Lesão Vascular Isolada + Tadalafila - LVI+T (n=10), os animais foram submetidos a lesão seletiva da artéria cavernosa bilateralmente e receberam tadalafila na dose de 5mg/Kg/dia.

A tadalafila foi administrada pelo período de 45 dias, quando os animais foram sacrificados e o corpo cavernoso foi removido em bloco para análise histomorfométrica. A organização e composição microestrutural do corpo cavernoso foram avaliadas por histomorfometria, imunohostoquímica e métodos bioquímicos. A análise estatística foi realizada pelo teste de comparações múltiplas de Bonferroni. RESULTADOS**:** Entre os grupos avaliados não houve diferença

entra as áreas de secção peniana, demonstrando homogeneidade entre os mesmos. O volume de músculo liso diminuiu significativamente no grupo com lesão neural (G2), quando comparado ao controle (12,87±1,90 vs 21,78±1,81 p< 0,05), o que não ocorreu no grupo com lesão neural tratado com tadalafila (G4). O volume de espaço sinusoidal diminuiu tanto no grupo com lesão vascular assim como no grupo com lesão neural (5,01±1,62 e 6,33±1,84 vs 9,80±3,66 p< 0,05), no G4 e G5 (LNI+T e LVI+T) não houve diferença estatística quando comparados ao controle. A densidade de vasos sanguíneos na trabécula (CD31) foi maior nos grupos lesados (G2 e G3) (29,32±4,13 e 20,80±2,47 vs 10,13±2,71 p<0,05). A densidade do colágeno foi significativamente maior nos animais com lesão vascular isolada quando comparados ao controle (93,76±15,81 vs 64,59±19,25 p<0,05), o que não ocorreu no G5 (LVI+T). Na análise qualitativa o colágeno mostrou-se menos organizado no G2 e G3, entretanto nos grupos tratados com tadalafila G4 e G5 o padrão foi semelhante ao controle. Houve grande desorganização das fibras elásticas nos grupos com lesão neuro vascula (G2 e G3), o que não ocorreu nos animais do G4 e G5. CONCLUSÃO: Apesar das alterações histomorfométricas da lesão nervosa serem maiores do que as produzidas pela injúria vascular, estudos de colágeno demosntraram maior fibrose no CC entre os animais com lesão vascular. A tadalafila é efetiva na prevenção da maioria das alterações histomorfométricas

Palavras-chave: Artéria cavernosa, Colágeno; Disfunção erétil; Elastina; Lesão isolada do nervo; Ratos.

# INTRODUÇÃO

O câncer de próstata (CaP) é o tumor sólido não cutâneo de maior incidência em homens acima dos 50 anos, correspondendo por aproximadamente 40% dos tumores que atingem o sexo masculino. Representa a segunda maior causa de mortalidade câncer específica nos Estados Unidos[1]. Atualmente o tratamento cirúrgico por meio da prostatectomia radical retropúbica (PRR) com preservação do feixe vásculo nervoso é o tratamento mais utilizado para a doença localizada [2]. A disfunção erétil (DE) pode ocorrer em 30 a 50% dos casos [3,4,5], com grande impacto na qualidade de vida destes pacientes [6].

A principal causa de DE pós a PRR é a neuropraxia do nervo cavernoso, causada pelo processo inflamatório, devido a tração excessiva durante a dissecção e exerese da próstata, ou então pela lesão térmica devido a utilização de eletrocautério[7]. Esta lesão nervosa pode persistir por até 24 meses, desencadeando uma reação em cascata, cujo principal fator é a hipóxia do corpo cavernoso (CC). Estas alterações podem ocasionar um desarranjo microestrututral do tecido erétil, caracterizado pelo aumento da produção de colágeno e remodelação venosa, com conseqüente diminuição da musculatura lisa [8,9,10]. Na lesão vascular pode ocorrer uma diminuição da oxigenação do tecido erétil, contribuindo para DE[11,12] .

Alguns autores [13,14] demonstram que o uso dos inibidores da fosfodiesterase E-5 (IPDE-5) diminuem a taxa de DE no pós operatório da PRR, porém, este fato não está bem elucidado. Esta droga age melhorando a oxigenação do corpo cavernoso (CC) evitando assim alterações as alterações teciduais [13,14].

Langworthy [15] , em 1965, estudou pela primeira vez a inervação pélvica em ratos, posteriormente foram descritos os nervos cavernosos, responsáveis pela ereção nestes animais[16]. Desta forma este modelo animal tornou-se o mais utilizado para o estudo das alterações estruturais que ocasionam DE pós PRR.

A lesão isolada seja do nervo ou artéria cavernosa na remodelação do corpo cavernoso não estão bem estabelecidos na literatura.

## OBJETIVO

O objetivo de nosso estudo foi avaliar o impacto no corpo cavernoso causado pela lesão isolada do nervo e artéria cavernosa, assim como determinar o efeito da tadalafila (IPDE-5) na remodelação tecidual.

## MATERIAL E MÉTODOS

50 ratos machos, da raça Whistar, pesando entre 250 e 350 gramas, foram aleatoriamente divididos, de forma randomizada em cinco grupos, assim discriminados:

Grupo 1 (Controle Cirúrgico - CCir, n=10), neste grupo os animais foram submetidos a intervenção cirúrgica com identificação do feixe vásculo nervoso peri prostático.

Grupo 2 (Lesão Nervosa Isolada - LNI, n=10), neste grupo os animais foram submetidos ao mesmo procedimento do Grupo 1, porém foi ressecado segmento de 3-5mm do nervo cavernoso bilateralmente, conforme descrito por Quinlan e User [16] (Figura 1).

Grupo 3 (Lesão Vascular Isolada - LVI, n=10) , neste grupo os animais foram submetidos ao mesmo procedimento do Grupo 1, porém com ligadura e secção da artéria cavernosa bilateralmente (Figura 1).

Grupo 4 (LNI + Tadalafila, n=10), neste grupo os animais foram submetidos ao mesmo procedimento do Grupo 2 (LNI), e receberam tadalafila na dose de 5mg/kg/dia a partir do terceiro dia de pós operatório, por um período de 45 dias. Esta dose de tadalafila corresponde a 20mg/dia em humanos [17].

Grupo 5 (LVI + Tadalafila, n=10), neste grupo os animais foram submetidos ao mesmo procedimento do Grupo 3 (LVI), e receberam tadalafila na dose de

5mg/kg/dia a partir do terceiro dia de pós operatório, por um período de 45 dias. Esta dose de tadalafila corresponde a 20mg/dia em humanos [17].

Houve aprovação do projeto pelo Comitê de Ética em Pesquisa Animal da Faculdade de Medicina de Botucatu - UNESP, protocolo número 756/2009.

Para a realização do procedimento cirúrgico foi realizado anestesia geral com quetamina (0,25-0,30 mg/kg) e xilazina (0,25-0,30 mg/kg) intramuscular. Para a identificação das estruturas foi utilizado magnificação de 6x, além de material de microcirúrgia.

A tadalafila foi diluída em glicose a 10% e administrada através de gavagem. A administração da medicação foi suspensa três dias antes do sacrifício para depuração da droga ("wash out").

Após o sacrifício o pênis foi completamente removido (Figura 2). O segmento proximal, que estende-se da porção óssea até a raiz prostática foi fixado em solução de formalina tamponada 10% durante 48 a 72 horas, e subsequentemente submetido a processamento histológico para inclusão em paraplast.

**Análise histomorfométrica:**

Após emblocamento foram realizadas secções transversais do corpo cavernoso com espessura de 5 micrometros e as lâminas coradas com Hematoxilina Eosina (HE) para análise de área da secção transversa, volume de fibras musculares e volume sinusoidal. O colágeno foi analisado utilizando-se a coloração do Picrosirius red, e as fibras elásticas foram caracterizadas pela imunofluorescência.

Após secção e fixação das lâminas em HE, as imagens foram digitalizadas com aumento final de 200x, utilizando uma câmera de vídeo acoplada a um microscópio óptico (LEICA DMLB) e um microcomputador com tela de monitor colorida. As medidas transversais para análise das fibras musculares lisas e espaço sinusoidal foram realizadas em cinco campos randomizados em grade de 100 pontos utilizando o programa de morfometria Image J versão 1.4 (NIH, Bethesda, USA), e expressa como percentagem. O espaço de referência utilizado foi todo o corpo cavernoso excluindo-se a túnica albugínea.

Para avaliação da densidade de nervos no septo fibroso do pênis foi utilizado o marcador tubulina beta-3 para axônio com aumento de 200x e grade de 100 pontos, através do programa Image J versão 1,4.

Na análise imuno histoquímica utilizamos o anticorpo anti CD-31 para marcação vasos sanguíneos na trabécula cavernosa, possibilitando avaliar a densidade dos vasos na trabécula. Os resultados foram expressos em num. vasos / $mm^2$ e foram excluídos da área total o espaço vascular e a túnica albugínea.

A proliferação celular no corpo cavernoso foi avaliada pelo marcador de proliferação celular (PCNA) , que marca núcleos. Os resultados foram expressos em núcleos positivos/mm2. A camada de músculo liso subendotelial foi incluída nesta avaliação.

Para a quantificação das fibras do colágeno, as imagens foram digitalizadas com aumento de 200 vezes, sendo analisados cinco campos randomizados para cada

animal. Foi medida a área de superfície ocupada pelas fibras de colágeno ($\mu m^2$), em cada campo, utilizando o programa de morfometria Image-J versão 1.4.

A densidade de área das fibras do sistema elástico foi obtido através de método qualitativo, por imunofluorescência [18], o que possibilitou avaliar a distribuição e organização das mesmas no CC.

**Análise estatística:**

As análises estatísticas foram conduzidas de acordo com Sokal e Rohlf (2011) [19]. Para a avaliação do colágeno foi utilizado análise de variância não-paramétrica, complementada com o teste de comparações múltiplas de Dunn [20]

Demais variáveis foi utilizada a técnica de análise de variância paramétrica, complementada com o teste de comparações múltiplas de Bonferroni [20]. Todas as comparações múltiplas foram analisadas consideradas o nível de 5% de significância.

**Principais resultados mensurados:**

Alterações estruturais no corpo cavernoso de ratos após lesão seletiva da artéria e nervo cavernosos e a influência de um IPDE-5 (tadalafila)

# RESULTADOS

Em nenhum animal notamos efeitos adversos da tadalafila como letargia ou priapismo. Durante o estudo os animais apresentaram ganho adequado de peso em todos os grupos.

A área de secção transversa do pênis foi semelhante nos diferentes grupos estudados, demonstrando uma adequada padronização no preparo do material histológico (Tabela 1).

A densidade de nervos na trabécula peniana foi significativamente menor no grupo CCir ($G_1$) em relação aos grupos LNI ($G_2$) e LNI + Tadalafila ($G_3$) ( Tabela 1). Não houve diferença estatisticamente significativa em relação aos demais grupos (Tabela 1).

O volume de músculo liso no corpo cavernoso foi significativamente maior no grupo CCir ($G_1$) em relação aos grupos LNI ($G_2$) e LVI ($G_3$). Porém, nos animais com lesão neuro vascular tratados com tadalafila ($G_4$ e $G_5$), este parâmetro foi semelhante ao grupo controle (Tabela 1).

O volume do lúmen sinusoidal foi significativamente menor no grupo LNI ($G_2$), quando comparado ao grupo controle ($G_1$). Entretanto, no grupo com lesão nervosa tratado com tadalafila ($G_4$) o volume sinusoidal foi semelhante ao grupo controle (Tabela 1). Não houve diferença estatisticamente significativa em relação ao demais grupos (Tabela 1).

A densidade de vasos sanguíneos na trabécula cavernosa foi significativamente menor no grupo CCir em relação aos grupos LNI ($G_2$) e LVI ($G_3$) (Tabela 1). Entretanto nos animais tratados com tadalafila ($G_4$ e $G_5$) apresentaram densidade de vasos sanguíneos semelhante ao grupo controle cirúrgico (Tabela 1).

A proliferação celular (PCNA) no CC foi significativamente maior no grupo LNI ($G_2$) em relação aos demais grupos (Tabela 1). A utilização da tadalafila associada a lesão de nervo isolada ($G_4$), mostrou uma proliferação celular significativamente maior em comparação ao grupo controle cirúrgico. Não houve diferença estatisticamente significativa no grupo controle em relação aos grupos $G_3$ e $G_5$.

A quantificação de colágeno no CC foi significativamente maior no grupo LVI ($G_3$) em relação aos grupos CCir ($G_1$) e LNI ($G_2$). Entretanto não houve diferença estatística nos animais tratados com tadalafila em relação ao CCir (Figura 3)(Tabela 1) .

Na avaliação qualitativa das fibras elásticas no CC foi observado diminuição e desorganização estrutural nos grupos LNI ($G_2$)e LVI ($G_3$) em comparação ao grupo CCir. Nos grupos tratados com tadalafila ($G_4$ e $G_5$) tiveram comportamento semelhante ao controle cirúrgico (Figura 4) (Tabela 1).

# DISCUSSÃO

Em nosso estudo houve um ganho adequado de peso nos animais, demonstrando assim que os grupos foram homogêneos. A análise da área de secção peniana nos grupos avaliados revelou que as secções transversas foram semelhantes, demonstrando não ter havido influência deste parâmetro nos resultados obtidos.

A análise dos nervos no septo peniano foi realizada com intuito de se confirmar a lesão isolada do feixe nervoso no grupo LNI ($G_2$). Desta forma notamos uma proliferação nervosa significativamente maior no Grupo LNI ($G_2$) e no grupo LNI+T ($G_4$) em relação ao controle cirúrgico. Este fato deve-se provavelmente a uma ação compensatória ocorrida nestes animais devido a lesão do plexo nervoso. Demonstrou-se também que a tadalafila não exerceu efeito protetor nos animais com lesão nervosa.

Diferentes autores tem demonstrado uma diminuição do volume da musculatura lisa e do volume de espaço sinusoidal no CC de pacientes com DE pós PRR, provavelmente pelo processo isquêmico desencadeado pela lesão do feixe peri prostático [21,22,23,24]. Em nosso estudo observamos uma diminuição significativa do volume de músculo liso no CC dos animais submetidos a lesão nervosa e vascular isoladas ($G_2$ e $G_3$). Notamos também uma ação protetora da tadalafila, tendo em vista que os grupos $G_4$ (LNI+T) e $G_5$ (LVI+T) tiveram comportamento semelhante ao grupo controle cirúrgico.

Em relação ao volume sinusoidal notamos uma alteração mais significativa no grupo submetido a LNI ($G_2$), em relação ao grupo controle cirúrgico, e a tadalafila exerceu um papel protetor, pois quando avaliamos o grupo LNI+T ($G_4$) encontramos valores próximos ao controle. Apesar de não ter havido uma diferença estatisticamente significante entre o grupo LVI ($G_3$) e o controle ($G_1$), os números absolutos mostraram um volume sinusoidal menor no LVI ($G_3$) em relação ao controle, indicando que um aumento amostral poderia demonstrar diferença estatística entre os grupos.

O volume de vasos na trabécula peniana foi significativamente maior nos grupos LNI ($G_2$) e LVI ($G_3$) em relação ao controle cirúrgico, provavelmente decorrente do processo compensatório pela isquemia do CC, ocasionado pela lesão do feixe peri prostático, observamos nestes grupos um efeito protetor da tadalafila. Estudos recentes tem afirmado que a vasodilatação decorrente da ação desta droga poderia proteger os pacientes das lesões intra cavernornosas pós prostatectomia radical [13,14,17]

Na proliferação celular notamos uma considerável influência da lesão do feixe nervoso peri prostático, demonstrando ter ocorrido uma remodelação estrutural nestes animais. A utilização da tadalafila não influenciou nestes resultados, entretanto, em números absolutos observamos uma diminuição na proliferação celular de 47% nos animais que utilizaram esta droga. Provavelmente estudos com amostra maior de animais poderiam demonstrar um efeito protetor dos IPDE-5.

Alguns autores tem demonstrado que o colágeno participa em até 63% da composição das trabéculas cavernosas em ratos normais [25]. Estudos controlados relatam um aumento de colágeno e diminuição de músculo liso em pacientes com DE[11, 26]. Em nosso estudo observamos aumento do colágeno com significância estatística no grupo LVI ($G_3$) em relação aos demais grupos. A tadalafila exerceu efeito protetor nos animais com LVI + T ($G_5$). Este fato vem corroborar que provavelmente a lesão vascular isolada do feixe peri prostático tem fator contributivo importante no desenvolvimento da fibrose do CC.

A composição das fibras elásticas do CC exercem importante função no processo de ereção [11, 21, 22]. Em nossa série houve uma maior desorganização estrutural destas fibras nos grupos com lesão neuro vascular ($G_2$ e $G_3$), porém a tadalafila exerceu papel importante, retornando esta organização a padrões semelhantes aos do grupo controle cirúrgico.

## CONCLUSÃO

Apesar das alterações histomorfométricas da lesão nervosa serem maiores do que as produzidas pela injúria vascular, estudos de colágeno demosntraram maior fibrose no CC entre os animais com lesão vascular. A tadalafila é efetiva na prevenção da maioria das alterações histomorfométricas

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**Tabela 1 -** Desrição das variáveis histomorfométricas nos diferentes grupos estudados.

| VARIÁVEIS | GRUPOS G1 (n = 10) | G2 (n = 10) | G3 (n = 10) | G4 (n = 10) | G5 (n = 10) | Análise Estatística |
|---|---|---|---|---|---|---|
| Área de Secção Transversa do pênis [(1)] | 8,17 (0,59) | 8,81 (1,24) | 8,75 (0,71) | 8,75 (1,04) | 8,26 (0,78) | p > 0,05 |
| Densidade de nervos na trabécula peniana [(1)] | 15,72 (1,82) [¥] | 22,62 (2,84) | 16,72 (2,08) | 19,53 (3,47) | 15,67 (2,61) | p < 0,001 |
| Volume do Músculo Liso no CC[(1)] | 21,78 (1,81) [#] | 12,87 (1,90) | 18,93 (1,51) | 19,49 (2,49) | 19,37 (1,89) | p < 0,05 |
| Volume do espaço sinusoidal no CC[(1)] | 9,80 (3,66) [&] | 5,01 (1,62) | 6,33 (1,84) | 8,01 (3,29) | 8,09 (2,41) | p < 0,001 |
| Densidade de vasos na trabécula peniana [(1)] | 10,13 (2,71) [θ] | 29,32 (4,13) | 20,80 (2,47) | 12,89 (4,63) | 9,89 (2,51) | p < 0,001 |
| Proliferação Celular (PCNA) [(2)] | 45,82 (11,21 ; 70,92) | 517,73 [β] (107,80 ; 788,65) | 121,99 (49,79 ; 262,41) | 269,65 (175,89 ; 560,28) | 35,46 (12,77 ; 130,50) | p < 0,001 |
| Dosagem de colágeno [(1)] | 64,59 (19,24) | 47,48 (15,31) | 93,76 (15,81) [$] | 76,26 (11,18) | 63,11 (21,11) | p < 0,001 |

[(1)] Média (desvio padrão)
[(2)] Mediana (valor mínimo; valor máximo)

[(1)] Mean (standard deviation)
[(2)] Median (minimum value; maximum value)

[¥] (p< 0.001) G1 X (G2, G4)
[#] (p< 0.05) G1 X (G2,G3)
[&] (p< 0.001) G1 X G2
[θ] (p< 0.001) G1 X (G2, G3)
[β] (p< 0.001) G1 X G2 X G3 X G4 X G5
$ (P< 0,001) G3 X (G1,G2)

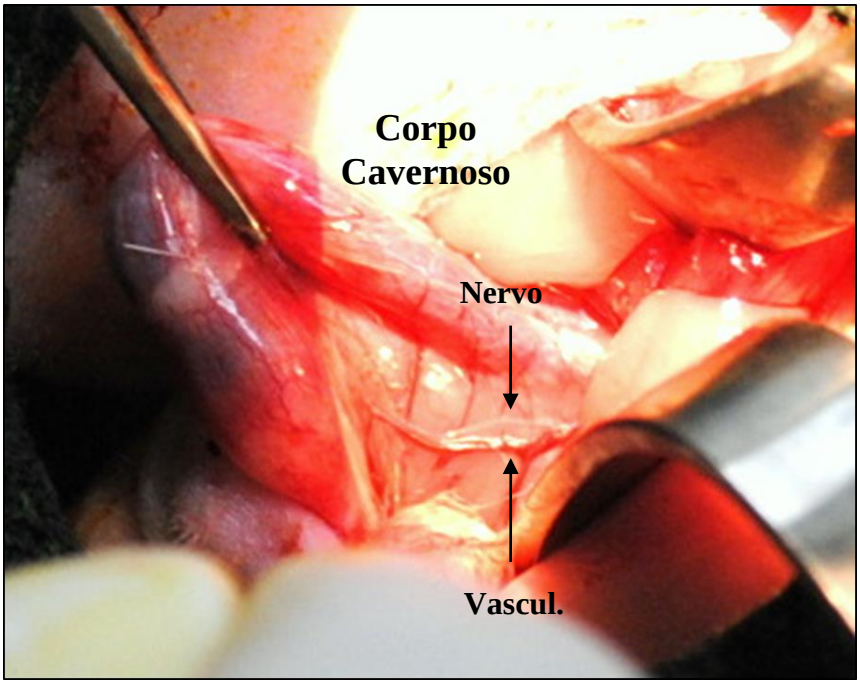



**Figura 1:** Demonstração do componente vascular e nervoso no feixe peri prostático em modelo experimental de ratos

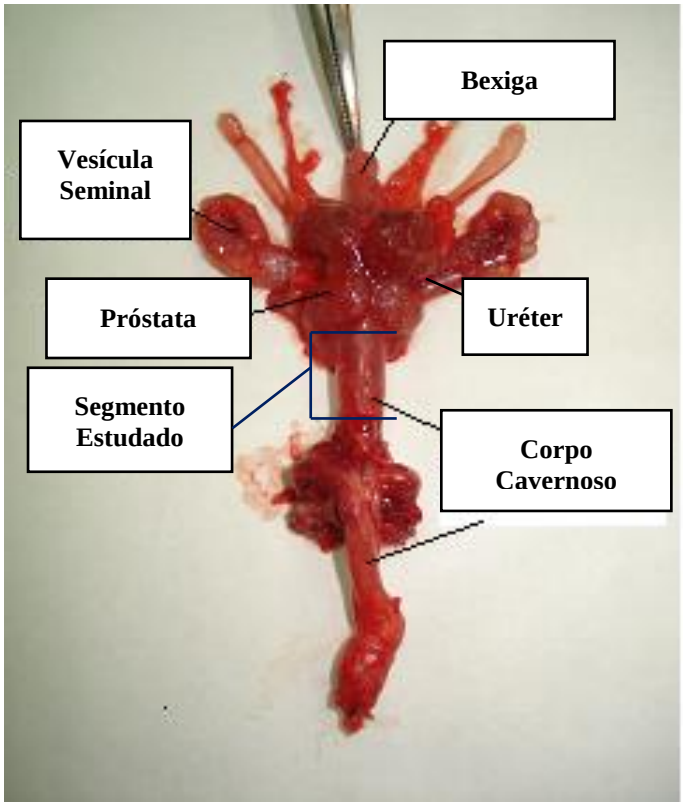



**Figura 2:** Ilustração do pênis de rato demonstrando as diferentes estruturas e segmentos que compõe o corpo cavernoso

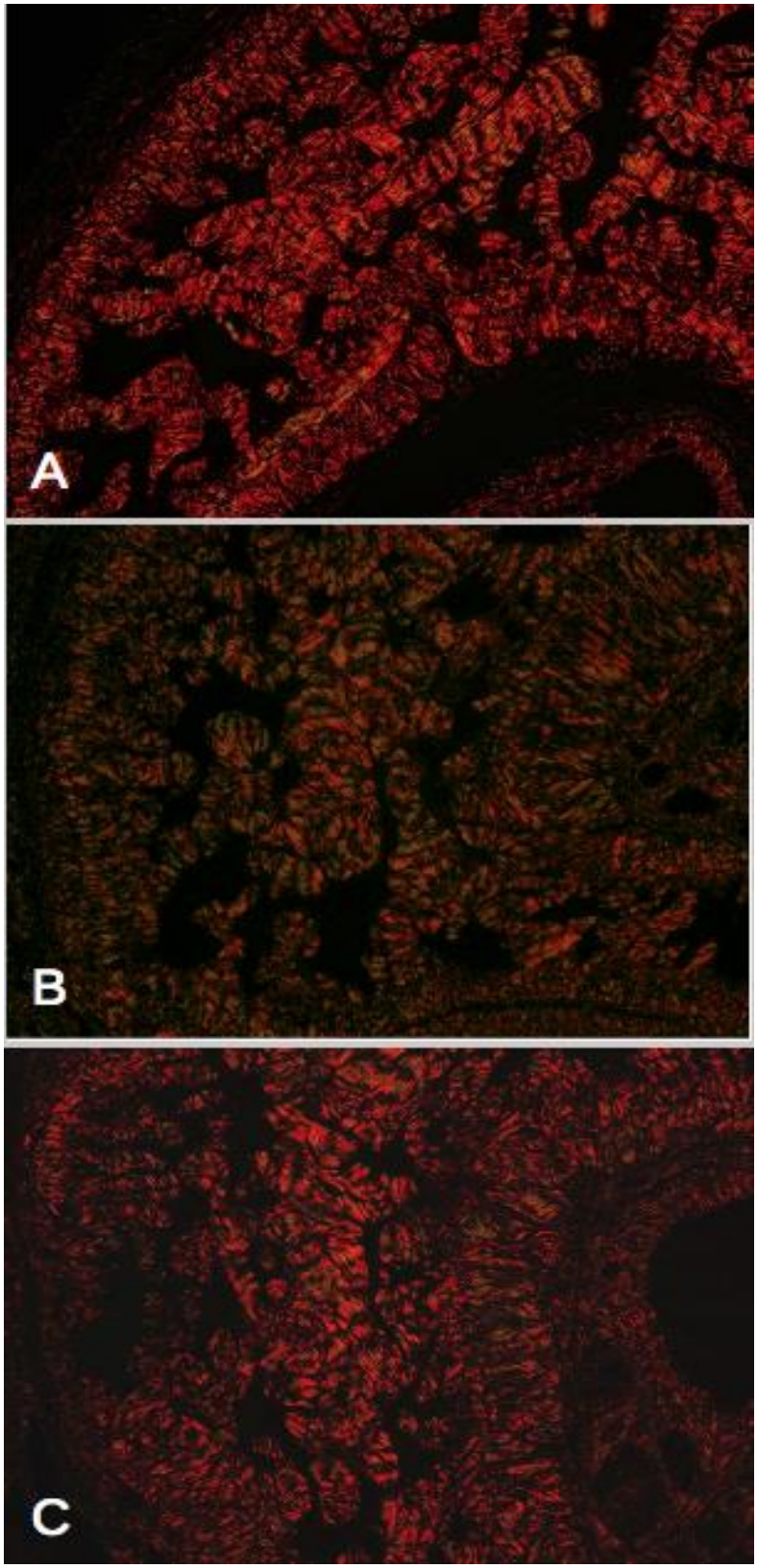



**Figura 3:** Análise da organização do colágeno nos grupos controle (A); lesão vascular isolada (B) e lesão vascular + tadalafila (C)

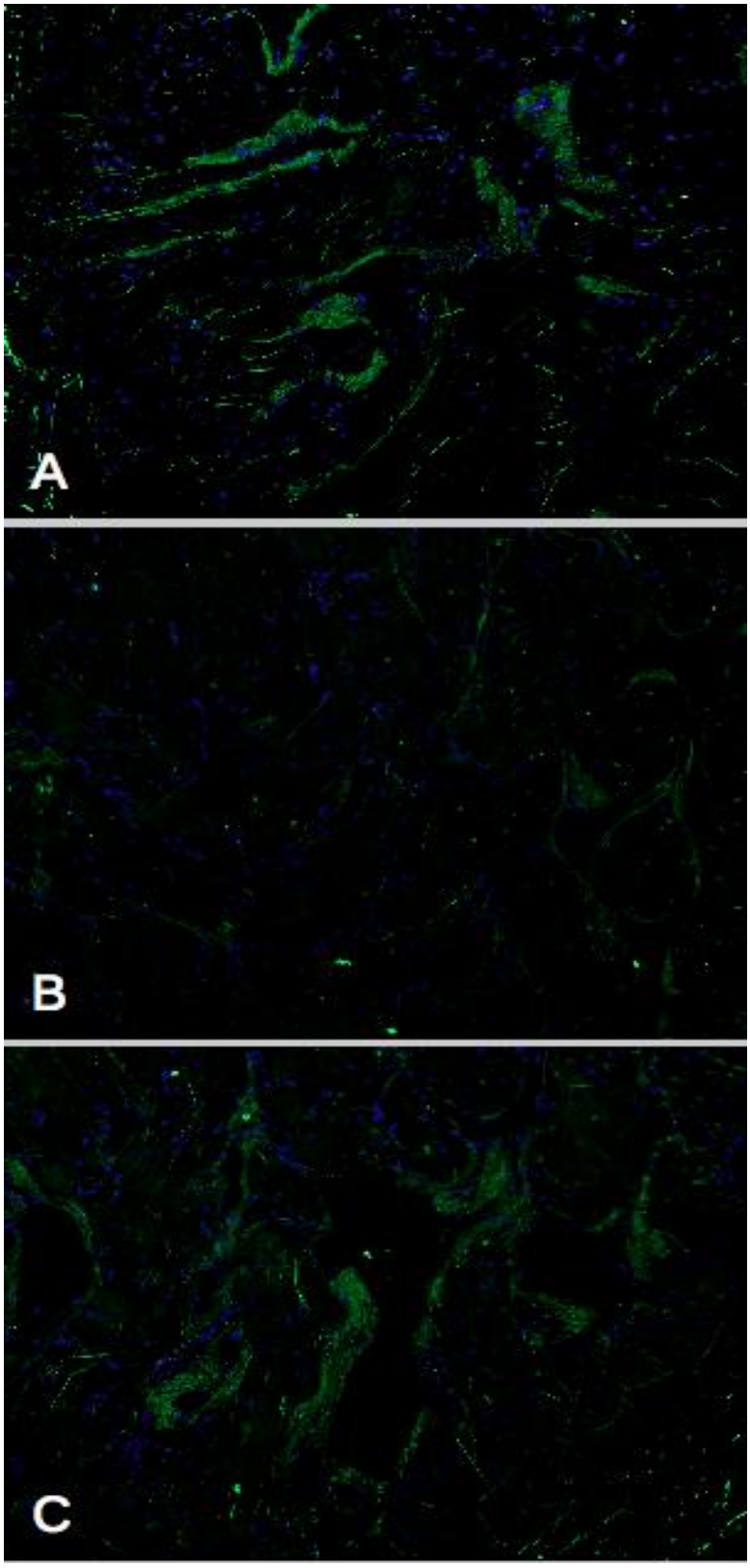
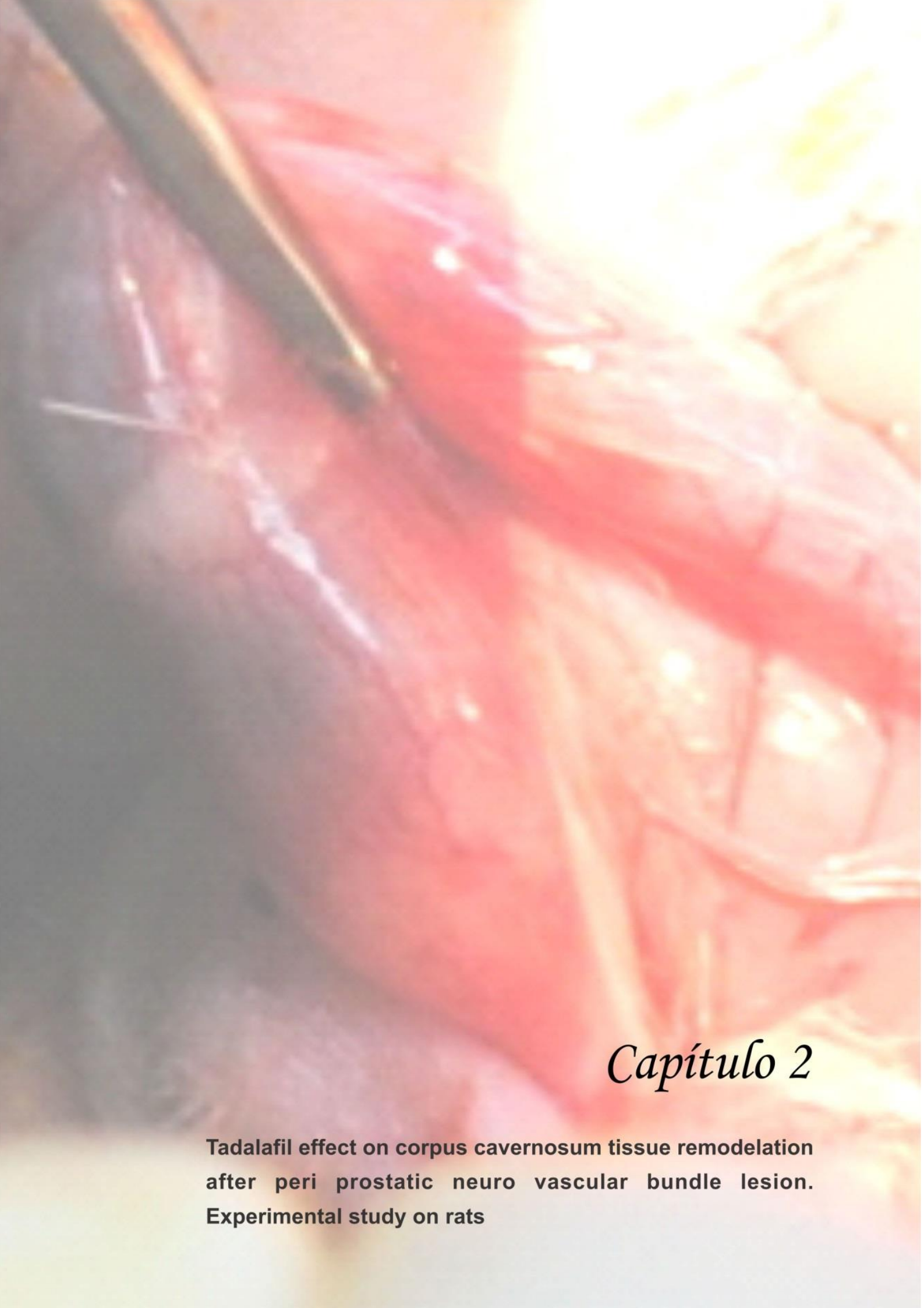



**Figura 4 -** Análisa de fibras elásticas nos grupo controle (A); lesão nervosa isolada (B) e lesão nervosa + tadalafila (C).



# *Capítulo 2*

**Tadalafil effect on corpus cavernosum tissue remodelation after peri prostatic neuro vascular bundle lesion. Experimental study on rats**

# Tadalafil effect on corpus cavernosum tissue remodelation after peri prostatic neuro vascular bundle lesion. Experimental study on rats

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## ABSTRACT

OBJECTIVE: This study aims to evaluate the impact on the corpus cavernosum caused by isolated injury of the cavernous nerve and artery, as well as determine the effect of tadalafil (IPDE-5) on tissue remodeling, using a rat experimental model.

Material and Methods: Fifty (50) male Whistar rats, weighing between 250 and 350 grams, were divided randomly into five groups. Group 1 (G1): surgical control - SCon (n=10) having undergone only exposure of the periprostatic neurovascular bundle. Group 2 (G2): Isolated Nerve Lesion - INL (n=10), the animals were subjected to selective cavernous nerve injury, bilaterally. Group 3 (G3): Isolated Vascular Lesion - IVL (n=10), the animals were subjected to selective cavernous artery injury, bilaterally. Group 4 (G4): Isolated Nerve Lesion + Tadalafil - INL+T (n=10), the animals were subjected to cavernous nerve injury, bilaterally, and received tadalafil in a dosage of 5mg/kg/day. Group 5 (G5): Isolated Vascular Lesion + Tadalafil - IVL+T (n=10), the animals were subjected to selective cavernous artery injury, bilaterally, and received tadalafil in a dosage of 5mg/kg/day. The tadalafil was administered for a 45 day period, then the animals were sacrificed and the corpus cavernosum was removed en bloc for histomorphometric analysis. The organization and microstructural composition of the corpus cavernosum were assessed through histomorphometry, immuno-histochemistry, and biochemical methods. Statistical analysis was performed using the Bonferroni multiple comparisons test. RESULTS: Among the groups evaluated, there was no difference between the penile section areas, demonstrating their

consistency. The volume of smooth muscle decreased significantly in the group with nerve injury (G2) when compared with the control (12.87±1.90 vs 21.78±1.81 $p<0.05$), which did not occur in the group with nerve injury treated with tadalafil (G4). Sinusoidal space volume decreased both in the group with vascular injury as well as the group with nerve injury (5.01±1.62 and 6.33±1.84 vs 9.80±3.66 $p< 0.05$); in the G4 and G5 (INL+T and IVL+T) there was no statistical difference when compared to the control. Blood vessel density in the trabecula (CD31) was higher in the injured groups (G2 and G3) (29.32±4.13 and 20.80±2.47 vs 10.13±2.71 $p<0.05$). Collagen density was significantly higher in the animals with isolated vascular injury compared with the control (93.76±15.81 vs 64.59±19.25 $p<0.05$), which did not occur in G5 (IVL+T). In the qualitative analysis, collagen proved less organized in G2 and G3, however in the groups treated with tadalafil, G4 and G5, the pattern was similar to the control. There was major disorganization of the elastic fibers in the groups with neurovascular injury (G2 and G3), which did not occur in the animals from G4 and G5. CONCLUSION: Although the histomorphometric changes from nerve injury were higher than those produced by vascular injury, collagen studies demonstrated greater fibrosis in the CC among animals with vascular injury. Tadalafil is effective in preventing most of these histomorphometric changes.

Keywords: Cavernous artery, Collagen, Erectile dysfunction, Elastin, Isolated nerve injury, Rats.

# INTRODUCTION

Prostate cancer (PCa) is the non-cutaneous solid tumor with the highest incidence in men over 50 years, accounting for approximately 40% of the tumors affecting males. It represents the second leading cause of cancer-specific mortality in the U.S.[1] Currently, surgical treatment by nerve sparing radical retropubic prostatectomy (RRP) is the most common treatment for the localized disease [2]. Erectile dysfunction (ED) can occur in 30-50 % of the cases[3,4,5], with a major impact on the quality of life of these patients[6].

The main cause of post-RRP ED is neuropraxia of the cavernous nerve caused by inflammation due to excessive traction during dissection and removal of the prostate, or afterwards by thermal injury due to the use of electrocautery[7]. This nerve damage can persist for up to 24 months, triggering a chain reaction, whose main factor is hypoxia of the corpus cavernosum (CC). These changes can cause a microstructural disorganization of erectile tissue, characterized by increased production of collagen and venous remodeling, with a consequent decrease in smooth muscle [8,9,10]. In the vascular lesion, a decrease in oxygenation of the erectile tissue can occur, contributing to ED[11,12].

Several authors [13,14] have demonstrated that the use of phosphodiesterase type 5 inhibitors (IPDE-5) diminishes the rate of RRP postoperative ED, however, this fact is not well elucidated. This drug works by improving oxygenation of the corpus cavernosum (CC) thus avoiding tissue changes[13,14].

Langworthy [15], in 1965, was the first to study pelvic innervation in rats, and subsequently the cavernous nerves, responsible for erections in these animals [16], were described. Thus, this animal model has become the one most used to study the structural changes that cause post-RRP ED.

Isolated injury, whether of the cavernous nerve or artery, in the remodeling of the corpus cavernosum, is not well established in the literature.

## OBJECTIVE

The aim of our study was to evaluate the impact on the corpus cavernosum caused by isolated damage of the cavernous nerve and artery, as well as to determine the effect of tadalafil (IPDE-5) on tissue remodeling.

## MATERIALS AND METHODS

Fifty (50) male Whistar rats, weighing between 250 and 350 grams, were divided randomly into five groups, categorized as follows:

Group 1 (Surgical Control - SCon, n=10), animals in this group underwent surgery with exposure of the periprostatic neurovascular bundle.

Group 2 (Isolated Nerve Lesion - INL, n=10), animals in this group underwent the same procedure as Group 1, however a 3-5mm segment of the cavernous nerve was ressected bilaterally as described by Quinlan and User [16] (Figure 1).

Group 3 (Isolated Vascular Lesion - IVL, n=10), animals in this group underwent the same procedure as Group 1, however with a section of the cavernous artery, bilaterally (Figure 1).

Group 4 (INL + Tadalafil, n=10), animals in this group underwent the same procedure as Group 2 (INL) and received tadalafil at a dosage of 5mg/kg/day starting on the third postoperative day, for a period of 45 days. This tadalafil dosage corresponds to 20mg/day in humans [17].

Group 5 (IVL + Tadalafil, n=10), animals in this group underwent the same procedure as Group 3 (IVL), and received tadalafil at a dosage of 5mg/kg/day starting on the third postoperative day, for a period of 45 days. This tadalafil dosage corresponds to 20mg/day in humans [17].

This project was approved by the Ethics Committee on Animal Research, School of Medicine, Botucatu - UNESP, protocol number 756/2009.

To perform the surgical procedure, general anesthesia with ketamine (0.25-0.30 mg/kg) and xylazine (0.25-0.30 mg/kg) was administered intramuscularly. To identify the structures, 6x magnification was used, as well as microsurgical materials.

The tadalafil was diluted with glucose at 10% and administered by gavage. The administration of the medication was discontinued three days before sacrifice, for elimination of the drug ("washout").

After sacrifice, the penis was completely removed (Figure 2). The proximal segment, which extends from the bony portion to the prostatic root was fixed in a solution of 10% buffered formalin for 48 to 72 hours, and subsequently subjected to histological processing for embedding in paraplast.

After embedding, cross-sections of the corpus cavernosum were made with a thickness of 5 microns and stained with hematoxylin and eosin (HE), for analysis of the cross-sectional area, volume of muscle fibers, and sinusoidal volume. Collagen was analyzed using Picrosirius red staining, and elastic fibers were characterized using immunofluorescence.

After sectioning and fixation of the slides in HE, the images were captured with a final magnification of 200x, using a video camera coupled to an optical microscope (LEICA DMLB) and a microcomputer with a color monitor. Transversal measures for analysis of smooth muscle fibers and sinusoidal space

were taken in five randomized fields in a grid of 100 points using the Image J version 1.4 morphometry program (NIH, Bethesda, USA), and expressed as a percentage. The reference space used was the entire corpus cavernosum excluding the tunica albuginea.

To evaluate the density of nerves in the fibrous septum of the penis, the tubulin beta-3 axonal marker was used at 200x magnification and with 100 grid points, using the Image J version 1.4 program.

In the immuno-histochemical analysis we used the anti CD-31 antibody for marking the blood vessels in the cavernous trabecula, enabling an evaluation of the density of the vessels in the trabecula. The results were expressed as number of vessels / $mm^2$, and the vascular space and the tunica albuginea were excluded from the total area.

Cell proliferation in the corpus cavernosum was assessed by the cell proliferation marker (PCNA), which marks nuclei. The results were expressed in positive nuclei / $mm^2$. The subendothelial smooth muscle layer was included in this evaluation.

To quantify the collagen fibers, the images were scanned with a 200x magnification, and five randomized fields for each animal were analyzed. The surface area occupied by collagen fibers ($microm^2$), in each field, were measured using the Image-J version 1.4 morphometry program.

The areal density of the elastic system fibers was obtained through a qualitative method, using immunofluorescence [18], which allowed us to evaluate their distribution and organization in the CC.

**Statistical analysis:**

Statistical analyses were conducted according to Sokal and Rohlf (2011) [19]. To evaluate collagen, nonparametric analysis of variance was used, complemented by Dunn's test for multiple comparisons [20].

For the other variables, parametric analysis of variance techniques were used, complemented by the Bonferroni multiple comparison test [20]. All multiple comparisons were analyzed considering a 5% level of significance.

## Main results measured

Structural changes in the corpus cavernosum of rats after selective lesion of the cavernous artery and nerve, and the influence of an IPDE-5 (tadalafil)

## RESULTS

In no animal did we note adverse effects of tadalafil, such as lethargy or priapism. During the study, the animals showed adequate weight gain in all groups.

The cross-sectional area of the penis was similar in the different groups studied, demonstrating an adequate standardization in the preparation of the histological material (Table 1).

The density of nerves in the penile trabecula was significantly lower in the SCon (G1) group in relation to the INL (G2) and INL + Tadalafil (G4) groups (Table 1). There was no statistically significant difference in relation to the remaining groups (Table 1).

The volume of smooth muscle in the corpus cavernosum was significantly higher in the SCon (G1) group in relation to the INL (G2) and IVL (G3) groups. However, in the animals with neurovascular injury treated with tadalafil (G4 and G5), this parameter was similar to the control group (Table 1).

The volume of the sinusoidal lumen was significantly lower in the INL (G2) group compared to the control (G1) group. However, in the group with nerve injury treated with tadalafil (G4), sinusoidal volume was similar to the control group (Table 1). There was no statistically significant difference in relation to the remaining groups (Table 1).

The density of blood vessels in the cavernous trabecula was significantly lower in the SCon group in relation to the INL (G2) and IVL (G3) groups (Table 1). However, the animals treated with tadalafil (G4 and G5) presented blood vessel densities similar to the surgical control group (Table 1).

Cell proliferation (PCNA) in the CC was significantly higher in the INL (G2) group compared to the other groups (Table 1). The use of tadalafil combined with isolated nerve lesion (G4), showed a significantly higher cell proliferation compared to the surgical control group. There was no statistically significant difference between the control group and groups G3 and G5.

The quantification of collagen in the CC was significantly higher in the IVL (G3) group compared to the SCon (G1) and INL (G2) groups. However there was no statistical difference in the animals treated with tadalafil compared to the SCon (Figure 3) (Table 1).

In the qualitative assessment of the elastic fibers in the CC, a reduction and a structural disorganization were observed in the INL (G2) and IVL (G3) groups compared to the SCon group. In the groups treated with tadalafil (G4 and G5), they had a behavior similar to the surgical control (Figure 4) (Table 1).

## DISCUSSION

In our study there was an adequate weight gain in the animals, thus demonstrating that the groups were homogeneous. Analysis of penile section area in the groups evaluated revealed that the cross-sections were similar, showing that this parameter had no influence on the results obtained.

Analysis of the nerves in the penile septum was performed to confirm the isolated injury of the nerve bundle in the INL (G2) group. Thus we note a significantly higher nerve proliferation in the INL (G2) group and the INL + T (G4) group in relation to the surgical control. This fact is probably due to a compensatory action that occurred in these animals due to injury of the nerve plexus. It was also demonstrated that tadalafil did not exert a protective effect in the animals with nerve injury.

Various authors have been demonstrating a decrease in the volume of smooth muscle and the volume of sinusoidal space in the CC of patients with post-RRP ED, probably through the ischemic process triggered by injury of the periprostatic bundle [21,22,23,24]. In our study we observed a significant decrease in the volume of smooth muscle in the CC of animals subjected to isolated nerve and vascular injury (G2 and G3). We also noticed a protective action from tadalafil, considering that the G4 (INL+T) and G5 (IVL+T) groups had behavior similar to the surgical control group.

With regard to sinusoidal volume, we noted a more significant change in the group having undergone INL (G2), in relation to the surgical control group, and tadalafil exerted a protective role, since in evaluating the INL+T (G4) group, we found values close to those of the control. Although there was no statistically significant difference between the IVL (G3) and the control (G1) groups, the absolute numbers showed a smaller sinusoidal volume in the IVL (G3) compared to the control, indicating that a sampling increase could demonstrate statistical difference between groups.

The volume of vessels in the penile trabecula was significantly higher in the INL (G2) and IVL (G3) groups compared to the surgical control, probably because of a compensatory process for the ischemia of the CC, caused by injury to the periprostatic bundle. In these groups we observed a protective effect from tadalafil. Recent studies have stated that the vasodilation resulting from the action of this drug could protect patients from intra cavernous lesions after radical prostatectomy[13,14,17].

In cell proliferation we noted a considerable influence from injury of the periprostatic nerve bundle, demonstrating that a structural remodeling had occurred in these animals. The use of tadalafil did not affect these results, however, in absolute numbers we observed a decrease in cell proliferation of 47% in the animals that used this drug. Probably, studies with a larger sample of animals could demonstrate a protective effect from IPDE-5.

A number of authors have shown that collagen participates in up to 63% of the composition of the cavernous trabeculae in normal rats [25]. Controlled studies have reported a collagen increase and smooth muscle decrease in patients with ED[11,26]. In our study we observed statistically significant increased collagen in the IVL (G3) group compared to the other groups. Tadalafil had a protective effect on the animals in IVL+T (G5). This fact has been corroborating the idea that isolated vascular lesion of the periprostatic bundle is likely an important contributing factor in the development of fibrosis of the CC.

The composition of the elastic fibers of the CC has an important function in the erection process [11,21,22]. In our series there was a higher structural disorganization of these fibers in the groups with neurovascular lesion (G2 and G3), but tadalafil played an important role, returning this organization to patterns similar to those of the surgical control group.

## CONCLUSION

Although the histomorphometric changes from nerve injury were higher than those produced by vascular injury, collagen studies demonstrated greater fibrosis in the CC among animals with vascular injury. Tadalafil is effective in preventing most of these histomorphometric changes.

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Table 1: Description of the histomorphometric variables in the different groups studied

| VARIABLES | GROUPS G1 (n = 10) | G2 (n = 10) | G3 (n = 10) | G4 (n = 10) | G5 (n = 10) | Statistical Analysis |
|---|---|---|---|---|---|---|
| Cross-sectional area of the penis (1) | 8.17 (0.59) | 8.81 (1.24) | 8.75 (0.71) | 8.75 (1.04) | 8.26 (0.78) | p > 0.05 |
| Nerve density in the penile trabecula (1) | 15.72 (1.82) ¥ | 22.62 (2.84) | 16.72 (2.08) | 19.53 (3.47) | 15.67 (2.61) | p < 0.001 |
| Volume of Smooth Muscle in the CC(1) | 21.78 (1.81) # | 12.87 (1.90) | 18.93 (1.51) | 19.49 (2.49) | 19.37 (1.89) | p < 0.05 |
| Volume of the sinusoidal space in the CC(1) | 9.80 (3.66) & | 5.01 (1.62) | 6.33 (1.84) | 8.01 (3.29) | 8.09 (2.41) | p < 0.001 |
| Vessel density in the penile trabecula (1) | 10.13 (2.71) θ | 29.32 (4.13) | 20.80 (2.47) | 12.89 (4.63) | 9.89 (2.51) | p < 0.001 |
| Cell Proliferation (PCNA)(2) | 45.82 (11.21 ; 70.92) | 517.73 β (107.80 ; 788.65) | 121.99 (49.79 ; 262.41) | 269.65 (175.89 ; 560.28) | 35.46 (12.77 ; 130.50) | p < 0.001 |
| Collagen level (1) | 64.59 (19.24) | 47.48 (15.31) | 93.76 (15.81) $ | 76.26 (11.18) | 63.11 (21.11) | p < 0.001 |

(1) Mean (standard deviation)
(2) Median (minimum value; maximum value)

¥ (p< 0.001) G1 X (G2, G4)
# (p< 0.05) G1 X (G2, G3)
& (p< 0.001) G1 X G2
θ (p< 0.001) G1 X (G2, G3)
β (p< 0.001) G1 X G2 X G3 X G4 X G5
$ (p< 0.001) G3 X (G1, G2)

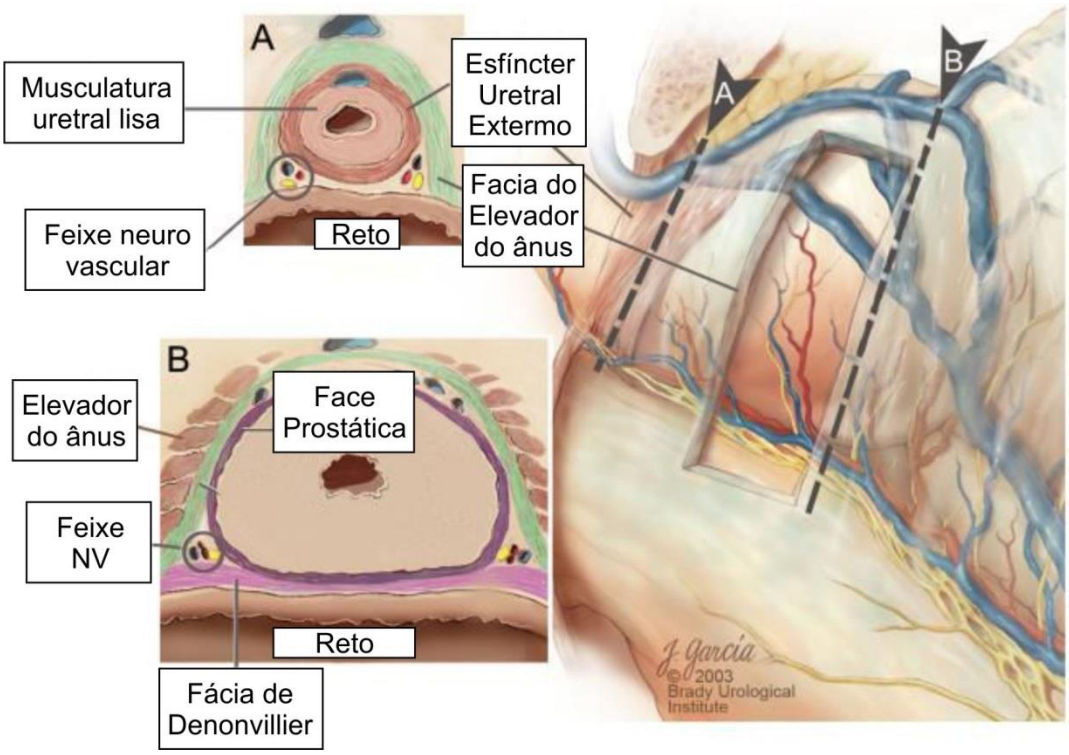



**Figure 1 -** Example of the neural and vascular component in the periprostatic bundle in an experimental rat specimen

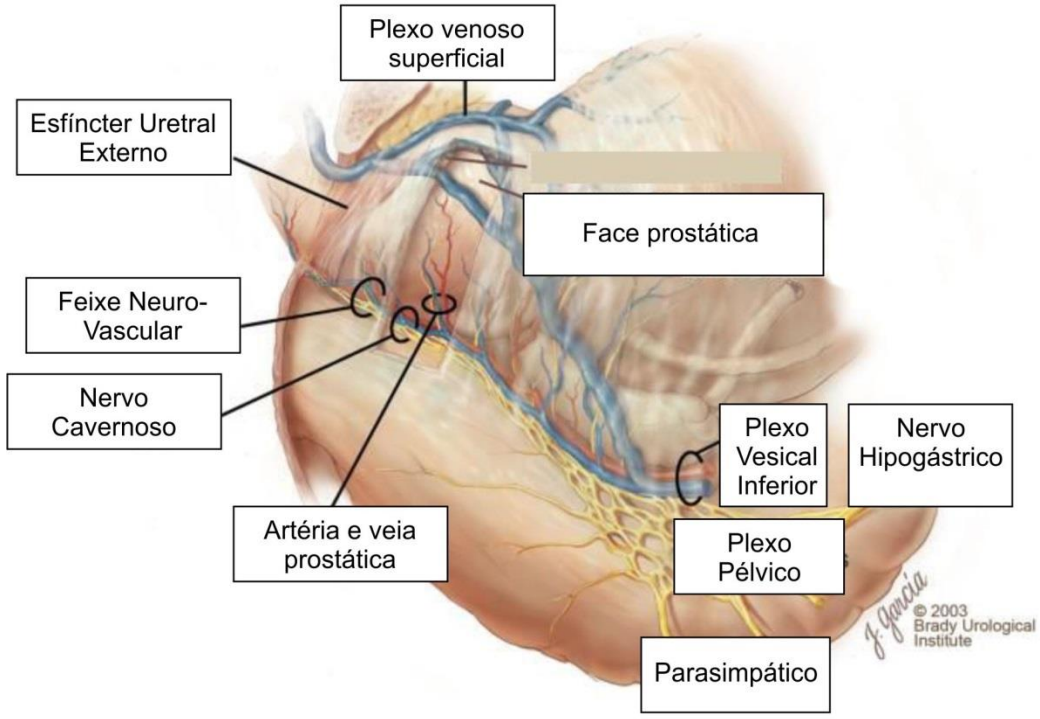



**Figure 2 -** Illustration of the rat penis, demonstrating the different structures and segments that make up the corpus cavernosum

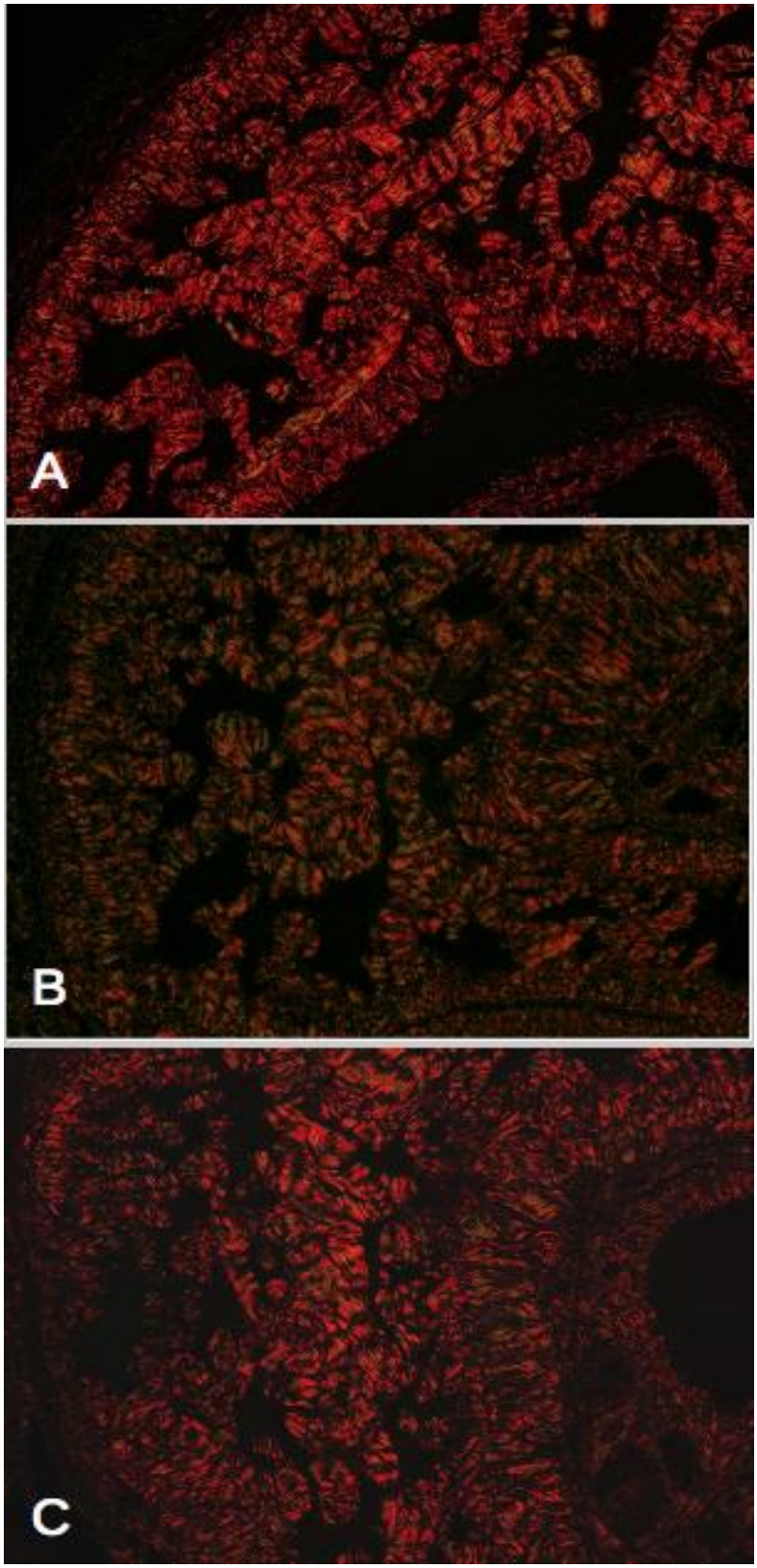



**Figure 3 -** Analysis of collagen organization in the control (A), isolated vascular injury (B), and vascular injury + tadalafil (C) groups

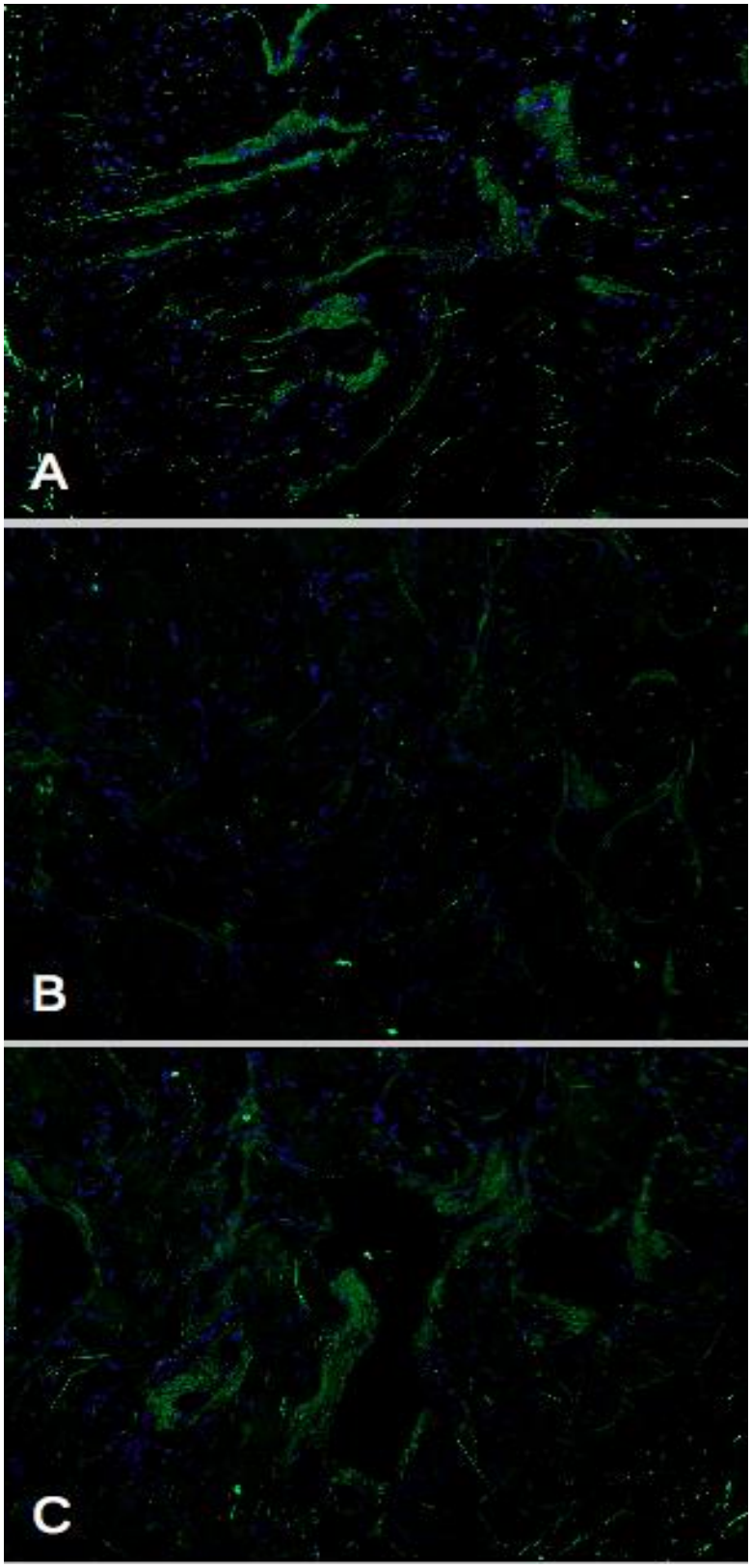
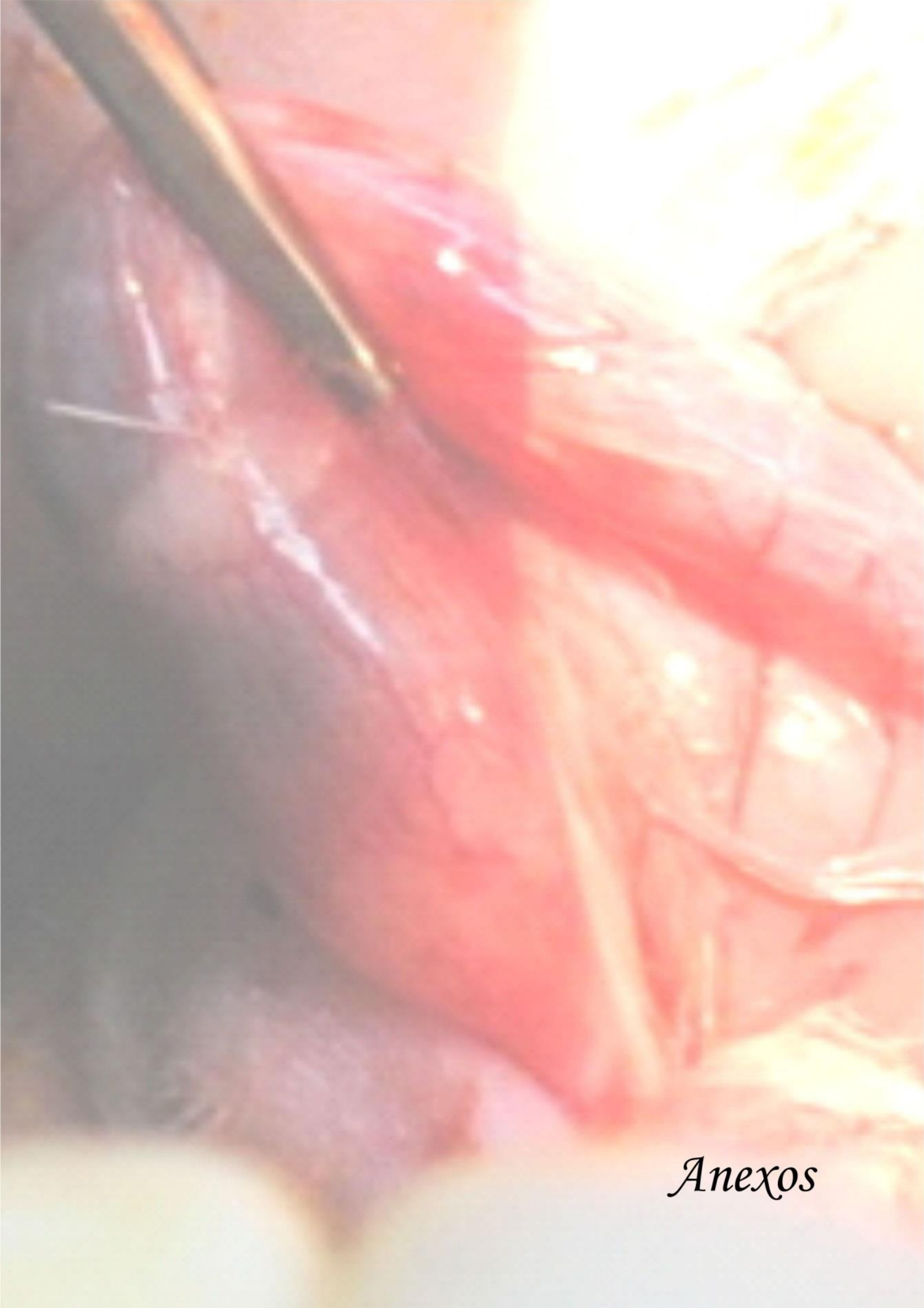



**Figure 4 -** Analysis of elastic fibers in the control (A), isolated nerve injury (B), and nerve injury + tadalafil (C) groups



# Anexos

**Anexo 1 -** Parecer do Comitê de Ética em Pesquisa.

unesp
**Universidade Estadual Paulista**
**Faculdade de Medicina de Botucatu**

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CEEA Ética
Comissão de Ética em Experimentação Animal

Botucatu, 15 de abril de 2010. Of. 005/10-CEEA

Ilustríssimo Senhor
Prof. Dr. João Luiz Amaro
Departamento de Urologia da
Faculdade de Medicina de Botucatu.

Caro Dr. Amaro,

De ordem da senhora presidente da Comissão de Ética em Experimentação Animal, informo que em 08/04/10 foi autorizado incluir o Prof. Dr. Hamilton Akihissa Yamamoto como colaborador do projeto de pesquisa "Avaliação das alterações histológicas e morfométricas do corpo cavernoso causadas pela lesão vascular e nervosa do feixe peri prostático de ratos – **(Protocolo CEEA 756-2009)** ", conduzido por Antônio Carlos Tonelli de Toledo, orientado por Vossa Senhoria com a colaboração dos Profs. Drs. Paulo Roberto Kawano, Luis Eduardo Macedo Cardoso e Oscar Eduardo H. Fugita, aprovado em 24/09/09.

Alberto Santos Capelluppi
Secretário da CEEA

**Anexo 2 -** Justificativa de Alteração de Título.

Fls. .....
Proc. .....
Rub. .....

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

***JUSTIFICATIVA DE ALTERAÇÃO NO TÍTULO DO PROJETO DE PESQUISA***

*Declaramos que o Projeto de Pesquisa* ***"Avaliação das alterações histológicas e morfométricas do corpo cavernoso causadas pela lesão vascular e nervosa do feixe peri prostático de ratos"****, aprovado pelo CEP em 24 / 09 / 2009, teve seu título alterado para* ***" Efeito da tadalafila na prevenção de alterações do corpo cavernoso após lesão vásculo-nervosa do feixe peri prostático. Estudo experimental em ratos"****, sem nenhuma alteração no seu conteúdo metodológico da época de apresentação para análise do CEP.*

*A presente alteração foi efetuada somente para adequação do título da Tese de Doutorado.*

*Botucatu, 26 de Novembro de 2013*

*Nome/Assinatura do(a) aluno(a)* ..... ANTÔNIO CARLOS TONELLI DE TOLEDO

*Nome/Assinatura do(a) orientador (a)* ..... JOÃO LUIZ AMARO

*Programa de Pós Graduação em Bases Gerais da Cirurgia*

**OBS.: Preencher formulário em 2 vias e protocolar no respectivo CEP**

Comitê de Ética em Pesquisa
Comissão de Ética em Experimentação Animal
Recebi aos 26 / 11 / 13
Alberto Santos Capelluppi
Secretário CEP/CEEA

**Anexo 3 -** Informação para Autores do The Journal of Sexual Medicine.





# The Journal of Sexual Medicine




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## Author Guidelines

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First click on the "Check for Existing Account" button on the opening page of the website (the bottom of the two buttons). If you do not already have an account, then create an account for yourself by clicking on the "Create an Account" button. You will then be able to submit and monitor the progress of your manuscripts. Follow the submittal instructions carefully.

At the end of a successful submission, a confirmation screen with manuscript number will appear and you will receive an E-mail confirming that the manuscript has been received by the journal. If this does not happen, please check your submission and/or contact the editorial office at jsm@issm.info (mailto:jsm@issm.info). Through individual Author Centers on this website, authors can view the status of their manuscripts as they progress through the review process.

Any major word processor software may be used, and both DOS-based and Macintosh operating systems are acceptable.

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*The Journal of Sexual Medicine* works together with the ISSM's new Open Access Journal, *Sexual Medicine*, to enable rapid publication of good quality research that we are unable to accept for publication in our journal. Authors, whose papers are rejected by *The Journal of Sexual Medicine*, may be offered the option of having the paper, along with any related peer reviews, automatically transferred for consideration by the Editors of *Sexual Medicine*. Publication decisions will be made a short time after the transfer takes place. The Editors of *Sexual Medicine* will accept submissions that report well-conducted research which reaches the standard acceptable for publication. *Sexual Medicine* is an Open Access journal and article publication fees apply. For more information please go to www.sm.issm.info

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Please note that if you are an author or reviewer for *The Journal of Sexual Medicine*, you automatically have an account with *Sexual Medicine* and you should use the same login information for submission to that journal that you use for the other journal.

**AIMS AND SCOPE**

*The Journal of Sexual Medicine* publishes multidisciplinary basic science and clinical research to define and understand the scientific basis of male, female, and couple's sexual function and dysfunction. As an official journal of the International Society for Sexual Medicine and the International Society for the Study of Women's Sexual Health, it provides healthcare professionals in sexual medicine with essential educational content and promotes the exchange of scientific information generated from experimental and clinical research.

*The Journal of Sexual Medicine* includes basic science and clinical research studies in the psychologic and biologic aspects of male, female, and couple's sexual function and dysfunction, and highlights new observations and research, results with innovative treatments and all other topics relevant to clinical sexual medicine.

The objective of *The Journal of Sexual Medicine* is to serve as an interdisciplinary forum to integrate the exchange among disciplines concerned with the whole field of human sexuality. The journal accomplishes this objective by publishing original articles, as well as other scientific and educational documents that support the mission of the International Society for Sexual Medicine.

**International Society for Sexual Medicine Mission**

Specifically, the ISSM aims:

- To establish a scientific Society to benefit the public by encouraging the highest standards of practice, education and research in the field of human sexuality;
- To develop and assist in developing scientific methods for the diagnosis, prevention and treatment of conditions affecting human sexual function;
- To promote the publication and encourage contributions to the medical and scientific literature in the field of sexual function.

**MANUSCRIPT TYPES**

*The Journal of Sexual Medicine* publishes several types of manuscripts. A brief description of each type follows:

- Original Research

- Reports and Brief Reports
- Reviews
- Editorials
- Continuing Medical Education
- Calendar

**Original Research**
Original research papers are scientific reports from original research in sexual medicine. As a general guideline, manuscripts should be 3,000 words in length; more extensive manuscripts will be considered and judged on merit; however, authors are urged to be as concise as possible. All manuscripts must include an abstract, a maximum of 7 tables and figures (total), and up to 50 references. More may be accepted if justified.

**Reports**
Reports usually describe one to three patients with pertinent conditions. Brief Reports are concise reports of cases, clinical experience, clinical studies, drug trials, adverse effects, or devices related to sexual medicine. Maximum length of text is 1,750 words; no more than 10 bibliographic references and one figure or table per case.

**Reviews**
Review articles are timely, in-depth treatment of an issue. Reviews are usually solicited by the editors, but unsolicited materials will be considered. The text should be approximately 5,000 words, with an abstract, a maximum of 7 tables and figures (total), and up to 75 references. More references may be accepted if justified. Review articles undergo the same peer-review and editorial process as all other manuscripts submitted to the journal.

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- Abstract
- Introduction
- Aims
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It is **strongly recommended**, where appropriate, that you ensure your manuscript conforms to a reporting guideline that best fits your type of manuscript. For example, a CONSORT statement should be completed and uploaded with your manuscript for a Randomized Controlled Trial. The International Society for Sexual Medicine (ISSM) Publication Reporting Guidelines (ISSM_Reporting_Checklists.pdf) detail the appropriate checklist(s) to use per study type.

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*The Journal of Sexual Medicine* requires that all prospective, randomized, controlled trials with patient enrollment starting on or after August 1, 2007, be registered in a public database that meets the requirements of the World Health Organization. Currently, such registries include the following: www.actr.org.au (http://www.anzctr.org.au/), www.clinicaltrials.gov (http://www.clinicaltrials.gov), www.ISRCTN.org (http://www.ISRCTN.org), www.umin.ac.jp/ctr/index/htm (http://www.umin.ac.jp/ctr/index/htm), and www.trialregister.nl (http://www.trialregister.nl).

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Authors of reports of diagnostic tests are encouraged to submit the STARD flow

diagram and checklist (Bossuyt PM, Reitsma JB, Bruns DF, et al. for the STARD Group. Towards complete and accurate reporting of studies of diagnostic accuracy: the STARD initiative. Clin Chem. 2003;49:1-18).

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To ensure the highest standards of quality and accuracy, *The Journal of Sexual Medicine* strongly encourages the authentication of cell lines used in the research submitted to the journal. Manuscripts based on research using cell lines must include a statement addressing the following points in the Methods section of the manuscript:
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2. Whether the cell lines have been tested and authenticated
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- Identify all statistical methods and verify the assumptions for all statistical tests
- Provide alpha (the probability of a Type I error) for all statistical tests
- Specify whether tests are one- or two-sided
- Report the descriptive statistics (n, mean, median, and standard deviation) for all continuous variables
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- Adequately explain complex statistical procedures such a multivariate logistic regression and the Cox proportional hazards regression model and verify the assumptions of each such procedure
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1. Jones, TH, Smith, ML, Land SW. Diagnosis and treatment of erectile dysfunction. J Urol 1986;135:922-927.
2. King, RE. Sexual dysfunction in men and women. Taylor and Francis: Philadelphia 1974, 86pp.
3. Stevens RA, Otis PN. Persistent sexual arousal syndrome. In: Johnson DA, ed. Female sexual dysfunction.. Little Brown and Company: Boston, 1976, pp 100-106.

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A list of acceptable abbreviations is published in the Uniform Requirements for Manuscripts submitted to Biomedical Journals (also known as the Declaration of Vancouver). For more information, refer to: International Committee of Medical Journal Editors. Uniform requirements for manuscripts submitted to biomedical journals. Ann Intern Med 1997;126:36-47. You may contact the Editor or publisher directly with questions.

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