Derek Andrew Rosenfield

Study on the perspective of non-lethal population control in capybara (*Hydrochoerus hydrochaeris*) through reversible immunocontraceptive methods

Sao Paulo

2019

DEREK ANDREW ROSENFIELD

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Thesis presented to the Graduate School in Animal Reproduction of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo as a requirement for the title of Doctor in Science.

Department:
Animal Reproduction

Concentration Area:
Animal Reproduction

Advisor:
Prof. Dr. Cristiane Schilbach Pizzutto

São Paulo
2019

DADOS INTERNACIONAIS DE CATALOGAÇÃO NA PUBLICAÇÃO

(Biblioteca Virginie Buff D'Ápice da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo)

T. 3853
FMVZ

Rosenfield, Derek Andrew
Study on the perspective of population control of capybaras (*Hydrochoerus hydrochaeris*) by reversible immunocontraceptive method / Derek Andrew Rosenfield. – 2019.
255 f. : il.

Título traduzido: Estudo sobre a perspectiva de controle populacional de capivaras (*Hydrochoerus hydrochaeris*) através de método imunocontraceptivo reversível.

Tese (Doutorado) – Universidade de São Paulo. Faculdade de Medicina Veterinária e Zootecnia. Departamento de Reprodução Animal, São Paulo, 2019.

Programa de Pós-Graduação: Reprodução Animal.
Área de concentração: Reprodução Animal.
Orientadora: Profa. Dra. Cristiane Schilbach Pizzutto.

1. Capivara. 2. Controle populacional. 3. Imunocontracepção. 4. Rickettsia. 5. Febre maculosa. I. Título.

Ficha catalográfica elaborada pela bibliotecária Maria Aparecida Laet, CRB 5673-8, da FMVZ/USP.

ERRATA

ROSENFIELD, D. A. **Study on the perspective of population control of capybaras (*Hydrochoerus hydrochaeris*) by reversible immunocontraceptive method**. 2019. 255 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2019.

Página	Onde se lê	Leia-se
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Resumo		ROSENFIELD, D. A. **Estudo sobre a perspectiva de controle populacional de capivaras (*Hydrochoerus hydrochaeris*) através de método imunocontraceptivo reversível**. 2019. 255 f. Tese (Doutorado em Ciências) - Faculdade de Medicina Veterinária e Zootecnia, Universidade de São Paulo, São Paulo, 2019.

CERTIFICADO

Certificamos que a proposta intitulada "Estudo sobre a perspectiva de controle populacional de capivaras (Hydrochoerus hydrochaeris) através de método imunocontraceptivo reversível", protocolada sob o CEUA nº 9553260816, sob a responsabilidade de **Cristiane Schilbach Pizzutto** *e equipe; Derek Andrew Rosenfield* - 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 03/07/2017.

We certify that the proposal "Study of capybara (Hydrochoerus hydrochaeris) from the perspective of population control by means of reversible immunocontraceptive method", utilizing 12 Brazilian wild species (males and females), protocol number CEUA 9553260816, under the responsibility of **Cristiane Schilbach Pizzutto** *and team; Derek Andrew Rosenfield* - 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 07/03/2017.

Finalidade da Proposta: Pesquisa

Vigência da Proposta: de 11/2016 a 10/2018 Área: Reprodução Animal

Origem:	Não aplicável biotério						
Espécie:	Espécies silvestres brasileiras	sexo:	Machos e Fêmeas	idade:	1 a 10 anos	N:	12
Linhagem:	Hydrochoerus hydrochaeris -Capivara			Peso:	20 a 90 kg		

Registro IBAMA/Sisbio/Etc: Número SISBio: 54634 O projeto já foi avaliado por SISBio, mas para sua aprovação final, fica na dependência da aprovação prévia do CEUAVet. A seguir, o pedido do SISBio: Motivo da devolução : Prezado pesquisador, Para proceder com a autorização da sua solicitação, será necessária a apresentação do documento do parecer da CEUA - Comissão de Ética no Uso de Animais, como anexo desta solicitação. Qualquer dúvida, por favor entrar em contato com atendimento.sisbio@icmbio.gov.br

Método de Captura: Contenção química Na etapa de tratamento os animais serão contidos quimicamente uma vez por mês para a realização de exames físicos (acompanhando do estado clínico e detecção de eventual problema que comprometa a saúde e/o bem-estar), ultrassonográficos, coleta de sêmen e eletroejaculação. Se algum animal, durante o trabalho apresentar qualquer sinal que comprometa sua saúde e/o bem-estar, o mesmo será retirado do trabalho.

Resumo: A superpopulação de capivaras (Hydrochoerus hydrochaeris) em áreas urbanas representa um importante problema de saúde pública, uma vez que são animais de relevância epidemiológica para a febre maculosa. A grande resistência das capivaras a condições ambientais adversas e sua alta capacidade de procriação aliadas à extrema redução de predadores naturais e proibição legal de sua caça, podem quadruplicar sua população em apenas um ano em áreas antrópicas, aumentando o risco de disseminação de doenças. Diante da possibilidade de utilizar um método contraceptivo seguro e eficaz para o controle populacional destes animais, objetivamos, neste projeto, o uso do produto GonaCon, produzido pela USDA (United States Department of Agriculture), como uma alternativa recente e promissora de um método anticoncepcional reversível baseado no conceito de imunocontracepção. Trata-se de uma vacina imunocontraceptiva recombinante, de dose única, aplicada via intramuscular, que provoca redução da fertilidade. Contando com o apoio da USDA e com sua autorização para o uso do GonaCon em nossa pesquisa, objetivamos provar a eficiência de um método de imunocontracepção e avaliar sua possível reversibilidade no período de 2 anos. Para tanto, utilizaremos 12 exemplares de capivaras (Hydrochoerus hydrochaeris) adultos, sendo 6 machos e 6 fêmeas; os animais serão divididos em 2 grupos (controle e tratados com vacina GonaCon). Mensalmente os animais serão contidos quimicamente para colheita de sangue (análise da concentração hormonal de testosterona, estradiol e progesterona, bem como testes de titulação de anticorpos anti-GnRH), de sêmen por eletroejaculação e obtenção de imagens ultrassonográficas das gônadas. Os resultados serão analisados comparando-se as etapas antes e durante o tratamento e realizando testes de correlação com as análises obtidas.

Local do experimento: Área do condomínio residencial Capital Ville, na região de Cajamar, estado de São Paulo.

São Paulo, 04 de julho de 2017

Profa. Dra. Denise Tabacchi Fantoni
Presidente da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

Roseli da Costa Gomes
Secretaria Executiva da Comissão de Ética no Uso de Animais
Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo

São Paulo, 17 de abril de 2019
CEUA N 9553260816

Ilmo(a). Sr(a).
Responsável: Cristiane Schilbach Pizzutto
Área: Reprodução Animal

Título da proposta: "Estudo sobre a perspectiva de controle populacional de capivaras (Hydrochoerus hydrochaeris) através de método imunocontraceptivo reversível".

Parecer Consubstanciado da Comissão de Ética no Uso de Animais FMVZ (ID 004581)

A Comissão de Ética no Uso de Animais da Faculdade de Medicina Veterinária e Zootecnia da Universidade de São Paulo, no cumprimento das suas atribuições, analisou e **APROVOU** a Notificação (versão de 28/setembro/2018) da proposta acima referenciada.

Resumo apresentado pelo pesquisador: "Conforme solicitado, estou anexando dois arquivos: - adendo com protocolo de castração para as capivaras - autorização do SISBIO para a realização da castração".

Comentário da CEUA: "Adendo com descrição de procedimento e SISBIO fornecidos.".

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

Ministério do Meio Ambiente - MMA
Instituto Chico Mendes de Conservação da Biodiversidade - ICMBio
Sistema de Autorização e Informação em Biodiversidade - SISBIO

Autorização para atividades com finalidade científica

Número: 54634-4	Data da Emissão: 17/04/2018 17:48	Data para Revalidação*: 17/05/2019

* De acordo com o art. 28 da IN 03/2014, esta autorização tem prazo de validade equivalente ao previsto no cronograma de atividades do projeto, mas deverá ser revalidada anualmente mediante a apresentação do relatório de atividades a ser enviado por meio do Sisbio no prazo de até 30 dias a contar da data do aniversário de sua emissão.

Dados do titular

Nome: DEREK ROSENFIELD	CPF: 228.027.458-24
Título do Projeto: Estudo sobre a perspectiva de controle populacional de capivaras (Hydrochoerus hydrochaeris) através de método imunocontraceptivo reversível	
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	Administração de vacinas ativas (anti-GnRH) e inativas (controle)	10/2016	10/2019
2	Coleta e transporte de amostras biológicos in situ	11/2016	10/2019
3	Captura de animais silvestres in situ	11/2016	10/2019
4	Coleta e transporte de espécimes da fauna silvstres in situ	11/2016	10/2019
5	Manutenção temporária de vertebrados silvestres em cativeiro	02/2017	01/2019
6	Procedimentos médico veterinários, confirme necessário (emergenciais, feridas, doenças, eutanásia)	01/2018	10/2019
7	Castração dos animais do grupo de pesquisa (análise efeito contraceptivo na histologia das gonâdas)	01/2018	10/2019

Observações e ressalvas

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EVALUATION FORM

Author: ROSENFIELD, Derek Andrew

Title: **Study on the perspective of population control of capybaras** (*Hydrochoerus hydrochaeris*) by reversible immunocontraceptive method

Thesis presented to the Graduate School in Animal Reproduction of the School of Veterinary Medicine and Animal Science of the University of Sao Paulo as a requirement for the title of Doctor in Science.

Date: ___/___/_____

Examination Board

Prof. Dr. ____________________ Institution: ______________________
Verdict: _____________________ Signature: _____________________

Prof. Dr. ____________________ Institution: _____________________
Verdict: _____________________ Signature: _____________________

Prof. Dr. ____________________ Institution: _____________________
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Prof. Dr. ____________________ Institution: _____________________
Verdict: _____________________ Signature: _____________________

Prof. Dr. ____________________ Institution: _____________________
Verdict: _____________________ Signature: _____________________

DEDICATION

Marcelo Alcindo de Barros Vaz Guimarães †

Who sent me on my path, unknown to where it would lead. Thank you for that.

Eros Yudji Tanaka e Silva †

Who has left earth much too soon. Although young in age, an inspiration and an example of how a human being should be.

Sergio Cipresso †

Who took me in. gave me so much and made it all possible!

ACKNOWLEDGMENTS

A research project of this magnitude is not a solitary path. To one degree or another, everybody needs support and assistance, - help that more often than not appears out of nowhere, - unexpected, - even by unknown. All of who have had one attribute in common, their willingness and enthusiasm to help when and where needed, while every so often, leaving a profound impact, leading to new-found friendships along the way. To all these fantastic individuals I would like to express my sincere gratitude for being part of my long journey during all academic research endeavors and indebt for sharing your wisdom with me.

To my wife, partner, and soulmate, Ligia Cipresso Rosenfield, - words cannot describe the appreciation and admiration I have for you. You have given me so much.

Sarah, Ellen, my inspirations!

My family and friends that stood by my side and provided support whenever possible.

Prof. Dr. Cristiane Schilbach Pizzutto, my project angel. One could not ask for a better academic supervisor. Grateful for all the devoted hours and hands-on efforts, being on my side and making it all possible! Thank you for your kindness and your dedication to me and my project. Through you, Marcelo was always a part of our projects. I think, we really moved something!

Elizabeth Ferreira, thank you for helping me to increase my academic library;

Sr. Nicolau Cassia, without whom I would have had a harder time, finding my entrance into the academic world.

Otília Tioko Sano, providing access to CEES Clara Mantelli and the possibility to study during vacation, in preparation for the entrance exam, 10 years ago, when the journey began. Watching over me, from collegial to doctorate.

Prof. Dr. César Augusto Dinóla Pereira, my professor, peer, and friend from day one of my academic career. Over the following decade, thank you for all the talks and being available when called upon.

Fernanda Nunes, appreciative for the friendship and all the support given, in all shapes and forms, always available when needed. We still have some ways to go, taking care of all the capybara populations.

Professor José Catão-Dias, honored and grateful for the interest you took in my research, always making yourself available to be part of the examining board, providing positive criticism and guidance.

Professor José Guerra, a privilege to have been a student of your pathology lectures. Thank you for your guidance throughout all post-graduate studies, allowing me to access a world of wisdom and competence.

In appreciation to the University of Sao Paulo, School of Veterinary Medicine and Animal Science, for allowing me to conduct my studies with the assistance of the School's Dean, Prof. Dr. José Antonio Visintin and the secretaries, Mrs. Thais Soto Longarço and Catia Silene do Carmo Pimenta . It was a great honor to have been here these past four years.

My gratitude to all the professors and colleagues of the Department of Animal Reproduction (FMVZ/USP) that provided me, not just with a home over the past 4 years but bounteous support to my research studies. Professor Pietro Sampaio Baruselli, thank you for the conservations and suggestions. Mayra Elena Ortiz D'Avila Assumpção, always available when needed. Specially in-debt to Prof. Dr. Marcilio Nichi and colleagues from the Andrology Lab, João D. A. Losano, Giulia Kawai, and Roberta F. Leite (Bobbie), for being hands-on, taking part of one of the most fundamental results for this study. My gratitude to Priscila Viau, Lab for Hormone Analysis, in her efforts to offer advice and support the project.
A very special thank you to my second post-graduate angel, Harumi Doi Shiraishi, secretary of the post-graduate program, department of animal reproduction, for all your assistance and friendship over the years.

To my peers and many helping hands:

The department of Veterinary Pathology (VPT), Professor Claudia Momo; and Professor Bruno Cogliati.

The department of Experimental Epidemiology Applied to Zoonoses (VPS). Especially Professor Labruna; Professor Oswaldo; Colleague Alfredo

The department of Veterinary Clinic: Professor Silvia; Professor Zoppa; Professor Stefano, for making yourself available with knowledge and equipment, trying to discover the applicability of ultrasound exams in capybaras. Nicole, thank you for your time and your competence during the capybara x-ray sessions, one day it will be published. Rosendo, for all the help. Very special thanks to my friends and colleagues Mário Antonio Ferraro Rego and Priscila Rocha Yanai, for generously participating in the project, and being available when most needed.

Guarulhos Municipal Zoo, to my friends and fantastic colleagues Claudia Igayara, Gilberto Penido, grateful for you taking part in this project. Without you, things would have been very different. And to all to all of the Zoo's great professionals for the opportunity to participate in wildlife handling and medicine, allowing me to learn so much from every one of you, Cristiane Bolochio, Hilari Hidasi, Camila Mazoni, Thais Sanches, Marcos Melo, Fernanda Magalhaes, Guilherme R de Azevedo, among others. I surely will miss my visits with my *Sogro*.

Grateful to Professor Tarcízio Antônio Rego de Paula, from the Federal University de Viçosa, Centrum of Biological Science and Health, Department of Veterinary Medicine, for sharing competence and knowledge, shedding light into the world of the capybara gonadal physiology.

To the prefecture of the University City of the University of São Paulo, I'd like to express my appreciation for the opportunity to conduct my research at the Raia Olímpica, allowing me access to the Capybara and providing support along the way, Daniela Vilela Lima, Environmental Health Service, dedicated to her work, and eager to help, thank you for your support. José Cícero da Silva & Wilson Wanderley de Aquino, thank you for all the assistance and all the fun while working at the field. Ricardo Borzan Mota, appreciating all the provided hands-on assistance during our field days, while guarding and liberating our special friends.

To all the veterinary students in participating as volunteers, providing an invaluable service to the project. Particularly Felipe Jose Corrado Kyomen and Flávia Sayeg Johansson, thank you!

FAPESP: Thankful to the São Paulo Research Foundation (FAPESP) for the research grant and scholarship (#2016/12549-5), providing the opportunity to pursue my doctorate in Brazil.

Project Sponsor: I am grateful to Mr. Blair Soares, President of Pneu-Dart, Inc., Williamsport, PA 17701 for becoming, unexpectedly, a project sponsor, contributing to the project very generously with equipment, material, knowledge and the best technology, when it comes to Remote Drug Delivery Systems, to the project, and Mrs. Donna Emick for assistance and support throughout.

Brazilian Ministry of Agriculture, Livestock and Food Supply (MAPA)
Thank you to: Divisão de Fiscalização de Produtos de Uso Veterinário (Ministério da Agricultura, Pecuária e Abastecimento). Cláudia Leite Barbosa, Auditora Fiscal Federal Agropecuária, DPF/CPV/DFIP/SDA/MAPA, and team.

National Wildlife Research Center (NWRC). United States Department of Agriculture (USDA). The Animal and Plant Health Inspection Service (APHIS), Fort Collins, CO, USA. Many thanks to Douglas C. Eckery, Ph.D. - Project Leader – Fertility Control and to Emilly W Ruell, John Eisemann. For the opportunity to provide a seminar about my research on Wildlife Population Control in Brazil, during y technical visit to Ft. Collins, Co, US, 2016. Also, for sharing with me everything to learn about GonaCon, especially grateful for Darcy Orahood Mora.

International Society of Wildlife Endocrinology (ISWE): Thank you to all organizer and peers for allowing me to share my work during International congress events. Special thanks to Jonathan Aaltonen, Camryn D Allen, and Cat Reburn-Wheaton, Mandi Wilder Schook, Andre Ganswindt, Janine Brown, Maria Ponzio.

Exército Brasileiro: Grato ao Coronel Adilson (DFPC), Diretoria de Fiscalização de Produtos Controlados. Quartel General do Exército. Setor Militar Urbano – Brasília – DF, e ao Coronel Alves Souza (SFPC), e seus membros oficiais da seção Serviço de Fiscalização de Produtos Controlados. 2ª Região Militar - Região das Bandeiras. Exército Brasileiro – São Paulo, SP

Receita Federal - EARE: Grato a Sr. Darlan Gonçalves da Rosa - Auditor-Fiscal da Receita Federal do Brasil, Chefe da Equipe de Controle de Regimes Aduaneiros Especiais – ERAE, e sua Equipe, de alfândega do Aeroporto Internacional de São Paulo/Guarulhos. Superintendência da Receita Federal do Brasil na 8ª Região Fiscal

Governo do Estado de São Paulo, Secretária do Meio Ambiente, Departamento de Fauna: Agradecimento profundo pelo de todos os membros: Sra. Vilma Clarice Geraldi, Sra. Thais Guimarães Luiz, Sra. Monicque Silva Pereira pelas oportunidades de apresentar minha pesquisa nas reuniões técnicas sobre Manejo de Capivaras e Febre Maculosa Brasileira.

Governo de Estado de São Paulo, Secretária de Estado da Saúde. Superintendência de Controle de Endemias (SUCEN): Agradeço o apoio para o projeto Adriano Pinter e Celso Eduardo de Souza, pelas oportunidades de representar meu trabalho nos Simpósios Estaduais de Doenças Transmitidas por Carrapatos em Indaituba, SP, 2017 e 2019.

Dr. Britto, D.V.M., Municipal Americana

And,

Dr. Daniel Chagas Dantas, in a different way, given me the possibility of continuing my research!

“It would be absolutely useless for any of us to work to save wildlife without working to educate the next generation of conservationists”,

“What you do makes a difference, and you have to decide what difference you want to make.

- Jane Goodall

ABSTRACT

In Brazil, the invasion of urban areas by capybaras (*Hydrochoerus hydrochaeris*) represents a severe public health problem because of their epidemiological importance as an amplifying host for *Rickettsia*, the causative agent of the tick-spread disease Brazilian Spotted Fever. This is in addition to the human–capybara conflicts (traffic accidents, agricultural damage, etc.). Capybaras' resistance to adverse environmental conditions, high proliferative rates that are concurrent with reduction by natural predators, and laws against hunting mean that their population can quadruple in one year, consequently, increasing the beforementioned problems in anthropic areas. In this thesis, a population control model, based on immunocontraception, was used for the first time in *H. hydrochaeris*. The anti-GnRH vaccine (GonaCon), a single-dose recombinant, was administered intramuscularly, causing prolonged infertility in both sexes. To study the antifertility effects in this species, multidisciplinary studies were conducted; for a period of 36 months, 20 adult capybaras (10 males and 10 females); divided into two groups (control and treated) were investigated. Prior to the first intervention, animals were observed for reproductive behavior, fertility, and social group dynamics. Furthermore, positive-reinforcement conditioning was used to facilitate physical and chemical restraint, minimizing capture-related stress. During the initial event, animals were health examined, ID marked, and biomaterials were collected, serving as a base-reference. Biomaterials were collected periodically (every 3–4 months), and investigations of contraceptive impacts on reproductive behavior and the group's integrity were carried out. Results showed: GonaCon-treated individuals ceased mating activity, with no direct births observed, confirmed by alterations to the reproductive physiology (spermatogenesis and folliculogenesis). Nevertheless, agonistic, courtship and alloparental behavior was preserved, maintaining the group's integrity. As a result, population growth was reduced, suggesting that immunization against GnRH is an effective long-term antifertility method in capybaras. Motivating critical discussions on non-lethal wildlife population control in Brazil, and the pressing issues on synanthropic species (capybaras), the study's intent was to provide an alternative strategy and, possibly, a national model to mitigate human-wildlife conflicts and zoonotic disease spread.

Keywords: Capybara; *Hydrochoerus hydrochaeris*; Population control; Rickettsia; Immunocontraception; Spotted Fever.

RESUMO

No Brasil, a invasão de áreas urbanas por capivaras (*Hydrochoerus hydrochaeris*) representa um grave problema de saúde pública que se deve ao seu papel epidemiologicamente importante como hospedeiro amplificador de *Rickettsia rickettsii*, provocador da doença Febre Maculosa, transmitida por carrapatos. Some-se a isso os possíveis conflitos humanos - capivaras (acidentes de trânsito, danos à agricultura). A resistência das capivaras às condições ambientais adversas e sua alta capacidade de procriação, combinadas com a redução de predadores naturais e leis contra sua caça, podem quadruplicar sua população em apenas um ano, aumentando, consequentemente, os problemas anteriormente mencionados nas áreas antrópicas. Nesta tese, foi usada pela primeira vez, em *H. hydrochaeris,* um modelo de controle populacional baseado em imunocontracepção. A vacina anti-GnRH, um imunocontraceptivo recombinante de dose única, foi administrado por via intramuscular, causando infertilidade prolongada em ambos os sexos. A fim de estudar os efeitos da antifertilidade nessa espécie, foram conduzidos estudos multidisciplinares por um período de 36 meses em 20 capivaras adultas (10 machos e 10 fêmeas); dividido em 2 grupos (controle e tratado). Antes da captura inicial, os animais foram observados em seu comportamento reprodutivo, fertilidade e dinâmica social do grupo. Além disso, condicionamento de reforço positivo foi utilizado para facilitar confinamento físico e químico, o que minimizou o stress relacionado à captura. Durante o evento inicial, a saúde dos animais foi examinada, receberam identificação, e biomateriais foram coletados, servindo como referência. Biomateriais foram periodicamente coletados (a cada 3-4 meses) e investigações sobre o impacto dos contraceptivos sobre o comportamento reprodutivo e integridade do grupo foram realizadas. Nas observações diretas, os resultados mostraram que nos indivíduos tratados com GonaCon não houve acasalamentos nem nascimentos a eles associados, o que foi confirmado por alterações na fisiologia reprodutiva (espermatogênese e foliculogênese). Por outro lado, o comportamento agonístico, de cortejo e aloparental foram mantidos, preservando, assim, a integridade do grupo. Resultado: o crescimento da população reduziu, sugerindo que a imunização contra GnRH é um método de antifertilidade prolongado efetivo em capivaras. Motivando discussões críticas sobre controle populacional não letal de animais silvestres no Brasil e sobre questões prementes em espécies sinantrópicas, o objetivo do estudo foi fornecer uma estratégia alternativa, e possivelmente um modelo nacional para mitigar os conflitos seres humanos-animais selvagens, e a disseminação de doenças.

Palavras-chave: Capivara; *Hydrochoerus hydrochaeris*; Controle populacional; Imunocontracepção; Rickettsia; Febre Maculosa.

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SUMMARY

CHAPTER PRESENTATION

This thesis is written throughout in English language and in format for International publication. Included published articles, in its principal presentation, followed the editor's guidelines. The work is organized into ten chapters and two appendices:

Chapter I provides a brief overview of the emerging issues of Human-Wildlife Conflicts, wildlife/feral population control, the One Health concept, and a concise review about the biological and ecological facts of *Hydrochoerus hydrochaeris* – the capybara.

Chapter II describes in detail, as a literature review, the influence of contraceptive methods on the reproductive physiology in mammals and questioning, if the concept of immunocontraception for wildlife population control would be indeed the most adequate choice. This chapter, in its final format, was published by the scientific journal Brazilian Journal of Veterinary Research and Animal Science, co-authored by Cristiane Schilbach Pizzutto.

Chapter III proposes an alternative to directly control the capybara natality rate, and indirectly, the maintenance and transmission of *R. rickettsii* (Brazilian Spotted Fever, port. *Febre Maculosa*) within a capybara population. This chapter is published in the Journal of the Brazilian Society of Tropical Medicine, co-authored by Gina Polo, and Cristiane Schilbach Pizzutto.

Chapter IV emphasizes the importance to maintain the alpha behavioral and sexual characteristics in male capybara, especially following an antifertility treatment. The manuscript was published at the Journal for Threatened Taxa, co-authored by Cristiane Schilbach Pizzutto.

Chapter V reports the findings of the principal study on field-testing a single-dose immunocontraceptive in free-ranging male capybara (*Hydrochoerus hydrochaeris*), explaining its effects on the reproductive physiology, secondary sexual characteristics, and agonistic behavior. This chapter was published by the journal Animal Reproduction Science (Elsevier), co-authored by Marcilio Nichi, João D. A. Losano, Giulia Kawai, Roberta F. Leite, Alfredo J. Acosta, Oswaldo Santos Baquero, and Cristiane Schilbach Pizzutto.

Chapter VI informs about the findings of the principal study in regard to the immunocontraceptive effects on free-ranging female capybaras, their reproductive, -and alloparental behavior.

Chapter VII explains the methodology of "conditioning by positive reinforcement", with the objective to minimize capture-related stress and to facilitate repetitive captures of selective animals. This article has been accepted for publication at the journal *Acta Scientiarum* - Biological Sciences, co-authored by Cristiane Schilbach Pizzutto.

Chapter VIII details the development of anesthetic protocols, used during the field-research. The manuscript has been submitted to the Brazilian Journal of Veterinary Medicine, co-authored by Mario Ferraro, Claudia Igayara, Silvia Renata Gaigo Cortopassi, and Cristiane Schilbach Pizzutto.

Chapter IX reports on the iatrogenic provoked risks of tympany, brought about by excessive use of bait and anesthetics administered. This article is accepted for publication at the Brazilian Journal of Veterinary Pathology. Co-authored by Mario Ferraro; Priscila Rocha Yanai; Claudia Igayara, and Cristiane Schilbach Pizzutto.

Chapter X provides the technical aspect of RDDS (Remote Drug Delivery Systems), and how it applies to long-distance vaccination, analyzing the temperature-dependent drug viscosity.

Chapter XI, a short discussion, concludes this thesis, reflecting on the findings, represented in each chapter, and its significance in the overall research objective: managing wildlife, and feral populations through an alternative non-lethal method, while controlling human-wildlife conflicts and epidemiological concerns.

Appendix XII registered the project's appearance in the media (TV and print)

Appendix XIII discloses the Governmental interest in the project's advancement

The number of chapters and the diversity of the topics investigated, although all-encompassing, to one degree or another, the same general research objective, demonstrates the multidisciplinary nature of the studies involved. Ranging from Animal Anatomy, Reproduction, Veterinary Medicine and Surgery, Anesthesiology, Experimental Epidemiology Applied to Zoonoses, to Pathology.

1. INTRODUCTION

1.1 HUMAN-WILDLIFE CONFLICTS AND THE ONE HEALTH CONCEPT

With a world human population closing in on 8 billion by the year 2020 ("World Population Clock," n.d.), anthropogenic activities diminished and polluted natural habitats, either invasion, or destruction, provoking large scale annihilation of wildlife species on one side, or forcing wildlife into rural and urban areas, invading public and private spaces, inevitably causing human-wildlife conflicts (HWC). As it is the case with some high-proliferative mammalian species in South-America, pertaining to the native fauna, like capybara (*Hydrochoerus hydrochaeris*), Peccary (*Pecari ssp*.), as well as emerging species, such as quati (*Nasua nasua*), opossum (*Didelphis* ssp.), bat (*Chiroptera* ssp.) and primates (*Callithrix* ssp.) in addition to exotic invasive and feral species, primarily Wild boar (*Sus scrofa*), with driving population numbers in common (Hemetrio, 2011; Marchini & Jr, 2015). Not including fauna species pertaining to aves, reptiles, and insects, for example, vulture, serpents, and scorpions, respectively, and equally important.

Brazil, unfortunately, is also champion in roadkill statistics, with more than half-a-billion killed animals yearly and associated consequences to human-life and economics.

However, the greatest concern being epidemiological, in form of direct and vector-borne zoonotic disease spreads (other than by insects), frequently associated with before mentioned species, namely leptospirosis, toxoplasmosis, rabies, rickettsioses,

Wildlife management is still in its infancy in Brazil and lacks nation-wide coordination. The biggest challenge in controlling human-wildlife confrontations to overcome is Brazilian's environmental law, basically prohibiting any culling and hunting, with exceptions to exotic invasive species, and in public health crisis.

Most recently, one such zoonotic and tick-borne crises was related to Brazilian Spotted Fever, one of the most lethal strains of the etiologic agent *Rickettsia rickettsii*, maintained in amplifying hosts, like the capybara, horses, and dogs, vectored by various types of *Ixodidae* (hard ticks) of the genus *Amblyomma* (Labruna, 2014; Meira & et al, 2013).

1.2 THE ONE HEALTH CONCEPT

One Health recognizes that the health of people is connected to the health of animals and the environment. The goal of One Health is to encourage the collaborative efforts of multiple disciplines-working locally, nationally, and globally to achieve the best health for people, animals, and our environment.

Importance: A One Health approach is important because 6 out of every 10 infectious diseases in humans are spread from animals (“One Health | CDC,” 2017).

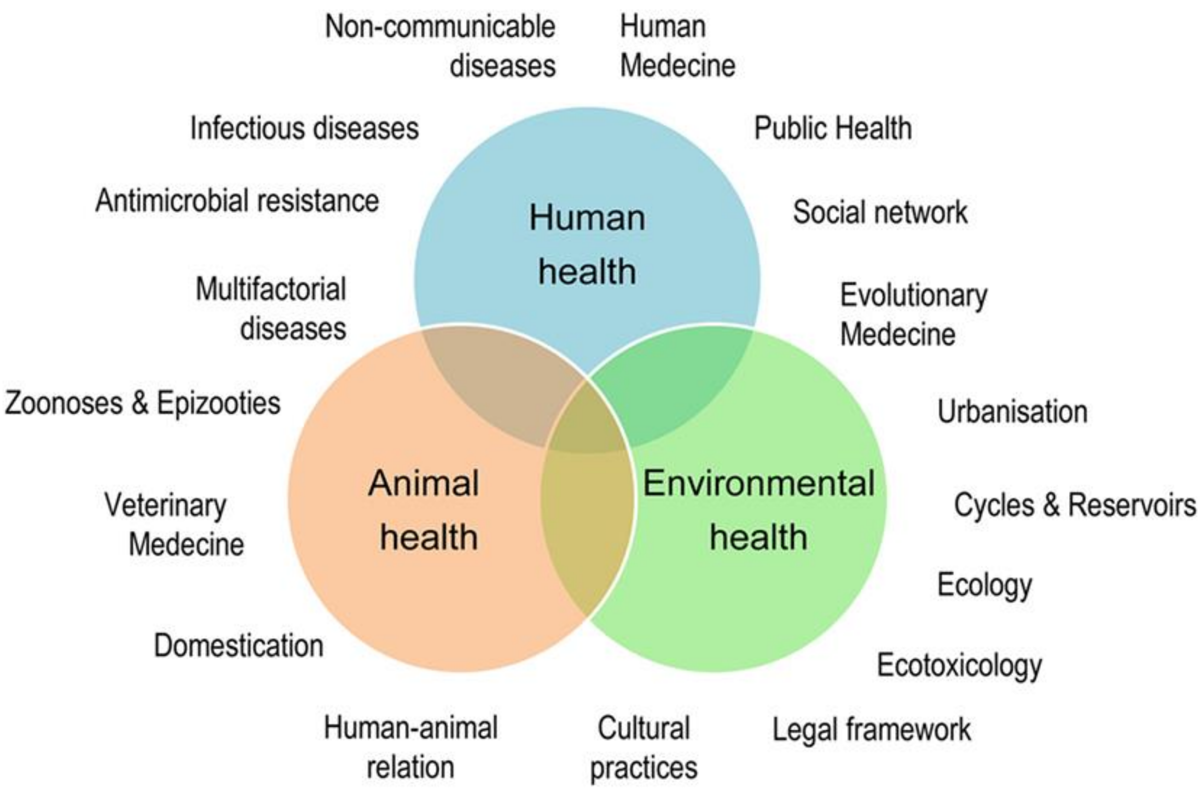

Figure1.1 - One Health Concept.

Source: Delphine Destoumieux Garzon, Cnrs Montpellier PD.

Naturally, wildlife-associated zoonotic diseases increase the public's negative perception of wildlife, progressing to abhorrence and aversion, rather than seeing benefits. One Health connects animal health, environmental health, and human health as a concept as well as in practice, intending to mobilize multiple disciplines and resources in a combined effort to most efficiently combat risk to public health while promoting environment and wildlife conservation. The interconnectedness of the One Health concept demands efforts in creating and increasing general public awareness and knowledge in order to gain public support (Buttke, Decker, & Wild, 2015).

1.3 BIOLOGICAL AND ECOLOGICAL CONSIDERATION ABOUT *Hydrochoerus hydrochaeris* - THE CAPYBARA

1.3.1 Scientific and popular names

Table 1.1 Scientific and vernacular nomenclature capybara

Scientific name	**Name English language Vernacular**	**Name other languages Vernacular**
Hydrochoerus hydrochaeris Greek “*hydro*” water & “*choerus*” hog	Capybara	Capivara (PT) Wasserschwein (DT) Chigüiro/Carpincho (ES) Ka'apiûara (Tupi) Quiuit (IT) Cabiaï (FR) カピバラ (JP)
Hydrochoerus isthmius Greek: “*hydro*” water & “*isthmius*” small	Lesser Capybara	

1.3.2 TAXONOMY

Kingdom: Animalia

Phylum: Chordata

Class: Mamalia

Order: Rodentia

Family: Caviidae

Subfamily: *Hydrochoerinae*

Genus: *Hydrochoerus*

Species: *Hydrochoerus hydrochaeris*

Common names: Capybara

Figure 1.2 - *Hydrochoerus hydrochaeris* male
Source: D. Rosenfield, 2017

1.3.4 Threat Status and Distribution (*Hydrochoerus hydrochaeris*)

Threat Status

The International Union for Conservation of Nature's Red List of Threatened Species

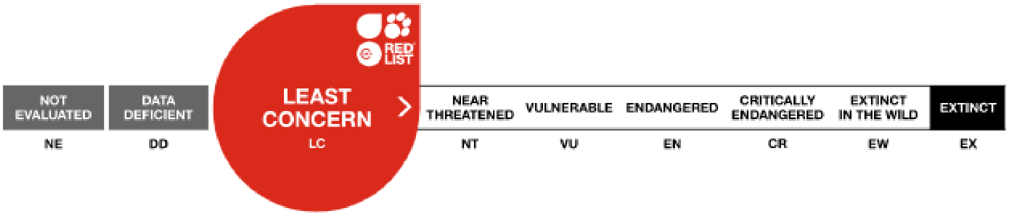

Figure 1.3 - Threat Status Source: IUCN Red List of Threatened Species 2016

Geographic distribution

Hydrochoerus hydrochaeris has a broad distribution throughout South America, including east of the Andes, Colombia, Venezuela, Ecuador, Peru, and Guyana, Brazil, eastern Bolivia, Paraguay, Uruguay, northwestern and eastern part of Argentina (Lacher, Slack, Coburn, & Goldstein, 1999).

Figure 1.4 - *H. hydrochaeris*, South America distribution map Source: USGS, NOAA, 2019

1.3.5. Threat Status and Distribution (*Hydrochoerus isthmius*)

Threat Status

The International Union for Conservation of Nature's Red List of Threatened Species

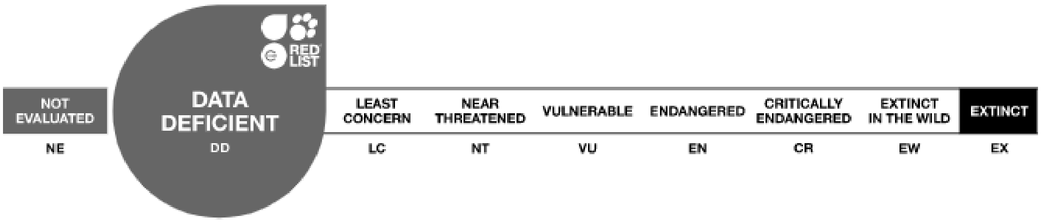

Figure 1.5 - Threat Status   Source: IUCN Red List of Threatened Species 2016

Geographic distribution

Hydrochoerus isthmius's distribution is concentrated to the North-Eastern and Central American Region, including some Islands, including western Colombia, and northwestern Venezuela, Panama and Trinidad/Tobago (Dinanath, 2017).

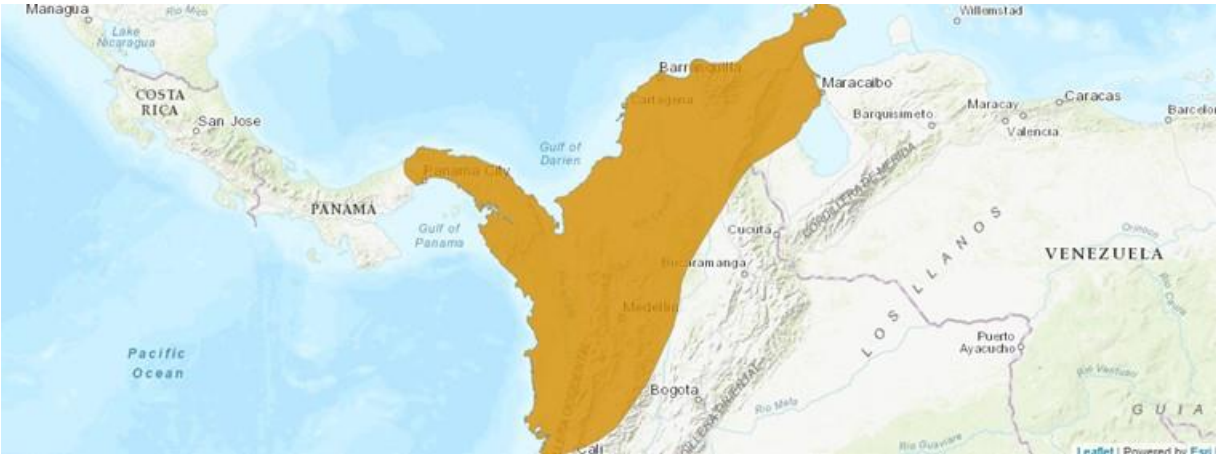

Figure 1.6 - *H. isthmius.* South America distribution map.   Source: USGS, NOAA, 2019

Capybaras are also maintained in zoo and parks throughout the world, as well as a trend to keep them as pets. Furthermore, there are speculations as to sightings of free capybaras in surrounding areas of the Everglades (Florida, USA), as an exotic-invasive species (EAS, 2019; Parker et al., n.d.; ROUS Foundation, 2019).

1.3.6 General Description (*Hydrochoerus hydrochaeris*)

Capybaras are the largest of rodent species, with a height reaching over 60cm and a length of over one meter. Their average body mass is between 50-60kg, while in synanthropic individuals can gain over 100kg (pers. obs., 2017). The color of their coarse and not dense fur range from light to reddish-brown, to black, with the ventral side being almost free of coat. As semi-aquatic animals, their anatomy has a number of characteristics adapted to life in and around water. Starting with the sturdy and barrel-shaped body (fig. 1.7), with a large proportion of adipose tissue, allowing for buoyancy and forward motion while swimming or diving, facilitated by a partially interdigital webbing, with four toes on their forefeet and three on their hindfeet (fig. 1.8 a/b).

Figure 1.7 - Adult male capybara. Source: D. Rosenfield, 2017

Figure 1.8 - Capybara feet.
a) forefeet. b) hindfeet with partial webbing (yellow arrow). Source: D. Rosenfield, 2017

The head is showing the most significant adaptations, with an elongated nasal bone where the nostrils, eyes, and ears are located near the top, allowing for surfacing while swimming. The ears are small and can be folded shut, to prevent water from entering.

Sexual dimorphism is only distinct when comparing sexual mature and dominant adults. Dominant males (alpha males) are identifiable by their enlarged protuberant nasal gland, which also exists in females, but much less developed and less secretion (fig. 1.9).

The male urogenital system remains intraabdominal (fig. 1.10 c), without a visual scrotum. Only in dominant males, the testicles migrate to the inguinal region (fig. 1.10 b), becoming slightly visible on the most upper-medial area, bilateral of the hind legs (Moreira, 2013; D. A. Rosenfield & Schilbach Pizzutto, 2019).

Figure 1.9 - Capybara sexual dimorphism

Blue arrow male, red arrow female nasal gland. Blue section enlarged male gland with oleos secretion. Source: D. Rosenfield, 2018

Figure 1.10 - Reproductive anatomy

a) exposed penis; b) Visible testis of an alpha male; c) male cloaca; d) female cloaca. Source: D. Rosenfield, 2018

Capybaras consume about 65,5kcal/kg/day of variations of grasses and aquatic plants (Ojasti 1973), and if accessible, crops such as sugar cane, corn, and banana leaves. They are not just the world’s largest rodent, but as monogastric herbivores, also the largest cecum fermenter, with 74% of the entire gastric tract (Borges et al., 1996).

As the large intestines are not able to adequately absorb the first passage of nutrient-rich cecal content, they depend on cecotrophy as an added digestive strategy, whereby the digesta is ingested directly from the anus, maximizing nutritional absorption during the second passage (Baldizan et al., 1983; Borges et al., 1996; Moreira, 2013). After this second passage, feces in the form of pallets are formed and excreted. The size of the produced pallets (fig. 1.11) can be used to identify age, body weight and quantity/occurrence can assist to estimate population number (Ojasti, 1973; Luciano M. Verdade, Moreira, & Ferraz, 2013).

Figure 1.11 - Capybara adult feces (pallets). Source: D. Rosenfield, 2017

Capybara has no canine, as their dental formula is highly specialized for selective grazing, showing 1/1 incisors with a wide diastema (gap) to the 1/1 premolars, followed by 3/3 molars. The very large and sharp incisors (fig. 1.12) allowing to feed on very short vegetation, which also serves as their principal weapon to fend off predators but also for fights among themselves, especially for dominance competing males, leaving very deep and potentially fatal wounds.

Figure 1.12 - Capybara two pairs of sharp incisors. Source: D. Rosenfield, 2017

1.3.7 Female Reproductive physiology

Sexual maturity: age 12 – 13 months   weight ≥20kg

Adult weight (non-synanthropic): 40 – 55 kg

Placenta: Chorioallantoic (principal placenta) and vitelline placenta

Seasonality: annual polyestrous

Estrous cycle: 7.5 ±days

Spontaneous ovulation: 8 hours

Gestation period: 147 – 156 days

Litter size: 2 – 8 young

(M. A. Miglino, Carter, Ferraz, & Machado, 2002; Maria Angélica Miglino, Santos, Kanashiro, & Ferraz, 2013)

1.3.8 Offspring

As it is typical for prey species, pups are born with their eyes open and able to interact with their environment instantly. Weight at birth: 700 gr – 1100 gr

Figure 1.13 - Newborn capybara pup. Source: D. Rosenfield, 2017

1.3.9 Social hierarchy and alloparental behavior

Capybara's polygynous society is heavily female-biased (fig. 1.12), made up of an alpha male, or principal breeder, fiercely defending its harem from any potential male intruder, or sexually maturing male, which are driven out of the group, remaining in a certain distance as satellite males. Followed by dominant adult females and adult females, gender-mixed adolescents and pups (fig. 1.13).

Dominant females become aggressive when it comes to feeding rights and are the principal breeders. Not all subordinate females breed, although sexually mature, with the alpha male, uncertain if this is caused by a potentially cyclic suppression, due to the presence of dominant females, or another kind of mechanism. On the other hand, subordinate females are observed to leave the group to mate with nearby satellite males, afterward returning to the group, or giving start to a new group formation.

A capybara surviving strategy includes alloparental conduct, whereby females do nurse pups indiscriminately, being their own, or from other females Reason why females nursing pups is not an indication of biological association. In addition, enlarged teats do not serve as an indication for birth [(Nogueira, Otta, Dias, & Nogueria-Filho, 2000), and own observations, 2016 - 20190].

Figure 1.14 - Capybara group. Females, adolescents, pups. Source: D. Rosenfield, 2016

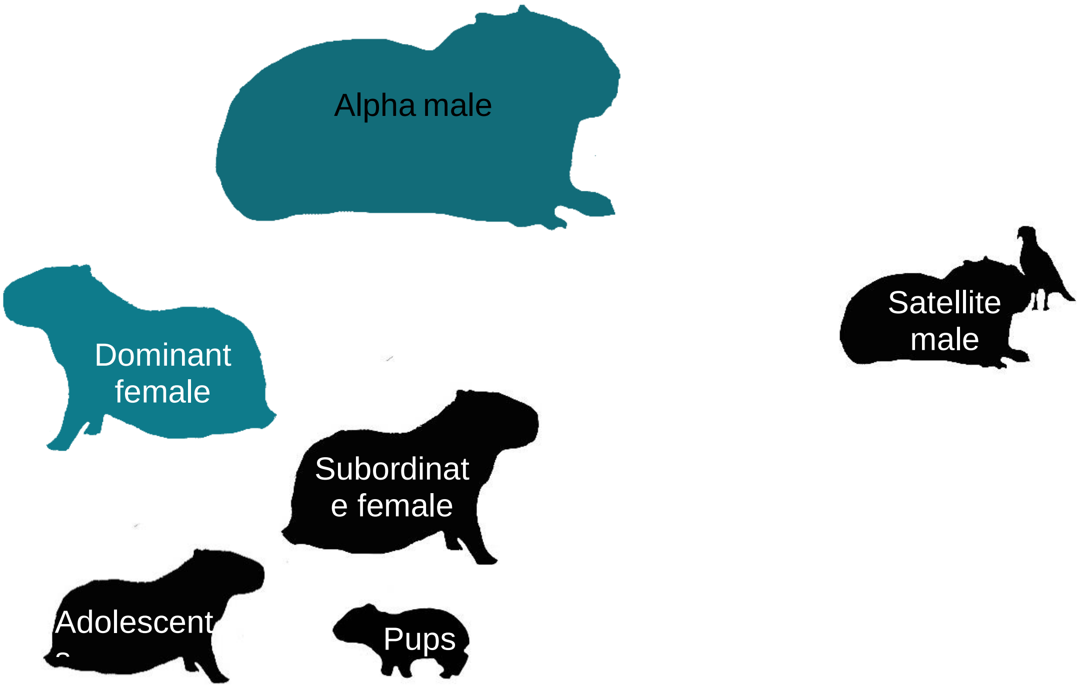

Figure 1.15 - Capybara linear hierarchy. Source: D. Rosenfield, 2016

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2. WILDLIFE POPULATION CONTROL - REPRODUCTIVE PHYSIOLOGY UNDER THE INFLUENCE OF CONTRACEPTIVE METHODS IN MAMMALIAN WILDLIFE, WITH EMPHASIS ON IMMUNOCONTRACEPTION: BEING THE BEST CHOICE?

2.1 ABSTRACT

Human-Wildlife-Conflicts, a growing and sad reality worldwide, making population control of wildlife and feral animals one of the biggest challenges in wildlife management, especially due to the rapidly expanding human population, and consequently, the ever-diminishing natural habitats of animals. Human activities and the destruction of nature forcing wildlife to move into urban and agricultural areas, inevitably, causing “conflicts”, like the risk of zoonosis, traffic accidents; as well as damage to crops in the search for food, whose losses reach millions of dollars. For decades, science been engaged in extensive efforts to develop methods of “humane” population control methods; and many techniques are being employed, to control wildlife population. In this article, we present an overview of applied contraceptive methods with simplified graphics demonstrations of their interactions with reproductive physiology; further, relating pro and contra of utilized antifertility agents. These are being compared to a set of desired characteristics for free-ranging wildlife for in-field applications, with emphasis on reversible immunocontraception, - concluding, therefore, the reasons why this concept is becoming the most appropriate and promising for free-ranging wildlife.

Keywords: Wildlife Population Control; Human-Wildlife Conflicts; Mammals; Reversible Immunocontraceptives

2.2 RESUMO

Os conflitos Humanos-Animais Selvagens são uma realidade crescente e triste no mundo inteiro, tornando o controle populacional da fauna silvestre e animais ferais o maior desafio, principalmente diante do crescimento da população humana e, consequentemente, da diminuição dos habitats naturais dos animais. As atividades humanas e a destruição da natureza, forçam os animais de vida livre a se dirigirem para áreas urbanas e agrícolas, inevitavelmente, causando conflitos, como o risco de zoonoses, acidentes de trânsito, bem como danos às plantações, quando em busca de alimentos, cujo prejuízo chega a milhões de dólares. Durante décadas, a ciência esteve empenhada em esforços extensivos para desenvolver métodos de controle populacional “humano”; e muitas técnicas foram utilizadas, a fim de se realizar um controle populacional destes animais silvestres. Neste artigo será apresentada uma visão geral dos métodos anticoncepcionais aplicados, com demonstrações gráficas simplificadas de suas interações com a fisiologia reprodutiva, bem como relacionando os prós e os contras dos agentes antifertilidade empregados; também serão comparados com um conjunto de características desejadas para as aplicações em fauna a campo, com ênfase em imunocontracepção reversível, concluindo assim, com as razões do porque este conceito torna-se o mais apropriado e promissor para animais silvestres de vida livre.

Palavras chaves: Controle de População Animal Silvestre; Conflitos Humanos-Animais Selvagens; Mamíferos; Imunocontraceptivos Reversíveis

2.3 INTRODUCTION

The biggest challenge in understanding wildlife reproductive physiology lies within its vast diversity of species. Just in the mammalian class alone, there are more than 5500 known species (IUCN, 2016), every single one with its anatomical and biological particularities. Any campaign to manipulate a species population by employing contraceptive methods, demands a dedicated understanding of its reproductive physiology and social makeup to warrant the overall health and well-being, of the individual animal as well as of its entire group. Which contraceptive method to choose depends on several aspects, ranging from the species to be treated; gender, age, reproductive biology; to environmental situations; among many others, and there is no “one-product-fits-all solution”! Moreover, most contraceptive products are developed and optimized for human use, and here, almost exclusively for women. Although approved by regulatory authorities, and tested for safety and efficiency, there are still many reported adverse effects in humans; let alone, potential health concerns in wildlife species. Modern contraceptives can manipulate biological processes at any point in the reproductive process, for instance, preventing hormone synthesis at the hypothalamus and pituitary glands by hindering receptor expression, or by blocking receptors; ceasing gametogenesis; impeding sperm motility; or block sperm-ovum diffusion, just to name a few (C. Asa, 2005; Cooper & Larsen, 2006; P. Delves, 2002; Kaur, Prabha, Kaur, & Prabha, 2014; J. F. Kirkpatrick, 2011; Kogan & Wald, 2014; Pickard & Holt, 2007).

But why is wildlife population control even an issue? Mainly, the attempt to control and minimize human-wildlife conflicts, like urban invasions; agricultural destructions; attacks on livestock and on humans; besides the spread of zoonotic diseases, among others. But also, for land management, or due to space limitation in captivity, furthermore, the noble efforts to guarantee species survival, and genetic variance come into play.

This review attends to the understanding of the dynamics of contraceptive methods and its interactions with the mammalian reproductive physiology and endocrinology, as well as associated adverse impacts. Emphasis is given on immunocontraceptive concepts, illustrating the attributes that make this strategy, for the time being, perhaps the most practicable alternative to control free-ranging wildlife population.

2.3 BRIEFLY - THE HYPOTHALAMIC–PITUITARY–GONADAL AXIS (HPG AXIS)

Even though the hypothalamus, the pituitary, and the gonadal glands are distinct entities, in their performance of controlling the reproductive function (among several other duties), they work together and depend on one another. The hypothalamus, an organ that regulates many homeostatic functions and responds to ambient influences (stimuli), drives many physiological and behavioral processes; to illustrate: a male perceives a female in heat by sensing her pheromones; sight, and physical contact, all stimuli that trigger the male's sexual behavior, exhibited by fights between males for dominance and mating rights; urging sexual arousal; mounting, intromission, and ejaculation.

The pituitary gland, or hypophysis, divided into three lobes, the anterior, or adenohypophysis (adeno = relating to gland), the intermediate, and the posterior lobe, synthesizing and secreting hormones responsible for regulating a wide range of physiological functions. Concerning reproduction, the gonadotropins Luteinizing Hormone (LH) and Follicular Stimulating Hormone (FSH), from the adenohypophysis, regulated by the hypothalamic Gonadotrophin-releasing Hormone (GnRH), are the chief reproductive hormones. These two glands are connected by the hypophyseal portal system, that supplies the blood, and therefore the passageway for hormones.

The hormones GnRH, LH, and FSH are identical in male and female mammals, with the same general regulatory role of gonadal activities (Figure 2.1) which is stimulating sex hormone synthesis and gametogenesis, although, not their only function.

These hormones are one of the principal targets regarding contraceptive agents and their applicability in both sexes. Reproductive hormones follow a very precise hierarchy, or pathway, referred to as the hypothalamic-pituitary-gonadal axis (HPG-axis), that functions as a cascade. Nonetheless, that does not imply a one-way signaling street only, as regulatory messengers do travel also in the opposite direction, called a positive or negative feedback loop, controlling the continuation of hormone synthesis and secretion. In the male, the HPG axis and feedback control are significantly simpler than in female, for obvious reasons (not having estrous cycles, pregnancy, and birth). During Positive Feedback, the GnRH stimulates the release of gonadotropins, and subsequently, LH and FSH stimulate Testosterone (T) synthesis mainly within the testis and spermatogenesis. During Negative Feedback, triggered by an increased plasma concentration of inhibin and peak T concentrations, secreted by the Sertoli and Leydig cells respectively, causes an inhibition of GnRH, LH, and FSH secretion; it is further believed that estrogen also plays a regulatory role in testosterone production (Abney, 1999; Cunningham & Klein, 2007; Johnson, 2013; McDonald, 2003; Nelson, 2005).

In females, feedback signaling is more complex, as it depends on the different phases of the estrous cycle (Figure 2.2) although distinct events, they depend on one another. In most mammalian species, leading from the follicular phase to ovulation, to the luteal phase, only interrupted by pregnancy; or cessation of the ovarian activity, either by menopause, pathologically; adverse ambient conditions; chronic stress; or, -suppression by contraceptive methods.

Feedback-loops during follicular phase: Positive feedback - GnRH stimulates the secretion of LH and FSH, which in turn, drives the ovarian follicle growth. Negative feedback - The ovarian follicles synthesize and secret Estradiol (E2); after reaching peak concentrations, GnRH decreases, keeping LH and FSH secretion to a minimum.

During ovulation: Positive feedback only - Continues release of GnRH maintains LH and FSH secretion, triggering ovarian follicles growth and eventually leading to the formation of dominant or matured follicle(s), starting to produce E2, subsequently, liberating an oocyte. Initially secreted E2 also promotes a thickening, and vascularization of the endometrium.

During the luteal phase: Positive feedback - After ovulation, the follicle’s tissue, under the influence of LH, transforms into a corpus luteum (CL), and together with FSH maintains Estrogen secretion. Negative feedback - The CL starts to secrete the Progesterone (P4). Increased plasma levels of P4 and E2 will act on the hypothalamus, as well as on the anterior pituitary, causing inhibition of GnRH, LH, and FSH, driving down follicle activity, simultaneously endometrial lining develops, preparing the uterus for a potential pregnancy. (Feldman, 2004; McDonald, 2003; Norris & Carr, 2013; Nussey & Whitehead, 2001; Squires, 2013).

2.4 LIGANDS AND RECEPTORS, THE “KEY” TO UNLOCK CONTRACEPTIVE MECHANISMS

Hormones, neurotransmitters, and pheromones are examples of ligands, because of their binding activity with a specific receptor protein, located either on the cell’s surface, within the cytoplasm or on the nucleus. Specific means that a ligand molecule must "fit" the receptor-like a key in a lock, and the better it does (rate of binding), the better the response. A binding prompts the receptor to a conformational change (shape), initiating the cell's signaling cascade, leading to cell response. Which is the biggest pharmacological challenge, designing a perfect ligand that only binds to its designated receptor. Pharmacological development is human-market-driven, leaving the development for wildlife species-specific contraceptives a low priority, and if at all, their focus application lies with domestic animals, leaving for wildlife management only what is generally obtainable, steroid hormone-based contraceptives being the classics and most frequently used. Nevertheless, as relayed by numerous published studies, these methods, depending on the species, carry a bad rep for serious side effects, provoked by unwanted activation of non-target receptors, causing several physiological, as well as behavioral side effects, especially in carnivore species. A typical example, in females, synthetic androgens may bind to P4 receptors, mimicking progesterone-like cell-signaling, consequently modulating endometrium function, leading to uterine pathologies; or synthetic P4s, known to induce hypersecretion of growth hormones, responsible for the development of cystic endometrial hyperplasia (Cheryl S. Asa et al., 2014; Bhatti et al., 2007; Gregoire, 2013; Lodish, 2000; Moresco, Munson, & Gardner, 2009).

Before a ligand can bind to a cell, its receptors must be adequately expressed (quantity) to bring about a biological effect, which depends on the physiological necessity. In some processes, the cell undergoes a "priming", or upregulating, describing the event of amplifying the number (expression) of receptors. Inverse to that, there is "downregulation", or a reduction of receptors.

2.5 AN EXAMPLE OF A CONTRACEPTIVE DRIVEN DOWNREGULATION, OR DESENSITIZATION

GnRH-stimulated gonadotropin secretion may be inhibited by using GnRH antagonists (physically blocking the receptor), or by application of a GnRH agonist (GnRH analog) that, due to constant exposure may provoke a desensitization (inactivation of receptors), or by a downregulation (decreased receptor expression), ultimately resulting in reduced gonadal activity. Noteworthy, in the case of a GnRH agonist, there is an initial “paradoxical” response, a flare effect, exhibiting an increase in plasma LH and FSH levels, followed by a sharp decrease (Finch, Caunt, Armstrong, & McArdle, 2009).

2.6 MALE REPRODUCTIVE PHYSIOLOGY UNDER THE INFLUENCE OF CONTRACEPTIVES

In males, the primary reproductive organs are the testis, responsible for steroidogenesis and spermatogenesis, controlled by LH and FSH, secreted in a pulsatile pattern, and regulated by the positive feedback of GnRH, and the negative feedback of Testosterone (Figure 2.3). Testosterone, the chief testicular androgen is synthesized from cholesterol, primarily within the Leydig cells of the testis, however, its synthesis also occurs in other organs, like the adrenal cortices in both sexes; furthermore, in female’s ovarian theca cells, the placenta during pregnancy; and even in the skin (Zouboulis, 2009).

Testosterone’s first androgenic effects take place during sexual differentiation in the developing fetus; during maturation of the male sex organs, during growth and puberty, and throughout adulthood to sustain spermatogenesis; plus, it's bearing on the development and maintenance of secondary sexual characteristics, physical and behavioral (aggression). In fact, many studies investigate antifertility drugs to control intramale aggression, a dilemma in several captive-held species (Ferrie, Becker, Wheaton, Fontenot, & Bettinger, 2011; Penfold et al., 2002; Rachel, 2012). Unfortunately, one of the undesired consequences, are the negative effects on male adornments, meaning the lion’s mane; elk or deer antlers; bird’s plumage; scent-marking glands, like the nasal Morillo of capybaras, and muscular appearance. Estrogen, although, thought of as a female sex hormone, is being synthesized from

Testosterone in the Sertoli cells, controlled by FSH, believed to play an inhibitory role in the secretion of testosterone from the Leydig cell (Abney, 1999). Likewise, there are several other cell-signaling molecules produced and co-regulating Testosterone synthesis. Sertoli cells also secrete a transport protein, called androgen-binding protein, important in its role to concentrate Testosterone in the seminiferous tubules and to carry it throughout the body, (Berger et al., 2007; Clarke & Pompolo, 2005; Cunningham & Klein, 2007; McDonald, 2003; Mead, Maguire, Kuc, & Davenport, 2007;

Reiter et al., 2009; Rotstein, 2011; Simpson et al., 1999; Tassigny & Colledge, 2010; Zouboulis, 2009).

2.7 PERSISTENT TESTOSTERONE LEVELS AFTER CHEMICAL, OR SURGICAL CASTRATION

We do know that successful inhibition of gametogenesis is achieved after castration, chemically, or surgically. We also understand that, despite castration, there is still a certain plasma Testosterone level present, maintained by adrenal androgen synthesis. The question remains, though, does Testosterone synthesis from androgens other than the testis reaches efficient plasma concentrations to have an impact on sexual/aggressive behavior or any other gender-specific characteristics? An important question, when considering Testosterone depending behavioral traits, that governs social group dynamics, such as establishing and maintaining the dominant hierarchical group structure, imperative for its very survival.

Reason being why in captive animals and in free-ranging individuals, or smaller groups vasectomy is the contraceptive method of choice, allowing for minute changes in the overall androgen plasma concentration. However, depending on species and physical location, not a feasible “in-the-field” alternative, especially on a greater scale (Chester-Jones, Ingleton, & Phillips, 2013; McDonald, 2003; Nishiyama, 2014).

2.8 FEMALE REPRODUCTIVE PHYSIOLOGY UNDER THE INFLUENCE OF CONTRACEPTIVES

Female endocrinology, in comparison, is far more complex because of the obvious: conception; embryo development; birth-giving, and the nourishment of the newborn. The two principal female gonadal steroid hormones are Estradiol (E2) and progesterone (P4), and like in males, synthesis and secretion are chiefly under the influence of GnRH and Gonadotropins. E2 is synthesized in the ovary's theca interna, - and granulosa cells; controlled by positive feedback mechanism of LH and FSH, aided by Activin; regulated by the negative feedback, when E2 reaches peak plasma concentrations, acting on GnRH, - and Inhibin on FSH synthesis (Figure 2.2). Moreover, depending on the species-specific reproductive characteristics, such as being seasonal or non-seasonal breeders; mono, - polyestrous; spontaneous, or induced ovulation, all of which have their unique endocrinological functioning. Analog to the male's Testosterone, Estrogen is responsible for developing the female's reproductive organs, and the maintenance of secondary sex characteristics, including sexual behavior (receptiveness for mating), also referred to as behavioral estrus. Other ovarian hormones are Oocyte Maturation Inhibitor (OMI), responsible for the preservation of the oocytes during the arrest stage. Inhibin, like in males, blocks the secretion of FSH, while Gonadocrinin influences the steroidogenesis in the theca cells (a layer of the ovarian follicles). In addition, Activin and Relaxin for ovarian activity and parturition, respectively. Ambient and physical conditions influences the female's cyclicity, which is why contraceptive effects on the reproductive physiology can be complex and irregular (Berger et al., 2007; Clarke & Pompolo, 2005; Cunningham & Klein, 2007; McDonald, 2003; Mead et al., 2007; Reiter et al., 2009; Rotstein, 2011; Simpson et al., 1999; Tassigny & Colledge, 2010; Zouboulis, 2009).

A simplified representation of estrus cycles under the influence of two types of contraceptives (Figure 2.5). **GnRH Analogue:** The administration of a GnRH analog implant, resulting in an initial flare response of LH and FSH secretion, and subsequently, a sharp drop, until an inhibitory effect on gonadotropin secretion, as long as the GnRH implant maintains its bioactivity (Finch et al., 2009).

2.9 STEROID-BASED CONTRACEPTION

Administration of the safer combo-contraceptive, like an estrogen and progesterone agent, functions by basically tricking the body into believing it is already pregnant. Plasma levels of estrogen and progesterone remain constant throughout the treatment (fig. 2.6). Resulting in failure to produce an estrogen and LH peak, consequently, no ovulation occurs, with an added effect of preventing fertilization mechanically, through a thickening of the cervical mucus, interfering with sperm motility (Martin, 2016).

2.10 CONTRACEPTIVE METHODS IN WILDLIFE POPULATION CONTROL

Not considering social and individual behavioral impacts, nor potential risks of adverse effects, contraceptive Methods available for Wildlife species can be divided into:

Reversible:

- **Physical separation:** The oldest, easiest, safest, and cheapest method, if space requirement is of no concern.
- **Surgical procedures, examples:** Vasectomy in males, technically, considered reversible but that depends on several factors to be done so successfully. In practice, however, and depending on the species treated, because of intensive inflammatory processes and formation of scar tissues, there, is a potential risk of non-reversibility. Tubal ligation in females. Under field conditions, all considered impractical; costly, often impossible, and dangerous, especially with a larger number of animals to be treated.
- **Chemical Contraception** (Exogenous hormone-based contraceptives) oral, injection, or implant, for male and females, steroid, and non-steroid hormones.
- Immunocontraceptive vaccines (male and female)
- **Chemical castration** (male and female), depending on treatment duration, concentration, and the agent used, considered reversible in many cases.
- **Mechanical:** By obstruction, like the Vas Plugin males, or the IUDs and sponges, some in combination with hormone preparations, for female application.

Non-reversible:

- **Surgical procedures:** orchiectomy (removal of the testis), for males, and ovary-salpingo-hysterectomy (removal of the ovaries, fallopian tubes, and the uterus) for females.

Which contraceptive method is being employed at the end, depends on the chosen strategy, regarding short or long-term effects; applicability in the field, - or in captivity; ease of administration; associated risks; logistic and costs involved, and of course, for what species,

2.11 THE “PERFECT” CONTRACEPTIVE FOR WILDLIFE SPECIES

Proposed desired attributes:

- first, and foremost, they should have no health risk for the individual (no adverse effects)
- with little impact on secondary sexual characteristics
- as little influence, as possible on the individual’s, - and the overall social group behavior
- in most circumstances, it is desired to offer 100% reversibility
- offering long-term anti-fertility effects
- applicable in both genders
- easy administration (especially long-distance)
- one-time (shot) only, meaning no refresher or booster needed
- economically feasible for any wildlife management program
- regarding environmental considerations, it should not pass through the food chain
- and shouldn’t turn into a major pollutant.

(J. F. Kirkpatrick & Rutberg, 2001; Liu & others, 2011)

2.12 HORMONE-BASED CONTRACEPTIVE AGENTS

Antifertility drugs that act on the endocrine system are based either on steroid, - or non-steroid (glycoprotein, peptide) hormones, are mainly developed for human application, and here, chiefly for women. Because of their relative easy application, (orally, injected, or implanted); their effectiveness, and not less important, relatively low costs, they are most commonly used in the treatment of domestic animals and captive wildlife (C.S. Asa & Porton, 2005; Garside, Gebril, Alsaadi, & Ferro, 2014; Liu & others, 2011; Pickard & Holt, 2007; Rosenfield, 2016). They may act at any given point along the hypothalamic-pituitary-gonadal axis, including feedback signaling. Either as an agonist or an antagonist, for example, inhibiting synthesis and secretion of GnRH, subsequently LH/FSH hormones, - and consequently cessation of gonadal activities, such as sex hormone synthesis and gametogenesis. Or it may modulate the function of the female reproductive apparatus, bringing about desired contraceptive effects, like obstructing the transport of the ovum, blocking sperm passage, and the impending implementation of the egg in the endometrium. Nevertheless, steroid hormone-based contraceptives are very potent and potentially bind to non-target receptors. For instance, a progestin, binding to an androgenic receptor (AR), causing androgenic effects, or an estrogenic effect, by binding to estrogen α and β receptors (ERs), possibly triggering several undesired cell responses, leading to serious side effects, (Cheryl S. Asa et al., 2014; C.S. Asa & Porton, 2005; Davtyan, 2012; Deligdisch, 2000; McAloose, Munson, & Naydan, 2007; McDonald, 2003; Munson, Gardner, Mason, Chassy, & Seal, 2002).

Most commonly used hormone contraceptives are progestin-only, or progestin-estrogen combination, requiring smaller dosages and are safer (Sitruk-Ware, 2006). As stated by Asa, 2005, MGA (melengestrol acetate) a progestin, is the most commonly used contraceptive in US zoos, mainly due to its availability and effectiveness in a wide range of species.

As this article focuses on free-ranging wildlife mammals, intrauterine devices are not being particularized, although frequently used in domestic and zoo environments.

2.13 THE IMMUNE SYSTEM – SELF, NONSELF, AND THE CONCEPT OF VACCINES

In general, the immunocontraceptive concept takes advantage of the body’s immune system and its protective mechanism to discriminate, by identifying indigenous (self), and to mark, attack and eliminate foreign (non-self) proteins, allowing to fight off infectious diseases. Nonself material, or antigens, are being marked by the presence of antibodies, - specific to a group of organisms, or very similar ones. When the body’s own defense mechanism acquires immunity, it is termed “active immunity” and may last many decades, even a lifetime. And if provided by external means, it is called “passive immunity”, either introduced with the first milk given by the mother to the offspring (colostrum), also transplacental, or, after being produced by a mammalian animal, harvested, and transferred as a vaccine, via bait or injection, to another animal. Although very effective protection, its shortcoming is its limited temporary activity, lasting normally only a few weeks to months. However, today less of an issue, with the development of slow-release technologies, capable of providing protection that may last years. Even though the strongest immune response is with live microorganisms. These antigens do not need to be alive to trigger a response. For safety reasons, vaccines containing foreign inactivated or killed pathogenic microorganisms that mimic a real infection, but without the infection and associated complications.

The concept of immunocontraception provokes the production of antibodies against, - and binding to the body’s own reproductive protein, hormone or receptor (autoimmune response), either causing a barrier, or the formation of large molecular structures (change of protein structure = change of function) thus, inhibiting their native role in the fertility mechanism as long as a sufficiently high concentration (titer) of antibodies is present (Barber & Fayrer-Hosken, 2000; Croy, 2014; P. J. Delves, 2004; Harrison & Rosenfield, 1996; Moser & Leo, 2010; Tizard, 2009).

2.14 THE ROLE OF ADJUVANTS

Besides the active ingredient, a vaccine is made up of adjuvants, (lat. *adiucare*: to aid), and are important additives to vaccines, improving the innate immune response (antibody and T lymphocyte) by increasing the inflammatory response as it would be a real infection, which in turn, is important for an optimized adaptive immune response. Unfortunately, adjuvants are known to have their own set of potential health risks and are often associated with local injection site reactions (Lyda, Hall, & Kirkpatrick, 2005; Munson et al., 2005).

Adjuvants may include organic components which may be liposomes, a spherical vesicle with a minimum of one lipid-bilayer, used as a vehicle for pharmaceutical drugs; or lipopolysaccharides (LPS), part of endotoxins; parts of a bacterial cell walls; RNAs, or DNA strands; alum (hydrated potassium aluminum sulfate); and emulsions (oil-in-water/water-in-oil mixtures), for instance, Freund’s Incomplete Adjuvant (IFA).

Some adjuvants also function as a delivery system, causing the formation of depots at the injections site that traps the antigens, and allowing only a slow release of the vaccine components, maintaining a prolonged immunogenic stimulus, while enhancing the overall immune response. A concept that permits the “one-shot” (meaning only one application), with long-term effects

(C. Asa, 2005; Miller, Gionfriddo, Fagerstone, Rhyan, & Killian, 2008; Reed, Orr, & Fox, 2013; Sitruk-Ware, 2006).

2.15 CONCEPTS OF IMMUNOCONTRACEPTION (IMMUNOLOGICAL CASTRATION)

Following the most commonly studied and employed contraceptives methods: female-only (PZP, Porcine Zona Pellucida), male-only (EPPIN, epididymal protease inhibitor), or female and male (anti-GnRH antibody), see figure 2.7.

Immunocontraceptive Vaccine

A non-cellular membrane surrounds the mammalian ovum, consisting of several glycoproteins, known as the zona pellucida (ZP). The glycoprotein ZP3 had been identified as the sperm-binding receptor (which permits the attachment of sperm to the ovum, to continue the next step of the fertilization process, leading to the diffusion of the sperm into the oocyte, creating a zygote). The pZP vaccine is produced by creating a zona pellucida antigen derived from porcine oocytes. Once the female is inoculated with the pZP vaccine, her immune system will respond by producing antibodies against the pig's oocyte antigen. The very same antibodies also bind to the sperm receptors on the Ovum's surface (Figure 2.8), which will cause a distortion of the egg's structure, thereby blocking sperms from attaching, rendering the egg infertile without any other side effects, or behavioral impacts (J. Kirkpatrick & Rutberg, 2012; Liu et al., 2005; Miller, Crane, Gaddis, & Killian, 2001).

The Mechanism of The GnRH Immunocontraceptive Vaccine

The synthetic GnRH peptide is coupled to a foreign protein, like a hemocyanin protein, purified from the hemolymph of limpets (aquatic snails), together with a killed pathogenic microorganism, to enhance antigenicity. The elicited antibodies in this autoimmune response, now mark native (self) GnRHs, forming large antibody-GnRH complexes. The inhibitory mechanism is not evidently elucidated, offering two assumptions 1) the newly formed protein complex has dimensions too large to diffuse through the capillary membrane of the hypophyseal portal system, thus, not able to reach GnRH receptors for LH and FSH synthesis at the adenohypophysis, and 2) the GnRH-Antibody structure prevents the binding to corresponding GnRH receptors in the pituitary gland, consequently inhibiting gonadotropin secretion, ultimately preventing sex hormone synthesis and gametogenesis, (Figure 2.9) (Fagerstone, 2006; Gray, Thain, Cameron, & Miller, 2010; Massei et al., 2008; Miller et al., 2008; Sharma, McDonald, Miller, & Hinds, 2014).

The concept of Immunocontraception, “next to perfect” for Wildlife application?

Although, several articles mention immunocontraception as a “truly novel”, concept, it is not quite the case, as first studies, using the body’s own immune system as an antifertility method, started in the early 1930s (Baskin, 1932). In-field application, procedures that require prior capture, tranquilization, and invasive procedures like implants, tubal ligation, and vasectomy, are less feasible, but, of course, there are exceptions. Also, antifertility vaccines that require booster dosages, are not practical.

2.16 DISCUSSION

To choose between the immunocontraceptive of pZP or GnRH vaccines depends on the overall strategy on population control of a specific species, are female, - male the targets, or both genders, among other factors. Regrettably, there is no population control without having any impacts on the individual or the group. Especially in species with strong hieratic social structures, targeting dominant male, or female for contraception will have an intensive impact on the group’s social behavior, perhaps even serious implications for its survival. The objective of free-ranging wildlife/feral population control is to avoid, or better, minimize human-wildlife conflicts, and circumventing senseless killings, therefore, leaving preoccupation about social impacts in second place. Anti-GnRH vaccines are not perfect, and there are real concerns about its use, nevertheless, recalling the wish list's criterions, for the time being, what else is there?

- Employable in both genders
- “One-shot” only
- Highly effective (depending on species)
- Long-term contraceptive effects (month to years, also depending on the species)
- Very little-known adverse effects (physiologic)
- No observed impacts on secondary sexual characteristics (lack of long-term studies)
- Safe and quick administration (perfect for long-distance)
- Last, but not least, reasonable cost to benefit ratio

2.17 CONCLUSION

Research conducted on traditional contraceptive methods, steroid hormone and non-steroid hormone agents show important improvements. Mainly by offering newer generations, and combinations of synthetic steroid hormones, that require lesser concentrations, hence, fewer side effects. Nevertheless, hormone treatments are problematic when it comes to the application in wildlife species, although, still the most frequently used, when it comes to population management for captive wildlife. Non-steroid hormones seem to be the next best alternative, as they offer long-term infertility effects, with fewer health risks, and provide better functionality when it comes application in free-ranging, - as well as captive wildlife.

Immunocontraception, by far the most studied subject and scrutinized infertility method for domestic, free-ranging wildlife and feral species over the last ten years, for several, before mentioned, reasons. Numerous empirical studies proved repeatedly its effectiveness in rendering various species infertile, and that for extended periods of time, as stated by (Miller, Fagerstone, Wagner, & Killian, 2009), in some cases up to 7 years. Unfortunately, many studies, due to time constraints, investigating reversibility was not always available. Observed adverse effects were a few, and if apparent, none were severe. Most frequent adverse effects were vaccine injection site injuries, and the development of some persistent granulomas, due to extensive inflammatory reactions to the adjuvants, considered a necessary "evil", allowing to stimulate the immune response effectively enough, while providing the slow-release effect, keeping the antibody titer adequately high. Also, maintaining contraception without the need for "booster" applications.

Looking at current and future research efforts, apparently, the focus stays on immunocontraceptives, with some promising new concepts to follow.

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2.19 FIGURES

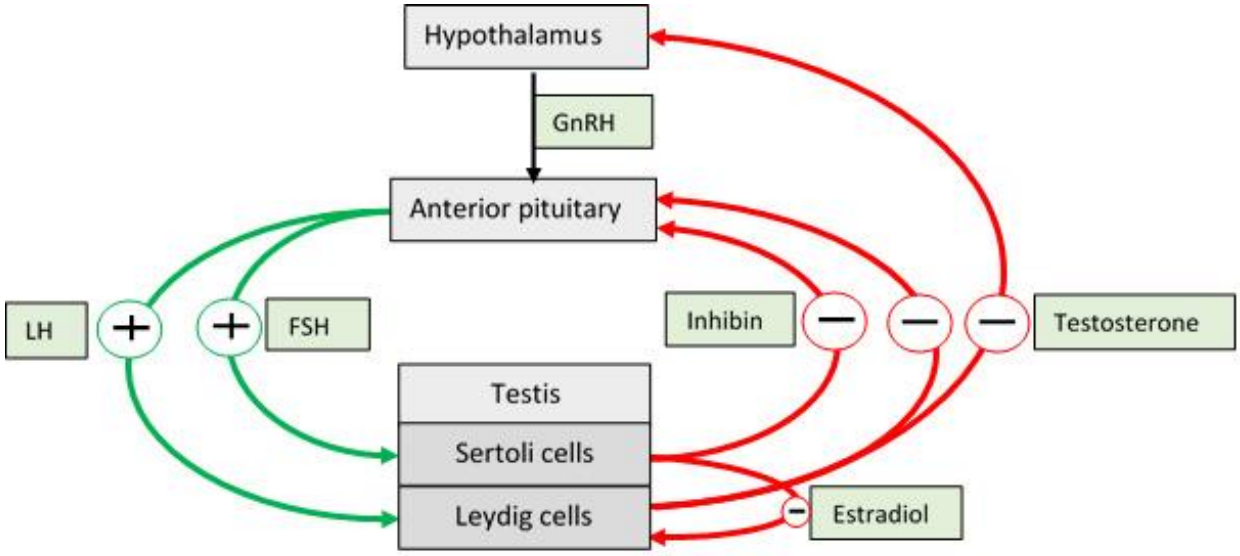

Figure 2.1 - Male Hypothalamic-Pituitary-Gonadal (HPG) axis and reproductive feedback loops.

Grey fields, location of hormone receptors and synthesis; Green fields reproductive hormones. Green arrows: positive feedback loop (stimulatory) and red arrows: negative feedback loops (inhibitory). Graphic: (Rosenfield 2016; Adopted Mac Hadley; Jonathan Levine, 2007; Source: D. Rosenfield, 2017

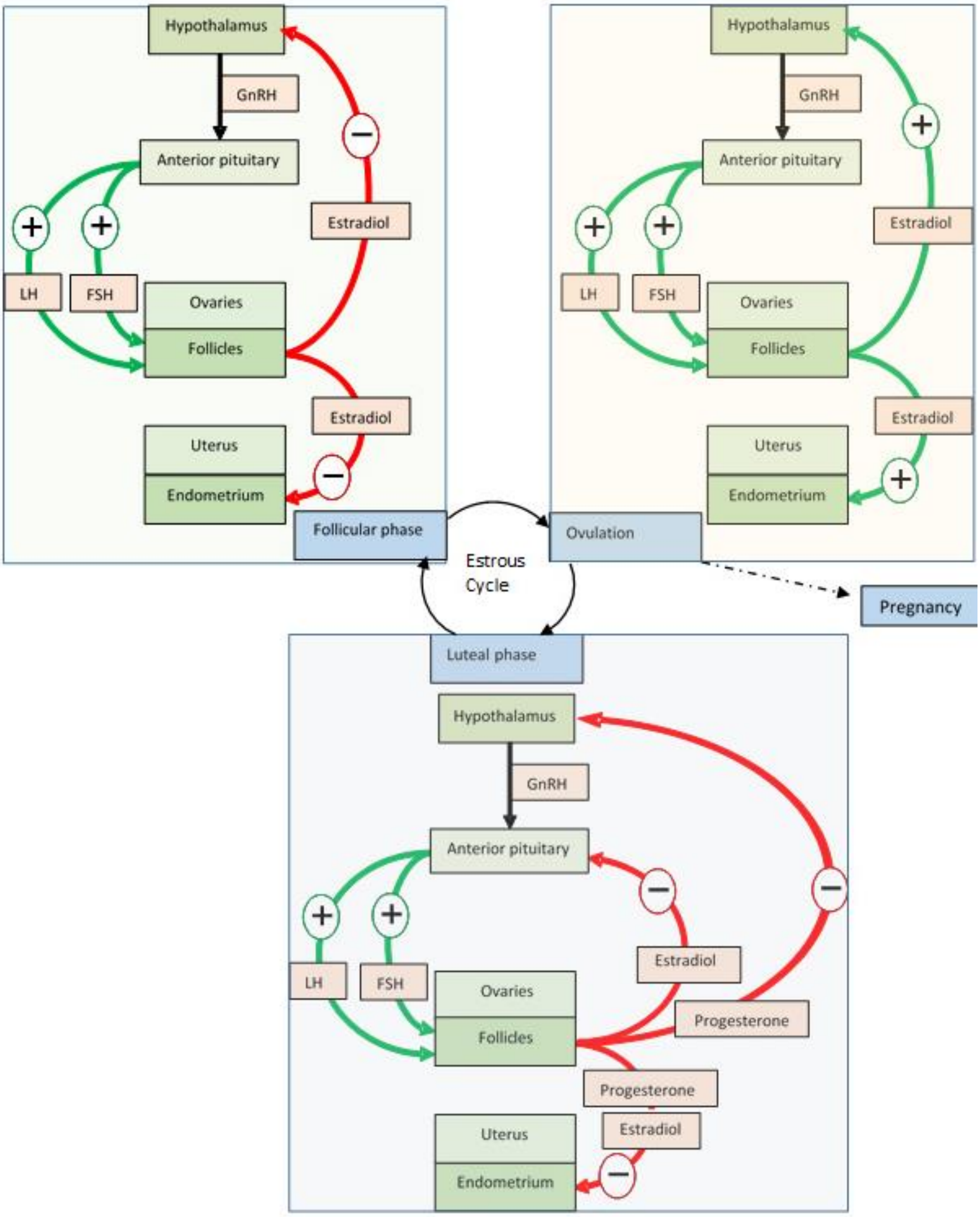

Figure 2.2 - Female HPG-axis and feedback loops
Depending on the phase of the estrous cycle. Graphic: Rosenfield, 2016; Adopted: Mac Hadley; Jonathan Levine, 2007; Rosenfield, 2016

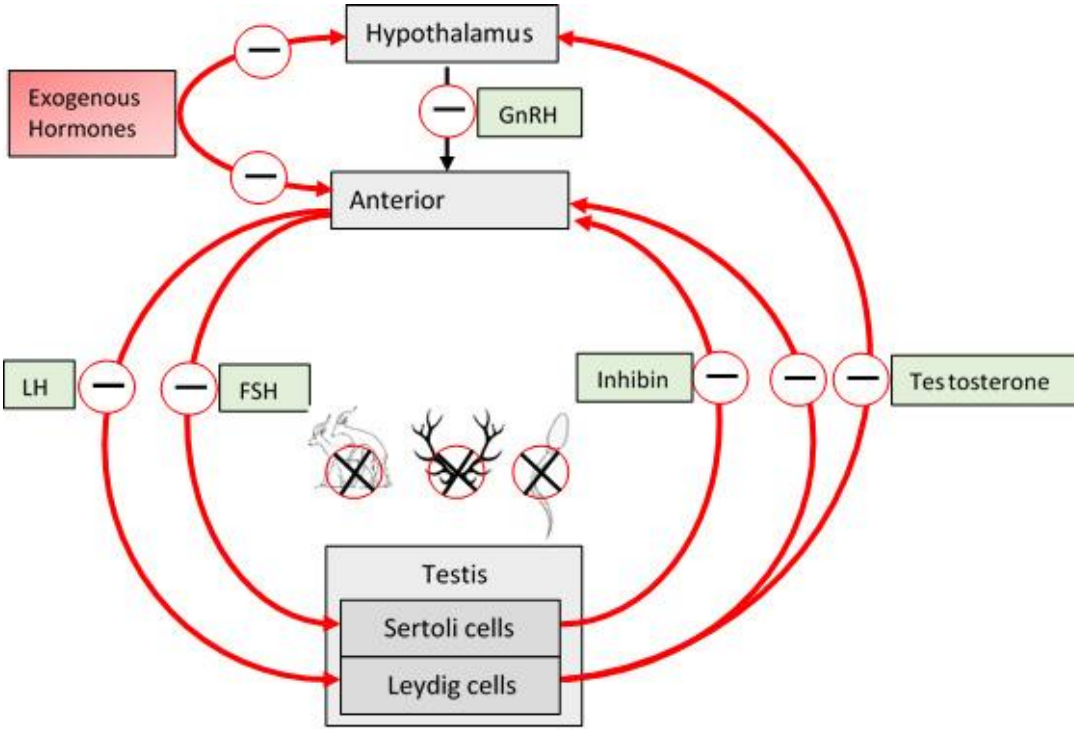

Figure 2.3 - Negative feedback signaling while under the influence of contraceptives. Source (Rosenfield 2016; Adopted Mac Hadley; Jonathan Levine, 2007; Rosenfield, 2016)

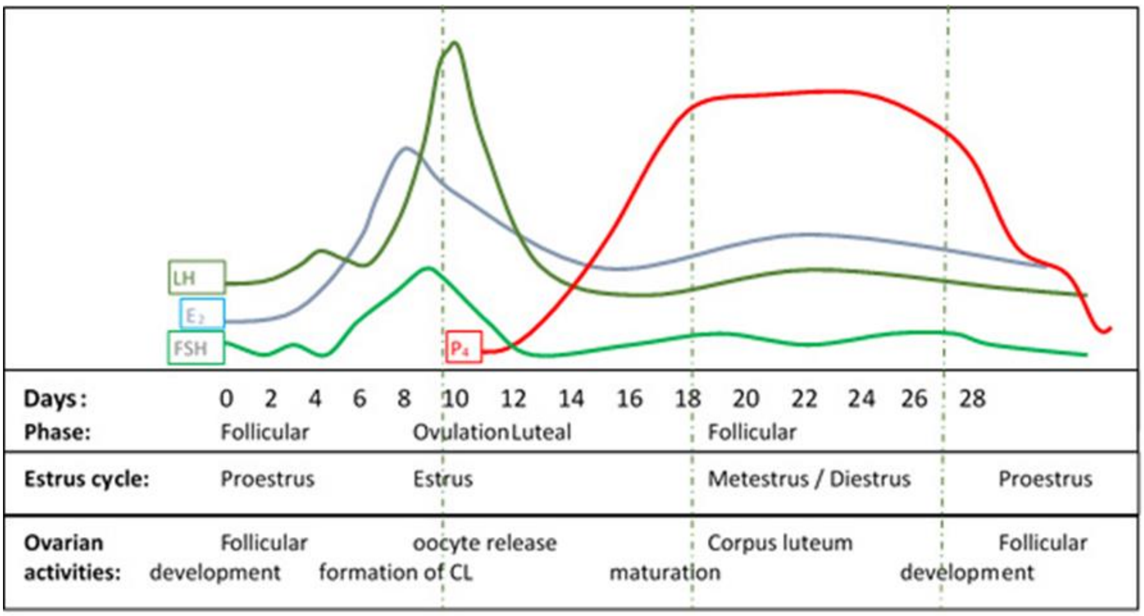

Figure 2.4 - Example of hormonal changes during an estrous cycle, without contraceptive influences
Source: Rosenfield, 2016; McDonald, 2003

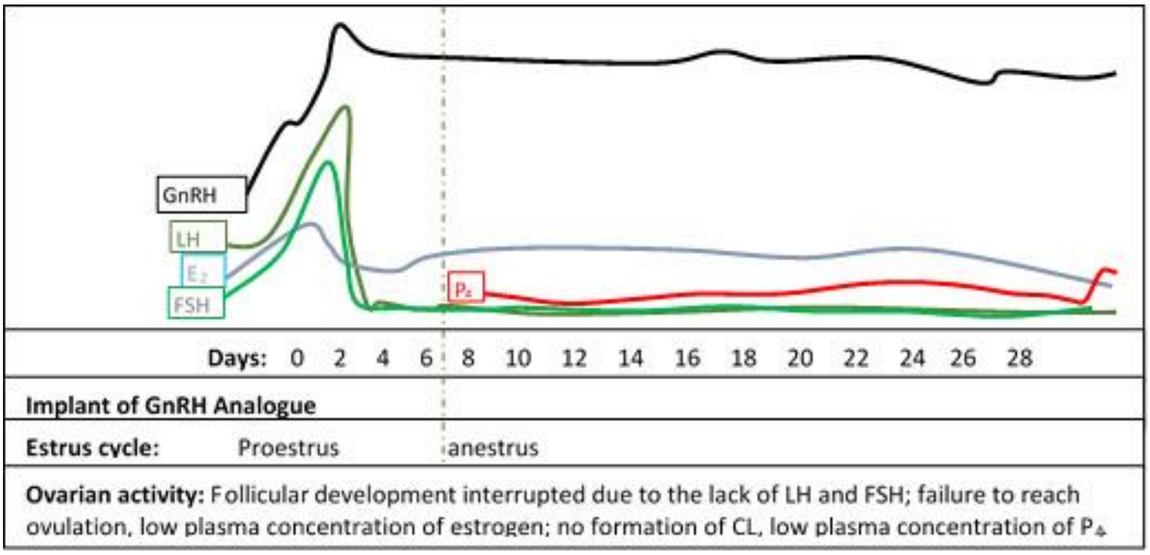

Figure 2.5 - Estrus Cycle under GnRH contraceptive analogue Influence.

Source: Rosenfield, 2016; McDonald, 2003

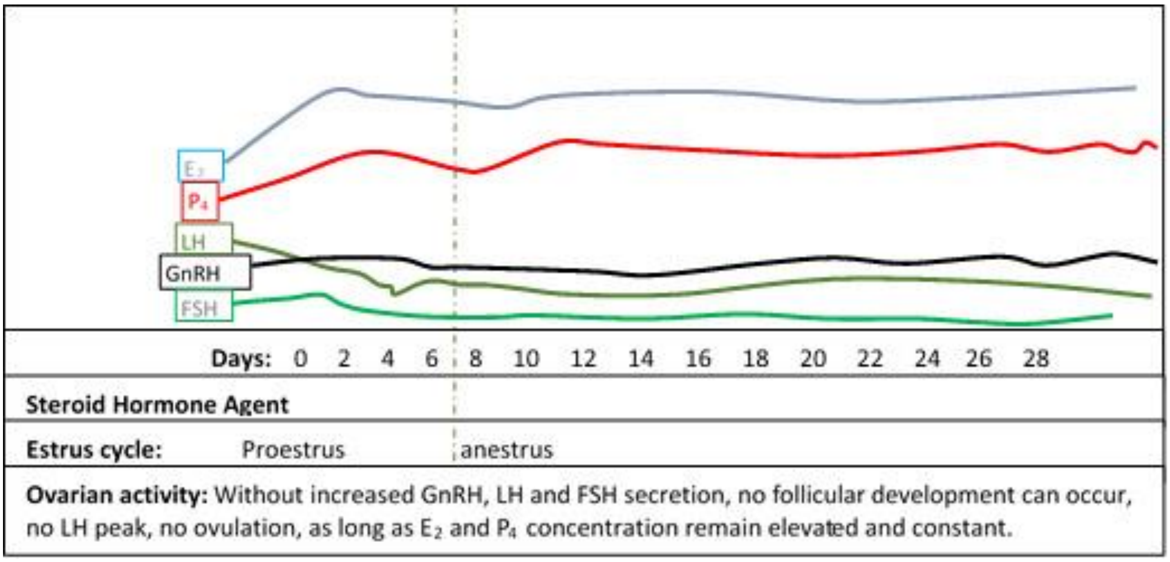

Figure 2.6 - Estrus cycle under steroid hormone contraceptive influence

Source: Rosenfield, 2016; McDonald, 2003

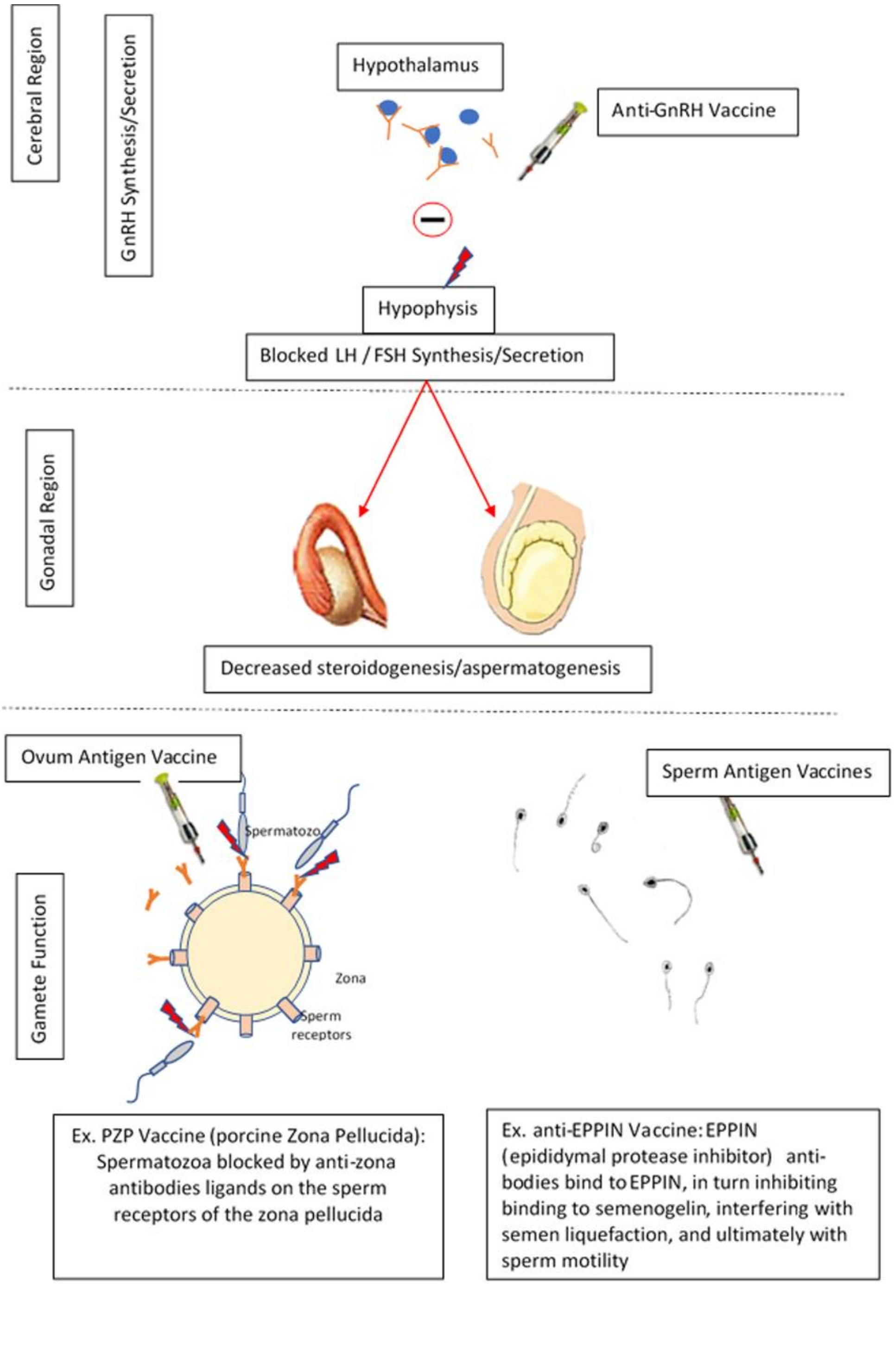

Figure 2.7 - Overview Immunocontraceptive Targets.

Source: Adopted Liu et al., 2005; Rosenfield, 2016)

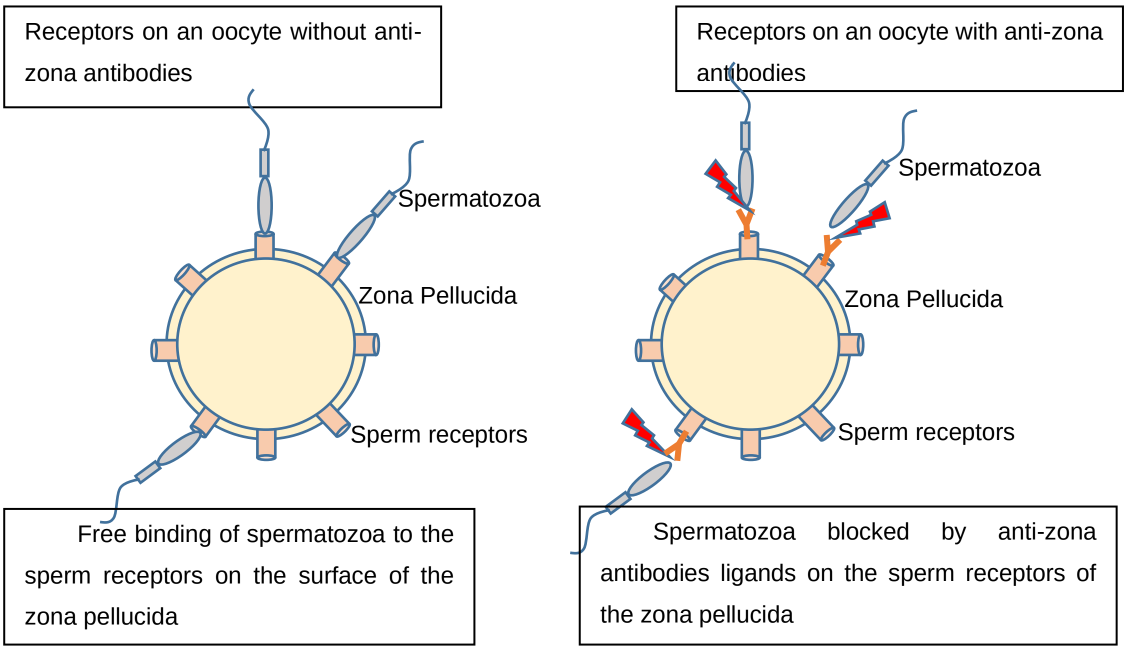

Figure 2.8 - PZP vaccine effect. ZP sperm-binding receptors with, and without anti-zona antibodies. Source: Adopted Liu et al., 2005; Rosenfield, 2016)

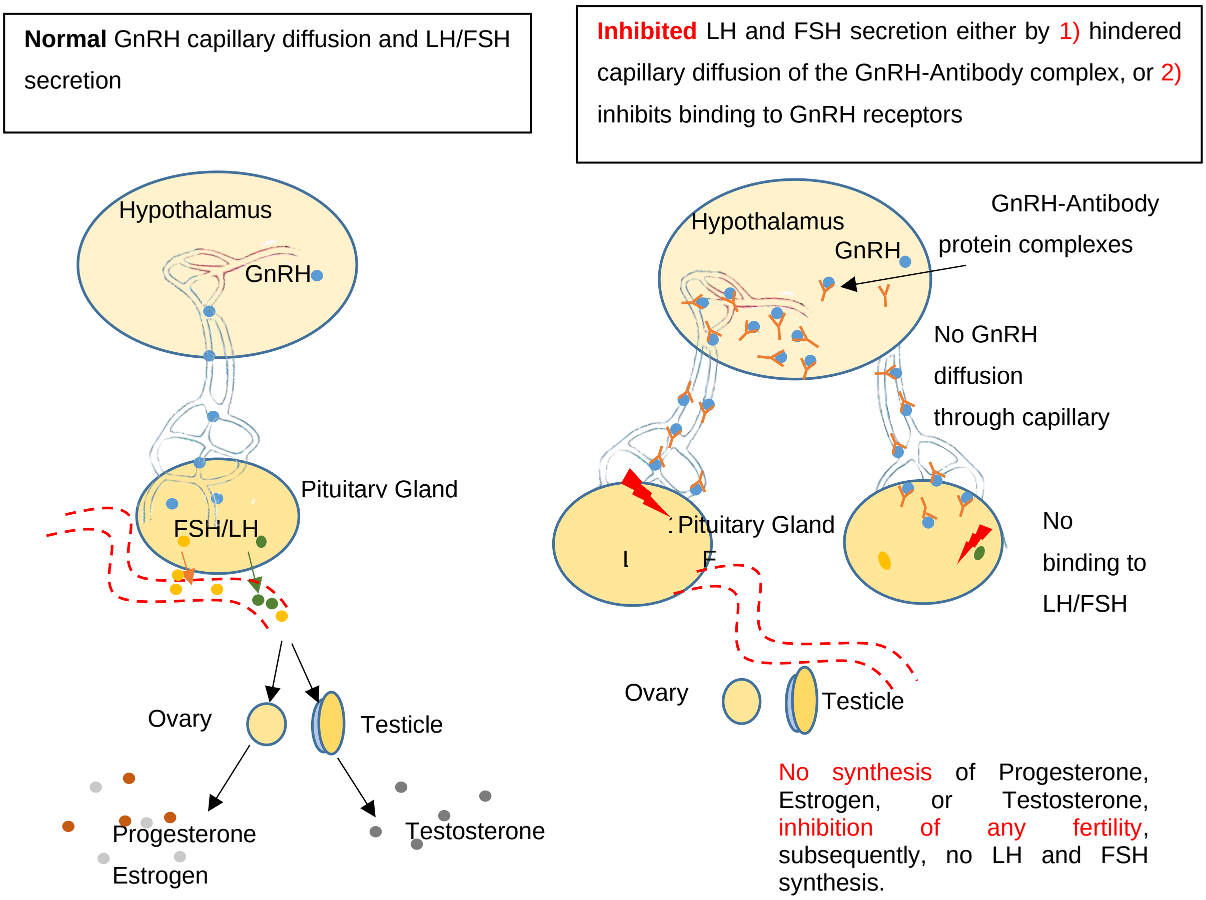

Figure 2.9 - anti-GnRH vaccine effect. Normal, - and inhibited by anti-GnRH antibodies. Source: adopted McDonald, 2003, Rosenfield, 2016

3. BRAZILIAN SPOTTED FEVER PREVENTION THROUGH A NOVEL NON-LETHAL CAPYBARA POPULATION CONTROL STRATEGY

3.1 ABSTRACT

Introduction: Brazilian spotted fever (BSF), a lethal tick-borne Rickettsioses (2000 - 2018 >600 human deaths) involving synanthropic capybara as host. Method: We introduced an alternative to mitigate human-capybara conflicts and epidemiologic concerns of BSF. Complex aspects like transmission dynamics, risk areas, host mobility, and birth rate control, were considered to develop a prevention strategy using an anti-GnRH vaccine. Results: The propositioned immunocontraceptive potentially remove and prevent the spread of BSF from endemic areas. Conclusion: We propose the anti-GnRH vaccine as a BSF prevention strategy based on these favorable results.

Keywords: Immunocontraception. anti-GnRH. GonaCon. *Hydrochoerus hydrochaeris*. *Rickettsia*. *Amblyomma sculptum*.

In this study, we addressed the fundamental issue pertaining to the prevention of BSF in humans, while considering the dynamics between the vertebrate hosts and ticks, and the environmental and anthropogenic conditions[1,2].

In São Paulo, one of the primary tick vectors for BSF is *Amblyomma sculptum*, a species of three-host parasite. During any growth stage, ticks can either become infected or infect the host via three different routes (fig. 3.1): by horizontal transmission, where the pathogen is transmitted to the tick by feeding on an infected host's blood containing sufficient bacteremia; by vertical transmission, where the pathogen is transmitted from an adult female tick to her eggs; and through transstadial transmission, where the pathogen is maintained during the molting passages (egg to larva); however, some routes are much less effective in sustaining the pathogens. Humans, if infected by a pathogen-carrying tick, are considered accidental, dead-end hosts and do not play a role in maintaining the bacterium in the ecosystem[3]. Due to anthropogenic activities and subsequently diminishing natural habitats, wildlife species will either become extinct or be driven into human habitats, where various species are capable of quick adaptation to the urban and agricultural areas. Currently, as synanthropic pests, they provoke numerous human-wildlife conflicts by devastating crops and cause epidemiological threats, such as the aforementioned BSF[4].

Capybaras (*Hydrochoerus hydrochaeris*), the world's largest rodent, are considered as potentially amplifying hosts for *R. rickettsii*, as they fulfill all the following necessary criteria: *i*) the potential host species must be abundant in the endemic area; *ii*) be a good host for ticks; *iii*) be susceptible to *rickettsia* infection; *iv*) be highly proliferative, ensuring the introduction of susceptible animals; and *v*) have enough bacteremia to infect feeding ticks[5,6]. In Brazil, reduction in the natural predators, mainly in areas of anthropological impacts, and abundant water and food sources, largely due to agricultural activities, provide favorable conditions for this highly proliferative species to turn into superpopulations[4,5].

Thus, to plan an effective BSF control strategy, several complex aspects should be emphasized: *i*) the modeling and understanding of the effects and contributions of capybaras and ticks in maintaining BSF transmission, including the associated effects of inter- and intra-species transmissions, births, and deaths; *ii*) defining the important reproductive parameters (e.g., Basic reproduction number R_0)[1,2], and calculating the speed of dissemination; *iii*) describing the spatial relationships between BSF in human cases, environmental/anthropogenic conditions, and host mobility effects on BSF transmission and therefore, comprehending/predicting risk zones of BSF; and *iv*) determining an adequate and species-specific population control strategy, focusing on the most important species in the maintenance and propagation of BSF.

Population control methods must be strategically employed to act as an effective BSF transmission management tool. As suggested by a transmission dynamic model of *R. rickettsii* involving *H. hydrochaeris* hosts and *A. sculptum* vectors[6], to eliminate the etiological agent from an endemic area, a sustained reduction of 80% of the capybara's birth rate is necessary, which can result in the disappearance of the infected individuals by the 4th year. Moreover, if a 90% birth rate decrease could be achieved, infected capybaras and ticks would also cease to exist by the 2nd year[6]. Furthermore, based on the next-generation matrix approach, elements corresponding to the number of infected attached nymphs and adult ticks produced by an infected capybara and vice versa have been identified as critical contributors to changes in the basic reproduction number ($R_0 \approx 1.7$)[7]. Additionally, high-risk seasons correspond to the nymph (July to October) and adult (October to March) stages of the tick, indicating that preventive strategies must be planned accordingly, namely, from April to July or during the larvae season.

As described, the capybara's migration plays an important role in BSF dissemination. Thus, to prevent migration, the chosen population control must achieve a minimum of 58% birth rate reduction. Although this would not eliminate the disease, it would prevent *R. rickettsii* from spreading outside an endemic area. Using hyperspectral moderate-resolution satellite imagery, capybara's migration has been shown to be associated with the widespread sugarcane plantations [1], and these areas, in turn, have been associated with high-risk areas for BSF. Accordingly, capybara's migration can be predicted, while risk areas for human BSF can be identified. Correlating this pattern with the reported distribution of BSF in humans , the host mobility model confirmed that regions wita h high density of sugarcane has a higher BSF dissemination velocity[2], and consequently efforts on population control should be focused on these high-risk (sugar cane) areas.

In this context, to eliminate capybara conflicts including their influence on disease propagation, their migration and birth rate must be controlled. However, any intervention involving direct manipulation of the target animal is influenced by ethical, economic, logistical, and legal concerns. Specifically, environmental laws protect native fauna from being hunted (Federal Law, Brazilian Fauna Protection Act, 1967), and any population control efforts are limited in Brazil. For this reason, several wildlife population control concepts are being evaluated, such as strategies that indirectly influence their birth rate and migration by affecting an area's carrying capacity. For example, sugar cane plantations, one of São Paulo's principal crop production areas close to water bodies, are likely to be raided by animals. Knowing that capybaras are usually found a few hundred meters away from the water bodies[8], one approach could be the delimitation of movement through riparian reforestation, a natural barrier, respecting a perimeter far enough from water resources, to hinder their access to the food supply. However, a reported mean dispersal distance of 3366 m for capybara[9] might make this strategy less feasible.

Another strategy involves the relocation or translocation of capybaras to an area of less potential conflict. This is a valuable technique for conservation efforts of threatened taxa; yet, when it comes to synanthropic capybaras, this practice is less recommended, as it brings more problems than solutions, especially epidemiologically, considering that one single infected individual, with a minimum of one attached tick, is enough to trigger BSF in a non-endemic area[1]. In addition, susceptible animals may be introduced into an infected area, thus, aiding the maintenance of an etiological agent within a population, given the long-term survival of unfed ticks[9]. An alternative concept is based on a direct control strategy, which manipulates the animal's reproduction capacities (sterilization). A myriad of wildlife contraceptive methods are available and is categorized into three methods: invasive, gonadectomy; less invasive, vasectomy in males or tubal ligation in females; and minimally invasive, chemical contraceptives. Within this approach, either hormone-based or employed immunocontraception is the most common antifertility method[10].

The majority of contraceptive concepts are applied to captive wildlife; however, the options are limited in free-ranging wildlife. Moreover, its "adequacy" is determined by the overall objective, species, and environment. Controlling capybara populations should consider making these hosts infertile for a longer duration and the impact of such interventions on the capybara's polygynous (harem-like) society. Group stability and procreation are driven by the dominant male's alpha attributes, such as secondary sexual characteristics that include a prominent nose with perianal glands for territorial marking, and agonistic conduct (vigilance, fighting), as well as courtship behavior[11]. Contraceptive methods that maintain the alpha male's dominant behavioral and phenotypical characteristics while rendering the animal infertile, are imperative to successfully managing the population growth[11]. Therefore, procedures that preserve the gonad quality to maintain any steroid-dependent characteristics of the male should be chosen. This makes vasectomy the most commonly employed male contraceptive for controlling the capybara population in Brazil. Reproductive control in capybaras through vasectomy and tubal ligation are highly effective techniques in rendering males or females infertile; nevertheless, the logistics (in-the-field execution, competence, and high costs) make these procedures less attractive, especially when required to be performed for larger numbers[12]. Additionally, and more importantly, an injured or sick capybara will distance itself from the group until recuperated[11]. This temporary absence of the alpha male (5–20 days) allows opportunistic solitary males to take over the group, undoing any population control efforts[11].

As mentioned, capybaras quickly escape into the water when threatened; therefore, prior to intervention, capture and containment are imperative to avoid death due to drowning. These capture-recovery-release events are cumbersome and time-consuming. Taking all these undesirable factors into consideration, seeking better and more appropriate alternatives become necessary, while respecting the animals' well-being and logistics. Ongoing research for alternative methods led to the concept of an anti-GnRH vaccine, believed to offer most of the desired contraceptive characteristics. This immunocontraceptive, given in a single-dose, is a long-term antifertility vaccine, which has been studied successfully, worldwide, in many species over the last decade [10].

Briefly, after immunization, the body produces anti-GnRH antibodies, which eventually bind to endogenous GnRH molecules forming a large immunocomplex. This ceases its bioactivity and consequently inhibits the synthesis and liberation of the gonadotropin luteinizing hormone and follicle-stimulating hormone, necessary for gonadal activities[13] (fig. 3.2). The overall reported high success rate in several species[13–15] initiated the very first study using an anti-GnRH vaccine (GonaCon™, APHIS, USDA, Fort Collins, CO, USA) in male and female capybaras. The proposed population control method using a single-dose immunocontraception has been successfully employed in male capybaras, providing a 100% prolonged, antifertility effect over a 24-month trial [16]. Besides rendering the capybara males infertile, it also preserves their alpha traits such as secondary sexual characteristics and agonistic behavior, attributes that are absolutely imperative for the success of any population strategy for this species . No remarkable adverse effects were reported, except for an abscess formation at the injection site, which is common. The results are corroborated by similar studies conducted in several other species, although a number of conflicting conclusions were observed regarding its impact on the male's agonistic behavior and secondary sexual characteristics[17]. This invites the hypothetical question: Is this a species-specific response?

In conclusion, human-wildlife conflicts are inevitable as the human population continuously grows, expanding their living spaces and activities. Consequently, threats to human health, especially vector-borne zoonotic diseases, will also increase; thus, population control of wild, feral, and synanthropic animals must be addressed. Each circumstance, environment, and species require a specific plan to manage their populations.

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3.2 FIGURES

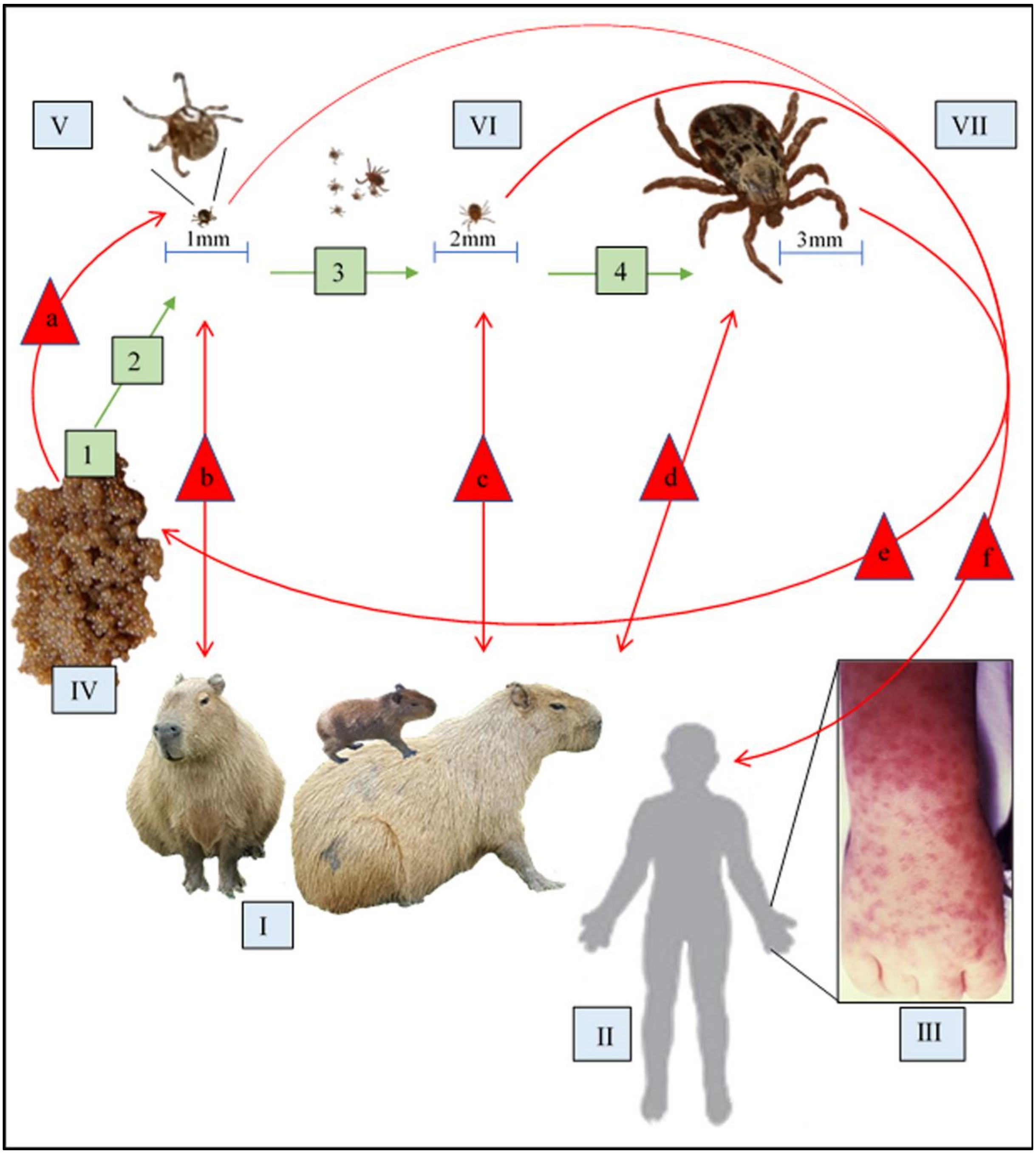

Figure 3.1 - The Three-Host Life-Cycle and the Dynamics of Pathogen Transmission.

I) Amplifying hosts; II) incidental (dead-end) hosts; III) characteristic symptom: petechial rash; IV) tick eggs; V) larvae; VI) Nymph; VII) adult tick; *Amblyomma* life-cycle stages, 1) eggs; 2) molt to larvae; 3) molt to nymph; 4) molt to adult; *Rickettsia rickettsii*, transmission dynamics: a) transstadial; b) larvae - host; c) nymph – host; d) adult – host; e) transovarial transmission. Source: D. Rosenfield, 2019

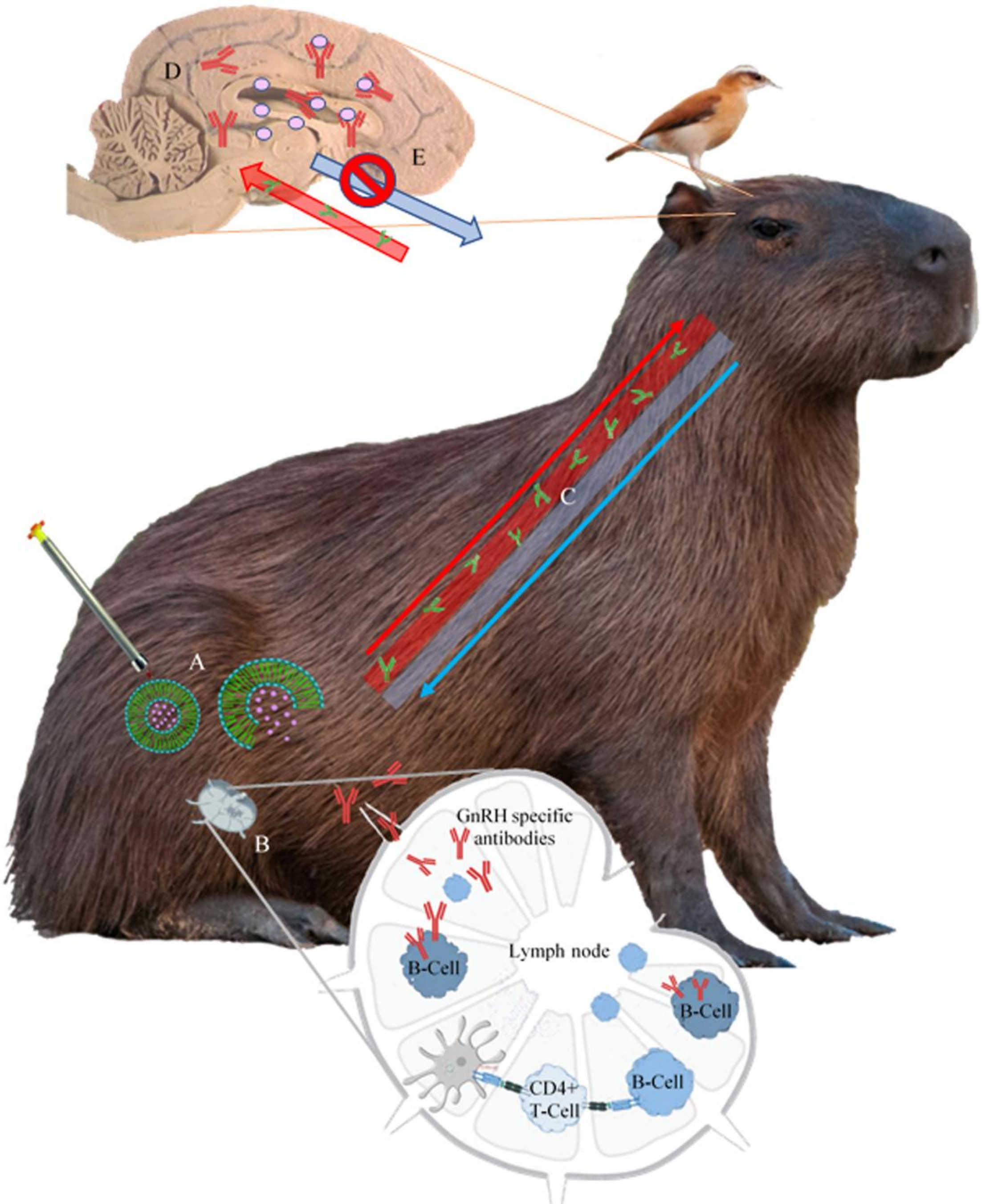

Figure 3.2 - Illustrated mechanism of action of the immunocontraceptive anti-GnRH.

A) Anti-GnRH Vaccine Dart mediated liberation of adjuvant and antigens. B) Immune response in the lymph node and formation of GnRH-specific antibodies. C) Hypothalamic-pituitary-gonadal axis, normal passage of gonadotropin hormones (LH, FSH) from the anterior pituitary gland to the gonads. After immunization, anti-GnRH antibodies are transported to the hypothalamic region. D) Anti-GnRH antibodies capture GnRH hormone, forming large immunocomplex molecules, inhibiting transfusion and ligation to GnRHR (receptors) in the pituitary region, ceasing LH and FSH synthesis and liberation. E) Impeding LH and FSH biological activities in the gonadal region (steroidogenesis and gametogenesis).
Source: D. Rosenfield, 2019

4. THE IMPORTANCE OF THE ALPHA BEHAVIOR INTEGRITY IN MALE CAPYBARA (*Hydrochoerus hydrochaeris*), FOLLOWING IMMUNOCONTRACEPTION TREATMENT.

4.1 ABSTRACT (EN)

As the human population continues to grow, habitat for wildlife shrinks, driving fauna either into extinction or into new habitats, including human-dominated areas. This, inevitably, creates problems on several levels. In Brazil, one native species, the capybara (*Hydrochoerus hydrochaeris*) in particular, is becoming a synanthropic pest by invading urban and agricultural areas, where they quickly reach super-population status, believed to be facilitated by abundant food supply and the lack of natural predators. But more importantly, it’s role as amplifying hosts of *Rickettsia rickettsii,* the pathogen of Brazilian spotted fever, a disease life-threatening to people. Thus, developing species-specific population management strategies, respecting public opinion, and considering animal-welfare are all required for the effective mitigation of this tick-borne zoonotic diseases. One consensus on how to potentially control the disease transmission is by directly controlling the host’s population, while indirectly removing the dynamics that allow for the pathogens to be maintained. As a polygynous society with a strong hierarchal organization that is upheld by a dominant male, the integrity of the hormone-driven secondary sexual characteristics and courtship behavior is crucial. For an intervention by contraceptive strategies to be a viable management tool, it is imperative to preserve the alpha male’s phenotypic and agonistic characteristics. Losing the dominant status would allow for the opportunistic entrance of a competitive male, consequently, leading to a failure of the intended population management. As part of a larger research project on the efficiency of an immunocontraceptive in free-ranging capybaras, the aim of this work was to observe its impact on the alpha male and the group’s social behavior. At the end of the 18-month-study, there were no recorded births involving the immunized animals. Concurrently, the alpha male’s characteristics were preserved and subsequently the integrity of the group. In conclusion, the results encourage the use of this anti-GnRH vaccine as an alternative population control tool in male capybara.

Keywords: Agonistic behavior, Secondary sexual characteristics, Gonacon, Wildlife population control, *Rickettsia*, Brazilian Spotted Fever,

4.2 ABSTRAKT (DE)

Mit ständiger weiter wachsende menschliche Bevölkerung somit auch der notwendige Lebensraum, unvermeidlich, schrumpft auch der natürliche Raum für wild lebende Tiere, dass treibt die Fauna entweder zum Aussterben oder in neue Lebensräume, einschließlich die von Menschen besätzten. Dies führt unweigerlich zu Problemen auf mehreren Ebenen. In Brasilien, wird insbesondere eine einheimische Wildtier Spezies für den Menschen immer lästiger. Der synanthropisch heranwachsende Capybara (*Hydrochoerus hydrochaeris*), der in seiner Natur stark proliferativ ist, entwickelt sich zu einer Superpopulation und wird zu einer Bedrohung für die menschliche Gesundheit, aufgrund seiner funktion als einer der wichtigsten Wirt-Tiere für *Rickttsia rickettsii*, der Pathogen für das Brasiliansche Flecken Fieber. Daher ist die Entwicklung von artengerechter Bevölkerungs-Kontrolle, unter Beachtung der öffentlichen Meinung und die Berücksichtigung des Tierschutzes, zur wirksamen Eindämmung von durch Zecken übertragenen Zoonoseerkrankungen erforderlich. Ein Konsens darüber, wie dieKrankheitsübertragung des möglicherweise kontrolliert werden kann, besteht darin, die Bevölkerung des Wirts direkt zu kontrollieren und gleichzeitig die Dynamik zu beseitigen, die die Aufrechterhaltung des Pathogens ermöglicht. Als polygynische Gesellschaft mit einer starken hierarchischen Organisation, die von einem dominanten Männchen aufrechterhalten wird, ist die Beibehaltung der Integrität der hormonabhängigen sekundären Geschlechtsmerkmale und des agonistichen Verhaltens von entscheidender Bedeutung. Damit ein Eingriff durch Verhütungsstrategien ein wirksames Managementinstrument in Capybara sein kann, ist es unbedingt erforderlich, die phänotypischen und Dominanzmerkmale des Alpha-Männchens zu erhalten. Der Verlust des dominanten Status würde den opportunistischen Einstieg eines „Satelliten"- Männchens ermöglichen und somit das angestrebte Ziel der Geburtenkontrolle verfehlen. Ziel dieser Arbeit war es, im Rahmen eines größeren Forschungsprojekts zur Effizienz des Immunokontrazeptivums Anti-GnRH (Gonacon ™) in Wild-Capybaras die Auswirkungen dieser Bevölkerungskontrollstrategie auf das alpha Männchen sowie dem sozialen Verhalten der Gruppe zu untersuchen. Beobachtungen, die über einen Zeitraum von 18 Monaten gemacht wurden, rapportieren eine Reduktion der Geburtenrate von 100% der immunisierten Tiere unter Beibehaltung der alpha-Merkmale des dominanten Männchens und somit die Aufrechterhaltung der sozialen Struktur der Gruppe.

Zusammenfassend lässt sich festhalten, dass die Ergebnisse die Verwendung dieses Immunokontrazeptivaimpfstoffs zur Populationskontrolle in frei-lebendem Capybara empfohlen werden kann.

Schlüsselwörter: Wasserschwein; Dominantz-Verhalten; Gonacon; Immunkontrazeption; Mensch-Wildtier Konflikt; Wildtier Bevölkerungskontrolle; Brasilianisches Flecken Fieber.

4.3 RESUMO (PT)

À medida que a população humana continua a crescer e expandir seu habitat, o espaço natural para a vida selvagem diminui, levando a fauna à extinção ou a novos habitats, incluindo áreas ocupadas por seres humanos. Isso, inevitavelmente, gera problemas em vários níveis. No Brasil, uma espécie nativa, em particular, está se tornando mais incômoda. A capivara sinantrópica (*Hydrochoerus hydrochaeris*), altamente proliferativa por natureza, atinge superpopulações, sendo uma ameaça emergente à saúde humana já que é um dos principais hospedeiros da *Rickettsia rickettsii*, patógeno da febre maculosa brasileira. Assim, o desenvolvimento de estratégias de gestão de populações específicas de espécies, respeitando a opinião pública e considerando o bem-estar animal são necessárias para a mitigação eficaz dessas doenças zoonóticas transmitidas por carrapatos.

Um consenso sobre como potencialmente conter a transmissão da doença é controlando diretamente a população do hospedeiro, enquanto indiretamente remove a dinâmica que permite que os patógenos sejam mantidos. Como uma sociedade polígina com forte organização hierárquica que é sustentada por um macho dominante, a integridade das características sexuais secundárias e do comportamento agonístico a hormônios é crucial. Para que uma intervenção das estratégias contraceptivas seja uma ferramenta de gestão viável, é imperativo preservar as características fenotípicas e agonísticas do macho alfa. Perder o status dominante permitiria a entrada oportunista de um macho competitivo, consequentemente, levando a uma falha do gerenciamento populacional pretendido. Como parte de um projeto de pesquisa maior sobre a eficiência de um imunocontraceptivo em capivaras de vida livre, o objetivo deste trabalho foi observar seu impacto sobre o comportamento do macho alfa e do grupo. No final do estudo de 18 meses, não houve registros de nascimentos envolvendo os animais imunizados. Concomitantemente, as características do macho alfa foram preservadas e subsequentemente a integridade do grupo. Em conclusão, os resultados encorajam o uso desta vacina anti-GnRH como uma ferramenta alternativa de controle populacional em capivaras machos

Palavras-chave: Capivara, Gonacon, Imunocontracepção; Conflito humano-animais silvestres, Controle populacional, Febre Maculosa.

4.4. INTRODUCTION

Hydrochoerus hydrochaeris, commonly known as capybara, is the world's largest rodent species, endemic to South America and are habitat generalists surviving in open grasslands and scrub vegetation. Capybaras are semi-aquatic so stay close to water, which is used as their principal getaway and serves as a place for thermoregulation, defecation, mating, as well as an important food source (Elias, 2013; Magnusson, 1998; Mones & Ojasti, 1986; Moreira, Ferraz, Herrera, & Macdonald, 2012). Intensive anthropogenic activities have dramatically changed the landscapes and habitats where capybara live. With diminishing natural space and an increase in agricultural and urban areas, these animals are re-occupying and thriving in their human-modified habitats.

Their social structure is based on polygyny (harem), with one dominant (alpha) male, females that are divided into dominant (breeding females) and subordinate females, male and female juveniles and, depending on the season, pups. In Brazil, a wild herd can reach up to 50 members (Macdonald, 1981). Isolated capybara, known as satellites, can often be seen maintaining a certain distance from the main group; these are sexually mature males forced out by the alpha male (fig. 4.1).

Subordinate females, although sexually mature, do not mate with the alpha male; their restraint is due to interactions with dominant females and their social stimuli, which is believed to cause reproductive suppression, either physically, endocrinologically, or by olfactory cues (Maldonado-Chaparro & Blumstein, 2008). However, some subordinate females have been observed leaving their group for short periods of time to seek out nearby satellite males to mate with, as observed during this present study.

Due to several contributing factors, such as the loss of natural predators, the capybara's ability to quickly adjust to agricultural and urban settings, their tolerance to human presence, the abundance of available foods, combined with their high proliferation rates, have allowed the capybara to become Brazil’s second most important pest-species, the other being wild boar (Pedrosa, Salerno, Padilha, & Galetti, 2015). Under these conditions capybaras are attaining large population sizes, with herd numbers that can reach over 100 individuals which creates traffic accidents, damage to private property, invasion of public and private spaces, and destruction to crops, particularly corn and sugarcane plantations (Abreu Bovo, Ferraz, Verdade, & Moreira, 2016; de Oliveira Vieira, Bernardes Filho, & Azulay-Abulafia, 2015; Felix et al., 2014; Ferraz, Lechevalier, Couto, & Verdade, 2003; Labruna, 2013; Labruna, Pacheco, Ataliba, & Szabó, 2007)

However, the main concern is the threat to human health as capybara are associated with the maintenance and spread of the tick-borne disease Brazilian spotted fever (port.: “febre maculosa”). Capybara are considered an amplifying host for this emerging vector-borne zoonosis caused by the potentially deadly bacterium *Rickettsia rickettsii*, which is spread by ticks of the genera *Amblyomma ssp.* (Brites-Neto, Brasil, & Roncato Duarte, 2015; Fortes et al., 2011; Labruna, 2013).

They fulfill five requirements to be considered a good amplifying vertebrate host for *R. rickettsia*:

(1) be abundant in the endemic area; (2) be a good host for the ticks; (3) be susceptible to *Rickettsia* infection; (4) have high population growth rates, and (5) have enough bacteremia counts to infect ticks (Labruna, Kamakura, Moraes-Filho, Horta, & Pacheco, 2009).

Although, there are other native wildlife species reported to host *R. rickettsii*, such as dog, horse, opossum, among others (Labruna et al., 2009; Milagres et al., 2010), there are a number of reasons that capybara are the major contributing factor for *R. rickettsii* infection in endemic areas. They exclusively occupy areas close to bodies of water and are move slowly making them very conducive for ticks to infest in large numbers and feed upon. Some tick-species, such as the *Amblyomma dubitatum*, are highly specific to capybaras and rarely feed on other host species. However, humans may become accidental hosts (Beati et al., 2013; Brites-Neto, Duarte, & Martins, 2015; Guglielmone et al., 2006; Labruna et al., 2007).

Several field studies and stochastic models have been developed that have reported spotted fever transmission dynamics, postulating that birth-rate reduction not just can directly control capybara population growth, but potentially slow disease transmission (Federico & Canziani, 2005; Labruna, Kasai, Ferreira, Faccini, & Gennari, 2002; Polo, Acosta, Labruna, & Ferreira, 2017; Polo, Labruna, Ferreira, & Brockmann, 2018; D. A. Rosenfield, Polo, & Schilbach Pizzutto, 2019; Sonenshine & Mather, 1994).

In an effort to control these fast-growing super-populations, several research projects are being conducted, seeking methods that are effective in managing populations while conforming to environmental protection laws and public opinion. Capybara, as it's categorized as Brazilian native fauna, is protected from hunting, slaughter, and abuse (Presidência da República, 1981; Rodrigues, 2013).

In Brazil, capybara potentially reproduces all year round, however, are constrained by environmental factors, food availability and human impacts. As the principal breeder, the alpha male protects the herd and mates with many females, focusing on sterilization of the dominant male could be a population control strategy of choice, provided that the procedure does not alter its dominant status (Alho & Rondon, 1987; Paula & Walker, 2013; Rodrigues, 2008). Capybara are fiercely territorial, protecting harem and habitat, driving out potential male intruders or uprising subordinate males that attempt to challenge the alpha male (Herrera & Macdonald, 1993). Thus, the importance to maintain testosterone production which influences their secondary sexual characteristics and dominance (agonistic) behavior. For this reason, vasectomy was considered initially a suitable intervention as sperm conduction is interrupted, yet, leaving the gonad function intact so a continuation of steroidogenesis is ensured (Meira & et al, 2013). If performed correctly, it is completely effective. On the downside, the logistics, cost, skill availability and access to the testes which is in an intra-abdominal position, are more challenging. The biggest dilemma, despite being considered a minimally invasive surgical procedure, is the time for recovery. Capybara, when injured, sick, or during labor, distance themselves from the group until healing is complete (pers. obs. Xi.2016 – xii.2018). Observations indicate that vasectomized males' distance themselves from the group for up to 10 days, potentially allowing competitors to move in and take over, jeopardizing the efforts to manage the population growth.

Additionally, subordinate males are known to breed opportunistically, even as much as 40% of the overall growth rate (Rodrigues, 2008), which is initiated by subordinate females temporarily leaving the main group (pers. comm.: Marcelo, Labruna; Fernanda Nunes; 20.ix.2018). In this case, an alternative method to consider is tubal ligation (tubectomy) in all sexually mature females. The concept is analogous to the deferentectomy procedure in males, with the intent to inhibit gamete transmission but preserve gonadal steroidogenesis and, hence, social behavior/group stability.

In general, we can organize the breeding hierarchy into one alpha male and several dominant females as the principal breeders. Subordinate females (believed to be in reproductive suppression due to the presence of dominant females), and/or their opportunistic mating with external (satellite) males (fig. 4.3), which postulates three distinct population control strategies:

Contraceptive Strategies:

The immunocontraceptive treated alpha male effectively maintains agonistic conduct and secondary sexual characteristics. Successfully defends against potential intruders. However, the alpha male does not mate with dominant females. Subordinate females opportunistically and temporarily leave the group to join nearby satellite males to mate (fig. 4.4). After the mating event, the now pregnant female returns to the group and remains there during gestation. Following birth, the pups are brought up in an alloparental manner, as commonly observed in capybaras (Nogueira, Otta, Dias, & Nogueria-Filho, 2000).

Due to the castration, the original alpha male loses agonistic conduct and secondary sexual characteristics. Growing males sexually mature, or dominant growing satellite males challenge the alpha male, leading to his defeat and consequently driving the ex-alpha male out of the group or even killing him (fig. 4.5). The new (untreated) dominant male will become the alpha male and re-start the mating process.

The treated alpha male does not leave the group post-treatment and maintains alpha associated conduct and secondary sexual characteristics. Alpha male, now infertile (fig. 4.6), but the group’s social structure is stable. Also, treating all satellite males and all sexually mature females will prevent opportunistic mating encounters with satellite males.

Illustrated Contraceptive Strategies:

Figure 4.5

Figure 4.6

Figure 4.7

In order to find alternative contraceptive methods that would address the weaknesses of currently employed population control strategies in capybaras, an intensive literature review on contraceptive methods in wildlife was conducted. The objective was to match most of the desired characteristics of a contraceptive agent, which would include antifertility effectiveness of more than 90%; long-term effect of more than 12 months; with very little to no adverse effects (physiological/behavioral).

Especially, considering a polygynous society, like capybara, the importance of maintaining the dominant male's agonistic behavior. Furthermore, is applicable in both sexes; represents no risks to pregnant females; potentially reversible; easy and safe application; allows for remote drug delivery (long-distance darting); does not provoke environmental pollution; does not have contraceptive effects when entering the food-chain, and lastly, is economically viable and available.

The anti-GnRH vaccine GonaCon™ was selected as it conformed to most of these conditions (Asa, 2005; Gionfriddo, Gates, DeNicola, Fagerstone, & Miller, 2008; Ajadi & Oyeyemi, 2015; D. A. Rosenfield, 2016). The objective of this research was to demonstrate the effectiveness of an anti-GnRH immunocontraceptive (Gonacon™) in reducing population growth in the capybara without interfering with the behavioral characteristics of the alpha male.

4.5 MATERIALS & METHODS

4.5.1 Location

A large man-made water pool ("Olympic lake", port.: Raia Olimpica) surrounded by diverse vegetation and extended grassy areas, used for the university's aquatic sports activities, was selected in Sao Paulo. The location is shown in fig. 4.7. The environmental conditions are very similar to the natural capybara habitats, allowing for a unique opportunity to observe free-ranging capybaras in an open confined setting.

4.5.2 Identifying males

At first sight, the urogenital apparatus is not easily distinguishable between male and female capybara, as the male penis is situated within a large anogenital invagination. In sub-adult males, gender can be confirmed by palpation and exposure.

Capybara alpha males have specialized androgen-driven secondary sexual characteristics, such as a prominently developed nasal and perianal glands for scent-marking (fig. 4.2 a/b). The testes, in immature males, are located subcutaneously in the inguinal region, whereas in dominant males, they migrate from the inguinal region towards the area of the inner/upper thigh, becoming slightly visible (fig. 4.2 c).

4.5.3 Veterinary intervention

Two groups were selected for the study and were based on their population size and pest status: Group 1 consisted of >40 individuals and Group II of seven individuals. The socio-sexual and reproductive behavior of male and female capybaras were recorded for approximately 100 hours pre- and >120 hours post-treatment using the continuous focal observation method (Martin & Bateson, 2007) between June 2016 and December 2018.

In Group I, 3 individuals (male n=1, female n=2) were treated with the anti-GnRH vaccine GonaCon™, and in group II, 6 individuals (male n=2, female n=4). The vaccine was administered intramuscularly in the larger muscle group of the hind leg. The rest of the population served as control.

This project-specific ethogram (tab. 4.1) was used to assist in identifying any treatment-associated alterations, allowing an interpretation of cues of a successful antifertility method and the integrity of the alpha male‘s agonistic behavior. This is essential for maintaining the group’s social stability and preventing an intruder from mating, and hence, providing an appropriate population growth management tool.

Contraceptive Effect Analysis

As part of the evaluation process of the anti-fertility effect, steroid-hormone, spermogram, biometry, and testicular morphology were employed. At the end of the study period, males were hemi-castrated for further histological investigation of the testicular parenchyma.

4.6 RESULTS

Ethogram

Table 4.2

Descriptive findings on population dynamics

Original alpha male of Group I: Expired during the second capture event, provoked by tympany (bloat), leaving the group without a leader.

Original alpha male of Group II: Almost immediately (the next day) left his group to investigate Group I, but initially remained very cautious to the possible return of the dominant male. Over the next few days, he took charge as the new alpha male of Group I.

Subordinate male of Group II: In the meanwhile, the subordinate male from Group II assumed the status as the group's new dominant male.

Satellite males: No satellite males got involved during this transitional phase.

Birthrate: Over the following four-month period, no births were recorded in either group.

Alpha male Group I: Eight months into the project, the new alpha male of Group I got severely injured, and typical for injured members, during recovery, he distanced himself from the group, which triggered the Group II's alpha male to leave his group, in an attempt to take over Group I, which was successfully completed. After the recovery, the ousted alpha male frequently challenged his opponent but failed to remove the invader. During one of these fights, the ex-alpha was injured, to such an extent that he died of sepsis.

Group II, without alpha male: Interim, and opportunistically, a satellite male (untreated and considered control) took over Group II as the new dominant male. During the following six months, Group II created seven pups, while Group I still showed no offspring.

4.7 DISCUSSION

The leadership dynamics observed of all involved males was very compelling, as it proved that the immunocontraception was effective in rendering the treated males infertile, while concurrently, preserved their alpha male behavior, thus the group's integrity. Contrary to the fertile control male, that took over Group II, which, during the same timeframe, did produce offspring.

Positive antifertility effects of the immunocontraceptive was confirmed in similar studies and various species, however, were the findings differ, are the observations that state the loss of agonistic behaviors in males (Donovan et al., 2013; Doughty, Slater, Zitzer, Avent, & Thompson, 2014; Snape, Hinds, & Miller, 2011) (refs) while others report no significant changes (Massei et al., 2008; Young, n.d.), including the present work.

Also important is the fact that the treated animals did not distance themselves from the group for recovery, hence, preventing any opportunity for a satellite male to invade.

Noteworthy, alpha males, given the right circumstance, would leave their group in order to take over a group with a larger number of females, as observed twice.

There were no significant phenotypic or behavioral alterations, nor any pathological adverse effects in the treated animals. Although the treated alpha males were considered infertile, their secondary sexual characteristics and agonistic behaviors appeared to be preserved, as well as the groups' overall social integrity, which is an important key fact to successfully manage the population of this species and exceeded all minimum expectations.

Other currently considered male fertility strategies, such as castration and vasectomy, which are 100% effective and do have their merits when employed in the right situation, seem less adequate when considering large-scale intervention in the field, considering logistics, as well as animal well-being.

Furthermore, as the findings suggest, injured or sick individuals tend to retreat from the group until recovered, which can take several weeks, representing a window of opportunity for a fertile rival male to take over the group, undoing any population control attempt.

In regard to the relevance to public health, specifically for Brazil and Spotted Fever, based on a stochastic model (Polo et al., 2017, D. Rosenfield, 2019), that indicated a birth-rate reduction of ≥ 90%, the etiological agent of the tick-borne zoonotic disease, hypothetically, could be controlled after two years of intervention, utilizing the alternative population control method for capybaras described in this work.

The observations conducted over the study period of 18 months suggests that the birth rate reduction needed to directly manage a capybara population, - and indirectly the dynamics involved in maintaining and spreading *R. rickettsii*, could be achieved.

4.8 CONCLUSION

When it comes to population control, there is no “one solution” fits all. Every case and every situation is unique and requires a specific study to choose the most appropriate solution. In free-ranging capybaras, being able to treat the alpha male, satellite males, as well as all dominant females, seems to be the most promising method for controlling their population growth with the highest success rate.

Nevertheless, it is important to understand that all efforts are temporary, and in order to maintain functioning population management, this method must be practiced continuously.

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4.10 TABLES

Table 4.1. Example of reproductive behavior ethogram.

Behavioral Ethogram of an Alpha Male	Description of Behavior	Alpha Male/Sexually Mature Males/Satellite Males	Observed Photographed Filmed	Dates (hours) Observed
Any Visual Treatment-Related Discomfort	Separating from the group; apathetic; no foraging; allowing an intruder to get close to the group/females			
Vigilance	Alpha male remains a certain distance in a sitting or ventral decubitus position watching over the group			
Relocation	Leading group to a different location (for better foraging grounds, or for safety)			
Scent Marking	Marking territory by rubbing with nasal gland surface or perianal gland surface over stationary objects; urinating onto the ground			
Courtship Behavior	Seeking physical contact with females. Testing receptivity for mating by sniffing urogenital region, pushing snout into female's flank, or snout. Putting head onto female's dorsal pelvic region.			
Mating	Male continuously follows the female. Frequent mounting attempts by placing upper torso onto female's lumbar/pelvic region, performing a thrusting motion with the pelvis.			
Agonistic (aggressive) Behavior	Attacking, fighting, and chasing the intruder			

Table 4.2. Ethogram - Recorded behavior.

Ethogram Alpha Male Behavior	Description of Behavior	Observations Control Alpha Male	Treated Alpha Male	Satellite Male
Any visual treatment-related discomfort	Separating from the group immediately after treatment; Apathetic; no foraging; allowing an intruder to get close to the group/females	n/a	Treated alpha did not distance himself at any moment post-treatment. All alpha related conducts remained intact.	n/a
Vigilance	Alpha male remained at a certain distance in a sitting or ventral decubitus position watching over the group	Confirmed	Confirmed	Observing the group. Infrequent contact with subordinate females
Relocation	Leading group to a different location (for better foraging grounds, or for safety)	Confirmed	Confirmed	n/a
Scent Marking	Marking territory by rubbing with nasal gland surface or perianal gland surface over stationary objects; urinating onto the ground	Confirmed	Confirmed	Confirmed
Courtship Behavior	Seeking physical contact with females. Testing receptivity for mating by sniffing urogenital region, pushing snout into female's flank, or snout. Putting head onto female's dorsal pelvic region.	Confirmed	Frequent contact (sniffing) but no mating conduct	Observed when approached by a female
Mating	Male continuously follows the female. Frequent mounting attempts by placing upper torso onto female's lumbar/pelvic region, performing a thrusting motion with the pelvis.	Confirmed	Not observed. Possible attempts, but infertile male.	Observed when approached by a female
Agonistic (aggressive) behavior	Attacking, fighting, and chasing the intruder	Confirmed	Confirmed	n/a

4.11 FIGURES

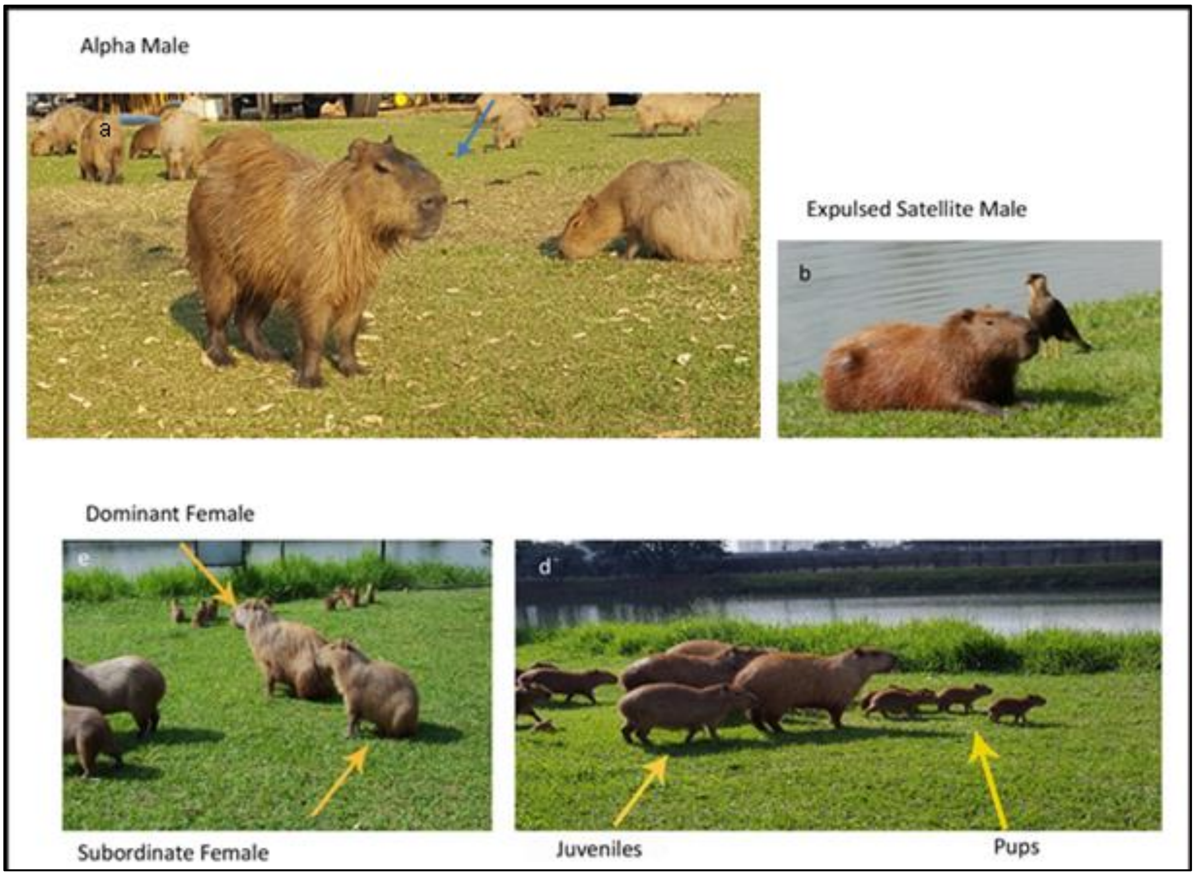

Figure 4.1 - Capybara hierarchal group organization.

(a) alpha male, blue arrow, prominent nasal gland, position, vigilant. ; (b) satellite male, isolated/distant from group; (c) dominant female and subordinate female; (d) juveniles and pups. Source: D. Rosenfield, 2018

Figure 4.2 - Secondary Sexual Characteristics.

a) nasal gland, red arrow; (b) scent marking using nasal gland, blue arrow; (c) visible testes, yellow arrows. Source: D. Rosenfield, 2018

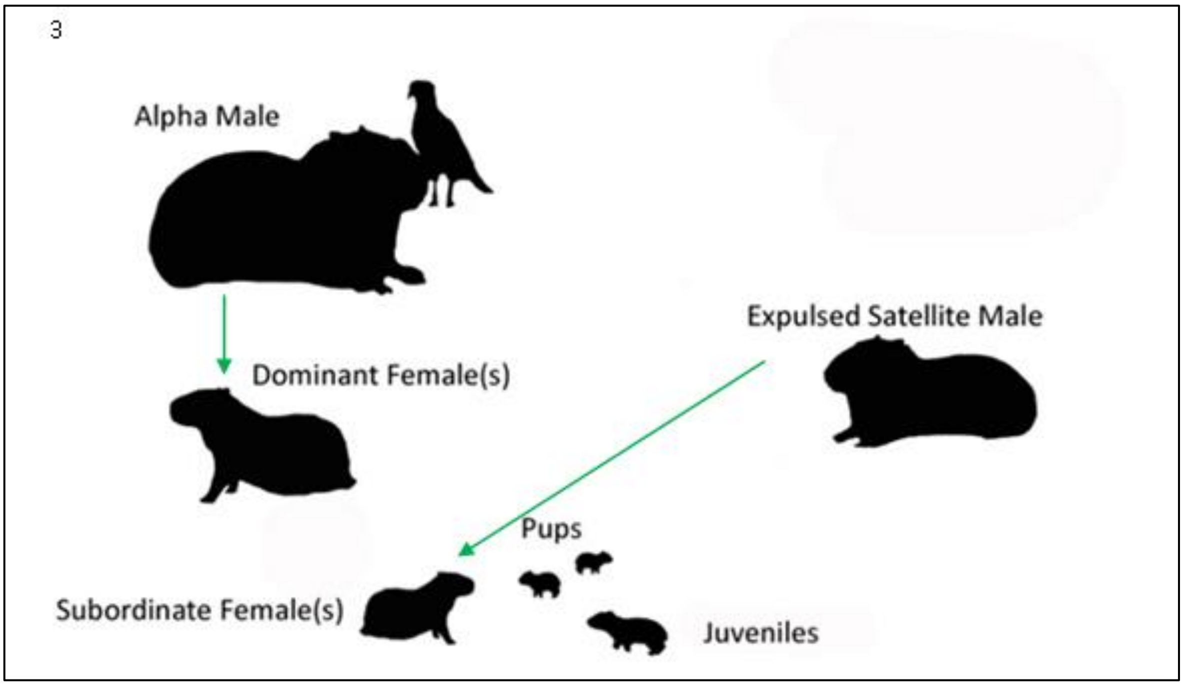

Figure 4.3 - Potential mating dynamics.

Green arrows: Alpha male, dominant females, subordinate female(s), male and female juveniles, pups. Expulsed satellite male (sexually mature). D. Rosenfield, 2019.

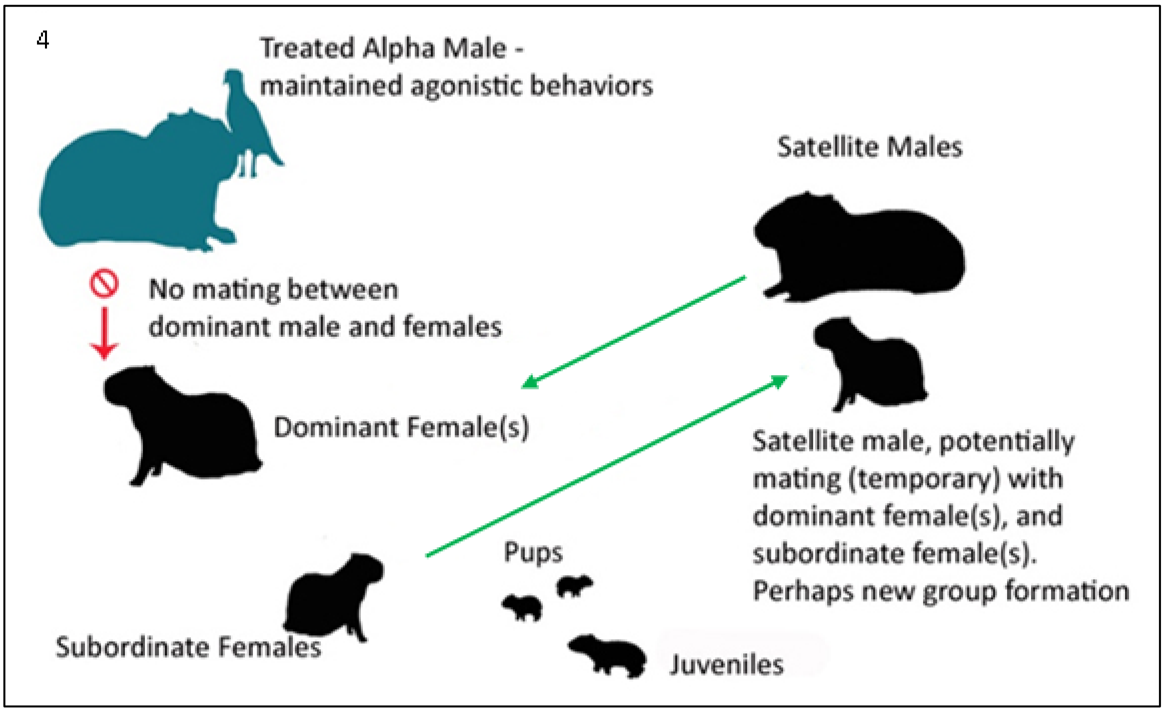

Figure 4.4 - Contraceptive strategy I

Treated dominant male, infertile but with preserved alpha characteristics/behavior. No mating between the treated dominant male and dominant females (red arrow). Opportunistic mating between satellite male and subordinate females (may leave the group temporarily).
Source: D. Rosenfield, 2019

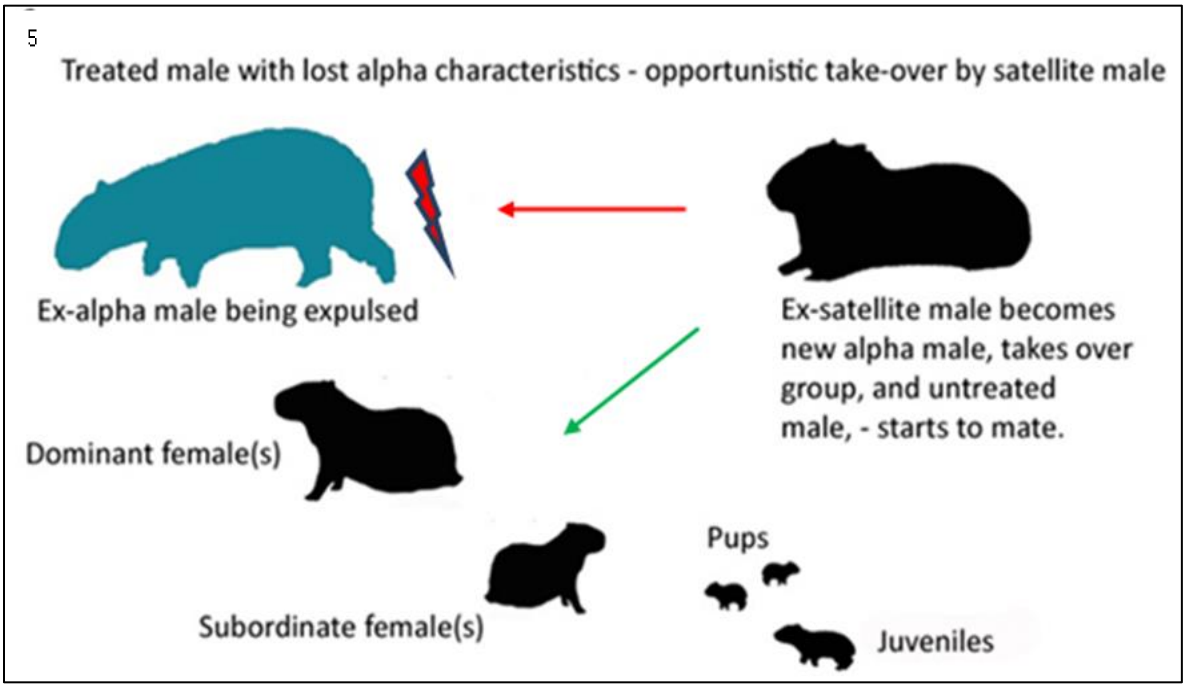

Figure 4.5 - Contraceptive strategy II

Effects of castration: Loss of male's alpha characteristics (mainly agonistic behavior), subordinate/satellite males challenge the alpha male for dominance, eventually taking over the group. Effects of vasectomy: Alpha male will distant himself from the group for a short period of time (recovery phase from surgical injury), leaving the group temporarily without an alpha male. Opportunistic window for a satellite male to invade/take over the group. D. Rosenfield, 2019.

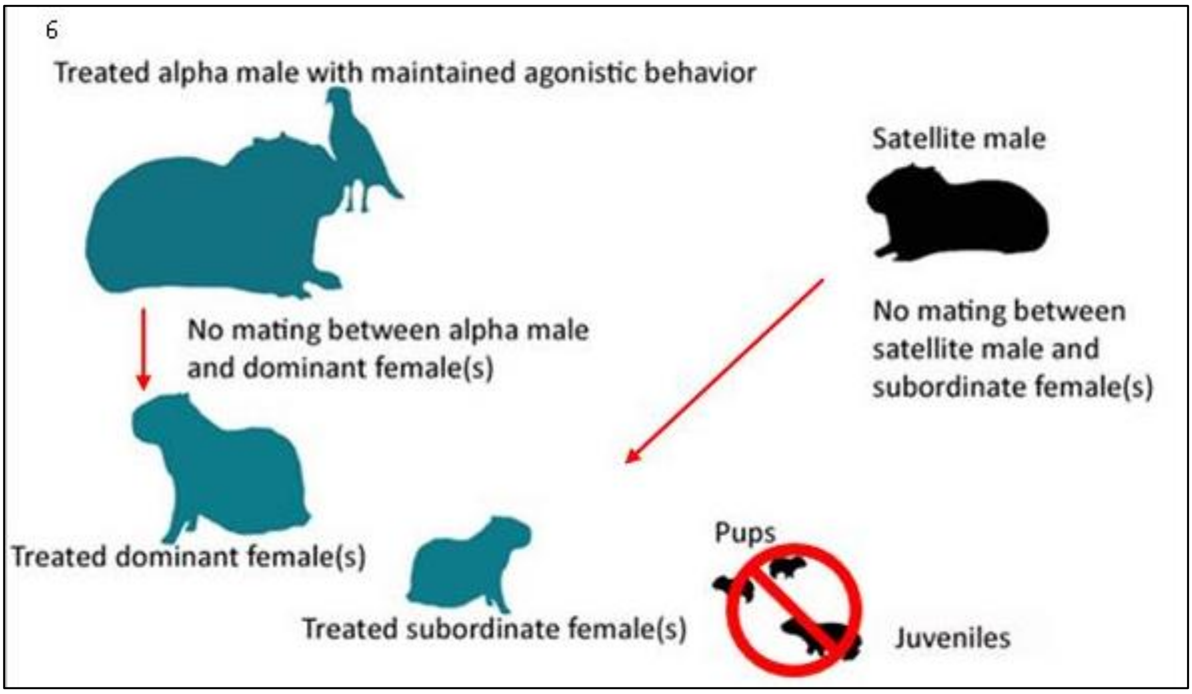

Figure 4.6 - Contraceptive strategy III

Treated dominant male with maintained alpha characteristics; treated dominant females and satellite males. No mating between alpha/satellite males and treated dominant females (red arrows); contraceptive strategy most indicated for capybaras. D. Rosenfield, 2019.

Figure 4.7 - Project Location - Map

(a) South America/Brazil, Sao Paulo State, Sao Paulo City; (b) Raia Olimpica; (c) direct view of the lake with a group of Capybaras. Source: D. Rosenfield, 2019.

5. FIELD-TESTING A SINGLE-DOSE IMMUNOCONTRACEPTIVE IN FREE-RANGING MALE CAPYBARA (*Hydrochoerus hydrochaeris*), EVALUATING ITS EFFECTS ON REPRODUCTIVE PHYSIOLOGY, SECONDARY SEXUAL CHARACTERISTICS, AND AGONISTIC BEHAVIOR)

5.1 ABSTRACT

Controlling wildlife populations to mitigate human-wildlife conflicts and the spread of zoonotic diseases is an ever-growing necessity. The objective of this study was to evaluate a single-dose anti-gonadotropin-releasing hormone vaccine (GonaCon, USDA/NWRC, Fort Collins, CO, USA) as a non-lethal alternative for population control in free-ranging synanthropic male capybara. In addition to infertility efficacy of this treatment, potential effects on the alpha male's secondary sexual characteristics and agonist behavior need to be assessed because any alterations in these factors could lead to population management failure. The treatment group (n = 3) received 1 mL of the anti-GnRH vaccine, intramuscularly, and the control group (n = 2) a 1 mL sham vaccine. Reproductive behavior and social group dynamics were monitored for 30 days prior to inoculation (June 2017) with continuous observations occurring during the study period. Antifertility effects were assessed by conducting exams of testicular morphology, semen characteristics, and histological analysis (after 270 days via hemi-gonadectomy). Compared to the control group, the testicles of the treated males had severe atrophy ($P < 0.05$), oligozoospermia and greater numbers of sperm cells in a static developmental phase. Courtship and agonistic alpha male behavior were not altered, and the group's social integrity was maintained. Results indicate there was 100% infertility in capybara males, observed throughout the study period of 18 months, and equally important, the male's alpha characteristics were not affected by the treatment, which is imperative for successful capybara population control efforts.

Keywords: Population control; Male capybara; GonaCon; Immunocontraception; Agonistic behavior; Anti-fertility

5.2 INTRODUCTION

In Brazil, where wildlife and feral animal population control is still in its early development ages, an emerging “problem” species is the capybara (*Hydrochoerus hydrochaeris*). Native to South America, these animals are the world’s largest rodent, and a very resilient and highly proliferative species of polygynous nature, with a well-defined hierarchy in its social structure. The decrease of its natural predators (jaguar, puma, alligator, and anaconda) and the capacity to adapt quickly to rural/urban settings, attracted by the abundant food supply, have made the capybara one of the most prevalent invasive synanthropic species in Brazil (Felix et al., 2014). Consequently, with capybara, there are all the classical human-wildlife conflicts (HWC), such as traffic accidents, area occupation, and crop destruction, among others. The primary concern, however, is that capybara represent a serious epidemiological threat to public health, specifically, capybaras are considered to be amplifying hosts for *Rickettsia rickettsii* the etiological agent of the tick-borne disease, Brazilian spotted fever (BSF), the most lethal rickettsiosis (Meira, 2013; Labruna, 2014). The disease is transmitted by ticks of the genus *Amblyomma.* In Brazil alone, this disease has caused more than 1900 confirmed human cases since the year 2000, and numbers are steadily increasing (Sinan, 2018).

Depending on the application in free-ranging or captive wildlife, contraceptive concepts range from simple physical separation to hormone antifertility agents and immunocontraceptives, each with its merits and disadvantages. Some of the desired contraceptive characteristics include being effective in a large variety of species and in both genders, applicable remotely and as a single-dose (no booster required), long-term effects that are reversible, safe for the animal and handler with no adverse effects (physiologic or psychologic), logistically straightforward in the field, non-polluting to the environment and food-chain, and inexpensive and readily available (Rosenfield, 2016).

One contraceptive approach that combines most of these relevant characteristics is the immunocontraceptive anti-GnRH vaccine. The use of the immunocontraceptive GonaCon (GonaCon, USDA/NWRC, Fort Collins, CO, USA) induces an immune response to the inoculated antigens (synthetic GnRH peptide and other foreign proteins) because of the formation of antibodies that consequently neutralize the actions of endogenous GnRH. The resulting antibody-endogenous GnRH immunocomplex leads to an alteration in the chemical structure of endogenous GnRH and an ultimate loss in biological function because of a lack of capacity to bind to the target receptors in the anterior pituitary. Consequently, there is an inhibition of the release of FSH and LH and subsequently a relatively lesser intra-gonadal synthesis of the sex steroid hormones testosterone and estrogen than would occur in non-immunized animals.

The general objective of this study was to provide an alternative strategy to control the rapidly-growing syntrophic capybara populations and to indirectly mitigate the increasing human-capybara problems. For this purpose, there was an examination of GonaCon as a potential non-lethal, non-invasive contraceptive for field-application in free-ranging capybaras, while also assessing any possible adverse effects and the effects of this treatment on the social-reproductive dynamics of capybara. There was a specific focus on the alpha male’s behavior as the alpha male from a reproductive perspective, which is particularly important when considering the polygyny (harem-like) social structure of capybaras. Having an alpha male lose its courtship capacity would only provoke subordinate or invading rival males to dominate the group and be the principal individual in the reproductive capacity of the polygynous group, thus, negating the capacity for population management using this treatment in these groups. Having a treated alpha male maintain the dominant behavior and social status within the polygynous group would maintain group stability and minimize the overall rate of reproduction within the group.

5.3 MATERIALS AND METHODS

The project was approved by the university’s ethics committee and the Brazilian Ministry of the Environments, SISBio: 54634-2. It was approved by the university’s Ethics Committee on the Use of Animals in Research (CEUAVET/FMVZ/USP, protocol # 9553260816).

5.3.1 Area of study

The study was conducted at a manmade water pool, of approximately 247,500 m^2, surrounded by trees and extended grassy areas, and used for water-based athletics. It is part of the University City, University of Sao Paulo, south-eastern region of Brazil (23.555202, -46.722433).

5.3.2 Animals

In 2013, wild capybara invaded the pool area, from a nearby river, through a breach in the water canalization system. In total, two adults (one male and one female) and five pups were reported, which grew within a 4-year period to greater than 40 animals. As of May 2016, when the present study commenced, Group I represented one dominant male, 14 females, and 15 juveniles. Group II represented one dominant male, one subordinate male, five females, and three satellite males. All had free access throughout the entire area. In total, there was a selection of five sexually mature capybara males that were assigned to one of two groups. The treatment group (n = 3; alpha males) was treated intramuscularly with a single-dose of 1000 µg anti-GnRH vaccine (GonaCon). The control group (n = 2; one alpha male, one sexually mature satellite male) was treated with a sham vaccine. Based on observational data, the alpha males had a proven history of fertility, and after a visual assessment, all individuals included in the study were considered to be healthy.

5.3.3 Identifying an alpha male

Although sexual dimorphism in capybaras is limited, especially until sexual maturity, alpha males have two distinct physical characteristics which are considered androgen-dependent (Costa and Paula, 2006; Herrera, 1992). The most prominent of these is the morillo, a nasal gland (fig. 5.1 a) that, along with the perianal gland, is used for territorial marking. Results from several studies are consistent with the male with the greatest concentration of testosterone and greatest sexual activity having the largest morillo (Herrera, 1992). The second characteristic is the testicles; male capybaras do not have a scrotum and the testicles are located subcutaneously in the inguinal region (Paula and Walker, 2013). Based on observations of those conducting the present study of sexually mature/dominant males, the testes migrate bilaterally in the mid-sagittal plane to the region of the upper medial thigh (fig. 5.1 b), becoming distinctively visible. These two secondary sexual characteristics were considered to be indicators of the effects of the immunocontraceptive treatment.

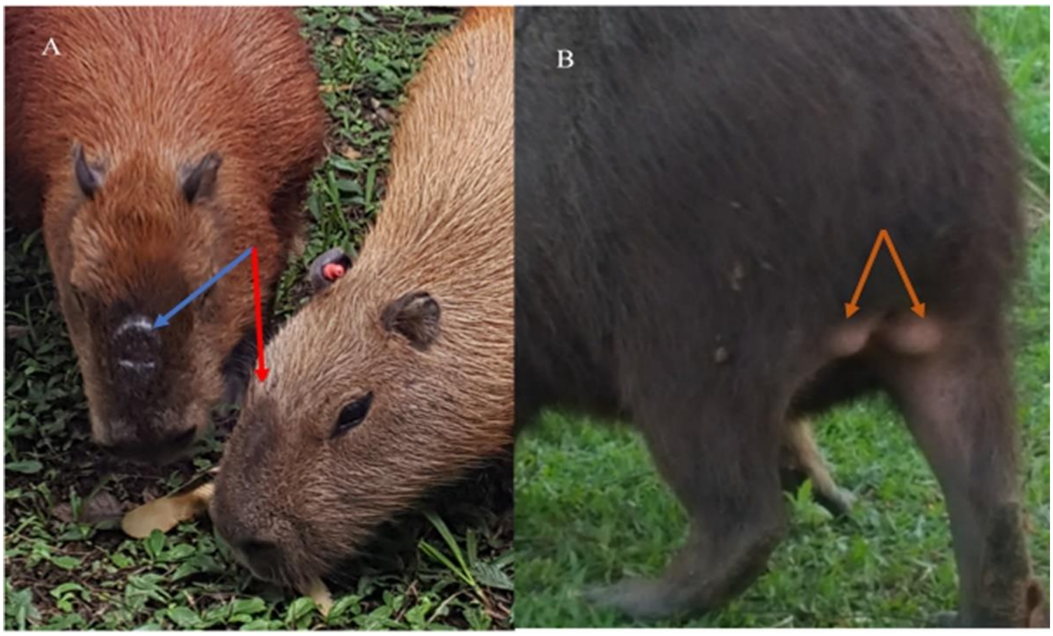

Figure 5.1 - Capybara Alpha Male Secondary Sexual Characteristics.

A) blue arrow, showing an alpha male’s nasal gland, red arrow, compared to a female’s nasal gland; B) yellow arrow, two visible testes at the caudal-cranial area of the inner thigh. Source: D. Rosenfield, 2019

5.3.4 Behavioral study

To serve as a baseline for comparing reproductive behavioral observations post-treatment, there was a recording of the adult males’ behaviors three times a week for a period of 1 month prior to any intervention. There was use of the continuous focal sampling method for 2 hours with observational sessions being distributed evenly between morning, afternoon, and evening, totaling > 20 hours. Post-treatment observations were conducted for 18 months, twice per month, using the same continuous focal sampling method for 2 hours with observational periods being distributed evenly between morning, afternoon, and evening periods, totaling > 40 hours of observation. To categorize behavioral traits, there was development of a simple but specific capybara alpha male agonistic and courtship behavior score that could be visually assessed.

5.3.5 Bio-material collection/analysis

On day 0 (first capture) there was recording of the animal's biometrics and identification of the animal with pre-prepared ear clips and an ID microchip implant, which were placed subcutaneously at the intra-scapular region. Preceding the histological analysis of the testicles, the epididymis was removed, and external dimensions were measured and there was weighing of the tissue.

5.3.6 Male fertility assessment

5.3.6.1 Semen collection

Sperm samples were collected either by electroejaculation or through pharmacologically induced urethral catheterization. Opportunistically, after hemicastration or necropsy, semen was collected by direct epididymal aspiration. The sample was transported in temperature-controlled conditions to the laboratory for immediate computer-assisted sperm analysis (CASA).

For the semen collection using urethral catheterization, there was specific selection of an anesthetic protocol consisting of ketamine (Syntec, Brazil) and dexmedetomidine (Zoetis, Brazil), a potent alpha-2 adrenergic agonist, which is believed to have relaxing effects on the smooth muscle of the ductus deferent, thereby promoting the release of semen into the urethra (Lueders et al., 2012; Pisu et al., 2017).

Semen collection by epididymal aspiration was performed in cases where a hemi-orchiectomy (in the field) was required. The removed testicles were taken to the laboratory for further processing. Spermatozoa were collected from the epididymal cauda using a slicing technique (Nichi, 2009).

5.3.6.2 Sperm analysis

Immediately after seminal collection, the samples were protected from light, stored at 37 °C, and transported to the laboratory for further processing and analysis. There was analysis of samples for spermatic kinetics, concentration, sperm morphology, evaluation of mitochondrial activity, and evaluation of plasma and acrosomal membrane integrity using procedures that were previously described: Evaluation of sperm kinetics (Goovaerts et al., 2006) and sperm morphology, using the wet chamber method and a differential interference contrast microscope (DIC, Nikon® Eclipse TE300, Tokyo, Japan), (Barth and Oko, 1989); plasma membrane integrity, using eosin-nigrosine staining (Barth and Oko, 1989); acrosomal integrity, using fast-green/rose bengal staining (Pope et al., 1991); and mitochondrial activity using the DAB (Diaminobenzidine) test (Hrudka, 1987).

5.3.7 Collection frequencies

On days 90 and 180 of the present study, there was repeated collections of the biomaterial and biometric data. On day 270, in addition to collecting blood/semen samples and biometrics, a hemi-orchiectomy was performed for morphological and histological studies.

5.3.8 Morphometric analysis of testes

Immediately post-castration, the testis mass (epididymis removed) was quantified to the nearest 0.5 g, and the dimensions were measured to the nearest 1 mm using a Vernier caliper.

5.3.9 Histology analysis

For the histological samples, there was preparation using the following methods described by (Júnior, 2012; Paula, 1999). Morphological changes of the treatment group were evaluated using a microscope and there was comparison with values from tissues collected from the control animals.

5.3.10 Variables of immunocontraceptive effects on the testes

To identify alterations due to the contraceptive effects, the values for the following variables were compared to those of untreated males: testicular morphology (gross morphology weight/dimensions); and evaluation of the testicular parenchyma, assessment of the parenchymal tissues including 1) evaluations for the presence and organization of seminiferous tubules (numbers and structural arrangements within the seminiferous tubule); 2) Sertoli cells (structural integrity); 3) Leydig cells (numbers, cellular organizations); 4) basal lamina structure; 5) germinal epithelium/germ cells/8-stages of differentiation (numbers, arrangements, stage, number of necrotic cells); 6) lymphoid space (size); and 8) lumen (size).

5.3.11 Population survey

To support the overall evaluation of the effectiveness of the immunocontraceptive vaccine for population control, there was monthly monitoring of the two population groups, double counting the group members by direct observation, from a distance of 10 to 50 meters, for a duration of 1 to 2 hours, and various morning, day, evening, and night sessions. Monitoring was initiated 1 month prior to the vaccination date and continued throughout the study period.

5.3.12 Statistical analysis

The statistical analysis performed in the present study to compare the groups was mainly descriptive because the number of experimental units (i.e., animals) available for the study was not great enough to make concise statements due to the experimental conditions. There were, however, significant differences observed among the groups that should have great applicability in future research or field conditions. In this way, the data were analyzed using the SAS System for Windows program (SAS Institute Inc., Cary, North Carolina, USA). Differences between the treatments were evaluated using the Student T-test considering there was normality of the values (Gaussian distribution) and homogeneity of the variances. The significance level used to reject the H0 (null hypothesis) was 5%, that is, for a level of significance less than 0.05; it was considered that statistical differences occurred between the values for the variables evaluated in this study.

5.4 RESULTS

5.4.1 Testicular morphology

At 9 months post-treatment, hemi-orchiectomies of the animals in the treatment (n = 3) and control (n = 2) were performed. The testis of the treated males had a 35% lesser ($P < 0.05$) - weight whereas the testicular volume was less ($P = 0.06$), compared to the volume of the control group (fig. 5.2 a/b). Values are reported as the mean ± S.E.M. (Table. 1).

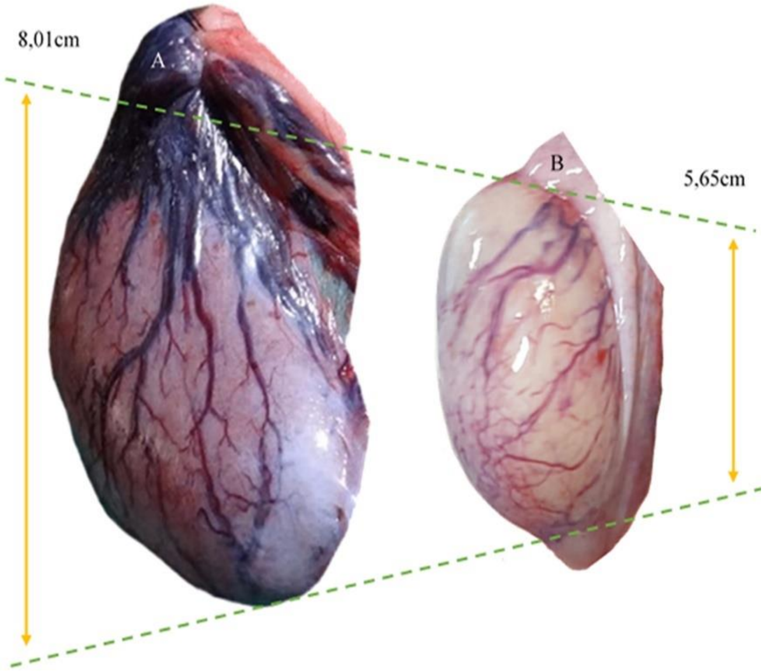

Figure 5.2 - Comparison Testicular Morphology.

A) Testicle – control male, and B) treated male, 270 days post-treatment.
Source: D. Rosenfield, 2019

5.4.2 Semen characteristics

Results from the analysis of semen (n = 5) of the animals in the control and treated group indicated there was a difference in spermatozoa concentration between groups ($P < 0.05$; Table 2). Furthermore, there was a greater percentage of total and primary spermatozoa morphology abnormalities in the animals of the treated compared with the control group ($P < 0.05$; Table 6.2), and there was a tendency for a greater percentage of spermatozoa with minor defects in animals of the treated compared with the control group ($P = 0.06$; Table 6.2).

Table 5.1. Testis weight and volume comparison – control and treated males 270 days post-treatment

	Treatment group	Control group	P
Testicular Weight (g)	16.33 ± 10.68	45.76 ± 8.47	0.0268
Testicular Volume (mL)	19.67 ± 0.80	62.29 ±11.71	0.0672

Values are mean ± S.E.M; Treatment group (*n* = 3) and control group (*n* = 2)

Table 5.2. Data for sperm collected from epididymis following unilateral orchiectomy – control and treated males 270 days post-treatment

Variables	Control Males	Treated Males	*P*
Concentration (Spz. x 10^6/mL)	146.25 ± 3.75	18.38 ± 10.68	0.0028
Total Motility (%)	49.00 ± 19.00	11.00 ± 9.00	0.2124
Progressive Motility (%)	13.50 ± 1.50	0.00	*0.0704
Rapid (%)	41.50 ± 0.50	9.00 ± 1.00	0.0012
Medium (%)	21.00 ± 5.00	6.00 ± 3.00	0.1439
Slow (%)	28.50 ± 0.50	50.50 ± 7.50	*0.0996
Static (%)	9.50 ± 5.50	38.50 ± 15.50	0.2199
VAP (average path velocity, µm/s)	53.10 ± 6.70	35.50 ± 7.10	0.2132
VSL (straight-line velocity, µm/s)	27.10 ± 4.80	17.15 ± 0.35	0.1746
VCL (curvilinear velocity, µm/s)	104.20 ± 17.40	65.10 ± 9.50	0.1873
ALH (amplitude of lateral head displacement, µm)	7.85 ± 0.15	0.00	0.0122
BCF (beat cross-frequency, Hz)	26.70 ± 0.10	35.55 ± 10.25	0.5466
STR (straightness, %)	52.00 ± 3.00	48.00 ± 6.00	0.6115
LIN (linearity, %)	27.50 ± 1.50	26.00 ± 3.00	0.6985
Major defects (%)	29.00 ± 1.00	41.33 ± 0.33	0.0007
Minor defects (%)	26.50 ± 0.50	37.67 ± 2.96	*0.0622
Total defects	55.50 ± 1.50	79.00 ± 2.64	0.0073
Plasmatic membrane integrity (%)	60.50 ± 0.50	59.67 ± 1.20	0.6376
Acrosomal membrane integrity (%)	84.50 ± 2.50	74.67 ± 10.41	0.5211
High mitochondrial activity	64.00 ± 4.00	60.67 ± 1.20	0.3944
Medium mitochondrial activity	24.00 ± 4.00	28.00 ± 1.52	0.3447
Low mitochondrial activity	7.00 ± 0.00	7.00 ± 1.00	1.0000
No mitochondrial activity	5.00 ± 0.00	4.33 ± 0.88	0.5286

Values are mean ± S.E.M; Treatment group (*n* = 3) and control group (*n* = 2); **P* values indicating tendency

5.4.3 Histological Analysis

Histological examination of the testicular parenchyma of animals in the control group indicated there was an abundance in number of Leydig cells and the assessment indicated there was a well-organized spermatogenic process (fig. 5.3 a), while basal lamina was undamaged as a result of treatment, and the luminal space was minimal (fig. 5.3 c), with spermatids of each developmental stage being present (fig. 5.3 e).

Examination of the testicular parenchyma of treated males indicated there were disruptions in the overall cellular organization (fig. 5.3 b) with a decrease/absence of germinal cells (oligozoospermia) (fig. 5.3 d). Consistent with this finding was the substantial number of seminiferous tubules (fig. 5.3 f) with degenerative characteristics of the germinal epithelium for which there were cytoplasmic vacuoles and spermatogonial multinucleate giant cells.

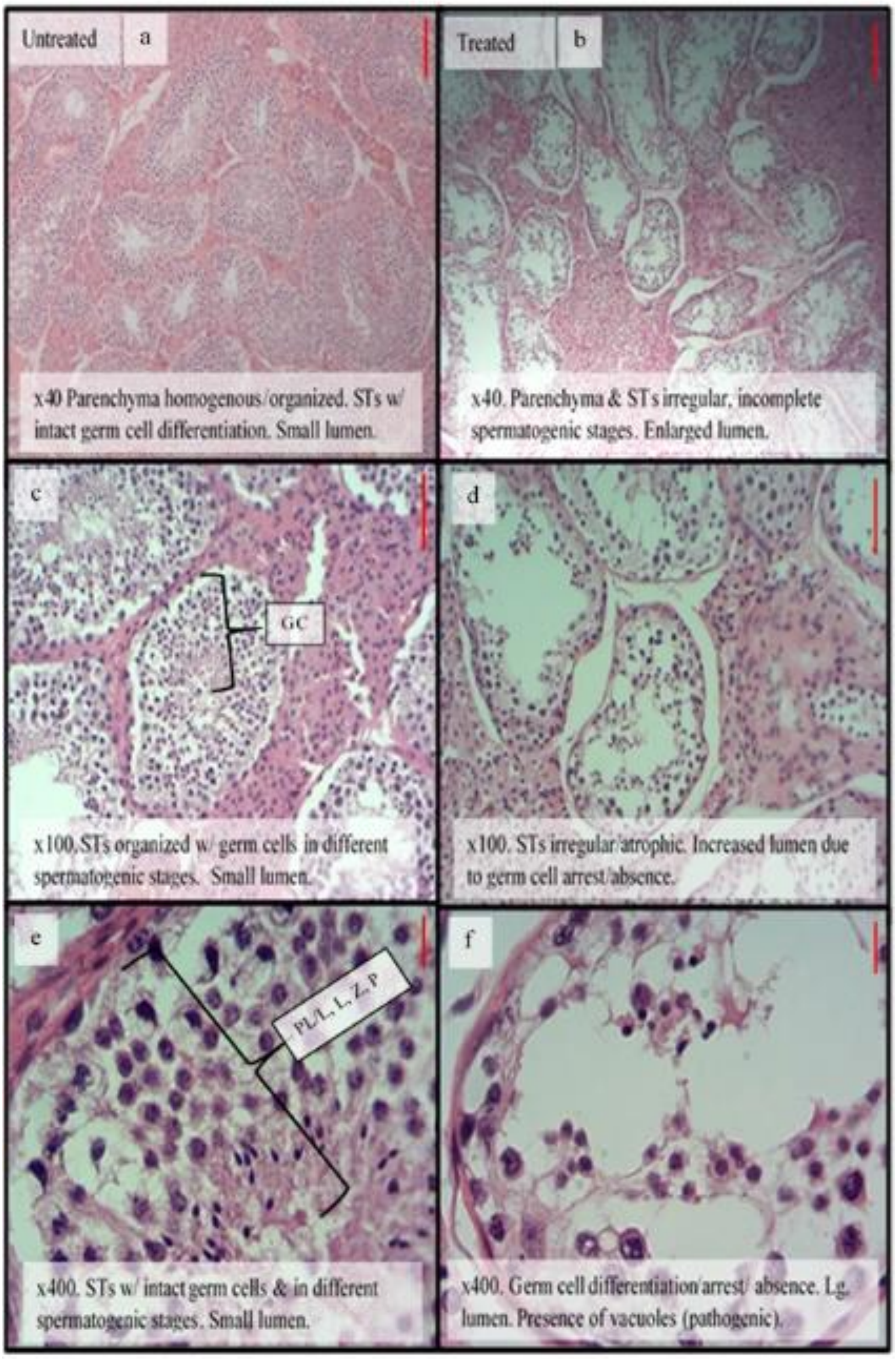

Figure 5.3 - Direct histological comparison of the testicular parenchyma

Control male (a, c, and e) and treated male (b, d, and f). Image a, b, c, d: Scale bar (red line) 100µm (a, b, c, and d) and 10µm (e and f). Source: D. Rosenfield

5.4.4 Alpha male behavior and secondary sexual Characteristics

None of the treated males had any loss of the behavioral characteristics that are typical for the alpha male and these males continued to mark the territory of the polygynous group. Agonistic behavior toward other males was maintained after the treatment was imposed on the alpha male. The only variant observed was part of the courtship behavior, whereby males continued to follow females and sniff their genitals but made very few or no attempts to mount.

5.4.5 Population dynamics

The actual population size of the polygynous group studied increased during the period when there were observations in this study between 2014 and 2017. The results from the sensitivity analysis indicated that the most important variables were the number of births and the capacity of the area where the polygynous group resided for nutrient procurement, which were twice as important as sterilization and mortality rates in affecting size of the polygynous group. The 2018 simulation scenario (58 animals) is greater than the values for data from the first year of observation (52 animals). Detailed information on the population dynamics is provided in the supplementary material for this manuscript.

5.5 DISCUSSION

To our knowledge, this present study is the first report on the use of GonaCon as a method of antifertility in *Hydrochoerus hydrochaeris*. Research findings in the present study confirm that there is a 100% effectiveness of this immunocontraceptive anti-GnRH by inhibiting the fertility of capybara alpha males over the entire study period of 18 months, without any indication that the treatment was becoming ineffective, while there was a sustained maintenance of the alpha characteristics of the dominant male, phenotypically as well as behaviorally. The research of the present study focused on three physiological fertility variables and one observational determinant to ascertain the contraceptive effects of the imposed treatment in capybara. The first and most obvious effect of the treatment was the changes in testicular weight and volume among treated males, compared with control males, indicating there was acute testicular atrophy. This testicular atrophy has also been observed in other studies where there was treatment with GonaCon in various species (D'Occhio, et al., 2001; Ghoneim et al., 2012; Wicks et al., 2013).

A second important finding was that the assessment of the semen characteristics confirmed there were similar mean spermatozoa concentrations in intact and fertile (free-ranging) males to the findings in previous studies with the same species (Rodríguez et al., 2012, in supplementary material), Using the reported values as a reference, together with the data from untreated control males in the present study that were considered fertile because of reproductive history and by having a similar mean sperm concentration as those in previous studies, the findings with use of the CASA assay on the semen characteristics indicated there was a marked decrease in the sperm concentration in GonaCon-treated males (literature reference male: 127 ± 59.01 Spz. x 10^6/mL compared with control male: 146.25 ± 3.75 Spz. x 10^6/mL compared with treated male: 18.38 ± 10.68 Spz. x 10^6/mL). The lesser than typical sperm count is suggestive of an infertile individual. Values for additional sperm variables also indicate there are significant differences in sperm motility, damage, and morphology between control and treated males.

The third important aspect of the physiological evidence is based on the histological findings in the present study for which there was comparison of the testicular parenchyma between control and treated males. Extensive histological studies on testicular morphology and spermatogenesis in intact capybara males (Moreira et al., 1997; Paula et al., 1999; Paula 2002; Costa et al., 2006) served as reference information for the control (intact and fertile) male capybaras in the present study.

Observations of the testicular parenchyma of GonaCon-treated males indicated the cells and structures of parenchyma and seminiferous tubules were abnormal as compared with those in the control animals and those described previously in capybara, with the lumen diameter being greater due to a cessation of spermatogenesis. These results indicate there is a lack of germ cell differentiation throughout all developmental stages, including preleptotene/leptotene (P/L), leptotene (L), pachytene (P), diplotene (D), and zygotene (Z), leading to a decrease in, or total absence of, rounded/elongated spermatids, resulting in oligospermia. This cessation of spermatogenesis and testicular degeneration that subsequently progresses until there is testicular atrophy was evident in the treated capybara of the present study. The lumen of seminiferous tubules was enlarged and irregular in shape due to the depletion of spermatids. In the present study, all of the histological findings were substantiated by the lesser sperm concentrations in the semen that was collected, and data obtained using computer-assisted sperm analysis as compared to values for sperm in the males of the control group.

The basal membrane appeared intact, an important fact considering the potential reversibility of the infertility effect. The presence of large numbers of Leydig cells is a species-specific normality (Paula, 2002). Furthermore, additional evidence of the effects on the parenchyma was evident as a result of the presence of multinucleated giant cells, greater numbers and size of the lymphatic space, and the presence of vacuoles. These findings are similar to those from other studies where there was use of an anti-GnRH vaccine in males from a variety of species (Ghoneim et al., 2012; Malmgren et al., 2010; Han et al., 2013).

In regard to the observational determinants, monitoring the population dynamics and comparing the numbers of the polygynous groups using the mathematical models (available in the supplementary materials, Oswaldo and Costa, 2019) was a valuable and effective non-invasive method to measure the treatment effectiveness. The population dynamics that were present as a result of the treatment with GonaCon were more desirable than anticipated when the study was initiated. It is thought that the conception of the offspring during the initial study period occurred prior to immunization or a female was inseminated by a male other than the treatment group’s alpha male. The second observational element, which is as important as the successful antifertility effect, is the preservation of the agonist behavior of the alpha male subsequent to treatment with GonaCon, including the secondary sexual characteristics (Rosenfield and Pizzutto, 2019). Any alterations to the alpha males’ phenotype or behavioral traits would eventually lead to the loss in the dominant position, consequently undoing any capybara population control effort as a result of the GonaCon treatment.

Besides a local injection site reaction and the formation of a small abscess, which is expected because of the immune response to the vaccine’s adjuvant, no other adverse effects were observed. This type of response has also been documented in most of the prior studies conducted using GonaCon.

Alternative measures such as surgical procedures, for example, castration and vasectomy, are 100% effective and in certain situations, the use of the approaches is warranted, but the use of these approaches are not feasible on a larger scale or for in-the-field applications. Furthermore, and perhaps even detrimental to the polygamous group's stability and overall goal of population control, the 7 to 14 days of recovery time for a male from surgical procedures (in this case post-operation trauma) provides opportunities for untreated rivals to ascend to the alpha male status.

5.6 CONCLUSIONS

Considering the consolidated data, the use of GonaCon results in a satisfactory anti-fertility effect on alpha male capybaras, without any significant adverse effects on secondary sexual characteristics, agonist behavior, or pathological occurrences. The capacity to administer a single dose via remote drug delivery systems makes this immunocontraceptive a valuable non-lethal population control approach for managing synanthropic capybaras. Inhibition of the overall population growth helps to mitigate the conflicts between humans and capybaras. More importantly, regarding public health, this treatment might be an effective strategy for preventing the spread of tick-borne pathogens such as *R. rickettsia* considering the outcomes from the present study.

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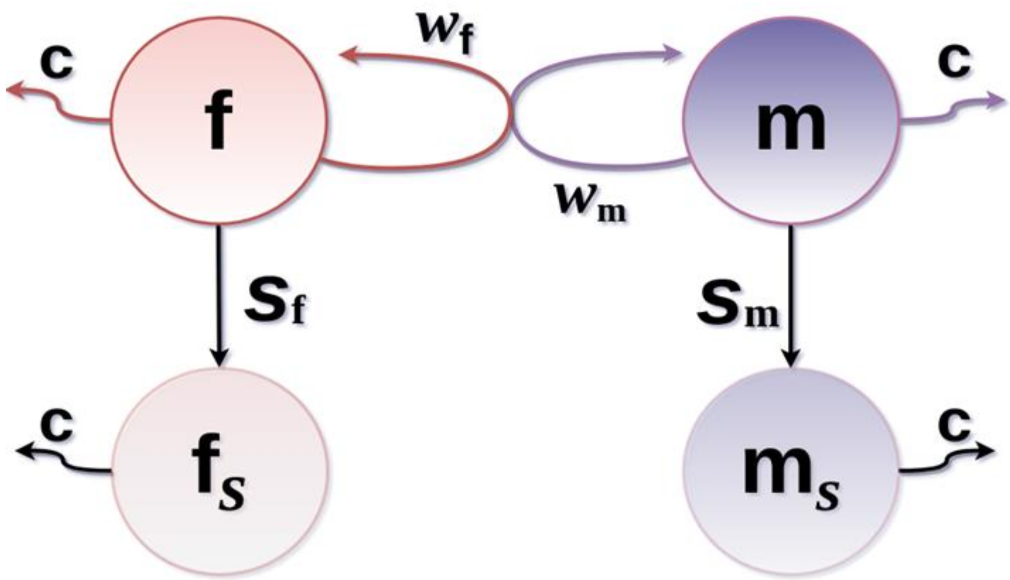
5.8 SUPPLEMENTARY MATERIAL

5.8.1 Mathematical Model of Population Dynamics

Compartmental model of population dynamics; the compartments were given by sex and reproductive status (fertile and sterilized), (fig. 5.9 I). The model was based on a system of coupled differential equations (tab. 5.9 I) adapted from (Santos Baquero et al., 2016). We assumed that all rates were constant, fertility and mortality were density dependent, and sterilization was lifelong.



Suppl. Figure 5.8 I - Compartmental model of *H. hydrochaeris* population dynamics.
f: intact females; m: intact males; fs: sterilized females; ms: sterilized males; w: birth function; c: mortality function; sf: female sterilization rate, sm: male sterilization rate.
Source: A. Acosta, Rosenfield, 2019

With the notation described, equations were given by:

$$n = f + fs + m + ms$$
$$y_f = \frac{b}{2f} = \frac{x\,m}{m + fh^{-1}}$$
$$w_f = y_f - (y_f - d_f)\frac{n}{k}$$
$$c_f = d_f + (w_f - d_f)\frac{n}{k}$$
$$\frac{df}{dt} = \left(wf - cf - s_f\right) * f(t)$$
$$\frac{dfs}{dt} = -cf * fs(t) + s_f * f(t)$$

were, *x* is the number of births per harem calculated form the second equality in the second equation, *h* is the harem mean size, and *d* the mortality rate. Equations for males were equivalent to female equations.

The differential equations were solved numerically using the fourth order method of Runge-Kutta (Soetaert, 2010) and a global sensitivity analysis was performed to identify the most influential parameters . Simulations were done using the capm v0.12.9 R package.

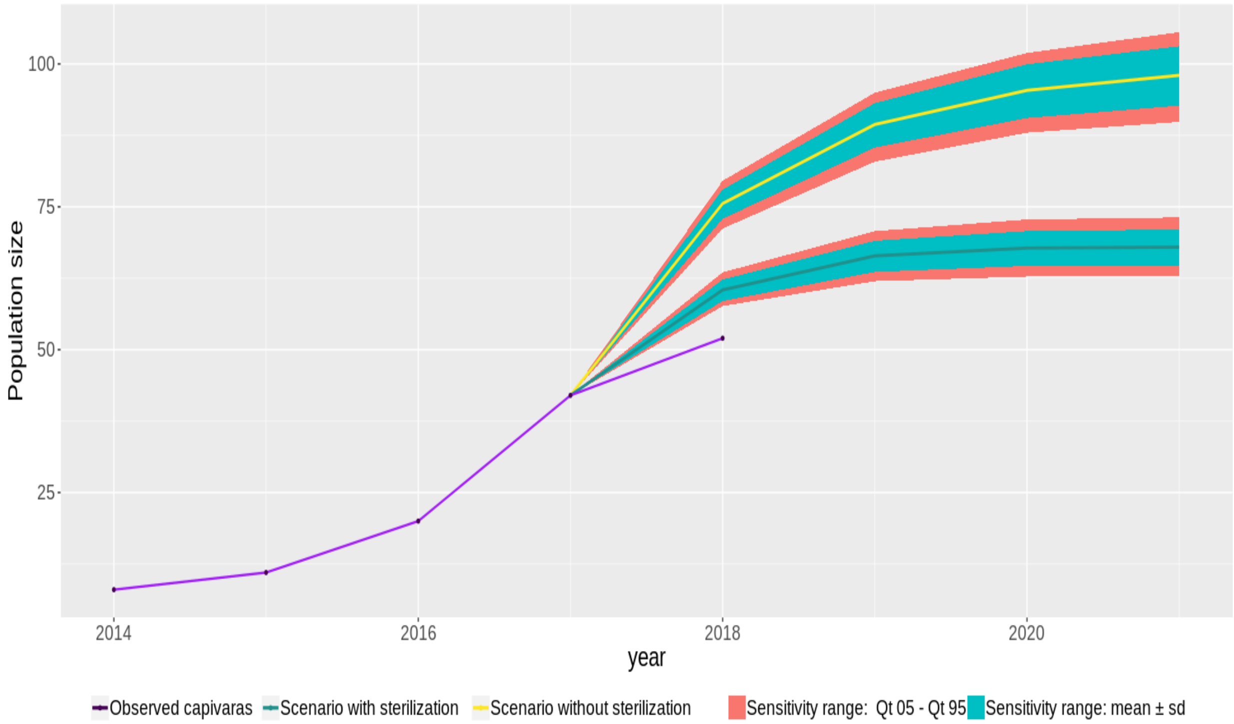
The parameters were obtained from field estimates, the literature or modeled (Table. II). The carrying capacity was calculated according to the estimated availability of vegetation according to the season and the reported capybara daily consumption. Furthermore, anthropological impacts on the environment were considered. The simulations ran from 2017 to 2021 and included two scenarios: with and without sterilization.

Suppl. Table 8 I

Initial conditions and parameters of the compartmental model

	Value	Description	Reference
Parameter			
b	83.43; 57.08	Births in the population	Modeled
lz	4.8	Litter size	(Tacutu et al., 2018)
bfy	1.25	Births / female / year	(Tacutu et al., 2018)
d	0.16; 0.14	Mortality / year (males and females)	(Tacutu et al., 2018)
sf	0.31	Female-sterilization rate	Modeled
sm	0.5	Male-sterilization rate	Modeled
h	0.45	harem mean size	Modeled
k	100,11	Carrying capacity	Modeled
Initial Conditions			
f	23	Intact females	Observational data (2017)
fs	6	Sterilized females	Observational data (2017)
m	10	Intact males	Observational data (2017)
ms	3	Sterilized males	Observational data (2017)

5.8.2 Population dynamics – monitored



Suppl. Figure 8 II - Population dynamics. Purple line representing actual (observed) population growth. (Santos Baquero, Costa. A., 2019). Additional reference: Tacutu, R., et al, (2018). Human Ageing Genomic Resources: new and updated databases. Nucleic acids research, 46(D1), D1083–D1090. doi:10.1093/nar/gkx1042. AnAge Database of Animal Ageing and Longevity. AnAge entry for *Hydrochoerus hydrochaeris*: HAGRID: 02498. http://genomics.senescence.info/species/entry.php?species=Hydrochoerus_hydrochaeris)

Suppl. Table 8 II

Reference parameters of *H. hydrochaeris* semen characteristics (Rodríguez, et al, 2017).

Measured Parameter	Mean Values
Volume (µL)	135.5 ± 93.5
pH	8.14 ± 0.37
Vigor (1–5)	3
Viability (%)	60.08
Concentration (Spz. x 10^6) / mL	127 ± 59.01
Mass motility (%)	32.6 ± 13.4
Individual motility (%)	34 ± 19.8
Estimated normal spermatozoids (%)	56.1
Head shapes (%)	32.6
Total abnormalities (%)	11.2

Values used for comparison with study findings.

5.8.3 Secondary sexual characteristics

Suppl. Figure 8 III - Male's alpha behavioral integrity - post-treatment. A) Yellow arrow: prominent nasal gland of an alpha male; B) red arrow, territorial marking by a treated alpha male, using the nasal gland. Source: D. Rosenfield

5.8.4 Alpha male agonistic behavior

Suppl. Figure 8 IV - Observed alpha male's agonistic behavior (chasing/fighting). Treated alpha male (yellow arrow), chasing rival male (red arrow) away from the group, over a large distance (± 1,0km). Source: D. Rosenfield

6. LONG-TERM STUDY ON A SINGLE-DOSE IMMUNO-CONTRACEPTIVE IN FREE-LIVING SYNANTHROPIC FEMALE CAPYBARAS

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6.1 ABSTRACT

Controlling synanthropic capybara population has become a wildlife management priority, as they grow into superpopulations, provoking several human-capybara conflicts, with emphasis on epidemiological concerns, namely the tick-born Brazilian Spotted Fever. The study's intent was to investigate the long-term antifertility effectiveness of the immunocontraceptive GonaCon in free-ranging, synanthropic capybara females, using a single-application, concurrently, evaluating its impact on the reproductive behavior and group's social integrity. Methods: Sexually mature females (n = 10), divided into control group (n = 4) and treatment group (n = 6), immunized with an anti-GnRH vaccine (1mL GonaCon), administered via hand-syringe IM. To evaluate the contraceptive's impact on the reproductive behavior and antifertility effectiveness, starting December of 2016, observations of reproductive behavior and social group dynamics were conducted prior to intervention and maintained during the entire study period of 32 months. Biometric data, ovarian morphology, and histopathological analysis were studied. Results: During the study period, no treated female demonstrated estrous behavior and no births were observed. Post-gonadectomy, the ovarian gross morphology of the treated females appeared atrophic, specifically ovary weight ($p<0.005$) when compared to the control. Histological findings exhibited folliculogenic activities in both groups, displaying follicles in varies developmental stages, from primary to Graafian, including corpus luteum. Treated females showed a lesser number of mature follicles, in addition to apparent dysmorphology and pathological alterations in antral follicles and oocytes. No negative impact on the overall group's social integrity, including alloparental conduct, were observed. Besides the commonly reported injection-site reaction, no other adverse effects were reported. In conclusion, the study's findings suggest the use of GonaCon as an effective and safe contraceptive agent in female capybara.

Keywords: Population control; Male capybara; GonaCon; Immunocontraception; Agonistic behavior; Anti-fertility.

6.2 INTRODUCTION

As reported in publications, capybara are an emerging synanthropic nuisance in numerous municipalities throughout the Southeastern region of Brazil (VERDADE; FERRAZ, 2006; BRITES-NETO; BRASIL; RONCATO DUARTE, 2015; ABREU BOVO et al., 2016; ROSENFIELD; POLO; SCHILBACH PIZZUTTO, 2019), by growing into superpopulations, private and public urban areas, traffic collisions, devastating crops in agricultural settings, and more importantly, giving rise to epidemiological concerns, such as the zoonotic and tick-borne disease Brazilian Spotted Fever (FERRAZ et al., 2003; KRAWCZAK et al., 2014; MARCHINI; JR, 2015; ABREU BOVO et al., 2016).

This growing capybara-problem, enhanced by public demand, prompted immediate interventions to control their populations (LABRUNA, 2013; KUNIY et al., 2018). A challenge that is not easily solved, capybara, pertaining to the Brazilian Fauna, are protected by State and Federal laws, making lethal population management next to impossible (FEDERAL LAW, BRAZILIAN FAUNA PROTECTION ACT, 1967, 1967). Furthermore, the search for the most adequate capybara population management strategy is still an ongoing endeavor, and several studies are underway to find quick and cost-effective measures (FEDERICO; CANZIANI, 2005; PEREIRA; ESTON, 2007; GUIMARÃES; RODRIGUES; SCOTTI, 2014; ROSENFIELD, 2016a; KUNIY et al., 2018). In the case of capybara, simply applying any effects antifertility methods is not adequate enough, as a polygynous society, with a strong female-based, treating one male versus numerous females, seems logistically and economically more feasible. In this regard, two principal antifertility methods are being studied. One, being based on vasectomy in males and ligature in females, thus, allowing the continuation of sex-hormone synthesis, necessary for an alpha male, being the principal breeder, to maintain his dominant sexual characteristics, and agonist behavior, which is imperative for a successful control strategy (ROSENFIELD; PIZZUTTO, 2019). However, logistics to execute these procedures are complex and demand competence. Also, initially opening a window of opportunity for competing males to take over the group, while the treated alpha male is recovering. A period of 10 - 14 days where the dominant male distance himself from the group.

Alternatively, option two, employing an immunocontraceptive method, that has been successfully tested in male capybara (ROSENFIELD et al., 2019), providing a number of desired characteristics of an antifertility agent, considering free-ranging capybara. As a vaccine, the agent can be administered remotely, using darts. Specifically, the anti-GnRH vaccine GonaCon (USDA's National Wildlife Research Center, Ft. Collins, Co, USA), which doesn't require a booster application and has proven effectiveness in studies on several species and both genders (GIONFRIDDO et al., 2008; KILLIAN et al., 2009; YODER; MILLER, 2010; COWAN et al., 2011; SNAPE; HINDS; MILLER, 2011; MILLER et al., 2013; FISCHER et al., 2018; WIMPENNY; HINDS, 2018), among others.

GonaCon provokes infertility by triggering an immune response against endogenous Gonadotrophin-releasing Hormones, inhibiting its biological function in controlling reproduction in both sexes. In females, impeding the synthesis and liberation of the Luteinizing Hormone and Follicle-stimulating Hormone, consequently ceasing ovarian activities (FERRO et al., 2004; MILLER et al., 2008a; SHARMA et al., 2014; ROSENFIELD, 2016b).

In South-eastern part of Brazil, capybara females are annual polyestrous with breeding peaks at the start of the rainy season (October - November). Their estrous cycle lasts about 8 days, with spontaneous ovulation and a receptive period of about 8 hours (MIGLINO et al., 2013). Females do not present any external signs of estrous, besides being receptive to the male, whereby the male touches the female with his head and scent gland, sniffing the female's genitals. The Copulation may occur on land, but in its majority, inside the water, with numerous repetitions.

The objective of this study was to evaluate GonaCon as an immunocontraceptive agent in free-ranging synanthropic female capybara and its impact on social, - and reproductive behavior.

6.3 MATERIAL AND METHODS

This study was approved by the Brazilian Ministry of Environment (SISBio, 2016); University of Sao Paulo's Ethics Committee for Use of Animals in Research (CEUAVET, 2016).

6.3.1 Study area

The study was conducted throughout all dry and rainy seasons (December 2016 to January 2019) at the main-made pool for aquatic athletics, campus University City, University of Sao Paulo, Sa Paulo, Brazil. 23º33'21S 46º43'14W, altitude 722 m (WGS84 EGM96 Geoid. The pool area is approximately 247 500 m2, surrounded by trees and extended grass areas. The city of Sao Paulo lies within the Atlantic Forest domains of the State of Sao Paulo. Temperatures in the summer months (December - March) can reach 30ºC, with an average of 25ºC, median rainfall of 170 mm. Whereas in the winter (June - September), temperatures can fall to below 12ºC, average temperature 19ºC and median rainfall of ≤ 50 mm (World Meteorological Organization, 2019).

6.3.2 Animals

Originally, one adult male and one female together with five pups found their way into the pool area (end 2013), growing to over 40 animals, divided into two groups, over a 4-year period, demonstrating a high proliferative breeding activity. Status as of December 2016, Group I: One dominant male, 14 females, and 15 juveniles. Group II: One dominant male, one subordinate male, five females; in addition to three satellite (isolated adult) males.

During the months May to August 2019 ten adult females, pertaining to two resident groups, were chemically restraint, using specialized tranquilizer darts (Pneu-Dart, Inc., Williamsport, Pa, USA) with an association of 9mg/kg $^{-1}$ Ketamine (Syntec, Brazil) and 5mg/kg $^{-1}$ Dexmedetomidine (Zoetis, Brazil). For dosage, an estimated bodyweight of 60kg was used.

All females were examined for health status and biometrics. In addition, the animal received a microchip and ear tag for identification.

The treatment group (n = 6) were immunized with 1mL GonaCon, injected intramuscularly into the hamstring muscle group, while the control group (n = 4) received a sham injection.

6.3.3 Evaluation of contraceptive efficacy

Reproductive behavior and social interaction were monitored throughout the study period, initiating 6 months prior to vaccination (December 2016) until the last capture event (August 2019). Observations were conducted from distances varying between 10 to >50 meters by direct observation with binoculars and digital video recording equipment.

6.3.4 Reproductive behavior was classified as:

- Receptiveness toward the male
- Mating
- Isolation from the group for a few days prior, - to a few days post-birth
- Nursing
- Udder (teats) (fig. 6.1) inspection was tested as a potential indicator of pregnancy status and nursing activity. Teats in an adult female, not about to give birth and not nursing, have five to six pairs of teats with a length of ± 10mm. When giving birth and during nursing, the teats become enlarged/swollen, reaching a length of ± 25mm and are visible from a distance.

Udder as birth/nursing indicator

Figure 6.1 - Udder condition as an indicator of reproductive status.

Red arrows: inactive; teats, a) ± 10mm; white arrow: active, prominent teat, b) ± 25mm.
Source: D. Rosenfield, 2018.

6.3.5 Morphometric analysis of the ovaries

At the end of the field study, bilateral gonadectomy was performed on treated and control females. The ovaries were quantified to the nearest 0.5g and dimensions measured to the nearest 1mm using a Vernier caliper.

After biometric measures, the sample tissues were placed for fixation in formalin for a minimum of 3 days.

6.3.6 Histology analysis

Histological samples were prepared following methods described by (Júnior, 2012; Paula, 1999). Morphological changes of the treatment group were evaluated using a microscope and compared with values from tissues collected from the control animals.

Investigation of the ovary included the existence of follicle development, presence of intact mature (Graafian) follicles, identification of atretic follicles and corpora lutea.

Atretic follicles were determined by the presence of degenerating oocytes, and corpora lutea, identified by large pale-staining granulosa lutein cells (WANG et al., 2010; TOWNSON; COMBELLES, 2012; VIDAL; DIXON, 2018).

6.3.7 Use of statistics

Differences between the treatments were evaluated using the two-sample t-test (Welch) considering there was normality of the values (Gaussian distribution) and homogeneity of the variances. The significance level used to reject the H0 (null hypothesis) was 5%, that is, for a level of significance less than 0.05; it was considered that statistical differences occurred between the values for the variables evaluated in this study.

6.4 RESULTS

6.4.1 Bodyweight

The body weight comparison between control and treated females was statistically not significant ($p>0.05$).

Table 6.1 Bodyweight

	***Control** (n = 3)	***Treated** (n = 6)	**p-value**	****Control literature reference**
Body weight	69.6±5.08	66.6±2.40	0.629	55

*Synanthropic females; **none-synanthropic (OJASTI, 1973; MIGLINO et al., 2013)

6.4.2 Adverse effects

Throughout the entire study period, no adverse reaction to the captures or procedures were observed and all females remain in good health. Only the expected injection site reaction of the vaccine administration was present in all treated individuals.

6.4.3 Reproductive behavior

During field observations, none of the immunized females demonstrated male receptiveness. Contrary, all control females should male-receptiveness and mating activity. Using prominent udder condition as an identifier of which female was about to, - or gave birth, was inadequate, as the presence of multiple offspring, and nursing activities were observed in both groups, due to their alloparental conduct.

6.4.4 Morphometric analysis of the ovaries

The ovarian weight difference between control and treated was significant (tab. 6.2; fig 6.3), suggesting ovarian atrophy.

Table 6.2. Ovarian dimensions and weight (values mean ± S.E.M.)

	***Control** (n = 3)	***Treated** (n = 6)	**p-value**	****Control literature reference**
Length (mm)	30.37±0.08	30.91±0.52	0.219	19
Width (mm)	16.92±1.77	16.24±1.22	0.683	15
Weight (g)	3.55±0.20	2.29±0.08	0.002	2.9

*Synanthropic females; **none-synanthropic (OJASTI, 1973; MIGLINO et al., 2013)

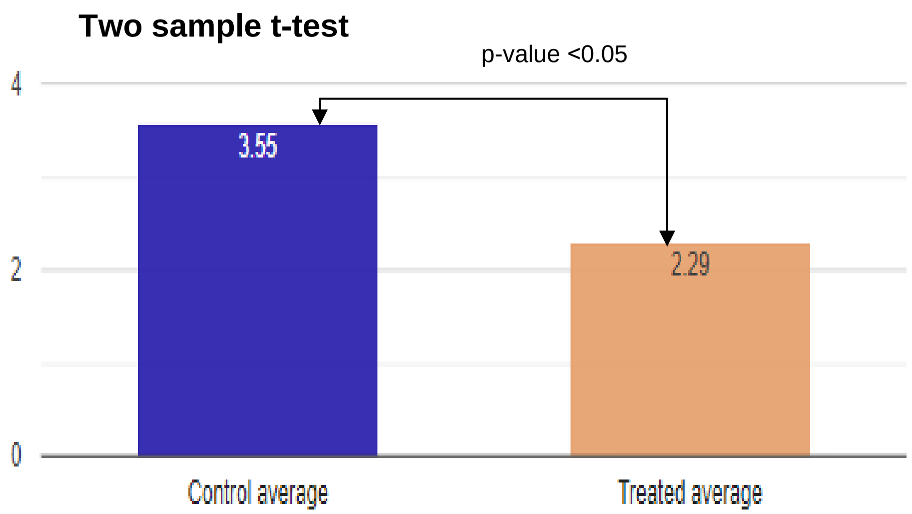

Figure: 6.2 - Ovary weight. Control to treatment comparison

6.4.5 Histopathologic analysis - ovarian parenchyma

6.4.5.1 Control female

Normal folliculogenic development and maturation were observed, demonstrating the presence of primary follicles to antral follicles (fig. 6.4), without notable pathological alterations. Additionally, Atretic follicles and corpora lutea were identified.

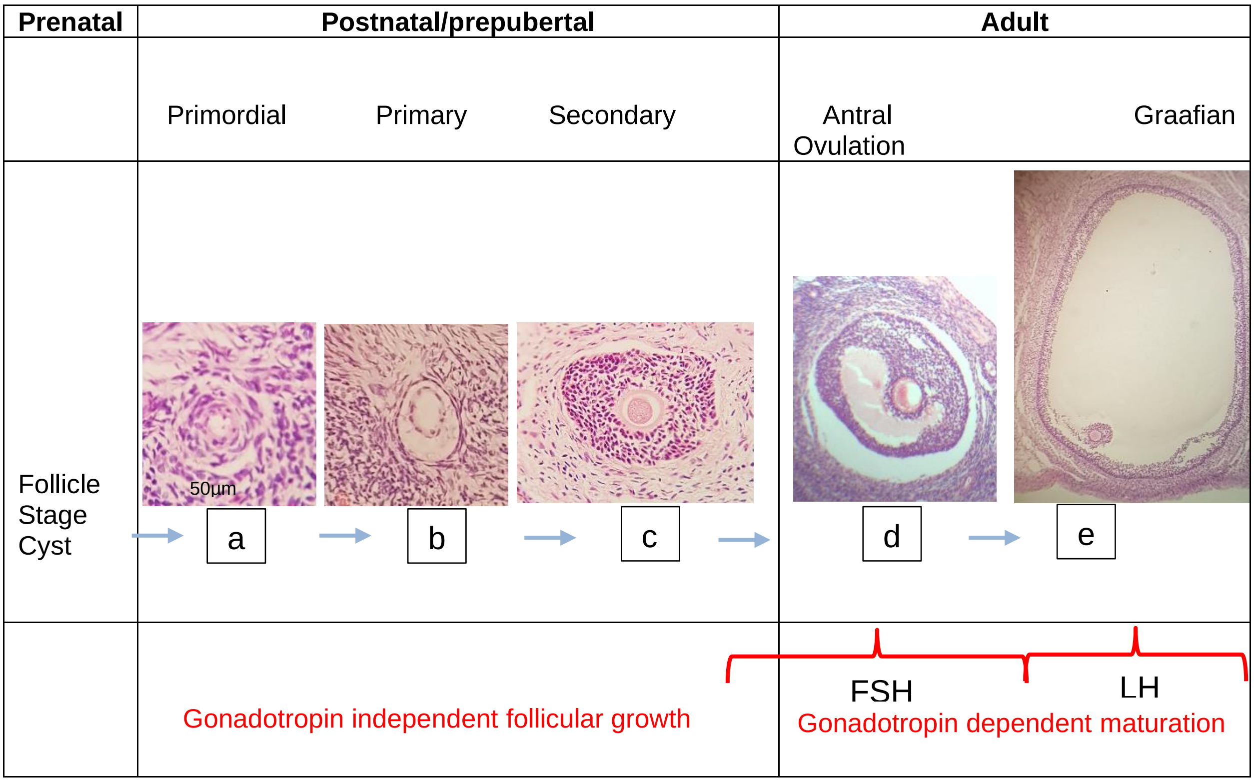

Figure 6.3 - Folliculogenesis

Depicting normal follicles from control females (n=3) in various developmental stages: a) primordial; b) primary; c) secondary; d) pre-antral; e) antral/Graafian.
Source: D. Rosenfield

Control Female

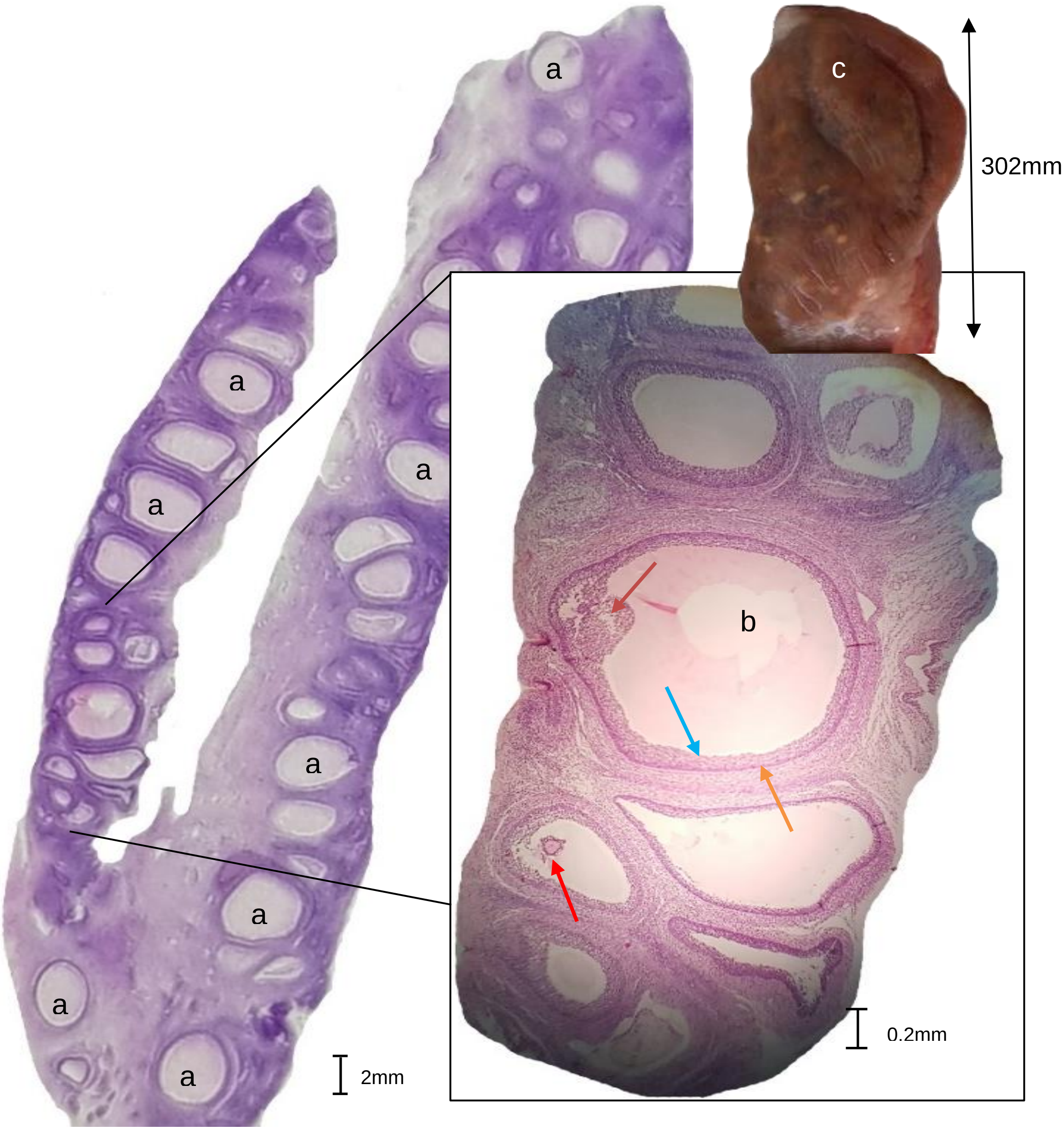

Figure 6.4 - Ovarian histology of a control female.

Longitudinal cut, hematoxylin and eosin staining. Depicting **a)** large number of antral follicles; **b)** magnified section, showing a tertiary follicle with well-defined follicular antrum; Red arrow indicating developing antral follicle with oocyte and visible corona radiata. Blue arrow: granulosa cells; green arrow: theca cells, and orange arrow: remanence of granulose cells, forming the cumulus oophorous and corona radiata, w/o oocytes in this section. **c)** elongated oval-shaped ovary with protrusions and an uneven surface. Source: D. Rosenfield

6.4.5.2 GonaCon treated females

Figures 6, 7, and 8 displaying ovaries of three different GonaCon treated females, with various degrees of folliculogenesis.

GonaCon treated female

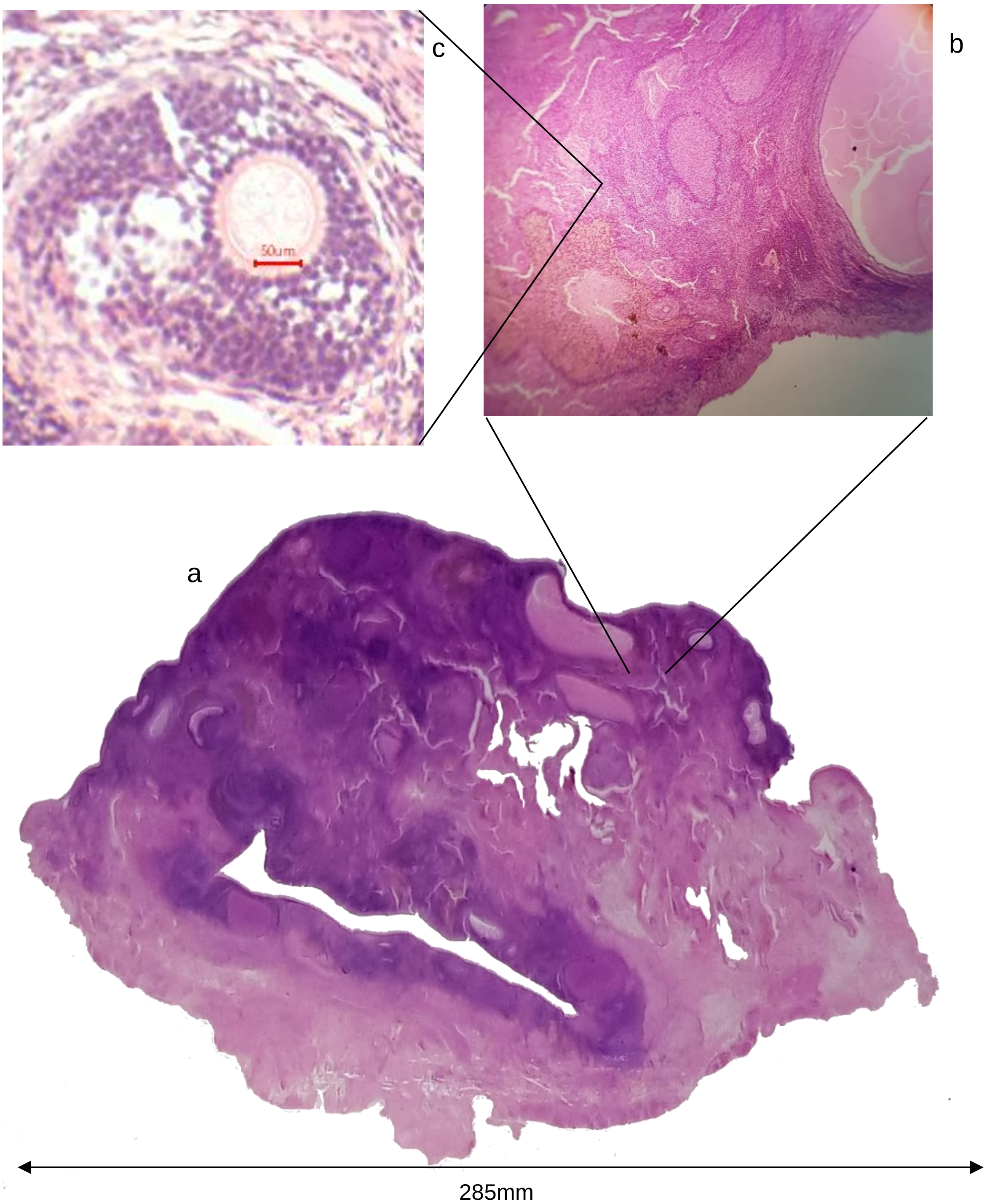

Figure 6.5 - a) Ovary of a treated female.

b) parenchyma, depicting a very low number of partial mature antral follicle and corpus luteum. c) secondary follicle with disorganized zona granulosa cells.
Source: D. Rosenfield

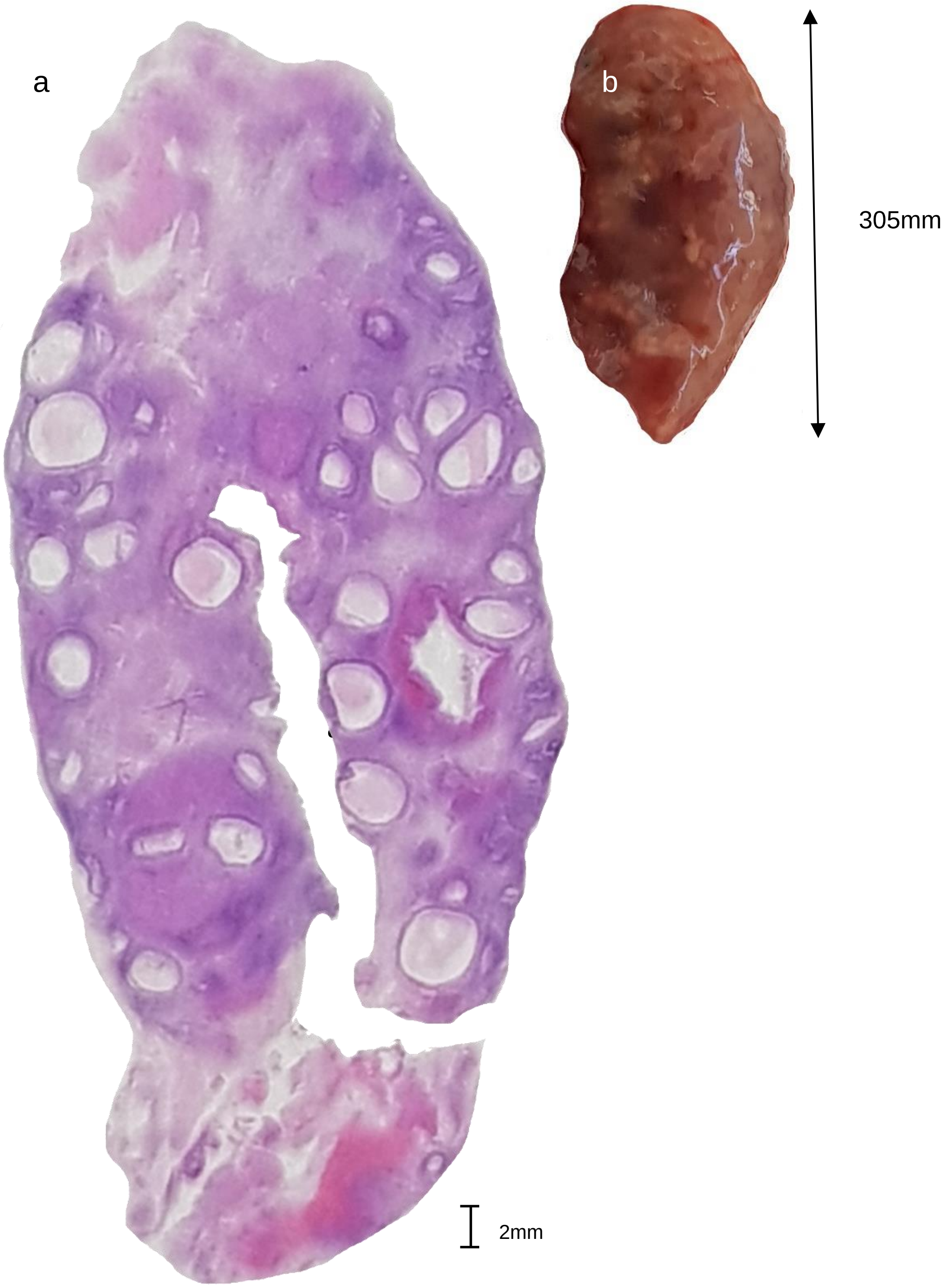

Figure 6.6 - a) ovarian parenchyma of a GonaCon treated *Hydrochoerus hydrochaeris*

Longitudinal cut; hematoxylin-eosin staining; including a) several antral follicles, b) ovary with smooth surface and no protrusions.
Source: D. Rosenfield

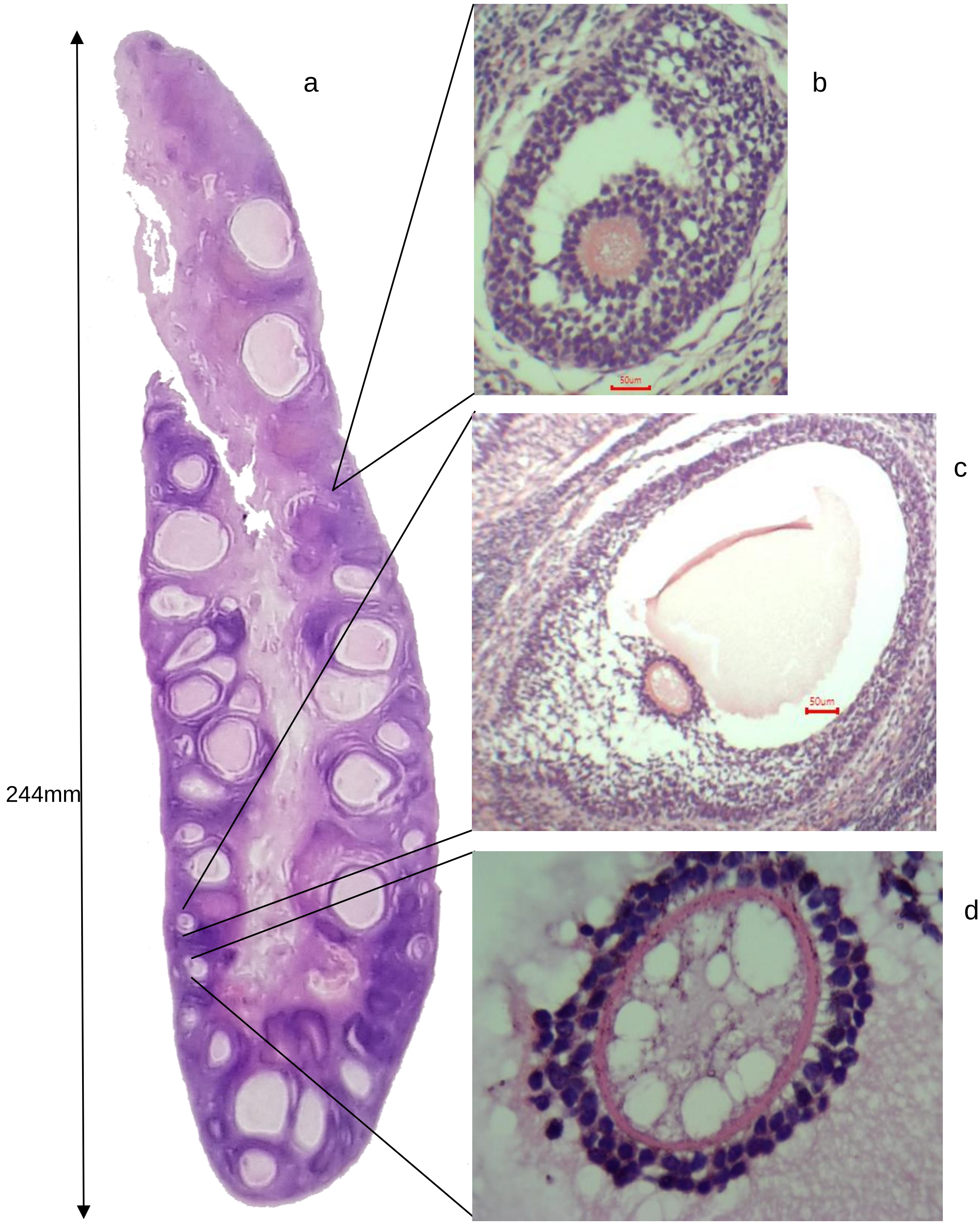

Figure 6.7 - a) Ovary of a treated female with several visible antral follicles

b) pre-antral follicle (x100), with disorganized zona granulosa with formed cumulus oophorous; c) antral follicle with present oocyte with visible inclusions within the cytoplasm, disintegrating granulosa membrane; d) oocyte with cytoplasmic dysmorphism/abnormalities. Visible corona radiata, intact zona pellucida (x400). Source: D. Rosenfield

6.5 DISCUSSION

Gonacon has been chosen for its desirable characteristics and high effectiveness as a contraceptive strategy in both sexes for wildlife population control, as proven in several studies with numerous species, including *H. hydrochaeris* males (MASSEI et al., 2008, 2015; MILLER et al., 2008b; YODER; MILLER, 2010; COWAN et al., 2011, 2019; LEVY et al., 2011; SNAPE; HINDS; MILLER, 2011; KRAUSE et al., 2014; POWERS et al., 2014; SOMGIRD et al., 2016; WIMPENNY; HINDS, 2018; YOUNG, 2018; ROSENFIELD et al., 2019).

Interpreting the data from direct observations collected over the study period, the results showed no GonaCon treated female demonstrated receptiveness toward the male, likewise, the male did not approach the treated female, and no mating activities were witnessed.

The initially considered use of udder investigation as a reproductive indicator was later dismissed as impractical due to the alloparental nature of capybara (NOGUEIRA et al., 2000). Observations confirm this conduct, including by treated females (fig. 6.9), where pups were taken on and nursed by any of the group's female members.

Figure 6.8 - Alloparental Conduct.

Red arrow: Ear tag of a GonaCon treated female with pups from another female member. D. Rosenfield, 2019

Folliculogenesis is a complex hormonally driven process. In regard to the involvement of gonadotropins, folliculogenesis can be divided into two principal stages, a) development of preantral follicles, which is gonadotropin independent, and b) development of antral follicles, which does require gonadotropin, in order to support growth. Specifically, FSH is needed for antral follicle survival and germ cell proliferation, luteinizing hormone receptor expression, and the synthesis of estradiol. LH is important for signaling-pathways, steroidogenesis control, and ovulation (NITZAN et al., 2016; VIDAL; DIXON, 2018).

Studying the control female's folliculogenesis, follicles of all stages were present, including corpus luteum and atretic follicles (fig. 6.4). The ovarian parenchyma presented the largest number of maturing antral follicles. Tertiary follicles showed a well-defined follicular antrum with oocyte presence and visible corona radiata. The granulosa membrane was intact with evenly distributed and thick follicular cells, bordering the inner layer of theca cells. The atresia occurrence of matured antral follicles, displaying disintegrating granulose membrane with remanence of the cumulus oophorous and corona radiata, w/o oocytes in these sections, are considered a normal occurrence (WANG et al., 2010; TOWNSON; COMBELLES, 2012).

The ovarian parenchyma of three different treated females (fig. 6.6, 6.7, 6.8) displayed mature antral follicles, although, to a much lesser degree than control. Specifically, the ovary in figure # 3, exhibits a severe lack of antral follicles (n = 2), similar to observations made by (SEEKALLU et al., 2010), reporting a complete cessation of waves, testing a similar immunocontraceptive.

Contrary to studies, like (POWERS et al., 2014), deducting the presence of larger numbers of small follicle recruitment, attributable to an inhibition of negative feedback from dominant follicles, as well as early regression caused by the lack of gonadotropins, a persistent development of antral follicles was observed.

In control and treated females, luteal tissue was present, differing again with reports of other studies (POWERS et al., 2014).

Observation of abnormalities in the form of atretic follicles and oocytes, displaying disintegrating granulosa membranes and corona radiata, and/or dysmorphic oocytes, showing cytoplasmic abnormalities in the form of vacuole-like structures (fig. 6.4 d) in GonaCon treated females were somewhat abundant. However, at this time, it can't be concluded if there was a higher incidence due to the contraceptive treatment.

Because of the blockage of gonadotropin synthesis and liberation, it can be hypothesized, that very little to no mature antral follicle should develop behind the stage of preantral. Subsequently, lacking an LH surge, no ovulation should occur, and consequently, no fresh parenchymal hemorrhage, formed corpus luteum, or albicans should exist. Findings, however, comparing histologically control to treated females, in both, folliculogenic activities can be observed, from primary follicles to maturing/antral (Graafian) follicles. According to the work by (HALPIN; CHARLTON; FADDY, 1986), one possible explanation for the continuous development of antral follicles might be due to intra-ovarian autoregulatory mechanism, independent of gonadotropin's availability, ensuring a constant supply of antral follicles. As reported in several studies on human folliculogenesis, atretic (degenerated) follicles and oocytes, with or without visible anomalies are indicator for severe intrinsic adverse effects during oogenesis and maturation (WANG et al., 2010; FANCSOVITS et al., 2011; TOWNSON; COMBELLES, 2012), demonstrating that a dysmorphic oocyte has a minimal chance of normal development.

Furthermore, because of the capybara alloparental behavior, considering udder alterations, due to nursing pups, as an indicator of birth is not a recommended method.

Interestingly, contradictory results were observed when comparing different studies that investigated the use of GonaCon in females, showing a variation in outcomes, with some being effective in provoking the formation of high antibody titers and contraceptive effect, while others, even with higher dosages and booster applications were less successful (OKON; LIVNI; KOCH, 1980; MILLER; TALWAR; KILLIAN, 2006; GIONFRIDDO et al., 2008; POWERS et al., 2011; DONOVAN et al., 2013; D'OCCHIO, 2013), showing a clear species-depended response (success).

Other contradictory findings exist when comparing reports on the behavioral impacts of immunocontraceptive treatments. On one side, intending to minimize unwanted aggression and estrus behavior, as frequently used in livestock creation or in elephants to control aggression in bulls, or minimize the undesired odor in pork meat (SOMGIRD; BROWN; THITARAM; CURTIS et al., 2008), on the other side, confirming the preservation of agonistic behavior in capybara alpha males in order to maintain the group's social integrity (ROSENFIELD; PIZZUTTO, 2019).

Nevertheless, observation of ovarian atrophy by weight (p-0,0061), a lower number of antral follicles in treated females, the presence of follicular atresia and oocyte dysmorphism and other abnormalities, lack of receptiveness toward the male and mating, are suggestive of an immunocontraceptive effect.

6.6 CONCLUSION

To date, the study's results suggest that GonaCon might be effective as a contraceptive agent in female capybara. Although, the principal evidence is based on direct observations and to a lesser degree in morpho,- and histopathological assessments, no mating, and no birth were observed in treated females. Nevertheless, to determine the exact contraceptive mechanisms in female gonads involved, further research is needed.

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7. POSITIVE-REINFORCEMENT STRATEGIES TO REDUCE CAPTURE-STRESS IN CAPYBARAS

7.1 ABSTRACT

Prior to the administration of any anesthetics, capturing the semi-aquatic capybara (*Hydrochoerus hydrochaeris*) o demands physical restraint, which presents immense challenges. Traditional methods, such as lassoing and traps, are prone to induce acute stress, may result in injury, and can even have fatal outcomes. As part of a larger population control project using contraceptive methods, frequent capture-induced stress and injury may directly affect normal reproductive physiology. Thus, choosing a less stress-inducing method was imperative. In this report, we describe methods of conditioning to enable frequent capture and manipulation, using bait as a positive reinforcement associated with a special click-sound, in a free-ranging population of 40 capybaras. The objectives were to attract, herd, and allow capybaras to voluntarily enter a coral. We evaluated the conditioning effect on individual and group behaviors, interpreting vocal and body language manifestations during the processes of conditioning, herding, capture, and recovery (post procedure/chemical restraint), with the aim of minimizing capture-related stress and injuries. Based on our observations, we report that conditioning, used as part of the capture strategies, noticeably facilitated physical restraint and manipulation throughout the procedures, while apparently maintaining the animals' overall welfare as it relates to conditioning and capture.

KEY WORDS: Animal welfare, baiting, conditioning, *Hydrochoerus hydrochaeris*, physical restraint, learning.

7.2 RESUMO

Antes da administração de quaisquer anestésicos, a captura da capivara, (*Hydrochoerus hydrochaeris*) animal semiaquático, requer confinamento físico, o que apresenta imensos desafios. Métodos tradicionais, como laço e armadilhas, têm a tendência de induzir estresse agudo, podendo resultar em ferimentos, ou até mesmo, algo fatal. Como parte de um projeto mais amplo de controle populacional usando métodos contraceptivos, ferimentos e o estresse induzido por frequentes capturas podem afetar diretamente a fisiologia reprodutiva normal. Assim, usar um método que provocasse menos estresse era imperativo. Neste relato, descrevemos um método de condicionamento para possibilitar capturas e manejo frequentes, usando ceva como reforço positivo associado ao som de um clique diferenciado, numa população de 40 capivaras de vida livre. Os objetivos eram atrair, pastorear e permitir que os animais entrassem voluntariamente no brete. Avaliamos o efeito condicionante em comportamentos individuais e em grupo, interpretando manifestações de linguagem vocal e corporal durante os processos de condicionamento, pastoreio, captura e recuperação (pós-procedimento contenção química), com o objetivo de minimizar stress e ferimentos relacionados à captura. Baseado nas nossas observações, reportamos que o condicionamento, usado como parte de estratégias de captura, notadamente facilita a contenção física e o manejo durante os procedimentos, além de contribuir, de uma forma geral, no bem-estar durante todo o processo da captura dos animais.

Palavras-chaves: Bem-estar animal, ceva, condicionamento, *Hydrochoerus hydrochaeris*, contenção física, comportamento

7.3 INTRODUCTION

Capturing wildlife species for research is a frequent necessity and is often accompanied by great challenges; as is the case with capybaras (*Hydrochoerus hydrochaeris*). The capybara is the world's largest rodent and native to South America. It has an average adult weight of 40kg, and in its natural habitat may live in groups of 5–20 animals (Alho & Rondon, 1987). This is different to synanthropic capybaras (fig. 7.2), which re-inhabit either agricultural or urban areas and have body weights that can reach over 100 kg. In addition, because of their high proliferative nature, groups can quickly reach large numbers, with over 100 members in some cases (M. Labruna, 2018). This is largely due to a lack of natural predators, the abundance of food, resistance to anthropogenic activities, and their ability to quickly adapt to urban dynamics (Marchini & Crawshaw, 2015).

One of the main reasons why studies on capybaras have increased is because of their potential threat to human health, being an amplifying host of *Rickettsia rickettsii*, the etiological agent of Brazilian Spotted Fever, a lethal tick-borne disease (Fiol, Junqueira, Rocha, Toledo, & Barberato Filho, 2010; Labruna, 2013). Thus, finding the most adequate method of population control concurrent with collecting material for epidemiological surveillance, has become a top priority (Abreu Bovo, Ferraz, Verdade, & Moreira, 2016; Felix et al., 2014; Labruna, 2013; Celso E. Souza, 2004). Controlling capybara populations is limited to non-lethal methods, pertaining to the native fauna, they are protected by law from hunting, trafficking, and abuse (Federal Law, Brazilian Fauna Protection Act, 1967).

Capybaras live in social groups composed of one dominant male (alpha), subordinate males, adult females, juveniles, and infants. Sexually mature males, expelled from the group by the dominant male, so-called satellite males, are commonly observed in close proximity to the group (fig. 7.1). The presence of these satellite males may lead to group division when females abandon their group (Macdonald, 1981), or they may simply assume leadership if the alpha male is injured, ill, or dies.

They are a semi-aquatic prey species for large felines, alligators, and anacondas. Besides using water for hydration, thermo-regulation, copulation, and defecation, it serves as the principal getaway in case of a predator attack (José Roberto Moreira, Ferraz, Herrera, & Macdonald, 2013). Capybaras are quite fast and agile animals; however, their stamina is short-lasting, which is why they stay in close proximity to water (fig. 7.2). As such, capturing capybaras, without putting their lives at risk, is notoriously difficult.

Figure 7.1 Satellite male.

Raia Olimpica, Campus University City, University of Sao Paulo, Brazil; Green arrow, pointing to a satellite male, close to the water, identified by secondary sexual characteristics of an adult male, solitary position, and through prolonged observation. ; The white arrow points to a group of about 30 members, crossing the water.
Source: D. Rosenfield, 2017

The literature describing detailed capture methods for capybaras is limited and the few studies that are available are not suitable for an English-speaking audience. However, capture methods can be divided into three groups: (1) lassoing, a traditional method commonly employed by Argentinian cowboys (Salas, Pannier, Galíndez-Silva, Gols-Ripoll, & Herrera, 2004); (2) baited heavy metal traps; and (3) corrals with bait, as described in several research projects (Kuniy et al., 2018; José Roberto Moreira et al., 2013; Celso Eduardo de Souza et al., 2008). The first two methods can potentially provoke intense stress and have an increased risk of injury or death and would make recapture almost impossible. Logistics are a great challenge when frequent capture is necessary, particularly for semi-aquatic capybaras that require prior physical confinement, as any anesthetized animal would almost certainly flee into the water, if not physically restrained, and inevitably drown after the onset of the anesthetic drug.

Furthermore, it is important to use capture methods that allow the capture outcome to be more controllable (targeting individuals or larger number of members, time independent), rather than relying on random capture using baited traps.

As part of a greater research project on population control using immunocontraception in free-ranging capybaras (Sao Paulo, State, Southeastern Brazil), we investigated different capture methods that would allow for frequent selective capture, throughout the year. Capybaras have a keen olfactory system when it comes to detecting food, especially sugary plants. Observing their attraction to specific foods, curiosity, and gregarious social structure, we recognized the potential for attracting, and even training, capybaras using methods based on classical and operant conditioning, reasoning that it would aid our overall project and allow for frequent physical restraint but minimize capture-related stress and injuries.

Adhering to wildlife conditioning and enrichment techniques (Pizzutto, Scarpelli, Rossi, Chiozzotto, & Leschonski, 2013), we aimed to describe the strategies used during this study period to attract and herd free-ranging capybaras into confinement and assess the classical and operant conditioning paradigms as a viable aid for capturing capybaras. Moreover, considering the research objectives of the principal project, evaluating contraceptive effects, low-stress captures might have the least impact on the animal's hemostasis, especially on their reproductive physiology. Other positive aspects of concern include the need for smaller tranquilizer dosages, smoother procedures, quicker recovery, and more importantly, the ability to make frequent recaptures possible and safer.

7.4 MATERIALS & METHODS

7.4.1 Study area

The study was conducted year-round, during wet and dry seasons (December 2016 to January 2019) at the "Raia Olimpica", a large man-made pool (fig. 7.2 b) on the campus of University City, University of Sao Paulo, Brazil. 23º33'21S 46º43'14W, altitude 722 m (WGS84 EGM96 Geoid. Google Earth Satellite Imagery, 2019). The pool area is approximately 247 500 m2, surrounded by trees and extended grass areas, in the midst of one of the world's largest megalopolises (fig. 7.2). The Pinheiros river, the principal corridor for capybara migration, one of two rivers partially surrounding the City, is separated by an intercity highway. The city of Sao Paulo lies within the Atlantic Forest domains of the Sao Paulo State. Peak temperatures in the summer months (December - March) reach 30ºC, with an average temperature of 25ºC and median rainfall of 170 mm. In the winter (June - September), temperatures can fall to below 12ºC, with an average temperature of 19ºC and median rainfall of ≤ 50 mm (World Meteorological Organization, 2019).

7.4.2 Animals

In 2013, free-living capybaras invaded the pool area from the nearby Pinheiros river through a constructional breach in the water canalization system. A total number of two adults and five pups were reported to have entered, which grew to 40 animals within a four-year period then divided into two major groups and several satellite males (status at end of 2016).

Figure 7.1 - Project location.

Google map adopted; a) South America; b) Metropolitan area of Sao Paulo ; c) Bird view: Raia Olimpica, a man-made water pool for aquatic sports; d) Group of synanthropic capybaras, close to the water. Source: D. Rosenfield, 2019

Group I: 1 dominant male (original alpha male), 14 adults (including the original adult female), and 15 juveniles.

Group II: 1 dominant male (a direct descendant of the original alpha male and female). After growing into a sexually mature male, he was expulsed by the alpha male, together with a second mature male (likely from the same litter). Over time, five adult females from group I joined the two males, forming group II.

Satellite males: distributed throughout the pool area; three sexually mature males that were driven out by the group I alpha male.

The polygynous society of capybaras represents a ridged linear hierarchy, where the alpha male agonistically protects his breeding rights. Nevertheless, subordinate females have been observed to leave the main group to reunite with satellite males, either temporarily or to form new groups. Likewise, subordinate males opportunistically attempt to mate with females from established groups (Herrera & Macdonald, 1993; José Roberto Moreira et al., 2013).

7.4.3 The corral

For group confinement, we constructed a budget-friendly flexible corral (fig. 7.3 a) using a scaffold frame (ea. element L: 150 cm x H: 100 cm). The leg extensions were forced into the ground and connected to one another using a metal wire (gauge 14). A continuous metal fence was then installed from the inside (1.5 m x 45 m in circumference, ± 127 m^2), fixed to each scaffold frame using the same metal wire. The top portion of the fence was curved inward and maintained in position by tightening the upper portion of the fence every three to four meters with metal wire. The aim of the fence's flexibility was to impede any push-throughs or possible jump or scaling escapes. Capture strategies for individual capybaras, namely satellite males, were twofold: (a) employ classical conditioning methods to lure the male into the corral, or (b) alternatively, use specially-constructed single-animal traps.

7.4.4 Cage-traps

A single piece of 2 m x 3 m (8 mm) steel net, with a 15 x 15 cm mesh, was folded to create a continuous U-shaped cage. The cage was placed over a pre-mounted steel frame and welded to all contact points. The structure was reinforced with steel rods. The U-shaped frame served as a track for the removable wood-plates, making the cage light enough to facilitate transport. The wooden front and back doors measured 100 cm x 115 cm x 1.2 cm, and the wooden floor measured 100 cm x 195 cm x 1.2 cm. The wood was made from a waterproof medium-density fiberboard (MDF) for external use. In addition, a wheel support was mounted onto each corner so that it could be rolled away. On location, these wheels were buried into the ground, assisting in keeping the cage in place. The trapping mechanism was based on two possible guillotine-door trigger concepts: by weight, pushing the floor down when entering, or by bait contact.

During habituation of the capybara satellite males to the traps, both sides of the doors remained open. Holes were placed on only one side of the wooden floor to allow the sugarcane to be placed upright (fig. 7.3 b). When it was determined, via observation, that the animal was comfortable enough to enter the cage and feed, the cage was set for capture. The back-side was closed, the front door remained in the open position, the bait was set, and a simple nylon string was attached directly to the bait or to the floor to trigger the guillotine-door to slide shut (fig. 7.3 c). The track also contained foam-cushion to minimize the noise of the door closing.

Figure 7.2 - Corral & cage-traps.

a) flexible corral design, b) single-trap, with removed doors for habilitation, c), depicting trapped capybara male (satellite). Source: D. Rosenfield

7.4.5 Behavioral observations

Thirty days prior to the first conditioning attempt (May 2017), behavioral observations were conducted *ad libitum*, following descriptions by Altmann (1974), for three-hour periods during the day (either 07:00–10:00 or 14:00–17:00) or during the night (19:00–22:00). Observations were carried out at distances 1–100 meters by direct observation or using visual magnifying aids and recording devices. The observations were conducted by a single investigator, studying the overall group dynamics; individual conduct; sleeping, resting, and eating periods; social interactions; and breeding behavior. The collected data served as a behavioral reference for observations after the contraceptive treatment.

7.4.6 Vocalization and body language as a stress indicator

To evaluate the success of our strategies in reducing capture and handling associated stress, we focused on direct behavioral observations (Aguiar & Moro-Rios, 2009; B.F. Skinner, 1938), such as: conduct, body language, and vocalization. Capybaras have a broad spectrum of vocal communication (Barros, Tokumaru, Pedroza, & Nogueira, 2011; Sánchez, 2003). Interpretations were done by one observer and were based on vocal (V) and body language (BL) expressions for an individual's, and group's emotional state, discriminating between three principal states: (1) general well-being, curiosity, and positive excitement (V: high-pitched purring-clicking, often contagious to the rest of the group. BL: inquiring, probing, sniffing closing-in); (2) distress, fear, and irritation (V: squeaking, whistle, bark, teeth-chatter. BL: neck-hair stands up, muscle tremors, urinating, fleeing); (3) agonistic behavior (V: teeth chattering, bark. BL: neck-hair stands up, sham-attacks, real attacks, biting). We created several benchmarks for the purpose of measuring the progress of classical conditioning and behavioral training.

7.4.7 Classical conditioning

Initially, we used the concept of classical conditioning (or Pavlovian conditioning), taking advantage of unconditional stimuli (food) that was natural to capybaras, specifically sugarcane. Once attracted by the smell and voluntarily approaching the food source, referred to as the unconditional response (not based on behavior), we accompanied each food offering with click-like oral sound. This subsequently created a classical conditioning scenario by combining the unconditional response with a conditioned stimulus, which over a short period of time, turned into a conditioned response, providing the fundamental principles to expand the strategy to operant conditioning (or, Skinner Principle).

Conditioning goals: habituation to humans; voluntary approximation, hand-feeding; habituation to corrals/traps; entering corrals/traps voluntarily (bait present); herding/entering corrals; and remaining calm during recovery. For each goal, the number/time of sessions needed for the first-time achievement was recorded.

Click-sound: starting with the first training session, a click-sound was used to achieve a conditional stimulus.

Positive experience: independent of scheduled training events, food was also placed in the corrals/traps to reinforce the association of the location with a pleasant experience.

Conditioning Sessions: fourteen days prior to the first capture event (June 2016), we initiated the conditioning process. Sessions lasted 30–60 minutes, three times a week, with continued behavioral observations conducted *ad libitum*.

7.4.8 Food for training reward/baiting

Capybaras are herbivorous, and although specific grass and aquatic plants are their staple food, they do have a great desire for sugary plants, especially sugarcane (lat. *Saccharum officinarum*) (Borges & Colares, 2007; De Barros Ferraz, 2007; Felix et al., 2014). Sugarcane was cut into approximately 2-foot-long pieces, and either hand-fed to the capybaras (fig. 7.4) or placed upright into the ground to attract the animals, which works much more effectively than leaving stalks uncut and flat on the floor (pers. obs., 2017).

7.4.9 Calling/herding

Starting with the first contact, one observer emitted a click-sound to accompany each event, when approaching the animals or animals approached the observer, during food presentation/hand-feeding during herding (fig. 7.4 a) and entering the corral (fig. 7 4 b), with emphasis before and during food offerings. The click-sound was not used if food was not immediately associable, which was to avoid the negative effect of “conditioning extinction”, thus, possibly undoing the training attempts.

7.4.10 Positive Reinforcements

Aside from direct training attempts, to eventually achieve an operant conditioning effect, efforts to complement the positive experiences with the event location were made. Food was placed periodically and independently from events, into the corral and traps, reinforcing their voluntarily entrance and pleasant experience during procedures and recovery (fig. 7.4 c–e).

7.4.11 Chemical Restraint

After the individual or group confinement, the target animal was anesthetized with a combination of ketamine and dexmedetomidine (9 mg/kg, Syntec, Brazil, and 5 µg/kg, Zoetis, Brazil, respectively) which was administered intramuscularly into the hind leg musculature, using specialized darts and a CO_2 projector (X-Caliber, Pneu-Dart, Inc., Williamsport, Pa.).

7.4.12 Clinical Procedures

After sedation, the animals were brought to our field clinic for biometric examination (weight, size, gender), ID marking, and health status evaluation. During different events, the procedures performed included administration of an immunocontraceptive, collection of semen, and in the final stage of the project, hemigonadectomy of the treated and control animals. After each procedure, the animal was returned to the corral, until fully recovered.

Figure 7.3 - Conditioning/Training/Reinforcement with sugarcane.

a) herding b) corral entry c) corral positive experience/reinforcement d) darted animal, while other members close by w/o agitation or agonistic behavior e) return of an treated animal, with members relocating towards the end of the corral, expressing no irritation or panic, and members outside the corral calmly grassing. (red arrows – sugar cane; yellow arrow – tranquilizer dart). Source: D. Rosenfield, 2017

This study was approved by the Brazilian Ministry of Environment (SISBio, 2016); University of Sao Paulo's Ethics Committee for Use of Animals in Research (CEUAVET, 2016).

7.5 RESULTS & DISCUSSION

In this work, our was to describe the consequences of developed capybara capture strategies that would permit frequent capture, without provoking severe discomfort to the animal in the form of stress or injury. Comparing this work with other literature is difficult because references relating to capybara capture methods are limited. One study describes a capture method using lassoing (Salas et al., 2004), which is the most dramatic form of live-capture besides culling and perhaps netting. With lassoing, three capture-dynamics are required: (1) the chase, (2) the actual capture by lasso, and (3) tying the legs. Although, within the report, capture-stress and risk of injury were not referred to, these actions must cause tremendous stress and a certain degree of injuries to the animals. However, considering the capture location and environment of Salas's study, this strategy was a necessary tool to capture target individuals. Several studies have been published in Portuguese that do mention corrals and baiting, even stating that it is the most appropriate method for capybara. They also provide some explanation of certain conditioning methods, types of bait used, and corral design, but do not elaborate on capture related animal-welfare, except death due to capture stress (K. M. P. M. B. Ferraz & Verdade, 2001; Rodrigues et al., 2017), One publication does describe, in length, corral design, trapping methods, efficiency, and types of bait, but does not mention anything about capture-related behavior and stress (K. Ferraz, Santos-Filho, Piffer, & Verdade, 2001). Several capybara-captures that used small-spaced, heavy-duty, closed-in, and steel-reinforced structures, with guillotine trap-doors, provoked capture-stress and panic behavior, causing injuries to the heads and legs of animals.

Depending on the country and region, hunting-pressure can be considered an influential factor on behavioral and habitat changes, thereby influencing capture outcomes (J. R. Moreira, Pinha, & Cunha, 2001; José Roberto Moreira, 2013; Verdade, 1996); however, within this specific area and study, this aspect is not relevant.

For the purpose of developing an alternative capture method, we initially employed the concept of classical conditioning (Grant, 1964; Owen & Amory, 2011) in association with specifically designed equipment. While assessing the theories of classical and operant conditioning, our goals were to attract and herd free-ranging capybaras into confinement, making efforts to provide a positive experience, and site of pleasure, for the animals when voluntarily entering the corral, and during capture events, using sugarcane as the main bait and positive reinforcer. A further important aspect, considering the objectives of the principal research project on contraceptive effects, was the quality of the study results, influenced by low-stress and injury-free captures. We hypothesized that the capture strategies that we developed might have the least impact on the animals' hemostasis, especially on their reproductive endocrinology. In turn, this allowed for a more accurate comparison of the reproductive physiology under normal conditions compared with conditions under the influence of the contraceptive treatment. Other theoretical facets include the need for smaller tranquilizer dosages, smoother procedures, and quicker recovery.

We used two physical restraining models: (1) the corral design, which served as the principal, and preferred, method of capture, as this represents the method with the lowest impact on the animals; and (2) the single trap. This method was thought of as an alternative tool, to be used if target animals could not be conditioned, or captured within the corral, or were too distant from the corral, so herding would not be feasible.

7.5.1 Corral Design

The large flex-corral withstood any escape attempt by the animals, usually achieved by probing the fence for weak spots (pushing the head into the fence at different locations). No scaling or jumping was observed. The fence's flexibility and the use of metal wire circumvented any breakage of the structure and prevented injuries to the animals. The corral's large oval shape allowed the trapped animals to relocate to different parts when humans entered to retrieve or return a sedated animal. We believe that this key design feature contributed to avoiding panic by providing enough 'safety-distance". The prior habituation efforts, in conjunction with the "pleasure" experience of finding reinforcing food in the corral and traps when entered voluntarily, contributed toward the calm capture, which was maintained throughout the duration of the animals' captivity.

7.5.2 Single-trap

After several cage-building trials, the final concept produced a cage/trap with a light-structure-design, allowing for easy relocation, as site adjustments were frequent. Although lightweight (33 kg), its structure was resistant enough to contain the trapped animal for prolonged periods of time. The trapping mechanism used was efficient in automatically trapping the animal.

7.5.3 Conditioned responses and behavioral targets

The first crucial task was to habituate the groups to our presence and for the animals to associate us and the click-sound with food. Surprisingly, very little time was needed to achieve this. The first session started with an initial distance of approximately 50 meters, slowly moving toward the group while using the click-sound for the first time. Then the investigator stopped and remained in a position approximately 20 meters away. The mood status of the animals was observed to be: (V) some animals barked; (BL) some animals got up, some were cautious or perhaps fearful, most of the group members turned and faced the direction of the water, and some juveniles dived into the water. Time invested at the location was approximately 20 minutes.

7.5.4 Voluntary approximation, hand-feeding

The second session, carried out on the following day, started from 50 meters, The mood status of the animals was observed: (V), an initial two or three barks. The investigator approximation was done in intervals of 5 meters, using a click-sound, until they remained approximately 10 meters from the first members of the group. Sugarcane was forced upright into the ground, while also holding two pieces in the hand. The food was positioned up-wind to aid in the olfactory detection of the sugarcane. (BL), the animals closest to the observer got up into a sitting position, while the other animals remained as they were. Within five minutes, two individuals started to move toward the bait and carefully started to feed. Within a further three minutes, most members had gotten up and walked toward the sugarcane. The original members turned toward the investigator, hesitantly and cautiously starting to accept the food by hand. (V), animals made the singing-like high-pitched purring-clicking sound that is associated with positive excitement. Throughout the entire session, the investigator emitted the click-sound intermittently. The goal of accepting human presence and hand-feeding was achieved in only two sessions and a total time of $\leq$ 50 minutes was invested.

7.5.6 Habituation /entering the corral/trap

During the second session, the corral and trap were prepared with several pieces of sugarcane (fig. 7.4 c). Some was placed at the entrance and some was distributed throughout the corral/trap. During the next visit on the following day, the sugarcane had been consumed, and there was evidence of capybara having visited, in the form of feces.

7.5.7 Herding attempts

Session three occurred two days after session two at approximately 09:00 and was the first capture event for procedures. The same investigator and two additional staff members, with sugarcane in hand, got close to the group. The distance between the group and the corral was approximately 220 meters. While closing in, using the click-sound, the investigators stopped at a distance of approximately10 meters from the group. Within 2 minutes, the first members (apparently the same individuals from session two) came straight toward the food offerings. (V), on their way, they were observed to be emitting the purring-sound, and shortly after, three more members followed. The investigators used alternating “stop” and “go” to lure the capybaras toward the corral, using the bait as a tease and periodically allowing the animals to chew for a few seconds on the sugarcane, until the corral was reached (fig. 7.4 a). Half-way through, some members started to stay behind, but they eventually reunited with the group. As the capybaras dictated the progress rhythm, the walk was cumbersome and excruciatingly slow; although, the goal of herding the animals over a lengthy distance was achieved.

7.5.8 Taking animals into the corral

After a 30-minute walk, the five individuals from the herding attempts would (BL) cautiously follow the investigator into the corral (the attraction of the bait seemed to be stronger than the uncertainty). (V), the animals would continue to emit the purring sound. The goal of entering the corral/first capture was achieved in one session, with the total time to capture being ≤ 20 minutes (fig. 7.4 b). We did recognize that the individuals initiating the approach, first to be hand-fed, and first to be herded and enter the corral, were not the dominant male or female (identified by body size and phenotypic characteristics). Another unexpected side effect, after taking the first five members into the corral, was that within the next 20 minutes the entire group, including the dominant adults, had gathered around the corral, trying to get in, with multiple members emitting the purring sound. During this first procedure event, small pieces of sugarcane were given to the members periodically, inside and outside the corral, to maintain their interest and to sustain the overall tranquil atmosphere.

7.5.9 Remaining calm during procedures and recovery

During the anesthetic application and animal removal (fig. 7.4 d) and after procedures, when returning sedated animals for recovery, the other group members relocated to the far-most side of the corral but remained tranquil (fig. 7.4 e). (V), after the initial capture, no further purring-sounds were emitted. (BL), other than some probing at the fence, sitting down, or chewing sugarcane leftovers, no apparent fearful or irritated behaviors were observed, nor were any agonistic behaviors (toward other group members or staff). The goal of animals remaining calm during manipulation and recovery was achieved. The total time of the first event time (time of intervention) was approximately six hours.

After the first event, food was offered by hand-feeding, accompanied by the click-sound biweekly, during 30-minute reinforcement sessions. In addition, the corral was prepared with bait to maintain/reinforce the positive association with the location.

At each consecutive conditioning session, the response to the click-sound was almost immediate. In less than five minutes (a fast response time for capybaras), most of the group members would approach the investigator to receive their food-treats, (V) making the purring-sound for the remainder of the feeding time. (BL), no stress-behavior was observed. Periodically, members and satellite males were observed inside the corrals, even without food present.

Over a period of 18 months, a total of 5 events were executed; each time the group members were successfully called and herded into the corral. Throughout all sessions and events, no agonistic or panic-like behavior was observed, except the occasional superficial attack by dominant animals toward subordinate members for the right to feed first.

7.5.10 Measuring behavior and stress

There are a myriad of techniques available to measure stress, which are mainly endocrinological and monitor stress-associated hormones or their metabolites. In addition, there are many well-defined ethological programs (Copeland, n.d.; DeNicola & Swihart, 1997; Romano et al., 2010). However, for this project, because of logistics, resources available, and the irregular capture of free-ranging capybaras, the observational interpretation of vocalizations, behavior, and body language, was considered the only viable form of measurement. Although aware of potential investigator bias and the risk of subjective interpretation, driven by strong expectations (Tuyttens et al., 2014), we believe that the results are clear and objective enough to avoid any misinterpretation.

All conditioning goals were achieved in a relatively short time, such as habituation to human presence; voluntary approximation, allowing to be hand-fed; habituation to, and voluntarily entering, corrals/traps; and allowing to be herded over distances and taken into the corrals. Equally important, the aims to maintain a calm atmosphere when handling the animals in the corral, removing a sedated animal, or retuning the same animals for anesthetic recovery, with other members present, were all achieved. The conditioning method allowed the investigators to execute the selective capture of target animals at any time when needed, compared with random trapping. As there is no available literature on similar methods or experience with this species, it is not possible to discuss the time investment or quality of the conditioning.

Capybaras are curious animals and, when there is no threat present, they are quite calm and approachable, especially if food is involved. How quickly they respond to offered food depends on the individual animal’s character and experience, as well as the transient group dynamics. As soon as one member breaks the contact barrier, the rest will follow shortly, suggesting a learning behavior by imitation. The capybaras quickly learned to associate the tongue-click sound with food, and after only a few repetitions, the approaching vehicle and individual staff members were associated with food and greeted with excitement.

Great caution is warranted to not feed excessive amounts of sugarcane to capybaras, as this may lead to intensive fermentation, potentially provoking tympany. In addition, working with hand-fed bait, a calm situation can quickly turn into a light feeding-frenzy when the animals get too close to each other, leading to fights of feeding-dominance.

Based on our behavioral observations, the capture strategies employed in this study appeared to provide several positive effects. Using the concept of classical conditioning was successful, with animals rapidly associating the click-sound with food offerings and approaching the investigator to be hand-fed. Animals could easily be herded into the corral while maintaining a calm demeanor throughout all capture events. These results demonstrate that the applied conditioning methods for capybaras are useful in facilitating frequent capture needs.

7.6 CONCLUSION

There is no “one-strategy fits all”, and each situation and species demand its own capture strategies. Nevertheless, for the purpose of frequent capture, we propose that for capybaras, where time and logistics permit, prior conditioning can be a beneficial strategy in preserving the capybaras’ overall welfare while facilitating the execution of frequent capture and procedures.

7.7 ACKNOWLEDGMENTS

We thank Pneu-Dart, Inc., Williamsport, Pa for their support and sponsored Remote Drug Delivery System Technologies. The research is funded by the São Paulo Research Foundation (FAPESP).

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8. FIELD-TESTING KETAMINE-DEXMEDETOMIDINE IMMOBILIZATION USING A REMOTE DRUG DELIVERY AND ATIPAMEZOLE REVERSAL IN FREE-RANGING SYNTROPHIC CAPYBARAS (HYDROCHOERUS HYDROCHAERIS)

8.1 ABSTRACT

Objective To evaluate a Ketamine/Dexmedetomidine association as a reversible chemical restraint alternative for free-ranging capybaras (*Hydrochoerus hydrochaeris*), seeking enhanced anesthetic and recovery characteristics while testing a specialized remote drug delivery system (RDDS). Study design Qualitative field research through direct observation. Animals 18 adult capybaras (male n=8; females n=10) (67.3 ± 9.45kg). Methods All animals, prior to chemical restraint, were physically confined in a corral, subsequently darted intramuscularly (IM) with 9 mg kg^{-1} ketamine and 0,005 mg kg^{-1} dexmedetomidine (KD). Post-intervention, 0,005 mg kg^{-1} atipamezole, administered IM, was used as a reversal agent (n=5). Anesthetic effects were recorded as latency period LP I (first observed effects) and LP II (lateral recumbency plus time to be able to handle the animal, or apt time). Time to recovery was divided into R1 (no reversal agent, fully recovered/ready for release), R2a/R2b (with reversal agent, time to ambulant position and time to full recovery), respectively. Heart rate (HR), respiratory rate (f_R), hemoglobin saturation (SpO_2), and body temperature (Cº) were recorded at a 15-minute interval. GraphPad Prism 8.1.1 was used to perform unpaired t-test, with a p-value < 0,05 considered significant. Results Mean LP I: 3 ± 1min.; LP II: 10 ± 2min. Duration of non-invasive procedures: 49 ± 5min. Recovery time without reversal agent (R1): 55 ± 15min, compared to 18 min. (R2a) to the ambulant position (accompanied by severe discoordination), requiring additional time to full recovery (ready for release): ± 45min (R2b). Total time, ready for release R2a/b: mean ± SD = 67 ± 13.85. Conclusion and clinical relevance: The association of Ketamine and Dexmedetomidine performed satisfactorily in capybaras, providing effective sedation and analgesia, relative short latency periods. Use of a reversal agent did not lessen the recovery time significantly (P-value = 0,7328). Adverse effects such as the risk of acute cecal tympany in capybara, due to the lack of pre-anesthetic fasting, concurrent to collateral effects of injectable and volatile anesthetics on the motility of the digestive

tract, as well as induced bradycardia and hyperthermia effects warrant anticipation and close monitoring. The employed RDDS provided reliable drug deposits in capybara under field conditions.

Keywords: RDDS, wildlife chemical restraint, atipamezole, capybara.

8.2 INTRODUCTION

Hydrochoerus hydrochaeris, commonly known as capybara, is the world's largest rodent. Although native to South America, capybaras can be found in zoos, as well as pets, around the world (ROUS Foundation, 2019). In Brazil, as a high-proliferative species with extraordinary resistance to anthropogenic activities and consequential pollution, the lack of natural predators, protection by law from hunting, and great food abundance in agricultural and urban areas, capybaras are steadily turning into a synanthropic pests, rapidly reaching superpopulations (Abreu Bovo et al., 2016; Felix et al., 2014; Macdonald, 1981; Marchini and Jr, 2015). Thus, bringing with them several

Human-Capybara Conflicts, but the main concern lies in their epidemiological role in the maintenance of the tick-borne disease, Brazilian Spotted Fever, being an amplifying host for the etiological agent *Rickettsia rickettsii* (Labruna, 2014, 2013; Rodrigues, 2013). In nation-wide efforts to control their population growth in order to mitigate conflicts, the numbers of studies increase, and with that, the necessity to frequently capture and recapture these animals for research-related procedures. As semi-aquatic animals, using water for protection, thermoregulation, and reproduction (Herrera and Macdonald, 1989; Mones and Ojasti, 1986); they always stay in very close proximity to water. In consequence, making capybara capture notoriously challenging, as any chemical restraint demands a series of precautions. For example, the physical confinement prior to anesthesia in order to prevent darted animals from escaping into the water, which would inevitably bring about death by drowning (Salas et al., 2004).

As part of an ongoing population control research on synanthropic capybaras, in a continuous effort of finding the most adequate anesthetic protocol for free-ranging capybaras, the aim of this study was to evaluate the Ketamine/Dexmedetomidine association as an alternative chemical restraint, testing specialized remote drug delivery system (RDDS). Specifically, seeking to achieve a shorter time of action (latency period), little adverse effects, and faster recovery, including the potential use of atipamezole as a potential reversal agent.

8.3 METHODS AND MATERIALS

This project was approved by the Ethics Committee on Animal Use in Science, School of Veterinary Medicine and Animal Science, University of Sao Paulo, CEUA: 9553260816, and by the Brazilian Ministry of the Environments, Institute of Biodiversity Conservation Chico Mendes, SISBio: 54634-2. It is CEUA's responsibility to comply with and enforce the provisions of Federal Law No. 11,794, of October 10, 2008, which establishes procedures for the scientific use of animals and State Law 11.977, of 08/25/2005, establishing the Code of Protection for Animals of the State of São Paulo and other rules applicable to the use of animals in didactic and scientific activity.

8.3.1 Location

The study was conducted at a large, man-made water pool, with trees and extended grass areas, used for water-based athletics. This pool is part of the University City, University of Sao Paulo, south-eastern region of Brazil (23º33’22” S 46º43’14” W, 726m altitude), known as the Olympic Ray resembles in its environmental condition the natural capybara habitats and very conducive for constant observations.

Captures took place year-around, during dry, - and rainy seasons, with temperatures ranging from 23 to 33ºC.

8.3.2 Animals

The animals that participated in the study on population control, are considered synanthropic, as they invaded, via tributary river systems, into inner-city areas. A differentiation, that comes to bare when comparing body conditions to non-synanthropic capybaras. Within the group, adult males and females were chosen for their role as principal breeders. Individuals captured: male (n=8) and female (n=10).

8.3.3 RDDS technology (Remote Drug Delivery System)

After physical restraint, the animals were anesthetized using a CO2 dart projector (X-Caliber, Gauged CO2 Long Range Projector, Pneu-Dart Inc, Williamsport, PA, USA) and specialized darts with various volume capacities, as well as a needle length suitable for intramuscular injection (Type P, with tri-port, gel collar, cannula with 4.44cm). Specifically chosen for their advanced technologies such as: explosive charge to drive the plunger forward to inject the drug load; a gel collar, which, after reaching subcutaneous tissues would remain for an extended time, before starting to soften (due to body temperature), eventually detaching itself; a slow-inject technology, injecting the drug at a lower speed to potentially minimize further tissue damage, in additionally, the tri-port concept would deposit the drug over a much larger surface (fig.10.1), augmenting the absorption rate, thus, shortening the latency period.

Capturing started early morning, to minimize the impact of ambient temperatures. The darts were projected from various distances, ranging from 5 to 30 meters, targeting preferentially the pelvic limb musculature (M. biceps femoris, M. semitendinosus/semimembranosus).

Drug delivery quality was confirmed by removing any post-darting drug residue, which then was compared to the original loaded volume.

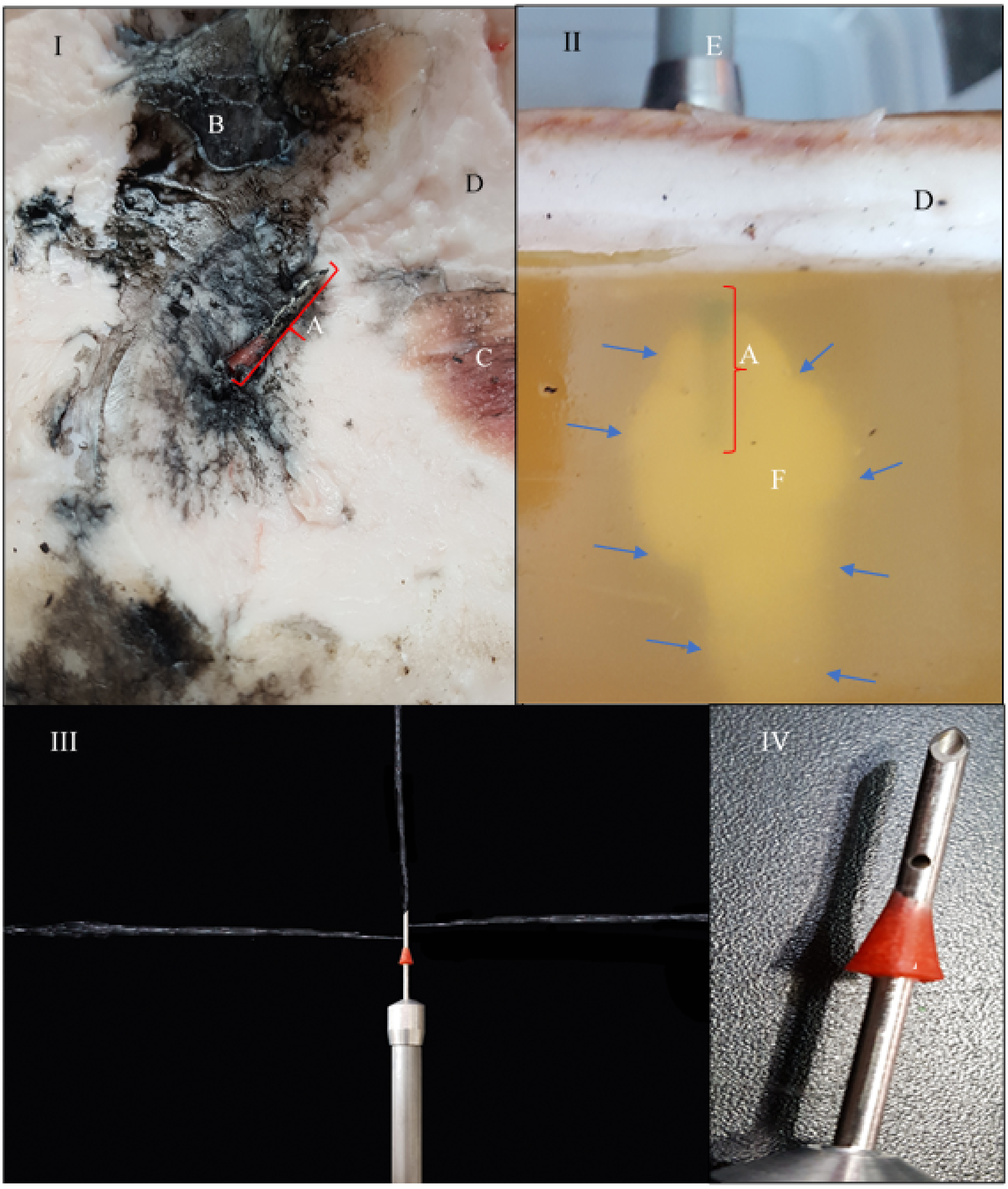

Figure 8.1 - Remote Drug Delivery System (RDDS) deposit quality

I) View of the ink-stained adipose layered side of a fresh pork hide, mounted on ballistic gel., depicting deployed dart with Tri-port effect .II) Pork hide, mounted on ballistic gel, in comparison, showing a deployed Single-port dart, with canula perforation through the adipose tissue, depositing the drug IM. Dart canula (red marker); Concentrated drug deposit (blue arrows). A) Dart canula with gel collar. B) Tri-port dispersed drug deposit (black ink) in adipose tissue layer. C) Start of muscular tissue layer. Red arrow: adequate dart canula length for IM injection. E) Dart. F) Single-port concentrated local drug deposit. III) Drug jet stream when forced through the Tri-port. IV) Tri-port and gel collar close-up.
Source: D. Rosenfield, 2018

8.3.4 Anesthetic protocol

Before any intervention, time was invested to classical condition the animals, allowing for frequent capture, while minimizing associated stress. Target animals were identified (principal breeder) and physically restrained by herding them into large corrals. For initial anesthetic dosage calculation, an estimated body weight of 70 kg was used for all animals. The employed anesthetic drugs were Ketamine Hydrochloride 10%, injectable, dosage 9 mg kg^{-1} (Syntec, Brazil), and Dexmedetomidine Hydrochloride (0.5 mg/mL), injectable, dosage 0,005mg kg^{-1} (Dexdomitor, Zoetis, Brazil), combined as a single-dose and filled into a 7 mL dart.

For ocular protection and to minimizing luminous stimuli, a moistened cloth was placed over the eyes.

8.3.4.1 Reversal agent

After the animal was returned to the corral for anesthetic recovery, the alpha-2 agonist effects were reversed with atipamezole 5.0mg/mL (Antisedan, Zoetis, Brazil), administered by hand-syringe, using a dosage equal 1:1 of the preceding dexmedetomidine dosage volume.

8.3.4.2 Evaluation of anesthetic effects

In capybara, achieving recumbency does not mean the animal is ready to be handled, in fact, there is a potential of being bitten for several minutes after it would achieve recumbent position. Therefore, the initial anesthetic effects were evaluated by measuring two latency periods: LP-I (onset of action), defined as the time from dart deployment to first observed incoordination, and LP-II, lateral recumbency/apt time, defined as the time from darting to lateral lying position and the moment when the animal could be safely manipulated/transported. Duration of action, defined as the time of procedure initiation (post-LP-II) until the time when the animal is being returned to the corral for recovery. Full recovery time without reversal agent (R1): defined as the time from lateral recumbency to ambulation, free of any visible anesthetic effects (incoordination), at which time the animal could be safely released. Full recovery time with reversal agent (R2a): defined as the time from recumbent to ambulant position, and (R2b) ambulant animal, without observed incoordination, time of safe release.

Anesthetic depth was assessed by performing different reflex tests, such as response to pain stimulation, pupillary light reflex, palpebral reflex, corneal reflex, eyeball position, and jaw tone.

8.3.4.3 Monitoring of physiological parameters

Normal – (not under anesthetic effect), were measured by luring the conditioned animals with bait into position, which would permit to perform light physical examinations, including direct thoracic auscultation for heart, - and respiratory rate (alternatively, by observing chest wall movements). Body temperature was measured using an infrared thermometer (Digital Laser, Model KP-8005, Knup, Brazil) in a distance of ± 10cm into the external acoustic meatus (ear canal).

Under Anesthetic Effect (UAE), vital physiological parameters were continuously monitored during none-invasive and semi-invasive procedures using a veterinary pulse oximeter (ARSTN, China), placing the sensors inter-digitally, or on the tongue. Pulse, rectal temperature and blood-oxygen saturation were recorded in intervals of 15 minutes, supported by intermittent auscultation exams for heart and respiratory frequency and peristalsis. During invasive procedures, heart frequency, respiratory rate, blood oxygen saturation, and rectal temperature were monitored using a DX 2022+ Multiparameter Monitor (Dixtal – Philips Healthcare, Brazil).

8.3.4.4 Body mass

After sedation took effect (save to be handled), the animals were placed on a stretcher and weighed on a digital hanging weighing scale (Mini Digital Crane Scale 300kg, Outmate, China).

Believed to be of relevance when capturing and working with capybaras suffering synanthropic influence on their behavior and physiology, attention is being drawn to the significant body mass variation between synanthropic capybaras (S), defined as groups inhabiting agricultural and urban areas, with access to abundant food sources, and limited threat from predators; and non-synanthropic capybaras (NS) defined as groups living in original habitats without any anthropic presence, or activities. Particularly, in this study, the consideration of the effects of anesthetic agents on capybaras, subject to obesity.

8.3.4.5 Data presentation and statistical analysis

The data are described as mean ±SD. Unpaired T-test (P-value <0.05; CI 95%) was performed using GraphPad Prism version 8.1.1 for Windows, GraphPad Software, USA.

8.4 RESULTS

8.4.1 Body mass comparison

Between studied synanthropic animals (S) and non-synanthropic animals (NS), shown in figure 10.2. Studied animals (synanthropic), mean 67.3 ± SD 9.45, whereas the body mass of non-synanthropic capybaras: mean 52.5 ± 4.58.

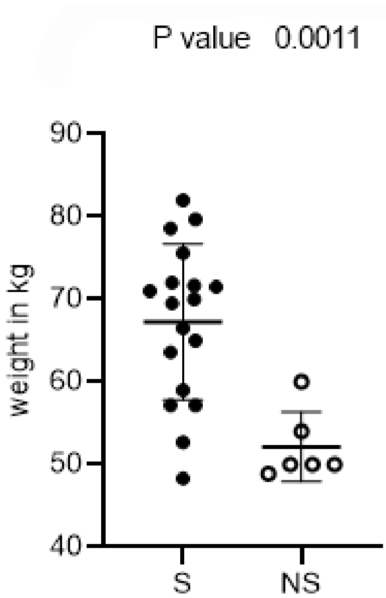

Figure 8.2 - Body mass comparison between synanthropic to in the wild capybaras.

8.4.2 Time of anesthetic latency effects (fig.10.3)

LP I, first observed effects: (n = 18; mean ± SD = 4 ± .83; range 3 – 6 minutes.

LP II, time to lateral recumbency/apt time: (n = 18; mean ± SD = 8 ± 1.68, range 6 – 12 minutes).

Anesthetic duration: (n = 18; mean ± SD= 53 ± 20.89, range 53 – 96 minutes). Time to full recovery (safe release) without, - and with anesthetic reversal agent se (fig. 10.4).

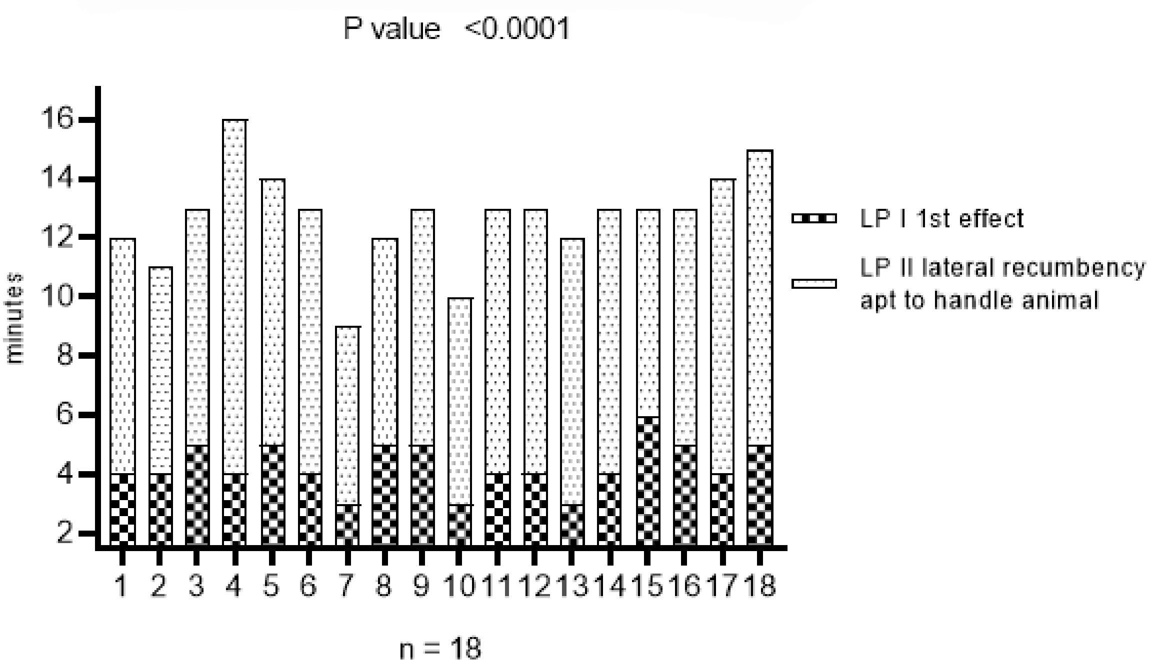

Figure 8.3 - Anesthetic latency effects

8.4.3 Recovery time

R1, time to full recovery/no reversal agent: (n = 13; mean ± SD = 67 ± 13.85; range 40 – 79 minutes). R2a, time to ambulant position/ use reversal agent: (n = 5; mean ± SD = 18 ± 15.60; range 13 – 20 minutes), plus R2b, time to full recovery: (mean ± SD = 49 ± 15.48; range 35 – 70 minutes).

R1 to R2a/b comparing total recovery time (safe to release): Mean R1 = 66.85, mean R2a/b = 64.40. Difference between means (B - A) ± SEM; -2.446 ± 7.042. 95% confidence interval: -17.37 to 12.48

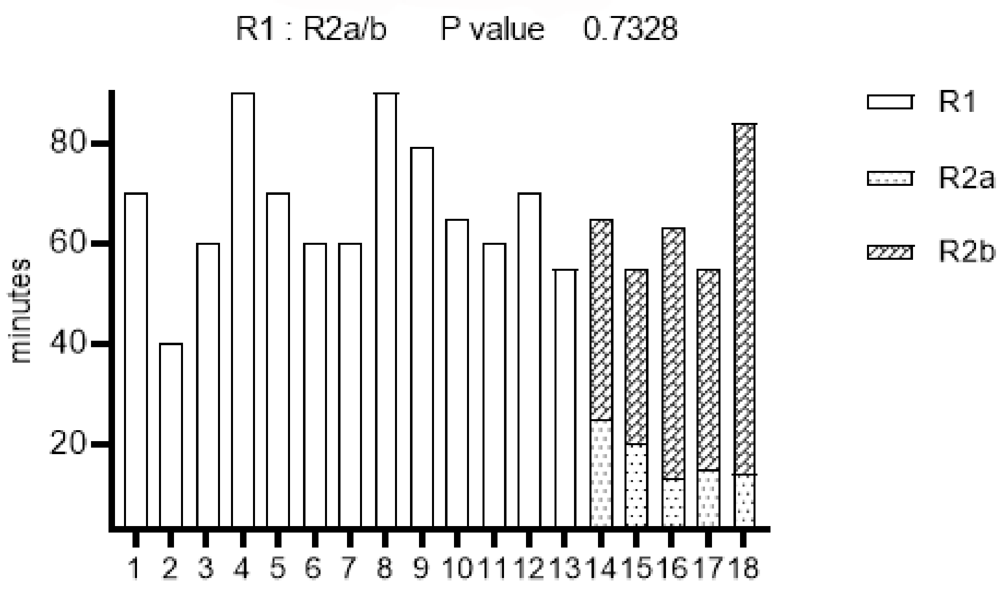

Figure 8.4 - Time to full recovery.
Data comparison, R1 w/o, - and R2a/b w. anesthetic reversal agent

8.3.5 Vital parameters

The as normal described vital parameters in this study (N) are defined as observed vital signs not under the influence of anesthetic effects, in contrast to in literature commonly described physiological parameters, which are recorded during anesthesia. Comparing the physiological parameters (fig.10.5), a significant difference was identified for heart rate, respiratory rate, and body temperature. Normal SpO_2 could not be collected under non-immobilized conditions.

Heart Rate a) UAE (n = 18) 68 ± SD = 7.86; range: 49 – 78. Heart Rate b) N (n = 18) 80 ± SD 3,81; range: 76 – 88. HR variance of significant difference UAE and N: P = 0,0001. 95% CI 7.148 – 15.63.

Respiratory Rate a) UAE (n = 18) 32 ± SD = 3.78; range: 25 – 38. Respiratory rate b) N (n = 18) 40 ± SD = 3,01; range: 35 – 48. RR variance of significant difference UAE and N: P = 0,0001. 95% CI 4.849 – 9,485.

Body temperature a) UAE (n = 18) 36.0 ± SD = 1.79; range: 33 – 38.2. Body temperature b) N (n = 18) 34.3 ± SD 0,90; range: 33.5 – 37.2. BT/^{o}C variance of significant difference UAE and N: P = 0,0001.

Peripheral oxygen saturation UAE SpO2 (%) 94 ± SD = 2.43, range: 90 – 98. SpO2 N: not available.

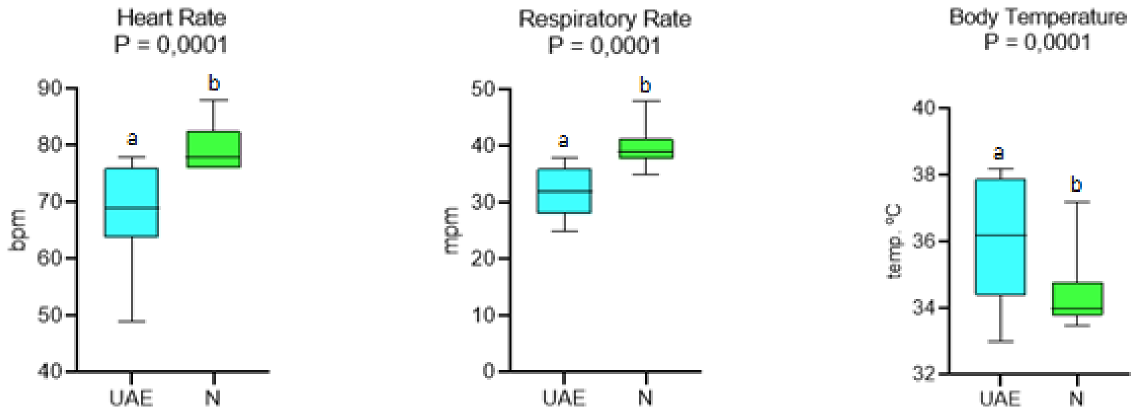

Figure 8.5 - Physiological parameters under anesthetic influence

UAE: Under Anesthetic Influence; N: Normal.
bpm = beats/minute; b/m = breaths per minute. Data presented in $\bar{x}$ ± SD. Physiological parameters

8.3.6 Mortality

The mortality rate was 5% (1:18). The cause of death was identified as acute cecal tympany and believed to have been caused by iatrogenic reasons, such as the lack of adequate fasting, anesthetic adverse effect on the gut motility with subsequent difficulty to liberate excessive fermentation gas, (case report submitted, 2019).

8.5 DISCUSSION

Due to the capybara's relative tranquil nature, employing remote injection, was uncomplicated. The specialized darts performed satisfactorily, full drug delivery, no bounce-backs. Being able to rapidly immobilize a capybara means safety for the animal, as vital signs can be monitoring sooner. Nevertheless, whenever possible, prior physical confinement is imperative to avoid any potential death by drowning, and the same holds true for anesthetic recovery.

Prior to establishing an anesthetic field-protocol for remote drug delivery, several anesthetic drug protocols were researched in available literature (tab. 8.1) with emphasis on chemical restraint in capybaras, (Cruz et al., 1998; Góngora et al., 2010; King et al., 2010; Nishiyama, 2006, 2003; Salas et al., 2004). Besides seeking enhanced anesthetic performances, assisting in collecting semen through urethral catheterization, as reported in several studies (Araujo et al., 2018; Lueders et al., 2012; Pisu et al., 2017), was of great interest, as andrological exams were part of the overall research project.

In the present study, the use of a ketamine/dexmedetomidine association, and atipamezole as the alpha-2 antagonist in free-ranging capybaras are being reported for the first time.

In this species, defining the latency period as the time of darting to achieved lateral recumbency, wouldn’t be adequate, as the true time (able to handle the animal) required considerably more time. The reason why in this study the latency period was divided into two sub-periods, LP I and LP II. The findings showed a relatively short latency period of $\bar{x}$ = 8 minutes (LPI and LPII), compared to 25 minutes (Cruz et al., 1998; Roberto et al., 2018), 10 to 13 minutes (Cruz et al., 1998; King et al., 2010; Salas et al., 2004), and 7,9 to 9,3 minutes (Cruz et al., 1998; Nishiyama, 2006), assuming, in this context, the reported latency meant animals were able to be handled.

Recovery periods, on the other hand, were still lengthy and cumbersome, independently of the use of atipamezole as an alpha-2 antagonist, showing no significant difference between the mean R1 = 66.85 (no reversal) and a mean R2a/b = 64.40, using atipamezole as a reversal agent. Contrary to most reported studies, using atipamezole, although different species (Arnemo et al., 2005; Granholm et al., 2007; Re et al., 2013; Tsuruga et al., 1999). In regard to the two studies found that used atipamezole in similar protocols in capybaras, also show extended recovery times (≥ 38 min.). Furthermore, based on observations made, regarding the quality of recovery, recovery without the reversal agent suggested a smoother recovery (less tumbling and falls), perhaps a fact that could be linked to residual anesthetic effects of ketamine (Bouts et al., 2010). In fact, working with species that have a higher corporal fat proportion, such as the capybara, even more so in synanthropic capybaras (private communication, 2018), that can reach obesity-lite status with more than 60% above average weight to non-synanthropics, ± 50kg (Moreira et al., 2012), anesthetic agents might deserve special attention, when it comes to action onset, recovery periods and adverse effects.

The duration for procedures, related to this research (mean ± SD= 53 ± 20.89), was adequate, including the analgesic effects for superficial treatments. For marking like ear-tags, an additional local analgesic (1mL lidocaine bolus) was administered.

Vital parameters: Comparing normal vital parameters (conscious animal) to parameters under anesthetic effects, significant difference was observed, suggesting bradycardia and respiratory depression: (HR-UAE 68 ± SD = 7.86 to HR-N 80 ± SD 3,81) and (RR-UAE 32 ± SD = 3.78; to RR-N 40 ± SD = 3,01), respectively, which is in agreement with previously reported adverse effects, when using alpha-2 agonists (Arnemo et al., 2005; Granholm et al., 2007; Grayson et al., 2017; Pascoe, 2015). Including the peripheral oxygen saturation of (SpO_2 % UAE 94 ± SD = 2.43), extra caution is warranted to prevent any adverse hemodynamic effects.

Pharmacological semen collection: As before mentioned, employing one of the latest generations of an alpha-2 agonist for pharmacological semen collection, dexmedetomidine was used with some success in male capybaras.

Adverse effects:

Hyperthermia - elevated rectal temperatures under anesthetic effects were recorded (36.0 ± SD = 1.79; range: 33 – 38.2 Cº), possibility contributed in part by high tropical ambient temperatures. Nevertheless, the use of an alpha-2 agonist is linked to hyperthermia as a collateral effect, as corroborated in studies on several species, including capybaras (Cruz et al., 1998; Grayson et al., 2017; King et al., 2010). When working with hyperthermia-prone capybaras, it is also recommended to have a couple of buckets with water in reach to help control any potential excessive body temperature.

Acute cecal tympany - All anesthetized animals showed varied intensity of abdominal extension (bloat), a condition that rapidly (within a few minutes) can turn into a life-threatening situation, if not identified in time. In one incidence, while continuous monitoring vital signs during recovery, an animal demonstrated an accelerated heart rate with flat respiration, together with a slight increase of the abdominal volume, - and taut abdominal wall. Shortly thereafter, due to cardiorespiratory arrest, the animal died, despite attempted cardiopulmonary resuscitation. Necropsy performed immediately at the location, an extreme dilation of the cecum, with evidence of ischemic mesentery and intestines was observed. The authors believe that the cause is of iatrogenic nature, brought about by several collective factors, including collateral anesthetic effects, absence of pre-anesthetic fasting, and high-sugary plants for baiting. Similar observations have been reported in a number of studies and in varied species (Abutarbush et al., 2005, 2005; Colorado State University, 2019; Kümper, 1994; Tanila et al., 1993; Torjman et al., 2005; Zullian et al., 2011)

It is important to note that attempting to immobilize free-living animals through long-distance darting comprises many challenges, especially the accurate estimation of the individual's body weight, which, when miss-calculated can lead to failures, a missed opportunity, and/or the need for additional darting, with the potential risk of overdosing.

Particularly true for synanthropic capybaras, that reach body weights of 40 – 50% higher, with a maximum reported 105kg (Labruna, personal communication, 2018), compared to their non-synanthropic counterparts, weighing an average of 40 – 50kg (Moreira et al., 2012).

Using adequate equipment, combined with the competent use of Remote Drug Delivery System (RDDS), has shown to provide an overall better project execution, and more importantly, maintain animal well-being, looking at alternative capture methods, as reported by (Salas et al., 2004), where the capture by a lariat. All animals captured by lasso afterward showed difficulties of movement, hyperventilation, and even death.

Working with capybara, the use of a corral for physical restraint is fundamental for the safety of the animals, avoiding escapes and any potential death by drowning of sedated individuals (Salas et al., 2004).

One aspect to consider that can bring great benefits, if time and logistics permits, is to apply classic conditioning of target individuals. Allowing to be called and herded into the corral voluntarily, facilitating time-independent, frequent and selective capture (versus aleatory success), provoking less capture-induced stress, reducing the risk of injury (even death), while requiring lesser amounts of anesthetics, producing smoother recovery, and improving overall team safety. Furthermore, it allows conducting health exams and vital parameter evaluation, such as auscultation for the heart, - respiratory rate, and body temperature (fig. 8.6).

Figure 8.6 - Monitoring normal vital signs,
Depicting heart frequency auscultation during voluntary exam. Source: D. Rosenfield

Regarding the baiting methods for capybaras, as said, using sugary plants, like sugar cane, as bait is certainly a great tool to lure, however, it is also the one with a certain risk if used excessively, as it can easily provoke a situation of acute cecal tympanism, an life-threatening emergency in capybaras, whose onset is not readily recognized, since signs are rather subtle, like the slight loss of visible lower rib-cage contour due to abdominal dilation.

Only in anticipation and through constant monitoring heart rate, blood pressure, and respiratory rate, and percussion/palpation of the abdominal wall, is it possible to identify tympany in time. It is, therefore, crucial to use sugar cane in very small quantities, just enough to draw attention. Alternatively, less sugar containing corn and banana leaves work quite well and are safer.

8.6 CONCLUSION/CLINICAL RELEVANCE

For the present study on free-living capybaras and intended procedures, the anesthetic protocol of a ketamine/dexmedetomidine association performed adequately. Using specialized RDDS provided satisfactory drug delivery quality. Nevertheless, more controlled studies are necessary to adequate KD dosage requirements, or testing alternative anesthetic, - and reversal agents, to improve recovery periods, with lesser collateral effects.

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8.8 TABLES

Table 8.1 Anesthetic protocols for capybaras reported in literature, (* with use of an reversal agent).

Anesthetic agents	Dosage respectively	t latency (min.)	t recovery (min.)	t anesth. effect (min.)	Authors
Ketamine - Xylazine	15mg/kg – 1mg/kg 10mg/kg – 0.5mg/kg 10mg/kg – 0.5mg/kg	7±1 8.2 n/a	167±14 31.7 n/a	75±5 95.4 ±90	Cruz et al., 1998; Monsalve-Buritica et al.,2013; Nishiyama, 2003
Ketamine - Midazolam	15mg/kg – 0.5mg/kg	12±10	91±10	8±1	CRUZ et al., 1998;
Ketamine - Medetomidine Atipamezol (dosage equivalent to half the medetomidine volume)	4mg/kg – 40mcg/kg	9±4.73	7±9*	n/a	Salas et al., 2004)
Ketamine - Romifidine	15mg/kg – 0.1mg/kg	25±5	129±13	25±5	Cruz et al., 1998)
Tiletamine/Zolazepam	5.0mg/kg 2.3/2.3mg/kg 5mg/kg	7.9 6.6 7	72.7 71.4	62.5	King et al., 2010; Monsalve-Buritica et al., 2013; Nishiyama, 2003
Tiletamine/Medetomidine – Atipamezol Tiletamine/Medetomidine – Butorphanol	0.8/0.8mg/kg 0.008mg/kg 0.8/0.8mg/kg 0.0075mg/kg 0.075	10.0 8.5	49* 44.3*		King et al., 2010
Tiletamine/Zolazepam - Levomepromazine	5.0mg/kg 0.5mg/kg	6.8	110.1	107,3	Nishiyama, 2003

9. ACUTE CECAL TYMPANY DURING CHEMICAL RESTRAINT IN FREE-RANGING CAPYBARA (*Hydrochoerus hydrochaeris*) - IATROGENIC CAUSE AND TREATMENT

9.1 ABSTRACT

The occurrence of acute tympany, probable cause and treatment are being discussed for the first time in *Hydrochoerus hydrochaeris*, commonly known as the capybara. As part of a capybara population control study, adult male and female (n=18), prior to intervention, were physically confined and subsequently chemically immobilized with a ketamine dexmedetomidine (KD) association. Procedures included health exams, biomaterial collection, and ID markings. For surgical events, the animals were maintained under volatile anesthesia with Isoflurane. One animal, during anesthetic recovery, entered into tachycardia and tachypnea, with cyanotic mucosa, and a low SPo2. During auscultation and percussion/palpation a taut abdominal wall was recognized. Shortly thereafter, the animal suffered a cardiorespiratory arrest and expired after failed resuscitation. Immediately performed necropsy suggested death by acute respiratory and circulatory failure due to exerted pressure against the diaphragm and compression of major blood vessels, leading to hypoxia and hypovolemic shock, secondary to cecal dilation. Three animals under general anesthesia demonstrated moderate bloating and were treated by trans-abdominal catheterization for cecal decompression, successfully preventing any potential development of severe tympany.

Conclusion: During chemical restraint all animals demonstrated bloat of varies degrees, suggesting iatrogenic cause brought about by adverse effects of anesthetic agents. Acute cecal tympany in sedated capybaras most be anticipated and closely monitored to prevent fetal outcome. Trans-abdominal catheterization into the cecum proved effective in treating bloat.

Keywords: Bloating, Cecal Decompression, trans-abdominal catheterization, rodent, anesthesia

This project was approved by the Ethics Committee on Animal Use in Science, School of Veterinary Medicine and Animal Science, University of Sao Paulo, CEUA: 9553260816, and by the Brazilian Ministry of the Environments, Institute of Biodiversity Conservation Chico Mendes, SISBio: 54634-2.

9.2 INTRODUCTION

Mammals carry an active population of micro-organisms (bacteria, fungi, and protozoa) in their digestive tract, especially in herbivores, these organisms are necessary to breakdown fibrous vegetation, aiding in the extraction and synthesis of nutrients (1). Capybaras are hindgut, - or cecal fermenters (2), same as rats, guinea pigs, rabbits, and horses. The later, used to compare with capybara (fig. 1) on the basis of tympany/bloat occurrence and comparable treatments. As part of a micro-organism driven fermentation process, large quantities of gas are produced, that under normal conditions, is being expelled continuously, either through eructation and/or through peristalsis-enabled flatulence. However, in hind-gut fermenters, gas is only liberated by flatulence. Especially capybaras, as semi-aquatic species are obligatory nose-breather (3), have a number of particular anatomic differences when it comes to the vomitus and eructation, including an atypical emetic reflex, which makes it impossible to vomited, nor eruct, similar to equines (4,5).

The terms tympany, bloat or bloating, meteorism, acute abdominal dilation/distention are terms that are used interchangeably in the literature, whereas the term tympany, in conjunction with a specific anatomic structure, may describe dilated organs, species, and/or age involved. I.e.: abomasal tympany, used to define accumulation of gas in the abomasum (fourth stomach of a ruminant), common in calves (6), or ruminal tympany (the first stomach of a ruminant), both specific for species with foregut or ruminal fermentation. In hindgut, or cecal fermenters, terms commonly used are cecal tympany, gastric bloat, - tympany, - or dilation, also used for other monogastric species, including carnivorous.

All terms refereeing to potentially life-threatening gastrointestinal emergencies, involving several species, as reported in the literature (6–11).

The etiology involved in acute cecal tympany (ACT), the term used in this report to specifically describe the excessive accumulation of gas within the capybara’s cecum.

In its pathophysiological dynamics, it is similar to cecal tympany in horses. However, the causes differ.

Whereas in equines the condition might be related to digestive tract diseases, parasitism, ingestion of improper types or quantities of food or sand, among others, causing impaction or volvulus, as well as being of iatrogenic nature, potentially incited during anesthesia (12–14). The latter, actually being the investigated reason for ACT in capybaras.

Acute cecal dilation is diagnosed by identifying a taut abdominal wall through palpation and performing a sharp finger percussion over the mesogastric region, including its corresponding lateral abdominal areas. The excessive gas filled cecum exerts pressure on all surrounding structures and organs, compressing and strangulating large blood vessels, liver and spleen, while obstructing the diaphragm's mobility, reducing intrathoracic space for lung and heart movement, compromising the cardiovascular and respiratory system, reflected by tachycardia and dyspnea respectively, cyanotic mucous membranes, sharp drop in SPo2 %, and an increases capillary refill time.

Since reports on the cecal disease in capybara and potential emergency procedures never have been published, no literature reference could be provided.

The purpose of this study was to report the occurrence of acute cecal tympany by examining the potentially iatrogenic etiology involved, such as adverse anesthetic effects, concurrent to the inevitable lack of pre-event fasting, and the use of sugar-rich bait. Furthermore, it was the intend to describe a treatment method of cecal decompression to alleviate bloat and to prevent acute cecal tympany in capybaras.

9.3 CASE REPORT I – CECAL DILATION UNDER ANESTHETIC EFFECTS

As part of a larger research project on capybara population control, 18 adult capybaras (males n=8 / females n=10), prior to chemical restraint, were physically confined. The anesthetic protocol employed was an association of Ketamine Hydrochloride 10%, injectable, dosage 9 mg kg-1 (Syntec, Brazil), and Dexmedetomidine Hydrochloride, 0.005 mg kg-1, injectable (Dexdomitor, Zoetis, Brazil), administered remotely as a single-dose (vol. 7mL, type P dart, and X-Caliber CO2 projector, Pneu-Dart, Inc. Pennsylvania, USA). The alpha-2 reversal agent, Atipamezole, 0.005 mg kg-1 (Antisedan, Zoetis, Brazil), administered via hand syringe (n=5), after the animals were returned into the corral for anesthetic recovery.

Continuous vital sign monitoring for non-invasive procedures was performed using an oximeter (ARSTN, China), and during general anesthesia a DX 2022+ Multiparameter Monitor (Dixtal – Philips Healthcare, Brazil).

Normal vitals: heart, - respiratory rate, and body temperature (Table 1), used as reference values, were taken from animals not under anesthetic effects, by direct auscultation, palpation, and observation. Body temperature was measured with an infrared thermometer (Digital Laser, Model KP-8005, Knup, Brazil), understood that the reading is not as accurate as a rectal inserted thermometer, thus, serving as an approximate value.

The animal, after achieved sedation, defined as the time of lateral recumbency without response to physical manipulation (palpebral reflex), was brought to the field clinic for general procedures (biometry, collection of biomaterials, and ID marking), and weight, using a digital hanging weighing scale (Mini Digital Crane Scale 300kg, Outmate, China). For surgical procedures, such as gonadectomy, the animal was maintained under general anesthesia, using Isoflurane (mean period 50 ± 20min.).

All animals showed changes in the vital signs to various degrees: Increase of the heart rate (>70b/m); Increase in the respiratory rate (> 30 b/m), and SPO2 drop (< 92%), concomitant to a tympany onset, identified by representing a slight taunt/dilated abdomen, loss of rib-cage contour.

Animals, where change of vital signs and onset of abdominal distention was evident, as a preventive measure, were prepared with a 70% alcohol or a povidone-iodine solution and cleaned with gazes, prior to catheterization. Following, a gauge 14 catheter (14Gx2 Safelet, Nipro Medical, LTDA, Brazil) was placed percutaneously through the abdominal wall into the cecum (fig. 2). The same procedure would be used in severe tympany intervention. The site of catheterization was identified by performing a finger percussion (tapping), noting tympanitic (drum-like) sounds typical for air-filled intestinal structures, compared to a dull sound of a solid or liquid-filled area (15).

Successful pressure relief was confirmed by the sudden presence of an intensive fermentation smell and improved vital signs. After the procedures, the cannula was removed, followed by a simple superficial wound treatment.

9.4 CASE REPORT II - ACUTE CECAL TYMPANY (SEVERE ABDOMINAL DISTENTION)

After executed procedures, one male capybara was returned into the corral for anesthetic recovery and positioned in lateral recumbency. Subsequently, 0,07mL Atipamezole was administered with the intent to revert the Dexmedetomidine effects. Within ten minutes the animal attempted to get up on its feet and walk around. However, motor discoordination made the animal fall over several times and finally remained in a lateral position. Hemodynamic monitoring showed a heart rate in beats/minute (HR □ 90 b/m), tachypnea, with a flat respiratory movement, measured in breath/minute (RR □ 80b/m), a peripheral capillary oxygen saturation (SPO2 < 92 %), cyanotic mucous membranes, and a refill time (CRT) of > 4 seconds. Within minutes, the animal went into cardiorespiratory arrest and expired after failed resuscitation attempts.

9.4.1 Post-mortem necropsy findings

Immediately performed necropsy provided clear evidence of an extensive dilated cecum, that quickly forced its way out of the abdominal cavity after incision. The ventral view shows the enlarged cecum over the entire abdominal region of the animal (fig. 3). Lifting the cecum, exposed the underlying viscera with an evidently altered appearance. Macroscopic diagnosis: severe dilation of the cecum and intestines due to accumulated fermentation gas. In turn, provoking compression of surrounding organs, resulting in severely congested and hypoxic mesenteric and intestinal blood vessels (dark purple discoloration, fig. 4) . During examination, no evidence of volvulus or torsion was found.

9.5 DISCUSSION

In capybaras, recognizing acute cecal tympany is not as straightforward, but diagnosis and preventive monitoring are relatively simple and fast to execute. In these case reports, ACT was considered a secondary condition, with its etiology believed to be of iatrogenic nature.

Under controlled conditions, any animal about to be anesthetized would have undergone minimum fasting of 6 to 12 hours, with the intent to prevent potential colic, caused by free gas formation (16). However, working with free-ranging wildlife, this preventive technique is often difficult if not impossible to implement.

As descript in several studies and species (14,17–20), using anesthetic agents for chemical restraint or to maintain the animal anesthetized during procedures, bloating is a common occurrence. Brought about by known adverse effects of some of the anesthetic agents used, primarily the decrease in intestinal motility, interfering with peristalsis, consequently losing the capability to eliminate excessive gas, as the findings of this report can collaborate.

In capybaras, the initial signs are rather insidious and undramatic. Although severe cecal dilation is associated with intensive pain, capybaras, during anesthetic recovery, would not vocalize any discomfort.

Anticipating ACT, closely monitoring of the vital signs (fig. 5), observing the subtle disappearance of the ribcage-contour, caused by the expansion of the abdominal volume, and confirmation of tympany by abdominal percussion and palpation will allow recognizing the right moment for intervention.

Anesthetic Reversal - Atipamezole - Alpha-2 antagonist

Atipamezole (Antisedan, Zoetis, Brazil) was administered in 5 animals, in a 1:1 dosage (0,7mL IM). Time from lateral recumbency to being on four feet was relatively fast (mean 18 minutes), nevertheless, the animal was not considered fully recovered, as maintained anesthetic effects were observed, chiefly ataxia, disorientation, intermittent falls. Even with the antagonist, time to full recovery exceeded 40 minutes (fig. 6).

9.6 TREATMENT

Cecal Decompression is a routine emergency procedure in horses, as previously described (21). The relatively simple and quick technique provided immediate improvements in the vital signs and lessening of the abdominal volume. Although no adverse effects from the procedure were observed postoperatively, attention should be paid to potential complication such as (septic) peritonitis (22).

Using this technique as a routine preventive procedure, when working with anesthetized capybaras, deserves consideration. Nevertheless, more research is required to confirm any associated long-term consequence.

The other major contributing factor to acute cecal tympany is baiting and luring with high-sugary plants capybaras (private correspondence, 2018). As fasting is not possible under field condition, the offered quantities must be held to an absolute minimum, not allowing for any real consumption in order to minimize intensive fermentation and excessive gas development.

When working with capybaras, where procedures include the necessity of chemical restraint, it is imperative for the medical professionals to anticipate ACT and are aware of cecal tympany symptoms, preventing therefore, any development into an acute condition, thus, avoiding any potential death.

ACKNOWLEDGMENTS

We thank the Pneu-Dart, Inc. for their cooperation and material contributions. The project was funded by FAPESP, Sao Paulo, Brazil.

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9.8 TABLES

Table 9.1 Normal vital signs

Heart rate (resting/low activity) (beats per minute)	Respiratory frequency (breath per minute)	body temperature* (ºC)
80 ± 8	40 ± 4	36,0 ± 3

Original reported vital signs of *H. hydrochaeris*, taken from non-sedated animals, during routine conditioning training. Activity intensity: resting/low. *Body temperature measure with a digital laser pointed into the outer ear canal).

Table 9.2 General overview – affected animals (n = 18)

# Animals	Signs of tympany	Treatment	Outcome
n = 1	ACT*	n/a	n = 1 expired
n = 2	Moderate bloat	Catheterization	Cecal depression, abdominal contour returned to normal; initial vital signs of elevated heart/respiratory rate, SpO2 drop, rapidly retuned to normal. Acute tympany prevented, further bloat avoided, n = 2 survived
n = 15	Mild bloat	n/a	No intervention necessary, n = 15 survived

* ACT (acute cecal tympany)

9.9 Figures

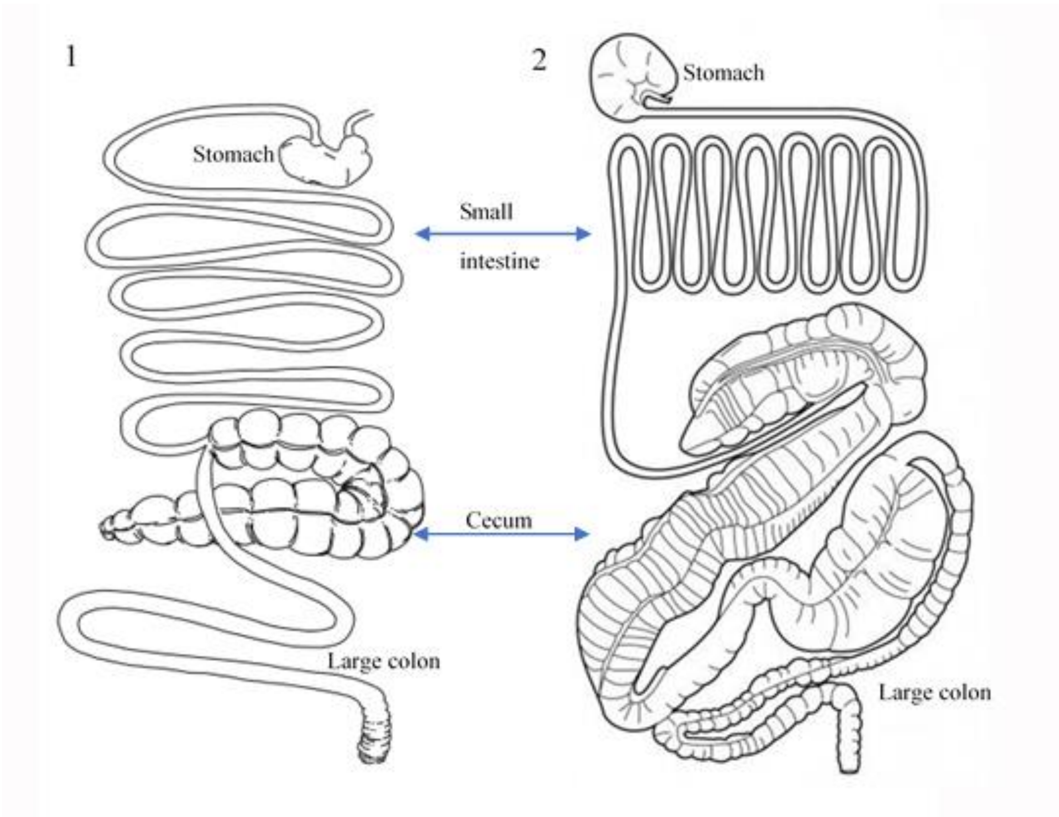

Figure 9.1 - Anatomy Capybara digestive tract

Stomach: ± 2 L; Sm intestine: ± 10 m; Cecum: ± 5 L; Lg. colon: ±1,2 m. 2) Equine digestive tract: stomach: 10 L; Sm intestine (jejunum) 19.5 m; Cecum: ≥ 30 L; Lg. colon: ≥ 7 m. Graphic credit (1) Luis Miguel Márquez), in Moreira et al. 2013, adopted. (2) KPPUSA. 2019, adopted.

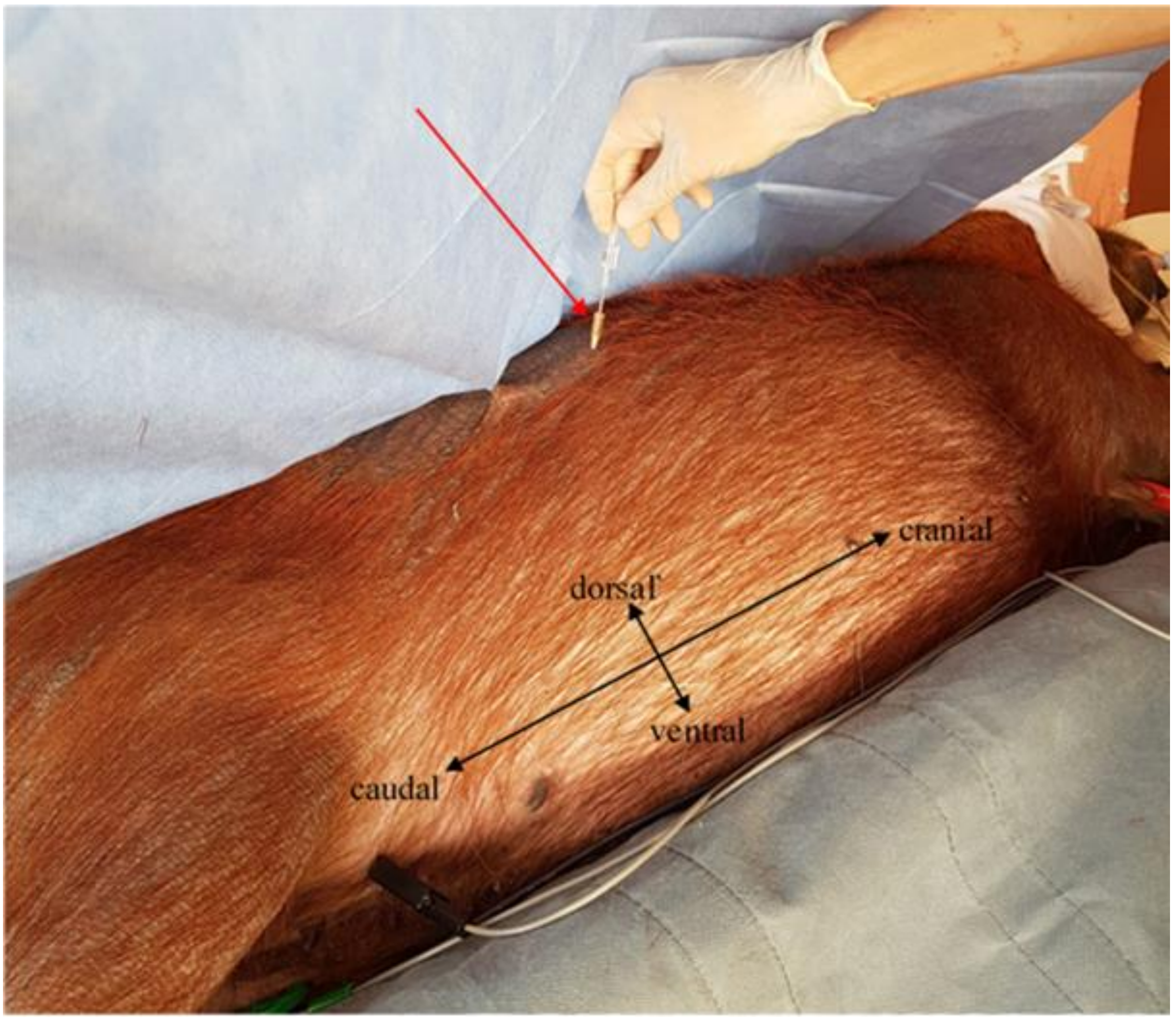

Figure 9.2 - Treatment: Cecal decompression by trans-abdominal catheterization

Lateral view. Red arrow: decompression with a large diameter cannula (G14), alleviating the gas pressure from the caecum, preventing potential acute tympany/death, in a prior surgically prepared site. Source: D. Rosenfield

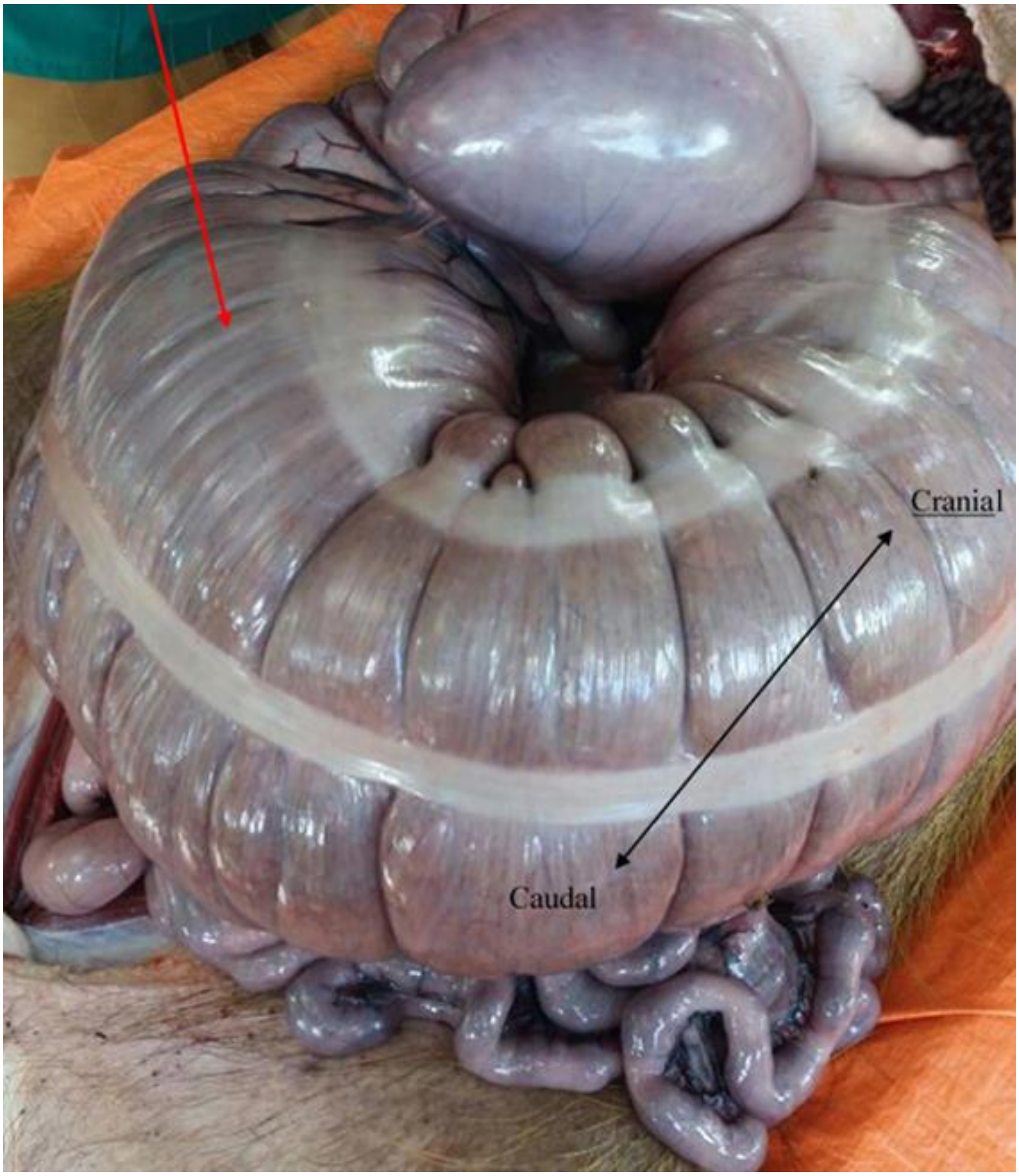

Figure 9.3 - Abdominal pathology I

Ventral view: Red arrow, depicting a pale extremely dilated cecum due to excessive gas formation. Cecal curvature in caudal direction.

Figure 9.4 - Abdominal pathology II

Ventral view: Open abdominal cavity, showing the increased volume of the cecum with intestinal and mesenteric congested and hypoxic blood vessels (purple discoloration). A) cecum; B) colon segment; C) sm. intestines; D) congested mesenteric blood vessels.

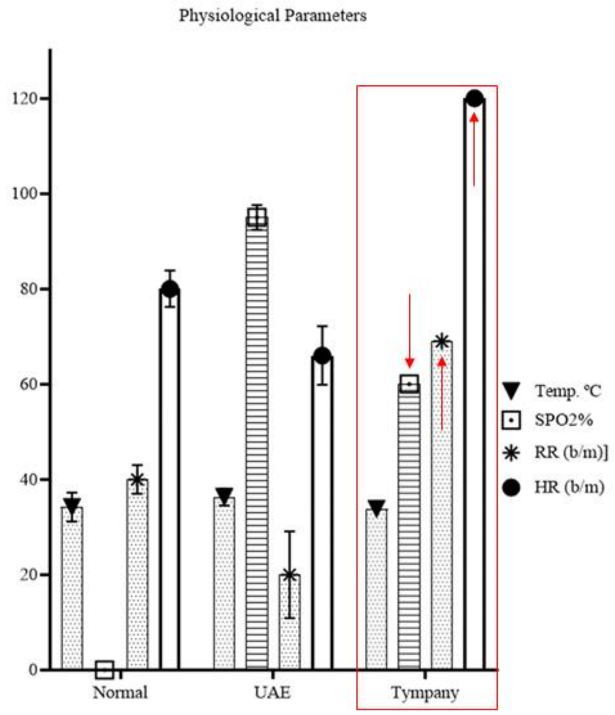

Figure 9.5 - Vital parameter comparison

Findings show significant vital sign differences between animals under normal condition (no anesthetic effects), animals chemically restrained (under anesthetic effects/UAE), and sedated animals, suffering acute cecal tympany, with red arrows indicating crucial vital parameter alterations. During the process from bloating to acute cecal tympany, heart rate and respiratory rate increased drastically, accompanied by a sharp SoO2% drop.

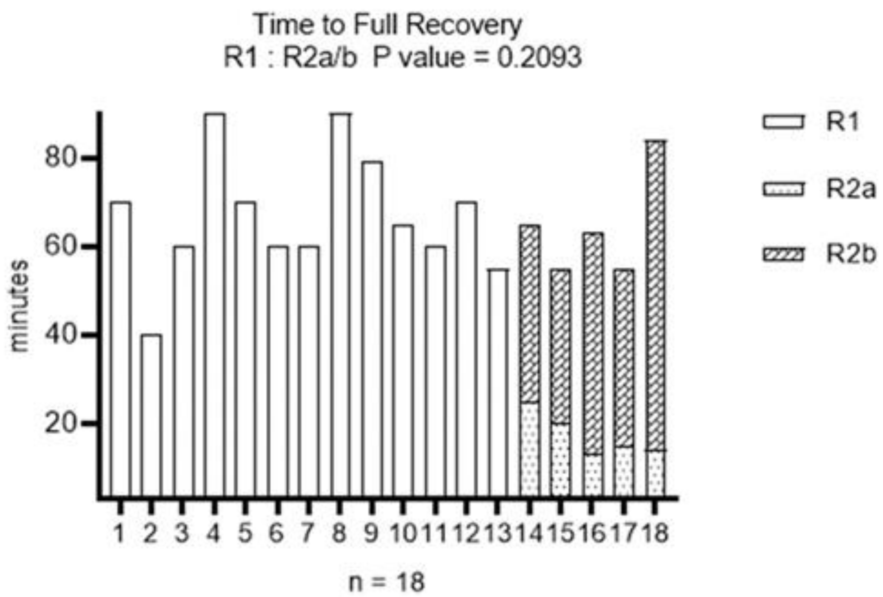

Figure 9.6 - Anesthetic recovery time comparison

* Normal SPO2 values are not available from conscious animals.

Comparing anesthetic recovery times, with and without the administration of Atipamezole as the reversal agent.

10. IMPACT OF VISCOSITY ON INJECTION QUALITY OF A VACCINE USING A REMOTE DRUG DELIVERY SYSTEM

10.1 ABSTRACT

We used remote drug delivery systems (RDDS) to evaluate how temperature (storage vs. ambient) and viscosity variance potentially affect delivery failure of an emulsion-based solution. To better understand Newtonian and non-Newtonian fluids and their temperature dependency, we investigated the dynamics of a water-in-oil emulsion (vaccine/adjuvant) and an aqueous solution (e.g. tranquilizer drugs). This was completed first in a lab-setting, for viscosimetric physics, and subsequently, under field conditions. Using dart minimum impact velocity for intramuscular injection (prior determined as ≥ 40 m/sec), we compared the quality of the dart-delivery system at two temperature variations (6.5ºC/20ºC) by examining drug deposit and measuring the total injected volume fired into a ballistic gel, covered with fresh pork hide. All executed dart-deployments performed satisfactorily, despite initial concerns that potential temperature-dependent viscosity would impact on the delivery quality. The only noteworthy drug loss we observed (> 10%) was associated with drug transfer and resulting residues in the syringe/dart dead-space and, depending on the drug/treatment, should be considered inaccurate dosing. Furthermore, due to the difficulty in reaching adequate impact velocity and the precision needed for a successful intramuscular (IM) injection, using a blowgun with specialized darts and emulsion-based products is discouraged.

Key words: Darts, emulsion, RDDS, remote drug delivery, tranquilizer, vaccines, wildlife

10.2 INTRODUCTION

The ability to treat animals in the wild from a distance and without the need for prior capture has many advantages, ranging from safety and easier logistics to lower costs and improved aspects of animal welfare. In our project—wildlife population control by immunocontraception—one of the vaccine-delivery strategies employed was a Remote Drug Delivery System (RDDS); specifically, a CO_2 projector and specialized darts. Although the concept seems straightforward and assumes a successful delivery, several important factors must be considered. For example, the user's ability to accurately use an RDDS (Cattet *et al.*, 2006), ambient influences, and the ballistic dynamics involved. This last factor is in part subject to the drug's specific characteristics, including the temperature-dependent viscosity, which can interfere with a complete drug deposit when using darts (Evans & DeNicola, 2015). In our study, we used an emulsion-based contraceptive vaccine for population control in free-ranging wildlife. Naturally, two crucial characteristics of such a treatment are to permit a single-dose application (no booster immunization needed), as the chances of finding the same individual again in the wild are very low. Moreover, it should have a long-term antifertility effect among components of high immunogenicity to stimulate a strong immune response (Miller *et al.*, 2008). These qualities are mostly found in an emulsion-based drug vehicle (adjuvant), which also have high viscosity. Hypothetically, an emulsion-based drug vehicle could provide the desirable characteristics, but at the same time, its viscosity may interfere with the drug deposit quality due to low flow characteristics. This is compared to an aqueous vehicle, as commonly used in tranquilizer drugs.

Today's RDDS technology has progressed significantly, and there are several high-quality products available, allowing for very specific application needs. For our field study, which used a water-in-oil emulsion-based vaccine, we opted for a single-port cannula dart to keep the vaccine localized intramuscularly. This is different from the administration of a tranquilizer, for which a tri-port cannula is indicated to promote an injection over a larger surface area, thereby promoting faster drug absorption and biological effects.

During a pilot study, the first of the vaccines were delivered by hand-syringes with a large diameter cannula (18 gauge), which were stored at the manufacturer's recommended storage temperature of 4–7ºC. When administering the first injection, it was realized that a heightened manual force was required to deposit the water-in-oil emulsion intramuscularly, prompting concern about potential delivery failure or incomplete volume delivery of the vaccine when using darts. Thus, this raised the question of what might cause such high resistance, and if it is just based on temperature, would it be necessary to bring the emulsion to an ambient temperature prior to deployment to achieve full delivery by an RDDS?

These questions led to the following side-project, with the aim to evaluate some key characteristics of fluid drugs. While there are a number of publications on the use of darts in wildlife, only a limited number address the dynamics and qualities of drug deposit, considering the possible influences of Newtonian and non-Newtonian fluid viscosities at variable temperatures. Furthermore, can specialized darts with vaccines be adequately deployed by blowguns?

10.3 OBJECTIVES

10.3.1 Lab

1) Compare Newtonian (aqueous) and non-Newtonian (emulsion) fluid behavior.
2) Identify differences in viscosity dynamics based on temperature variance.

10.3.2 Field

1) Classify the minimum impact velocity (MIV) necessary for adequate dart penetration, with minimal potential for tissue damage, for:
    a. CO_2 projector (20 meters)
    b. Blowgun (three meters)
3) Evaluate the quality of emulsion-based vaccine deposits (by weight), using specialized darts, by comparing the viscosity-behaviors under various temperature conditions (storage temperature, 2–7ºC; ambient temperature, 18–20ºC).
4) Identify any additional drug loss due to dead-space residue.

10.4 MATERIALS AND METHODS

10.4.1 Statistics

To evaluate the differences between the means for viscosity samples, a bi-caudal Welch's t-test was used considering unequal sample sizes. R software was used.

10.4.1 Lab work

Behavior of aqueous (Newtonian) and emulsion (non-Newtonian) fluids and their temperature-based viscosity dynamics.

To test the viscosity of both fluids a Programmable Rheometer Brookfield DV-III and a Cone Plate Version Viscometer with sample cone and different spindles were used.

10.4.1.1 WATER-IN-OIL EMULSION (NON-NEWTONIAN FLUID)

To test the immunocontraceptive vaccine, which is a water-in-oil based emulsion, we used the original sham vaccine/adjuvant (USDA, NWRC, Fort Collins, USA). We performed a viscosity test at two different temperatures. As the emulsion was already maintained at the manufacturer's recommended storage temperature of about 7ºC, this was used for the first test. For the second test, the temperature was increased to an ambient temperature of 20ºC using a circulating temperature bath, which was part of the Rheometer’s flow system. Subsequently, the viscosity was measured with different spindle sizes and rotation velocities. Each test was then repeated.

10.4.1.2 AQUEOUS SOLUTION (NEWTONIAN FLUID)

In order to simulate a tranquilizer drug, we prepared three different aqueous solutions: distilled water, saline (0.9%), and saline/ethylene glycol (90% v/v to 10% v/v). As these drugs are normally stored at an average ambient temperature of 20ºC, the viscosity test for all three fluid types was performed at the same ambient temperature.

10.4.2 IN-THE-FIELD

Prior to any shooting, we established the minimum impact velocity (MIV) required for adequate tissue penetration for an intramuscular injection with enough force to detonate the dart's explosive charge. The dart impact was kept to a minimum, thereby minimizing potential tissue damage.

10.4.2.1 BASIC SET-UP

A semi-outdoor area was used with fixed distances of 20 m for the CO_2 projector and 3 m for the blowgun. A special rifle shooting-support (Caldwell, USA); was placed on a sturdy table. A precision chronographer (Ballistic Precision Speed Chronograph with Caldwell Ballistic Chronograph app for digital read-outs); was placed 30 cm from the target field, capable of detecting object velocities in m/sec (accuracy +/- 0.25%). The target was a 112 mm thick piece of fresh pigskin, with an intact layer of adipose tissue and some visible areas of connected muscular tissue that served as an indicator for intramuscular (IM) injection. The pigskin was mounted onto a block of 10% ballistic gel, prepared by weight. To maintain the physical characteristics throughout the testing period, the gelatin block was surrounded with ice packs.

10.4.2.2 SHOOTING EXECUTION

The same shooter executed all shots. Distance darting was performed using a high-precision CO_2 projector (X-Caliber, 50 cal. [12.3 mm] Pneu-Dart, Inc., Williamsport, PA, USA) with a mounted scope. The projector-scope was zeroed-in at 20 m. The barrel was cleaned before each new shooting. Initial pressure settings and posterior pressure adjustments were based on dart size, distance, and manufacturer recommendations, using type P 1cc practice darts (Pneu-Dart, Inc.). For the drug delivery tests, type P 1cc darts were used: 1.25 in length cannula, gel collar, single-port, with an explosive charge (Pneu-Dart, Inc.). Initial practice tests were carried out using 1cc type P practice darts with a blowgun (23 in length by 12.3 mm diameter [Pneu-Dart, Inc.]), with a target distance of 3 m.

10.4.2.3 MINIMUM IMPACT VELOCITY

MIV refers to the lowest pressure setting for the dart to reach its target, resulting in an explosive charge and adequate intramuscular cannula penetration, but without bounce-back or drug volume loss and with minimum tissue damage. For the CO_2 projector, we identified the MIV for a distance of 20 m by determining the penetration depth. Optimal depth was considered when the dart's gel collar was positioned on the far-side of the adipose tissue, allowing for the cannula to reach muscle tissue. This was accomplished by gradually increasing the gas pressure of the CO_2 projector, measuring the projectile velocity, and grouping the impact results into the following two criteria: (1) needle penetration quality: Full = gel collar past skin/fat layer; ½ = gel collar stuck within skin/fat layer; F = failure of gel collar to penetrate skin, and (2) flight behavior, impact, and deposit quality. The setting with the lowest pressure that resulted in full penetration was used as the new MIV.

10.4.2.4 DRUG DELIVERY QUALITY

The quantity of drug deposited by the RDDS at two different temperatures was evaluated. The recommended vaccine storage temperature of 4–7ºC and the ambient temperature of 18–22ºC were evaluated to determine potential delivery failure (drug volume loss) due to temperature-dependent fluid dynamics (viscosity), as well as any potential loss of material dead-space. The vaccines were originally available in prefilled 1mL syringes and needed to be transferrred to the darts. Preceding execution, all syringes and darts were sorted and identified. A precision digital scale (500g x 0.01 g) was used to weigh (1) the syringes containing vaccine, (2) the empty darts, (3) the darts after being loaded, and (4) the darts after deployment. The difference in weight between the original vaccine-loaded syringe and the weight of the vaccine-filled dart after deployment was considered the net drug weight deposited. Any weight difference not being equal to the original vaccine-syringe weight was considered to be the amount of drug lost.

10.5 RESULTS

10.5.1 Lab work

The rheological behavior of the SHAM vaccine, a water-in-oil emulsion, indicated that there is no linear relationship between tension and strain rate. It is, therefore, a liquid with non-Newtonian behavior. As for the temperature, it was observed that the increase in temperature implies a greater fluidity of the emulsion, a behavior that is typical of viscous liquids (Figure 10.1). The emulsion’s viscosity was significantly different at two different temperatures, 6.5 vs. 19.7ºC (Bi-caudal Welch's t-test [$p = 0.04052$; 95% CI, 598.00 – 20160.65]). The aqueous solution presented a linear relationship between the stress and the rate of deformation, i.e. Newtonian behavior. Therefore, viscosity can be defined as being constant for a given temperature at temperatures of 7ºC and 20ºC (Figure 10.3). The aqueous viscosity was also significantly different at two different temperatures, 7ºC vs. 20ºC (Bi-caudal Welch's t-test [$p = 0.0000156$; 95% CI, -7.634565 – -5.850435]).

10.5.2 Field work

After determining the minimum force (velocity) necessary for complete pork skin penetration (fig. 10.4) using 1cc darts, we started with the manufacturer’s recommendations and subsequently adjusted the pressure settings.

10.5.3 Blowgun

We tested 1cc type P darts using a blowgun and showed that adequate dart penetration was difficult and infrequently achieved. Due to incomplete penetration and recurrent bounce-backs, the blowgun application was considered to be unsatisfactory and eliminated from further tests.

10.5.4 Drug volume loss during transfer from the original vaccine syringe to the dart

The difference was 0.01 g or 1% of the total drug weight (residue from syringe/needle dead space).

10.5.5. Dart weight-difference between pre-loaded (empty) and post-deployment

The final drug volume injected into the animal. The difference was 0.10 g, but it could not be determined whether the weight difference was purely the result of residue in the dart's dead space, and/or the type of propellant used. There was a statistically significant difference between the empty dart weight and post-deployment dart weight (mean, 0.1 g; $p < 0.01$; 95% CI, 0.076–0.123).

10.5.6 Post-darting weight difference in deposited volume due to temperature differences

When the deposited drug weight difference due to temperature (6°C vs. 22°C) were compared, the results showed no statistically significant difference ($p = 0.3194$).

10.5.7 Overall drug loss

Considering the original weight of 1 mL vaccine (approx. 1 g) and the accumulative residue losses due to transfer of the drug from the syringe to the dart and the drug volume injected (dart to animal), the total amount of drug loss observed was statistically significant (0.114 g; $p = 0.00613$; 95% CI, 0.081–0.146).

10.5.8 Drug volume differences due to dead-space residues

The accumulated residue from dead spaces depends on the make and model, cannula size (length and inner diameter) used for drug transfer (from syringe, or ampulla to the dart), and the size of the dart cannula (inner diameter) (fig. 10.5) and could be quite substantial. For example, a 1 mL vaccine may lose as much as 0.142 mL when using an 18G (0.84 mm ID*) x 3 in (7.62 mm) syringe needle and a 14G (1.6 mm ID) x 1.25 in (3.17 mm) dart cannula (Sigma-Aldrich, 2018), and an estimated syringe dead space (hub) volume (Küme *et al.*, 2012; Uc Davis & Ysp, 2016). **Inner canula diameter*. Using the following formulae:

$V = \pi \, r \, 2 \, h = \pi \cdot 0.422 \cdot 7.62 \approx 4.22 \text{ mm}^3$ for this syringe and needle (0.00426 mL)

$V = \pi \, r \, 2 \, h = \pi \cdot 0.82 \cdot 3.17 \approx 6.37 \text{ mm}^3$ for this dart and cannula (0.00639 mL)

The estimated syringe/dart hub space, based on a mean 1 mL syringe/dart is: 0.066 mL x 2 = 0.132 mL - total estimated residue volume = 0.142 mL.

10.6 DISCUSSION

The first by-hand-injections of an emulsion-based drug that we carried out corroborated our concerns about potential drug delivery failure because of increased fluid resistance. In the lab set-up, temperature dependent rheological behavior was evident, contrary to initial concerns about the quality of drug delivery. However, the field test resulted in satisfying drug delivery without the need to warm the emulsion to ambient temperatures beforehand. Nevertheless, a noteworthy drug volume loss was observed, caused by dead-space residues, mainly within the transfer needle and the dart cannula. In the case of sensitive dosage requirements, special consideration should be given to this fact. The use of a blow-gun is discouraged due to factors such as minimum dart velocity (adequate perforation), use of specialized darts, cc fill-amounts, drug characteristics, and factors dependent on the individual's skill set and ability that could potentially lead to failures regarding target-precision and bounce-back.

10.7 CONCLUSION

Each situation is unique, requires specific planning, and adequate equipment. Drug delivery with a specialized dart using any kind of propellant is certainly a traumatic option, especially when considering potential tissue damage, the initial pain of the dart injection, and the type of drug being administered. However, compared to alternatives such as physical restraint (net, trap, culling), which are associated with stress and risk of injury, employing remote drug delivery systems, with their high precision and reliability of complete drug delivery, may outweigh these concerns and take the welfare and safety of the animal into consideration.

10.8 ACKNOWLEDGMENTS

We thank the Pneu-Dart, Inc. for their cooperation and material contributions.
The project was partially funded by FAPESP, Sao Paulo, Brazil.

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10.10 FIGURES

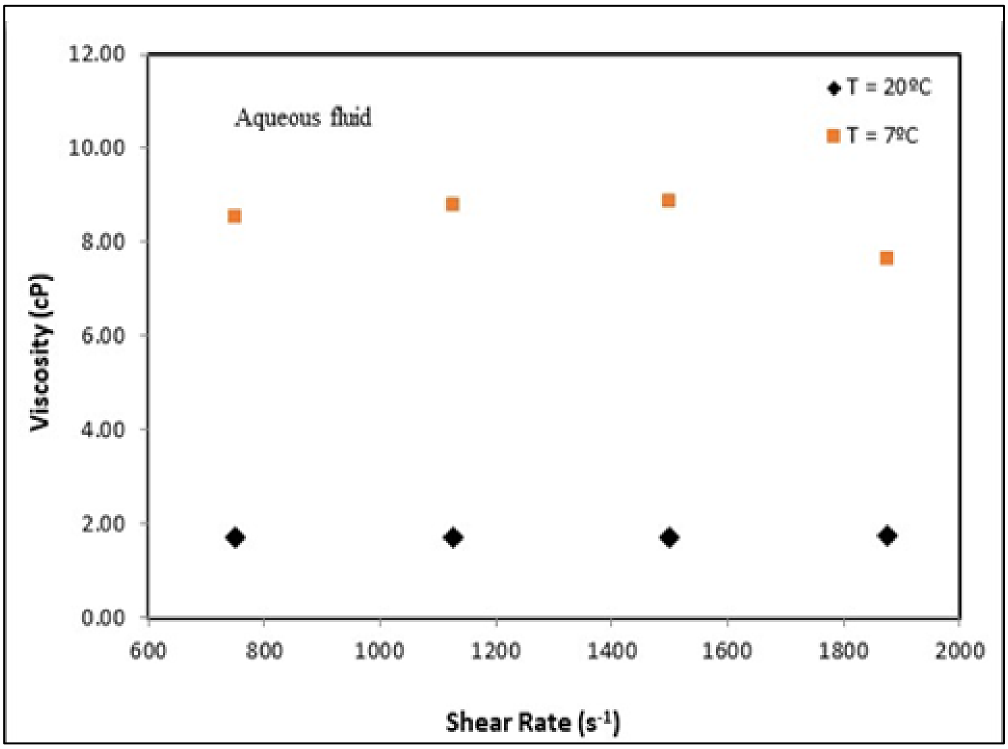

Figure 10.1 - Shear stress test of the aqueous solution at different temperatures.

The aqueous viscosity was also significantly different at two different temperatures, 7 ºC vs. 20 ºC (Bi-caudal Welch's t-test [p = 0.0000156; 95% CI, -7.634565 / -5.850435]).

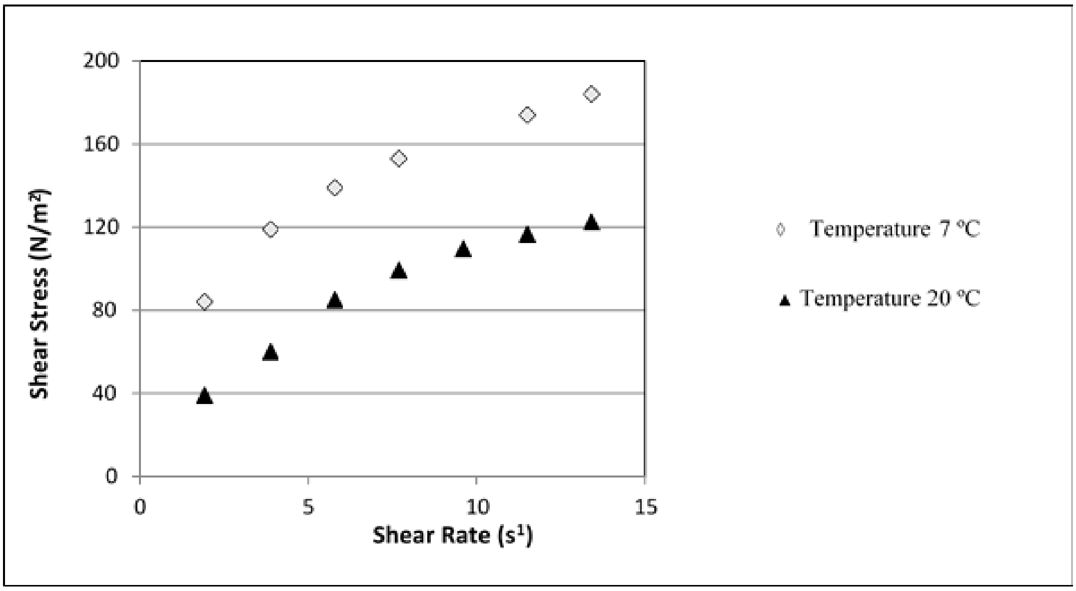

Figure 10.2 - Shear stress test of the emulsion-based solution at different temperatures

The emulsion's viscosity was significantly different at two different temperatures, 7 ºC vs. 20 ºC (Bi-caudal Welch's t-test [p = 0.04052; 95% CI, 598.00 – 20160.65]).

Figure 10.3 - Darting - Drug Deposit Evaluation

A) Pork skin mounted on a block of ballistic gel with two darts showing full penetration of the dart's cannula; B) dart needles with adequate penetration, reaching muscle tissue, secured by the gel collar at the far side of the adipose tissue layer (blue arrows); C & D) the quality of the drug deposits in the form of clouds within the ballistic gel [red arrows]. Source: D. Rosenfield, 2019

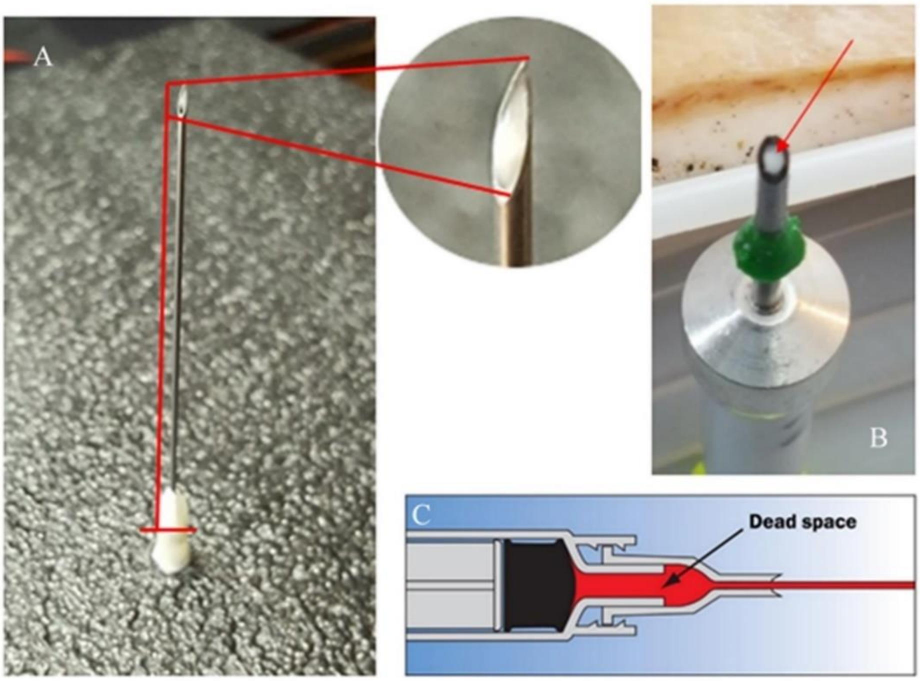

Figure 10.4 - Dead space evidence
Post-application A) red indicator, residue in a 3 in transfer needle dead space;
B) drug residue in the dart's 1 ¼ in cannula; C) illustrated dead space (UNC, 2018).
Source: D. Rosenfield, 2019.

11. FINAL CONSIDERATIONS

There is no “one-strategy-fits-all” for controlling wildlife and feral populations. In fact, successful management must be a species and environment-specific approach.

Although, the objective of the principal study was to evaluate the efficacy of an immunocontraceptive anti-GnRH vaccine, the multiplicity of the topics presented in the chapters indicate the diversity of factors to consider, resulting in several side-projects, each with their own complexity.

This thesis introduced a non-lethal immunocontraceptive method as part of a *Hydrochoerus hydrochaeris*-specific strategy to, not just effectively, but with utmost adequacy, control their population growth. Adequacy refers to taking into account the target-species' reproductive physiology and behavior, the environmental dynamics it inhabits, the antifertility agent's mechanism of action, and how it provides the desired contraceptive properties with the fewest number of adverse effects.

Initiating the study with a broad investigation on the necessity to control wildlife and feral populations subsequently led to the demand for a solution to combat associated zoonotic and tick-borne diseases being recognized. In this study, with emphasis on the capybara and its role in the maintenance and dissemination of Brazilian Spotted Fever, a proposal for a strategy to reduce, and perhaps even eradicate, the disease from an endemic area was presented.

During the study's intensive observational period, the capybara's social make-up and reproductive behavioral characteristics were identified and understood, which was imperative to preserve the groups integrity. This served as a basis for evaluating the reproductive conduct under the influence of the contraceptive agent.

The assessment of the anti-GnRH vaccine’s effectiveness as a contraceptive agent in *H. hydrochaeris* showed promising results. In addition to provoking > 90% infertility in males, it also preserved the alpha male's agonistic behavior, and therefore, the overall group's social integrity, while preventing male intruders from mating with the group's females, as long as they stayed within the group. Regarding the contraceptive effects in female capybara, observations showed no mating activity, with histopathological indications of altered folliculogenesis; however, their alloparental behavior was preserved.

To facilitate and maintain the animal's well-being throughout all events, a strategy of conditioning though positive reinforcement was successfully developed and employed.

Results from the side projects allowed the testing of a capybara-specific anesthetic protocol, which not only promoted quick chemical restraint and anesthetic recovery but facilitated semen collection through catheterization. In addition, it allowed us to become familiar with the risk of tympany, its avoidance, and treatment.

In summary, the research objectives were accomplished, rendering the capybara infertile for prolonged periods of time, while maintaining the group's integrity. With this, a strategy can be recommended that may be adequate to manage capybara population, permitting the mitigation of human-capybara conflicts, and more importantly, preventing zoonotic disease.

Besides providing the basis to create a national model in the future, the additional projects that were created seem conducive to indorse a consequential study, testing the hypothesis on reverting a *R. rickettsii*-endemic area, as proposed in chapter three, solely by reducing the inhabiting capybara population's natality rate.

12. APPENDIX I - THE PROJECT IN THE MEDIA

Media/Journalist	Type	Date Interview	Date Published	Title
Jornal do Campus USP Repórter: Laura Barrio (11) 9 8223 6936 http://www.jornaldocampus.usp.br/index.php/2019/03/usp-pretende-controlar-capivaras-na-raia/	Via telephone Printed Digital journal	March 23rd 2019	March 28th	USP pretende controlar capivaras na raia
Rede Globo Jornalista: Fabiane Leite (11) 9 9937 6412 https://globoplay.globo.com/v/7561412/	Filmed report at Raia	April 9th/19th 2019	April 23rd	Programa: Bem Bicho- Capivaras As capivaras mais vigiadas do Brasil
Jornal Estadão SP Paula Felix (11) 9 7694 4984 https://sao-paulo.estadao.com.br/noticias/geral,americano-estuda-capivaras-na-marginal,70002808363	Interview at Raia w/ photos Printed newspaper	April 23rd 2019	April 29th	Americano estuda capivaras na marginal
Jornalismo Tv Cultura Jornalista: Eugenio Araujo https://tvcultura.com.br/playlists/62_jornal-da-cultura-primeira-edicao-jornal-da-cultura-1a-edicao_CYyQYBMAQ-8.html	Film report at Raia	May 4th 2019	May 5th 2019	Sem titulo
TV Brasil Produtora: Thaís (11) 9 8303 3608 Jornalista: Raquel (11) 9 7303 5352 http://tvbrasil.ebc.com.br/reporter-brasil/2019/05/usp-faz-pesquisa-para-controlar-populacao-de-capivaras-nas-cidades	Film report at Raia	May 5th 2019	May 6th 2019	USP faz pesquisa para controlar população de capivaras nas cidades
TV Record Fala Brasil Jornalista: Eduardo Souza (11) 9 9782 5040 https://recordtv.r7.com/fala-brasil/videos/usp-testa-metodo-para-conter-febre-maculosa-no-brasil-080520191	Film report at Raia	May 8th 2019	May 12th 2019	USP testa método para conter febre maculosa no Brasil
Jornal Folha* Repórter: Guilherme (11) 9 8402 7366	Interview at Raia w /photos	May 6th 2019	May 14th, 2019	
Em inglês				

Researcher Tests Vaccine that Sterilizes Large Rodents Living Near Body of Water on USP Campus https://www1.folha.uol.com.br/internacional/en/scienceandhealth/2019/05/researcher-tests-vaccine-that-sterilizes-large-rodents-living-near-body-of-water-on-usp-campus.shtml				
Em espanhol Un investigador prueba en los exteriores de la Universidad de São Paulo una vacuna que esteriliza capibaras https://www1.folha.uol.com.br/cotidiano/2019/05/pesquisador-testa-vacina-que-esteriliza-capivaras-na-raia-da-usp.shtml		Folha.com (edição em espanhol) - Publicado em 14 maio 2019		
TV Record Domingo Espetacular Produtora: Mayella Itié (11) 9 7262 6422 Jornalista: Felipe (11) 9 7107 5923	Film report at Raia	8_5_19	8_5_19	

Fonte principal	**Média usando**		**Data**
Folha	Gaucha ZH Geral	https://gauchazh.clicrbs.com.br/geral/noticia/2019/05/pesquisador-testa-vacina-que-esteriliza-capivaras-na-raia-da-usp-cjvn0za6o038x01ohct5y7hr2.html	
	Agora São Paulo	https://namidia.fapesp.br/veterinario-testa-vacina-contra-a-fertilidade-das-capivaras/185250	14/5/19
Estadual	UOL Notícias	https://noticias.uol.com.br/ultimas-noticias/agencia-estado/2019/04/29/americano-estuda-capivaras-na-marginal.htm	29/4/19
	FAPESP Na Média	https://namidia.fapesp.br/guarulhosweb/367/0	
	Via FAPESP na Média	Veterinário testa vacina contra a fertilidade das capivaras Agora São Paulo - Publicado em 14 maio 2019 Americano estuda capivaras na Marginal Mix Vale - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Correio do Papagaio online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Portal do Holanda - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal	

		Diário de Notícias online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Tribuna do Sertão - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Jornais Virtuais - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Folha da Região (Araçatuba, SP) online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Tarobá News - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Tribuna PR - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Meon - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Guarulhosweb - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Diário do Sudoeste (Pato Branco, PR) online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Tribuna do Interior online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal The World News (Ucrânia) - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Massa News - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Metro News - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Tribuna do Agreste - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal TV Vertentes - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Portal R7 - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Folha de Valinhos online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Jornal da Cidade (Bauru, SP) online - Publicado em 29 abril 2019	

		Americano estuda capivaras na Marginal Repórter Diário - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Jornal do Oeste (Toledo, PR) online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Leia Já - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Diário da Jaraguá - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Associações Hoje - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal A Semana News - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal O Estado de S. Paulo - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal A Tarde (BA) online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Diário do Grande ABC online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal UOL - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Estado de Minas online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal BOL - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal IstoÉ Dinheiro online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal A Crítica (MS) online - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Estadão.com - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal Terra - Publicado em 29 abril 2019 Americano estuda capivaras na Marginal IstoÉ online - Publicado em 29 abril 2019 Gazeta de S. Paulo online - Publicado em 13 maio 2019 A pesquisa de Derek Andrew Rosenfield propõe método de	

		Pesquisador testa vacina que esteriliza capivaras na raia da USP BN - Bahia Notícias - Publicado em 14 maio 2019 Pesquisador testa vacina que esteriliza capivaras na raia da USP Portal Salvador Dez - Publicado em 14 maio 2019 Pesquisador testa vacina que esteriliza capivaras na raia da USP Yahoo! - Publicado em 13 maio 2019 Pesquisador testa vacina que esteriliza capivaras na raia da USP Bem Paraná online - Publicado em 13 maio 2019 Pesquisador testa vacina que esteriliza capivaras na raia da USP Zero Hora online - Publicado em 13 maio 2019 Americano estuda capivaras na Marginal Tribuna do Sertão - Publicado em 29 abril 2019	

13. APPENDIX II - GOVERNMENTAL INTEREST

GOVERNO DO ESTADO DE SÃO PAULO
SECRETARIA DE INFRAESTRUTURA E MEIO AMBIENTE
SUBSECRETARIA DO MEIO AMBIENTE
COORDENADORIA DE BIODIVERSIDADE E RECURSOS NATURAIS

São Paulo, 14 de janeiro de 2019.

À Diretoria da FAPESP

Tendo tomado conhecimento da proposta **"Controle da População de Capivaras por Imunocontracepção"**, a ser submetida à Fundação de Amparo à Pesquisa do Estado de São Paulo para captação de recurso pelo pesquisador Derek A. Rosenfield, Doutorando em Reprodução Animal/Silvestres pela Faculdade de Medicina Veterinária e Zootecnia (FMVZ) da Universidade de São Paulo (USP), reconhecemos a importância do projeto para a erradicação da circulação da bactéria *Rickettsia rickettsii*, agente etiológico da doença Febre Maculosa Brasileira, em determinadas áreas endêmicas no estado de São Paulo.

Este Departamento de Fauna (DeFau/CBRN/SMA/SIMA) desde 2011 desenvolve políticas públicas junto à Superintendência de Controle de Endemias do Estado de São Paulo (SUCEN/SES) visando à prevenção e controle da Febre Maculosa Brasileira, principalmente no que diz respeito ao estabelecimento de diretrizes voltadas ao manejo populacional da espécie *Hydrochoerus hydrochaeris* (capivara) como medida estratégica, conforme evidenciado na Resolução Conjunta SMA/SES n° 01/2016.

Ressaltamos, assim, a grande relevância de pesquisas científicas envolvendo métodos para o manejo reprodutivo de capivaras, cujo resultado é de total interesse ao Departamento de Fauna.

Esperamos que os esforços no desenvolvimento deste projeto tragam resultados efetivos para prevenção e redução de casos humanos de Febre Maculosa Brasileira nos municípios paulistas afetados, bem como diminuam os casos em que o manejo de retirada (abate assistido) de capivaras seja necessário.

Ficamos à disposição para eventuais esclarecimentos adicionais.

Atenciosamente,

Vilma Clarice Geraldi
Diretora do Departamento de Fauna
CBRN/SMA/SIMA

SECRETARIA DE ESTADO DA SAÚDE
SUPERINTENDÊNCIA DE CONTROLE DE ENDEMIAS
"SUCEN"

São Paulo, 26 de Dezembro de 2018

Venho por meio deste e a quem posso interessar externar a importância de estudos e desenvolvimento técnico e científico quanto a estratégias para o controle populacional de capivaras no Estado de São Paulo.

As capivaras são animais silvestres que têm se beneficiado da ocupação humana e em muitos locais no Estado têm apresentado populações supranumerárias com grupos que podem ultrapassar 40 indivíduos formando populações com mais de 300 animais em regiões como Piracicaba, Campinas e Marília.

As capivaras são parte fundamental no ciclo epidemiológico da bactéria *Rickettsia rickettsii* agente etiológico da Febre Maculosa Brasileira, responsável por mais de 700 casos humanos (mais de 300 óbitos) da doença nos últimos 10 anos no Estado de São Paulo. A bactéria é transmitida pelo carrapato *Amblyomma sculptum* (Carrapato-Estrela) e capivaras, em especial os filhotes no primeiro ano de vida, são responsáveis pela amplificação horizontal do agente entre os vetores.

A estabilização das populações de capivaras através da diminuição da capacidade reprodutiva é a principal estratégia para a redução da circulação da bactéria. Embora estratégias para esse fim sejam conhecidas para animais domésticos, a utilização em animais silvestres é complexa e carente de conhecimento técnico.

Assim, a proposta de desenvolvimento técnico científico para a utilização de imunocontraceptivos é de grande importância para o Sistema de Saúde Paulista em especial para as instituições que trabalham no controle e prevenção de endemias.

Respeitosamente,

Adriano Pinter dos Santos.

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