PRISCILA ROCHA YANAI

Salpingectomia e deferentectomia em capivara (*Hydrochoerus hydrochaeris* Linnaeus, 1766): estudo experimental comparativo entre as abordagens cirúrgicas videolaparoscópica e aberta

São Paulo
2020

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Dissertação apresentada ao Programa de Pós-Graduação em Clínica Cirúrgica Veterinária da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Mestre em Ciências.

Departamento:
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Orientador:
Prof. Dr. Luis Claudio Lopes Correia da Silva

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CERTIFICADO

Certificamos que a proposta intitulada "Salpingectomia e deferentectomia em capivara (Hydrochoerus hydrochaeris, L. 1766): estudo experimental comparativo entre as abordagens cirúrgicas videolaparoscópica e aberta ", protocolada sob o CEUA nº 7590270218 (ID 005386), sob a responsabilidade de **Luís Cláudio Lopes Correia da Silva** *e equipe; Priscila Rocha Yanai; Silvia Renata Gaido Cortopassi* - que envolve a produção, manutenção e/ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto o homem), para fins de pesquisa científica ou ensino - está de acordo com os preceitos da Lei 11.794 de 8 de outubro de 2008, com o Decreto 6.899 de 15 de julho de 2009, bem como com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi **aprovada** pela Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo (CEUA/FMVZ) na reunião de 15/08/2018.

We certify that the proposal "Salpingectomy and deferentectomy in capybara (Hydrochoerus hydrochaeris, L. 1766): experimental and comparative study between laparoscopic and open surgical approaches ", utilizing 40 Brazilian wild species (males and females), protocol number CEUA 7590270218 (ID 005386), under the responsibility of **Luís Cláudio Lopes Correia da Silva** *and team; Priscila Rocha Yanai; Silvia Renata Gaido Cortopassi* - which involves the production, maintenance and/or use of animals belonging to the phylum Chordata, subphylum Vertebrata (except human beings), for scientific research purposes or teaching - is in accordance with Law 11.794 of October 8, 2008, Decree 6899 of July 15, 2009, as well as with the rules issued by the National Council for Control of Animal Experimentation (CONCEA), and was **approved** by the Ethic Committee on Animal Use of the School of Veterinary Medicine and Animal Science (University of São Paulo) (CEUA/FMVZ) in the meeting of 08/15/2018.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 03/2018 a 02/2020 Área: Clínica Cirúrgica Veterinária

Origem:	Não aplicável biotério						
Espécie:	Espécies silvestres brasileiras	sexo:	Machos e Fêmeas	idade:	7 a 144 meses	N:	40
Linhagem:	Hydrochoerus hydrochaeris			Peso:	20 a 100 kg		

Registro IBAMA/Sisbio/Etc: SISBio

Método de Captura: Os animais serão contidos quimicamente por meio do disparo de dardos anestésicos com a associação de cloridrato de cetamina (5mg/kg) e cloridrato de xilazina (1,0mg/kg). Em seguida, os animais serão encaminhados para o centro cirúrgico onde serão mantidos sob anestesia geral com isofluorano diluído em oxigênio. Serão avaliadas as variáveis de frequência cardíaca, frequência respiratória, pressão arterial, saturação da oxihemoglobina e temperatura retal. Todos os parâmetros serão avaliados a cada 15 minutos. Os animais receberão criterioso acompanhamento médico até que consigam se manter em posição quadrupedal, com recuperação pós-anestésica segura para a soltura.

Local do experimento: Centro cirúrgico do Criadouro Científico da Associação Mata Ciliar, Jundiaí/SP.

São Paulo, 22 de janeiro de 2019

Profa. Dra. Anneliese de Souza Traldi
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretária
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

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Dados do titular

Nome: Luis Claudio Lopes Correia da Silva	CPF: 083.396.138-12
Nome da Instituição: Faculdade de Medicina Veterinária e Zootecnia USP	CNPJ: 63.025.530/0019-33

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Procedimento cirúrgicos nos animais	04/2018	03/2020

Equipe

#	Nome	Função	CPF	Nacionalidade
1	MARIO ANTONIO FERRARO REGO	Pesquisador colaborador	305.714.968-57	Brasileira
2	Priscila Rocha Yanai	Pesquisadora colaboradora	324.974.378-07	Brasileira
3	SILVIA RENATA GAIDO CORTOPASSI	Pesquisadora colaboradora	179.464.048-75	Brasileira
4	CRISTINA HARUMI ADANIA	Pesquisadora colaboradora	041.118.578-09	Brasileira

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Dados do titular

Nome: MARIO ANTONIO FERRARO REGO	CPF: 305.714.968-57
Título do Projeto: Avaliação cardiovascular da anestesia inalatória em capivara (Hydrochoerus hydrochaeris) submetidas a salpingectomia e deferentector	
Nome da Instituição: Faculdade de Medicina Veterinária e Zootecnia USP	CNPJ: 63.025.530/0019-33

Cronograma de atividades

#	Descrição da atividade	Início (mês/ano)	Fim (mês/ano)
1	Captura e cirurgia dos animais	06/2019	05/2021
2	Análise dos resultados	06/2021	08/2021
3	Confecção do Relatório	08/2021	09/2021

Equipe

#	Nome	Função	CPF	Nacionalidade
1	Priscila Rocha Yanai	Médica Veterinária	324.974.378-07	Brasileira
2	SILVIA RENATA GAIDO CORTOPASSI	Orientadora	179.464.048-75	Brasileira

FOLHA DE AVALIAÇÃO

Autor: YANAI, Priscila Rocha

Título: **Salpingectomia e deferentectomia em capivara (*Hydrochoerus hydrochaeris* Linnaeus, 1766):** estudo experimental comparativo entre as abordagens cirúrgicas videolaparoscópica e aberta

Dissertação apresentada ao Programa de Pós-Graduação em Clínica Cirúrgica Veterinária da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo para obtenção do título de Mestre em Ciências.

Data: _____/_____/_____

Banca Examinadora

Prof. Dr._____

Instituição:_____ Julgamento:_____

Prof. Dr._____

Instituição:_____ Julgamento:_____

Prof. Dr._____

Instituição:_____ Julgamento:_____

DEDICATÓRIA

À minha mãe.

Aos meus amados sobrinhos: Leonardo, Gustavo e Rafaela.

A todos que foram meus Professores.

À Tina, Loba, Ula, Nenê, Amelie e ao Scooby.

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Às capivaras deste estudo.

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O presente trabalho foi realizado com apoio da Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Código de Financiamento 001.

RESUMO

YANAI, P.R. **Salpingectomia e deferentectomia em capivara (*Hydrochoerus hydrochaeris* Linnaeus, 1766):** estudo experimental comparativo entre as abordagens cirúrgicas videolaparoscópica e aberta. 2020. 95 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

A capivara é um roedor silvestre e potencial disseminador da Febre Maculosa Brasileira. Os métodos mais adequados para o seu controle populacional são a deferentectomia e a salpingectomia. Visto que as técnicas videolaparoscópicas geralmente apresentam vantagens sobre as correspondentes abertas, objetiva-se comparar estas duas abordagens para os procedimentos de salpingectomia e deferentectomia em capivaras. Para tanto, quatro técnicas cirúrgicas foram desenvolvidas, descritas e avaliadas: deferentectomia por técnica aberta (DA); deferentectomia por videolaparoscopia (DV); salpingectomia por técnica aberta (SA); e salpingectomia por videolaparoscopia (SV). Cinco cadáveres de capivara foram preliminarmente utilizados para estudo anatômico. Trinta e nove capivaras hígidas, adultas, machos e fêmeas, oriundas de vida livre, foram distribuídas entre as quatro técnicas, sendo os filhotes excluídos do estudo. Os animais foram contidos quimicamente, intubados e mantidos sob anestesia geral com isofluorano. A soltura ocorreu no mesmo dia do procedimento cirúrgico, após constatada a recuperação anestésica. O tempo de ligadura do ducto deferente ou tuba uterina, tempo cirúrgico total, tamanho de incisão, complicações anestésicas e cirúrgicas, estado clínico após uma semana de pós-operatório e a qualidade de cicatrização da ferida foram analisados. Foram realizadas 12 SA, 11 SV, 9 DA e 7 DV. Todas as técnicas cirúrgicas propostas, definidas após a investigação do acesso cirúrgico mais adequado, mostraram-se exequiveis e seguras. Timpanismo e hipotensão arterial foram as principais complicações transoperatórias e ocorreram em todas as técnicas. Não houve diferença clínica perceptível na recuperação pós-operatória entre as abordagens. Com relação ao tempo cirúrgico total dos procedimentos propostos, porém, os realizados por videolaparoscopia (35.6 ± 4.2 e 82.3 ± 29.1 minutos para DV e SV, respectivamente) apresentaram a vantagem de terem sido efetuados em menor tempo que os procedimentos abertos (71.7 ± 27.1 e 91.2 ± 23.7 minutos para DA e SA, respectivamente).

Palavras-chave: Laqueadura. Vasectomia. Controle reprodutivo. Videocirurgia.

ABSTRACT

YANAI, P.R. **Salpingectomy and deferentectomy in capybara (*Hydrochoerus hydrochaeris*):** laparoscopic versus open surgical approach. 2020. 95 f. Dissertação (Mestrado em Ciências) – Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2020.

Capybara is a wild rodent and potential disseminator of Brazilian Spotted Fever. The most suitable methods for its population control are deferentectomy and salpingectomy. Since videolaparoscopic techniques generally have advantages over open techniques, the objective was to compare these two approaches for salpingectomy and deferentectomy procedures in capybaras. For that, four surgical techniques were developed, described and evaluated: deferentectomy by open technique (DA); deferentectomy by videolaparoscopy (DV); salpingectomy by open technique (SA); and salpingectomy by videolaparoscopy (SV). Five capybara cadavers were previously used for anatomical study. Thirty-nine free-ranging capybaras, adults, males and females, were distributed among the four techniques, and the cubs were excluded from the study. The animals were chemically restrained, intubated and kept under general anesthesia with isofluorane. The animal release occurred on the same day of the surgical procedure, after verifying anesthetic recovery. The time of ductus deferens or tubal ligation, total surgical time, incision size, anesthetic and surgical complications, clinical status after one-week postoperative and wound healing were analyzed. Twelve SA, 11 SV, 9 DA and 7 DV were performed. All the proposed surgical techniques, defined after the investigation of the most appropriate surgical access, proved to be feasible and safe. Tympanism and arterial hypotension were the main intraoperative complications and occurred in all techniques. There was no evident clinical difference in postoperative recovery between the approaches. Regarding the total surgical time of the proposed procedures, however, those performed by videolaparoscopy (35.6 ± 4.2 and 82.3 ± 29.1 minutes for DV and SV, respectively) had the advantage of having been performed in less time than the open procedures (71.7 ± 27.1 and 91.2 ± 23.7 minutes for DA and SA, respectively).

Keywords: Tubal ligation. Vasectomy. Reproductive control. Videolaparoscopy.

SUMÁRIO

1 INTRODUÇÃO GERAL

Um mundo, uma só saúde. O conceito de saúde-única parte do princípio de que o estado sanitário dos seres humanos está relacionado com a saúde dos animais, que ambos afetam o meio-ambiente e são igualmente afetados por ele (KING et al., 2008). Um bom exemplo disso é o caso da disseminação da Febre Maculosa Brasileira (FMB). Nos humanos, a FMB é a doença transmitida por carrapatos mais letal do hemisfério ocidental, com taxa de letalidade entre 50-80% no sudeste no Brasil (WALKER, 2002; ANGERAMI et al., 2006, 2009; DE OLIVEIRA et al., 2016). Desequilíbrios no meio-ambiente causados por queimadas, desmatamentos e a caça de espécies de níveis tróficos mais altos, por exemplo, propiciam a reprodução descontrolada de populações de capivaras (*Hydrochoerus hydrochaeris*) e a sua aproximação de áreas periurbanas (OJASTI, 1973; PAGLIA, 1997; MOREIRA; PIOVEZAN, 2005; VERDADE; FERRAZ, 2006; FERRAZ et al., 2007). Estes animais são hospedeiros do carrapato-estrela (*Amblyomma sculptum*), vetor da bactéria *Rickettsia rickettsii*, causadora da FMB (VIEIRA et al., 2004; NAVA et al., 2014).

A capivara é uma espécie de roedor herbívoro que ocupa diversos tipos de ambientes naturais, vastamente distribuída na América do Sul (OJASTI, 1973), possui alto grau de adaptabilidade à presença humana e elevado potencial reprodutivo. Em casos de risco à saúde pública, o controle populacional desta espécie faz-se necessário. Técnicas de remoção de tais populações, através da caça, eutanásia ou pela simples transferência para áreas afastadas da população humana são de baixa eficácia, uma vez que pode ocorrer a migração de novos indivíduos oriundos de outras áreas (MOREIRA; PIOVEZAN, 2005), e a taxa de natalidade pode inclusive se elevar, dada a maior disponibilidade de recursos e consequente redução na competição entre indivíduos das populações remanescentes das áreas manejadas.

Geralmente não existe uma estratégia única e o controle populacional pode ser atingido de outras formas. No entanto, considerando-se que métodos para controle reprodutivo utilizados em outras espécies, como implantes hormonais e administração de imunocontraceptivos, ainda não foram efetivamente validados ou tiveram a sua eficácia comprovada a longo prazo nas capivaras (ROSENFIELD et al., 2019), a contracepção cirúrgica através de vasectomia (ou deferentectomia) e laqueadura (ou salpingectomia) parece ser ainda a estratégia mais eficaz no momento.

As capivaras são animais territorialistas e formam grupos familiares que apresentam uma forte hierarquia social (ALHO; CAMPOS; GONÇALVES, 1987). Por isso, a deferentectomia e a salpingectomia são preferíveis à castração como método contraceptivo, por não interferirem nos níveis hormonais do animal, evitando-se a sucessão na hierarquia familiar e comprometimento da estabilidade do grupo, situação esta que tornaria totalmente ineficaz o controle reprodutivo com finalidade de supressão da disseminação da FMB.

As técnicas cirúrgicas abertas de deferentectomia e salpingectomia já foram utilizadas em capivaras com sucesso (RODRIGUES et al., 2017; NUNES et al., 2020), porém o enfoque destes estudos não foi a descrição da técnica cirúrgica e, portanto, questões relevantes referentes aos procedimentos não foram tratadas. Deferentectomia e salpingectomia por cirurgia videolaparoscópica já foram desenvolvidas para outras espécies animais (SILVA et al., 1993; OLIVEIRA JR et al., 2003; HERNANDEZ-DIVERS et al., 2007; FERANTI et al., 2013; MARAIS et al., 2013; YOON; CHUNG; LEE, 2014; HARTMAN et al., 2015a, 2015b; HEIDERICH; SCHILDGER; LIERZ, 2015; YU et al., 2015), porém não há estudos publicados acerca da utilização de tais técnicas em capivaras.

Com base no exposto, este presente estudo tem, portanto, o intuito de desenvolver, descrever e avaliar as técnicas videolaparoscópicas e abertas de deferentectomia e salpingectomia em capivaras, para fornecer ao médico veterinário atuante em programas de controle populacional de capivaras, informações claras de como executá-las. Além disso, comparar estas abordagens, afim de avaliar se as vantagens usualmente verificadas quando da utilização de procedimentos videolaparoscópicos em outras espécies silvestres reflete-se igualmente na capivara a ponto de justificar a sua recomendação.

2 APRESENTAÇÃO DOS ARTIGOS

Esta dissertação é apresentada em formato de artigos. Os textos foram escritos na língua inglesa para publicação internacional e seguindo as diretrizes do periódico *Veterinary Surgery* para submissão. Este periódico permite a publicação das versões *preprint* (versão de submissão, antes da avaliação por pares) em dissertações de mestrado depositadas em repositórios universitários de acesso aberto, sem a necessidade de período de embargo (Anexo B). Mario Antônio Ferraro Rego, Andressa de Fátima Kotleski Thomaz de Lima, Silvia Renata Gaido Cortopassi e Luis Claudio Lopes Correia da Silva são coautores em todos os artigos.

Para que o objetivo do trabalho fosse atingido, qual seja, a comparação entre as abordagens cirúrgicas aberta e por videolaparoscopia, foi necessário previamente, no entanto, desenvolver as técnicas cirúrgicas a serem comparadas. Assim, entre os capítulos 3 a 6 estão divididos os 4 artigos, cada qual abordando um dos quatro procedimentos cirúrgicos estudados.

O primeiro artigo encontra-se no capítulo 3. Nele, é discutido a viabilidade de execução da laqueadura aberta em capivaras a partir de 3 acessos cirúrgicos diferentes: celiotomia mediana ventral, laparotomia unilateral ou laparotomia bilateral. Além disso, são apresentadas questões relevantes sobre a anatomia cirúrgica.

No capítulo seguinte, o procedimento cirúrgico abordado é a vasectomia aberta em capivaras. Neste caso, quatro locais de acesso cirúrgico são avaliados com relação a viabilidade de execução: acesso pré-púbico pela linha mediana, acesso bilateral sobre o canal inguinal, acesso bilateral sobre os testículos e acesso bilateral sobre o cordão espermático.

O desenvolvimento das técnicas de salpingectomia por videolaparoscopia e de deferentectomia videoassistida é referido nos capítulos 5 e 6, respectivamente.

Ao final do trabalho, nas considerações finais, partindo dos dados levantados nos artigos, é realizada a comparação entre as abordagens.

3 TUBAL LIGATION IN CAPYBARAS (*HYDROCHOERUS HYDROCHAERIS*): DESCRIPTION OF SURGICAL ANATOMY AND TECHNIQUES

3.1 Abstract

Objective: Identify the main anatomical structures accessed or serve as reference for the appropriate surgical approach to the uterine tube, develop and describe the most feasible surgical technique for salpingectomy in capybaras.

Study Design: Experimental study.

Animals or Sample Population: Cadaveric (n=3) and healthy capybaras (n=12).

Methods: Dissection of 3 capybara cadavers were performed for anatomical study purposes. Three surgical accesses were tested in vivo: ventral midline celiotomy (n=1); and unilateral or bilateral laparotomies (n=11). Recordings of body weight, tubal ligation time, total surgical time, incisions size, surgical and anesthetic complications, one-week postoperative clinical status and incisional healing were analyzed.

Results: The surgical access by ventral midline celiotomy was difficult, required a long time to be executed (surgical time, 142 minutes) and was therefore discontinued. The bilateral laparotomy (mean surgical time, 91.2 ± 23.7 minutes) was successfully performed in all capybaras. Unilateral access to both tubas was aborted in all specimens on which it was tested and had to be converted to bilateral laparotomy. Skin incisions healed without complications.

Conclusion: The surgical access for salpingectomy with minimal incisions was technically challenging in capybaras, because of the thickness of the abdominal wall and surround tissues topography. The bilateral laparotomy was the most feasible approach for adult capybaras, allowing exposure of the uterine tube without compromising vital structures or causing excessive intraoperative pain.

Clinical Significance (or Impact): The bilateral laparotomy proposed for tubal ligation must be considered in the population control of free-ranging adult capybaras to prevent perioperative complications.

O manuscrito apresentado neste capítulo foi submetido ao periódico *Veterinary Surgery* em 19 de outubro de 2020, sob a identificação VSU-20-326 (Anexo A).

3.2 Introduction

Capybara population control is becoming one of the most serious conservation problems in Brazil. Habitat fragmentation, absence of predators, a high degree of adaptability to human presence and different environmental conditions associated with its high reproductive capacity and potential to spread Brazilian Spotted Fever (BSF), increases the public health concerns about this species.[1–4]

Epidemiological, legal and ethical precepts are strong obstacles for the slaughter and translocation of capybaras. Removal techniques are of low efficacy since the migration of new individuals from other areas may occur[5] and because of the strong hierarchical characteristic in this species. The simple removal of capybara in endemic areas of BSF may also aggravate the risk of transmission, due to the increase of susceptible animals and consequent amplification of *Rickettsia rickettsii* in ticks.[6]

There are no reports of the use of hormonal implants in this species for contraceptive purposes. The study of the use of immunocontraceptives in capybaras is recent[7] and has not yet been validated for females. Thus, the most appropriate methods for population control of capybaras are still vasectomy and tubal ligation (or salpingectomy). Such techniques have already been used on the management of capybaras populations,[8,9] but a detailed description of the surgical procedure has never been given.

Because of increased recognition of issues discussed above, salpingectomy may become a more frequently performed procedure in campaigns of capybaras population control. The potential application of this procedure for definitive contraception along with the complexity of the execution of this procedure in the field warrant a comprehensive description of the surgical anatomy, approaches and technique. Anatomic study of the body wall and the topography of tissues closely related to the reproductive system is essential before undertaking the surgical procedure. Equally important, the evaluation of different accesses to salpingectomy allows comparing the feasibility between them and choosing the most appropriate for each situation.

Therefore, the aim of this study was to identify the main anatomical structures accessed or used as a reference for the appropriate surgical approach of the uterine tube, and develop and describe the most feasible surgical access for salpingectomy in capybaras.

3.3 Materials and methods

3.3.1 Animals

This study was approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo (CEUA FMVZ/USP; No. 7590270218) and Brazilian Biodiversity Information and Authorization System (SISBIO No. 62352-1 and 70016-1).

In order to study the myology of the abdominal wall, the anatomical topography of the reproductive system and surgical accesses to the uterine tube, cadavers of intact female capybaras (one juvenile and two adults) were used. These animals, whose deaths were unrelated to the study, were kept frozen at -20°C until its use and had no history of abdominal surgery. Three accesses to uterine tube were studied: unilateral laparotomy, bilateral laparotomy and ventral midline celiotomy (in this sequence). This investigation consisted of finding a reference for skin incision, identifying the muscles through the incision and testing the viability of the uterine tube exposure.

Following this preliminary study, those three surgical accesses were then evaluated in free-ranging capybaras (n=12). The ventral midline celiotomy was performed in only one specimen. In 11 animals, unilateral access to both uterine tubes was first attempted and, when not feasible, the procedure was converted to bilateral laparotomy. Cubs (body weight < 10 kg) were excluded from this study at the request of SISBIO.

3.3.2 Anesthesia

Capybaras were conditioned to enter a corral using a bait of sugar cane minutes before the surgical procedure. Under these conditions, it was not possible to establish a previous fasting. The animals were chemically restrained by intramuscular darts with a combination of ketamine (7 mg/kg) and dexmedetomidine (10 µg/kg). Anesthesia was induced with propofol (4–6 mg/kg IV) and maintained with isoflurane in oxygen via a circle rebreathing circuit. Lactated Ringer solution at 5 mL/kg/hour was administered intravenously during surgical procedure. Anesthesia was monitored by electrocardiogram, indirect and direct arterial blood pressure, pulse oximetry, and capnography. A single dose of oxytetracycline (20 mg/kg IM) was administered at the

beginning of the procedure. Postoperative analgesia was provided with flunixin meglumine (1.1 mg/kg IM), sodium dipyrone (25 mg/kg IM) and tramadol hydrochloride (2mg/kg IM).

After being weighed, the capybara was positioned in lateral or dorsal recumbency and the surgical site was clipped, scrubbed, and draped for surgery. All animals received microchip implant and ear tag prior to anesthetic recovery. At the end of the surgical procedure, capybaras were weaned off the ventilator when necessary, transported back to the corral, extubated, and allowed to recover under constant supervision of the anesthetic team. The capybaras were released on the same day once adequate deambulation was observed.

3.3.3 Surgical Procedures

3.3.3.1 Salpingectomy by ventral midline celiotomy

This access was performed in one live adult capybara positioned in dorsal recumbency (Figure 1). The opening of the abdominal cavity on the midline with an 8-cm pre-umbilical incision, started 3-cm caudally to the level of the last rib, progressing toward the umbilical scar, continuing through the cutaneous muscle and linea alba. The abdominal cavity was inspected and the uterine body was identified dorsally to the bladder. Following the uterine horns, as a referential structure, it was possible to identify the ovaries and then the uterine tubes. Traction of these structures toward the midline was performed after moving and putting out parts of the cecum and large colon. The uterine tubes were isolated from the mesosalpinx by blunt dissection using an hemostatic forceps. Tubal ligation was performed by applying two medium/large size (closed length, 8.99-mm) titanium clips (Ethicon Endo-Surgery, Cincinnati, OH) in each uterine tube. About 1-cm segment of both uterine tubes were excised between the clips. The linea alba was closed with 2-0 nylon by using a simple interrupted pattern. The cutaneous muscle and subcutaneous layer were sutured together in a simple continuous pattern with 2-0 nylon. The skin was approximated with an intradermal pattern and closed using a simple interrupted pattern with 2-0 nylon.

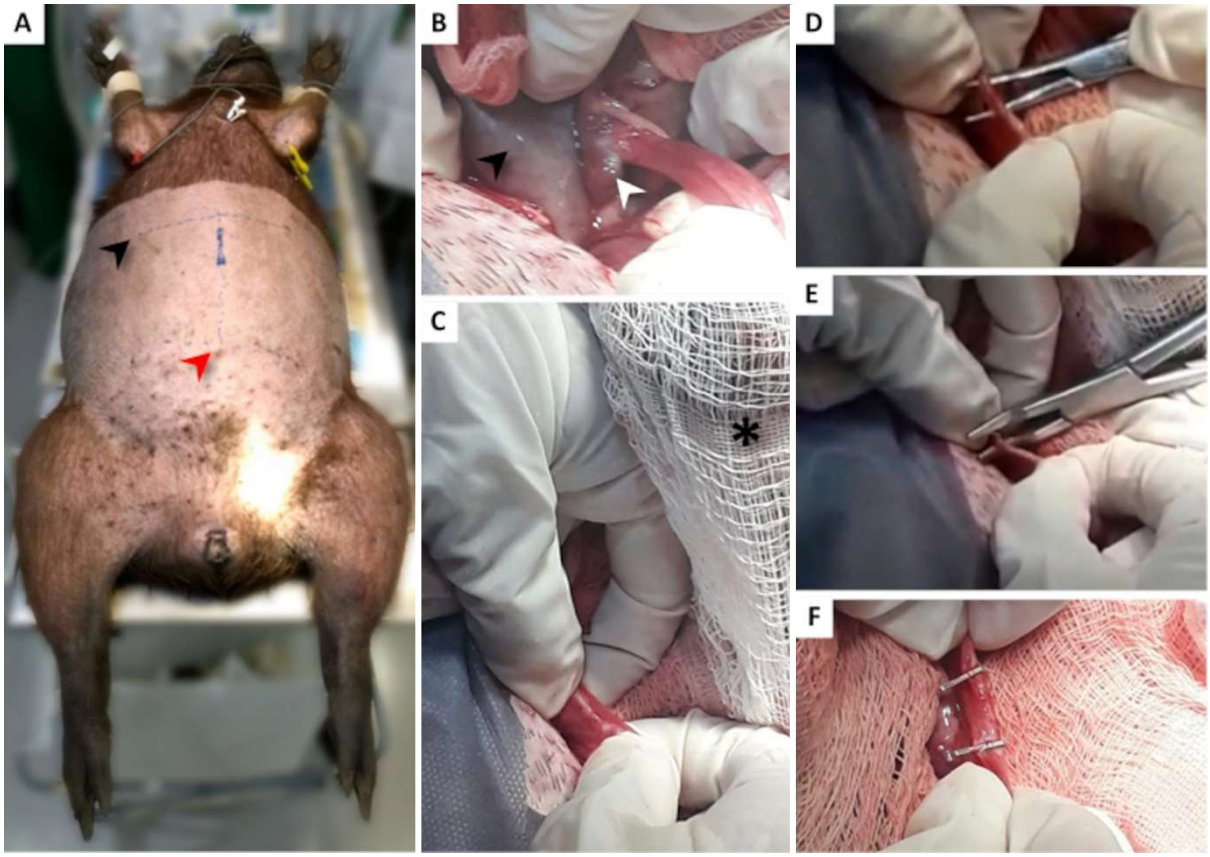

FIGURE 1 Salpingectomy in capybara by ventral midline celiotomy. **A**, Incisional site planning: references of the last rib (black arrowhead) and umbilical scar (red arrowhead). **B**, Traction of the uterus to locate and expose the left ovary; ovarian bursa (white arrowhead) and stomach (black arrowhead). **C**, Uterine tube isolation; note the exteriorization of part of the intestines (asterisk) under the surgical sponge. **D**, Divulsion of the uterine tube from the mesosalpinx. **E**, First clip placement. **F**, Uterine tube double ligated.

3.3.3.2 Salpingectomy by unilateral and bilateral flank celiotomy (laparotomy)

The animals (n=11) were positioned in lateral recumbency, a flank incision was initiated approximately 2-cm from the ventral border of the lumbar iliocostalis musculature, extending toward the ventral midline. This incision of 2 to 3 centimeters in size was centered between the wing of ilium and the last rib (Figure 2). Blunt dissection was performed from the underlying muscle layers to the transverse fascia and peritoneum. After sectioning of the peritoneum, palpation of the abdominal structures was performed to locate the ipsilateral ovary suspensory ligament, as a referential, then the ovary and uterine horn. These structures were positioned just below the incision, and a castration hook was used to expose them out of the abdominal cavity (Figure 3). The sequential procedures performed for tubal ligation were the same as those described for ventral midline celiotomy. Traction of the contralateral uterine horn or suspensory

ligament toward the incision site was performed in an attempt to access the contralateral uterine tube. In cases where the opposite uterine tube could not be apprehended through the unilateral laparotomy, the incision site was closed and the animal moved to the contralateral recumbency to proceed with the bilateral laparotomy technique. The synthesis of the abdominal muscles and subcutaneous tissue layers were performed separately with a simple continuous pattern using 3-0 poliglecaprone 25. The skin was closed with 3-0 nylon using intradermal and simple interrupted patterns.

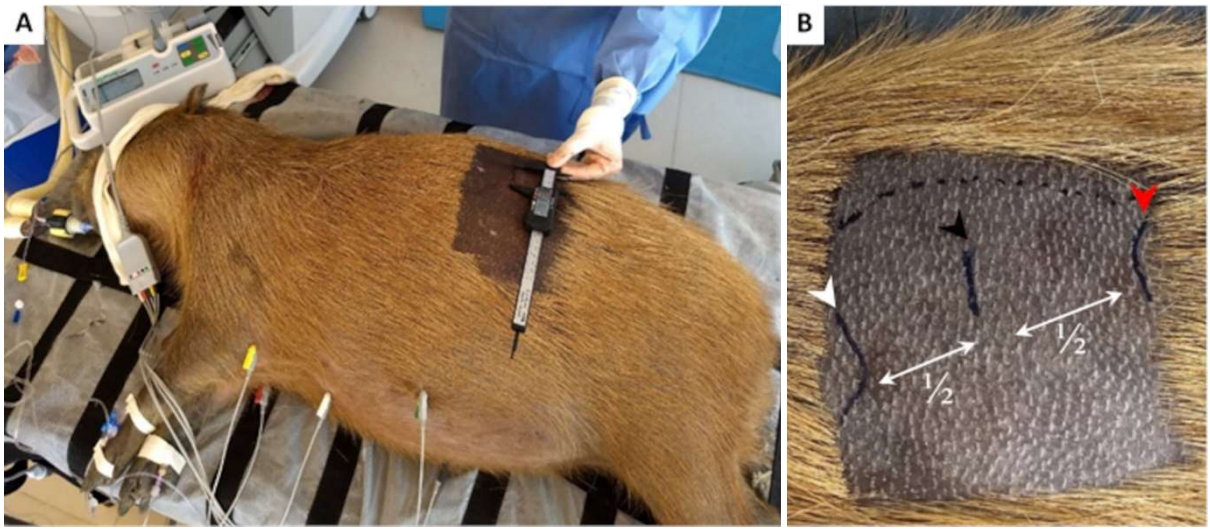

FIGURE 2 Planning of salpingectomy in capybara by bilateral laparotomy. **A**, Animal positioned in lateral recumbency. **B**, References of last rib (white arrowhead), wing of ilium (red arrowhead) and ventral border of iliocostalis muscle (dashed line) found by palpation; incisional site (black arrowhead).

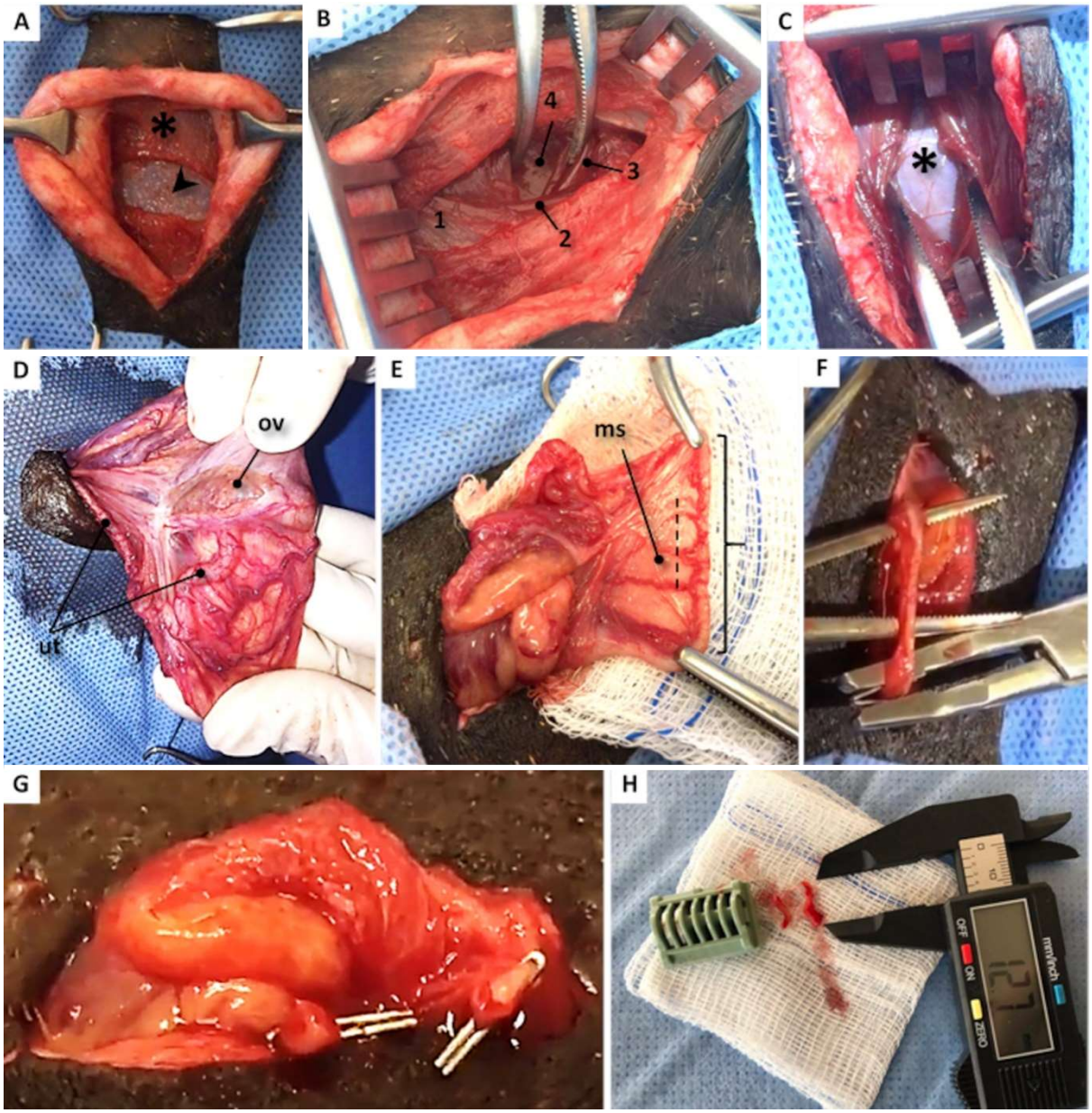

FIGURE 3 Salpingectomy in capybara by bilateral laparotomy. **A**, Skin and cutaneous muscular layer incised (*); thoracolumbar fascia exposed (black arrowhead). **B**, Thoracolumbar fascia (1), external abdominal oblique (2) and internal abdominal oblique muscles. (3) blunt dissected; M. transversus abdominis (4) exposed. **C**, M. transversus dissected and transversalis fascia exposed (*) prior to opening the peritoneum. **D**, Exteriorization of the ovary (ov) and uterine tube (ut). **E**, isolation of the uterine tube (ut); incisional site (dashed line) in mesosalpinx (ms). **F**, Clipping of the uterine tube. **G**, Uterine tube ligated and excised. **H**, One-centimeter fragment of both uterine tubes removed.

3.3.4 Outcome measures

The body weight, tubal ligation and total surgical times, incisions size, intraoperative and postoperative complications were recorded for all procedures. Capybaras were recaptured 1-week after surgery to evaluate clinical status and incisional healing. Tubal

ligation time was considered from incision to clip ligation for each side. Total surgical time corresponded to the time elapsed between the first incision and the last suture of the skin.

3.3.5 Statistical Analysis

All data were subjected to Shapiro-Wilk normality test. Data are mean ± SD and range. A paired *t* test was used for comparison between the left and right sides approached for the parameters: tubal ligation time and incision size. Statistical significance was set as $P < .05$. All statistical analyses were performed using software RStudio version 0.99.903 (RStudio Inc, Boston, MA).

3.4 Results

3.4.1 Description of the surgical anatomy

The following significant anatomic structures were present on the abdominal wall (Figure 4): external oblique abdominal muscle, rectus abdominis muscle, internal oblique abdominal muscle, transversus abdominis muscle, transversalis fascia and peritoneum. In adult capybaras, the skin had a thick dermis approximately 1-cm deep, and the cutaneous muscle was robust in all its extension, from its thoracic limit to the inguinal region. The great omentum did not appear covering the intestines; it was under the cecum and small intestine when the cavity was accessed ventrally. The uterine horns had a dorsal location, with the interposition of the well-developed intestinal tract between them and the ventral abdominal wall. The stomach and/or part of the large intestine were the structures that overlap the left ovary, while the small intestine was concentrated around the right ovary. Both ovaries were located in the middle third of the space between the last rib and the wing of ilium, supported by a fibrous, inelastic and relatively short suspensory ligament.

FIGURE 4 Anatomical topography of the capybara female reproductive tract. **A**, Cadaver of an adult specimen in lateral recumbency (30-cm scale beside). **B**, Dorsal recumbency, showing umbilical scar (white arrowhead). **C**, External appearance of the skin fold where the terminal portions of the rectum, reproductive and urinary tracts are found. **D**, Skin reflected exposing the m. cutaneous (1); external abdominal oblique muscle (2) and its aponeurosis (3). **E**, Abdominal cavity opened; external abdominal oblique muscle reflected, exposing the internal abdominal oblique muscle (4). **F**, Cecum (5); stomach (6); small (7) and large (8) intestines. **G**, Small intestines reflected to expose de right ovary (9); bladder (10) caudally reflected to expose the uterine body (11). **H**, Stomach reflected to expose the left ovary (12).

3.4.2 Surgical Approaches

The mean body weight of the capybaras undergoing salpingectomy was 51.3 ± 11 kg, which corresponds to adult animals. The approach by bilateral laparotomy was successfully performed in all capybaras without any intraoperative complications. The access to both tubas by unilateral laparotomy was only possible during the preliminary studies with the cadaver of a juvenile capybara (estimated body weight, 25 kg). In live and larger animals, all unilateral approaches had to be converted to bilateral laparotomy. In this case, mean surgical time was 91.2 ± 23.7 minutes and means of incision sizes were 2.9 ± 0.6 and 3.1 ± 0.7 centimeters for right and left side, respectively, taking an average of 26 ± 9.2 minutes to ligate the right uterine tube and 22.3 ± 11.3 minutes to the left (Table 1). No statistical differences were observed between the sides regarding incision size (P = .478) or tubal ligation time (P = .376). The procedure started from the right side in 5 capybaras and the left in 6.

TABLE 1 Summary data of 11-female capybaras (*Hydrochoerus hydrochaeris*) undergoing salpingectomy by bilateral laparotomy.

Capybara	Weight (kg)	First side operated	Tubal ligation time (min)		Total surgical time (min)	Incision size (cm)	
			Right	Left		Right	Left
1	36	Left	31	47	149	3.22	3.19
2	59	Left	19	26	104	3.14	4.42
3	38	Right	34	15	96	3.75	3.46
4	44.7	Right	26	19	84	3.25	3.16
5	43.2	Left	24	16	79	3.4	2.38
6	54.6	Left	21	20	83	2.06	3.15
7	44.5	Right	24	38	101	3.5	2.76
8	65.7	Left	45	23	97	2.5	3.1
9	59.8	Right	33	12	81	2.14	2.1
10	68.4	Right	16	20	73	2.28	2.43
11	50	Left	13	9	56	2.82	3.7

The capybara submitted to ventral midline celiotomy weighed 59.6 kg. This procedure lasted 142 min and the incision site had to be enlarged caudally to 12-cm in order to be able to properly expose the ovary by removing part of the cecum from the abdominal cavity. Due to these difficulties, the ventral midline approach was performed only once and then was discontinued.

During the time between chemical restraint and surgical recovery, all animals presented tympanism in different degrees and frequencies, which required intervention through the introduction of a percutaneous catheter to perform gastric or intestinal decompression. Some animals developed severe arterial hypotension and ephedrine administration was necessary. The animal accessed by the ventral midline required the administration of fentanyl for pain control when traction of the ovarian pedicle was performed. Slight increases in heart rate and systolic blood pressure were also evident when the contralateral uterine tube was tractioned to try unilateral access to both tubes. Hemorrhage was minimal and easily controlled for all approaches.

All capybaras recovered well from surgery. No signs of abnormal behavior were observed in the immediate postoperative period and the animals walked actively within 2 hours. One-week after the surgical procedure, all incisions showed satisfactory healing and all animals were clinically healthy and did not show any major or minor complications associated with the procedure (Figure 5).

FIGURE 5 One-week postoperative status of capybaras subjected to salpingectomy. **A**, Salpingectomized capybara showing normal behavior and comfortable posture. **B**, Surgical wound healing without signs of infection or complications.

3.5 Discussion

In this study, only approaches by ventral midline celiotomy and bilateral laparotomy proved viable for salpingectomy of adult capybaras. In contrast with a previous study,[10] that suggested the left unilateral laparotomy as the best approach for tubal ligation in capybaras, this technique was practicable only in the cadaver of a juvenile capybara in our study. In addition, based on our anatomical study and on attempts made on adult individuals (body weight > 36 kg), we hypothesized that unilateral laparotomy is viable only in cubs and juvenile individuals (body weight < 30 kg) using a proportionally larger and diagonally angled incision.

Other recent study[9] recommended the ventral midline access. However, just like the study carried out by Rodrigues et al,[10] some pertinent points were unclear, such as, which age group the individuals undergoing this procedure belonged to and how extensive was the incision used to make this access possible. Although we had tested ventral midline celiotomy in only one animal and found that it is feasible, the requirement of accentuated traction of the suspensory ligament and the exteriorization of part of the intestines to have adequate access to the uterine tube, make this procedure less recommended than the bilateral laparotomy for adult capybaras salpingectomy.

There was no difference between the times for right and left tubal ligation in the bilateral approach, including similar access incision sizes, which suggests that the difficulty of access by both sides is equal. The total surgical time and tubal ligation time to perform salpingectomy in capybaras were relatively longer than that observed in other species.[11,12] However, the purpose of counting the time in this study was only descriptive, there was no concern in relation to stopping the count to record images of the procedure or to deal with small anesthetic interventions such as resuming a lost arterial access, for example. It is believed that in a non-scientific situation in the field, without the need for more accurate records and monitoring, salpingectomy by bilateral laparotomy can be performed in capybaras in a significatively shorter time.

As previously described, [13] the uterine horns, ovaries and uterine tube have a shape and topography similar to the guinea pig,[14] a specie of the same family. Due to the short suspensory ligament and the presence of cecum and large colon that protrude out of the cavity at the time of the incision, it is known that the exteriorization

of the ovaries during the castration of guinea pigs through the linea alba access is more difficult compared with the bitch and we found the same occurring in capybaras.[15]

Other relevant anatomical variances were found. Capybaras, like guinea pigs,[16] does not have the greater omentum extensively covering the intestine,[17] which facilitates the identification and apprehension of the suspensory ligament of the ovary using castration hooks and allows the use of smaller incisions. A specific characteristic of the muscle morphology was the presence of a robust cutaneous muscular layer. Because they are semi-aquatic animals, this may be an advantageous attribute in relation to the closure of the abdominal cavity, since it presents as another barrier to the penetration of water during the healing of the surgical wound. Considering this, it is also suggested the use of non-absorbable monofilament suture in the skin closure, since the animals usually search for places with water or mud as a point of escape during the postoperative period.

It is possible to perform salpingectomy on any portion of the uterine tube, however, as the uterine horn is more easily drawn out of the abdominal cavity than the ovarian suspensory ligament, it is recommended to execute this procedure on the caudal portion of the uterine tube, close to the uterine horn, to minimize pain caused by excessive traction of the pedicle.

The animals showed to be stressed when kept for many hours in the corral, with escape attempts and fights. For this reason, we chose to perform the capture only at the time of the procedure, and this strategy made it impossible to perform a previous fast. It is not certain whether the lack of fasting contributed to the occurrence of tympanic episodes in the intraoperative period, or if it was a reaction to the drugs used in chemical restraint as discussed by Rosenfield et al,[18] or even a combination of both. The fact is that this issue deserves attention and further studies.

The animals evaluated about a week after the surgical procedure showed a satisfactory evolution of the surgical wound healing, despite diving in the water in the immediate postoperative period. The use of a single dose of oxytetracycline at the beginning of the procedure seems to be sufficient to prevent infection, but it may even be unnecessary, if minimal surgical access and non-absorbable suture are used to close the skin. This would be an important point to be explored in the future, since we should always try to minimize the use of antibiotics. As these animals urinate and defecate in the water, concern must be given to the contamination of the environment and its effects on the ecosystem.

Finally, this was the first study with focus on surgical anatomy and development of surgical techniques for the capybara species. It is found that salpingectomy by bilateral laparotomy seems to be the best approach in adult individuals because allows less exposure of internal tissues, an important condition when performing surgical procedures in the field; requires less traction of the ovarian pedicle causing minor intraoperative pain; and allows access to uterine tubes relatively easier and faster. However, a limitation of our study was the impossibility to test all these approaches in vivo on smaller animals, because all the captured animals were adults and the SISBIO permission restricted the performance of surgeries in cubs. Thus, further investigations are needed to assess whether unilateral laparotomy and ventral midline celiotomy are better alternatives in younger age groups.

3.6 Acknowledgments

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3.7 Disclosure

The authors report no financial or other conflicts related to this report.

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4 VASECTOMY IN CAPYBARAS (*HYDROCHOERUS HYDROCHAERIS*)

4.1 Abstract

Objective: Develop, describe and evaluate a feasible open surgical access for vasectomy in capybaras.

Study Design: Experimental study.

Animals or Sample Population: Cadaveric (n=2) and healthy capybaras (n=9).

Methods: Dissection of 2 capybaras cadavers were performed for anatomical study purposes. Four different incision sites in the inguinal region were tested in 9 animals. The capybaras were chemically restrained, blind intubated, and placed under general anesthesia. Recordings of body weight, ductus deferens ligation and total surgical times, incisions sizes, surgical and anesthetic complications, and one-week postoperative clinical status were analyzed.

Results: The best approach proposed for vasectomy was the bilateral inguinal access over spermatic cord (mean surgical time, 71.7 ± 27.1 minutes) and was successfully performed in all capybaras on which it was tested. With the exception of the prepubic midline access, the other accesses evaluated were also viable to reach the ductus deferens, but with disadvantages. Episodes of arterial hypotension and tympanism were observed during all procedures. Two animals died in the postoperative period of causes unrelated to the procedure. Remaining animals showed satisfactory recovery after one-week follow-up.

Conclusion: The choice of the incision site is crucial to perform the least invasive vasectomy procedure in adult capybaras. Bilateral inguinal access over the spermatic cord was the most feasible approach, allowing easier and faster access to the ductus deferens without unnecessarily accessing and exposing other structures.

Clinical Significance (or Impact): The bilateral inguinal access over spermatic cord proposed for vasectomy must be considered in the population control of free-ranging adult capybaras.

4.2 Introduction

The capybara, widely distributed in South America,[1] is a species of herbivorous rodent that occupies several types of natural and peri-urban environments.[1–4] Its high degree of adaptability to human presence associated with its high reproductive potential and the absence of predators determines changes in the landscape and an important public health problem: the spread of Brazilian Spotted Fever (BSF).[5]

These animals form family groups that have a strong social hierarchy, with the alpha male being responsible for most copulations in the group. Therefore, in the case of alpha male orchiectomy, there is a reduction in serum testosterone levels and a consequent loss of dominance behavior, with rapid succession in the hierarchy and compromised group stability, rendering reproductive control totally ineffective. Vasectomy, as it does not interfere with hormonal levels, is a more viable option for male contraception without destabilizing the family group.[6] Such techniques have already been used on the management of capybaras populations,[7,8] but a detailed approach to the surgical issues has never been given.

There are no reports of the use of hormonal implants in this species for contraceptive purposes. The study of the use of immunocontraceptives in capybaras is recent[9] and further studies are needed in order to attest if this method it is permanent or transitory.

Thus, based on the potential application of this procedure for definitive contraception along with the complexity of the execution of this procedure in the field, the aim of this study was to identify the main anatomical structures accessed or which serve as reference for the appropriate surgical approach to the ductus deferens, and develop and describe the most feasible surgical access for vasectomy in capybaras.

4.3 Materials and methods

4.3.1 Animals

The Brazilian Biodiversity Information and Authorization System (SISBIO No. 62352-1 and 70016-1) and Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo (CEUA FMVZ/USP; No. 7590270218) approved this study.

In order to study the gross anatomical topography of the male reproductive system, cadavers of intact capybaras (two adults) were used. These cadavers were kept frozen at -20°C until its use. Following this preliminary study, four surgical accesses to ductus deferens were evaluated in free-ranging capybaras (n=9): the attempt to access both ductus deferens through a single-incision in the midline of the prepubic region (n=1); bilateral inguinal access over inguinal canal (n=1); bilateral inguinal access over testicles (n=1); and bilateral inguinal access over spermatic cord (n=7). The inguinal accesses by the prepubic midline and over the inguinal canals were evaluated in the same specimen. Cubs (body weight < 10 kg) were excluded from this study at the request of SISBIO.

4.3.2 Anesthesia

Capybaras were captured minutes before the surgical procedure without a previous fasting or hydric restriction. A combination of ketamine (7 mg/kg) and dexmedetomidine (10 µg/kg) was used to chemically restraint, administrated by intramuscular darts. Anesthesia was induced with propofol (4–6 mg/kg IV) and maintained with isoflurane via a circle rebreathing circuit. The cephalic vein was used to keep an intravenous infusion of Lactated Ringer solution at 5 mL/kg/hour during the surgical procedure. Anesthesia was monitored by electrocardiogram, indirect and direct arterial blood pressure, pulse oximetry, and capnography. A single dose of oxytetracycline (20 mg/kg IM) was administered at the beginning of the procedure. All animals received microchip implant and ear tag prior to anesthetic recovery. Postoperative analgesia was provided with flunixin meglumine (1.1 mg/kg IM), sodium dipyrone (25 mg/kg IM) and tramadol hydrochloride (2 mg/kg IM). At the end of the surgical procedure, capybaras were transported back to the corral, extubated, and allowed to recover under constant supervision of the anesthetic team. The capybaras were released on the same day once adequate deambulation was observed, with the exception of the first 3 capybaras at the beginning of the study which were left in the corral for 24 hours to monitor post-surgical recovery before the release time.

4.3.3 Surgical Procedure

4.3.3.1 Vasectomy by prepubic midline access and bilateral inguinal access over the inguinal canal

The capybara (n=1) was positioned in dorsal recumbency. A skin incision of approximately 3-cm was made in the midline of the prepubic region (Figure 6). Then, the underlying subcutaneous tissue was divulsed laterally toward the spermatic cord using Metzenbaum scissors, but it was not possible to reach it from this point. For this reason, this procedure was aborted and converted to the second approach proposed: the bilateral inguinal access over inguinal canal. In this case, an about 3-cm skin incision was performed parallel to the first midline incision, in a place where it was possible to feel the passage of the testis by palpation. After blunt divulsion of the subcutaneous tissue and the underlying muscular layers that form the inguinal canal, it was possible to identify the passage of the cremaster. The cremasteric layer and tunica vaginalis were opened by a ventral and longitudinal incision of approximately 2 centimeters. The ductus deferens was usually found in a dorsomedial location. The isolation of approximately 2.5-cm of the ductus deferens from the mesorchium was performed by blunt divulsion using an hemostatic forceps. The ductus deferens was ligated in two points apart about 2-cm with medium/large size (closed length, 8.99-mm) titanium clips (Ethicon Endo-Surgery, Cincinnati, OH). One-centimeter fragment of the ductus deferens was removed between the clips. The synthesis of the cremasteric and tunica vaginalis layers was performed together using a simple continuous pattern with 3-0 polyglecaprone 25. The muscular layers were closed as the subcutaneous tissue, using simple continuous pattern with 3-0 polyglecaprone 25. Intradermal and simple interrupted patterns with 2-0 nylon were used to close the skin. The same procedure was repeated for the contralateral duct.

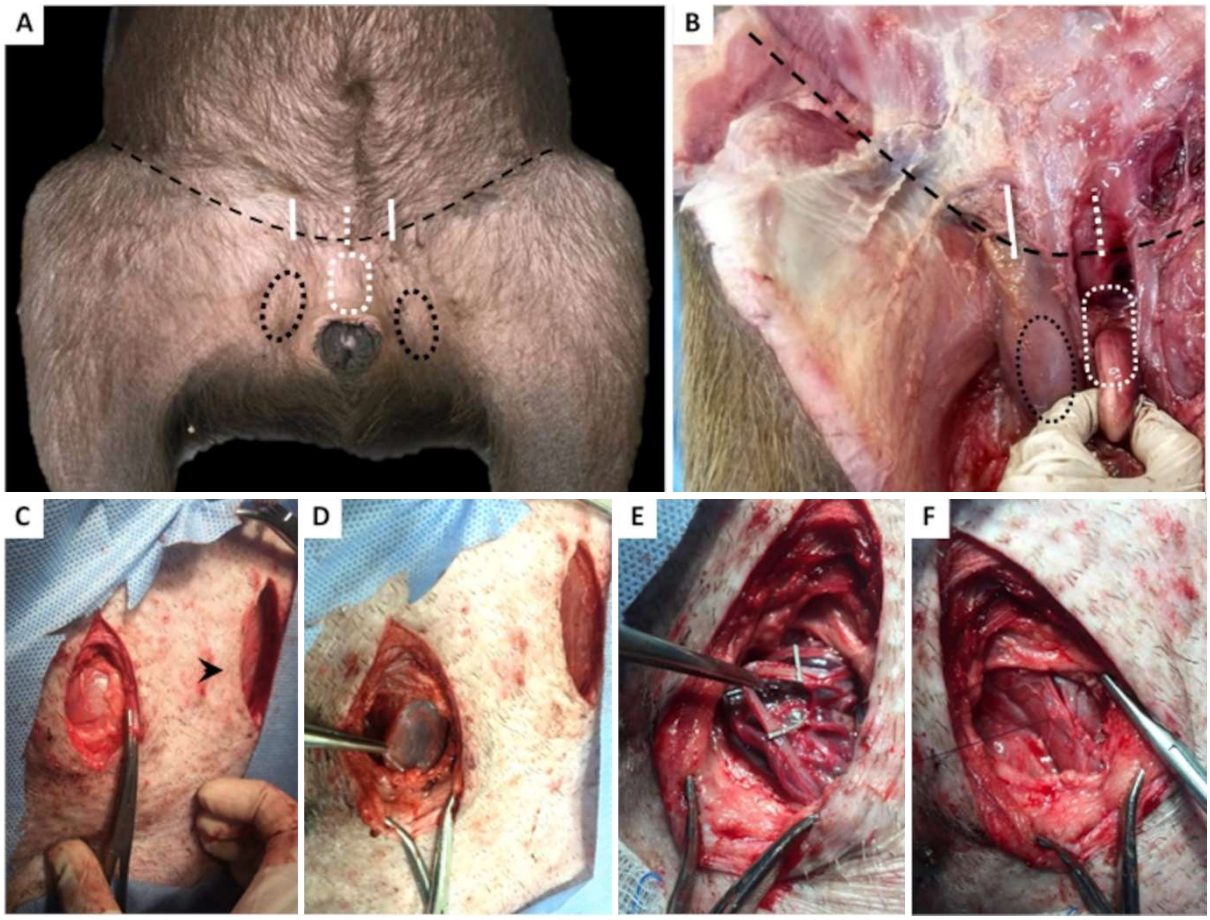

FIGURE 6 Capybara vasectomy by prebubic midline and over inguinal canals approaches. **A** and **B**, Cadaveric study of the incision sites: prepubic midline access site (white dashed line); invaginated penis site (white rectangle); bilateral over inguinal canal incision sites (white line); testis (black ellipse). **C**, Prepubic midline access aborted (black arrowhead); cranial displacement of the right testis to locate the spermatic cord passage after inguinal canal muscles divulsion. **D**, Cremasteric layer held by a anatomical forceps prior to spermatic cord opening. **E**, Ductus deferens ligated; note the tissue trauma to the surround muscle layer. **F**, Cremaster and tunica vaginalis synthesis.

4.3.3.2 Vasectomy by bilateral inguinal access over testicles

With the capybara (n=1) positioned in dorsal recumbency, the testis was positioned as caudally as possible and a skin incision of approximately 3-cm was made over it (Figure 7). The incision continued through the cremaster and tunica vaginalis to exteriorize the testis and the most distal portion of the spermatic cord. The ductus deferens was usually found in a dorsal location. The isolation of approximately 2.5-cm of the ductus deferens from the mesorchium was performed by blunt divulsion using an hemostatic forceps. The ductus deferens was ligated in two points apart about 2-cm with medium/large size titanium clips. One-centimeter fragment of the ductus deferens was removed between the clips. The synthesis of the cremasteric layer and

tunica vaginalis were performed after cranial displacement of the testis, using simple continuous pattern with 3-0 polyglecaprone 25. The subcutaneous tissue was closed with simple continuous pattern with 3-0 polyglecaprone 25. Intradermal and simple interrupted patterns with 2-0 nylon were used to close the skin. The same procedure was repeated for the contralateral duct.

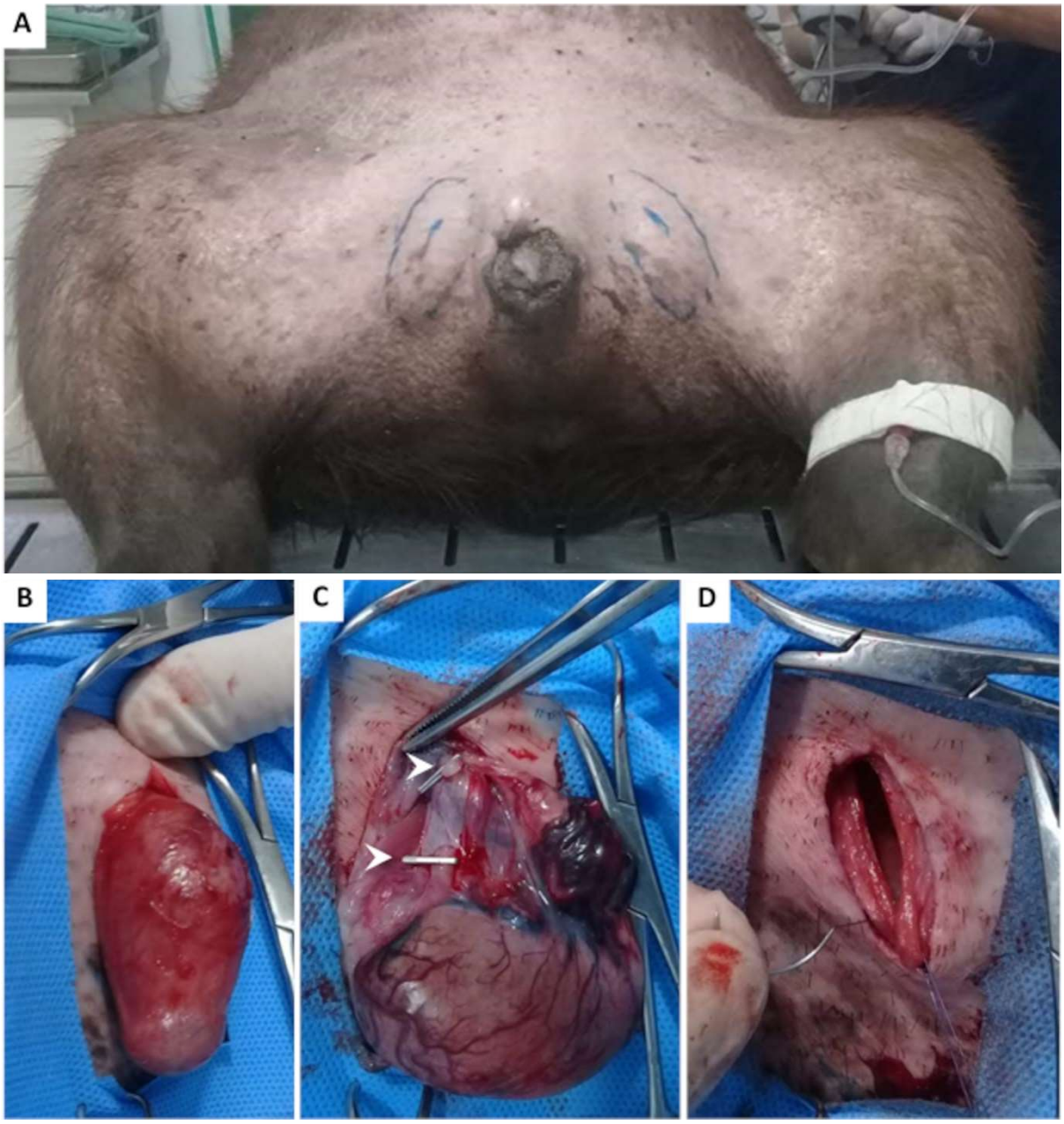

FIGURE 7 Capybara vasectomy by bilateral inguinal access over testicles. **A**, Incision site planning. **B**, Testis exposed, covered by cremaster and tunica vaginalis. **C**, Tunica vaginalis reflected, showing the deferentectomy site with a double ligature (white arrowheads). **D**, Cremaster and tunica vaginalis synthesis after cranial displacement of the testis.

4.3.3.3 Vasectomy by bilateral inguinal access over spermatic cord

This access was performed in capybaras (n=7) positioned in dorsal recumbency (Figure 8). A support (towel) was placed under the dorsal portion of the sacrum area to slightly raise the inguinal region in relation to the abdomen. Due to the great testicular mobility in the subcutaneous inguinal region, in order to precisely locate the incision site, the testicles were intentionally displaced in the craniocaudal and caudocranial direction. With this maneuver, it was possible to identify the probable inguinal location of the spermatic cords. Following, the testis was positioned as caudally as possible and the skin incision started from its most cranial edge, extending about 3-cm cranially, over the previously found location of the spermatic cord. After individualization of the spermatic cord by blunt divulsion of the subcutaneous tissues, the cremaster and tunica vaginalis were opened by a ventral and longitudinal incision of approximately 2 centimeters. The ductus deferens was usually found in a dorsomedial location. The isolation of approximately 2.5-cm of the ductus deferens from the mesorchium was performed by blunt divulsion using an hemostatic forceps. The ductus deferens was ligated in two points apart about 2-cm with medium/large size titanium clips. One-centimeter fragment of the ductus deferens was removed between the clips. The synthesis of the cremaster and tunica vaginalis was performed together with simple continuous pattern using 3-0 polyglecaprone 25. The subcutaneous tissue was closed with simple continuous pattern with 3-0 polyglecaprone 25. Intradermal and simple interrupted patterns with 2-0 nylon were used to close the skin. The same procedure was repeated for the contralateral duct.

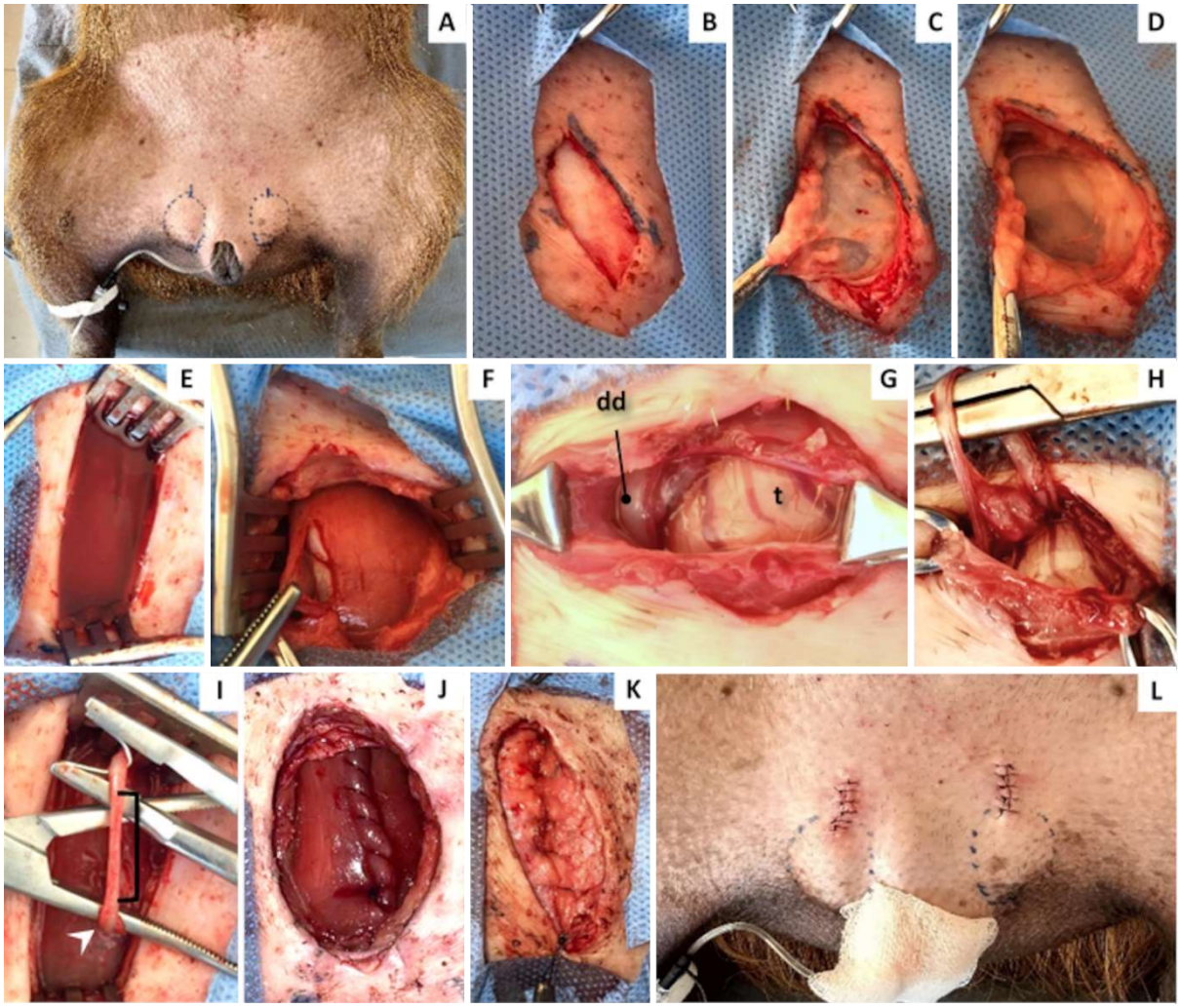

FIGURE 8 Capybara vasectomy by bilateral inguinal access over spermatic cord. **A**, Incision site planning. **B** to **D**, Dieresis of skin and subcutaneous tissue to reach the spermatic cord. **E**, Note peritoneal fluid coming from the abdominal cavity after incision of the cremaster and tunica vaginalis. **F**, After drying the fluid, it was possible to visualize the internal structures. **G**, Testis (t) and the ductus deferens (dd) showed. **H**, Ductus deferens dissected from mesorchium using an hemostatic forceps. **I**, Ductus deferens ligation using a titanium clip; second ligation site (white arrowhead); fragment to be excised (bracket). **J**, **K** and **L,** Synthesis of the spermatic cord, subcutaneous layer and skin, respectively.

4.3.4 Outcome measures

The body weight, ductus deferens ligation and total surgical times, incisions size, perioperative complications were recorded for all procedures. Capybaras were distance assessed 1-week after surgery for clinical status and behavior. Ductus deferens ligation time was considered from incision to clip ligation for each side. Total surgical time corresponded to the time elapsed between the first incision and the last suture of the skin.

4.3.5 Statistical Analysis

All data were subjected to Shapiro-Wilk normality test. Data are mean ± SD and range. A paired Student *t* test was used for comparison between the right and left sides approached for the parameters: ductus deferens ligation time and incision size (animals 1 and 3 were excluded). Statistical significance was set as $P < .05$. All statistical analyses were performed using software RStudio Version 0.99.903 (RStudio Inc, Boston, MA).

4.4 Results

The mean body weight of the capybaras undergoing vasectomy was 53.1 ± 8.1 kg, which corresponds to adult animals. The bilateral approaches were successfully performed in all capybaras without any intraoperative complications. In this case, mean surgical time was 71.7 ± 27.1 minutes and means of incision sizes were 2.6 ± 0.5 and 2.4 ± 0.8 centimeters for right and left side, respectively, taking an average of 20 ± 13.9 minutes to ligate the right ductus deferens and 22.3 ± 13.7 minutes to the left (Table 2). No statistical differences were observed between the sides regarding incision size ($P = .548$) or ductus deferens ligation time ($P = .757$). The procedure started from the right side in 1 capybara and the left in 6.

TABLE 2 Summary data for 9-male capybaras (*Hydrochoerus hydrochaeris*) undergoing vasectomy.

Capybara	Weight (kg)	First side operated	Ductus deferens ligation time (min)		Total surgical time (min)	Incision size (cm)	
			1st	2nd		Right	Left
1[a]	54.3	Right	37	17	125	5.7	4
2	41.3	Left	35	33	98	3.2	3
3[b]	63.3	Left	32	9	123	3.54	3.62
4	60	Left	40	17	96	3.13	3.78
5	53.2	Right	10	9	43	2.13	1.98
6	56.8	Left	18	23	68	2.47	2.19
7	48	Left	34	8	63	1.77	1.50
8	40.8	Left	13	43	100	2.85	1.59
9	60	Left	7	6	34	2.15	2.62

[a]Two techniques: prepubic midline access; and bilateral inguinal over inguinal canal access.
[b]Bilateral inguinal access over testicles.

The attempt to access both ducts through a single incision in the prepubic midline had to be aborted halfway. Due to the presence of the subcutaneously invaginated penis, the position of the incision site had to be set cranially to the pubis. From this position, it was impracticable to lift the spermatic cord due to the lack of elasticity of the skin; and because of the location of the spermatic cord in deeper planes, under the layers of the abdominal muscles, with very low ventral and lateromedial mobility.

In order to complete the vasectomy procedure in the animal in which the midline access was attempted, two paramedian incisions were performed, just lateral to the first midline incision. However, it was found that in order to gain access to the spermatic cord it was necessary to cross some abdominal muscle layers. In this case, access to the ductus deferens was performed over the inguinal canal, very close to the external inguinal ring.

At the third tested access site, the access was performed over the testicles, in their most caudal position in the inguinal region. In this place, as there were no layers of cutaneous or abdominal muscles between the skin and the testis, access to the ductus deferens required less tissues trauma. On the other hand, in order to have adequate access, there was an inevitable need to fully expose the testicles.

The last approach tested was the bilateral incision on the inguinal location of the spermatic cord, just cranial to the testicles. This access site, in addition to allowing the spermatic cord to be reached without penetrating the muscle planes, did not expose the testicles unnecessarily. However, it was noted that when special attention was not given to placing the testicles in their most caudal position, the testicles tended to move cranially and therefore the reference to the incision site. In this case, the ideal access point, where there is no presence of muscle layers, was lost (Figure 9).

A peculiar characteristic observed when opening the cremasteric layer was the overflow of a large volume of peritoneal fluid. This fluid had to be dried with a surgical sponge because it hid the structures inside the spermatic cord and the location of the ductus deferens. In contrast, it was observed that when a support, like a towel, was placed under the dorsal portion of the sacrum region, the resulting tilt was sufficient to force the fluid into the abdominal cavity and improve the visualization of the structures.

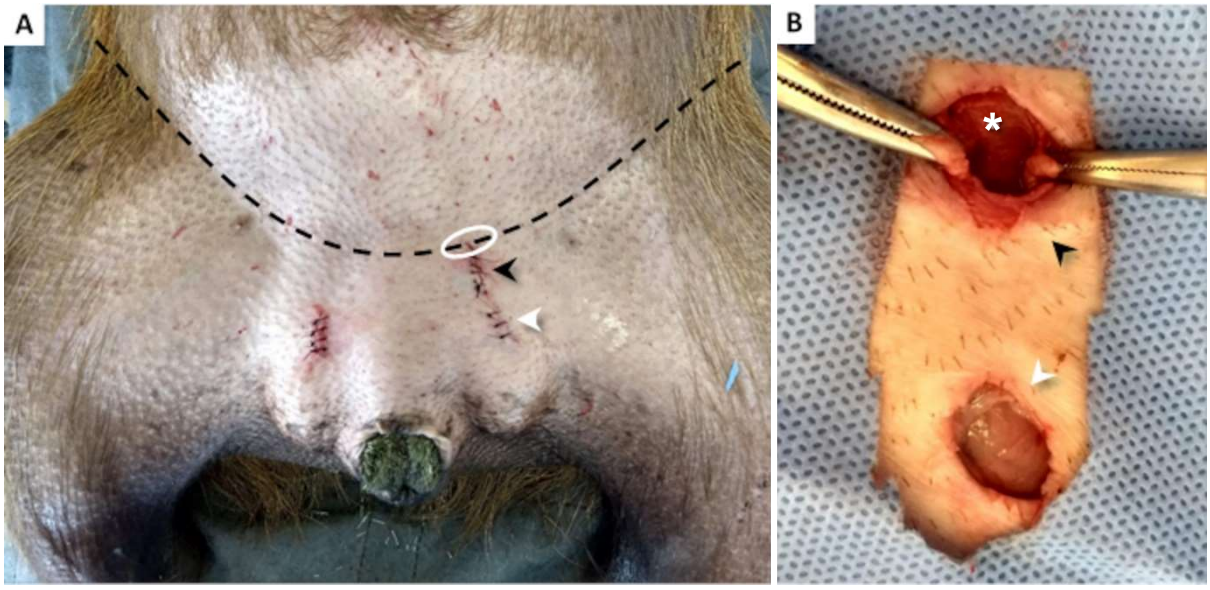

FIGURE 9 Proper positioning of the testicles to locate the incision site. **A**, With the testis as caudal as possible: the proposed incision site (white arrowhead) and the incision site performed without careful positioning (black arrowhead); caudal limit of the abdominal muscles (dashed line); location of the external inguinal ring (white ellipse). **B**, Detail of the dieresis layers: skin and subcutaneous in the proposed incision site (white arrowhead); skin, subcutaneous and muscles of the external inguinal ring (*) in the mistaken incision site (black arrowhead).

The size of the clips used in this study seems to have been adequate, allowing sufficient occlusion of the ductus deferens without interfering with the surrounding tissues.

Hemorrhage was minimal and easily controlled for all approaches.

All animals presented tympanism in the perioperative period, which required intervention through the introduction of a percutaneous catheter to eliminate flatus. Transitory episodes of arterial hypotension was the main anesthetic complication.

All capybaras recovered well from surgery. No signs of abnormal behavior were observed and the animals walked actively within 2 hours. However, capybaras 1 and 3 were found dead in the capture corral the day after the procedure. The necroscopic examination did not reveal the cause of death due to autolysis status, but no sign of hemorrhage was found at the surgery site. After 1-week of surgery, the remaining animals were clinically healthy and showed normal behavior (Figure 10).

FIGURE 10 One-week postoperative follow-up. **A**, Incision site healing of the only recaptured male. **B**, General status of the animal assessed at a distance (males were tagged in the left ear).

4.5 Discussion

In contrast with a previous study, that suggested an over testicles access,[8] in this study, the bilateral inguinal over spermatic cord approach proposed showed to be the most feasible technique for vasectomy of adult capybaras, especially in a field condition. In another study in which a bilateral inguinal access is cited,[7] some pertinent points were unclear, such as the age group of the individuals submitted to these procedures, how extensive was the incision and where exactly the incision site was located on the inguinal region.

The vasectomy procedure proposed was performed in a relatively longer total surgical time than those already reported for other species.[10–13] However, it must be emphasized that, in addition to the fact that an anesthetic experiment which required accurate monitoring of several parameters was being performed concurrently, the purpose of recording and reporting these measured times was only descriptive. It is believed that in a non-scientific situation in the field, without the need for more accurate records and monitoring, this procedure can be performed in a shorter time. There was no difference between the times for right and left ductus deferens ligation in the bilateral inguinal over spermatic cord approach, using similar access incision sizes, which suggests that the difficulty of access by both sides is equal.

Vasectomy is a procedure commonly performed for the population control of wild animals, especially in gregarious ones, such as capybaras that have a marked social hierarchy, since orchiectomy could cause changes in sexual behavior and

interfere in the social dynamics of the group. The incision sites reported for vasectomy in these species include access to both ductus deferens through a single incision in the midline[12,14–16] and bilateral inguinal access over the spermatic cords.[16]

Although the protuberance of the testicles can be seen in an adult male when in the standing position, capybaras do not have a differentiated scrotal sac as seen in other species.[17] Perhaps, because of this characteristic, when the incision was performed over the testicles to access the ductus deferens, the testicles tended to come out. As the ductus deferens was dorsally positioned, when this access was performed, the testicles need to be rotated in order to gain access to the ductus deferens, which induced painful stimuli.

Contrary to what has been described for the guinea pigs,[18] a species from the same family as the capybara, it was found in our study that only the youngest capybaras have functional cryptorchidism. In adults, the inguinal ring remains open but not enough to pass the testicles into the abdominal cavity. In addition, the epididymal fat pad involving the ductus deferens was not seen in any of the studied specimens. This structure, usually present in other species of rodents, prevents the herniation of abdominal organs through the inguinal canal.[19]

A limitation of our study was the restriction of performing the vasectomy of younger animals, which did not allow us to ascertain whether the proposed technique would also be viable in this age group due to testicular mobility or if it would be better to approach the abdominal ductus deferens by ventral midline celiotomy. Another relevant anatomical characteristic found, previously described by Rodrigues et al, was the presence of a robust cremaster muscle.[7] This can explain the quite limited lateromedial mobility of the spermatic cord observed in our study.

The cutaneous muscle does not cover the entire inguinal area, especially in the region suggested for the incision; therefore, after crossing the skin any contaminant easily reaches the spermatic cord and the abdominal cavity. Considering this, we advocate giving preference to the use of non-absorbable sutures to close the skin. Although we have not tested the use of absorbable suture in this layer, the fact that capybaras seek escape in water or mud immediately after release, already supports this concern.

The animals showed to be stressed when kept for many hours in the corral, for this reason, we chose to perform the capture only at the time of the procedure, thus, it was impossible to perform a previous fast. It is not certain whether the lack of fasting

contributed to the occurrence of tympanic episodes in the perioperative period, or it was a reaction to the drugs used in chemical restraint as discussed by Rosenfield et al, [20,21] or even a combination of both. The same concern about stress was taking into account in the release time. At the beginning of the study, the protocol was the maintenance of the animals for 24 hours in the capture corral before release. However, two capybaras were found dead in the capture corral the day after the procedure. Analysis of video images that recorded the immediate postoperative period showed that both deaths occurred after moments of stress, with the animals trying to escape of an external threat. This fact made us rethink the management of animal retention in the postoperative period, so the animals started to be released immediately after anesthetic recovery and no other deaths occurred.

Finally, this was the first study with focus on surgical anatomy and development of a less invasive surgical technique for vasectomy of the capybara species. The bilateral inguinal over spermatic cord access seems to be the best approach in adult individuals. All overcame obstacles and techniques reported in this study may have applications for capybara population control programs and as a model for other surgical and anesthetic procedures in this species.

4.6 Acknowledgments

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4.7 Disclosure

The authors report no financial or other conflicts related to this report.

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Tympany During Chemical Restraint in Free-Ranging Capybara (Hydrochoerus hydrochaeris)-Iatrogenic Cause and Treatment. *Braz J Vet Pathol*. 2019;12(3):117-122. doi:10.24070/bjvp.1983-0246.v12i3p117-122

5 LAPAROSCOPIC SALPINGECTOMY IN CAPYBARAS (*HYDROCHOERUS HYDROCHAERIS*)

5.1 Abstract

Objective: To evaluate the viability and safety of laparoscopic salpingectomy of free-ranging capybaras.

Study Design: Experimental study.

Animals or Sample Population: Capybaras (n=11).

Methods: The capybaras were chemically restrained, blind intubated, and placed under general anesthesia. A ventral abdominal approach was tested in one animal. The 3-port technique was used bilaterally in both flanks for laparoscopic salpingectomy in 10 capybaras. Recordings of body weight, tubal ligation and total surgical times, surgical and anesthetic complications, one-week postoperative animal clinical status and incisional healing were analyzed.

Results: The ventral abdominal approach had to be finished by a video-assisted technique. The bilateral flank approach was successfully performed in all animals: the mean surgical time was 82.3 ± 29.1 minutes; and the means of tubal ligation times were 18.6 ± 7.7 and 23 ± 13.9 minutes for right and left sides, respectively. Seven of the 11 capybaras recaptured 1-week after the procedure were clinically healthy, with satisfactory wound healing and no signs of infection. Two animals were diagnosed as pregnant during the procedure and were not recaptured, but showed normal behavior. One animal died in the immediate postoperative period. One procedure had to be converted for technical reasons.

Conclusion: Laparoscopic salpingectomy can be safely performed in capybaras.

Clinical Significance (or Impact): The bilateral laparoscopic technique proposed for salpingectomy of capybaras must be considered in the population control programs as an alternative to the open approach, by providing through small incisions: visualization of the abdominal cavity and non-exposure of internal tissues.

O manuscrito apresentado neste capítulo foi submetido ao periódico *Veterinary Surgery* em 19 de outubro de 2020, sob a identificação VSU-20-324 (Anexo A).

5.2 Introduction

Brazilian Spotted Fever (BSF) is an emerging disease in South America and its occurrence is connected to the presence of capybaras, amplifier hosts of *Rickettsia rickettsii* and primary hosts of the tick *Amblyomma sculptum*, main vector of the agent in endemic areas.[1,2] In southeastern Brazil, both capybaras and BSF occurrences have increased significantly over the last three decades,[3] and along with that, the need for the establishment of population control programs of this species.

Contrary to what happens with other wild species,[4–9] there are still no published studies on the use of hormonal implants or immunocontraceptives in female capybaras. Due to the hierarchical characteristic of this species and the dynamics of BSF dissemination, castration including the complete removal of the gonads is contraindicated.[10] Thus, among the methods used for contraceptive management of free-living capybaras populations, vasectomy and tubal ligation are still the most effective.

It is widely known that, whenever possible, the use of laparoscopic procedures is highly recommended in wild animals because of the difficulty in assisting and maintaining its postoperative care.[11] The main benefit in this case, in addition to those already known in the case of humans and domestic species,[12–16] is the possibility to practically release the animal back into the environment immediately after anesthetic return, with the assurance that they will experience a satisfactory recovery with low pain and minor or no complications. Therefore, our purpose is to describe the laparoscopic salpingectomy technique in capybaras; its feasibility, obstacles and the solutions found to overcome them.

5.3 Materials and methods

5.3.1 Animals

After approval from the animal research ethics committee (Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo - CEUA FMVZ/USP; No. 7590270218) and Brazilian Biodiversity Information and Authorization System (SISBIO No. 62352-1 and 70016-1), 11 free-ranging female adult capybaras were captured for this study.

5.3.2 Anesthesia

The chemical restraint were achieved by intramuscular dart with a combination of ketamine (7 mg/kg) and dexmedetomidine (10 µg/kg). All capybaras were conditioned to enter a corral minutes before the surgical procedure. Under these conditions, it was not possible to establish a previous fasting. Propofol, administrated intravenously (4–6 mg/kg), was used to induce anesthesia. Isoflurane in oxygen via a circle rebreathing circuit maintained the surgical anesthesia stage. Peripheral venous access in the cephalic vein was used for intravenous administration of Lactated Ringer fluid at 5 mL/kg/hour during surgery. Anesthesia monitoring included: electrocardiogram, indirect and direct arterial blood pressure measurement, pulse oximetry, and capnography. A single dose of oxytetracycline (20 mg/kg IM) was administered at the beginning of the procedure. Postoperative analgesia was provided with flunixin meglumine (1.1 mg/kg IM), sodium dipyrone (25 mg/kg IM) and tramadol hydrochloride (2mg/kg IM).

In order to facilitate the identification and recapture of animals after the procedure, all animals received microchip implant and ear tag prior to anesthetic recovery. At the end of the surgical procedure, capybaras were transported back to the corral, extubated, and allowed to recover under constant supervision of the anesthetic team. The capybaras were released on the same day once adequate deambulation was observed.

5.3.3 Surgical Procedures

5.3.3.1 Laparoscopic-assisted salpingectomy by ventral abdominal approach

All surgical procedures were led by the same surgeons team. The capybara (n=1) was initially placed in supine Trendelenburg position. The ventral abdominal area was clipped and aseptically prepared. A 15-mm incision in the umbilical scar was performed and the subcutaneous tissues were dissected. An 11-mm Endotip cannula (Karl Storz, Tuttlingen, Germany) with a 10-mm forward-oblique laparoscope 30° (Karl Storz) inside were used to enter the abdominal wall. The pneumoperitoneum was created by insufflation of CO_2 at a pressure of 6 mmHg and a rate of 1-2 L/minute,

using the side port of the Endotip cannula. With a prior skin incision, a 5-mm cannula with its trocar (Bhio Supply, Esteio, Brazil) was introduced paramedially in the middle third of the middle abdominal region at the level of the flank fold, guided by the laparoscope. After inspecting the abdominal cavity and visualizing the ovary positioned dorsally, the animal was repositioned to 0° inclination and laterally rotated about 30°. A 5-mm Maryland grasper (Bhio Supply) was used to move the gastrointestinal tract away from the target surgical region and to manipulate the uterine tube toward the abdominal wall. A small enough incision for the passage of an hemostatic forceps was made in the abdominal wall at the estimated position of the ovary. After blunt dissection with an hemostatic forceps, an Allis forceps was introduced to apprehend the uterine tube and pull it out of the abdominal cavity. The mesosalpinx was perforated and isolated from the uterine tube with an hemostat. Two medium/large size (closed length, 8.99-mm) titanium clips (Ethicon Endo-Surgery) were applied to the uterine tube about 20-mm apart using a clip applier (Bhio Supply). The uterine tube was sectioned between the clips and a fragment of approximately 1-cm was excised (Figure 11). The absence of relevant hemorrhages was verified after returning the remaining uterine tube to the abdominal cavity, which was then deflated. The grasper port and the incision used to externalize the uterine tube were closed. The muscular and subcutaneous layers were sutured separately with 3-0 polyglecaprone in a sultan pattern, and the skin with 2-0 nylon in a simple interrupted pattern. The animal was rotated to the opposite side so that the same procedure could be performed in the contralateral tuba. The procedure was registered with a TelePack Vet X (Karl Storz).

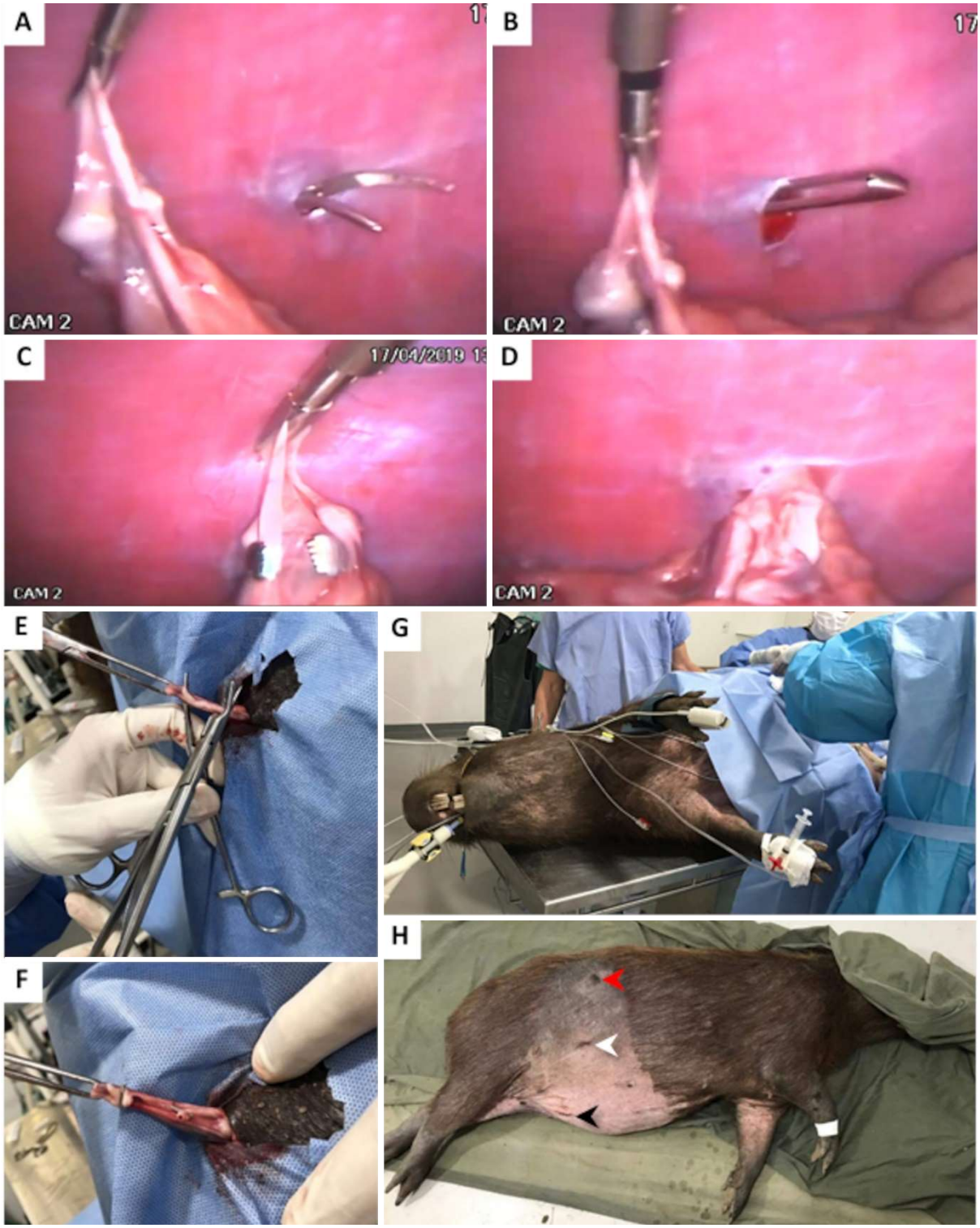

FIGURE 11 Laparoscopic-assisted salpingectomy in capybaras, with the laparoscope port inserted in the umbilical scar. **A**, An hemostat was used for blunt divulsion of the musculature and enlarging the incision in the region of the lumbar fossa, close to the apprehended ovary. **B** to **D**, An Allis was introduced and used to hold and pull ovary and uterine tube out. **E**, Tubal ligation performed outside the body. **F**, Uterine tube double ligated and 1-cm fragment resected. **G**, Animal positioned in right lateral recumbency, tilted laterally about 30° to improve tubal visualization. **H**, Site disposition of uterine tube access (red arrowhead), laparoscope port (black arrowhead) and 5-mm port (white arrowhead).

5.3.3.2 Laparoscopic salpingectomy by bilateral flank approach

The capybaras (n=10) were placed in lateral recumbency. The flank area was clipped and aseptically prepared. A 15-mm paramedian skin incision at the level of the ovaries was performed and the subcutaneous tissues were dissected. An 11-mm Endotip cannula with a 10-mm forward-oblique laparoscope 30° inside were used to enter the abdominal wall. The pneumoperitoneum was created by insufflation of CO_2 at a pressure of 6 mmHg and a rate of 1-2 L/minute, using the side port of the Endotip cannula. With a prior incision, an 11-mm cannula with its trocar was introduced next to the last ribs, guided by the laparoscope, to avoid underlying organs, especially the tympanic bowels. Similarly, a 5 mm port was created next to the wing of ilium (Figure 12).

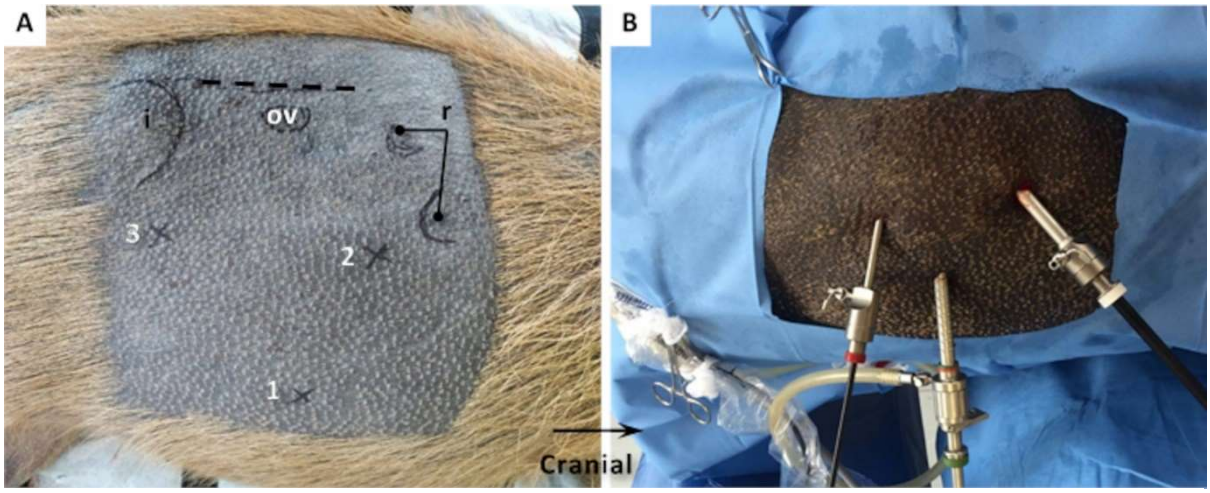

FIGURE 12 Planning of laparoscopic salpingectomy in capybaras by bilateral flank approach, repeated in the opposite side. **A**, Animal positioned in left lateral recumbency; laparoscope port (1); 11-mm port (2); 5-mm port (3); the wing of ilium (i); penultimate and last floating ribs (r); estimated position of the right ovary (ov); ventral limit of the iliocostalis muscle (dashed line). **B**, Instrumentals disposition during surgery.

After inspecting the abdominal cavity and visualizing the ovary positioned dorsally, a 10-mm Babcock (Bhio Supply), 5-mm Maryland or 5-mm grasping forceps (Bhio Supply), introduced through the 11-mm cannula, was used to move the gastrointestinal tract away from the target surgical region and to manipulate the uterine tube. The mesosalpinx was sectioned to isolate the uterine tube with a 5-mm endoscopic Metzenbaum scissors, introduced through the 5-mm cannula. A 5-mm forceps to hold the uterine tubes then replaced the Metzenbaum scissors. Two

medium/large size titanium clips were applied to the uterine tube about 20-mm apart using a 10-mm endoscopic clip applier introduced through the 11-mm cannula. The clip applier was replaced by Metzenbaum scissors, the uterine tube was then sectioned between the clips and a fragment of approximately 1-cm was excised (Figure 13). After verifying the absence of relevant hemorrhages, the abdominal cavity was deflated and the muscular and subcutaneous layers were closed with 3-0 polyglecaprone in a sultan pattern, and the skin with 2-0 nylon in a simple interrupted pattern. The contralateral side was operated in the same way. The procedure was registered with a TelePack Vet X.

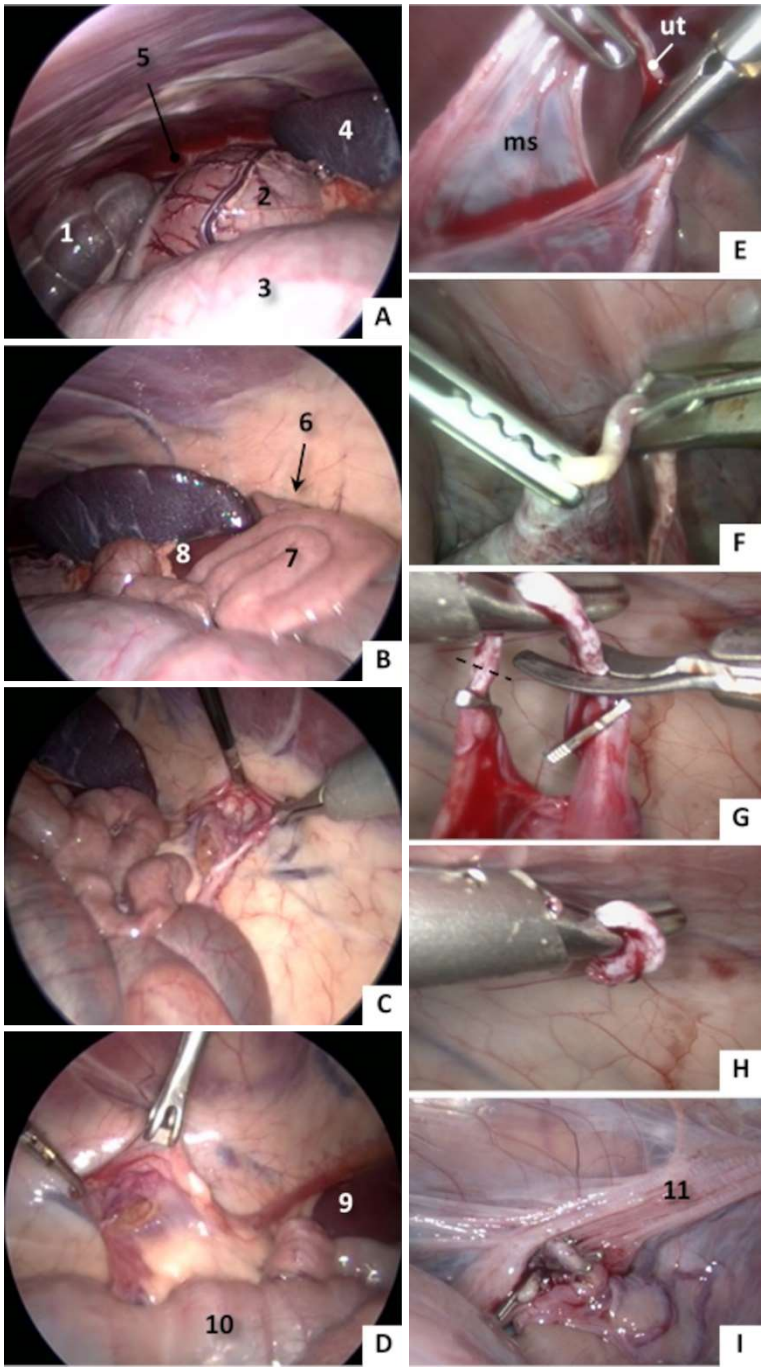

FIGURE 13 Laparoscopic salpingectomy in capybaras by bilateral flank approach. **A**, Initial cavity inspection under pneumoperitoneum; cecum (1); stomach (2); large intestine (3); spleen (4); diaphragm (5). **B**, Looking for topography of the left ovary (6), below the intestinal loops (7) and caudally to the kidney (8). **C**, Suspension of the left ovary by the uterine tube, after removing the intestine. **D**, Topography of the right uterine tube when the procedure is performed on the opposite side; kidney (9) and intestines (10). **E**, Blunt divulsion of the mesosalpynx (ms) to isolate part of the uterine tube (ut). **F**, Application of titanium clips. **G**, Excision of a uterine tube fragment between the ligatures; second cut site (dashed line). **H**, 1-cm uterine tube fragment removed through the working port. **I**, Checking for bleeding before deflation and abdominal cavity closure; suspensory ligament (11).

5.3.4 Outcome measures

The body weight, tubal ligation and total surgical times, intraoperative and postoperative complications were recorded for all procedures. Tubal ligation time was considered from incision to first clip applying for each side. Surgical time corresponded to the time elapsed between the first incision and the last suture of the skin. Capybaras were recaptured about 1-week after surgery: animal clinical status and incisional healing were evaluated.

5.3.5 Statistical Analysis

All data were subjected to Shapiro-Wilk normality test. Data are mean ± SD and range. A paired *t* test was used for comparison between the right and left sides approached for the parameter tubal ligation time (animals 1 and 8 were excluded). Statistical significance was set as $P < .05$. All statistical analyses were performed using software RStudio Version 0.99.903 (RStudio Inc, Boston, MA).

5.4 Results

The mean body weight of the 10 capybaras undergoing laparoscopic salpingectomy by bilateral approach in this study was 56.4 ± 7.1 kg, which corresponds to adult animals. Despite of one converted procedure (8; Table 3), the laparoscopic salpingectomy proposed were successfully performed in all capybaras without major intraoperative complications. Mean surgical time was 82.3 ± 29.1 minutes, and the means of tubal ligation times were 18.6 ± 7.7 and 23 ± 13.9 minutes, respectively for the right and left sides (Table 3). No statistical differences were observed between the sides regarding tubal ligation time ($P = .329$). The procedure started from the right side in 5 capybaras and the left in 6.

In the first animal (1; Table 3), the procedure was initially attempted with the laparoscope positioned in the umbilical scar, with the animal in supine Trendelenburg position. However, the uterine tube was not appropriately visualized because the intestinal bowels were blocking the view of the surgical area. In addition, episodes of severe hypotension and bradycardia were observed. After repositioning to lateral recumbency and 0° inclination, hemodynamic parameters improved, but the bowels

continued to hide the ovaries; the animal had to be rotated laterally about 30° and the procedure was finished by a video-assisted technique with external tubal ligation.

TABLE 3 Summary data for 11-female capybaras (*Hydrochoerus hydrochaeris*) undergoing laparoscopic salpingectomy.

Capybara	Weight (kg)	First side operated	Tubal ligation time (min)		Total surgical time (min)
			Right	Left	
1[a]	78	Right	32	9	123
2	46.4	Left	35	39	144
3	54.7	Left	17	14	72
4	59.9	Left	20	31	96
5	58	Left	14	19	68
6	48.1	Right	11	14	59
7[b]	57	Right	22	15	78
8[c]	64.4	Right	16	-	-
9[d]	50	Right	23	17	83
10	56.8	Left	10	8	42
11[b]	69	Left	15	50	99

[a]Laparoscopic-assisted tubal ligation using umbilical access for laparoscope port.
[b]Diagnosed as pregnant during the procedure.
[c]Laparoscopic procedure converted to open surgery.
[d]Death in the immediate postoperative period after fight with another animal.

During the laparoscopy of one of the animals (8; Table 3), a failure in the CO_2 supply occurred and it was decided to convert the procedure after the ligation of the first uterine tube. One capybara died after being attacked by another capybara that invaded the corral in the immediate postoperative period, while still recovering from the anesthetic procedure (9; Table 3). Pregnancy was diagnosed during two of the procedures (7 and 11; Table 3). In these cases, the surgery was performed without complications, however, the enlarged uterus made it difficult to locate the uterine tube (Figure 14).

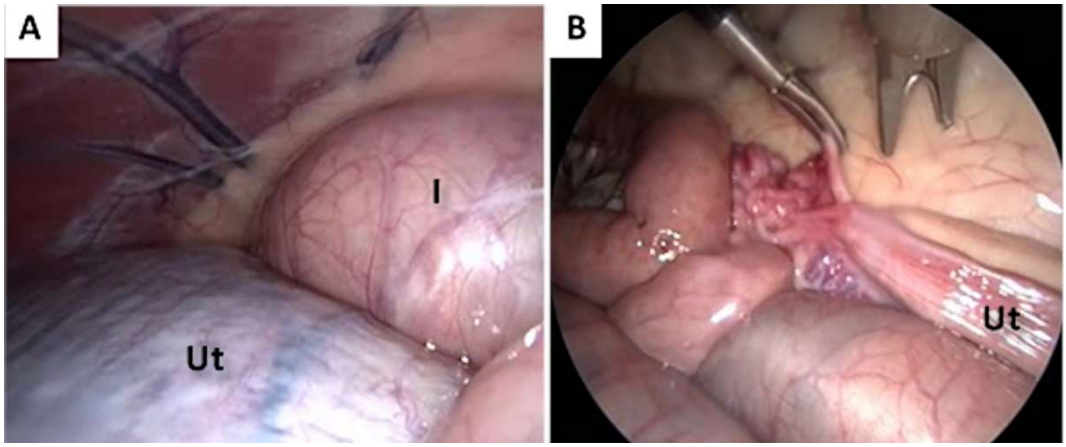

FIGURE 14 Laparoscopic diagnosis of pregnancy in capybara. **A**, Appearance of pregnant uterus (Ut); intestines (I). **B**, Appearance of a non-pregnant uterus (Ut).

The pneumoperitoneum of 6 mmHg provided a suitable working space while allowing comfortable triangulation of the instruments. Manipulation of the intestines was required to locate the uterine tubes even in lateral recumbency and bilateral approach, but no complications were observed. No problem was found to remove the excised uterine tube fragment through the 5-mm portal. None of the animals developed subcutaneous emphysema. Minimal self-limiting bleeding was noted during surgery. The medium/large size titanium clips were effective at sealing the uterine tubes.

Introduction of a percutaneous catheter to eliminate flatus was necessary in all animals. Manifestation of tympanism occurred in different degrees and frequencies during the time between chemical restraint and surgical recovery.

Anesthetic complications included hypercapnia, hyperthermia and hypotension. All capybaras recovered well from surgery and showed normal activity in the immediate postoperative period, walking actively within 2 hours. After 1-week of surgery, all incisions of the recaptured animals healed without complication, the animals were clinically healthy and did not show any major or minor complications associated with the procedure (Figure 15).

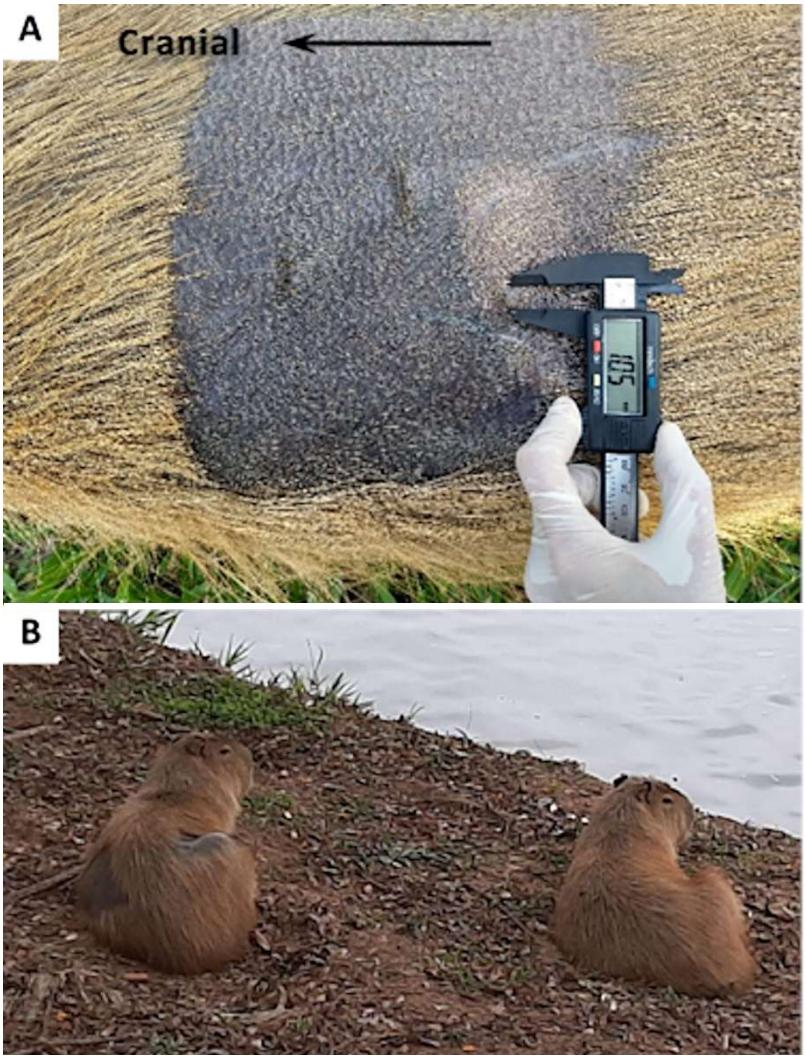

FIGURE 15 One-week postoperative status of capybaras subjected to laparoscopic salpingectomy by bilateral approach. **A**, Animal positioned in left lateral recumbency; surgical wound healing without signs of infection or complications. **B**, Salpingectomized capybara (left) next to another non-operated one, showing normal behavior and comfortable posture.

5.5 Discussion

Based on our results, laparoscopic salpingectomy can be safely performed in capybaras using a 3-port technique and bilateral approach. This procedure has already been reported for wildlife animals,[17–23] however, to the best of our knowledge, this is the first report describing laparoscopic salpingectomy in capybaras. Although using different techniques and considering that the purpose of counting time in this study was only descriptive, the total surgical and tubal ligation times to perform laparoscopic salpingectomy in capybaras were relatively longer to those previously described in African lions,[17] cheethas[17,18] and leopards[19]; and similar to that achieved in white-tailed deers.[23] In addition, there was no difference between the times for right and left tubal

ligation in the bilateral approach, using similar access incision sizes, which suggests that the difficulty of access by both sides is equal.

Lateral recumbency and separate bilateral access were required for suitable visualization of the uterine tubes in adult capybaras. Similar to other species,[18,24] the Trendelenburg position were not beneficial to access ovaries. In capybaras, this inclination did not provided enough improvement in ventral displacement of the intestines away from the target surgical area; and worst, showed to be dangerous if the patient were not intubated, because of the need for assisted ventilation in this case. In addition, despite the possibility of using fewer portals with the laparoscope port inserted in the umbilical scar or next to it, this triangulation strategy did not allowed easier visualization of the uterine tube in capybaras as expected. The attempt to keep captive free-ranging capybaras, in order to establish a previous fast, was frustrated at the beginning of the experiments due to the remarkable stress caused in these animals. It was not possible to conclude, therefore, if the absence of fasting also contributed notably to the difficulty in visualizing the structures and to the occurrence of hemodynamic changes observed intraoperatively in this ventral approach.

The capybara abdomen has a robust gastrointestinal system, with a cecum and colon that are relatively larger than other rodents[25,26]; and sometimes even more voluminous because of tympanism.[27,28] Regardless of concerns about a sufficient working space, while higher pressures usually used caused greater hemodynamic damage in capybaras, a pneumoperitoneum of 6 mmHg showed to be enough when using the bilateral approach. But, laparoscopic cannulas should be introduced carefully so that intestinal puncture does not occur.

The animals in the current study were clinically healthy. As they are free-ranging animals, it may justify the fact that no excessive abdominal fat was observed. As reported in lion[24] and cheetahs,[18] fat was minimal in the ovarian pedicle and it did not vary markedly between capybara individuals.

As previously described[29] and also observed in our study, chemical restraint with the ketamine-dexmedetomidine mixture has adverse effects such as the risk of acute cecal tympanism and the potential induction of bradycardia and hyperthermia, and therefore, deserves extra care in capybaras.

It is well known that carbon dioxide insufflation in the peritoneum can produce systemic acidosis and hypercapnia because of the absorption through the peritoneal surface of CO_2 into the circulation. In addition to the absorption of insufflated CO_2,

increases in intra-abdominal pressure limit diaphragm excursion, contributes to an increase in $PaCO_2$ and $ETCO_2$, which leads to fatigue and the need for mechanical ventilation.[30] All animals in our study received mechanical ventilation when required, but nothing is yet known about the peculiarities of the physiology and respiratory mechanics of these semi-aquatic species. It is possible that the hypercapnia observed in our study is, therefore, the result of both inadequate ventilation and CO_2 insufflation.

The abdominal distension caused by a laparoscopic procedure itself may cause pain.[31] Unfortunately, there is no validated way to assess pain in capybaras. Therefore, future investigations can be considered to assess pain after laparoscopic surgery in capybaras. Studies comparing laparoscopic and open surgical approaches can point out advantages to postoperative recovery.

The requirement for advanced training and the cost of the equipment are other potential disadvantage of a laparoscopic approach. Of course, the use of laparoscopic sealer devices, for example, can shorten the steps and turn easier performing the proposed procedure and thus decrease surgical time and bleeding, but it is generally more expensive equipment. Some teaching and research institutions, private hospitals and veterinary centers, however, already have laparoscopy equipment available for other uses, in addition to trained personnel, which can be used, without being necessary to acquire them for this purpose.

A limitation of our study was the restriction of capturing younger specimens. As the size ratio between the abdominal organs varies between capybaras of different age groups,[32] further investigations are necessary to assess whether the ventral approach is feasible in smaller capybaras. In conclusion, the laparoscopic salpingectomy technique by bilateral approach proposed in this study may have clinical applications for contraception of adult capybaras and serve as a reference for other future laparoscopic procedures in this species.

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5.7 Disclosure

The authors report no financial or other conflicts related to this report.

5.8 References

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6 LAPAROSCOPIC-ASSISTED DEFERENTECTOMY IN CAPYBARAS (*HYDROCHOERUS HYDROCHAERIS*)

6.1 Abstract

Objective: To assess the feasibility and safety of laparoscopic-assisted deferentectomy of free-ranging capybaras.

Study Design: Experimental study.

Animals or Sample Population: Capybaras (n=7).

Methods: Male adult capybaras were chemically restrained, intubated, and placed under general anesthesia with isoflurane. Deferentectomy was performed by ventral abdominal approach. A 11-mm laparoscope port was placed in the umbilical scar to facilitate localization of the abdominal portion of the ductus deferens. The abdomen was insufflated with CO_2 to a pressure of 6 mmHg. The ductus deferens were externalized through an about 8-mm incision in the prepubic midline using an 18-cm hemostatic forceps. The ductus deferens ligation occurred extracorporeally using titanium clips and 1-cm segment was excised. Behavior were observed after 1-week postoperatively.

Results: Laparoscopic-assisted deferentectomy was successfully performed in all capybaras with low complications. Episodes of arterial hypotension and tympanism were detected during all anesthetic procedures. The mean surgical time was 35.6 ± 4.2 minutes, and the means of ductus deferens ligation times were 16.4 ± 4.2 and 5.2 ± 2.8 minutes for first and second sides, respectively. Six of the 7 capybaras showed normal behavior 1-week after the procedure. One animal died of unknown cause in the immediate postoperative period.

Conclusion: Laparoscopic-assisted deferentectomy can be safely performed in capybaras.

Clinical Significance (or Impact): The laparoscopic-assisted technique proposed for deferentectomy of capybaras must be considered as an alternative to open deferentectomy in the population control programs due to minimal trauma and low rate of perioperative complications.

6.2 Introduction

Capybara is the largest known rodent,[1] host of the tick *Amblyomma sculptum* and potential disseminator of Brazilian Spotted Fever (BSF).[2] This species is not in The International Union for Conservation of Nature's Red List, on the contrary, due to environmental degradation and a decrease in its predators, it has increasingly proliferated and approached urban centers. In this situation of risk to public health, population control of capybaras becomes necessary.

A single and recent study[3] showed that the use of the anti-gonadotropin-releasing hormone vaccine was able to lead to infertility in 3 male capybaras over a period of at least 18 months without affecting the male's alpha characteristics, which is imperative for successful capybara population control efforts. However, it is not yet known whether it is a permanent or transitory result, requiring further studies to be carried out in order to attest if this method can be recommended and applied. Therefore, among the methods used for contraceptive management of free-living male capybaras in BSF endemic areas, vasectomy is still considered the most effective.

Although the interest in use of minimally invasive laparoscopic techniques is increasing in free-ranging wildlife, because the option of confinement, monitoring, and repeat immobilization is often not available for this animals,[4] to our knowledge, laparoscopic techniques has never been described in capybaras.

Thus, considering the lack of reports in the literature involving vasectomy in capybaras, mainly with regard to the application of videosurgical procedures, the aim of this study was to describe the surgical technique developed for laparoscopic-assisted deferentectomy in free-ranging capybaras.

6.3 Materials and methods

6.3.1 Animals

The study was performed under the permit of the Brazilian Biodiversity Information and Authorization System (SISBIO No. 62352-1 and 70016-1) and was approved by the Ethics Committee of the School of Veterinary Medicine and Animal Science of the University of São Paulo (CEUA FMVZ/USP; No. 7590270218). Eight free-ranging adult male capybaras were studied.

6.3.2 Anesthesia

A previous fasting was not established because all animals were captured minutes before the surgical procedure. The capybaras were chemically restrained by intramuscular dart with a combination of ketamine (7 mg/kg) and dexmedetomidine (10 µg/kg). Anesthesia was induced with intravenous propofol (4–6 mg/kg). Endotracheal intubation was performed blindly, and general anesthesia was maintained with isoflurane in oxygen via a circle rebreathing circuit. Assisted ventilation was performed when required. Lactated Ringer solution at 5 mL/kg/hour was administered intravenously during surgery. Anesthesia was monitored by electrocardiogram, indirect and direct arterial blood pressure, pulse oximetry, and capnography. Antibiotic prophylaxis was performed with a single dose of oxytetracycline (20 mg/kg IM) administered at the beginning of the procedure. Postoperative analgesia was provided with flunixin meglumine (1.1 mg/kg IM), sodium dipyrone (25 mg/kg IM) and tramadol hydrochloride (2mg/kg IM).

After weighing, the capybara was positioned in dorsal recumbency; surgical site was clipped, scrubbed, and draped for surgery. All animals were identified by applying microchip and ear tag prior to anesthetic recovery. At the end of the procedure, capybaras were transported back to the corral, extubated, and allowed to recover under constant supervision of the anesthetic team. Releasing was performed on the same day of the procedure after adequate deambulation has been achieved.

6.3.3 Surgical Procedures

The animals (n=7) were placed in the dorsal recumbency with the caudal portion of the body slightly elevated (approximately 5°). Urethral catheterization (10Fr) was performed to maintain the bladder empty during the procedure. After the preparation of the surgical site, a skin incision of 15-mm was made in the umbilical scar to introduce the 11-mm Endotip cannula (Karl Storz, Tuttlingen, Germany) with a 10-mm forward-oblique laparoscope 30° (Karl Storz) through the abdominal wall. The pneumoperitoneum was created by insufflation of CO_2 at a pressure of 6 mmHg and a rate of 1-2 L/minute, using the side port of the Endotip cannula. Due to the presence of branches of the deep caudal epigastric vessels converging and running close to the linea alba, a 21G hypodermic needle was introduced through the abdominal wall to

confirm the exact location of the second incision. Taking advantage of the bevel of the needle, an incision was made in the peritoneum to facilitate the entry of the instrument and prevent the peritoneum detachment. This about 8-mm incision in the prepubic midline was used to insert an 18-cm hemostatic or Allis forceps, guided by the laparoscope. The ductus deferens was blunt dissected from mesorchium with an hemostat and ligated extracorporeally using two medium/large size (closed length, 8.99-mm) titanium clips (Ethicon Endo-Surgery, Cincinnati, OH) applied about 20-mm apart using a clip applier (Bhio Supply, Esteio, Brazil). One-centimeter segment of each ductus was excised (Figure 16). After verifying the absence of relevant hemorrhages, the abdominal cavity was deflated and the muscular and subcutaneous layers were closed separately with 3-0 polyglecaprone in a sultan pattern, and the skin was closed with 2-0 nylon in a simple interrupted pattern. The procedure was registered with a TelePack Vet X system (Karl Storz).

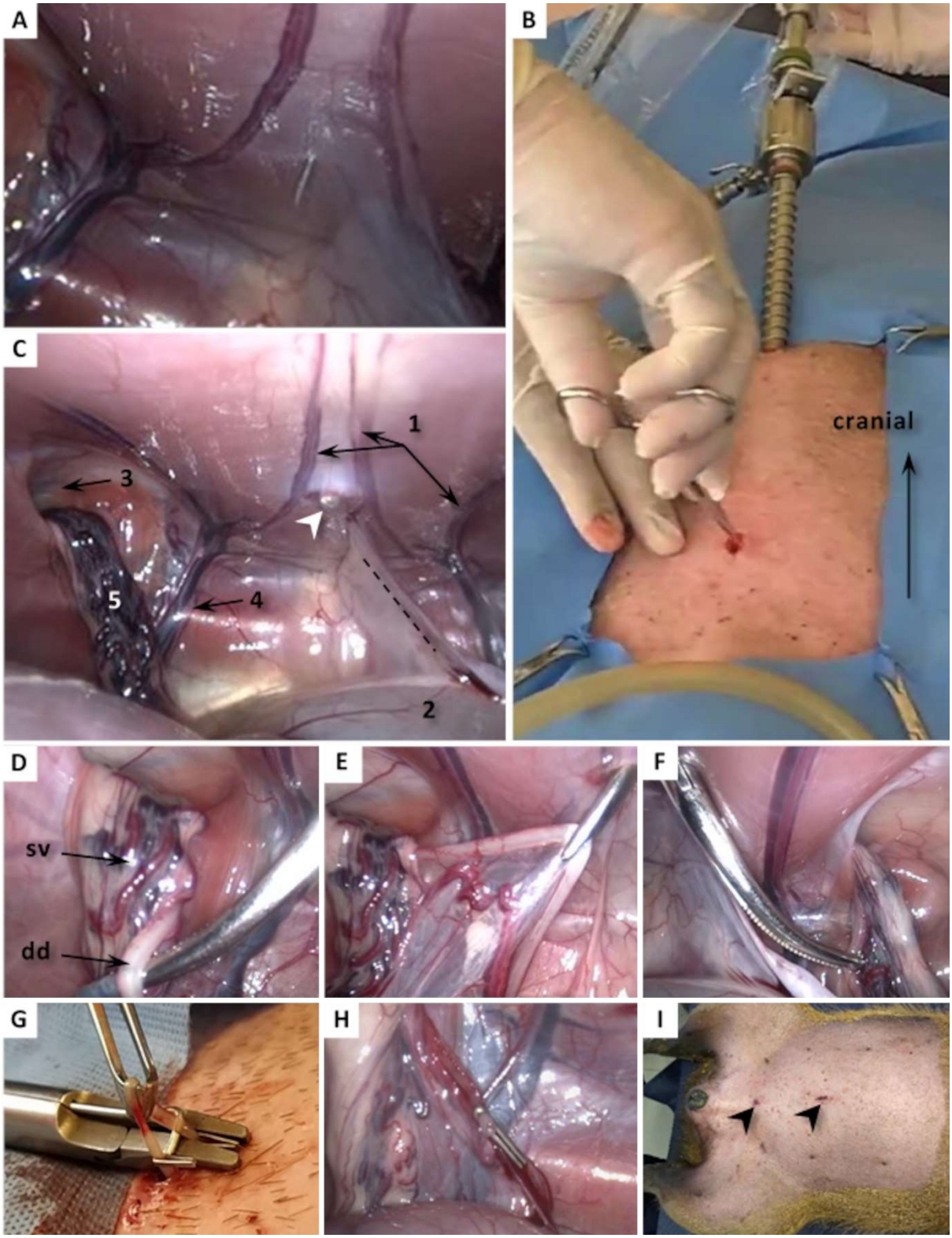

FIGURE 16 Laparoscopic-assisted deferentectomy in capybara. **A**, A hypodermic needle was used to determine the location of the forceps insertion, guided by the laparoscope inserted in the umbilical scar. **B**, The forceps was inserted in the midline, cranial to the pubis. **C**, Working space: deep epigastric vessels branches (1); bladder (2) and its ventral median ligament (dashed line); internal inguinal ring (3); iliac vessels (4); spermatic vessels (5); forceps (white arrowhead). **D**, Showing the caudomedial position of the left ductus deferens (dd) in relation to spermatic vessels (sv). **E** and **F**, Traction of the left and right ductus deferens, respectively. **G**, Clipping of the ductus deferens. **H**, After excision of a 1-cm fragment, the ductus deferent was returned to the cavity. **I**, Incisions closed, relative size and site.

6.3.4 Outcome measures

The body weight, ductus deferens ligation and total surgical times, intraoperative and postoperative complications were recorded for all procedures. All the animals were followed-up for a period of 1-week after surgery. Clinical status was evaluated at a distance. The first ductus deferens ligation time was considered from incision to first clip ligation; and the second ductus deferens ligation time, from the first clip ligated to the ligation of the contralateral ductus. Total surgical time was corresponded to the time elapsed between the first incision and the last suture of the skin. Data are mean ± SD and range.

6.4 Results

Laparoscopic-assisted deferentectomy was successfully performed in all capybaras. The mean total surgical time was 35.6 ± 4.2 minutes and the means of ductus deferens ligation times were 16.4 ± 4.2 and 5.9 ± 4.4 minutes for first and second sides, respectively. The procedure started from the right side in 1 capybara and the left in 6. The mean body weight of the animals was 45.5 ± 12.7 kg, which corresponds to adult animals (Table 4).

TABLE 4 Summary data for 7-male capybaras (*Hydrochoerus hydrochaeris*) undergoing laparoscopic-assisted deferentectomy.

Capybara	Weight (kg)	First side operated	Ductus deferens ligation time (min)		Total surgical time (min)
			1st	2nd	
1	57.3	Right	16	3	37
2	56.3	Left	24	7	41
3	62.8	Left	18	2	33
4	37	Left	14	5	35
5	32.3	Left	14	6	37
[a]6	38	Left	18	10	38
7	34.9	Left	11	3	28

[a]Death in the immediate postoperative period.

One capybara (6; Table 4) suddenly recovered from chemical restraint before the start of surgery and had to be restrained chemically again. This animal evolved into severe bradycardia during the intraoperative period and required the administration of adrenaline. During the recovery time, already back in the corral, this animal died. The cadaver was kept refrigerated until necropsy. This procedure was performed one day after his death and there were no signs of relevant hemorrhage in the surgical site or macroscopic alterations that justified the death.

The ductus deferens were more friable in younger animals (lower body weight), requiring more delicate handling during exteriorization. It is suggested that the apprehension of the ductus deferens be carried out in its most proximal portion using an Allis forceps in order to avoid excessive traction and rupture. In the lightest animal in this study, it was also observed that the testicles could be moved from the inguinal region to the abdominal cavity, which was not possible to be performed in the other heavier animals (Figure 17).

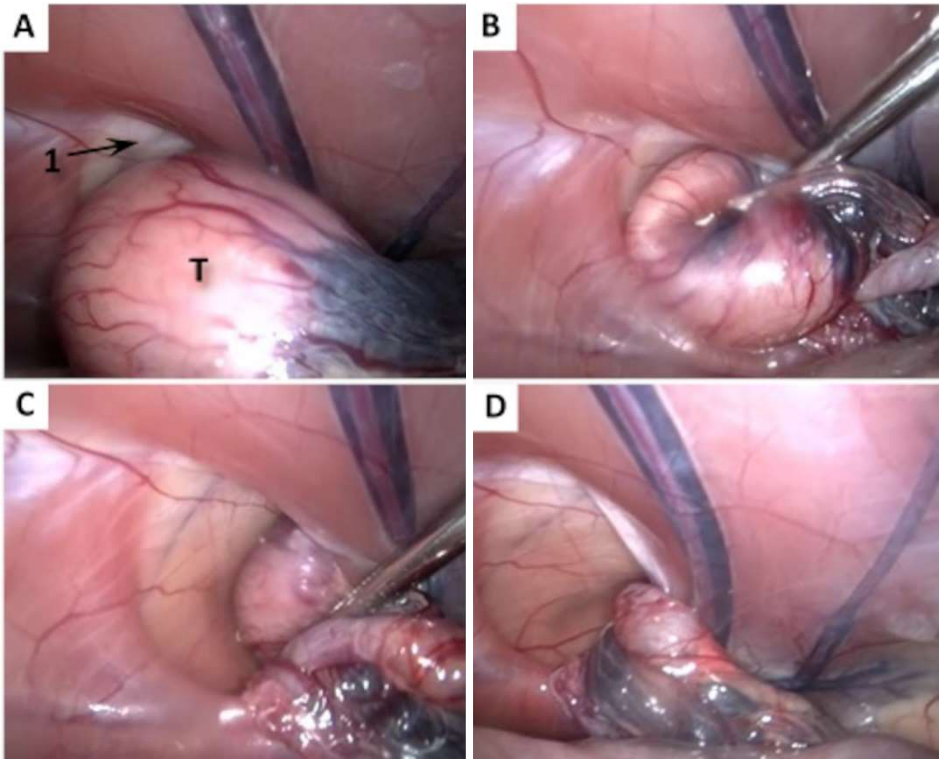

FIGURE 17 Testicular mobility in younger capybaras. **A** to **D**, Laparoscopic view of testis in abdominal cavity being repositioned in the inguinal region; internal inguinal ring (1); testis (T).

Manifestation of tympanism occurred in all animals in different degrees and frequencies during the time between chemical restraint and surgical recovery, requiring

a percutaneous cecum catheterization to eliminate flatus. Anesthesia complications included bradycardia, hypercapnia, and hyperthermia. With the exception of the capybara 6, the animals recovered well from surgery and showed normal behavior in the postoperative period, walking actively within 2 hours.

The initial intention to recapture capybaras 1-week following the surgical procedure was frustrated after the recapture of the first individual. In this case, we observed that males, unlike females, exhibit more frequent and intense escape behavior during the darting moment. As the group used in the study was not always close to the capture corral, it was decided not to recapture the remaining males due to the risk of drowning and death after escape from chemical restraint. However, after 1-week of surgery, all animals showed similar behavior to the other individuals in the group who were not operated. Based on this, we infer that all animals were clinically healthy and did not show any major or minor complications associated with the procedure (Figure 18).

FIGURE 18 One-week postoperative status of an capybara submitted to laparoscopic-assisted deferentectomy. No abnormal behavior or posture was detected.

6.5 Discussion

To our knowledge, this is the first study on the use of laparoscopic technique for capybaras vasectomy. The means of total surgical time and ductus deferens ligation time for laparoscopic-assisted deferentectomy in capybaras was similar to that already studied in other species,[5–10] although performed by different approaches. The assisted technique developed in this study was based on the anatomical exploration carried out in the first capybara captured. It was found that, probably due to having an open inguinal ring, the capybaras ductus deferens are easily isolated and extensible, and can be pulled out of the cavity without difficulty through the midline. The advantage of requiring less laparoscopic instruments and one less portal was also taken into account.

A common concern regarding laparoscopic procedures in the anterior portion of the abdomen in humans is the possibility of trauma to the superficial and internal epigastric vessels when the portals are inserted.[11] Although abdominal wall vessel injury is not a commonly reported complication of veterinary laparoscopic surgery,[12,13] it is related to the occurrence of subcutaneous hematoma formation, hemoperitoneum, compromised visualization of the surgical field, and increased surgical time.[12] The deep caudal epigastric vessels is only vaguely described in the veterinary literature as running along the rectus abdominis muscle,[14] without a detailed anatomical location. In the case of humans, studies indicate that the safe zone for insertion of trocars is in the midline region, closer to the linea alba.[15] However, this may not reflect what is observed in every species of animals. In the present study, we identified a branch of the deep epigastric vessel running millimetrically parallel to the linea alba and capable of being located laparoscopically. This finding must be taken into account when planning the introduction of portals or even for open surgical access in the caudal abdomen of capybaras.

Routine urethral catheterization is not recommended for laparoscopic pelvic surgery in humans, as it predisposes to urinary tract infection and increased postoperative discomfort.[16] In this case, it is suggest that the patient be asked to urinate before being anesthetized. Our study proposes the catheterization of capybaras undergoing laparoscopic-assisted deferentectomy in order to prevent bladder injuries when inserting a laparoscopic port and avoid obstruction of the pelvis view due to the bladder filling, which would also make prepubic access with forceps impossible.

However, it was not evaluated whether the previous emptying by abdominal massage alone would be sufficient to achieve the same result.

The Trendelenburg position was used in other animal species to perform laparoscopic vasectomy,[9,10,17–19] although not always necessary.[20] In the case of capybaras, for a pressure of 6 mmHg, maintaining a position of 5° in Trendelenburg proved to be necessary and sufficient to displace the bladder cranially and remove the large volume of the capybaras intestines. As an assisted procedure was proposed, and no laparoscopic grasping forceps was used, this maneuver is crucial to achieve clear visibility of pelvic structures. Despite performing cecal catheterization to treat tympanism in the intraoperative period as recommended by Rosenfield et al,[21] the pressure exerted by the gastrointestinal tract on the great vessels and diaphragm worsened the hemodynamic and cardiorespiratory condition of the patient and contraindicates the use of greater positioning angles.

Most mammals have testicles permanently lodged in the scrotum. In capybaras,[22] as well as other rodents,[23,24] the testicles are located in the inguinal region subcutaneously, the inguinal canal remains open and allows communication with the peritoneal cavity. In contrast to the rabbit,[25] whose testicles have free mobility between the scrotum and the abdominal cavity throughout life, it was observed in the capybaras of this study that this mobility to the abdominal cavity seems to occur only in younger individuals (lower body weight). Due to the restrictions imposed by SISBIO on the use of juvenile animals or cubs in this study, it was not possible to test the hypothesis that the use of laparoscopic technique could be more advantageous than open surgery in this case and, therefore, further investigations are needed.

In conclusion, even with some anesthetic complications, laparoscopic-assisted deferentectomy proved to be feasible in capybaras, using a 2-port technique, with few technical difficulties or associated complications.

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6.7 Disclosure

The authors report no financial or other conflicts related to this report.

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7 CONSIDERAÇÕES FINAIS

Esta dissertação introduz novas abordagens cirúrgicas para a vasectomia e laqueadura de capivaras, alternativas às técnicas já descritas, e aprimora o seu uso com foco na melhor recuperação pós-operatória dos animais.

Neste sentido, percebemos que a possibilidade de realizar o estudo anatômico prévio, para o melhor desenvolvimento das técnicas cirúrgicas, mostrou-se de suma importância para que pudéssemos chegar a abordagens menos cruentas e potencialmente de menor trauma cirúrgico.

Os acessos cirúrgicos abertos propostos nesta pesquisa apresentaram vantagens sobre os já anteriormente publicados e reforçam que a simples extrapolação de técnicas a partir de outras espécies pode não ser suficiente para se atingir o melhor resultado, e que as particularidades anatômicas da capivara interferem na escolha da melhor abordagem.

As técnicas por videolaparoscopia mostraram-se exequíveis nesta espécie, com tempo total de cirurgia menor que os respectivos procedimentos abertos de deferentectomia e salpingectomia. Considerando-se ainda que a recuperação clínica dos animais no pós-operatório não apresentou diferenças visualmente perceptíveis entre os grupos, podemos inferir que a abordagem por videolaparoscopia, neste tocante, seja mais vantajosa.

Um outro importante ponto a se ressaltar é o conhecimento adquirido sobre a anatomia através da exploração laparoscópica, a partir do qual torna-se possível o desenvolvimento de outras técnicas cirúrgicas abertas com mínima incisão, reduzindo tempo e trauma cirúrgicos.

Há que se considerar, no entanto, que a técnica videolaparoscópica exige o uso de infraestrutura e equipamentos mais custosos, conhecimento técnico diferenciado e a presença de um cirurgião auxiliar. Já o contrário acontece com as técnicas abertas, sendo razoável executá-las apenas com um cirurgião e instrumental cirúrgico básico. Sendo assim, as técnicas abertas talvez sejam, portanto, a escolha de melhor valia numa situação a campo, ao passo que as técnicas por videolaparoscopia possam ser a opção ideal se for possível o usufruto de um ambiente mais controlado. O que fica claro é que a escolha da abordagem ideal, por videolaparoscopia ou convencional, dependerá de cada situação, dos objetivos e recursos disponíveis.

A assincronia entre a disponibilidade de animais, infraestrutura e recursos financeiros para esta pesquisa não permitiu que concluíssemos uma etapa importante, previamente planejada para este estudo: a análise laboratorial de cortisol, haptoglobina, albumina, fibrinogênio, leucograma, perfil bioquímico renal e hepático, bem como a avaliação do etograma gerado a partir de imagens de vídeo-monitoramento. Estes dados coletados nos momentos pré e pós-operatório serão avaliados num futuro próximo e constituirão um outro artigo científico cujo objetivo principal será a comparação do trauma cirúrgico decorrente do uso das abordagens aberta e por videolaparoscopia para as técnicas de salpingectomia e deferentectomia em capivaras.

Os óbitos ocorridos durante os experimentos não tiveram correlação direta com o procedimento cirúrgico utilizado, mas aparentemente com problemas decorrentes do manejo pré ou pós-operatório. Apesar de termos tentado evitá-las ao máximo, estas intercorrências foram essenciais para aprimorar o manejo dos animais, e certamente o conhecimento gerado a partir delas poderá servir de embasamento para estratégias futuras de manejo desta espécie.

A limitação do uso de animais mais jovens impediu a validação das abordagens propostas também para esta faixa etária, e devem ser alvo de investigações futuras.

Finalizando, considerando a importância que o controle reprodutivo de capivaras vem adquirindo nos últimos tempos, este estudo contribui sobremaneira no que tange ao aprimoramento das técnicas de esterilização atualmente utilizadas e fornece base para o desenvolvimento de outros procedimentos cirúrgicos que se façam necessários nesta espécie futuramente.

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ANEXOS

ANEXO A – Comprovantes de submissão dos artigos

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Authors
Yanai, Priscila
Ferraro, Mario A. R.
Lima, Andressa
Cortopassi, Silvia Renata
Silva, Luis

Date Submitted
19-Oct-2020

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Title
Laparoscopic salpingectomy in capybaras (*Hydrochoerus hydrochaeris*)

Authors
Yanai, Priscila
Ferraro, Mario Antonio
Lima, Andressa
Cortopassi, Silvia Renata
Silva, Luis

Date Submitted
19-Oct-2020

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