MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA
DOUTORADO EM SAÚDE E PRODUÇÃO ANIMAL NA AMAZÔNIA

GESSIANE PEREIRA DA SILVA

AVALIAÇÃO DOS PARÂMETROS MORFOFISIOLÓGICOS COMO BIOMARCADORES EM PRIMATAS NEOTROPICAIS RELEVANTES PARA PESQUISA BIOMÉDICA

BELÉM

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2023

Dados Internacionais de Catalogação na Publicação (CIP)
Bibliotecas da Universidade Federal Rural da Amazônia
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PEREIRA DA SILVA, GESSIANE
AVALIAÇÃO DOS PARÂMETROS MORFOFISIOLÓGICOS COMO BIOMARCADORES EM PRIMATAS NEOTROPICAIS RELEVANTES PARA PESQUISA BIOMÉDICA / GESSIANE PEREIRA DA SILVA. - 2023.
112 f. : il. color.

Tese (Doutorado) - Programa de Pós-Graduação em Saúde e Produção Animal na Amazônia (PPGSPAA), Campus Universitário de Belém, Universidade Federal Rural Da Amazônia, Belém, 2023.
Orientador: Prof. Dr. FREDERICO OZANAN BARROS MONTEIRO
Coorientador: Prof. Dr. RAFAELA SAYURI CICALISE TAKESHITA.

1. 1. Medicina laboratorial; 2. Morfofisiologia comparada; 3. Bem-estar em animais silvestres; 4. Sanidade animal; 5. Primatas Não-Humanos. I. OZANAN BARROS MONTEIRO, FREDERICO, *orient.* II. Título

CDD 571.1

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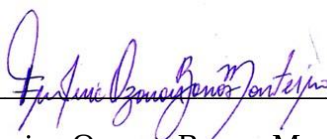

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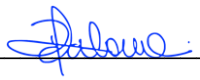

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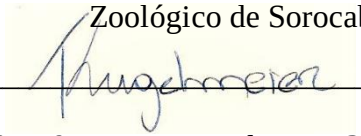

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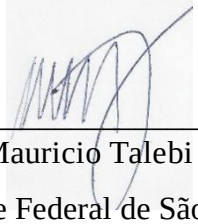

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AGRADECIMENTOS

A Deus, por me guiar nesta jornada desde o princípio, por ser um bom Pai e não abandonar aqueles que O buscam. A minha família, em especial minha mãe Zefina Pereira e as minhas irmas Luzia, Jessica e Rose, por todo apoio, incentivo, paciência e por estarem comigo em todos os momentos.

Ao meu orientador, Frederico Monteiro (Fred) por ser uma das pessoas mais generosas que de bom coração que já conheci! Obrigada por ser um grande entusiasta deste trabalho, e por me "adotar" no mundo científico, por seu apoio incondicional e amizade. Sou uma pessoa muito afortunada por tê-lo conhecido.

A minha coorientadora Rafaela Takeshita (Sayuri), por ter me acolhido desde o início em seu projeto, orientado, incentivado e trabalhado incansavelmente para a realização deste trabalho. Agradeço também a Sayuri e ao Wilson Chung (my best friend) por seu acolhimento durante o doutorado sanduíche, em que abriram as portas de seus laboratórios e de sua casa, me ensinaram e auxiliaram. Jamais esquecerei o que fizeram por mim nesse período

Ao Centro Nacional de Primatas (CENP), que cedeu seu espaço de campo e laboratório para a realização desta pesquisa. Aos amigos que fiz ao longo do trabalho, e que foram essenciais para o desenvolvimento da pesquisa: as amigas Josi Melo e Ana Karolyna, que estiveram comigo durante as etapas de coleta, processamento e dosagens. Aos técnicos do CENP que atuaram de forma imprescindível neste trabalho: Ricardo, Amauri, Potira, Lilian, Obadias, Sheila, Osvaldo, Flaudemir, Viana, Monique, Alfredo, Cledja, Keyla, Karol. E aos médicos veterinários que auxiliaram na avaliação dos animais e coleta dos dados: Rafael de Andrade, Aline Imbeloni e Ramiro Dias.

Aos professores e pesquisadores: Paloma Arakaki, Mauricio Talebi e Rodrigo Teixeira por todo auxílio e suporte na logística de coletas e processamento de amostras, e pelos dias de trabalho intenso em São Paulo! A Tatiana Kugelmier, pelas sugestões e melhorias neste trabalho.

Aos amigos da Ufra: Thyago Haber, Alana Lorena, Flavia Matos, e Sandy Matos por sempre serem prestativos e acolhedores! Aos amigos que tive o prazer de conhecer na Kent State University: Emilee Hart, Daniele Jones, Salan Ghaju e Fanan Aboalrob e Greta Siler, por todo apoio e pelos dias de muita diversão!

Ao Jayme Santos, melhor secretário da Pós-Graduação! Obrigada por todo suporte que nos dá, desempenhando sua função com excelência! Ao Programa de Pós-Graduação em Saúde e Produção Animal na Amazônia (PPGSPAA/UFRA) e ao Procad Amazônia. E as agências de financiamento CAPES e CNPq pelo suporte e apoio a ciência brasileira

SUMÁRIO

RESUMO

Os primatas não humanos (PNH) compõem um grupo bastante variado e têm diversas semelhanças comportamentais e fisiológicas com os humanos. Por esse motivo, são excelentes modelos comparativos para estudos biomédicos, evolutivos, e do envelhecimento. Assim, é fundamental o acompanhamento da sanidade dos PNH por meio de biomarcadores de saúde e senescência, que incluem exames de rotina como hemograma, bioquímica sérica, dosagens hormonais e exames de imagem. O principal grupo de hormônios para avaliar estresse e bem estar são os glicocorticóides (GC), mas tem-se buscado associar os GC com outros marcadores como o do sulfato de deidroepiandrosterona (DHEAS), para uma avaliação mais precisa da condição desses animais. Dessa forma, o Capítulo 1 é uma revisão de literatura a respeito do uso do macaco-de-cheiro (gênero *Saimiri*) como modelo experimental para estudos de desordens pélvicas na mulher, discutindo as principais semelhanças e diferenças encontradas. O Capítulo 2 trata da validação do ensaio de DHEAS em bugio (*Alouatta caraya*), macaco-da-noite (*Aotus azarai*) e macaco-prego (*Sapajus apella*), em amostras de fezes e sangue. O Capítulo 3 consiste na avaliação renal por meio da ultrassonografia e exames laboratoriais em bugios (n = 20) e macacos-prego (n = 21), considerando os efeitos do sexo e idade. E o Capítulo 4 considerou os resultados dos exames de hemograma e bioquímica sérica também focados em bugios (n = 20) e macaco-prego (n = 21). De forma geral, observou-se que o macaco-de-cheiro é um bom modelo comparativo para estudo dos distúrbios pélvicos na mulher. Além disso, foi possível validar o ensaio de DHEAS nas amostras de sangue e fezes das três espécies estudadas, confirmadas pelo aumento da produção dos hormônios-alvo, e pelos testes de paralelismo e precisão, com maiores concentrações observadas nos bugios. Na avaliação renal, observou-se diferenças entre sexos, idade e espécie, sugerindo que o bugio possui um mecanismo de envelhecimento mais rápido do que o macaco-prego, no entanto, altas demandas metabólicas neste último. Nos exames hematológico e da bioquímica sérica observou-se efeito da espécie, sexo, idade e nível de parasitismo em diversos parâmetros. Portanto, os PNH, em especial os platirrinos, são animais de grande importância nos esstudos biomédicos e da morfofisiologia comparada. As diferenças encontradas nos biomarcadores dessas espécies podem indicar como elas se adaptam às condições de habitat e manejo, e as implicações desta para a sobrevivência desses animais, além de serem clinicamente relevantes para avaliar a saúde animal e a adequação da criação desses programas.

Palavras-chave: Medicina laboratorial; Morfofisiologia comparada; Bem-estar em animais silvestres; Sanidade animal; Primatas Não-Humanos.

ABSTRACT

Non-human primates (NHP) are a varied group and have many behavioral and physiological similarities with humans. For this reason, they are excellent comparative models for biomedical, evolutionary, and aging studies. Thus, it is essential to monitor the health of NHP using health and senescence biomarkers, which include routine tests such as hemograms, serum biochemistry, hormone levels, and imaging tests. The main group of hormones used to assess stress and well-being are glucocorticoids (GC), but attempts have been made to associate GC with other markers, such as dehydroepiandrosterone sulfate (DHEAS), for a more accurate assessment of the condition of these animals. Thus, Chapter 1 is a review regarding the use of the squirrel monkey (genus *Saimiri*) as an experimental model for studies of pelvic disorders in women, discussing the main similarities and differences found. Chapter 2 approaches the validation of the DHEAS assay in howler monkeys (*Alouatta caraya*), owl monkeys (*Aotus azarai*), and capuchin monkeys (*Sapajus apella*), in feces and blood samples. Chapter 3 consists of renal evaluation using ultrasound and laboratory tests in howler monkeys (n = 20) and capuchin monkeys (n = 21), considering the effects of sex and age. And Chapter 4 considered the results of blood tests and serum biochemistry and focused on howler monkeys (n = 20) and capuchin monkeys (n = 21). In general, it was observed that the squirrel monkey is a good comparative model for the study of pelvic disorders in women. In addition, the validation of DHEAS assay in blood and feces samples from the three studied species was confirmed by the increase in the target hormones, the parallelism, and precision tests, showing higher concentrations in howler monkeys. In the renal evaluation, differences between genders, ages, and species were found, suggesting that howlers have a faster aging mechanism than capuchin monkeys, however, high metabolic demands in the latter. In the hematological and serum biochemistry tests, the effect of species, sex, age, and level of parasitism on several parameters was observed. Therefore, the NHP, especially the platyrrhines, are important animals in biomedical and comparative morphophysiology studies. The differences found in the biomarkers of these species may indicate how they adapt to habitat and management conditions, and the implications of this for the survival of these animals and demonstrate the clinical relevance for assessing animal health and the adequacy of the creation of these programs.

Keywords: Comparative morphophysiology; welfare in wild animals; adrenal steroids; animal health.

1 INTRODUÇÃO

Os primatas não humanos (PNH) têm várias semelhanças comportamentais e fisiológicas com os humanos, e por esse motivo são muito utilizados em estudos evolutivos (URLACHER *et al*., 2022) e pesquisas biomédicas voltadas a genética (LEE *et al*., 2018; VALLENDER *et al*., 2023), neurociência (MUNIZ *et al*., 2021), doenças infecciosas (PRATT-RICCIO *et al*., 2021) e morfofisiologia comparada (FALK, 2015; SILVA *et al*., 2021). Os PNH são também excelentes modelos comparativos para o estudo do processo de envelhecimento, o que tem auxiliado na compreensão de seus mecanismos básicos em humanos (TARDIF *et al*., 2014; DIDIER *et al*., 2016; ROSS; SALMON, 2019; SILVA *et al*., 2022).

Assim, o acompanhamento da sanidade desses animais por meio de exames laboratoriais é fundamental para a manutenção das colônias, pois auxiliam na avaliação geral do estado de saúde e no exame do curso da doença, juntamente com a eficácia da terapia aplicada (CANALES-ESPINOSA *et al*., 2015). Essa avaliação geralmente se dá mediante a análise de biomarcadores, tais como moléculas, índices ou comportamentos, que podem indicar o funcionamento fisiológico atual e são frequentemente usados para monitorar a saúde e diagnosticar doenças (WAGNER *et al*., 2016; EDES *et al*., 2022).

Diversos fatores influenciam nos biomarcadores, como a espécie (PALME *et al*., 2005, 2019), o estado clínico (de MELO *et al*., 2019; TAKESHITA *et al*., 2022), fatores genéticos (REGE *et al*., 2019), ambiente (TAKESHITA *et al*., 2013; SPENCER; DEAK, 2017), estado reprodutivo (TAKESHITA *et al*., 2017; 2016; URLACHER *et al*., 2022), sexo (PANNKUK *et al*., 2015; WHITHAM, 2020) e idade (TAKESHITA *et al*., 2013; WHITHAM, 2020). No entanto, para vários desses biomarcadores, como os hormonais, existem poucos ensaios validados e a falta de informações sobre os parâmetros em espécies selvagens dificultam a aplicação prática (EDES *et al*., 2022).

Para PNH, a avaliação da saúde geralmente inclui exames de rotina como hemograma e bioquímica sérica (de MELO, *et al*., 2019; GONÇALVES *et al*., 2019; CARDOSO *et al*., 2021a, 2021b; ROVIROSA-HERNÁNDEZ *et al*., 2022) e avaliação do estresse por mensuração de cortisol e seus metabólitos (PALME *et al*., 2005; WHITHAM, BRYANT, MILLER, 2020; URLACHER *et al*., 2022). Com relação ao estudo da senescência, as pesquisas são bem mais desenvolvidas na parvordem Catarrhini (primatas do Velho Mundo - PVM), mas o interesse na parvordem Platyrrhini (primatas do Novo Mundo - PNM ou neotropicais) tem aumentado (DIDIER *et al*., 2016). Tal interesse está relacionado ao fato de que esses animais apresentam maior facilidade de manejo em ambientes artificiais ou ex situ,

quando comparado 10 aos Catarrhini, como exemplo o menor porte e possibilidade de colônias com grande número de indivíduos (SILVA *et al*., 2021; PRATT-RICCIO, 2021). No entanto, os Platyrrhini são um grupo bastante heterogêneo e as diferenças inerentes a cada espécie devem ser consideradas antes de estabelecer os estudos experimentais ou a extrapolação interespecífica.

Dessa forma, esta pesquisa visou abordar a importância biomédica dos primatas Neotropicais, e avaliar os potenciais biomarcadores que indicam o estado de sanidade e senescência, por meio de exames clínicos e laboratoriais, que possam elucidar as adaptações dessas espécies a seus respectivos estilos de vida.

2 REVISÃO DE LITERATURA

A ordem Primates apresenta grande variedade de animais, com mais de 600 espécies. Esse numero tem aumentado devido as contínuas descobertas. Esses animais estão concentrados principalmente em países como Brasil, Madagascar, Indonésia, Republica do Congo, Colômbia e Peru. A ordem é divida em duas subordens. A subordem Haplorrhini e por sua vez, dividi-se nos Tarsiformes ou Primatas da Ásia e da Indonésia, Platyrrhini ou Primatas do Novo Mundo ou Neotropicais e Catarrhini ou Primatas do Velho Mundo. A subordem Strepsirrhini ou Prossímios contém os lêmures de Madagascar, lóris da África, Índia e Sudeste Asiático) (MITTERMEIER; WILSON; RYLANDS, 2013; MAYOR; PLANA, 2021). A figura 1 detalha a divisão da ordem Primates, baseada na análise de filogenia genômica.

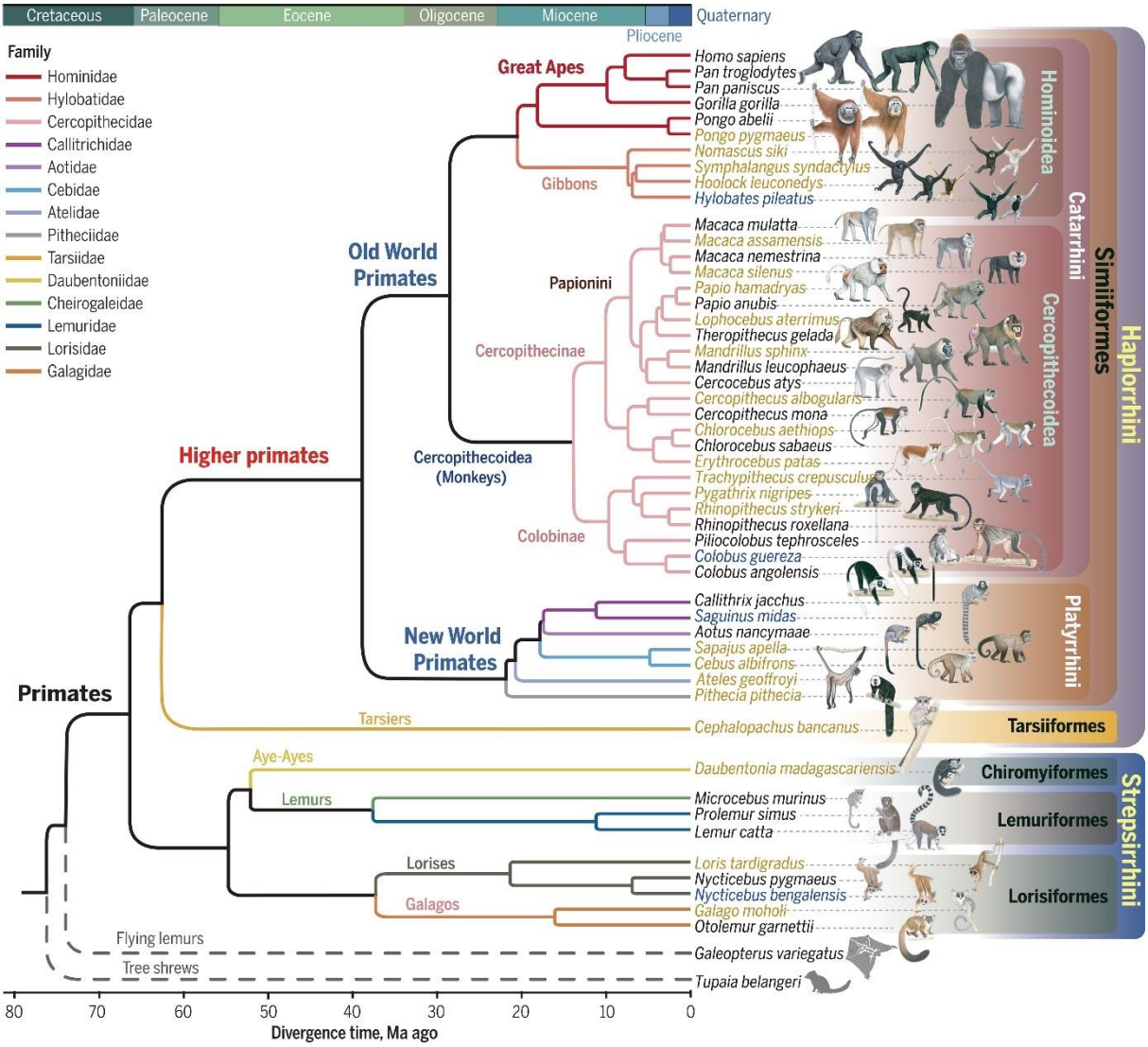

Figura 1. Filogenia genômica de primatas. Fonte: SHAO *et al*. (2023).

1.1 A diversidade dos platirrinos

Considerando a distribuição geográfica de PNH, o Brasil é o país com o maior número de primatas conhecidos. Segundo dados da Sociedade Brasileira de Primatologia (SBP, 2014) e do Instituto Chico Mendes de Conservação da Biodiversidade (ICMBio, 2022) são reconhecidos 19 gêneros, divididos em 5 famílias, com cerca de 140 táxons considerando espécies e subespécies, dos quais 83 são endêmicos do Brasil. No entanto, cerca de 40% dessas espécies encontram-se ameaçadas de extinção (SBP, 2014). Na edição de 2022-2023 da lista dos primatas mais ameaçados do mundo segundo o IUCN (*International Union for Conservation of Nature*) *Species Survival Comission Primate Specialist Group* encontram-se quatro espécies brasileiras, sendo elas: sagui-da-serra (*Callithrix flaviceps*), macaco-caiarara (*Cebus kaapori*), macaco-zogue-zogue (*Plecturocebus grovesi*) e macaco-bugio-ruivo (*Alouatta guariba*).

Os primatas neotropicais estão distribuídos em todos os biomas brasileiro, sendo algumas espécies endêmicas da Floresta Amazônica e Mata Atlântica (OPPENHEMER, 1981; FRESSE; BICCA-MARQUES; SILVA; GOMES, 2006; KIERULFF *et al*., 2007). Quase todas as espécies possuem hábitos diurnos, sendo o gênero *Aotus* o único considerado noturno, apesar de algumas espécies dentro deste gênero apresentarem adaptação aos hábitos diurnos (REIS *et al*., 2015). Em relação ao tamanho do grupo, os Platyrrhini podem apresentar grande variação, desde os pequenos como os dos calitriquídeos (3-10 indivíduos) (VERONA; PISSINATTI, 2014), ou grandes grupos que chegam a 35 indivíduos, como os formados por macacos-prego (gênero *Sapajus*) (BICCA-MARQUES; SILVA; GOMES, 2006). Outro aspecto relevante é a possibilidade de animais de gêneros (e portes) diferentes conviverem em um mesmo fragmento, como ocorre com espécies que conseguem consumir folhas jovens em borda de matas, enquanto outras se alimentam predominantemente dos insetos disponíveis (BOLT *et al*., 2021; JESUS *et al*., 2023).

Esses animais apresentam grande variabilidade nas características fenotípicas e biológicas. Nesse sentido, na natureza os primatas consomem grande variedade de alimentos de origem animal e vegetal, no entanto, as proporções dos tipos de alimento podem diferir bastante entre as espécies, desde as extremamente frugívoras como o gênero *Ateles* (REIS *et al*., 2015, BOLT *et al*., 2021), onívoros como os gêneros *Cebus* e *Sapajus*, e os majoritariamente folívoros como o gênero *Alouatta* (REIS *et al*., 2015). A variedade de possibilidades alimentares implica em diferenças morfofisiológicas e adaptativas dessas espécies tais como a dentição (TALEBI *et al*., 2016), tamanho do trato gastrintestinal

(MAYOR; PLANA, 2021; JESUS *et al*., 2023), taxa metabólica e tempo de trânsito intestinal (~35 h para o bugio versus ~3,5h em macaco-prego) (MILTON, 1984; PALME, 2019).

A massa corpórea é outro importante fator variável entre as espécies de platirrinos. Neste aspecto, a família *Callitrichidae* comporta os menores primatas conhecidos, como o sagui-leãozinho (*Cebuella pygmaea*) que possui massa corpórea média de 120 gramas, enquanto a família *Atelidae* é composta com os maiores primatas das Américas como os muriquis (gênero *Brachyteles*), que pesam até 15 kg (TALEBI *et al*., 2011; BICCA-MARQUES; SILVA; GOMES, 2006; Di FIORE; CAMPBELL, 2007). Essa diferença na massa corpórea em algumas espécies está ligada ao sexo (dimorfismo sexual), presente no bugio, em que as fêmeas pesam em média 5,7 kg comparadas aos machos 7,5 kg, enquanto outros primatas, como os macacos-da-noite não apresentam dimorfismo sexual aparente sem diferença significativa entre o peso de machos (média de 0,96 kg) e fêmeas (média de 0,85 kg) (MILTON, 1980; BAER, 1994; FERNANDEZ-DUQUE, 2011; REIS *et al*., 2015).

A reprodução desses animais também apresenta grande variabilidade. No que diz respeito às estruturas de união, eles podem viver em casais monogâmicos fixos; pequenos grupos familiares poligâmicos, com presença de dominância entre as fêmeas, como ocorre no gênero *Callithrix*, em que as dominantes inibem as fêmeas subordinadas. É possível observar também grupos aleatórios de primatas, sem a formação de casais (MITTERMEIER; WILSON; RYLANDS, 2013). Os sistemas de acasalamento também são diversificados, e podem ser classificados em multimacho/multifêmea como ocorre em nos gêneros *Ateles*, *Brachyteles*, *Cebus*, *Lagothrix* e *Saimiri*, com fêmeas copulando com mais de um macho, ou ainda monogâmico como ocorre com *Aotus*, *Callicebus*,*Callithrix*, *Pithecia* e *Saguinus*, no qual as fêmeas copulam com um ou poucos machos (DIXON, 1998; LEÃO *et al*., 2020). Em relação aos machos, existem variações do fluido seminal, com animais que apresentam sêmen de consistência fluida até aqueles que apresentam o plug copulatório, sendo essas diferenças relacionadas ao sistema de acasalamento da espécie (DOMINGUES *et al*., 2011), e devem ser considerados para a adequeação dos protocolos de biotecnologias reprodutivas e (ARAKAKI *et al*., 2019a,b; LEÃO *et al*., 2020). Além disso, existem diferenças na morfologia das glândulas acessórias, como a vesícula seminal que se apresenta maior nas espécies com acasalamento do tipo multimacho/multifêmea, e menor nas espécies monogâmicas (DIXON; ANDERSON, 2002).

Em relação a morfofisiologia reprodutiva das fêmeas, algumas espécies apresentam ciclo menstrual, como macaco-prego e macaco–da-noite (MAYOR *et al*., 2019; de LIMA CARDOSO *et al*., 2021) e bugio (KUGELMEIER *et al*., 2011), ou ciclo estral, como saguis

(HEARN, 1983). O período médio da gestação varia de 117-159 dias no gênero *Aotus* (de LIMA CARDOSO *et al*., 2021) a 230-255 dias no *Brachyteles* (TALEBI *et al*., 2011) com nascimento de apenas um filhote por parto, à exceção dos calitriquídeos que geralmente apresentam partos gemelares (HEARN, 1983). O desmame ocorre de 2 meses em calitriquídeos a 24 meses em atelídeos (TALEBI *et al*., 2011; MITTERMEIER; WILSON; RYLANDS, 2013; de ANDREADE *et al*., 2018), o que demonstra que, apesar de serem animais altriciais, com longo período de cuidado parental pós-natal, apresentam diferentes graus de altricialidade (DERRICKSON, 1992; de ANDRADE *et al*., 2018). Ademais, em algumas dessas sociedades, o cuidado parental se dá tanto pelas fêmeas quanto pelo macho, assim como pelos demais membros do grupo, e dessa forma as fêmeas podem voltar a se reproduzir novamente (HEARN, 1983)

A expectativa de vida também é bastante relativa nessas espécies. Dados advindos da criação sob cuidados humanos demonstram que alguns primatas apresentam longevidade média de 12 anos para sagui, 20 anos no macaco-da-noite e até 50 anos no caso dos macaco-prego (WALKER *et al*., 2009; NISHIJIMA *et al*., 2012; RAPOSO *et al*., 2015). Dessa forma, esses animais são importantes fontes de pesquisas para estudos sobre os efeitos da senescência, uma vez que é mais fácil observar os sinais do processo de envelhecimento em um período menor do que em seres humanos (DIDIER *et al*., 2016; ROSS; SALMON, 2019; SILVA *et al*., 2022).

Consequentemente, as diferenças apontadas podem influenciar nos marcadores biológicos, como os valores hematológicos, bioquímica sérica e concentrações hormonais. Assim, é importante considerar tais diferenças ao avaliar esses parâmetros biológicos, para a correta interpretação dos valores e comparação com animais de vida livre (CARDOSO *et al*., 2021a,b; PALME, 2019).

1.2 Importância de PNH nos estudos biomédicos e da morfofisiologia evolutiva

A ocupação de diferentes habitats e a formação de grupos dinâmicos faz dos platirrinos animais interessantes no estudo de adaptação morfofisiológica ao meio em que vivem, organização social e evolução (GRABOWSKI *et al*., 2022; JESUS *et al*., 2023). Nesse sentido, Garber e Kowalewski (2015) compilaram estudos com o gênero *Alouatta* com foco evolutivo, ecológico e comportamental, tais como as adaptações do sistema locomotor, dentição, visão tricromática, e uso de dieta de baixa qualidade, destacando a importância desses fatores para entender o comportamento e morfologia de primatas vivos e fósseis.

No que diz respeito ao estudo biomédico, os macacos-de-cheiro (gênero *Saimiri*) são bastante utilizados, seja na avaliação da resposta imune a doenças infecciosas (CHEN *et al*., 2020; TOUGAN *et al*., 2018), seja na influência de doenças na reprodução dessas espécies (IMBELONI *et al*., 2021). Outra área de estudo envolve os aspectos morfológicos do trato reprodutivo de fêmeas, sendo modelos bastante úteis no estudo de distocia e alterações no suporte pélvico (KRAMER *et al*., 2006; PIERCE *et al*., 2007; BRACKEN *et al*., 2011; JOYCE *et al*., 2014; LINDO *et al*., 2015), que também sofre efeito do envelhecimento (WILLIAMS, 2008). Além disso, os impactos do tamanho do feto e sua má apresentação em macaco-de-cheiro são semelhantes aos da mulher, tornando-o um bom modelo de comparação morfológica no estudo das desordens pélvicas na espécie humana. Esses fatores são discutidos no Capítulo 1, onde aborda-se o uso de fêmeas de macaco-de-cheiro como modelos para pesquisa de transtornos do assoalho pélvico em mulheres (SILVA *et al*., 2021a).

Em relação à morfofisiologia evolutiva, muitos avanços ocorreram a partir de estudos da conformação craniana, estruturas encefálicas e seus respectivos índices. Nesse sentido, Jerison (1973) estudou as diferenças interespecíficas de PNH e humanos quanto ao grau de encefalização (massa encefálica/massa corpórea), gerando índices que variaram na escala de 1,38 no bugio (*Alouatta villosa*), de 2,33 no macaco-aranha (*Ateles paniscus*), de 2,81 no macaco-de-cheiro (*Saimiri sciureus*), de 4,79 no macaco-prego (*Cebus albifrons*) e 7,79 em humanos. Outra pesquisa nesta área realizada por Aldridge (2012), avaliou os padrões de diferenças na morfologia cerebral humana em comparação com chimpanzés, bonobos, gorilas, orangotangos e gibões e observaram que existem padrões que diferenciam exclusivamente a morfologia humana da observada em outras espécies de PNH, indicando que a reorganização neural ocorreu na divergência evolutiva de cada um desses grupos. Ademais, um levantamento de literatura realizado por Pereira *et al*. (2021) a respeito da anatomia encefálica comparada entre humanos e PNH, incluindo macacos-prego, bugios e macaco-aranha, apontou particularidades no trajeto dos vasos sanguíneos no encéfalo e a presença de diferentes seios venosos da dura-máter, sendo características importantes para compreender a organização encefálica dos PNH.

No entanto, apenas valores da morfologia neural não são capazes de predizer as diferenças nos graus de inteligência e comportamento observados entre PNH e humanos 16 (FALK, 2015), sendo importante considerar a influência de questões ecológicas, fatores sociais, comportamentais, fisiológicos e sensoriais (GRABOWSKI *et al*., 2022). Assim, foi observado que as variações entre as espécies de primatas se correlacionam melhor com as diferenças no número de neurônios corticais, sinapses e na velocidade de processamento de

informações, em que o cérebro humano possui um grande volume cortical, densidade neuronal relativamente alto, e alta velocidade de condução (ROTH; DICKE, 2012). Nesse sentido, Grabowski *et al*. (2022) avaliaram como a dieta e a sociabilidade afetam a evolução do tamanho do cérebro dos primatas. Nesse estudo, observou-se que cada táxon tem a própria história evolutiva, e os cérebros são flexíveis e capazes de se adaptar aos desafios do ambiente. No grupo dos platirrinos, a dieta, o sistema de acasalamento e a massa corporal explicaram 98% da variação do tamanho do cérebro, o que pode refletir em sua estreita faixa ecológica e relativa radiação adaptativa recente (GRABOWSKI *et al*, 2022). Esses estudos demonstram a importância de se considerar conjuntos dos aspectos morfofuncionais na avaliação evolutiva.

Além disso, o aumento do cérebro em humanos e outros PNH conferiu benefícios cognitivos, mas trouxe custos associados ao crescimento e manutenção desse tecido, considerado energeticamente caro (MILTON, 1998; DUNBAR, 1998; 2009; GRABOWSKI, 2022). Nesta linha de estudo, Hartwing *et al*. (2011) investigaram os volumes relativos do cérebro, intestinos e a relação desses com a evolução em platirrinos. Nos resultados, o gênero *Alouatta* apresentou combinação de cérebros relativamente pequenos com intestinos relativamente grandes e estômago diferenciado, e gêneros como *Cebus* e *Saimiri* apresentam conformação oposta, sendo o *Cebus* com o menor coeficiente de diferenciação intestinal. Esses dados sugerem que a encefalização evoluiu várias vezes em paralelo entre platirrinos, em que cada um desses grupos exibem estratégias socioecológicas relativamente derivadas dentro de seus respectivos clados.

Além dos estudos envolvendo as relações da obtenção de energia e o tamanho cerebral e cérebro-intestino, muito se tem interesse nas adaptações morfológicas ao tipo de alimentação entre os primatas. Jesus *et al*. (2023) publicaram dados que demonstram alta diferenciação na estrutura dos órgãos digestórios entre os gêneros, separando *Alouatta* sp. dos demais, com características gástrica, colônica e retal, provavelmente ligadas à fermentação do conteúdo vegetal. Em contraste, os gêneros *Sapajus*, *Cebus*, *Saimiri* e *Cacajao* apresentaram quocientes de intestino delgado semelhantes, o que é esperado devido às suas altas taxas de consumo de matéria animal. Essas adaptações refletem os diferentes padrões alimentares, que podem permitir a coexistência geográfica de diferentes espécies. Esse estudo demonstrou também que o desempenho digestório dos cebídeos é semelhante ao esperado para platirrinos ancestrais, com baixa capacidade de fermentação.

Assim, as pesquisas apresentadas demonstram a necessidade de estudos sobre os fatores morfofisiológicos em conjunto, a fim de obter respostas mais robustas em termos

evolutivos e adaptativos. Nesse contexto, os zoológicos e centros de criação de primatas representam um excelente local para o desenvolvimento dessas pesquisas. Além de fornecer o material biológico necessário a investigação científica, esses locais desempenham um papel protagonista na conscientização e educação do público, proporcionando uma contribuição consciente na conservação *ex situ* e na pesquisa científica (COSTA, 2014).

1.2 Biomarcadores de saúde e senescência

Os biomarcadores são indicadores encontrados no corpo que podem auxiliar na avaliação do estado fisiológico, monitorar a saúde e bem-estar, diagnosticar doenças e sinalizar o nível de senescência. Esses marcadores podem ser hormônios, vitaminas, eletrólitos, proteínas, células presente na circulação, alterações no DNA, tamanho de telômeros e marcadores imunológicos, ou massa corpórea, dentre outros, com diferentes graus de sensibilidade e especificidade (WAGNER *et al*., 2016; WEY *et al*., 2019; EDES, 2022).

No que diz respeito ao estudo da senescência, as espécies mais utilizadas são macacos Rhesus (*Macaca mulatta*) e macaco-cinomolgo (*M. fasicularis*). No entanto, os estudos com platirrinos tem crescido, em sua maioria utilizando sagui-de-tufo-branco (*Callithrix jacchus*), mas também saguis-cabeça-de-algodão (*Saguinus oedipus*) e macacos-de-cheiro (*Saimiri sciureus*) (DIDIER *et al*., 2016). Os saguis têm sido espécies consideradas promissoras, devido ao tamanho relativamente pequeno e expectativa de vida curta, tornando-os modelos mais práticos. Além disso, estudos em outras espécies podem ser úteis para fornecer informações sobre os mecanismos básicos do envelhecimento animal e humano (MATTISON; VAUGHAN, 2017; WEY *et al*., 2019).

Existem diversos estudos em primatas a respeito dos biomarcadores encontrados nos exames hematológicos e de bioquímica sérica, descritos para variadas famílias, incluindo Callitrichidae (CARDOSO *et al*., 2021a), Cebidae (NÚÑEZ *et al*., 2007; WIRZ; TRUPPA; RIVIELLO *et al*., 2008; LIMA *et al*., 2014; FAVARETO *et al*., 2016; LINS *et al*., 2017; ABREU SOUSA *et al*., 2020; CARDOSO *et al*., 2021b), Atelidae (FLAIBAN *et al*., 2008; SÁNCHEZ-SARMIENTO *et al*., 2014; GARCÍA-FERIA *et al*., 2017; GONÇALVES *et al*., 2019) e Aotidae (MONTEIRO *et al*., 2009; TAKESHITA *et al*., 2011).

Os estudos têm demonstrado que os exames de hemograma e bioquímica sérica realizados na rotina dos centros de criação de primatas são de extrema valia para avaliação clínica desses animais. Dessa forma, é possível estabelecer e comparar valores de referência (WIRZ; TRUPPA; RIVIELLO, 2008; TAKESHITA *et al*., 2011; de MELO *et al*., 2019; GONÇALVES *et al*., 2019; CARDOSO *et al*., 2021a,b; ROVIROSA-HERNÁNDEZ *et al*.,

2022), avaliar a condição parasitária (MONTEIRO *et al*., 2009), enfermidades infecciosas (de MELO *et al*., 2019; SANCHEZ-FERNANDEZ *et al*., 2022; TEIXEIRA *et al*., 2022), e doenças crônicas (NUNAMAKER, LEE, LAMMEY, 2012). No entanto, os resultados das pesquisas são muitas vezes contraditórios, pois os indivíduos foram criados sob diferentes manejos ou o efeito do sexo, idade e presença de parasitas não foram avaliados (CARDOSO *et al*., 2021a,b; SILVA *et al*., 2022; ROVIROSA-HERNÁNDEZ *et al*., 2022). Esses fatores podem afetar os valores hematológicos e bioquímicos, por isso as análises precisam incluir múltiplas variáveis para melhor adequação dos resultados

Além disso, apesar dos diversos estudos a respeito de exames laboratoriais em platirrinos, a avaliação hormonal como marcador de alterações relacionadas à sanidade e senescência ainda é escassa. O perfil hormonal varia com a idade e pode indicar senescência em algumsas espécies (MATTISON; VAUGHAN, 2017). Dessa forma, métodos hormonais não invasivos são preferenciais e permitem monitorar o estresse em PNH, por meio de coleta e análise de amostras biológicas em fezes (TAKESHITA *et al*.., 2013; TAKESHITA *et al*., 2018; BUTI *et al*., 2018), pelo (CARLITZ *et al*., 2016; YAMANASHI *et al*., 2016; 2017), saliva (BEHRINGER *et al*., 2013; BROCHE Jr *et al*., 2019), e urina (CHEN *et al*., 2017; BEHRINGER *et al*., 2020). Para isso, é imprescindível a validação dos ensaios a serem utilizados, principalmente ensaios usando as fezes, para determinar a especificidade e adequação do anticorpo para a espécie, uma vez que apenas os metabólitos estão presentes nessas amostras (RANGEL-NEGRÍN *et al*., 2014; HEISTERMANN *et al*., 2006; PALME, 2019).

Assim, os principais hormônios usados para avaliar o estresse em animais mantidos sob cuidados humanos ou vida livre são os glicocorticóides (GC). Porém, o uso de GC apresenta limitações devido a fatores relacionados ao indivíduo (DANTZER *et al*., 2014; TAKESHITA *et al*., 2018; MULLER *et al*., 2021) e ao ambiente (WEINGRILL *et al*., 2004; TAKESHITA *et al*., 2014; 2017). Mais recentemente, tem-se optado pela associação com outros hormônios adrenais, como a dehidroepiandrosterona (DHEA) e a sua forma sulfatada (DHEAS) no estudo do estresse em humanos (DU *et al*., 2011; KAMIN *et al*., 2017), PNH (TAKESHITA *et al*., 2019), bovinos (ALMEIDA *et al*., 2008) e animais aquáticos como focas (GUNDLACH *et al*., 2018), golfinhos e baleias (MILLER *et al*., 2021). O DHEAS tem sido associado ao estresse, uma vez que tem ação antagonista aos GC (PRALL *et al*., 2017). No entanto, a maioria das pesquisas sobre a função e o padrão de secreção do DHEAS foram realizadas em primatas do velho mundo (MUEHLENBEIN *et al*., 2003; TAKESHITA *et al*.,

2013) e grandes símios (BEHRINGER *et al*., 2012; BERNSTEIN *et al*., 2012), que apontam para possível relação desse hormônio na evolução entre as espécies.

Em relação aos platirrinos, apesar dos diversos estudos com GC, estão limitados aos gêneros *Callithrix* (PATTISON *et al*., 2005; 2007), *Saimiri* (WIEBE *et al*., 1984; 1988), *Aotus* (BARDI *et al*., 2014) e *Sapajus* (TORRES-FARFAN *et al*., 2004), com poucos dados disponíveis a respeito dos hormônios DHEA e DHEAS (referidos juntos como DHEA/S). Dessa forma, a compreensão do padrão de secreção desses hormônios nas espécies neotropicais possibilitará o estudo do índice GC/DHEAS na avaliação do estresse e a comparação com os primatas do Velho Mundo em termos evolutivos.

O DHEA/S também é considerado um potencial biomarcador para investigar questões relacionadas à saúde e bem-estar (EDES *et al*., 2022). São hormônios esteróides produzidos em grandes quantidades pelas adrenais em primatas humanos e alguns PNH (MUEHLENBEIN *et al*., 2003), e servem como precursores de esteróides sexuais (MUEHLENBEIN *et al*., 2003; LEOWATTANA, 2004; REGE *et al*., 2016). Nas adrenais, o DHEA é convertido em DHEAS nas células da zona reticular, por meio da enzima desidroepiandrosterona sulfotransferase (SULT2A1), com concentração sérica cerca de 250 vezes maior e com maior tempo de meia-vida que a do DHEA, sendo, por esse motivo, a forma mais adequada para representar a disponibilidade de DHEA secretado na corrente sanguínea (KROBOTH *et al*., 1999). Dentre os mecanismos de ação destacam-se os efeitos genômicos, efeitos de modulação de canais e efeitos mediados pelos metabólitos de DHEA (STÁRKA; DUSKOVÁ; MARTIN HILL, 2015). A partir desses efeitos, eles têm sido associados a funções imunoestimulantes (HAZELDINE *et al*., 2010), biomarcadores do envelhecimento (MUEHLENBEIN *et al*. 2003) e neuroprotetores (MANINGER *et al*., 2009; SRIPADA *et al*., 2014; KAMIN; KERTES, 2017). No cérebro, o DHEA é metabolizado em testosterona, diidrotestosterona e estradiol, e outros os metabólitos que também possuem cooperação na função cerebral (STÁRKA; DUSKOVÁ; MARTIN HILL, 2015).

O uso do DHEA/S como biomarcador da idade em primatas tem sido atrelado a dinâmica adrenal nas fases da vida: (1) no desenvolvimento fetal; (2) na maturação da adrenal durante o período da pré-adolescência (denominado de adrenarca); e (3) no declínio constante que segue o pico pós-adrenarca (denominado de adrenopausa, relacionado à senescência) (CONLEY; PATTISON; BIRD, 2004; QUINN *et al*., 2018). Durante o período fetal, esses 20 hormônios são transferidos para a mãe pela placenta e convertidos em estrogênios, para a manutenção da gestação normal (RAINEY; CAR, 2004; TORRES-FARFAN *et al*., 2004; XING *et al*., 2015). Em humanos e PNH gestantes, o desenvolvimento adequado e função do

córtex adrenal fetal são essenciais para a maturação fetal e sobrevivência perinatal (QUINN *et al*., 2018), sendo, portanto, marcadores hormonais úteis para o monitoramento da gestação, e em baixas concentrações, no final da gestação, podem indicar morte fetal (RAINEY; CAR, 2004; TAKESHITA *et al*., 2016).

Devido a importância das análises hormonais e do DHEAS, e aos poucos estudos em platirrinos, o Capítulo 2 tratou da validação do ensaio de DHEAS em amostras fecais e séricas em *Alouatta caraya*, *Sapajus apel*la e *Aotus azarai*. Além disso, exames que avaliem a forma e função dos órgãos em primatas, como ultrassonografia, hemograma, e bioquímica sérica contribuirá para o monitoramento da saúde dessas espécies, fornecendo dados para comparação com outros PNH, estudos de efeitos ecológicos e adaptações evolutivas. Dessa forma, o Capítulo 3 descreve a avaliação renal de *Alouatta caraya* e *Sapajus apella* por meio da ultrassonografia e exames laboratoriais, e o Capítulo 4 descreve e discute os valores de hemograma e bioquímica sérica nessas espécies. Ambos os capítulos discutem as possíveis implicações dos achados para a saúde e adaptação dessas espécies.

3. OBJETIVOS

3.1 Geral

Abordar o uso de primatas neotropicais como modelos na pesquisa biomédica e de alguns parâmetros morfofisiológicos como biomarcadores de saúde e senescência nesses animais, de forma comparada

3.1 Específicos

- Descrever o uso do macaco-de-cheiro (*Saimiri* sp.) como modelo na pesquisa biomédica obstétrica.
- Validar o ensaio hormonal para detectar sulfato de dehidroepiandrosterona (DHEAS) em fezes e sangue de bugios-preto (*Alouatta caraya*), macaco-da-noite (*Aotus azarae infulatus*) e macaco-prego (*Sapajus apella*).
- Avaliar os resultados ultrassonográficos, de hemograma e bioquímica sérica como biomarcadores de saúde e senescência em bugios-preto e macaco-prego.
- Avaliar o efeito da idade, espécie, sexo e nível de parasitismo intestinal nos biomarcadores estudados.
- Comparar os resultados dos exames laboratoriais (hemograma e bioquímica sérica) e suas implicações para a fisiologia adaptativa de bugios-preto e macaco-prego comparada

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Review Article

Laboratory Animals
0(0) 1–10

DOI: 10.1177/00236772211032506
journals.sagepub.com/home/lan

Female squirrel monkeys as models for research on women's pelvic floor disorders

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Abstract

Animal models enable research on biological phenomena with controlled interventions not possible or ethical in patients. Among species used as experimental models, squirrel monkeys (*Saimiri* genus) are phylogenetically related to humans and are relatively easily managed in captivity. Quadrupedal locomotion of squirrel monkeys resembles most other quadrupedal primates in that they utilize a diagonal sequence/diagonal couplets gait when walking on small branches. However, to assume a bipedal locomotion, the human pelvis has undergone evolutionary changes. Therefore, the pelvic bone morphology is not that similar between the female squirrel monkey and woman, but pelvic floor support structures and impacts of fetal size and malpresentation are similar. Thus, this review explores the pelvic floor support structural characteristics of female squirrel monkeys, especially in relation to childbirth to demonstrate similarities to humans.

Keywords

Reproduction, nonhuman primates, biomodels, pelvic floor support

Date received: 23 December 2020; accepted: 22 June 2021

Introduction

Animal experimental models in biological research are necessary because they allow the study of biological phenomena. The choice of model is fundamental and must, therefore, be judicious. Animal models can improve understanding of human diseases and developmental injuries with controlled interventions, which is generally not possible with human subjects.[1] Although no animal model is perfect, some species are particularly suitable, such as rodents, pigs, and nonhuman primates (NHPs).[2] Animals have been used in research on infectious diseases, such as malaria,[3,4] Zika virus,[5] and the pathogenesis of COVID-19, Middle East respiratory syndrome (MERS), and severe acute respiratory syndrome (SARS).[6] Recently, these authors concluded that SARS-CoV-2 causes COVID-19-like disease in macaques and provided a new model for testing preventive and therapeutic strategies. NHPs are good models for research on human diseases and have enabled remarkable scientific progress by elucidating cardiovascular, endocrinal, neurological, and reproductive diseases.[7–9] However, the use of NHPs is costly and requires specialized resources. The ethics of using animals for research is another challenge.

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In particular, animal models have improved understanding of some pelvic disorders in women.[7,10] They are vital to basic and applied research on human female reproduction, prenatal development, and women's health more generally.[11] Squirrel monkeys (*Saimiri* sp.) are New World primates that have been used in research on dystocia and changes in pelvic support.[1,12–17] Knowledge of the pelvic floor support and delivery mechanism in these monkeys may help to improve understanding of women's reproductive disorders with the potential to prevent debilitating injuries. Thus, this review aims explore the morphophysiological characteristics of the female squirrel monkey pelvic support structures. We also discuss the mechanics of childbirth to demonstrate similarities between this species and women.

Use of *Saimiri* sp. in human obstetrical research

Squirrel monkeys are neotropical primates of the Cebidae family. They have a short, thick coat, rounded head, short and black snout, rounded ears, and black-tufted tail.[18] These animals are insectivores and frugivores, and their food includes arthropods, mollusks, small vertebrates, fruits, and seeds.[19]

Squirrel monkeys are among the most used neotropical primates in biomedical research, with the first studies reported by Klüver,[20] who provided information on their experimental use and management in captivity. Because of their small size (with a body mass of approximately 1 kg), squirrel monkeys can be kept in smaller spaces than necessary for other model NHPs. They acclimate well to captivity[21] and are easily handled because of their small size. Additionally, similarities in the development of pelvic changes in squirrel monkeys and humans make these monkeys good models for research on human obstetrics.[22] Another advantage of using squirrel monkeys as models is their reproductive longevity – the prime reproductive ages are between 3 and 13 years old.[23]

Squirrel monkeys have a gestation period of about 150 days, with a range of 141–154 days,[24,25] which enables one pregnancy per year to be monitored in captivity for experimental studies.[17] They are seasonal primates, and, in their native habitats, they reproduce from July to September, with births occurring from December to February; seasonality in this regard has also been observed in the northern hemisphere.[26] The average body mass of squirrel monkeys during the reproductive season is 914.58 ± 13.78 g for males and 752.5 ± 74.6 g for females.[27]

Comparative morphology of female squirrel monkey and human reproductive organs and pelvis

Quadrupedal locomotion of squirrel monkeys resembles most other quadrupedal primates in that they utilize a diagonal sequence/diagonal couplets gait when walking on small branches.[28] However, to assume a bipedal locomotion, the human pelvis, forelimb, hindlimb, scull, and spine have undergone evolutionary changes. Therefore, the pelvic bone morphology is not that similar between the female squirrel monkey and woman, but pelvic floor support structures and impacts of fetal size and malpresentation are similar.

Squirrel monkey genital morphology has been described by Branco et al.[29] and Mayor and Plana.[30] Figure 1 shows the gross morphology of the genital organs of squirrel monkeys, and Figure 2 shows how these organs can be measured via ultrasound. Some similarities exist between the genital morphology of squirrel monkeys and women, such as the presence of a simple uterus with less prominent uterine horns. The ovaries are large and ellipsoid, have a smooth surface, and produce visible follicles, but the corpus luteum is not clearly distinguishable by ultrasound. The uterine tubes are long and straight muscular structures that are included in the mesosalpinx. These tubes merge caudally into a single small uterine body, where pregnancy occurs. The mesovarium and ovary participate in the formation of the ovarian bursa, which does not fully involve the ovary. The cervix is short and characterized by a thick and well-developed muscle wall. The vagina extends from the cervix (external uterine orifice) to the exit of the urethra (external urethral orifice). The vaginal vestibule is shared by the genital and urinary tracts. The vulva is formed by two lips that meet at the dorsal and ventral vulvar commissures and has a vertically elongated vulvar sulcus. Finally, the clitoris is located ventrally in the fossa, which is well-developed.

The intrapelvic musculature of squirrel monkeys is similar to that of humans. For instance, the levator ani muscle consists of the pubocaudal and iliocaudal muscles, respectively, analogous to the pubococcygeus and iliococcygeus muscles in humans. In addition, the endopelvic fascia has connective tissue condensations that correspond to uterosacral and cardinal ligaments in women.[31] In women, the pelvic floor consists of muscles, ligaments, and fascia, whose contraction provides resistance to gravity and intra-abdominal pressure, and support the abdominal and pelvic organs, such as the uterus, vagina, urinary bladder, urethra, and rectum. Loss of muscle contraction and injury to ligaments, connective tissue, or nerves have been associated with the occurrence of pelvic organ prolapse (POP)[32] and similarly in squirrel monkeys.[14]

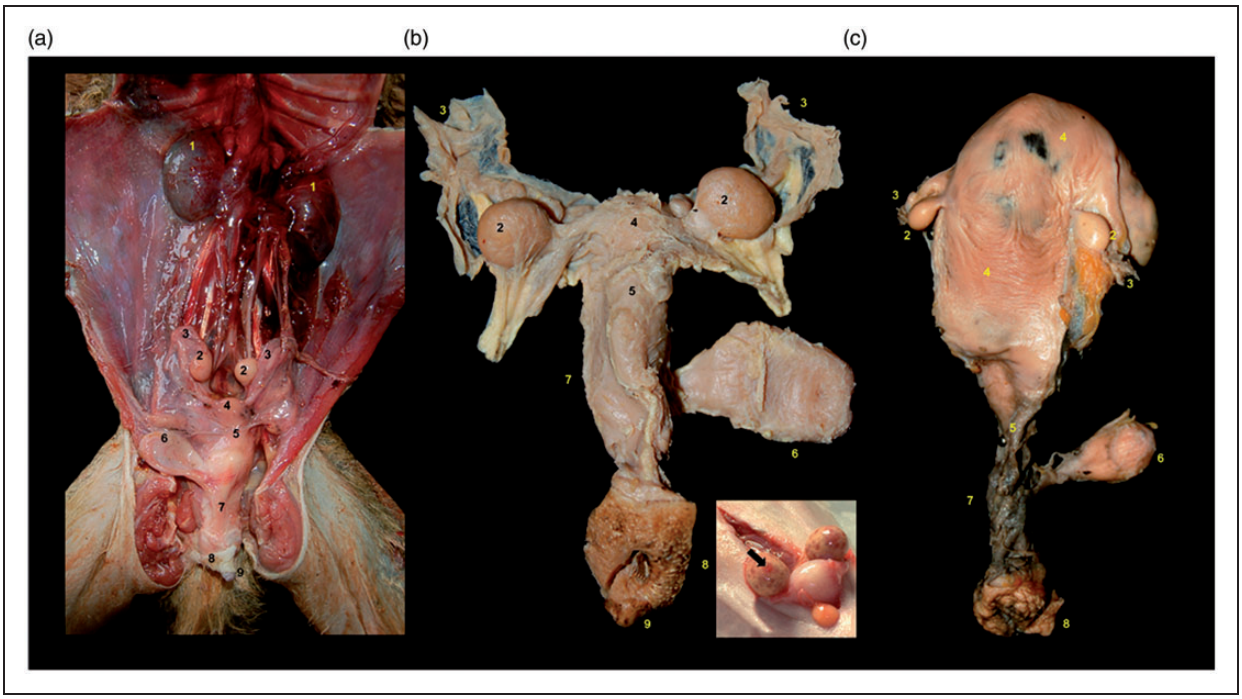

Figure 1. Gross anatomy of squirrel monkey (*Saimiri* sp.) genital organs. (a) In situ ventral view of nonpregnant; (b) dissected dorsal view of nonpregnant female and detail of ovary follicles (black arrow); (c) dissected dorsal view of pregnant female. 1. Kidneys, 2. Ovaries; 3. Uterine tubes; 4. Uterine body; 5. Cervix; 6. Urinary bladder; 7. Vagina; 8. Vulva; 9. Clitoris. Adapted from Mayor and Plana (2019).[30]

Two main muscle groups line the pelvic floor in women: the pelvic and urogenital diaphragms. The pelvic diaphragm lines the lower and lateral region of the pelvis, extends from the pubis to the coccyx, and consists of the levator ani and coccygeus muscles. For most of the pelvic floor, the levator ani muscle is divided into the puborectalis, pubococcygeus, and iliococcygeus muscles, which connect and support the vagina, urethra, anus, and coccyx. The coccygeus muscle originates from the sciatic spine and inserts at the lower end of the sacrum and upper end of the coccyx. Therefore, coccyx flexion is important during defecation and fetal expulsion, in addition to assisting the levator ani muscle in supporting pelvic organs.[33]

The urogenital diaphragm is in the superficial and distal layer of the pelvic floor and supports the distal portion of the vagina and urethra to the bony pelvis by fixing the ischiocavernosus, bulbocavernosus, and transverse muscles of the perineum. Muscles of urogenital diaphragm also stabilize the perineal body between the urogenital and the anal triangles. In the perineal body, the transverse muscle of the perineum supports the vagina and rectum and maintains urinary and fecal continence.[32]

Fasciae and ligaments are formed by dense connective tissue and support the organs by connecting bones and muscles. The endopelvic fascia consists of connective tissue and is composed of two parts: a visceral layer, located below the peritoneum, which surrounds the pelvic diaphragm and supports the organs; and a parietal layer, which comprises ligaments and a septum, fixing the pelvic floor and enabling vascularization and local innervation. Ligaments originate from points of fiber condensation in the endopelvic fascia and actively participate in pelvic visceral support. The main ligaments are the uterosacral and cardinal ligaments, which support the vagina and uterus, and the pubovesical, pubourethral, and anococcygeus ligaments, which support the bladder, urethra, and anus.[34]

Based on human anatomic dissections, the support of the vagina and uterus can be segmented into three levels. The upper level consists of the parametrium and paracolpium, which promote lateral support of the uterus and vaginal apex. In the second level, the cardinal and uterosacral ligaments act on the cervix, together with the pubocervical and rectovaginal fasciae, which laterally support the structures. In the lower level, the levator ani muscle and adjacent connective tissue support the vagina.[32]

In humans, the pubovesical ligament supports the bladder by fixing the detrusor muscle of the bladder to the tendinous arch of pelvic fascia and pubis, while the pubourethral and puborectalis ligaments act on the bladder neck. The lateral ligaments of the bladder

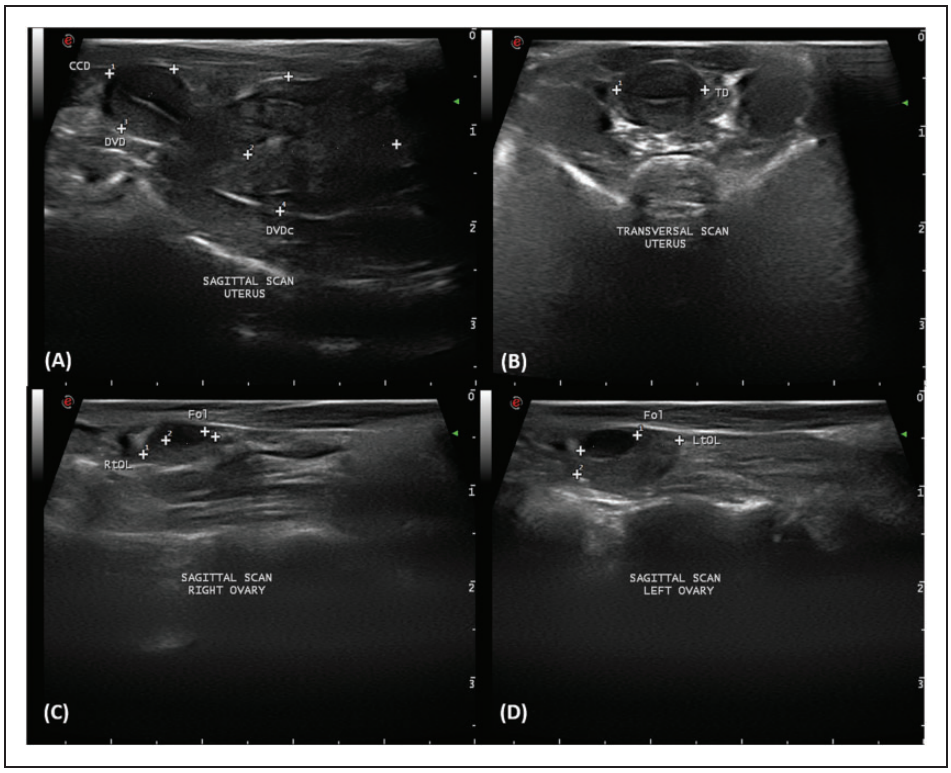

Figure 2. Uterine and ovaries images in adult of *Saimiri sciureus* (10 years old and five deliveries). (a) Uterine variables at the sagittal scan (non pregnant female). (b) Transversal diameter (TD) at the transversal scan (non pregnant female). Hyperechogenic line in the central uterine region indicates the endometrium internal surfaces (arrow). In both ovaries the variable length was obtained at the sagittal scan (c) and (d), represented here by the variables right and left ovary length (RtOL and LtOL, respectively). The largest diameter of the follicles was measured at the sagittal or transversal scan. CCD: cranio-caudal diameter; DVD: dorso-ventral diameter; DVDc: cervix dorso-ventral diameter; Fol: follicles.

support the bladder trigone, while the pubocervical fascia maintains the base of the organ, suspended in the tendinous arch.[33] The bladder in squirrel monkeys is typically higher in the pelvis prior to pregnancy and lower post-partum with longer urethra relative to size than in humans. The urethra is about the same length as in humans. This is interesting considering the great difference in body size (Dr Kuehl, personal communication, 2020). In women, the urethra is supported by the surrounding tissues that are attached to the pelvic bones. The main tissues responsible for fixing periurethral tissues are the pubourethral ligaments, located in the proximal urethral region, and the levator ani muscle.[32]

The anorectal fascia supports the rectum, laterally by the lateral ligaments of the rectum, anteriorly by the rectovaginal fascia, and posteriorly by fixing the presacral fascia to the sacrum. The anus is laterally supported by the pubovisceral and superficial transverse muscles of the perineum, while the perineal body and anococcygeus ligament support the anterior and posterior regions, respectively.[34]

Regarding squirrel monkeys as models for research on the physiopathology of pelvic relaxation and prevention of loss of pelvic support in humans, the similarities and differences in vulvar and pelvic anatomy should be assessed, as this can inform obstetrics and the delivery mechanism in these species.[1] Comparative anatomy of the pelvic muscles, innervation, and connective tissue ligaments should also be considered. In this respect, some pioneering work has been reported.[14,31,35,36] The main difference is the presence of the tail and muscle bundles in squirrel monkeys, which originate from the sacrum, located posteriorly in the pelvis and anterior to the sacral vertebrae.[12] The role of these muscles in the pathophysiology of POP remains unclear.

In women, the levator ani muscle plays an important role in supporting the pelvic organs. Comparative anatomy has indicated that this muscle in humans evolved from the tail muscles. Consequently, possible changes in pelvic muscles in squirrel monkeys have been evaluated, and the number of deliveries was found to promote changes in the structures of the levator ani muscle[13,14] and coccygeus muscle.[15]

Squirrel monkeys are dolichopelvic, and their pelvises have an oval cranial portion and are flattened laterally. The ischium is grossly excavated and arched ventrally at its caudal end, like that observed in ruminants and pigs.[37,38] Aksel and Abee[39] measured squirrel monkey pelvises using lateral and anteroposterior radiographic projections. Significant differences were subsequently observed between the superior and inferior bi-iliac diameters of females with livebirth neonates (1.84 ± 0.09 and 1.9 ± 0.13 cm, respectively) and stillbirths (1.87 ± 0.08 and 1.92 ± 0.10 cm, respectively). However, a more recent study evaluated pelvimetric data in squirrel monkeys[40] and found superior and inferior bi-iliac diameters of 1.71 ± 0.08 and 1.67 ± 0.09 cm for adult females and 1.59 ± 0.07 and 1.63 ± 0.13 cm for subadult females, respectively.

Childbirth behavior and implications for pelvic biomechanics

Regarding behavior and posture at the time of delivery, female squirrel monkeys rest on their feet and base of their tail. The pelvis does not come into direct contact with the ground and remains suspended. This position can predispose the laboring female to prolapse due to gravity's effect on the muscles that support the abdominal organs or during labor stress.[41] Furthermore, fetal rotation in female squirrel monkeys is similar to that in women, where the mean submentobregmatic diameter was significantly smaller than the mean occipitofrontal diameter.[22] Occipital–posterior presentation involves the fetal face facing in an opposite direction to that of the maternal face, which hinders the passage of the head and requires greater effort during delivery.[41]

Dystocia in NHPs can be caused by cephalopelvic disproportion. Figure 3 shows the dimensions of the fetal cranium and those of the pelvic inlet in humans and NHPs, including squirrel monkeys.[42] Squirrel monkeys have a high incidence of dystocia because large fetal head sizes lead to losses due to stillbirths or neonatal mortality.[43] Approximately 12% of captive female squirrel monkeys, especially primiparous females, experience dystocia.[22] According to Hartwig,[44] the squirrel monkey cranium has a unique dolichocephalic shape, presumably to accommodate the relatively large infant brain while minimizing cranial breadth. While 90% of squirrel monkey deliveries are with the longitudinal presentation and mentum anterior position, other presentations lead to dystocia (Dr Ruiz, personal communication, 2020), which represents a difference compared to humans and may lead

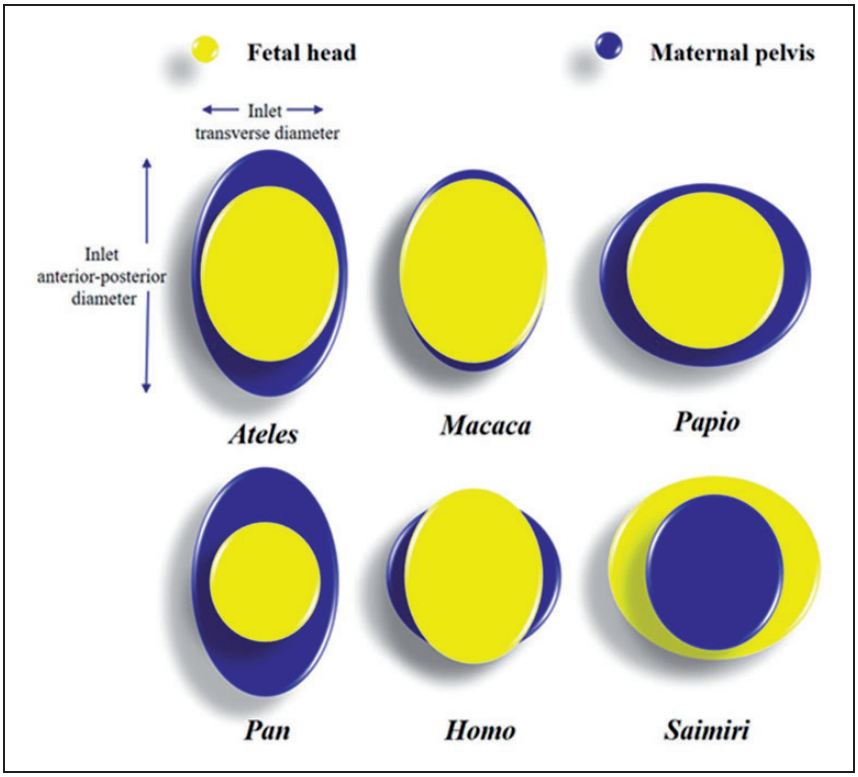

Figure 3. Schematic drawing illustrating the proportion of fetal head with maternal pelvis (transverse and antero-posterior diameters of pelvic inlet) in human (*Homo*) and non-human primates (*Ateles*, *Macaca*, *Papio*, *Pan*, and *Saimiri*). Adapted by Dr Ruiz from Rosenberg KR and Trevathan WR (1996).[42]

to an increase in the frequency of POP as first reported by Coates et al.[1] and Stoller.[22] In humans, discrepancies between the fetal size and maternal pelvis have also been reported and are associated with the evolutionary relationship between larger brain size and the bipedal position.[41]

Despite the elongated cranium in squirrel monkeys, the presenting diameter of the infant cranium is still larger than the maternal pelvic in-/outlet (Figure 4). Furthermore, these monkeys have high perinatal mortality due to this disproportion, which impairs the reproductive performance of captive individuals.[39] Aksel and Abee[39] developed a pelvimetric method, radiographing pregnant females, as a predictor of pregnancy outcomes in squirrel monkeys, according to which 95.2% of live births and 92.1% of stillbirths could be predicted. This calculation can, therefore, be a useful tool in predicting pregnancy outcomes by

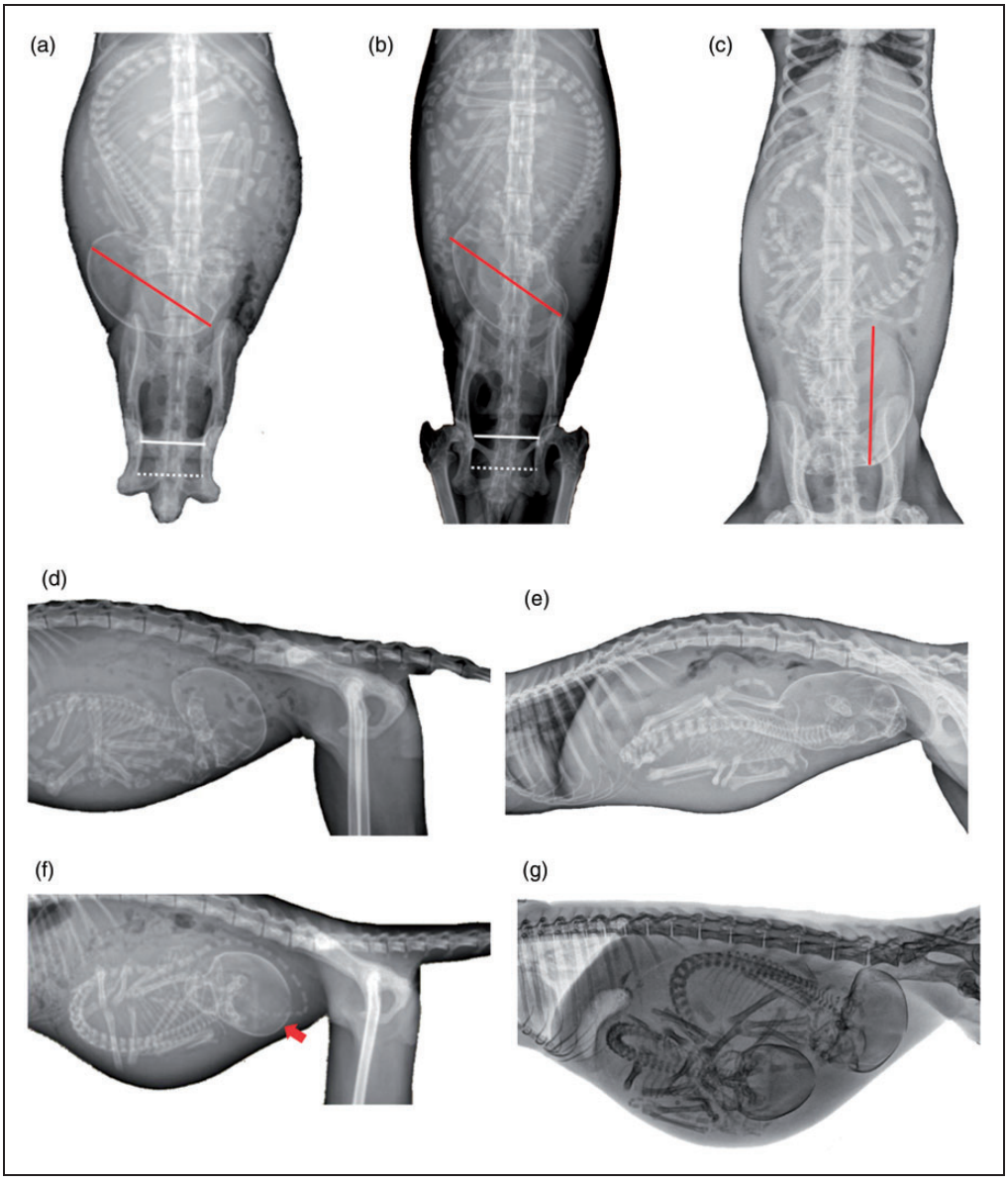

Figure 4. Ventrodorsal (a)–(c) and latero-lateral (d)–(g) radiographic views during delivery in *Saimiri boliviensis*. (a)–(e) Note that cranium diameter (red line) is still larger than the maternal inlet (white line) and outlet (dotted white line) pelvic; normal presentation (cephalic, vertex, or headfirst). (c)–(e) Fetal head is markedly extended with the face presenting. Fetal rotation as labor begins, the fetal neck is fully extended, and the sagittal axes of the mother and fetus are aligned ((d) and (e)). (f) Vertex presentation, longitudinal lie, occiput posterior position (red arrow). (g) Rare case of twin pregnancy in squirrel monkey (*Saimiri sciureus*). X-ray images by Dr Ruiz, JC.

observing narrow pelvic points in females who have stillbirths. Therefore, presentation is so critical in the squirrel monkey. Also, there are no squirrel monkey obstetricians to rotate/reposition the head during the delivery process. This leads to the need for a C-section/surgical intervention to salvage the mother as fetuses are usually lost by the time of discovery.

The squirrel monkey is one of the neotropical primates with the largest brain size.[45] The development of the facial bones and neurocranium is mainly responsible for neonate size, which can be up to 18% of the non-pregnant female weight, larger than that for other neotropical primates.[46] Neonates are approximately one-sixth the size of the mother's body weight and, thus, larger as a percentage than that reported in humans. The delivery of large neonates may increase the risk of perineal trauma, nerve damage, and prolapse.[12] Newborn squirrel monkeys have a large cephalopelvic disproportion at birth. In addition, the postnatal maturation of cranial bones is rapid, which is unusual for New World primates.[47]

The length of the newborn squirrel monkey's skull is substantially greater (136%) than the sagittal dimension corresponding to the maternal pelvic inlet. Furthermore, these monkeys have closely apposed orbits and a relatively large brain, reaching precocial prenatal development, a reflex in obstetric constraints imposed by the absolute limits of the pelvic inlet.[44] Favoretto et al.[40] described squirrel monkey pelvimetry using pelvic radiographs in ventrodorsal projections and compared the pelvis measurements of adult and sub-adult females to evaluate the occurrence of dystocia. It was observed that the latter had a smaller upper bi-iliac diameter and pelvic area than the latter did, suggesting a higher risk of dystocic birth.

In addition to trauma during labor, decreased estrogen levels with advancing age are associated with reduced strength and elasticity of the pelvic ligaments and muscles in women.[48] In squirrel monkeys, older females (>12 years old) have reduced hormone levels during the reproductive season of compared to those of younger ones.[49] Thus, the number of deliveries, newborn size, and aging may contribute to progressive denervation and subsequent pelvic prolapse in both squirrel monkeys and humans.

POP

A recent systematic review of the literature showed that several animal models have been used in the study of the pathophysiology of POP, such as lagomorphs, rodents, sheep, and NHPs.[50] The authors concluded that in several species there are measurable effects of pregnancy, delivery, and iatrogenic menopause, but there is not a single uniform pattern. However, only squirrel monkeys develop clinical POP spontaneously. Women and squirrel monkey females can develop disorders in their pelvic support muscles that may be related to age and number of previous deliveries. Coates et al.[12] found that almost 50% of older females in a squirrel monkey population showed pelvic changes compared to younger females. Multiparous females also had a higher occurrence of POP (4.0 versus 1.6).

Moreover, infant size and birth canal structure may render the pelvic floor more susceptible to injury and contribute to the occurrence of dystocic births.[1] Stratford et al.[51] first reported similarities in myogenic and neurogenic changes in the puborectalis muscle between women and squirrel monkeys via magnetic resonance evaluation. After delivery, no changes were observed in the volume of the levator ani and internal obturator muscles, but that of the coccygeus muscles increased, suggesting postpartum injuries.[15] Thus, the authors concluded that squirrel monkeys are suitable for comparative studies regarding women's pelvic floor support.

In the histological evaluation of the levator ani muscle and paravaginal ligaments in squirrel monkeys with and without POP, no gross ruptures were observed in the aforementioned muscle and its innervation. Myogenic changes were more frequently observed in their pubocaudal muscles and were correlated with aging. In addition, in the paravaginal ligaments, increased apoptosis was associated with parity.[14] On the other hand, in the evaluation of the function of and defects in the levator ani muscle in women with and without POP, magnetic resonance images demonstrated that women affected by POP more frequently had defects in their levator ani muscle.[52]

Joyce et al.[16] noted that the size of the pelvic outlet diameter was not related to POP occurrence in squirrel monkeys, but the number of deliveries was a risk factor. This finding was consistent with observations in women, with the number of births being the main predisposing factor for prolapse occurrence.[53] However, reduced pelvic support, related to pelvic musculature, is associated with age and number of births, which may be associated with obstetric complications. These observations inform ongoing research into the nature and cause of spontaneous pelvic relaxation in squirrel monkeys and support the potential use of these primates as animal models.[12]

In women, prolonged births have been associated with pelvic denervation injuries, which can be a risk factor for future prolapse.[54] Therefore, childbirth is a risk factor associated with POP, since postpartum changes are detected, but long-term assessment and randomized research are difficult in humans. Consequently, the selection of an experimental animal

model should be based on morphofunctional similarity and management practicality.[21] Based on this concept, the first randomized controlled trial of scheduled prelabor C-section was performed in squirrel monkeys.[17] Animals in the control group demonstrated descent of pelvic structures and bladder similar to those subsequently reported in primiparous women undergoing their first vaginal delivery.[55,56] However, animals undergoing scheduled C-section prior to the onset of labor did not show these changes.[17] Thus, there is the potential that such an intervention might reduce the impact of childbirth on human pelvic floor disorders. As this is a very hotly debated issue, follow-up and additional experiments are warranted.

Despite the progress made in recent decades in research conducted with the *Saimiri* genus, especially in captivity, substantial gaps in our knowledge remain. The relatively easy management of these monkeys in comparison with other primates, can improve the monitoring of birth-related disorders in women. Thus, squirrel monkeys can be used in studies that may inform the development of POP intervention in human obstetrics. Therefore, it is necessary that state-of-the-art imaging and assessments be carried out to understand the similarities between female squirrel monkeys and women. Such research is expected to advance scientific knowledge of reproductive research related to NHPs and human beings.

Declaration of Conflicting Interests

The author(s) have no conflicts of interest to declare.

Funding

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This was supported by the Coordination for the Improvement of Higher Education Personnel (CAPES, Coordination of Scholarships Abroad, grant number BEX 9255/13-3; National Academic Cooperation Program in the Amazon – PROCAD/Amazônia, grant number 1696/2018); and the National Council of Technological and Scientific Development (CNPq, grant number 305821/2017-2). We are also especially grateful to the Keeling Center for Comparative Medicine and Research at The University of Texas MD Anderson Cancer Center (SMBRR/NIH, grant number P40-OD010938-40).

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Les singes écureuils femelles sont des modèles de recherche sur les troubles pelviens du plancher chez les femmes

Résumé

Les modèles animaux permettent de mener des recherches sur les phénomènes biologiques par le biais d'interventions contrôlées qui ne seraient ni possibles ni éthiques à mener chez des patients. Parmi les espèces utilisées comme modèles expérimentaux, les singes-écureuils (genre *Saïmiri*) sont à la fois phylogénétiquement apparentés aux humains et relativement faciles à gérer en captivité. La locomotion quadrupède des singes-écureuils ressemble à celle de la plupart des autres primates quadrupèdes en ce qu'ils utilisent une démarche de couplets diagonaux/séquences diagonales lorsqu'ils se déplacent sur de petites branches. Pour supposer une locomotion bipède, le bassin humain a cependant subi des changements évolutionnaires. La morphologie osseuse pelvienne de la femme n'est par conséquent pas semblable à celle du singe-écureuil femelle, mais les structures de soutien du plancher pelvien et les impacts de la taille fœtale et d'une mauvaise présentation du fœtus sont semblables. Ainsi, cet examen explore les caractéristiques structurelles de soutien du plancher pelvien des singes-écureuils femelles, en particulier en ce qui concerne l'accouchement, pour démontrer des similitudes avec les humains.

Weibliche Totenkopfaffen als Modelle zur Erforschung von Beckenbodenstörungen bei Frauen

Abstract

Tiermodelle ermöglichen die Erforschung biologischer Phänomene mit kontrollierten Eingriffen, die bei Patienten nicht möglich oder ethisch nicht vertretbar sind. Unter den als Versuchsmodelle verwendeten Arten sind Totenkopfaffen (Gattung *Saimiri*) phylogenetisch mit dem Menschen verwandt und sie lassen sich relativ leicht in Gefangenschaft halten. Die Fortbewegung von Totenkopfaffen auf allen vieren ähnelt den meisten anderen vierfüßigen Primaten insofern, als dass sie beim Gehen auf Zweigen eine diagonale Sequenz/diagonale quadrupedale Gangart verwenden. Das menschliche Becken hingegen hat sich evolutionär hin zu bipedaler Fortbewegung entwickelt, weshalb in der Morphologie der Beckenknochen von weiblichen Totenkopfaffen und Frauen kaum Ähnlichkeiten bestehen. Die Stützstrukturen des Beckenbodens und die Auswirkungen auf fetale Größe und Lageanomalien sind jedoch durchaus vergleichbar. Daher werden in dieser Übersichtsarbeit die Merkmale der Stützstruktur des Beckenbodens bei weiblichen Totenkopfaffen untersucht, insbesondere im Zusammenhang mit der Geburt, um Ähnlichkeiten zum Menschen aufzuzeigen.

Monos ardilla hembra como modelos de investigación para enfermedades del suelo pélvico en mujeres

Resumen

Los modelos animales permiten realizar investigaciones sobre fenómenos biológicos cuando las intervenciones controladas no son éticas o no pueden realizarse en humanos. Entre las especies utilizadas como modelos de experimentación, los monos ardilla (*Saimiri* genus) están filogenéticamente vinculados a los humanos y pueden controlarse con moderada facilidad en cautiverio. La locomoción cuadrúpeda de los monos ardilla se parece a la mayoría de primates cuadrúpedos al utilizar una secuencia diagonal/movimiento diagonal al andar sobre ramas pequeñas. Sin embargo, con el movimiento bípedo, la pelvis de los humanos ha pasado por varios cambios evolutivos. Por ello, la morfología del hueso pélvico no es tan parecida entre el mono ardilla hembra y las mujeres, pero la estructura del suelo pélvico y los impactos de tamaño fetal así como la presentación fetal anómala son similares. Este estudio explora las características estructurales del suelo pélvico de los monos ardilla hembra, especialmente en relación al parto para demostrar las similitudes existentes con los humanos.

International Journal of Primatology
https://doi.org/10.1007/s10764-021-00239-x

Validation of a Dehydroepiandrosterone-Sulfate Assay in Three Platyrrhine Primates (*Alouatta caraya*, *Aotus azarae infulatus*, and *Sapajus apella*)

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Received: 8 April 2021 / Accepted: 15 July 2021/Published online: 16 August 2021

Abstract

The hormone dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are the most abundant circulating steroids in human and some nonhuman primates, and have been implicated in development, aging and in stress modulation. We aimed to validate a commercially available enzyme immunoassay kit to measure DHEAS in feces and serum of three platyrrhine primate species (*Alouatta caraya*, *Aotus azarae infulatus*, and *Sapajus apella*) in captivity. We collected serum samples from one male and one female from each species. To validate the kit for fecal samples, we conducted a physiological validation by administering DHEA orally to one adult female of each species. We also measured fecal DHEAS levels in four female *Alouatta caraya* individuals during the third semester of gestation and in two females following parturition. We obtained a total of 54 fecal samples and 6 serum samples from 10 individuals. We validated the assay analytically by testing parallelism and accuracy tests in both fecal and serum extracts for all species. We observed a peak in DHEAS 24 h following oral DHEA administration in all three species, with *A. caraya* presenting the strongest response and highest baseline concentrations. DHEAS levels were elevated in pregnant *A. caraya* (57,843.86 ± 37,160.31 ng/g) and declined after parturition (1,539.07 ± 2,894.74 ng/g). Our results demonstrated that these platyrrhines secrete measurable concentrations of DHEAS, with *A. caraya* secreting levels comparable to those of catarrhines. The EIA kit is valid for quantification of fecal and serum DHEAS, and it is useful for studies on stress and primate evolution.

Keywords Animal welfare · DHEA/S · Hormonal analysis · Stress monitoring

Handling Editor: Joanna M. Setchell

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Introduction

The hormone dehydroepiandrosterone (DHEA) and its sulfate ester (DHEAS) are produced in large quantities by the adrenal glands in primates and serve as precursors to sex steroids (Leowattana, 2004; Muehlenbein *et al.,* 2003). Both hormones (DHEA/S) decline with aging (Muehlenbein *et al.,* 2003) and play several functions as immunostimulants (Hazeldine *et al.,* 2010), neuroprotective hormones (Maninger *et al.,* 2009), and glucocorticoid antagonists (Prall *et al.,* 2017). These hormones are also important for parturition (Rainey *et al.,* 2004) and pregnancy maintenance, which makes them useful for gestational monitoring (Takeshita *et al.,* 2016). DHEA/S levels increase at late gestation (Takeshita *et al.,* 2016, 2019) owing to the development of a transient layer in the fetal adrenal known as the fetal zone. This layer secretes high levels of DHEA/S, which are transferred to the mother via the placenta and converted to estrogens (Kaludjerovic & Ward, 2012). The fetal zone regresses quickly after birth, so neonates have a steep decline in DHEA/S levels in the first weeks postpartum (Rainey *et al.,* 2004; Walsh *et al.,* 1984).

Following birth, the age-related pattern of DHEA/S secretion varies with species. A postnatal increase in DHEA/S secretion has been reported and defined as adrenarche in *Homo* (Enomoto *et al.,* 2008; Orentreich *et al.,* 1984), *Pan* sp., *Gorilla gorilla* (Bernstein *et al.,* 2012), and *Pongo pygmaeus* (Prall *et al.,* 2015; Takeshita *et al.,* 2019). In contrast, Afro-Eurasian monkeys, such as *Macaca mulatta* (Kemnitz *et al.,* 2000; Muehlenbein *et al.,* 2003), *Macaca nemestrina*, *Papio cynocephalus* (Muehlenbein *et al.,* 2003), and *Macaca fuscata* (Takeshita *et al.,* 2013), have a continuous age decline in DHEA/S levels, although a short increase in DHEA and DHEAS secretion has been reported in the first weeks post-birth in *M. mulatta* (Conley *et al.,* 2012). Few studies have reported the pattern of DHEA/S in platyrrhines. Studies of *Callithrix jacchus* revealed overall lower DHEA/S secretion than in catarrhines and no evidence of adrenarche. Sex differences are also observed in this species: while the male adrenal gland does not produce DHEA/S, ovariectomized females (but not intact females) showed a significant increase in circulating DHEA levels after adrenocorticotropic hormone (ACTH) stimulation, indicating that adrenal secretion of DHEA in females is regulated by ovarian activity (Pattison *et al.,* 2005, 2007). Whether this pattern is unique to *C. jacchus* is unknown. Comparative studies using other platyrrhine species are crucial to elucidate the role of DHEAS in primate evolution.

Recent studies have highlighted the use of DHEA/S in combination with glucocorticoids (GCs) to monitor animal welfare because of their roles in reducing stress, boosting the immune system, and improving mood (Pluchino *et al.,* 2015; Prall *et al.,* 2017). To minimize or avoid stress to the animal, collection of noninvasive biomaterials, such as feces, urine, and saliva, are the preferred methods of sample collection (Andrabi & Maxwell, 2007; Schwarzenberger *et al.,* 1996). Assays using feces must be carefully validated to determine the antibody specificity and suitability for the species, because only metabolites are present in feces (Heistermann *et al.,* 2006; Palme, 2019; Peter *et al.,* 1996; Schwarzenberger *et al.,* 1996). Although several studies have measured DHEA/S levels noninvasively (Behringer *et al.,* 2012; Seraphin *et al.,* 2008; Takeshita *et al.,* 2013, 2014, 2016, 2018a, b, 2019), only two have validated DHEA/S assays physiologically or biologically for use with feces (Takeshita *et al.*, 2018a, 2019).

We aimed 1) to validate a commercially available EIA to measure DHEAS in feces of three species of platyrrhine via a pharmacological challenge and 2) to test the effect of pregnancy on fecal DHEAS levels in *Alouatta caraya*. If the assay detects biological differences in DHEAS, we predicted that we would observe higher DHEAS levels in the final third of gestation compared to those in the postpartum or nonpregnant/nonlactating periods. We chose DHEAS because it is more stable than DHEA and present in higher concentrations in the circulation (Kroboth *et al.,* 1999).

Methods

Experimental Subjects

We studied 10 adult animals, based on age classifications established for each species: five female and one male *Alouatta caraya* (7.4 ± SD 3.43 years, range 4–12 years; Rímoli *et al.,* 2012), one female and one male *Aotus azarae infulatus* (14 ± SD 2.82 years, range 12–16 years; Aquino & Encarnación, 1994), and one female and one male *Sapajus apella* (15 ± SD 7.07 years, range 10–20 years; Fragaszy *et al.,* 2004). The animals belong to the breeding colonies of the National Primate Center (Centro Nacional de Primatas, CENP) in the district of Ananindeua, Pará, Brazil, 1°38′26″, 48°38′22″). They were kept in enclosures positioned in a north–south orientation to receive ≤12 h of natural light. The *A. a. infulatus* were housed in individual enclosures (1.5 m *D* × 1.0 m *W* × 2.0 m *H*), and had visual, olfactory, and auditory contact with other members of the colony. The other two species lived in groups of *ca.* 10 individuals. The enclosures measured 3.75 m *D* × 2.2 m *W* × 2.4 m *H* (*A. caraya*) and 3.85 m *D* × 2.6 m *W* × 2.5 m *H* (*S. apella*). Inside the enclosures, there were two wooden walkways, access to food bowls, and external water bottles for drinking. The animals were fed according to CENP's standard management practices. Their diet contained different types of fruits and vegetables, eggs, and commercial primate food with 18% crude protein (Cebidae P18 Megazoo, portion Megazoo, Betim, Minas Gerais, Brazil). We also provided daily supplements of amino acids, vitamins, macro and micro minerals, and 0.5 g of Aminomix Pet® (Vetnil Ind. Veterinary Products Ltda. Louveira, São Paulo, Brazil) per kg of body mass. Water was offered *ad libitum*.

Experimental Procedure

Physiological Validation In compliance with the ethics standards to reduce the use of animals in research, we conducted the pharmacological challenge in only one individual of each species. We selected females for this challenge because, in the only platyrrhine primate studied (*Callithrix jacchus*), males do not secrete DHEAS from the adrenal gland (Pattison *et al.,* 2007).

To validate the DHEAS assay, we administered one dose of DHEA (Youthful You™ DHEA, available in 5-mg capsules) to each female (day zero), by mixing the contents of the capsules with the fruit in the animals' regular diet. Although we did not measure the precise concentrations of the DHEAS powder, we used each capsule as estimates of 5 mg. We calculated the dosages for each species based on a validation in *Macaca fuscata* (Takeshita *et al*., 2018a) and adjusted according to body mass: 35 mg

for *Alouatta caraya* (7.5 kg), 5 mg for *Aotus azarae infulatus* (1.0 kg), and 7.5 mg for *Sapajus apella* (2.7 kg). The animals ingested all the DHEA administered in the fruits.

Sample Collection We transferred the two *Alouatta caraya* and *Sapajus apella* individuals to single cages to facilitate the experimental procedures. We started the experiment 10 days after the transfer to acclimatize the animals and to avoid the confounding effects of stress from the transfer (Takeshita *et al.,* 2014). We did not move the *Aotus azarae infulatus* animals because they were already housed individually. We collected fecal samples for 4 days before the DHEA administration (challenge) to determine DHEAS baseline levels in all species (days −4 to −1). Following DHEA administration, we conducted daily sampling on the challenge day (day 0) and for 5 days post-challenge (days 1–5), totaling 10 samples per animal. All sampling occurred between 08:00 h and 12:00 h to minimize the effect of circadian rhythms on hormonal concentrations. We stored each sample in plastic bags labeled with animal ID, date, and time of collection at −80°C within 1 h of collection. We discarded all samples contaminated with urine or remnants of materials from the enclosures.

After 10 days of fecal sampling, we collected a blood sample from each of the three females to determine their serum DHEAS levels. To mitigate stress from blood collection, we restrained the subjects chemically by administering a combination of 10% ketamine hydrochloride (3–5 mg/kg), dexmedetomidine (0.01 mg/kg), and midazolam (0.2 mg/kg) intramuscularly. We collected 1–4 mL of blood from the femoral vein, using sterile syringes and needles (gauge 14–21). We transferred the samples to a tube and centrifuged them at 4000 rpm for 7 min. We transferred the serum to clear microtubes and stored it at −80°C.

Although the males did not participate in the challenge, we collected three fecal samples and one blood sample from each (totaling nine fecal samples and three serum samples) using the same protocols as above. We combined serum samples from males and females to validate the assay analytically for each species and to provide preliminary data for each species' hormonal levels, given that the literature often reports the average for males and females (Bernstein *et al.,* 2012; Rege *et al.,* 2019).

Effect of Gestation We examined the effect of gestation opportunistically, as there were four pregnancies in the *Alouatta caraya* colony during the study. For the safety of the animals, we collected only fecal samples from these females in their own enclosure. To identify samples, we separated the females from the group temporarily on *x* nonconsecutive mornings. To do this, we moved them to an adjacent area through a sliding door. They rejoined the group as soon as the samples were collected. We collected 1–3 samples per female (total 10), on days ranging from 90 to 1 day before parturition. Following the births, we obtained 5 samples from 2 of these females (2–3 samples/female), 6–16 days postpartum.

Hormonal Analyses

Fecal Extraction We lyophilized fecal samples (L101, Liobras Ltda, São Carlos, Brazil) and extracted them by adding 2 mL of absolute ethanol to 0.2 g of sample. We homogenized the samples in a multivortex (TS - 100 Thermo-Shaker, Biosan SA, Riga, Latvia) at 1000 rpm for 30 min, then centrifuged them at 5000 rpm for 15 min (5427 R Eppendorf, Hamburg, Germany). We stored the supernatant at −80°C until hormonal analyses.

Preparation of Standards, Controls, and Samples We prepared five calibrators by serially diluting the top standard in assay buffer from 60,000 pg/mL to 96 pg/mL, according to the manufacturer's instructions (Arbor Assays EIA Kit, K054-H5; Ann Arbor, MI, USA). To determine the best dilution for each species, we serially diluted a pool of fecal extracts containing six samples (three from the female pre-challenge, and three from the male, for each species) at 1:5, 1:25, 1:125, 1:625, and 1:3125 in the provided assay buffer. For the serum, we serially diluted three pools containing male and female samples from each species at 1:2, 1:4, 1:8, 1:16, 1:32, and 1:64. We prepared the control at a concentration of 600 pg/mL.

Parallelism and Accuracy We performed parallelism tests for feces and serum using the serially diluted pool. We confirmed parallelism between the curves generated by the optical density (OD) of each pool and the standard curve, visually and using an F test. We conducted an accuracy test by adding the pools of fecal extracts and serum to equal parts of known quantities of calibrators (12,000, 2400, 480, 96 ng/mL). We calculated recovery as the observed/expected values based on unspiked samples and expressed as a percentage.

Assay Procedures We performed hormonal analyses using the DHEA-S Arbor Assays EIA kit (K054-H5) and a plate reader (Thermo Scientific ™ Varioskan ™, Vantaa, Finland), following the manufacturer's instructions. We analyzed the absorbance results in the Microplate Manager v6 software in a four-parameter curve.

The cross-reactivity of the antibody used in the DHEAS kit is 100% DHEAS, 162% for DHEA, 44.5% for epiandrosterone, 28.4% for androsterone, 15.2% for androstenedione, 0.5% for DHT, 0.4% for adrenosterone, 0.4% for testosterone, 0.2% for deoxycorticosterone and progesterone, and <0.1% for the other steroids.

Data Analysis

We carried out all analyses using GraphPad Prism Software (version 7.0, GraphPad Software Inc., San Diego, CA, USA). For the pharmacological challenge, we calculated baseline fecal DHEAS concentrations for each species as the mean of DHEAS concentrations obtained before the challenge. We defined the time lag between hormonal secretion and excretion in metabolites as the time between DHEA administration and the hormonal peak. We tested the effect of gestation by comparing fecal DHEAS levels before and after parturition. We used the fecal DHEAS levels of the female *Alouatta caraya* from the DHEA challenge for DHEAS levels in a female that was not pregnant or lactating. We did not perform statistical analyses because of our small sample size. We assessed analytical validation using accuracy and parallelism tests in a pooled sample of each species to exclude matrix effects in both serum and fecal extracts. Pooled samples are often used to determine the dilution rate for a species because it represents the mean hormonal levels of a given species (Behringer *et al.,* 2012; Bernstein *et al.,* 2012; Rege *et al.,* 2019; Takeshita *et al*. 2018a). We report the value obtained from the pooled (male and female combined) serum of each species at the dilution rate detected at 50% binding for comparison with findings for other species.

We report the mean ± standard deviation (SD) as measures of central tendency and variation. We set α at 0.05.

Ethical Note

The project followed all the guidelines contained in the resolutions of the National Council for the Control of Animal Experimentation – Ministry of Science and Technology (CONCEA-MCT, Brazil), and approved by the Ethics Committee on the Use of Animals (CEUA no. 43/2019) of the Institute Evandro Chagas (IEC), Ananindeua, Pará, Brazil. The authors declare that they have no conflict of interest.

Data Availability The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Results

Parallelism and Accuracy of the Assay

The ideal dilutions for measuring DHEAS in the three species ranged from 1:50 to 1:200 for fecal extracts and from 1:4 to 1:64 for serum (Table I).

The dilutions performed for the DHEAS assays in the three species were parallel to the standard curve (Fig. 1), and *F* tests showed that the curves were not significantly different (Table II). This indicates no matrix effects in both feces and serum pools. The mean intraassay coefficient of variation for the DHEAS test was 9.22% ($N = 29$) and the interassay coefficient was 14.95% ($N = 3$).

The overall mean recovery for fecal extracts was 114% ± 20 ($N = 4$) for *Alouatta caraya*, 110% ± 2 ($N = 4$) for *Sapajus apella*, and 107% ± 7 ($N = 4$) for *Aotus azarae infulatus*. The mean recovery for serum was 92% ± 21 ($N = 4$) for *A. caraya*, 5% ± 18 ($N = 4$) for *S. apella*, and 120% ± 23 ($N = 4$) for *A. a. infulatus*.

Pharmacological Challenge

The pharmacological challenge (oral administration of DHEA) resulted in an adrenal response in all three individuals. In *Alouatta caraya*, fecal DHEAS concentrations were 13,281.15 ± 4092.38 ng/g ($N = 4$) before the challenge and peaked 24 h after it—an increase of 672% (89,276.27 ng/g) (Fig. 2a). In *Aotus azarae infulatus* fecal DHEAS concentrations were 1217.78 ± 642.03 ng/g ($N = 4$) before the challenge, with two peaks, one at 24 h, with an increase of 308% (3753.84 ng/g) ($N = 4$), and a second peak at 72 h after administration, with an increase of 758% (9227.58 ng/g) (Fig. 2b). In *Sapajus apella* fecal DHEAS

Table I Dilutions of fecal extracts and serum for DHEAS in three species of platyrrhine housed at the National Primate Center, district of Ananindeua, Pará, Brazil, January 2020

Species	Fecal extracts	Serum
Alouatta caraya	1:200	1:64
Aotus azarae infulatus	1:50	1:16
Sapajus apella	1:100	1:4

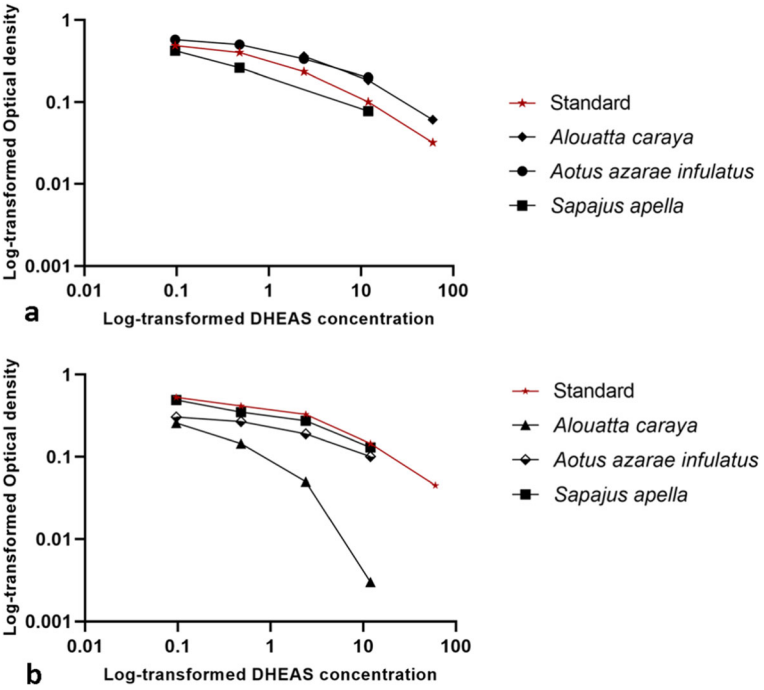

Fig. 1 Log-transformed optical density of DHEAS standard curve and a combined (**a**) fecal extract and (**b**) serum samples in three platyrrhine primate species (housed at the National Primate Center, in the district of Ananindeua, Pará, Brazil, in January 2020), diluted serially in assay buffer.

concentrations were 359.91 ± 255.00 ng/g ($N = 4$) before the challenge and peaked 24 h after administration, with an increase of 341% (1227.92 ng/g) (Fig. 2c).

Effect of Gestation

The mean fecal DHEAS levels in pregnant *Alouatta caraya* (57,843.86 ± 37,160.31 ng/g; $N = 10$) were above those for the female that was not pregnant or lactating (13,281.15 ± 4092.38 ng/g; $N = 4$), and samples from to late gestation had the highest DHEAS levels. After delivery, fecal DHEAS levels were 37-fold lower (1539.07 ± 2,894.74 ng/g; $N = 5$) than mean pregnancy levels (Fig. 3).

Table II Results of F tests for parallelism for DHEAS assays in three species of platyrrhine housed at the National Primate Center, district of Ananindeua, Pará, Brazil, January 2020

Species	Fecal extract			Serum		
	F	df	P	F	df	P
Alouatta caraya	1.64	4, 2	0.826	3.07	4, 3	0.384
Aotus azarae infulatus	1.31	4, 3	0.860	4.78	4, 3	0.229
Sapajus apella	1.29	4, 2	0.974	1.72	4, 3	0.684

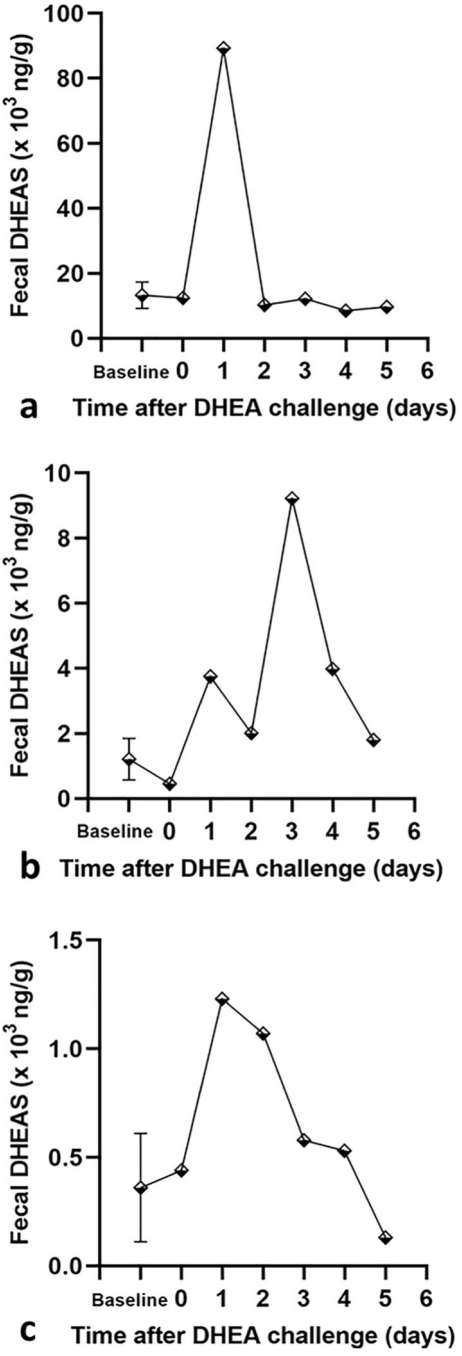

Fig. 2 Changes in fecal concentrations of DHEAS (× 10^3) over time in a female (**a**) *Alouatta caraya*, (**b**) *Aotus azarae infulatus*, and (**c**) *Sapajus apella* (housed at the National Primate Center, in the district of Ananindeua, Pará, Brazil, in January–March 2020) after oral administration of DHEA (day 0). Error bars indicate standard error of three samples collected before the challenge.

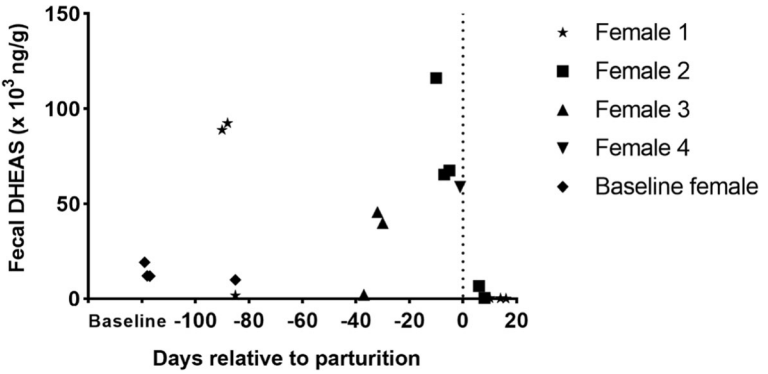

Fig. 3 Comparison of DHEAS concentrations (× 10^3) in *Alouatta caraya* (housed at the National Primate Center, in the district of Ananindeua, Pará, Brazil, in January–April 2020) during pregnancy ($N = 4$) and lactation ($N = 2$) with baseline levels (three samples from one nonpregnant/nonlactating female). Day 0 = day of parturition.

Serum DHEAS Concentration

The mean serum DHEAS levels obtained from the combined samples (one male + one female) for each species were 258.93 ng/mL in *Alouatta caraya*, 57.37 ng/mL in *Aotus azarae infulatus*, and 13.30 ng/mL in *Sapajus apella*.

Discussion

The accuracy and parallelism tests demonstrated that neither serum nor fecal samples exhibited matrix effects, indicating that these samples can reliably quantify DHEAS levels. The physiological validation showed that the assay measured DHEAS levels from fecal metabolites. The increase in DHEAS concentrations after oral administration of DHEA is expected due to conversion of DHEA to DHEAS, which occurs through the action of the enzyme DHEA sulfotransferase (SULT2A1) (Rainey *et al.,* 2004).

The DHEAS excretion time lag varied from 24 h to 72 h across species. The three species also differed in the time taken to return to baseline levels, with *Alouatta caraya* doing so faster than the other species. This is surprising, given that *A. caraya* has the slowest metabolism and highest degree of folivory of the three species (Glander, 1980; Milton, 1980). *Sapajus apella* have relatively higher metabolism and feed on a variety of foods (Fragaszy *et al.,* 2004). The gastrointestinal transit time of *Alouattas* sp. is approximately eight times greater than that of *Sapajus* sp. (Milton, 1984, 1993), which is related to the higher amount of fiber in the diet of folivores. However, the animals described in this study live in captivity, and their diets contain fruits and vegetables, which may have decreased the species differences in gut retention time and fecal frequency (Palme, 2019). Other factors that could have contributed to our findings are species-specific differences in the metabolic route, enzymatic activity of gastrointestinal bacteria, and differences in body mass (Bahr *et al.,* 2000; Goymann, 2012; Möstl & Palme, 2002).

The DHEAS fecal excretion time observed in *Alouatta caraya* is similar to the excretion of fecal GC metabolites in *A. caraya* challenged with ACTH (Buti *et al.,*

2018). This suggests that 24 h may be the standard time of secretion of adrenal steroids in this genus. Although *Sapajus apella* and *Aotus azarae infulatus* had similar peaks at 24 h, they had a slower decrease in DHEAS concentrations, taking 96 h to return to baseline levels.

The results of the DHEA challenge in *Sapajus apella* and *Aotus azarae infulatus* were similar to those in female *Macaca fuscata*, in which fecal DHEAS concentrations increased at 24 h and returned to baseline at 72 h after oral DHEA administration (Takeshita *et al*., 2018a). In addition, there were two peaks in *A. a. infulatus*, with the second peak being higher than the first. The explanation for the second peak is unclear, but it might be related to 1) gut reabsorption (Palme, 2019) or 2) further conversion of the DHEA to DHEAS in other tissues (Klinge *et al.,* 2018). It is also possible that the individual experienced acute stress during the experimental procedure (e.g., during feeding procedures or from visual communication with conspecifics), which could have elicited a DHEAS response (Takeshita *et al*. 2018, 2019). Furthermore, considering that *Callithrix* has a peculiar adrenal mechanism not observed in other species (Pattison *et al.,* 2005; 2007), the owl monkey adrenal may also have a different mechanism. Further studies are needed to test these hypotheses.

We observed an increase in DHEAS concentrations in pregnant females in relation to a female that was not pregnant or lactation, followed by a decrease after parturition. These findings are consistent with those for *Macaca fuscata* and *Pongo pygmaeus*, which detected high fecal DHEAS levels at the end of pregnancy (Takeshita *et al.,* 2016, 2019). Gestation lasts a mean of 180 days in *Alouatta caraya* (Calegaro-Marques & Bicca-Marques, 1993; Kowalewsky & Zunino, 2004), and we found an increase in DHEAS concentrations in the final third of pregnancy, *ca.* 30 days before parturition and at 83% of the gestational period, with the highest concentrations close to parturition, at about 95% of the gestational period. The increase observed at the end of pregnancy and decline after parturition may be associated with the development of the fetal adrenal gland. In *Sapajus* sp., which have a 155-day gestation period (Rylands & Mittermeier, 2013), the adrenal gland of the fetus increases in size significantly between 90 and 141 days (from 58% to 90% of the gestational period) (Torres-Farfan *et al.,* 2003). The development of the fetal zone at the end of pregnancy contributes to the high concentrations of DHEAS as a source of estrogens necessary for parturition, which plummet to lower levels after birth due to the regressing fetal zone (Klinge *et al.,* 2018; Rainey *et al.,* 2004; Walsh *et al.,* 1984). A study in *M. fuscata* suggested that low DHEAS levels at this stage may indicate fetal death, which makes this method useful for monitoring reproductive success in free-ranging primates noninvasively (Takeshita *et al.,* 2016).

Alouatta caraya showed higher baseline concentrations of DHEAS in feces and serum, followed by *Aotus azarae infulatus*, with lower concentrations in *Sapajus apella*. The concentration of DHEAS is highly variable among primates and is related to differences in the expression of adrenal enzymes involved in the conversion of DHEA to DHEAS (Rege *et al.,* 2019). In addition, circadian rhythm may have influenced these differences (Hucklebridge *et al.,* 2005). We collected samples in the morning, but the circadian rhythm of the nocturnal *A. a. infulatus* is likely to differ to that for the two diurnal species and could have influenced comparisons (Pieper & Lobocki, 2000). In addition, *A. a. infulatus* were housed singly, which increases DHEAS levels in *Macaca fuscata*, in comparison to socially living individuals

(Takeshita *et al.,* 2014). Future studies controlling for these two variables are needed to compare DHEAS levels across these species.

Serum DHEAS levels in our *Alouatta caraya* sample were higher than those reported in most catarrhine primates, such as *Cercopithecus* (134.87 ng/mL), *Macaca* (186.03 ng/mL), *Symphalangus* (116.19 ng/mL), and *Pongo* (109.06 ng/mL), comparable to that described in *Gorilla* (227.55–355 ng/mL) (Bernstein *et al.,* 2012; Edes, 2017) and lower than that described in the genera *Pan* (669.46 ng/mL) and *Homo* (1206.7 ng/mL) (Bernstein *et al.,* 2012). Previous studies did not consider the ability of platyrrhine primates to secrete DHEAS from the adrenal gland based on studies on *Callithrix*¸ which secretes low or nondetectable levels of DHEAS (Pattison *et al.,* 2005; Pattison *et al.,* 2007). However, due to the great diversity of platyrrhine primates, comparative studies that include other platyrrhine species with different ecology, behavior, and life histories are needed to understand the possible role of DHEAS in their evolution. This is supported by our data on the interspecies variation in DHEAS levels, and by the fact that *A. caraya* had levels comparable to those of catarrhine primates.

Serum DHEAS concentrations in *Aotus azarae infulatus* were similar to those described in *Colobus* (35.54 ng/mL) (Bernstein *et al.,* 2012). Only one study has measured DHEA in *Aotus* sp., noninvasively (from urine samples), to investigate the effect of reproductive experience on the DHEA/cortisol ratio in mated pairs (Bardi *et al.,* 2014). The authors found that mated pairs with reproductive experience showed more efficient foraging responses than those without reproductive experience, and a higher DHEA/cortisol ratio, suggesting that behavioral and social factors influence DHEA, and consequently, DHEAS levels in this species. Further studies and a larger sample size that controls for social factors are needed for interspecies comparison in DHEAS secretion.

Sapajus apella had the lowest DHEAS concentration among the three platyrrhine primates we evaluated. Its concentration was also low relative to those in catarrhines. However, the number of samples presented in this study is limited in comparison to studies of catarrhines. We used only adult animals and combined the sexes in our sample, and the lack of infants and juveniles in our dataset and potential sex differences may have influenced the overall DHEAS levels of this species. One study reports serum DHEAS concentrations in fetuses of *S. apella* (Torres-Farfan *et al.,* 2003) but we cannot compare our data with the data in that study because given that fetuses have significantly higher DHEAS levels due to the fetal adrenal (Raineyet *al.,* 2004; Takeshita *et al.,* 2013, 2016).

In addition to the enzymes involved in DHEAS biosynthesis, the percentage of free and bound hormone in the serum can influence the results (Barsano & Baumann, 1989). Other factors that need to be considered are related to the metabolism of DHEA and DHEAS, which varies according to sex and age, as well as species-specific metabolic clearance rates (Whitham *et al.,* 2020). Furthermore, DHEA and DHEAS can be metabolized in several tissues, such as the placenta, ovary, testicles, prostate, adipose tissue, liver, and brain, which are tissues associated with processes directed by a nuclear sex hormone receptor (Klinge *et al.,* 2018). All these factors may have influenced the interspecies variation in DHEAS levels found in this study.

In summary, we validated a noninvasive method to measure DHEAS in three platyrrhine species. Differences in DHEAS levels among these species suggest that

some platyrrhine species secrete DHEAS at levels comparable to catarrhines. To better understand the functions and age-related patterns of DHEAS secretion and excretion in primate evolution, comparative research involving platyrrhines is needed. These studies may help us to develop platyrrhine models for studying the effects of DHEAS on cognition and neonatal development, age-related disorders, and on the evolution of the adrenal gland among primate species (Campbell, 2020; Takeshita *et al*., 2018b). Furthermore, the GC/DHEAS index can be used as part of a multifaceted and more accurate assessment of stress (Takeshita *et al.,* 2019; Whitham *et al.,* 2020) and can be applied to monitor primate welfare and reproduction.

Acknowledgments We thank the staff from the National Primate Center (CENP) and from the Federal Rural University of the Amazon (UFRA) for providing animal care and for their assistance during data collection. We would like to express our gratitude to Dr. Anthony Tosi for editing the manuscript and to the journal editor and two anonymous reviewers for the very useful comments that improved this manuscript. This study was funded by the National Council of Technological and Scientific Development (CNPq Nos. 201475/2017-0 and 155324/2018-7), the Coordination for the Improvement of Higher Education Personnel (CAPES-Procad Amazônia No. 21/2018), the Wenner-Gren Foundation (No. 10088), and the National Science Foundation (Award No. 212357).

Author Contributions RSCT and FOBM conceived and designed the study. GPS, JTM, and ANPF performed the experiments and collected data. GPS and RSCT analyzed the data. GPS, RSCT, and FOBM wrote the manuscript; the other authors provided editorial advice.

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Received: 22 April 2022 | Revised: 24 May 2022 | Accepted: 25 May 2022

DOI: 10.1111/jmp.12599

ORIGINAL ARTICLE

WILEY

Effect of age and sex in renal function by ultrasound and serum chemistry in two primate species (*Alouatta caraya* and *Sapajus apella*)

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Funding information
National Science Foundation, Grant/ Award Number: 212357; Wenner-Gren Foundation, Grant/Award Number: 10088; Coordination for the Improvement of Higher Education Personnel, Grant/ Award Number: 21/2018; National Council of Technological and Scientific Development, Grant/Award Number: 316750/2021-2, 400881/2019-5 and 305821/2017-2

Abstract

Background: Comparative studies of kidney morphophysiology in nonhuman primates can help us investigate interspecies differences in growth and aging patterns.

Methods: We tested the effect of age and sex in renal morphophysiology in 21 *Alouatta caraya* and 21 *Sapajus apella* (age range = 0.5–26 years) by ultrasound, red blood cell (RBC) count, and kidney function tests.

Results: *A. caraya* had greater growth rate and absolute renal volume than *S. apella*, but the latter showed a greater relative renal volume and RBC count. There was a negative relationship between RBC and age, a positive relationship between creatinine and body mass, and an apparent negative relationship between creatinine and age only in *S. apella*.

Conclusions: Our results indicate that *A. caraya* has a faster aging mechanism than *S. apella*, and the higher relative kidney volume in *S. apella* is suggestive of high metabolic demands in this species.

KEYWORDS
aging, nonhuman primates, renal morphophysiology, ultrasonography

1 | INTRODUCTION

Kidneys are important metabolic organs due to their function in excretion, filtration, water balance, blood pressure, and in the production of erythropoietin (EPO) to stimulate red blood cell (RBC) production.[1–3] When the kidneys are damaged, their function may be compromised, which could result in anemia, high blood pressure, or even renal failure,[2,4] which can be fatal. Assessment of renal function is therefore essential in clinical settings as a screening tool and for monitoring disease progression in primate husbandry.

While renal function tests commonly include urea and creatinine, early detection of renal alterations are useful in preventing aggravation of the clinical condition. One potential indicator of renal alteration in humans is low renal volume,[5,6] which can indicate a reduction in the number of nephrons and predict risk of renal diseases.[5,7] However, ultrasound data showed variations in renal biometric parameters with advancing age in humans and nonhuman primates (NHP), such as a progressive growth into adulthood, followed by size reduction in elder *Aotus azarae infulatus*[8] and humans.[6] To account for these differences, we must establish reference values for renal biometric parameters per age class for each species.

In addition to the clinical importance of monitoring renal function, comparative studies can help us understand species differences in aging mechanisms. The renal aging process is marked by the loss of nephrons and a reduction in the glomerular filtration rate, leading to a loss of functioning renal mass.[9] In addition, macroscopic changes such as reduction in cortical volume, greater medullary volume, and the occurrence of cysts have been reported in elder humans.[6,9] Furthermore, age can influence renal function tests in NHP,[8,10,11] the responsiveness to EPO, and consequently, RBC count, which has been associated to increased rates of anemia in elderly patients.[12,13] However, whether these age-related changes correlate with changes in renal morphology remains to be investigated.

Nonhuman primates are excellent comparative models for study the aging process due to their phylogenetic similarities with humans, but also due to their great diversity in life-history traits. For example, the genus *Alouatta* (howler monkeys) and *Sapajus* (capuchin monkeys) are platyrrhine primates from the families Atelidae and Cebidae, respectively. *A. caraya* weights about 5 kg for females and 7.8 kg for males,[14] with a mean gestational period of 6 months, an inter-birth interval of 15.8 months,[15,16] and a longevity of approximately 26 years in captivity.[17] *S. apella* are relatively smaller, weighing approximately 3 kg for females and 4 kg for males,[18,19] but have similar reproductive traits, with a mean gestational period of 5 and 20 months of interbirth interval.[20–22] Despite the smaller body size and similar reproductive traits, *S. apella* has a longevity of up to 50 years in captivity,[23] which is almost double that of *A. caraya*. Considering this remarkable difference in longevity, the comparative study of age-related changes in kidney morphology and their effect in renal function tests in NHP can help us understand evolutionary mechanisms of senescence.[24]

Previous studies have characterized kidney size in different age classes in NHP such as *Callithrix jacchus*,[25] *A. azarae infulatus*,[8] *Saimiri collinsi*,[26] and *Macaca fascicularis*.[27] Although a few studies have described the renal ultrasonography in *Alouatta fusca*,[28] and *S. apella*,[29,30] these studies were limited to adults. The goal of this study was to evaluate the kidney by ultrasound in infant, juvenile, and adults *A. caraya* and *S. apella* to (1) describe age-related changes in renal ultrasonographic appearance, (2) establish the renal biometric parameters per age group, (3) compare species differences in renal volume and kidney growth, and (4) to test potential correlations between renal volume and age, renal function tests (urea and creatinine) and RBC.

2 | METHODS

2.1 | Humane care guidelines

The experimental project followed the guidelines of the Brazilian Council for the Control of Animal Experimentation—Ministry of Science and Technology (CONCEA-MCT, Brazil), and was approved by the Ethics Committee for the Use of Animals (CEUA no. 43/2019 and 24/2021) of the Institute Evandro Chagas (IEC), Ananindeua, Pará, Brazil and by the Biodiversity Authorization and Information System of the Chico Mendes Institute of Biodiversity (Sisbio/ICMBio, protocol 38529-9).

2.2 | Subjects

The animals belonged to the breeding colony of the National Primate Center (Centro Nacional de Primatas—CENP, Ananindeua, Pará, Brazil, 1°38′26″, 48°38′22″). We identified each animal using a three-letter code tattooed on the right thigh and a microchip placed in the interscapular area.

The subjects were 21 *A. caraya* (12 females and nine males), with a mean ± SD body mass of 5.13 ± 3.4 kg (0.9–14.1 kg) and 21 *S. apella* (11 females and 10 males), with a mean ± SD body mass of 2.12 ± 0.79 kg (0.9–3.95 kg). We classified the animals in three age groups, according to the literature available for each species. For *A. caraya*, there were three infants (<1 year; 6.6 ± 0.58 months), 10 juveniles (1.5–4 years; 2.6 ± 0.74), and eight adults (6–26 years, 12.5 ± 6), based on the classification proposed by Rímoli.[31] For *S. apella*, there were four infants (<2 years; 11 ± 4.12 months), 10 juveniles (2–4 years; 2.7 ± 0.95), and seven adults (5–21 years, 16.85 ± 6), based on the classification proposed by Fragaszy et al.[23]

All primate colonies at CENP are submitted to annual health screenings, which include physical examination, hemogram, and biochemical tests, in addition to deworming treatment. None of the animals used in this study had a history of infectious diseases as per their last health screening (2 months before data collection).

2.3 | Husbandry

All individuals lived in family groups of up to 10 individuals. They were kept in sheds and positioned in a north–south orientation to receive ≤12 h of natural light, in enclosures measuring 3.75 m × 2.2 m × 2.4 m (*A. caraya*), and 3.85 m × 2.6 m × 2.5 m (*S. apella*). The enclosures had external and internal water bottles and multiple bowls for food provisioning. The animals were fed according to CENP's standard management practices. Their diet contained different types of fruits and vegetables, eggs, and commercial primate food with 18% crude protein (Cebidae P18 Megazoo, portion Megazoo, Betim, Minas Gerais, Brazil). We also provided daily supplements of amino acids, vitamins, macro and micro minerals, and 0.5 g of Aminomix Pet® (Vetnil Ind. Veterinary Products Ltda.) per kg of body mass. Water was offered ad libitum.

2.4 | Capture and sample collection

Following an 8 h fasting period, the animals were contained physically with the aid of nets and chemically by intramuscular administration of a combination of ketamine hydrochloride (5 mg/kg), dexmedetomidine (0.01 mg/kg), and midazolam (0.2 mg/kg). With the animal contained, we collected between 0.5 and 3 ml of blood from the femoral vein with sterile syringes and needles (14–21 G, depending on the species and age of the animal). Blood samples were equally divided in two and transferred to two tubes: one containing ethylenediaminetetraacetic acid (EDTA) for the hemogram procedure and one without anticoagulants for clinical chemistry (see below).

After blood collection, a trained veterinarian conducted a clinical evaluation in each animal by inspection, auscultation, palpation, and percussion.

2.5 | Ultrasound exams

To prepare the animals for the ultrasound exams, we shaved the hair in their abdominal region to avoid hair-product artifacts and applied an acoustic transmission gel (Carbogelt, São Paulo, São Paulo, 04143-010, Brazil) to the shaven area to enhance ultrasonographic images. The exams were performed with an ultrasound system (Esaote® model Mylab Gamma), equipped with a linear and multifrequency electronic probe transducer of 4–13 MHz, in mode B. The animals were placed in three distinct positions (supine, right, or left lateral) to examine the entire abdominal region outlined by three limits: the last pair of ribs and the xiphoid cartilage (cranial limit), the transverse process of lumbar vertebrae (lateral limit), and the inguinal region (caudal limit).[29] First, to exclude the possible presence of other diseases that could affect laboratorial exams, we examined the liver and adrenal gland for potential alterations (e.g., cysts, gallstones, steatosis). In sequence, we evaluated the shape, echotexture, and size of both kidneys following the protocol previously reported in *A. azarae infulatus*[8] and *S. collinsi*.[26] Renal length (L) and height (H) were measured using sagittal scans, and renal width (W) was measured at the hilus using transverse scans (Figure 1). Renal volume was calculated (cm^3) by approximating the spheroidal geometric model from the 3 linear measurements ($L \times H \times W \times \pi/6$, where π is 3.1416) for each kidney. To determine the relative renal volume per individual, we calculated the mean renal volume from the left and right measurements, then divided the value obtained by the body mass. The growth rate was calculated per species for all biometric parameters (length, height, width, absolute volume). First, we calculated the mean values of each parameter for infant and adult classes. We then subtracted the infant means from the adult means and divided this value by the infant means. The results were expressed in percentage.

2.6 | Laboratory tests

Hemograms were performed with an MS4+blood analyzer (Melet Schloesing GmbH Central & Eastern Europe company, Sudstadtzentrum 1, Top 8, 2346 Maria Enzersdorf-Su¨dstadt, Austria) to determine Red Blood Cell (RBC) count, Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Hemoglobin (MCH), Mean Corpuscular Volume (MCV), Mean Corpuscular Hemoglobin Concentration (MCHC), White Blood Cell (WBC) count, and platelets. The clinical chemistry tests were performed on Sistema Vitros DTSC II, DT60 and DTE2 (Johnson & Johnson Medical Argentina), to determine urea and creatinine. For statistical analyses, we used only urea, creatinine, and RBC. The other parameters were used to exclude any potential illness (infection, anemia) that could be associated with kidney alterations.

2.7 | Statistical analysis

All statistical tests were performed in R software (3.3.0). The means obtained from the right and left kidneys were compared by Student's *t* test. General Linear Models (GLM) were used to test the effects of age, sex, and species, on biometric and blood parameters. First, the possibility of multicollinearity was excluded by calculating the Variance Inflation Factor (VIF) with the "car" package. Since all factors had VIF < 2, none were considered problematic in the model. To assess the equality of variances of categorical fixed factors (sex and species), we used Levene's test.[32] All values are expressed as the mean ± standard deviation. Differences with a *P* value of ≤.05 or less were considered significant.

Five models were built to the following variables (response factors): absolute kidney volume, relative kidney volume, RBC, creatinine, and urea. For the biometric parameters, we initially included age, body mass, species, sex, and interactions as fixed factors. As for blood parameters, age, body mass, species, sex, and relative renal volume were initially included as fixed factors. Following Burnham and Anderson,[33] we sequentially removed fixed factors to select the model with the lowest Akaike Information Criterion with correction for small sample sizes (AICc). If the AICc difference between two models (ΔAICc) was less than 2, both models were discussed.

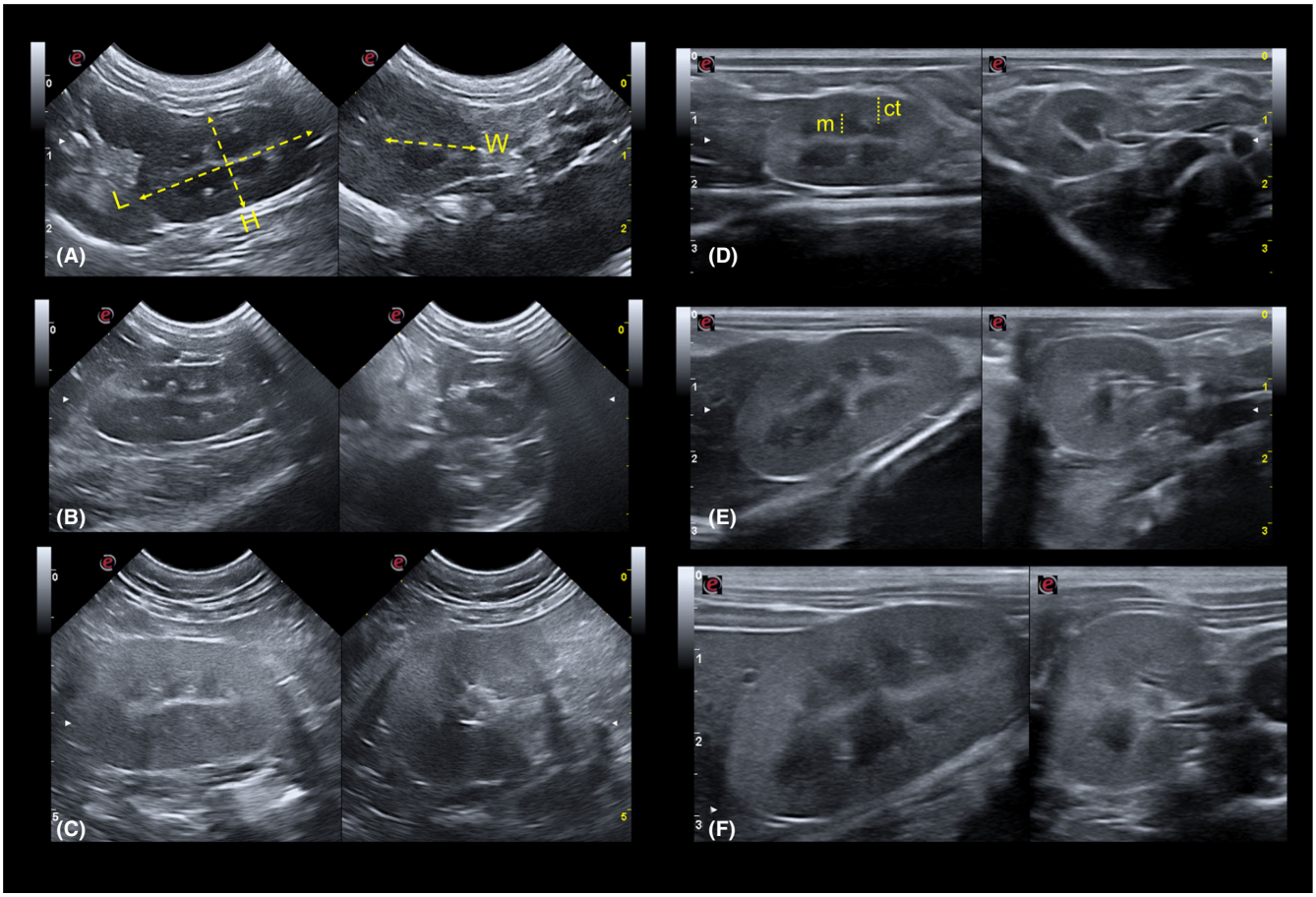

FIGURE 1 Ultrasonographic images demonstrating sagittal and transverse sections for measuring length (L) and height (H) were measured using sagittal scans, and renal width (W) Cortex (ct) and Medullary Pyramid (m) by age group [infant (A and D), juvenile (B and E), and adult (C and F)] in *Alouatta caraya* and *Sapajus apella*, respectively. Note increased thickness and echogenicity of the cortical region, more evident in adult animals of *A. caraya*

3 | RESULTS

3.1 | Ultrasound exams

None of the animals had alterations in kidney topography. The right kidney was more cranial than the left, and both kidneys were characterized by a homogeneous cortical echotexture with preserved corticomedullary differentiation, and a cortex/medulla thickness ratio of approximately 2:1. In both species, the medulla was more evident due to a lower echogenicity in infants. We observed an increase in the thickness and echogenicity of the cortical region with advancing age, which was more evident in adult *A. caraya* (Figure 2).

3.2 | Kidney biometrics

The biometric parameters of the right and left kidneys for each species are summarized per age group in Table 1. There were no significant differences between the measurements of the right and left kidneys for neither *A. caraya* length, $t(39) = 0.003$, $P = .99$, width, $t(39) = 0.33$, $P = .73$, height, $t(39) = 1.25$, $P = .21$, volume, $t(39) = 0.68$, $P = .49$, cortex/medulla, $t(39) = 1.25$, $P = .21$, nor for *S. apella* length, $t(41) = 0.21$, $P = .83$, width, $t(41) = 0.52$, $P = .60$, height, $t(41) = 1.08$, $P = .28$, volume ($U = 192$, $P = .48$), and cortex/medulla, $t(39) = 1.09$, $P = .27$.

The mean growth from infant to adult classes was more pronounced in *A. caraya* than in *S. apella* for all biometric parameters: length = 54.53% and 37.01%; height = 47.64% and 43.03%; width = 55.43% and 22.12%; and volume = 264.52% and 147.26%, respectively.

3.3 | Absolute renal volume

The best model that tested absolute kidney volume as a response factor revealed significant effects of species, sex, and age. *S. apella* had a lower mean renal volume (GLM: -0.02 ± 0.006, $t = -3.52$, $P < .001$) than *A. caraya* (Figure 2A), and the mean renal volume was greater in males (GLM: 0.01 ± 0.006, $t = 2.35$, $P = .02$) than in females (Figure 2B). Moreover, we observed a significant, positive

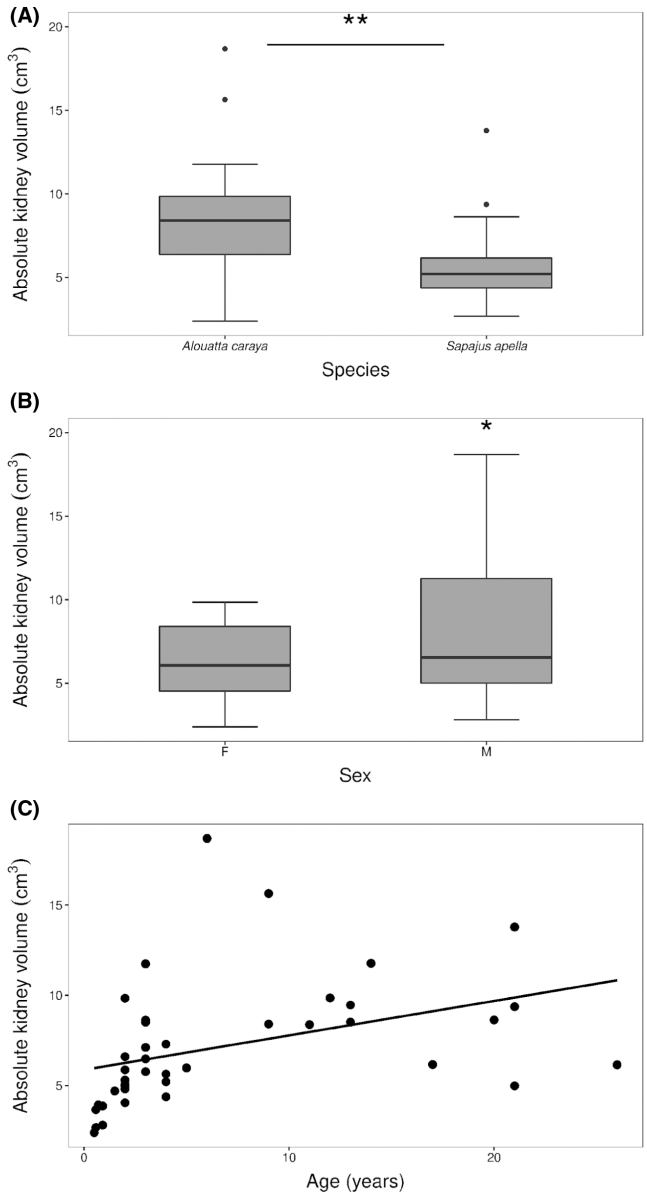

FIGURE 2 Effect of species (A), sex (F—Female; M—Male) (B), and age (C) on the absolute renal volume in *Alouatta caraya* ($N = 21$) and *Sapajus apella* ($N = 21$), housed at the National Primate Center, district of Ananindeua, Pará, Brazil, January 2020. $^{*}P < .05$, $^{**}P < .01$

relationship between volume and age (GLM: 0.001 ± 0.0004, $t = 3.90$, $P = .0004$), controlling for species and sex (Figure 2C).

3.4 | Relative kidney volume

We built a second model using the relative kidney volume as response factor, initially testing the same fixed factors as in the previous model. The best model revealed a significant effect of species, with a higher relative volume in *S. apella* (GLM: 0.32 ± 0.048, $t = 6.74$, $P < .001$) than in *A. caraya* (Figure 3). There was no effect of age and sex in none of the models, so these factors were excluded from the final model.

3.5 | Red blood cells, creatinine, and urea

Finally, we built three additional models to investigate the effect of RBC, creatinine, and urea on sex, age, species, and renal relative volume. For the model including RBC as a response factor, we observed a significant negative effect of age (GLM: -0.03 ± 0.01, $t = -2.3$, $P = .03$) (Figure 4A). We were unable to test the effect of sex due to heteroscedasticity ($F_{1,38} = 6.07$, $P = .02$), but we found a strong effect of species (GLM: 1.15 ± 0.24, $t = 4.77$, $P < .0001$), with higher RBC in *S. apella* ($5.60 \pm 0.49 \times 10^6/mm^3$) than in *A. caraya* ($4.37 \pm 0.58 \times 10^6/mm^3$) (Figure 4B).

When testing creatinine as a response factor, the best model showed a significant, positive correlation with body mass (GLM: 0.04 ± 0.01, $Z = 2.60$, $P = .01$; Figure 5A). We also observed an interaction between species and age, with a steeper negative relationship between creatinine and age only in *S. apella* (GLM: -0.03 ± 0.01, $Z = -3.33$, $P = .002$; Figure 5B). The models for urea as a predictor did not differ from the null model, indicating no effect of body mass, species, sex, or age on urea levels. Mean relative renal volume did not influence any of the blood parameters.

4 | DISCUSSION

The ultrasound appearance of the kidneys in both species was similar to that described previously in other Platyrrhines, including *A. fusca*,[28] *S. apella*,[29,30] *A. azarae infulatus*[10] and *S. collinsi*,[26] in which both kidneys had an elliptical shape, echogenicity and homogeneous cortical echotexture with preserved corticomedullary differentiation. One study in *C. jacchus*, reported a poor corticomedullary distinction in the kidneys,[25] which could have been due to the small size of these primates. Other studies reported a triangular shape when evaluating the left kidney in *Saguinus ursulus*[34] and in some *M. fascicularis* individuals.[35] This emphasizes the importance of characterizing the kidney anatomy for each species.

We found no differences between the right and left kidneys for all biometric parameters, which is similar to previous studies in *S. apella*[29,30] and *Macaca mulatta*,[7] but contrasts with studies in other species. For instance, studies in the platyrrhine primates *A. azarae infulatus*[8] and in *C. jacchus*[25] reported that the left kidney had a greater height and length than the right kidney. Other studies in the catarrhines *M. fascicularis*[35,36] and *Chlorocebus sabaeus*,[37] as well as in the platyrrhine *S. collinsi*,[26] reported the opposite trend, with a greater volume or length in the right kidney when compared to the left. The inconsistency in the literature may be associated with methodological differences, but it may be related to interspecies differences, with no clear trend between platyrrhine and catarrhine species.

The higher growth rate of biometric parameters observed in *A. caraya* in relation to *S. apella* may be associated with the fact that the former reaches a higher body mass than the latter, with approximately 5–7.8 kg in adult *A. caraya*[14] and 3–4 kg in *S. apella*.[18,19] Although we did not find an effect of body mass on renal biometry,

TABLE 1 Ultrasonographic measurements of the right and left kidneys (mean ± standard deviation) between the age groups of *A. caraya* and *Sapajus apella*, housed at the National Primate Center, district of Ananindeua, Pará, Brazil, January 2020

		Right kidney					Left kidney				
Species (*N*)	Age group (*n*)	Length (cm)	Height (cm)	Width (cm)	Cortex/medulla	Volume (cm^3)	Length (cm)	Height (cm)	Width (cm)	Cortex/medulla	Volume (cm^3)
Alouatta caraya (*N* = 21)	Infant (*n* = 3)	2.71 ±0.37	1.32 ±0.08	1.59 ±0.14	1.61 ±0.05	3.01 ±0.72	2.67 ±0.29	1.38 ±0.03	1.57 ±0.16	1.43 ±0.08	3.04 ±0.58
	Juvenile (*n* = 10)	3.73 ±0.42	1.64 ±0.18	2.13 ±0.21	1.68 ±0.48	6.94 ±1.73	3.63 ±0.24	1.75 ±0.25	2.39 ±0.36	1.57 ±0.29	8.16 ±2.85
	Adult (*n* = 8)	4.09 ±0.36	2.02 ±0.37	2.40 ±0.37	1.88 ±0.66	10.68 ±4.03	4.22 ±0.31	1.97 ±0.46	2.52 ±0.45	1.62 ±0.54	11.40 ±4.37
Sapajus apella (*N* = 21)	Infant (*n* = 4)	2.52 ±0.20	1.35 ±0.04	1.79 ±0.37	1.83 ±0.33	3.21 ±0.71	3.03 ±0.31	1.22 ±0.16	1.74 ±0.19	1.63 ±0.07	3.43 ±0.99
	Juvenile (*n* = 10)	3.35 ±0.17	1.61 ±0.08	1.92 ±0.11	1.67 ±0.11	5.45 ±0.62	3.23 ±0.31	1.61 ±0.11	1.79 ±0.22	1.67 ±0.23	4.90 ±1.06
	Adult (*n* = 7)	3.81 ±0.37	1.87 ±0.20	2.19 ±0.33	1.74 ±0.23	8.46 ±2.72	3.79 ±0.40	1.80 ±0.29	2.13 ±0.31	1.67 ±0.23	7.95 ±3.43

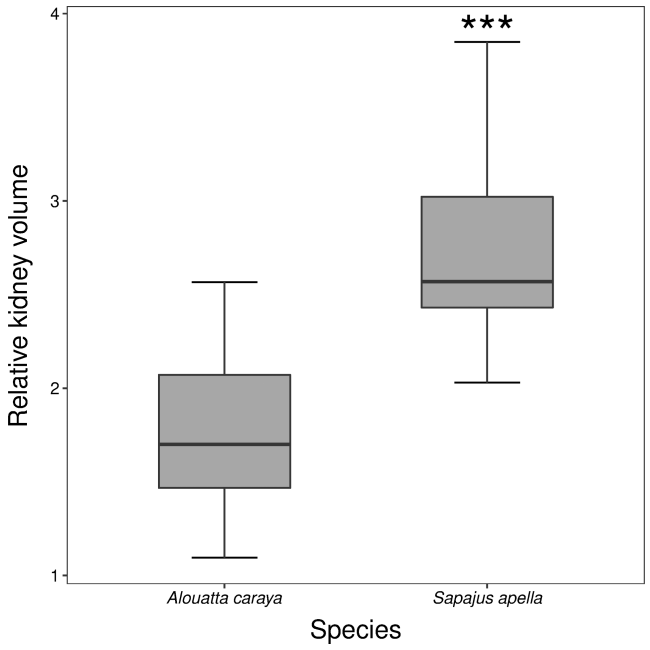

FIGURE 3 Relative renal volume in *Alouatta caraya* and *Sapajus apella*, housed at the National Primate Center, district of Ananindeua, Pará, Brazil, January 2020. ***$P < .001$

we found a greater absolute kidney volume in *A. caraya* than *S. apella* and in adult males in both species. Previous studies have reported a positive correlation between body mass and renal biometric variables in *A. azarae infulatus*,[8] *S. collinsi*[26] and *M. mulatta*.[7] In addition, renal volume in *M. fascicularis* was positively correlated with body mass,[35,36] and in humans the renal length was positively correlated with body mass and body mass index.[38] The lack of significance between body mass and absolute renal volume in this study was probably related to our multi-factorial analyses, and shows that species, sex, and age were better predictors of kidney volume than body mass.

The effect of sex in the literature is also mixed. One study in adult and elder humans (18–80 years old) found no sex differences in renal length or cortical thickness.[38] A post-mortem study in *M. fascicularis* showed that the absolute kidney mass was greater in older than in young animals of the same sex, but this effect was more pronounced in males.[27] In *S. collinsi*, the total kidney volume had an interaction between body mass and sex, being greater in heavier males.[26] In *A. azarae infulatus*, no differences in biometrics parameters were observed between males and females.[8] These interspecies differences are likely associated with the role of sexual dimorphism in the evolution of primate societies. *A. a. infulatus* are monogamous and characterized by little or no sexual dimorphism, given that males do not need to compete for accessing females,[39] and no sex differences are expected in absolute renal volume. In contrast, sexual dimorphism is present in *S. collinsi* and *M. fascicularis*,[39] as well as in both species examined in this study.[40] Consequently, males in these species have larger body mass than females, which reflects their multi-male/multi-female social system.[39,41] Thus, sex differences in morphophysiology appears to be

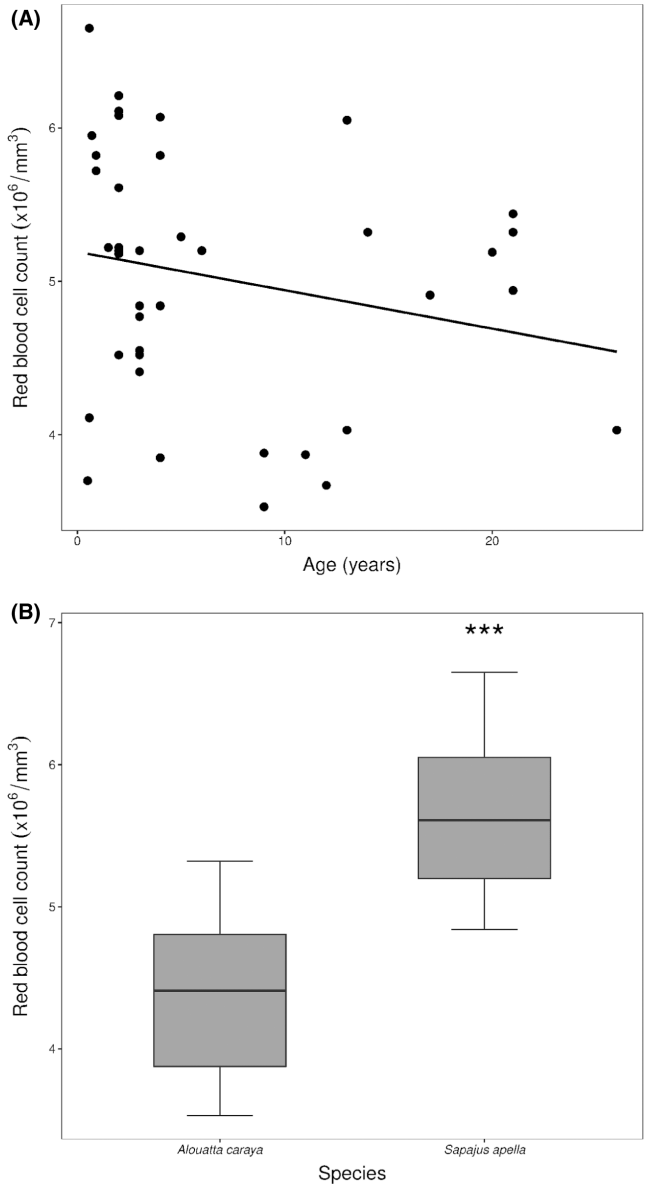

FIGURE 4 Effect of age (A) and species (B) on red blood cell count in howler monkey (*Alouatta caraya*) and capuchin monkey (*Sapajus apella*), housed at the National Primate Center, district of Ananindeua, Pará, Brazil, January 2020. ***$P < .001$

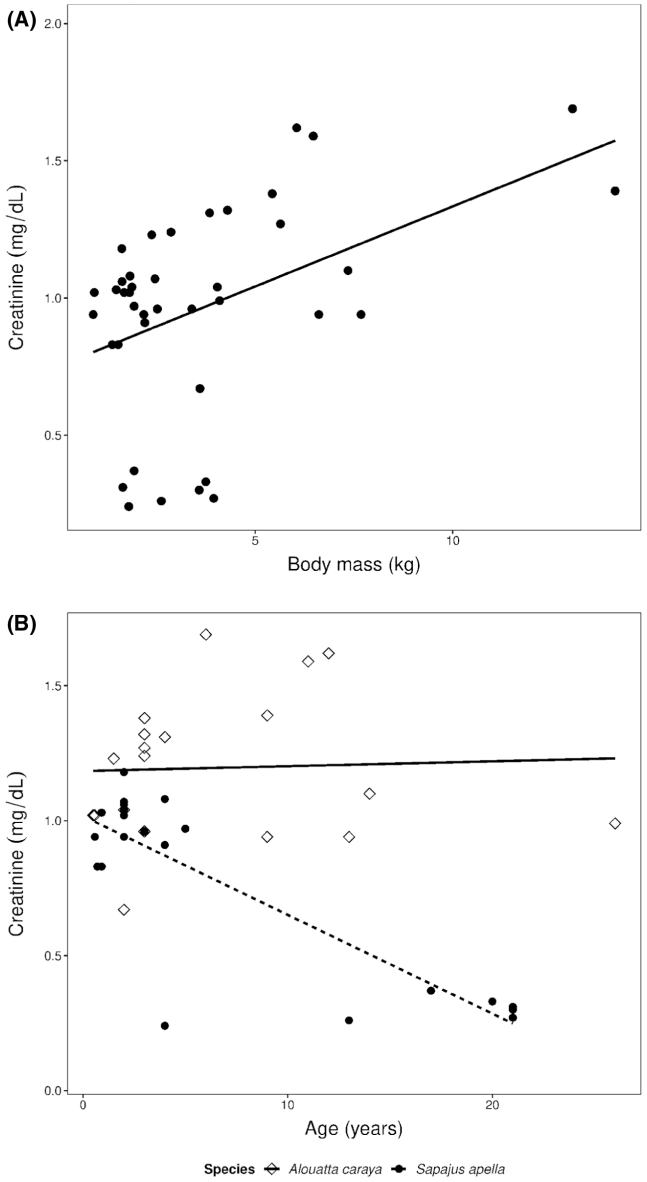

FIGURE 5 Effect of body mass (A) and the interaction between species and age (B) on creatinine values in *Alouatta caraya* and *Sapajus apella*, housed at the National Primate Center, district of Ananindeua, Pará, Brazil, January 2020

a characteristic of polygamous primate society, including what was observed for absolute renal volume.

The cortex/medulla ratio in both species was approximately 2:1 for all age groups. A similar result was described in another study in *S. apella*,[29] but it differs from the renal pattern previously described by ultrasound and postmortem evaluation in *A. fusca* (1:1).[28] In the catarrhine *M. fascicularis*, one study with histological and postmortem evaluations reported the cortex/medulla ratio as approximately 1:1 and associated this finding to the smaller size of the renal papilla in this species, which makes the medulla slightly larger than the cortex,[27] but a recent study using ultrasound in the same species reported a smaller cortex/medulla ratio (1:4).[35] Postmortem evaluation is considered a more accurate method to evaluate the cortex/medulla ratio than ultrasound, thus the inconsistency in the literature may be related to specie-specific or methodological differences. Comparative studies using postmortem evaluation are needed to clarify if there are species differences in the cortex ratio within primates.

With regard to changes in echogenicity, our ultrasound images revealed an age-related increase in the echogenicity of the cortical region compared to the medullar area in both species. The anechoic aspect of the renal medulla in young animals has been reported in humans and animals[42,43] and has been associated with the larger volume of medulla in the infants and the presence of dilute urine in the tubule.[42] Although echogenicity was quantified subjectively in this study, the age-related increase in echogenicity

was more evident in *Alouatta caraya*, which is visible by the lower degree of corticomedullary contrast in adults. One possible mechanism related to these changes may be a decline in prominence of medullary pyramids with age,[42] but additional studies are needed to elucidate this mechanism. Nonetheless, this finding suggests that the aging process in *A. caraya* is faster than in *S. apella* and may be one of the reasons by which the latter species have a remarkable longevity than expected for its body size. Similarly, corticomedullary contrast did not change with age in humans,[44] which supports this hypothesis. However, our study is limited by the lack of a quantitative echoic index to accurately assess renal echogenicity, that could detect renal changes.[45] Further comparative studies including other species with different life-history traits would be extremely relevant to confirm this hypothesis.

Although the absolute renal volume was greater in *A.caraya* as expected, the relative renal volume was greater in *S. apella*. This indicates that the latter has a larger kidney for their expected body mass, and it may explain the lack of correlation between absolute kidney volume and body mass in our study. Within the Primates order, the genus *Sapajus* has the second highest encephalization quotient (brain/body mass ratio), lower only than in humans.[46] This particularity of *Sapajus* indicates that these animals, like humans, undergo rapid neurological changes during the first years of life.[47] Consequently, some hypotheses have linked this trait with high cognitive abilities demonstrated by this genus.[47,48] In contrast, the genus *Alouatta* has a relatively small brain size compared to *Sapajus*.[49] The brain size differences have been associated with social factors such as group size as a potential factor that demands high cognition,[50,51] or with diet, proposed by Parker and Gibson[52] and Milton[53] in the "Ecological-Intelligence Hypothesis" that frugivorous primates, such as the *Sapajus* genus, evolved larger brains due to their higher energetic intake when compared to folivorous primates, such as the genus *Alouatta*.[49,54] Our data show that the brain is not the only organ larger than expected in *Sapajus*, and it suggests that this genus might have undergone dwarfism, with a reduction in the body size while maintaining important metabolic organs unaltered. Another hypothesis is that kidney enlargement in this genus co-evolved with their high encephalization quotient, given that a larger brain might increase oxygen demands.[55,56] Kidneys are important sources of RBC, which are necessary for oxygen transportation in the body.[2] Since the brain tissue is metabolically expensive to grow and to maintain,[55] a larger kidney may have evolved to attend those demands.

This hypothesis is supported by the species differences in RBC count in the present work and in a previous study in wild populations[57] that showed higher RBC count in *Sapajus lidibinosus* compared to *A. caraya*. Although we were unable to compare RBC count between sexes due to heteroscedacity, previous studies have reported a greater RBC count in males than females in innumerous primate species, including *S. apella*,[58] *S. lidibinosus*,[59] *Alouatta guariba clamitans*,[60] *A. azarae infulatus*[10] and *S. collinsi*.[11] These sex differences have been associated with the stimulatory effect of testosterone on erythropoiesis, and the inhibitory effect of estrogen.[61–63]

We also observed an aging effect on RBC count in both species, suggesting a decline in hematopoiesis in older individuals. Similar results have been reported in *Sapajus libidinosus*[59] but contrasts with another study in *S. apella* in which adult males had a higher RBC count than adult females and juvenile males.[58] This contrast may be related to differences in the age ranges of the subjects studied. Testosterone may have a strong effect in erythropoiesis in younger than older males. Age-related changes in the hematopoietic system have been characterized by intrinsic changes in erythroid progenitor cells, in the cell hematopoietic microenvironment and by humoral changes, such as individual response to erythropoietin, testosterone, growth hormone, and inflammation.[64]

Relative renal volume did not correlate with any of the laboratory tests, probably because these parameters accuse changes only after the kidney function is compromised.[9,65] Similarly, in humans, despite a significant decline in creatinine clearance with aging, a corresponding reduction in renal length was not observed.[38] Alternatively, kidney tests may be related to the number of nephrons instead of the renal volume. In humans and NHP, nephrogenesis occurs prenatally, and the kidney development after birth is limited to the differentiation of the nephrons already present.[3,66,67] Therefore, our data showed that kidney function tests do not correlate with renal volume, but whether a reduction in renal volume precedes nephropathologies remains to be investigated.

We found that creatinine had a significant, positive increase with body mass, and an interaction between species and age, indicating an apparent negative relationship with age in *S. apella*, but not in *Alouatta caraya*. The effect of body mass was similar to a previous report in *S. collinsi*[26] and is associated with the fact that creatinine is produced in the muscles.[68] However, the effect of age found in our study contrasts with those found in the other study with *S. apella*[68] and other primates, including *M. fascicularis*[69] and *A. azarae infulatus*[10] in which serum creatinine was higher in adult animals and in males. This contrast could be related to species differences in creatinine clearance rates. In humans, an increase in creatinine levels in elderly patients has been associated with a decline in clearance rates,[38] but further studies are needed to investigate species differences in creatinine clearance rates. Alternatively, the interaction found between these species could be related to the age ranges of our subjects. Primates of the genus *Sapajus* can live up to 50 years in captivity, but their mean lifespan in the wild is of approximately 25 years.[70] Therefore, *S. apella* over 25 years old may experience a decline in muscle mass that could contribute to their reduced survival chances in the wild. Our sample size included four *S. apella* and only one *A. caraya* over 20 years-old, which may explain the interaction observed in our findings. Further data on muscle mass in these animals is needed to confirm this hypothesis.

We found no effect of species, sex, age, or body mass on urea concentrations. The literature on this marker in NHP is mixed. In *S. collinsi*, there was no influence of sex, age, or body mass on urea concentrations.[26] In contrast, another study in the same species found higher urea concentrations in females than in males.[11] In *M. fascicularis*, one study showed age-related differences, with lower

concentrations in adults, but no sex differences.[27] In *A. azarae infulatus*, urea was significantly higher in males, but unrelated to age.[10] In addition to intrinsic factors, extrinsic factors such as diet, or kidney diseases can influence urea concentrations,[71] which may explain the discrepancies reported in the literature.

In general, the two species evaluated showed an increase in all renal biometric parameters with age. *A. caraya* showed greater growth rate when compared to *S. apella* and greater absolute renal volume. However, *S. apella* had greater relative renal volume, which could indicate higher RBC demands, as supported by the higher RBC production in this species. RBC and creatinine values were also influenced by age and body mass, respectively, and urea was not correlated with age, sex, or body mass in either species. The results described are useful for kidney assessment by ultrasonographic evaluation in different age groups in the two species. They can help us to understand the postnatal renal growth pattern in two neotropical primates and provide evolutionary insights on aging processes and metabolism among neotropical primates.

ACKNOWLEDGMENTS

The authors thank the staff from the National Primate Center (CENP) and Federal Rural University of the Amazon (UFRA) for providing animal care and for their assistance during data collection. This study was funded by the National Council of Technological and Scientific Development (CNPq Nos 305821/2017-2, 400881/2019-5 and 316750/2021-2), the Coordination for the Improvement of Higher Education Personnel (CAPES-Procad Amazônia No. 21/2018), the Wenner-Gren Foundation (No. 10088), and the National Science Foundation (Award No. 212357).

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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How to cite this article: da Silva GP, de Souza Pereira TH, Imbeloni AA, et al. Effect of age and sex in renal function by ultrasound and serum chemistry in two primate species (*Alouatta caraya* and *Sapajus apella*). *J Med Primatol*. 2022;00:1-11. doi: 10.1111/jmp.12599

Received: 7 December 2022 | Revised: 18 March 2023 | Accepted: 5 April 2023

DOI: 10.1111/jmp.12644

ORIGINAL ARTICLE

WILEY

Hematological and serum biochemistry evaluation in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*): A comparative study

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Funding information
Coordination for the Improvement of Higher Education Personnel; Council of Technological and Scientific Development; National Science Foundation; Wenner-Gren Foundation

Abstract

Background: Evaluation of blood parameters in captive non-human primates (NHPs) is crucial for monitoring their health and ensuring that their environment meets their physiological requirements.

Methods: We performed hemogram, serum biochemistry, and parasitological exams in 20 howler monkeys and 21 capuchin monkeys.

Results: In both species, over 50% of the individuals presented at least one parasite. There was a negative effect of age on red blood cell (RBC), white blood cell, platelets, total protein, globulin, and alkaline phosphatase, and a positive effect on the A:G ratio, gamma-glutamyl transferase, and mean platelet volume (MPV). Capuchin monkeys presented the highest platelets and alanine aminotransferase (ALT) values and howler monkeys presented the highest MPV, aspartate aminotransferase, ALT, amylase, glucose, bilirubin, and triglycerides values. We observed an interaction between species and sex on RBC, Htc, mean corpuscular hemoglobin concentration, and cholesterol.

Conclusions: Species differences found in blood parameters may reflect differences in physiological adaptations associated with ecological and morphological traits and are clinically relevant for evaluating animal health and the suitability of breeding programs.

KEYWORDS
hemogram, non-human primates, platyrrhines, serum chemistry

1 | INTRODUCTION

Non-human primates (NHPs) have various behavioral and physiological similarities to humans and are useful in evolutionary studies and in biomedical research.[1] Among platyrrhine primates, capuchin monkeys (genus *Sapajus*) stand out in pharmacology and neuroscience studies,[2,3] while howler monkeys (genus *Alouatta*) have been used in studies on infectious and parasitic diseases.[4,5]

Although both species have sexual dimorphism,[6] they differ in average body mass (~5 kg for females and ~7.8 kg for male howler monkeys; ~3 kg for females and ~4 kg for male capuchins),[7] the average lifespan in captivity (26 years for howlers; up to 50 years for capuchins),[8,9] and diet. Howler monkeys are mainly folivores, with a diet rich in young leaves, shoots, buds, and different types of fiber and supplemented with fruits, seeds, and insects.[10–12] In contrast, capuchin monkeys have greater dietary diversity, with a

diet composed mainly of fruits and insects, but can include larvae, seeds, roots, fossorial arthropods, small vertebrates, and eggs.[13–15] Considering that species have evolved specific physiological and morphological adaptations to different ecological niches, breeding programs must consider these differences when designing husbandry protocols for each species and conduct regular evaluation of physiological parameters to monitor their health and adaptability to captive conditions.

In this context, hematological and biochemical evaluations are standard laboratory tests in every animal facility and have been described in many platyrrhines such as capuchin monkeys,[16–19] howler monkeys,[20–22] owl monkeys (*Aotus azarae infulatus*),[23] squirrel monkeys (*Saimiri collinsi*),[24] black-tufted marmoset (*Callithrix penicillate*),[25,26] and spider monkeys (*Ateles geoffroy*).[27] However, these parameters can vary with sex, age, parasites, or if individuals are reared under different environmental conditions, which can interfere with the results.[28]

In our recent study, we investigated the effect of age and sex in kidney morphology and function, as well as differences in red blood cell (RBC) count. We found a higher absolute kidney volume in howlers, but higher relative kidney volume and RBC in capuchin monkeys. We also found a negative relationship between age and RBC in both species and a decrease in creatinine with age only in capuchins, suggesting that intra- and inter-specific factors can alter animal physiology and may illustrate differences in metabolic demands, the aging process, and general life strategies between these species.[29]

The aim of this study was to extend these analyses to compare the hemogram and serum biochemistry in captive howler and capuchin monkeys, considering the potential effect of species, age, sex, and the presence of intestinal parasites on blood parameters.

2 | METHODS

2.1 | Humane care guidelines

The experimental project followed the guidelines of the Brazilian Council for the Control of Animal Experimentation–Ministry of Science and Technology (CONCEA-MCT), and it was approved by the Ethics Committee for the Use of Animals (CEUA nos. 43/2019 and 24/2021) of the Institute Evandro Chagas (IEC), Ananindeua, Pará, Brazil and by the Biodiversity Authorization and Information System of the Chico Mendes Institute of Biodiversity (Sisbio/ICMBio, protocol 38529).

2.2 | Subjects

The subjects were 20 howler monkeys (*Alouatta caraya*—11 females and nine males), with a mean ± standard deviation (SD) body mass of 5.08 ± 3.48 kg (0.9–14.1 kg) and age range between 6 months and 26 years, and 21 capuchin monkeys (*Sapajus apella*—11 females and 10 males), with a mean ± SD body mass of 2.12 ± 0.79 kg (0.9–3.95 kg) and age range between 7 months and 21 years.

The animals were housed at the breeding colony of the National Primate Center (Centro National de Primatas–CENP), located at Ananindeua, Pará, Brazil (1°38′26″, 48°38′22″). We identified each animal by a three-letter code tattooed on the inner right thigh and a microchip placed in the interscapular area. All primate colonies at CENP are submitted to annual health screenings, which include physical examination, hemogram, and biochemical tests, in addition to deworming treatment. None of the animals used in this study had a history of infectious diseases as per their last health screening (2 months before data collection).

2.3 | Husbandry

All individuals lived in family groups of up to 10 individuals. They were kept in sheds and positioned in a north–south orientation to receive ≤12 h of natural light, in enclosures of dimensions 3.75 m × 2.2 m × 2.4 m (howler monkeys), and 3.85 m × 2.6 m × 2.5 m (capuchin monkeys). The enclosures had external and internal water bottles and multiple bowls for food provisioning. The animals were fed according to CENP's standard management practices. Their diet contained different types of fruits and vegetables, eggs, and commercial primate food with 18% crude protein (Cebidae P18 Megazoo, portion Megazoo). We also provided daily supplements of amino acids, vitamins, macro, and micro minerals, and 0.5 g of Aminomix Pet® (Vetnil Ind. Veterinary Products Ltda) per kg of body mass. Water was offered ad libitum.

2.4 | Fecal sample collection

We collected one fecal sample per animal for fecal parasitology tests shortly after defecation. The samples were stored in sterilized plastic containers labeled with an individual ID. Parasitology tests were performed according to a standard protocol established by CENP's parasitology laboratory, using direct examination techniques, as well as flotation and sedimentation.[30,31]

2.5 | Capture and blood collection

Following an 8-h fasting period, the animals were contained physically with the aid of nets, and chemically by intramuscular administration of a combination of ketamine hydrochloride (5 mg/kg), dexmedetomidine (0.01 mg/kg), and midazolam (0.2 mg/kg). With the animal contained, we collected between 2 and 3 mL of blood from the femoral vein with sterile syringes and needles (14–21G, depending on the species and age of the animal). Half of the sample was transferred to a tube containing ethylenediaminetetraacetic acid (EDTA) for hemogram and the other half was transferred to a tube without anticoagulants for clinical chemistry.

2.6 | Laboratory tests

The cell blood count was performed with an MS4+ blood analyzer (Melet Schloesing GmbH Central & Eastern Europe company, Sudstadtzentrum 1, Top 8) to determine RBC count, hemoglobin (Hb), hematocrit (Hct), mean corpuscular hemoglobin (MCH), mean corpuscular volume (MCV), mean corpuscular hemoglobin concentration (MCHC), white blood cell (WBC) count, differential WBC count (segmented, lymphocytes, monocytes, eosinophils, basophils), platelets, and mean platelet volume (MPV). Biochemistry tests were performed on sistema Vitros DTSC II, DT60, and DTE2 (Johnson & Johnson Medical Argentina), to evaluate the total protein (TP), albumin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), bilirubin (BIL), glucose, amylase, lipase, triglycerides, and cholesterol. Globulin value was calculated by subtracting albumin from TP values, then albumin/globulin ratio (A:G ratio) was calculated. All values were reported and followed by the respective reference values from the literature. Kidney function (urea, and creatinine) was evaluated and discussed in our previous study.[29]

2.7 | Statistical analysis

All statistical tests were performed in R software (3.3.0). General linear models (GLMs) were used to test the effects of age, sex, species, and parasitism on blood parameters. First, the possibility of multicollinearity was excluded by calculating the variance inflation factor (VIF) with the "car" package. Since all factors had VIF < 2, none were considered problematic in the model. To assess the equality of variances of categorical fixed factors, we used Levene's test.[32] Models that showed non-normality of residuals were power-transformed using *boxcox* function from the package *MASS*.[33] However, the residuals for the models MCV and MCH were not normally distributed even after transformation. Thus, they were excluded from the statistical analyses, but we reported their descriptive statistics.

We built seven models for blood count parameters (predictor variables: RBC, Hb, Hct, MCHC, WBC, platelets, and MPV). The differential WBC data were not submitted to statistical analyses as some of these parameters were zero-inflated but given the importance of the data in interpreting alterations in WBC count, we calculated their mean ± SD per species, sex, and age and included the data in the results. We also built 14 models for biochemical parameters, (predictor variables: TP, ALT, ASP, amylase, GGT, lipase, glucose, ALP, bilirubin, albumin, globulin, A:G ratio, triglycerides, and cholesterol). For all parameters, we initially included as fixed factors: age, body mass, species, sex, and parasitism (individuals infected with parasites were considered as "positive" and individuals that were not infected with parasites were considered as "negative"), and their interactions. Following Burnham and Anderson,[34] we sequentially removed fixed factors to select the model with the lowest Akaike information criterion with correction for small sample sizes (AICc). If AICc difference between the two models (ΔAICc) was less than 2, both models were discussed. We reported the mean ± (SD) and range (minimum–maximum) values for all parameters described in this study by species, sex, and age (Tables S1 and S2), and by sex only to compare our data with reference values reported in previous studies (Tables S3 and S4). Due to the limited data available in the literature, we included references that matched our study subjects at the genus level (*Alouatta*[21,22,35,36] and *Sapajus*[16,37]).

3 | RESULTS

3.1 | Parasitology tests

We were not able to collect fecal samples from infant howler monkeys ($n = 3$) due to their constant close contact with the mother. Among the remaining individuals of this species, we found that 47% (8/17) were positive for parasites. Specifically, we detected *Giardia lamblia* in 22% (4/17), *Entamoeba coli* in 17.6% (3/17), *Pentatrichomonas hominis* in 11.7% (2/17), and *Strongyloides stercoralis* in 5.8% (1/17). In capuchin monkeys, a total of 66.7% (14/21) individuals were parasitized, with *Ancylostoma* spp. in 57.1% (12/21), *S. stercoralis* in 47.6% (10/21), *P. hominis* in 9.5% (2/21), and *Entamoeba histolytica* in 4.7% (1/21).

3.2 | Hemogram and serum chemistry exams

All GLM models are shown in detail in Tables 1 and 2 (hemogram) and (serum chemistry).

The models including RBC as a predictor revealed a significant negative effect of age, and an interaction between sex and species, with a lower value in male capuchins than females, and the opposite effect in howler monkeys. For models including Hb, Hct, and MCHC as a predictor, we found an interaction between sex and species similar to the effect found for RBC, with lower values in male capuchins compared to females, but the opposite trend in howler monkeys, but no effect of age or parasitism (Figure 1).

For WBC, we found a significant negative effect of age and interaction between parasitism and species, in which positive capuchin monkeys had higher values than negative conspecifics, but the opposite trend in howler monkeys (Figure 2). Based on this result, we calculated the mean ± SD values of the differential WBC count by species and parasite condition (Table 3), which shows that eosinophils were higher in positive capuchin monkeys but fell below or undetectable levels in negative capuchin monkeys and in all howler monkeys studied. Positive capuchin monkeys also presented an increase in segmented, lymphocytes, monocytes, and basophils in comparison with negative conspecifics and any howler monkey group, whereas positive and negative howler monkeys presented similar results.

The model for platelets showed a significant effect of species, with a higher platelet count in capuchins than in howler monkeys.

TABLE 1 Generalized linear models (GLM) investigating the effects of species, sex, age group, and presence of parasites in hematological parameters in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*).

Hematologic parameter	Effect	Estimate	Standard error	*Z* value	*p*-value
Red blood cells ($\times 10^6$ per mm)	Intercept	4.43	0.19	23.34	<0.001
	Age	−0.03	0.01	−2.46	0.02
	Species_sex (*Sapajus apella*_male)	−0.06	0.31	−2.10	0.04
Hemoglobin (g/dL)	Intercept	14.32	0.51	27.90	<0.001
	Species_sex (*Sapajus apella*_male)	−2.18	0.85	−2.57	0.01
Hematocrit (%)	Intercept	42.62	1.60	26.77	<0.001
	Species_sex (*Sapajus apella*_male)	−6.67	2.63	−2.53	0.02
Mean corpuscular hemoglobin concentration (%)	Intercept	13.80	0.42	32.37	<0.001
	Species_Sex (*Sapajus apella*_male)	−1.84	0.88	−2.08	0.04
White blood cells ($\times 10^3$ per mm)	Intercept	209.12	9.82	21.28	<0.001
	Age	−1.18	0.56	−2.11	0.04
	Parasites_specie (positive_*Sapajus apella*)	55.30	16.60	3.33	0.002
Platelets ($\times 10^3$ per mm)	Intercept	216.60	16.33	13.26	<0.001
	Species (*Sapajus apella*)	31.46	15.45	−2.07	0.04
	Sex (male)	−31.24	15.21	−2.05	0.04
	Age	−4.50	1.07	−4.20	<0.001
Mean platelet volume (%)	Intercept	12.83	0.53	24.36	<0.001
	Species (*Sapajus apella*)	−2.76	0.50	−5.49	<0.001
	Age	0.11	0.03	3.07	0.004

We also observed an effect of sex, with lower platelet count in males compared to females, as well as a significant negative effect of age. The model for MPV revealed an effect of species and age, with lower MPV in capuchin monkeys than in howler monkeys and a positive relationship with age (Figure 3).

The models including TP and globulin as response variables showed a negative effect of age and an effect of parasitism, with higher TP and globulin in positive animals. However, there was an opposite trend for the A:G ratio as a response variable, with a positive relationship with age and lower values in parasitized animals (Figure 4).

In hepatic enzyme models, the AST model showed an effect of species, with lower values in capuchins than in howler monkeys. The ALT model, however, showed the opposite effect, with significantly higher values in capuchins than in howler monkeys. In addition, there was an effect of parasitism, with lower ALT in positive than negative animals (Figure 5). The GGT model revealed a positive relationship with age, and the ALP model revealed the opposite effect, with a significant negative effect of age. In the bilirubin model, there was an effect of species, sex, and the presence of parasites, with higher BIL in howler monkeys, males, and positive animals, respectively (Figure 6).

Both models for amylase and glucose showed an effect of species, with lower levels in capuchins compared to howler monkeys (Figure 7). In relation to lipidogram parameters, the model including cholesterol as a predictor showed an interaction between species and sex, with lower levels in male capuchin monkeys compared to female conspecifics, but the opposite trend in howler monkeys. In the triglyceride model, we found an effect of species, with lower levels in capuchins than in howler monkeys (Figure 8).

For lipase and albumin, the models including fixed factors did not differ from the null model, demonstrating that age, sex, parasitism, and species did not interfere with these parameters.

4 | DISCUSSION

Research centers that maintain NHP must adhere to safety and sanitation protocols to ensure animal health, and quality of research, and to avoid pathogen transmission between animals and keepers.[38] In the present study, parasites were present at an incidence rate above 50% in both NHP species investigated. Thus, periodical clinical examination, coproparasitological examinations, and medical therapy are essential to diagnose and control helminth dissemination in captivity, especially in the Amazon, where climatic conditions favor pathogen multiplication.[39]

Among the parasites found in this study, *Giardia lamblia* is an intestinal parasite commonly transmitted by water and infecting humans, birds, marsupials, small rodents, and carnivores.[40,41] Its main hosts are NHP, with several cases reported in platyrrhine species, including squirrel monkeys (*Saimiri sciureus*), spider monkeys (*Ateles fusciceps*), cotton-top tamarins (*Saguinus oedipus*), and howler monkeys (*Alouatta* spp.).[42,43] Captive primates generally have higher infection rates compared to free-ranging animals, as the confined environment allows *Giardia* cysts to spread more easily.[44,45] The genus *Entamoeba* is composed of protozoa with high zoonotic potential

TABLE 2 Generalized linear models (GLM) investigating the effects of species, sex, age group, and presence of parasites in serum biochemistry parameters in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*).

Serum biochemistry	Effect	Estimate	Standard error	*t*-value	*p*-value
Total proteins (g/dL)	Intercept	8.24	0.30	27.23	<0.001
	Age	−0.10	0.02	−5.17	<0.001
	Parasites (positive)	1.05	0.29	3.59	0.001
Albumin (null model)	Intercept	16.93	0.49	34.37	<0.001
Globulin	Intercept	4.36	0.19	23.43	<0.001
	Age	−0.09	0.01	−5.74	<0.001
	Parasites (positive)	1.07	0.23	4.66	<0.001
Albumin/globulin ratio	Intercept	0.98	0.05	20.43	<0.001
	Age	0.02	0.004	5.08	<0.001
	Parasites (positive)	−0.22	0.06	−3.93	<0.001
AST (U/L)	Intercept	2.47	0.02	93.18	<0.001
	Species (*Sapajus apella*)	- 0.19	0.02	−7.61	<0.001
ALT (U/L)	Intercept	15.52	3.06	5.07	<0.001
	Species (*Sapajus apella*)	24.80	2.90	8.53	<0.001
	Parasites (positive)	−7.70	2.95	−2.61	0.014
Amylase (U/dL)	Intercept	346.06	28.09	12.32	<0.001
	Species (*Sapajus apella*)	−149.88	25.89	−5.79	<0.001
GGT (U/L)	Intercept	-9.13×10^{-4}	1.28×10^{-4}	−7.15	<0.001
	Age	2.50×10^{-5}	8.24×10^{-6}	3.03	0.005
Lipase (U/L) (null model)	Intercept	9.38	1.13	8.28	<0.001
Glucose (mg/dL)	Intercept	124.51	12.19	10.21	<0.001
	Species (*Sapajus apella*)	−38.43	11.57	−3.32	0.002
Bilirubin (mg/dL)	Intercept	−31.47	5.89	−5.34	<0.001
	Species (*Sapajus apella*)	−25.23	5.93	−4.25	<0.001
	Sex (male)	15.79	6.12	2.58	0.02
	Parasites (positive)	15.30	6.18	2.48	0.02
	Intercept	135.94	12.50	10.83	<0.001
Cholesterol (mg/dL)	Species_sex (*Sapajus apella*_male)	−52.88	22.63	−2.33	0.02
	Intercept	4.42	0.12	36.92	<0.001
Triglycerides (mg/dL)	Species (*Sapajus apella*)	−0.64	0.14	−4.62	<0.001
ALP (U/L)	Intercept	−0.1	0.07	−1.40	0.174
	Age	−0.01	0.00	−3.27	0.003

Abbreviations: ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transferase.

that cause intestinal diseases and extraintestinal abscesses, with *E. coli* being one of the species most excreted by NHPs.[46,47] The increase in primates infected by this protozoan is usually related to the greater resistance of uninucleated cysts to amoebicidal drugs and the lack of treatment, as they rarely present clinical manifestations of intestinal lesions.[48,49]

In capuchin monkeys, the parasites reported were *Ancylostoma* spp., *Strongyloides stercoralis*, *Pentatrichomonas homini*, and *Entamoeba histolytica*. This genus has infections that may be prevalent due to their omnivorous diet, frequent contact with soil, large group sizes, and active social behavior.[50] The genus *Ancylostoma* is one of the most common in NHPs, along with strongylida, and the infection occurs by transcutaneous transmission by larvae that migrate to the gastrointestinal tract.[51] In captive primates, the incidence of this parasite has been associated with poor hygienic conditions. Thus, keeping the animals dewormed and in clean cages with filtered water and balanced food reduces contamination.[52,53] *E. histolytica* occurs in several species of NHPs, being more common in platyrrhine species because they are more sensitive. The strains are identical to humans strains and can be transmitted via the fecal–oral route through food and water contaminated with cysts.[54]

In this study, *S. stercoralis* and *P. hominis* were found in both species. *Strongyloididae* is one of the most prevalent groups of

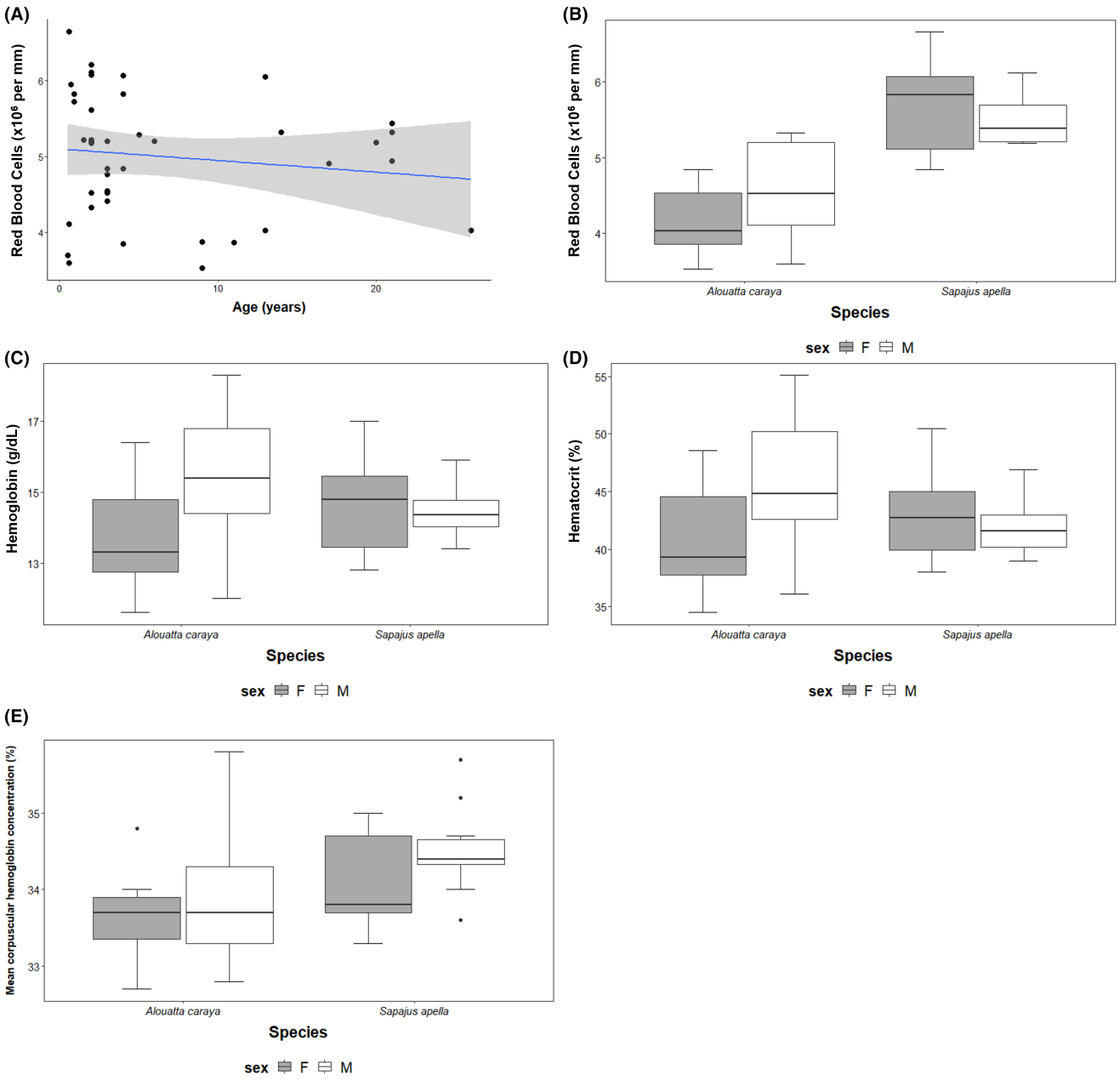

FIGURE 1 Negative effect of age in red blood cells (A); interaction between species and sex (F—female; M—male) in red blood cells (B), hemoglobin (C), hematocrit (D) and mean corpuscular hemoglobin concentration (E), in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

parasites,[51] found in hot and humid climates, and can infect hosts through skin penetration or when ingested.[55,56] This parasite reproduces asexually in the host's intestinal wall, contributing to high rates of infection.[57,58] *P. hominis* is a trichomonad that is commonly found in the intestinal tract of domestic animals and primates and may pose a risk of zoonotic and anthroponotic transmission.[59,60] The main form of transmission is the fecal–oral route, through the ingestion of contaminated food and water or through direct contact from one host to another, as the flagellated form does not survive long in the environment.[60,61] This protozoan is considered a non-pathogenic opportunistic agent and is generally not the main agent of intestinal lesions in NHPs.[62]

4.1 | Blood parameters

The age effect on RBC count in both species, suggests a decline in hematopoiesis in older individuals. Likewise, Núñez et al.[63] and Ferreira et al.[64] found higher values in young animals compared to adult capuchin monkeys (*Sapajus apella* and *S. libidinosus*). These results could be associated with changes in erythroid progenitor cells, in the cell hematopoietic microenvironment, and in humoral changes.[65] In addition, the bone marrow of young animals and humans has a higher percentage of red bone marrow, which is hematopoietically more active than yellow bone; this last one contains more adipose tissue and is more abundant in adults.[66]

The interaction between sex and species on RBC count, Hb, Hct, and MCHC revealed that while male howler monkeys had higher values than females for these parameters, female capuchin monkeys had higher values than their male conspecifics. However, the interaction does not indicate whether these differences are significant or not, so it is possible that the interaction was a product of our sample size. The sex differences observed in howler monkeys are consistent with previous reports in platyrrhine species, including other howler monkey species,[21,22,67] capuchin monkeys,[16,63,64,68,69] owl monkeys,[23] squirrel monkeys,[24] black-tufted marmoset,[25,26] spider monkeys,[27] and humans.[70] These sex differences have been associated with the stimulatory effect of testosterone on erythropoiesis, and the inhibitory effect of estrogen[16,37,70,71] but also related to genetic differences, such as the difference between males and females in erythropoietin gene and its receptor.[72]

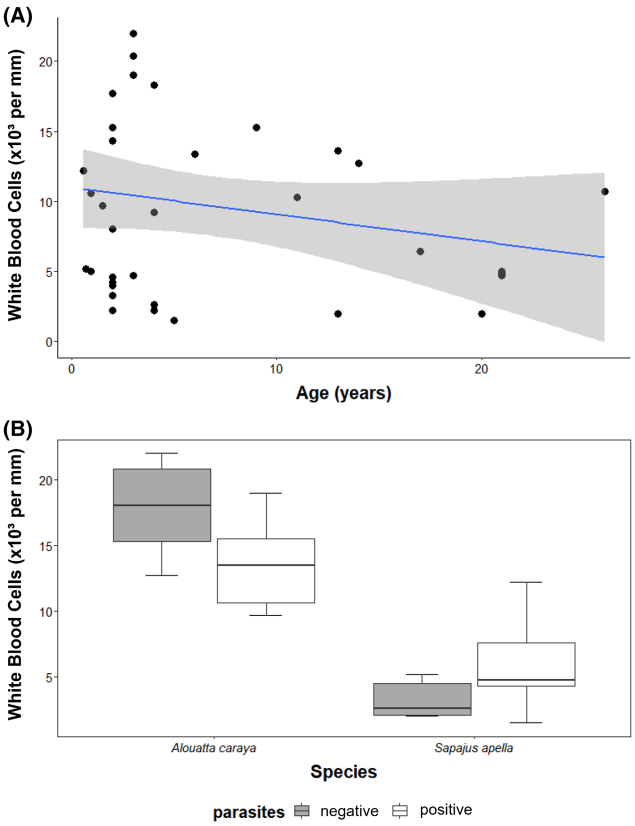

FIGURE 2 Negative effect of age (A) and interaction between species and parasite presence (B) in white blood cells in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

The negative effect of age on WBC in our study was consistent with a previous study in capuchin monkeys,[64] in which juveniles had higher WBC compared to adults. In infants, the bone marrow is hematopoietically active in all bones, whereas in adults and elderly, only the sternum, femur, and flat bones are hematopoietically active.[73,74] Another possibility is the major propensity of young animals to release epinephrine due to excitement or fear, causing neutrophilia, eosinophilia, and lymphocytosis due to leukocyte mobilization.[75,76]

We also observed an interaction between parasitism and species on WBC, in which higher values of WBC were found in positive capuchin monkeys but the opposite trend in howler monkeys. Changes in WBC depend on parasite load, the intensity and pathogeny of infection, and immune response. *Strongyloides stercoralis* is one of the most clinically important pathogenic species in NHP.[58] This parasite species was found in 10 capuchin monkeys but only in one howler monkey in the present study. Therefore, the interaction found in this result may have been a product of different parasite species eliciting different immune responses. Interestingly, we found that in the differential WBC, eosinophils were elevated only in positive, but not in negative, capuchin monkeys, nor in any howler monkey studied. Eosinophilia is commonly associated with parasite infection,[77] and our results for differential WBC suggest that the degree of pathogenicity of parasitosis may vary by both parasite and host species. This result highlights the importance of analyzing differential WBC count to determine and establish the diagnosis of each clinical condition. Although the WBC values were in accordance with the reference values available in the literature for capuchins,[16] were not for howler monkeys[21] (Table S3), thus we must consider intraspecific variations in WBC due to antigenic stimulation, stress during animal handling, and anesthesia.[21,77]

In this study, males had significantly lower platelet counts than females in both species, which was similar to results described recently in spider monkeys.[78] Previous studies, although without significant difference, reported lower values for platelets in males in other platyrrhine species such as howler monkeys,[21,35,67] capuchin monkeys,[19,69,79] spider monkeys,[27] and in catarrhines including vervet monkeys (*Chlorocebus aethiops sabaeus*)[80] and long-tailed macaques (*Macaca fascicularis*) and rhesus monkeys (*M. mulatta*).[81] Furthermore, in humans, the higher platelets observed in women[82,83]

TABLE 3 Differential WBC count (mean ± SD) by species and parasite condition in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*).

Parameter (×10³ per mm³)	*Alouatta caraya*		*Sapajus apella*	
	Negative (*n* = 9)	Positive (*n* = 8)	Negative (*n* = 7)	Positive (*n* = 14)
Segmented	8.93 ± 4.13	5.64 ± 3.34	1.57 ± 0.96	3.50 ± 2.24
Lymphocytes	7.02 ± 1.58	6.83 ± 2.48	1.37 ± 0.69	1.69 ± 1.07
Monocytes	1.05 ± 0.36	1.10 ± 0.39	0.26 ± 0.24	0.40 ± 0.39
Eosinophils	0	0	0.003 ± 0.007	0.11 ± 0.15
Basophils	0.2 ± 0.05	0.17 ± 0.05	0.04 ± 0.005	0.13 ± 0.14

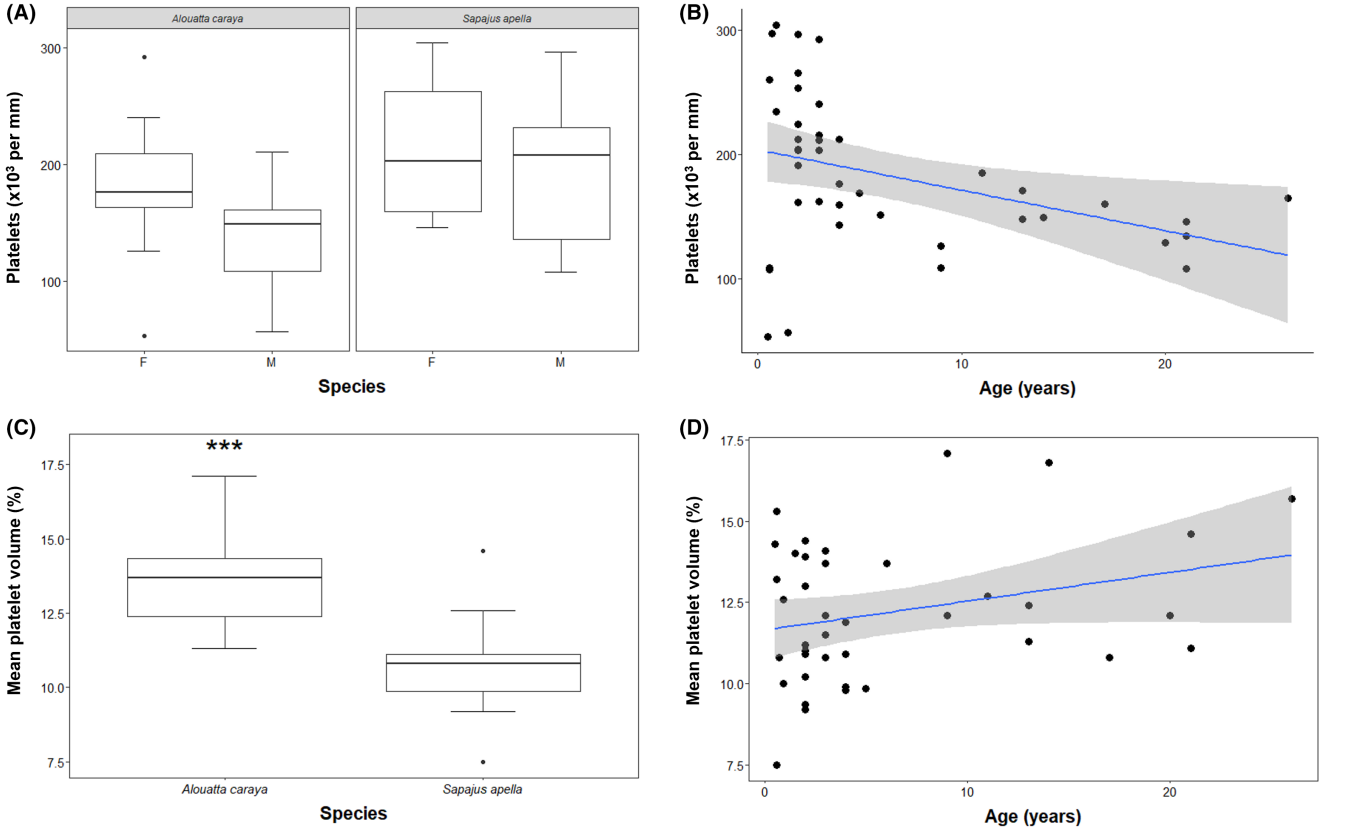

FIGURE 3 Effect of species (A) and age (B) in platelet count; effect of species (C) and age (D) in mean platelet volume in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

can be associated with the importance of platelet aggregation as possibly a mechanism to prevent heavy menstrual bleeding.[84] Since menstrual cycles have been described in both howler and capuchin monkeys,[85,86] this result may indicate an early adaptation in female primates.

The higher platelet count for capuchin monkeys found in our study corroborates with other studies in captivity and in wild.[16,18,19,21,22,35,63,79,87] We also found a higher RBC count in capuchin monkeys that was related to the higher metabolic demands in capuchin monkeys,[29] and platelet count seems to follow the same trend in these species.

We also observed a negative effect of age on platelet count. Other studies showed the same trend, though not significant, in capuchin monkeys,[16,63] marmosets (*Callithrix* sp.),[26] owl monkeys,[23] and spider monkeys.[27] This differs from one study in humans, in which the platelets had a significant positive correlation with age, though the authors highlighted that the impact of age seemed to be of minor relevance when compared with other factors such as sex and MPV.[83] Another possible explanation is the interaction of platelets and WBC by forming platelet–WBC aggregates in inflammatory responses.[88] Considering that age had a negative effect on WBC, it is possible that platelets followed the same pattern due to higher inflammatory responses in younger individuals.

MPV was significantly lower in capuchin monkeys. MPV is a possible determinant of platelet function and aggregability, and large platelets are more active than normal-sized platelets[89,90]; these results can be explained by the fact that howler monkeys presented a lower value of platelets compared to capuchin monkeys in this and in previous studies.[21,22,35,79] The higher value for MPV can be a compensatory mechanism in this species for a high platelet volume as a trade-off to a low platelet count. This is consistent with the positive effect of age on MPV, which is the opposite of what we found for platelet count. In normal physiological conditions, MPV is inversely proportional to the platelet count, which is associated with hemostatic maintenance and preservation of constant platelet mass[91]; thus, an increase in the production of platelets is accompanied by a reduction in their mean volume.[92]

4.2 | Biochemistry tests

The negative effect of age on TP and globulin values observed in our study was similarly described by Rodriguez et al.[93] and Scobar[94] in woolly monkeys (*Lagothrix lagotricha*), in which juveniles had higher TP values than sub-adults, suggesting that younger animals have higher globulin concentrations due to greater demand during growth. These results differ from those observed in chimpanzees (*Pan troglodytes*), which experienced an increase in globulin and a decrease in albumin with age.[95] In contrast, we did not observe any significant effect on albumin values in our study. We also observed an increase

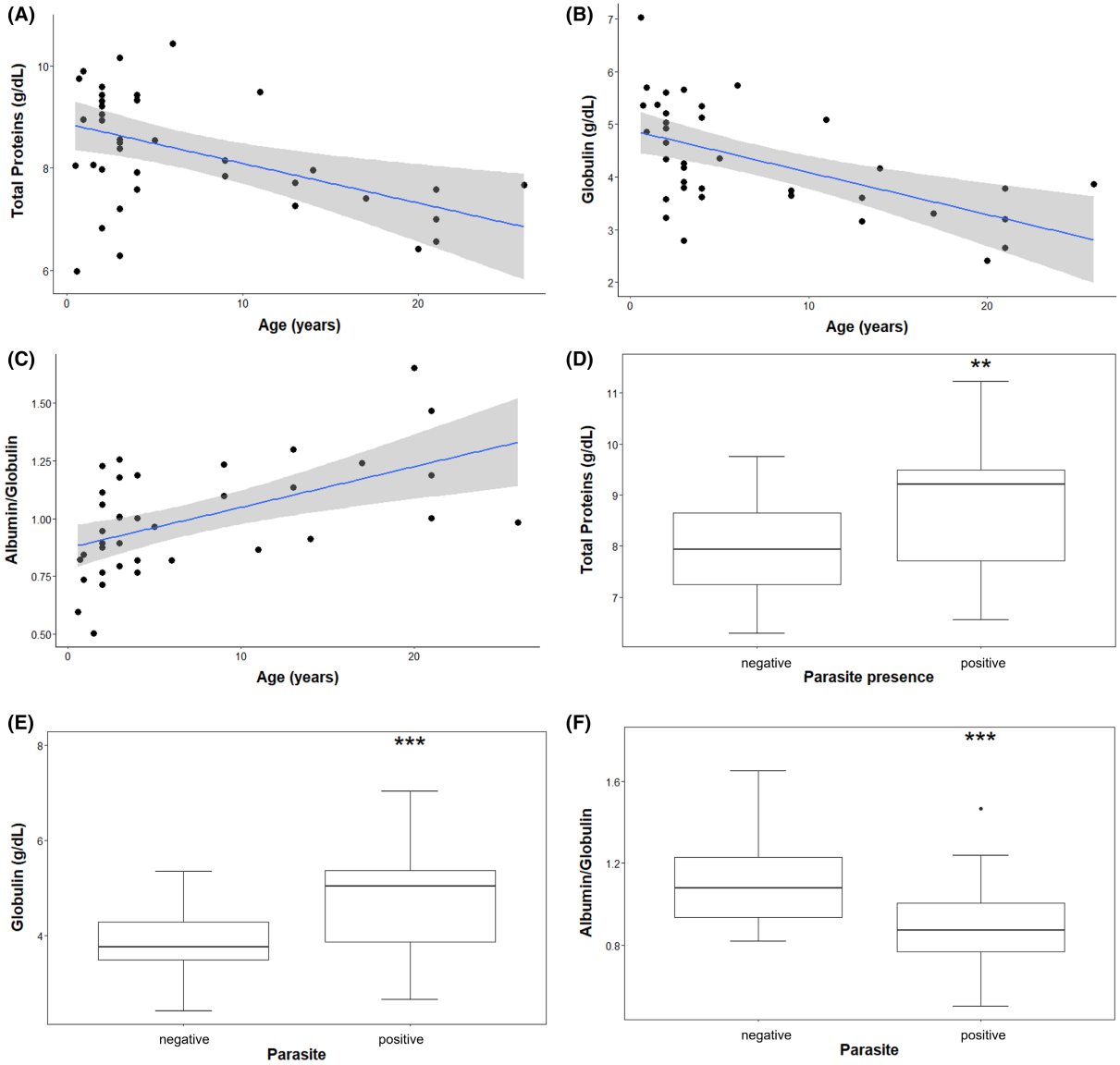

FIGURE 4 Negative effect of age in total protein (A), globulin (B) and albumin: globulin ratio (C); effect of parasite presence in total proteins (D), globulin (E), and albumin: globulin ratio (F) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

in TP and globulin in parasitized animals. Some liver proteins are carriers of molecules to promote enzymatic activity or participate in the innate immune response and are considered one of the earliest markers for any pathologic process or disease.[96] The increase in globulin in positive animals can be associated with the host's innate immune system in response to parasite infection,[97,98] as globulins are considered positive acute phase proteins (APPs), whereas albumin is considered a negative APP that decreases in infection cases.[96] Even though no changes were observed in albumin with parasitosis, we found that the A:G ratio was lower in positive animals, suggesting that the A:G ratio is a better index than albumin alone to diagnose parasite infection due to its higher sensitivity.

In relation to liver enzymes, we found lower AST levels in capuchin monkeys than in howler monkeys, which is consistent with results reported previously in these species.[16,22,35,63,87] In primates, AST is found in the mitochondria of the hepatocyte, and an elevation in its activity can be associated with liver damage.[99,100] Other sources of AST are the heart, skeletal muscle, kidneys, brain, pancreas, lungs, leucocytes, and erythrocytes.[101] However, none of the animals in our study presented alterations related to liver or muscular/cardiac function on clinical/ultrasound tests nor historic therapy with hepatotoxic drugs. Another reason for the increase in AST is related to muscular activity and degeneration. Muscles present two main types of fibers: Type I (slow twitch) and Type II (fast twitch). Type I fibers receive more glucose and oxygen than Type II and can compete for resources with the brain.[102] One study showed that larger-brained primates such as capuchin monkeys have fewer muscle fibers Type I than primates with smaller brains,[103] thus lower AST levels in capuchin monkeys may be a product of differences in muscle fiber. Considering that capuchin monkeys have a higher encephalization index than howler monkeys,[103,104] this result supports the expensive tissue hypothesis (ETH), aaa which postulates that an

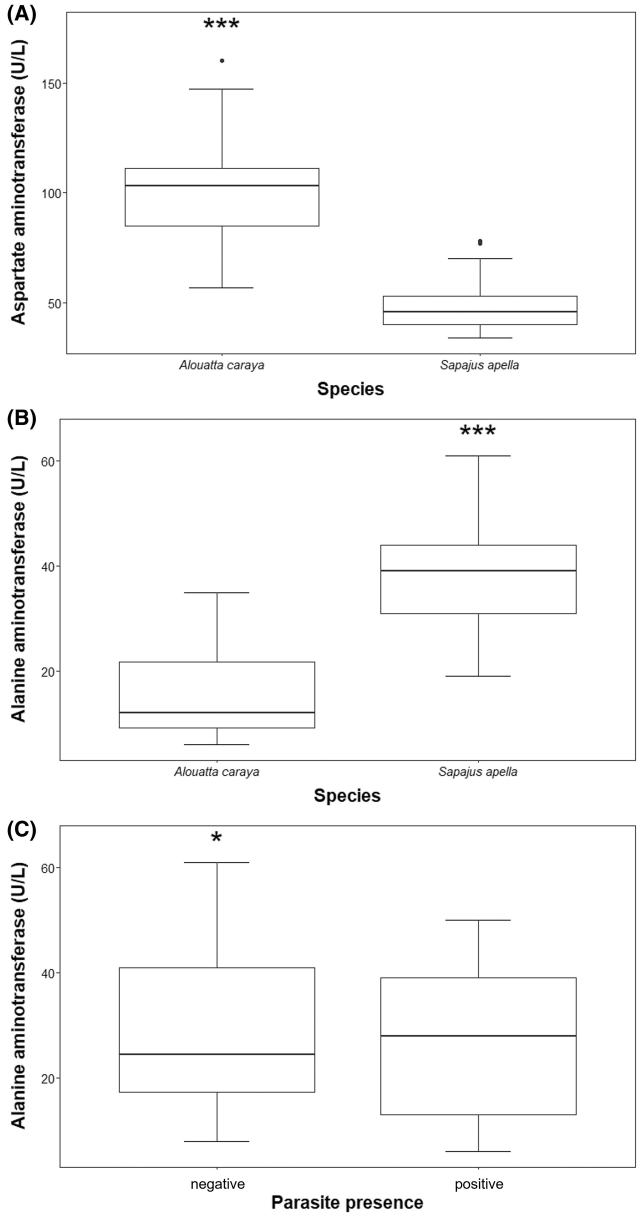

FIGURE 5 Effect of species in aspartate aminotransferase (A) and alanine aminotransferase (B); effect of parasite presence alanine aminotransferase (C) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

increase in the brain mass is compensated by a reduction in the mass of metabolically costly tissues such as the gastrointestinal tract[105] and skeletal muscle, though further comparative studies in other species are warranted to test this hypothesis.

In contrast with AST, we observed higher ALT levels in capuchin monkeys, which is similar when comparing the mean values reported in previous studies with these species.[16,18,21,22,35,63,87] The enzyme ALT is a transaminase hepato-specific in carnivores and has been useful as a marker of liver damage as it is released by injured hepatocytes.[102,106] We also observed that ALT was lower in parasitized animals in both species, but this result might not be clinically relevant, given the large variation in normal ALT values reported in previous studies.[16,21,37,69] Additionally, this effect may depend on parasite load and species, which were not evaluated in the present study.

In relation to GGT, we found a positive relationship with age, which is consistent with another study in capuchin monkeys.[16] Also, Núñez et al. (2008)[63] reported a higher value in adult capuchins compared to juveniles, though the difference was not significant, probably due to sample size. Interestingly, previous studies have found that GGT affects RBC integrity. Glutathione metabolism mediated by high concentrations of GGT can give rise to pro-oxidant substances when chelated transition metals are present. This results in the production of reactive oxygen species, which induces lipid peroxidation, and pore formation in the cell membranes of RBC, causing hemolysis.[107] This may explain our results on age decline in RBC count. Thus, GGT function is a promising biomarker of aging. Likewise, in humans, serum GGT has been reported as a remarkable predictor for a multitude of age-related diseases and chronic conditions such as liver disease and bile duct disorders, cardiovascular disease, metabolic syndrome, obesity, diabetes, and cancer,[108] which are linked to the presence of the enzyme GGT in bile ducts, kidneys, pancreas, and intestine.[101]

For ALP, the significant negative effect of age observed in our study was similar to previous reports in howler monkeys,[109] capuchin monkeys,[16] woolly monkeys,[93] and owl monkeys.[23] This enzyme is used for the evaluation of liver or bone diseases, given that more than 80% of serum ALP originates from these tissues.[101] Thus, higher serum activity in younger animals may be related to the increased metabolic activity of osteoblasts during bone development.[110,111]

For bilirubin, we observed that capuchin monkeys had lower levels than howler monkeys, and bilirubin was higher in males than females. The species effect was similar when comparing bilirubin levels in previous reports of these species[16,35] and the sex effect was also observed in woolly monkeys.[94] These results may be linked with differences observed in hemoglobin since 80% of bilirubin is made from the breakdown of hemoglobin in heme products released in senescent RBC.[112] Furthermore, a higher value was observed in parasitized animals in both species, which could indicate that the parasites present in the study affected the hepatobiliary system and that bilirubin may be an early marker of helminthic infestation. However, further analyses that account for parasite load and pathogenicity are needed to determine the clinical accuracy of bilirubin in the diagnosis of parasite infection.

Glucose and amylase were only affected by species, with lower values in capuchin monkeys than in howler monkeys. Glucose values found in our study differ from those reported in other studies in capuchins,[8,16,18] which are generally higher than those reported in howler monkeys.[22,35,67] Although hyperglycemia is associated with diabetes mellitus, is also observed in anesthetized animals (liver glycogen mobilization for circulation), and in pancreatitis,[113,114] which may be associated with metabolic and dietary differences between capuchins (omnivorous) and howler monkeys (folivorous).[115,116] The unexpected higher results in howler monkeys may be related to the low activity of this genus in captivity and the provisioning with fruits in captivity which leads to increased sugar intake and reduced

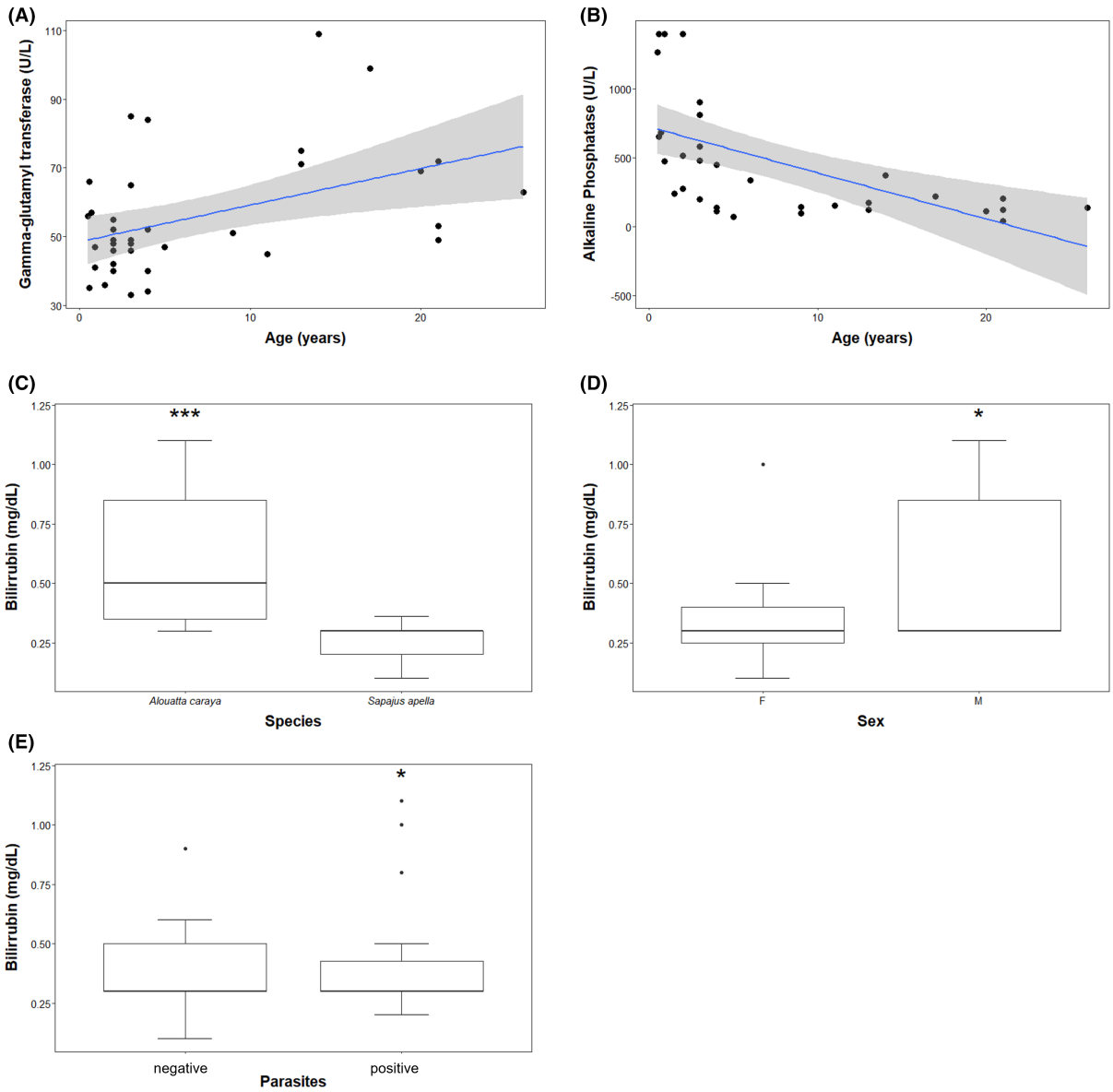

FIGURE 6 Effect of age in gamma-glutamyl transferase (A) and alkaline phosphatase (B); effect of species (C), sex (D), and parasite presence (E) in bilirubin in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

energetic expenditure, which predisposes to diseases such as diabetes. In fact, one howler monkey from the colony was excluded from this study because it presented a diagnosis of diabetes, which supports our hypothesis.

For amylase, the higher value in howlers than in capuchin monkeys is consistent with comparisons using data from other studies in these species.[16,35] Amylase is necessary for starch digestion in the small intestine due to its important function in hydrolyzing the glycosidic bonds in starch molecules and converting complex carbohydrates to simple sugars.[117,118] Furthermore, Campos et al. (2010)[119] reported an increase in amylase associated with acute necrotic pancreatitis in howler monkeys and associated this result with long-term inadequate nutritional management. Thus, considering the high values of amylase reported in our study and others in the literature, as well as glucose, it is important to monitor the pancreatic biomarkers in these species in order to avoid metabolic diseases, particularly in folivore species such as howler monkeys, and increment their diet with a wide variety of fiber sources, such as leafy greens and natural browse, to promote healthy natural gut microbiota and digestion.[115]

On lipidogram, we observed an interaction between species and sex for cholesterol, with lower cholesterol levels in male capuchins compared to females, but the opposite in howler monkeys. Cholesterol is found in cell membranes and is a precursor of bile acids and steroid hormones.[101] Our results for capuchins are in accordance with other studies in capuchin monkeys (*Cebus* spp.),[37] squirrel monkeys,[24] and humans.[120] Although capuchin monkeys have a sexual dimorphism in body mass in which males are heavier than females,[121] Edwards et al.[122] reported higher body fat percentages in females (21.2 ± 1.3%) compared to males (18.2 ± 1.8%), which can explain the higher cholesterol values in females observed in our

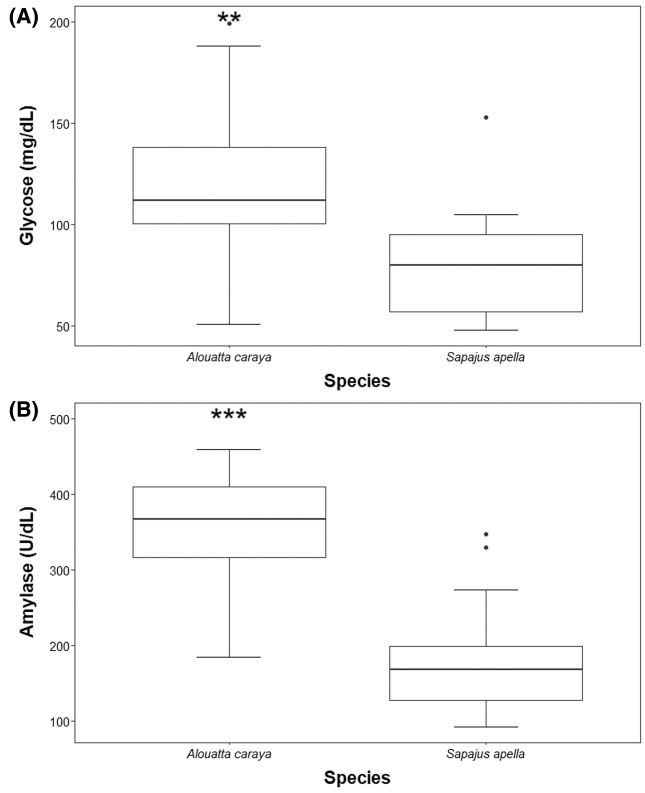

FIGURE 7 Effect of species in glucose (A) and amylase (B) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

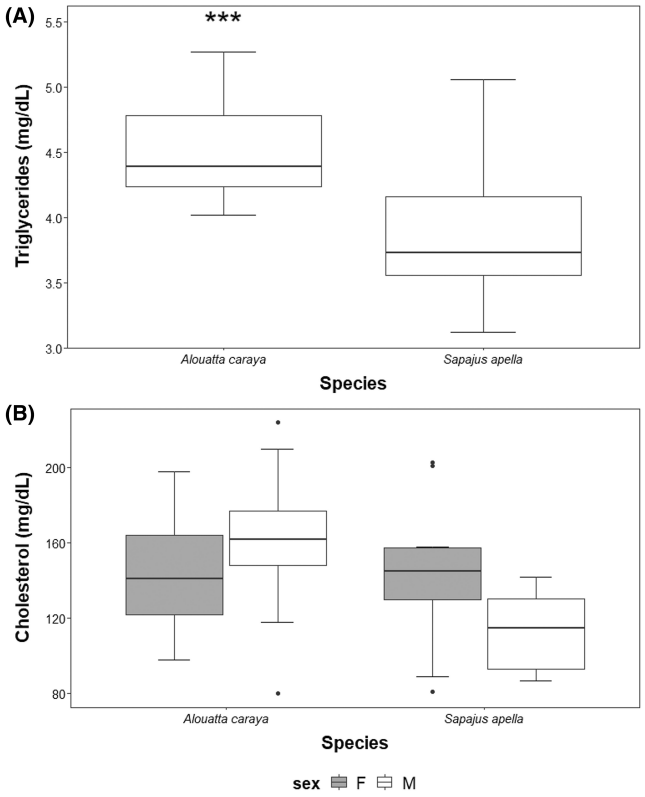

FIGURE 8 Effect of species in triglycerides (A) and interaction between species and sex (F—female; M—male) in cholesterol (B) in capuchin monkeys (*Sapajus apella*) and howler monkeys (*Alouatta caraya*).

study. Our results for howlers were similar to those of other studies in this species, where females had lower cholesterol levels compared to males.[68,79] Since howler monkeys also have sexual dimorphism in body mass as described in capuchin monkeys,[121] our results may suggest that males in this genus have higher fat percentages than females, but further studies are necessary to confirm this hypothesis.

Differences in fat percentages can also explain the lower triglyceride values in capuchins than in howler monkeys in the present study, which contrasts with previously reported data in these species.[35,37,87] Triglycerides are fatty acid esters of glycerol and represent the main lipid component of dietary fat and fat deposits in animals. They act as energy storage and transport energy from the small intestine and liver to peripheral tissues.[79,102,123] The body mass averages 2.12 ± 0.79 kg in capuchin monkeys and 5.08 ± 3.48 kg in howler monkeys, and this difference is greater when considering lean body mass.[124] Moreover, considering the slow metabolism of howlers when compared to capuchin monkeys,[125,126] the effect of captivity on reduced energy expenditure and increased caloric intake may have contributed to the accumulation of fat reserves in our howler monkey population, which may explain the contrast between our data and other reports in the literature, but future comparative studies that monitor lean body mass across captive conditions are warranted.

Lastly, we found that lipase and albumin were not affected by age, sex, parasitism, and species. This finding may suggest that these proteins are more stable and less affected by intra- and inter-specific factors. Nevertheless, we recommend that the evaluation of these proteins is evaluated in conjunction with other examinations and clinical signs, as they could indicate specific conditions that were not present in our study.

In summary, our results showed that parasitism affected WBC and liver proteins and enzyme values. Controlling for species and age, sex affected platelets, and bilirubin concentration. Most parameters were affected by species or interaction between this factor and sex or parasites, as expected, indicating different physiological adaptations in two primate species characterized by distinct ecology and body size. Age affected RBC, WBC, platelet and MPV, and some liver protein and enzyme values, such as TP, globulin, A:G ratio, GGT, and ALP. We highlight the importance of characterizing parasite prevalence in primate populations, which is essential for monitoring their health, the efficacy of deworming procedures, and for management of their diet as well as hygienic measures to reduce pathogen transmission within the colon. Moreover, our comparative data suggest that hemogram and biochemistry parameters can provide valuable information associated with body mass, aging, and ecology, and will enable comparative studies with other platyrrhine primates to investigate interspecies differences in blood parameters as physiological adaptations and the role they played in human evolution.

ACKNOWLEDGMENTS

We thank the staff from the National Primate Center (CENP) and the Federal Rural University of the Amazon (UFRA) for providing animal care and for their assistance during data collection. This study was funded by the National Council of Technological and Scientific Development (CNPq Nos. 305821/2017-2, 400881/2019-5, and 316750/2021-2), the Coordination for the Improvement of Higher Education Personnel (CAPES-Procad Amazônia No. 21/2018), the Wenner-Gren Foundation (No. 10088), and the National Science Foundation (Award No. 212357).

CONFLICT OF INTEREST STATEMENT

The authors declare that there is no conflict of interest regarding the publication of this article.

DATA AVAILABILITY STATEMENT

The datasets during and/or analyzed during the current study are available from the corresponding author on reasonable request.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

How to cite this article: da Silva GP, Pereira THdS, de Melo JT, et al. Hematological and serum biochemistry evaluation in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*): A comparative study. *J Med Primatol*. 2023;00:1-16. doi:10.1111/jmp.12644

CONCLUSÕES E PERSPECTIVAS

Esta pesquisa mostrou que os primatas neotropicais são espécies com grande potencial para estudo da morfofisiologia comparada e evolutiva, auxiliando na compreensão das adaptações que ocorreram na Ordem Primates ao longo de seu desenvolvimento.

No contexto da pesquisa com macaco-de-cheiro como modelo biomédico, o manejo relativamente fácil desses animais e a semelhança morfofisiológica nas estruturas de suporte pélvico com a mulher dão subsídios para estudos em obstetrícia humana. Tais estudos podem auxiliar na compreensão do mecanismo e monitoramento de distúrbios pélvicos e relacionados ao parto em primatas humanos e PNH.

A validação hormonal em espécies platirrinas, abrem espaço para entender o padrão de secreção do DHEAS, sua influência na fisiologia animal e seu potencial uso junto aos glicocorticoides como biomarcadores de estresse. Esses resultados tem colaborado como parte inicial dos projetos de avaliação da senescência e níveis de estresse em platirrinos, e do papel do DHEAS na evolução dos primatas. Será possível ainda, comparar a produção de DHEAS entre as diferentes espécies de PNH e os humanos, e entender o papel da endocrinologia evolutiva na história de vida dos primatas.

Os estudos de morfofisiologia por meio de exames laboratoriais e de diagnóstico por imagem auxiliam na avaliação clínica geral e a obtenção de biomarcadores de sanidade e senescência. Os resultados desses exames apontam diferença de acordo com a espécie, sexo e idade e a prevalência de parasitos, sendo importante incluir a avaliação conjunta desses fatores. Ademais, esses biomarcadores permitem compreender o desenvolvimento renal pós-natal em espécies selvagens e dão *insights* evolucionários sobre o envelhecimento e as necessidades metabólicas em primatas neotropicais.

Portanto, a colaboração entre universidades nacionais e internacionais, zoológicos e centros mantenedouros de primatas, por meio da concessão de amostras, e dos estudos a campo e em laboratório, são essenciais para a execussão de projetos de monitoramento de biomarcadores de saúde e senescência. Os resultados fazem parte de pesquisas e parcerias que tem avançado na área de endocrinologia e biologia molecular em diversas espécies de PNH, o que possibilitará determinar o padrão de secreção de DHEAS, bem como a análise da expressão de enzimas envolvidas na produção desse esteroide em tecidos adrenais e cerebrais.

APÊNDICE 1

Material suplementar referente ao Capítulo 4 - Hematological and serum biochemistry evaluation in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*): A comparative study.

TABLE S 1 Mean ± SD and range [minimum-maximum] values for hemogram and serum biochemistry parameters in 20 howler monkeys (*Alouatta caraya*).

Parameters	Total (n=20)	Infants (n=3)	Juveniles (n=9)	Adults (n=8)
Red Blood Cells ($x10^6$ per mm^3)	4.37 ± 0.57 [3.53-5.32]	3.80 ± 0.27 [3.60-4.11]	4.70 ± 0.32 [4.33-5.22]	4.21 ± 0.66 [3.53-5.32]
Hemoglobin (g/dL)	14.59 ± 1.88 [11.60-18.30]	12.93 ± 1.29 [12.00-14.40]	15.56 ± 1.00 [13.80-16.80]	14.13 ± 2.31 [11.60-18.30]
Hematocrit (%)	43.25 ± 5.70 [34.50-55.10]	38.40 ± 3.64 [36.10-42.60]	46.20 ± 3.05 [41.90-51.20]	41.75 ± 7.06 [34.50-55.10]
MCV (fL)	98.94 ± 2.88 [93.30-104.00]	100.90 ± 2.72 [98.80-104.00]	98.28 ± 2.92 [93.30-102.00]	98.93 ± 2.90 [96.60-104.00]
MCH (pg)	33.40 ± 1.51 [30.50-37.00]	33.97 ± 0.99 [33.30-35.10]	33.12 ± 1.65 [30.50-35.50]	33.50 ± 1.58 [32.10-37.00]
MCHC (%)	33.77 ± 0.74 [32.70-35.80]	33.70 ± 0.35 [33.30-33.90]	33.69 ± 0.81 [32.70-34.80]	33.88 ± 0.84 [33.10-35.80]
Leukocytes ($x10^3$ per mm^3)	15.95 ± 4.12 [9.70-22.70]	16.47 ± 5.44 [12.70-22.70]	17.71 ± 4.02 [9.70-22.00]	13.47 ± 2.74 [10.30-18.30]
Segmented ($x10^3$ per mm^3)	6.54 ± 3.67 [1.82-14.30]	3.68 ± 1.93 [1.82-5.68]	8.39 ± 4.19 [2.43-14.30]	5.52 ± 2.51 [2.78-10.25]
Eosinophils ($x10^3$ per mm^3)	0.00 ± 0.00 [0.00-0.00]	0.00 ± 0.00 [0.00-0.00]	0.00 ± 0.00 [0.00-0.00]	0.00 ± 0.00 [0.00-0.00]
Basophils ($x10^3$ per mm^3)	0.20 ± 0.07 [0.10-0.36]	0.21 ± 0.06 [0.14-0.25]	0.20 ± 0.06 [0.14-0.36]	0.18 ± 0.08 [0.10-0.31]
Lymphocytes ($x10^3$ per mm^3)	7.73 ± 2.79 [2.68-15.44]	11.64 ± 3.66 [8.13-15.44]	7.52 ± 2.35 [4.07-10.58]	6.51 ± 1.64 [2.68-7.75]
Monocytes ($x10^3$ per mm^3)	1.06 ± 0.36 [0.64-1.83]	0.94 ± 0.37 [0.70-1.36]	1.18 ± 0.32 [0.86-1.71]	0.96 ± 0.40 [0.64-1.83]
Platelets ($x10^3$ per mm^3)	161.71 ± 58.36 [53.20-292.00]	89.73 ± 31.65 [53.20-109.00]	192.60 ± 64.64 [57.00-292.00]	154.00 ± 25.91 [109.00-185.00]
Mean Platelets Volume (%)	13.73 ± 1.66 [11.30-17.10]	14.27 ± 1.05 [13.20-15.30]	13.34 ± 1.04 [11.50-14.40]	13.91 ± 2.31 [11.30-17.10]
Total Proteins (g/dL)	7.95 ± 1.03 [5.98-10.44]	7.02 ± 1.46 [5.98-8.05]	7.73 ± 0.85 [6.29-8.56]	8.40 ± 1.01 [7.67-10.44]
Albumin (g/dL)	4.00 ± 0.52 [2.70-4.70]	3.80 ± 0.57 [3.40-4.20]	3.84 ± 0.64 [2.70-4.60]	4.21 ± 0.32 [3.80-4.70]
Globulin	3.95 ± 0.80	3.22 ± 0.90	3.89 ± 0.77	4.19 ± 0.80

(g/dL)	[2.58-5.74]	[2.58-3.85]	[2.79-5.37]	[3.61-5.74]
A:G Ratio	1.05 ± 0.20	1.20 ± 0.16	1.02 ± 0.24	1.03 ± 0.16
	[0.50-1.32]	[1.09-1.32]	[0.50-1.25]	[0.82-1.23]
Bilirrubin	0.62 ± 0.36	0.35 ± 0.07	0.63 ± 0.31	0.68 ± 0.45
(mg/dL)	[0.30-1.60]	[0.30-0.40]	[0.30-1.00]	[0.30-1.60]
AST	101.70 ± 28.24	128.00 ± 26.87	110.00 ± 29.77	86.75 ± 20.40
(U/L)	[57.00-160.00]	[109.00-147.00]	[70.00-160.00]	[57.00-112.00]
ALT	15.28 ± 8.39	16.50 ± 14.85	16.38 ± 10.24	13.88 ± 5.52
(U/L)	[6.00-35.00]	[6.00-27.00]	[6.00-35.00]	[9.00-24.00]
ALP	581.90 ± 488.80	1334.00 ± 94.05	751.50 ± 469.10	224.50 ± 137.00
(U/L)	[93.00-1400.00]	[1267.00-1400.00]	[196.00-1400.00]	[93.00-448.00]
GGT	71.44 ± 46.92	61.00 ± 7.07	50.88 ± 16.77	94.63 ± 62.68
(U/L)	[33.00-230.00]	[56.00-66.00]	[33.00-85.00]	[45.00-230.00]
Amylase	348.60 ± 81.54	407.00 ± 7.07	367.00 ± 88.98	315.50 ± 74.88
(U/dL)	[184.00-459.00]	[402.00-412.00]	[184.00-459.00]	[186.00-411.00]
Lipase	15.33 ± 3.69	16.00 ± 2.83	15.25 ± 3.85	15.25 ± 4.13
(U/L)	[9.00-22.00]	[14.00-18.00]	[10.00-22.00]	[9.00-22.00]
Glucose	122.60 ± 41.69	150.50 ± 53.03	111.00 ± 16.34	127.30 ± 56.14
(mg/dL)	[51.00-199.00]	[113.00-188.00]	[96.00-140.00]	[51.00-199.00]
Cholesterol	150.20 ± 38.60	164.00 ± 4.24	153.00 ± 34.85	144.00 ± 47.90
(mg/dL)	[80.00-224.00]	[161.00-167.00]	[80.00-198.00]	[98.00-224.00]
Triglycerides	125.90 ± 60.22	81.50 ± 2.12	108.50 ± 46.48	154.50 ± 69.29
(mg/dL)	[66.00-261.00]	[80.00-83.00]	[66.00-206.00]	[78.00-261.00]

Abbreviations: MCV. mean corpuscular volume; MCH. mean corpuscular hemoglobin; MCHC. mean corpuscular hemoglobin concentration; A:G. albumin:globulin; AST. aspartate aminotransferase; ALT. alanine aminotransferase; ALP. alkaline phosphatase; GGT. gamma-glutamyl transferase

TABLE S 2 Mean ± SD and range [minimum-maximum] values for hemogram and serum biochemistry parameters in 21 capuchin monkeys (*Sapajus apella*).

Parameters	**Total** (n=21)	**Infants** (n=4)	**Juveniles** (n=10)	**Adults** (n=7)
Red Blood Cells ($x10^6$ per mm^3)	5.60 ± 0.50 [4.84-6.65]	6.04 ± 0.42 [5.72-6.65]	5.63 ± 0.49 [4.84-6.21]	5.31 ± 0.38 [4.91-6.05]
Hemoglobin (g/dL)	14.56 ± 1.06 [12.80-17.00]	14.73 ± 0.86 [13.60-15.70]	14.77 ± 0.86 [13.40-15.90]	14.16 ± 1.42 [12.80-17.00]
Hematocrit (%)	42.44 ± 3.10 [38.00-50.50]	42.95 ± 1.72 [40.90-45.10]	43.06 ± 2.48 [39.00-46.90]	41.26 ± 4.35 [38.00-50.50]
MCV (fL)	76.56 ± 3.98 [71.90-88.30]	74.25 ± 2.61 [71.90-77.50]	76.68 ± 4.77 [72.30-88.30]	77.70 ± 3.20 [73.80-83.50]
MCH (pg)	26.27 ± 1.33 [24.10-29.90]	25.43 ± 1.20 [24.10-26.90]	26.32 ± 1.51 [25.10-29.90]	26.67 ± 1.01 [24.90-28.10]
MCHC (%)	34.30 ± 0.61 [33.30-35.70]	34.25 ± 0.66 [33.30-34.70]	34.32 ± 0.58 [33.50-35.20]	34.31 ± 0.72 [33.60-35.70]
Leukocytes ($x10^3$ per mm)	4.97 ± 2.90 [1.50-12.20]	8.25 ± 3.70 [5.00-12.20]	4.50 ± 2.37 [2.20-9.20]	3.77 ± 1.90 [1.50-6.40]
Segmented ($x10^3$ per mm^3)	2.86 ±2.10 [0.71-7.73]	4.46 ±2.00 [2.20-6.68]	2.72 ±2.41 [0.90-7.73]	2.14 ±1.29 [0.71-3.97]
Eosinophils ($x10^3$ per mm^3)	0.07 ± 0.13 [0.00-0.42]	0.10 ± 0.20 [0.00-0.41]	0.07 ± 0.14 [0.00-0.42]	0.06 ± 0.10 [0.00-0.28]
Basophils ($x10^3$ per mm^3)	0.10 ± 0.13 [0.00-0.49]	0.25 ± 0.23 [0.05-0.49]	0.07 ± 0.04 [0.04-0.16]	0.07 ± 0.08 [0.00-0.24]
Lymphocytes ($x10^3$ per mm^3)	1.59 ± 0.95 [0.63-4.64]	2.63 ± 1.54 [0.88-4.64]	1.37 ± 0.58 [0.67-2.44]	1.31 ± 0.68 [0.63-2.18]
Monocytes ($x10^3$ per mm^3)	0.35 ± 0.35 [0.03-1.59]	0.84 ± 0.64 [0.03-1.59]	0.27 ± 0.08 [0.11-0.40]	0.19 ± 0.06 [0.14-0.28]
Platelets ($x10^3$ per mm^3)	202.80 ± 60.38 [108.00-304.00]	273.80 ± 32.79 [234.00-304.00]	217.00 ± 46.15 [143.00-296.00]	142.00 ± 20.39 [108.00-169.00]
Mean Platelets Volume (%)	10.75 ± 1.48 [7.49-14.60]	10.22 ± 2.12 [7.49-12.60]	10.32 ± 0.73 [9.19-11.20]	11.81 ± 1.65 [9.85-14.60]
Total Proteins (g/dL)	8.70 ± 1.29 [6.42-11.23]	9.96 ± 0.94 [8.96-11.23]	9.21 ± 0.66 [7.59-10.16]	7.26 ± 0.71 [6.42-8.55]
Albumin (g/dL)	4.15 ± 0.23 [3.80-4.60]	4.23 ± 0.13 [4.10-4.40]	4.24 ± 0.26 [3.80-4.60]	3.99 ± 0.16 [3.80-4.20]
Globulin (g/dL)	4.55 ± 1.16 [2.42-7.03]	5.74 ± 0.93 [4.86-7.03]	4.97 ± 0.58 [3.79-5.66]	3.27 ± 0.65 [2.42-4.35]
A:G Ratio	0.97 ± 0.26 [0.60-1.65]	0.75 ± 0.11 [0.60-0.84]	0.86 ± 0.11 [0.71-1.06]	1.26 ± 0.24 [0.97-1.65]
Billirrubin (mg/dL)	0.26 ± 0.07 [0.10-0.36]	0.29 ± 0.07 [0.20-0.36]	0.28 ± 0.05 [0.20-0.30]	0.24 ± 0.08 [0.10-0.30]
AST	49.14 ± 12.81	55.75 ± 14.55	42.90 ± 8.66	54.29 ± 14.27

(U/L)	[34.00-78.00]	[45.00-77.00]	[34.00-61.00]	[39.00-78.00]
ALT	38.19 ± 10.04	36.50 ± 9.15	33.20 ± 8.59	46.29 ± 7.99
(U/L)	[19.00-61.00]	[27.00-47.00]	[19.00-44.00]	[39.00-61.00]
ALP	345.60 ± 359.00	801.30 ± 409.90	258.80 ± 184.20	134.90 ± 67.23
(U/L)	[41.00-1400.00]	[471.00-1400.00]	[111.00-513.00]	[41.00-220.00]
GGT	53.95 ± 16.51	45.00 ± 9.38	49.30 ± 13.77	65.71 ± 18.15
(U/L)	[34.00-99.00]	[35.00-57.00]	[34.00-84.00]	[47.00-99.00]
Amylase	220.00 ± 188.70	188.30 ± 102.30	148.60 ± 31.01	340.00 ± 293.40
(U/dL)	[92.00-989.00]	[92.00-329.00]	[110.00-200.00]	[143.00-989.00]
Lipase	14.19 ± 7.07	12.50 ± 4.66	17.83 ± 9.20	11.67 ± 5.16
(U/L)	[3.00-30.00]	[7.00-18.00]	[3.00-30.00]	[5.00-19.00]
Glucose	80.52 ± 25.17	104.00 ± 35.00	71.40 ± 22.35	80.14 ± 15.61
(mg/dL)	[48.00-153.00]	[70.00-153.00]	[48.00-105.0]	[50.00-102.00]
Cholesterol	129.00 ± 34.40	143.80 ± 16.21	112.60 ± 26.30	144.00 ± 43.81
(mg/dL)	[81.00-203.00]	[126.00-158.00]	[81.00-148.00]	[89.00-203.00]
Triglycerides	66.71 ± 44.22	44.00 ± 4.97	64.80 ± 39.89	82.43 ± 59.01
(mg/dL)	[25.00-207.00]	[37.00-48.00]	[25.00-144.0]	[38.00-207.00]

Abbreviations: MCV. mean corpuscular volume; MCH. mean corpuscular hemoglobin; MCHC. mean corpuscular hemoglobin concentration; A:G Ratio. albumin:globulin ratio; AST. aspartate aminotransferase; ALT. alanine aminotransferase; ALP. alkaline phosphatase; GGT. gamma-glutamyl transferase

TABLE S 3 Comparative hemogram and serum biochemistry parameters (mean ± SD and range [minimum-maximum]) of the present study with data reported from the literature in *Alouatta caraya*, *A. pigra*, *A. clamitans* and *A. palliata*. Data includes infants. juveniles. and adults (with exceptions†)

Parameters	*Alouatta caraya*		*Alouatta caraya, A. pigra*		*Alouatta guariba clamitans*		*Alouatta palliata*††	
	Present study		Melo et al. (2019); Garcia-Feria et al. (2017)††		Gonçalves et al. (2019)†		Canales-Espinosa et al. (2015)	
	Females (n = 11)	Males (n = 9)	Females (n = 12)	Males (n = 15)	Females (n = 11)	Males (n = 19)	Females (n = 16)	Males (n = 12)
RBC (x10^6 per mm)	4.18 ± 0.44 [3.53-4.84]	4.60 ± 0.65 [3.60-5.32]	4.20 ± 0.40 [3.58-4.91]	4.47 ± 0.59 [3.73-6.16]	4.21 ± 0.31 [3.56-4.82]	5.06 ± 0.37 [4.49-5.86]	3.73 ± 0.31 [2.545.17]	3.61 ± 0.38 [2.545.17]
Hemoglobin (g/dL)	13.79 ± 1.55 [11.60-16.40]	15.57 ± 1.85 [12.00-18.30]	11.93 ± 1.17 [10.50-13.80]	13.13 ± 1.93 [10.60-17.50]	10.74 ± 0.61 [9.5-12]	13.02 ± 1.19 [11.3-15.5]	9.91 ± 0.91 [7.9−12.3]	10.24 ± 0.90 [7.9−13.3]
Hematocrit (%)	40.98 ± 4.49 [34.50-48.60]	46.02 ± 6.02 [36.10-55.10]	35.22 ± 3.18 [29.30-38.70]	39.40 ± 5.90 [30.90-54.00]	33.57 ± 1.99 [30.1-38.3]	41.45 ± 3.66 [35.8-48.6]	31.65 ± 2.78 [23.3−42.2]	32.50 ± 2.73 [23.3−41.2]
MCV (fL)	97.95 ± 2.46 [93.30-102.00]	100.10 ± 3.04 [95.60-104.00]	84.28 ± 7.06 [74.00-98.00]	87.85 ± 6.01 [80.00-99.00]	79.94 ± 2.91 [74.8-84.6]	81.7 ± 3.75 [81.7 ± 3.75]	84.99 ± 5.53 [65.5−100]	89.69 ± 4.10 [67.7−100]
MCH (pg)	32.95 ± 1.32 [30.50-35.50]	33.94 ± 1.62 [31.90-37.00]	28.66 ± 2.59 [25.10-35.30]	29.71 ± 3.00 [26.50-35.90]	25.56 ± 0.85 [23.8-26.8]	25.66 ± 1.34 [23-28.9]	26.60 ± 1.78 [20.3−34.0]	28.24 ± 1.68 [21.3−34.3]
MCHC (%)	33.65 ± 0.57 [32.70-34.80]	33.91 ± 0.93 [32.80-35.80]	33.97 ± 1.76 [30.20-36.00]	33.37 ± 2.18 [30.60-37.40]	32 ± 0.67 [30.8-33.3]	31.4 ± 0.70 [30.2-33.2]	31.2 ± 0.88 [24.0−31.7]	31.52 ± 0.78 [24.5−33.7]
Leukocytes (x10^3 per mm^3)	16.87 ± 4.30 [10.30-22.70]	14.69 ± 3.74 [9.70-22.00]	11.06 ± 2.90 [7.90-19.40]	13.20 ± 5.10 [5.90-22.90]	7.35 ± 2.23 [5.3-13.3]	9.97 ± 3.09 [6-18.6]	12.49 ± 4.08 [4.2−24.1]	12.49 ± 3.04 [3.2−24.1]
Segmented (x10^3 per mm^3)	7.67 ± 3.66 [2.72-14.30]	5.15 ± 3.35 [1.82-12.57]	6.90 ± 2.10 [2.60-10.40]	7.30 ± 3.80 [2.00-12.70]	6-18.6 [3.18-10.24]	3.18-10.24 [3.84-10.6]	N/A	N/A
Eosinophils (x10^3 per mm^3)	0.00 ± 0.00 [0.00-0.00]	0.00 ± 0.00 [0.00-0.00]	2.10 ± 1.70 [0.00-5.10]	2.30 ± 2.00 [0.00-8.00]	0.07 ± 0.09 [0-0.27]	0.09 ± 0.12 [0.09 ± 0.12]	N/A	N/A
Basophils (x10^3 per mm^3)	0.20 ± 0.06 [0.10-0.31]	0.20 ± 0.08 [0.13-0.36]	0.00 ± 0.00 [0.00-0.00]	N/A	0.02 ± 0.03 [0.02 ± 0.03]	0.08 ± 0.18 [0-0.77]	N/A	N/A
Lymphocytes (x10^3 per mm^3)	7.86 ± 3.37 [2.68-15.44]	7.58 ± 2.06 [4.07-11.34]	2.80 ± 1.20 [1.00-5.40]	4.60 ± 2.00 [2.00-10.40]	2.06 ± 0.78 [2.06 ± 0.78]	2.50 ± 1.29 [1.18-7.25]	N/A	N/A
Monocytes (x10^3 per mm^3)	1.15 ± 0.38 [0.72-1.83]	0.95 ± 0.33 [0.64-1.65]	N/A	N/A	0.43 ± 0.33 [0.17-1.33]	0.47 ± 0.21 [0-0.78]	N/A	N/A
Platelets (x10^3 per mm^3)	180.80 ± 61.32 [53.20-292.00]	138.30 ± 47.64 [57.00-211.00]	287.43 ± 135.20 [66.00-532.00]	258.80 ± 139.20 [44.00-538.00]	170.64 ± 42.54 [94-234]	256.74 ± 74.37 [97-382]	193.58 ± 69.46 [150−369]	219.66 ± 41.48 [158.9−399]
Mean Platelets Volume (%)	13.62 ± 1.85 [11.30-17.10]	13.84 ± 1.52 [12.10-16.80]	N/A	N/A	N/A	N/A	N/A	N/A

Total Proteins (g/dL)	7.91 ± 0.84 [6.29-9.49]	8.00 ± 1.30 [5.98-10.44]	7.45 ± 0.51 [7.00-8.10]	6.38±0.55 [5.40-6.80]	5.95 ± 1.11 [4.1-7.7]	6.39 ± 1.46 [3.95-9.05]	7.88 ± 0.5 [5.3−8.4]	7.44 ± 0.75 [5.6−8.0]
Albumin (g/dL)	4.06 ± 0.38 [3.40-4.60]	3.93 ± 0.68 [2.70-4.70]	2.68±0.29 [2.20-3.00]	2.48±0.22 [2.30-2.80]	3.83 ± 0.77 [2.29-4.96]	4.08 ± 0.55 [3.27-5.68]	4.60 ± 0.43 [3.5−5.6]	4.55 ± 0.62 [3.7−5.6]
Globulin (g/dL)	3.85 ± 0.57 [2.79-5.09]	4.07 ± 1.06 [2.58-5.74]	4.78±0.46 [4.30-5.40]	3.82±0.51 [3.10-4.30]	2.39 ± 0.56 [1.51-2.97]	2.22 ± 0.38 [1.57-3.08]	3.28 ± 0.31 [1.7−3.94]	2.89 ± 0.32 [1.7−3.2]
A/G Ratio	1.07 ± 0.13 [0.86-1.25]	1.02 ± 0.27 [0.50-1.32]	0.60±0.12 [0.50-0.70]	0.64±0.09 [0.50-0.70]	1.75 ± 0.73 [0.79-3.2]	1.89 ± 0.41 [1.26-2.75]	1.40 ± 0.18 [1.32−1.96]	1.52 ± 0.29 [1.32−2.41]
Bilirrubin (mg/dL)	0.46 ± 0.21 [0.30-1.00]	0.81 ± 0.43 [0.30-1.60]	1.23±2.53 [0.17-6.40]	0.19±0.08 [0.07-0.28]	N/A	N/A	N/A	N/A
AST (U/L)	92.40 ± 24.19 [57.00-138.00]	113.30 ± 30.15 [70.00-160.00]	76.20±13.83 [62.00-96.0]	93.00±33.12 [70.00-158.00]	85.20 ± 16.74 [69.8-130.1]	81.54 ± 13.42 [64.4-111.75]	N/A	N/A
ALT (U/L)	14.50 ± 7.31 [6.00-27.00]	16.25 ± 10.01 [6.00-35.00]	27.60±3.71 [23.00-33.00]	27.50±4.14 [22.00-33.00]	24 ± 5.89 [15.7-37]	24.41 ± 8.91 [12.2-51.2]	N/A	N/A
ALP (U/L)	437.60±391.00 [93.00-1267.00]	762.40 ± 562.90 [141.00-1400.00]	349.17±199.06† [90.00-669.00]	236.17±62.64† [124.00-297.00]	326.88 ± 131.69 [185.2-661.85]	296.82 ± 119.94 [183.8-580.45]	256.87 ± 90.64 [125.0−575.0]	274.46 ± 181.34 [125.0−700.0]
GGT (U/L)	57.30 ± 15.17 [33.00-85.00]	89.13 ± 66.38 [36.00-230.00]	51.00††	36.25±26.09 [3.00-59.00]	44.87 ± 15.05 [22.8-75.65]	65.14 ± 25.9 [22.8-141]	N/A	N/A
Amylase (U/dL)	355.80 ± 77.66 [186.00-459.00]	339.50 ± 90.67 [184.00-443.00]	376.00††	317.75±93.64 [183.00-399.00]	N/A	N/A	N/A	N/A
Lipase (U/L)	14.70 ± 3.77 [9.00-22.00]	16.13 ± 3.68 [10.00-22.00]	75.00††	82.33±10.41 [74.00-94.00]	N/A	N/A	N/A	N/A
Glucose (mg/dL)	117.00 ± 44.27 [51.00-187.00]	129.60 ± 40.01 [97.00-199.00]	84.80 ± 28.15 [37.00-112.00]	99.20±20.91 [75.00-130.00]	63.33 ± 12.8 [41.25-86.45]	67.28 ± 22.24 [31.9-114.45]	N/A	N/A
Cholesterol (mg/dL)	142.40 ± 31.88 [98.00-198.00]	160.00 ± 46.00 [80.00-224.00]	81.00±12.47 [66.00-95.00]	105.67±34.13 [66.00-167.00]	158.73 ± 23.22 [133.6-194.4]	182.99 ± 38.77 [129.8-255.2]	N/A	N/A
Triglycerides (mg/dL)	116.60 ± 58.06 [67.00-261.00]	137.60 ± 64.74 [66.00-231.00]	117.83±86.92 [38.00-278.00]	63.67±22.99 [35.00-87.00]	N/A	N/A	N/A	N/A

Abbreviations: RBC. red blood cells; MCV. mean corpuscular volume; MCH. mean corpuscular hemoglobin; MCHC. mean corpuscular hemoglobin concentration; A/G Ratio. albumin/globulin ratio; AST. aspartate aminotransferase; ALT. alanine aminotransferase; ALP. alkaline phosphatase; GGT. gamma-glutamyl transferase; N/A. data not available. †Only adult animals; †† free-ranging animals; ††† n = 1.

TABLE S 4 Comparative serum chemistry parameters (mean ± SD and range [minimum-maximum]) of the present study with data reported from the literature in *Sapajus apella*. Data includes infants. juveniles and adults (with exceptions[†]).

Parameters	*Sapajus apella*					
	Present study		**Wirtz. Truppa and Riviello (2008)**		**Favareto et al. (2016)**	
	Females (n = 11)	**Males (n = 10)**	**Females (n = 24)**	**Males (n = 20)**	**Females (n = 10)**	**Males (n = 10)**
RBC ($x10^6$ per mm^3)	5.69 ± 0.60 [4.84-6.65]	5.51 ± 0.36 [5.19-6.11]	5.37 ± 0.43 [4.08-6.47]	5.92 ± 0.45 [4.40-7.05]	5.29±0.63 [3.92–6.36]	5.49±0.46 [4.48–6.24]
Hemoglobin (g/dL)	14.59 ± 1.29 [12.80-17.00]	14.52 ± 0.81 [13.40-15.90]	13.07 ± 1.01 [10.00-15.40]	14.04 ± 1.10 [10.70-16.70]	13.40±1.08 [10.80–14.30]	13.62±1.02 [12.00–15.40]
Hematocrit (%)	42.81 ± 3.65 [38.00-50.50]	42.03 ± 2.49 [39.00-46.90]	38.77 ± 3.57 [28.00-47.10]	42.58 ± 3.54 [29.00-52.00]	44.00±3.12 [37–47]	45.50±3.14 [41–52]
MCV (fL)	76.73 ± 5.23 [71.90-88.30]	76.37 ± 2.18 [73.80-80.30]	70.96 ± 2.01 [65.00-80.00]	71.63 ± 2.26 [65.00-79.00]	83.58±5.22 [73.90–94.39]	83.04±4.28 [77.06–91.52]
MCH (pg)	26.17 ± 1.71 [24.10-29.90]	26.37 ± 0.80 [25.10-27.70]	23.59 ± 0.79 [20.00-26.00]	23.47 ± 0.85 [20.00-26.00]	N/A	N/A
MCHC (%)	34.11 ± 0.60 [33.30-35.00]	34.52 ± 0.59 [33.60-35.70]	33.47 ± 1.38 [29.00-38.00]	32.81 ± 1.78 [25.00-38.00]	30.43±0.69 [29.19–31.78]	29.93±0.59 [29.11–30.85]
Leukocytes ($x10^3$ per mm^3)	5.40 ± 3.58 [1.50-12.20]	4.50 ± 1.99 [2.00-9.20]	7.37 ± 1.76 [2.80-4.60]	7.21 ± 1.68 [3.00-15.30]	8.68±3.71 [5.20–17.60]	7.26±3.16 [4.00–13.70]
Segmented ($x10^3$ per mm^3)	3.08 ±2.30 [0.71-6.68]	2.61 ±1.96 [0.95-7.73]	N/A	N/A	5.01±2.43 [2.73–9.30]	3.38±1.56 [1.85–7.39]
Eosinophils ($x10^3$ per mm^3)	0.07 ± 0.14 [0.00-0.41]	0.08 ± 0.13 [0.00-0.42]	N/A	N/A	0.81±1.71 [0.00–5.63]	0.24±0.38 [0.00–1.23]
Basophils ($x10^3$ per mm^3)	0.13 ± 0.16 [0.00-0.49]	0.08 ± 0.06 [0.04-0.24]	N/A	N/A	N/A	N/A
Lymphocytes ($x10^3$ per mm^3)	1.67 ± 1.18 [0.63-4.64]	1.49 ± 0.68 [0.67-2.45]	N/A	N/A	2.26 ± 1.61 [0.67–5.63]	3.08 ± 1.76 [1.12–7.40]
Monocytes ($x10^3$ per mm^3)	0.48 ± 0.45 [0.14-1.59]	0.21 ± 0.10 [0.03-0.37]	N/A	N/A	0.50 ± 0.36 [0.15–1.41]	0.53 ± 0.54 [0.12–1.92]
Platelets ($x10^3$ per mm^3)	211.20 ± 60.57 [146.00-304.00]	193.60 ± 62.01 [108.00-296.00]	385.73 ± 56.93 [271.00-560.00]	394.80 ± 60.46 [254.00-527.00]	394.90 ± 74.61 [273–490]	409.60 ± 120.95 [264–577]
Mean Platelets Volume (%)	10.41 ± 1.49 [7.49-12.60]	11.09 ± 1.47 [9.36-14.60]	N/A	N/A	N/A	N/A
Total Proteins (g/dL)	8.73 ± 1.40 [6.56-11.23]	8.66 ± 1.22 [6.42-10.16]	7.41 ± 0.37 [6.30-8.80]	7.36 ± 0.41 [6.30-8.60]	7.53±0.39 [7.0–8.2]	7.4±0.36 [6.70–7.9]

Albumin (g/dL)	4.18 ± 0.23 [3.80-4.60]	4.12 ± 0.25 [3.80-4.50]	4.48 ± 0.24 [3.80-5.10]	4.40 ± 0.29 [3.80-5.40]	4.0.9±0.45 [3.3–4.8]	4.04±0.45 [3.5–4.6]
Globulin (g/dL)	4.55 ± 1.29 [2.66-7.03]	4.54 ± 1.07 [2.42-5.66]	N/A	N/A	N/A	N/A
A/G Ratio	0.98 ± 0.26 [0.60-1.47]	0.96 ± 0.28 [0.71-1.65]	1.51 ± 0.14 [1.11-1.90]	1.64 ± 0.57 [1.06-1.95]	N/A	N/A
Bilirrubin (mg/dL)	0.25 ± 0.08 [0.10-0.36]	0.30 ± 0.00 [0.30-0.30]	0.33 ± 0.13 [0.01-0.74]	0.34 ± 0.13 [0.03-0.64]	N/A	N/A
AST (U/L)	47.00 ± 11.48 [36.00-78.00]	51.50 ± 14.36 [34.00-77.00]	56.15 ± 14.67 [23.00-125.00]	49.85 ± 15.46 [27.00-116.00]	32.60±23.52 [13–86]	31.89±24.02 [9–85]
ALT (U/L)	39.00 ± 12.44 [19.00-61.00]	37.30 ± 7.10 [27.00-49.00]	45.10 ± 14.57 [17.00-111.00]	41.01 ± 12.68 [22.00-86.00]	6.40±3.63 [0–12]	9.11±7.70 [2–27]
ALP (U/L)	321.00 ± 239.90 [41.00-683.00]	394.80 ± 563.10 [111.00-1400.00]	223.26±191.09† [31.00-835.00]	279.96 ± 193.91† [35.00-891.00]	145.60±118.05 [41–456]	169.67±107.44 [73–402]
GGT (U/L)	56.36 ± 20.50 [34.00-99.00]	51.30 ± 11.14 [40.00-72.00]	71.65 ± 24.97 [33.00-132.00]	62.15 ± 13.42 [23.00-94.00]	56.11±27.75 [25–95]	47.30±21.29 [23–97]
Amylase (U/dL)	155.40 ± 43.56 [92.00-233.00]	291.00 ± 257.60 [121.00-989.00]	253.19 ± 60.78 [38.00-771.00]	233.77 ± 110.86 [37.00-918.00]	N/A	N/A
Lipase (U/L)	13.00 ± 5.26 [5.00-19.00]	15.11 ± 8.42 [3.00-30.00]	91.50 ± 15.50 [51.00-164.00]	82.05 ± 7.41 [52.00-124.00]	N/A	N/A
Glucose (mg/dL)	90.09 ± 27.91 [50.00-153.00]	70.00 ± 17.54 [48.00-98.00]	93.26 ± 27.98 [43.00-181.00]	82.74 ± 17.49 [41.00-132.00]	N/A	N/A
Cholesterol (mg/dL)	144.10 ± 38.12 [81.00-203.00]	112.40 ± 20.74 [87.00-142.00]	145.33 ± 38.44 [62.00-223.00]	138.42 ± 18.74 [74.00-206.00]	236.20±37.20 [186–311]	205.90±34.00 [158–253]
Triglycerides (mg/dL)	74.18 ± 54.38 [35.00-207.00]	58.50 ± 30.29 [25.00-125.00]	93.51 ± 29.63 [19.00-197.00]	90.83 ± 17.64 [13.00-214.00]	141.50±67.41 [65–248]	113.22±25.51 [79–150]

Abbreviations: RBC. red blood cells; MCV. mean corpuscular volume; MCH. mean corpuscular hemoglobin; MCHC. mean corpuscular hemoglobin concentration; A/G Ratio. albumin/globulin ratio; AST. aspartate aminotransferase; ALT. alanine aminotransferase; ALP. alkaline phosphatase; GGT. gamma-glutamyl transferase; N/A. data not available; † Juveniles and adults *Sapajus apella*.

APÊNDICE 2 - RELATÓRIO FINAL REFERENTE ÀS ATIVIDADES DESENVOLVIDAS DURANTE O DOUTORADO SANDUICHE NO EXTERIOR (EDITAL: 21/2018 – PROCAD/CAPES)

Curso de Doutorado: Programa de Pós-graduação em Saúde e Produção Animal na Amazônia-PPGSPAA/UFRA

Instituição/departamento no exterior: Kent State University, Departamentos de Antropologia e Biologia

Período: 01/03/2022 a 29/12/2022

Orientador no Brasil: Frederico Ozanan Barros Monteiro

Co-orientador(a) no exterior: Rafaela S.C. Takeshita

Título do projeto: "MONITORAMENTO DA SENESCÊNCIA E DE NÍVEIS DE ESTRESSE EM PRIMATAS NEOTROPICAIS"

1. Introdução

Primatas não humanos (PNH) apresentam consideráveis semelhanças genéticas, morfofisiológicas e comportamentais com humanos, e são considerados excelentes modelos em estudos comparativos sobre a evolução humana. Dados recentes da União Internacional para Conservação da Natureza (IUCN) apontam, entre espécies e subespécies, a existência de 199 primatas neotropicais que vivem em território sul-americano (IUCN, 2018). Desse número expressivo, 15 ocorrem na Mata Atlântica e 11 são amazônicas. Nesse contexto, o monitoramento do bem-estar animal é essencial para garantir que as condições ambientais dos primatas sejam adequadas para a reprodução e, consequentemente, para a preservação da espécie, em cativeiro ou em vida selvagem. Atualmente, o cortisol e outros glicocorticóides (GC) são os hormônios mais utilizados como indicadores de estresse em animais selvagens, e apesar de serem bons indicadores de estresse agudo, não são confiáveis em casos de estresse crônico, pois podem ser afetados por diversos fatores, relacionados ao próprio animal e ao ambiente (Hodges et al. 2010; Takeshita et al. 2014; Takeshita et al. 2016; Takeshita et al. 2017). Assim, tem-se buscado para avaliar níveis de estresse pela associação de GCs com deidroepiandrosterona (DHEA) ou a forma sulfonada, sulfato de deidroepiandrosterona (DHEAS), precursores de hormônios sexuais produzidos pela adrenal, que têm sido associados

com estresse em seres humanos (Du et al. 2011; Kamin & Kertes 2017) e primatas não humanos (Maninger et al. 2010; Goncharova et al. 2012; Takeshita et al. 2014).

O DHEA é a forma ativa, e é convertido em DHEAS por meio da enzima sulfotransferase (Kroboth et al. 1999) com concentração cerca de 250 vezes maior que a do DHEA, sendo, por esse motivo, a forma mais adequada para representar a disponibilidade de DHEA secretado na corrente sanguínea (Kroboth et al. 1999). Estudos demonstraram que o DHEAS age como antagonista de GCs, para combater os efeitos deletérios do estresse (como aumento da resposta imunológica). Dessa forma, a associação GC/DHEAS tem sido considerada como um melhor índice na avaliação da resposta ao estresse em humanos (Cruess et al. 1999), e se mostrou um índice promissor da resposta ao estresse em primatas devido à baixa variação intra-individual e sua aplicabilidade nas comparações intergrupos (Takeshita et al., 2019). O DHEAS também tem sido associado à senescência em seres humanos (Orentreich et al. 1984) e primatas não humanos (Muehlenbein et al. 2003; Bernstein et al. 2012; Takeshita et al. 2013). Em macacos do velho mundo como *Macaca* sp. e *Papio* sp., observou-se que os níveis plasmáticos do DHEAS diminuem com a idade (Kemnitz et al. 2000; Muehlenbein et al. 2003; Takeshita et al. 2013), enquanto que em humanos (Sizonenko & Paunier 1975) e grandes símios (Cutler et al. 1978; Behringer et al. 2012; Bernstein et al. 2012), há um aumento pré-puberal de DHEAS, um fenômeno endócrino denominado adrenarca (Campbell 2006). Essa variação na secreção de DHEAS entre as espécies indica que a adrenarca está relacionada com evolução, e possivelmente está associada à longevidade, uma vez que humanos e grandes símios possuem expectativa de vida mais longa que macacos do velho mundo (Ingram et al. 2001).

Apesar das diversas investigações dos níveis de DHEAS com a idade em grandes símios e em macacos do velho mundo (Bernstein et al. 2012; Prall & Muehlenbein 2015; Muehlenbein et al. 2003; Takeshita et al. 2013), a única espécie de primatas do novo mundo estudada foi o sagui-de-tufo-branco (*Callithrix jacchus*) (Pattison et al. 2005; Pattison et al. 2007), que apresenta expectativa de aproximadamente 12 anos e devem apresentar um padrão de secreção hormonal diferente ao de primatas do velho mundo. Assim, outros modelos de primatas neotropicais podem ser utilizados, como os macacos-prego (*Sapajus* sp.), que possuem um sistema social complexo, cérebros relativamente grandes, e demonstra grandes habilidades cognitivas (Visalberghi 1997; Ottoni et al. 2005), podem viver até 50 anos em cativeiro (Raposo et al. 2015), similar à longevidade de grandes símios, como orangotangos (55 anos;

Shumaker et al. 2008) ou chimpanzés (52 anos; Herndon et al. 1999). Comparativamente, macacos-da-noite e os bugios apresentam expectativa de vida de aproximadamente 20 anos em cativeiro, que seria intermediária entre os saguis e macacos pregos. Os muriquis (*Brachyteles* sp.) e o macaco-aranha (*Ateles* sp.) possuem muitas características semelhantes aos grandes símios, tais como idade de maturidade sexual, expectativa de vida de até 40 anos em vida livre, e estrutura social caracterizada pela transferência de fêmeas do grupo. Curiosamente, o comportamento dessas duas espécies do novo mundo é distinto com relação à organização social, com os muriquis sendo igualitários, e os macacos-aranha sendo hierárquicos e mais agressivos. Por esses motivos, essas são as espécies-chave para elucidar se o padrão de secreção de DHEAS com a idade está relacionado com a filogenia ou se está ligado ao comportamento dos primatas. Nesse contexto, tem-se destacado a necessidade de validar adequadamente os ensaios hormonais para a espécie que se deseja estudar (Heistermann; Palme; Ganswindt, 2006), principalmente para primatas neotropicais, para o monitoramento do estresse e nos programas de conservação em cativeiro e vida livre (Buti et al., 2018).

2. Justificativa

O projeto propôs analisar o padrão de secreção do DHEAS com a idade em espécies de primatas neotropicais, levando em consideração o sexo, estado de saúde e reprodução, por meio de análises hormonais, e exames clínicos e coproparasitológicos e o exame ultrassonográfico. Dessa forma, os métodos desenvolvidos irão fornecer um índice de monitoramento da resposta da adrenal ao estresse, que pode ser aplicado em outras espécies em vida livre ou em cativeiro. Outras contribuições desta proposta foram a validação de ensaios hormonais para espécies neotropicais em matrizes não invasivas, como as fezes, e a investigacao da expressão de enzimas ligadas a produção do DHEAS nos tecidos adrenais e recebrais de saguis, macaco rhesus, e humanos. Tais resultados servirão como base para outras pesquisas de monitoramento de estresse e senescência e estudos comparativos da evolução com os seres humanos.

3. Objetivo:

Objetivo geral

Investigar a função do DHEAS no desenvolvimento e no processo de senescência em primatas e estabelecer um novo índice de estresse por meio da razão entre dois hormônios adrenais (GC/DHEAS).

Objetivos específicos

- Validar o ensaio hormonal para detectar DHEAS em fezes de espécies de primatas neotropicais;
- Determinar a relação entre os níveis de DHEAS com a idade;
- Correlacionar o índice GC/DHEAS com o estado de saúde por meio exames clínicos, ultrassonográficos coproparasitológicos;
- Correlacionar o índice GC/DHEAS com estado reprodutivo (gestação, lactação e ciclo estral);
- Confecção de placas de ELISA para dosagem de DHEAS;
- Avaliar a expressão de enzimas referentes a produção de DHEAS em adrenal e cérebro de primatas;
- Contribuir com a formação e qualificação de recursos humanos para desenvolvimento de trabalhos na área de medicina e biologia de primatas neotropicais.

4. Material e métodos

O projeto foi sendo desenvolvido por meio de parcerias interinstitucionais entre a Universidade Federal Rural da Amazônia (UFRA/Belém, PA), o Centro Nacional de Primatas (CENP/Ananindeua, PA, Brasil), e mais 7 Zoológicos/Centros de pesquisas nacionais (Zoológico de Sorocaba, Fundação Parque Zoológico de São Paulo, Centro de Primatologia do Rio de Janeiro, Zoológico de Curitiba, Passeio Público, Zoo Pomerode e Zoo Itatiba) e a Kent State University (Kent, Ohio, USA). O projeto experimental seguiu as orientações contidas nas resoluções do Conselho Nacional de Controle de Experimentação Animal - Ministério da Ciência e Tecnologia (CONCEA-MCT, Brasil), sendo devidamente cadastrado no Sistema de Autorização e Informação em Biodiversidade (SISBio, protocolo 38529-7) e submetido com aprovação da Comissão de Ética no Uso de Animais (CEUA) do Instituto Evandro Chagas (IEC, protocolo 43/2019). Além disso, foi aprovado para coleta de amostras fecais de animais provenientes do Zoológico de Cleveland (Ohio, USA).

4.1. Atividades no Centro Nacional de Primatas (Brasil)

As amostras para análises laboratoriais (hormonais, hemograma e bioquímica) foram obtidas com o animal devidamente contido, realizados no laboratório de análises clínicas do CENP, no qual determinamos o perfil dos hormônios DHEAS e GCs em amostras de soro

sanguíneo. As concentrações de DHEAS foram medidas seguindo o protocolo descrito previamente em macacos japoneses (Takeshita et al. 2013). Os níveis de GC foram medidos de acordo com o método descrito por Mendonça et al. (2016) e Takeshita et al. (2018b). Os ensaios foram validados para cada espécie por meio de testes de precisão e paralelismo, nos quais se observa se as diluições são paralelas às curvas dos padrões de referência. A reprodutibilidade dos ensaios foi calculada determinando a variação inter e intra ensaio. Dessa forma foram realizadas as validações fisiológicas (desafio hormonal com DHEA oral ou hormônio adrenocorticotrófico - ACTH) e biológicas (coleta de fezes de animais gestantes, não gestantes e lactantes) dos ensaios com as amostras dos animais provenientes do CENP. Observamos aumento dos níveis de DHEAS após o desafio hormonal, e aumento desse hormônio nas fêmeas gestantes em comparação com fêmeas não gestantes e lactantes, conforme descrito para macacos do velho mundo (Takeshita et al. 2016).

4.2. Atividades no Departamento de Antropologia, Laboratório da dosagens hormonais (Kent State University, USA)

As atividades foram desenvolvidas em dois em dois laboratórios: Laboratório de Endocrinologia Comportamental do Departamento de Antropologia, sob orientação da Dra Rafaela Takeshita, e no laboratório de Fisiologia Reprodutiva do Departamento de Biologia, sob supervisão do Dr. Wilson Chung. No laboratório da dra Takeshita pude aprimorar as técnicas em ELISA, prepararando ensaios internos para medir hormônios esteróides de amostras biológicas. Dentre as espécies utilizadas para validação no Brasil, o *Alouatta caraya* apresentou maiores concentrações de DHEAS, comparáveis a espécies do Catarrinas. Por esse motivo, foram colhidas amostras fecais adicionais desta espécie, de animais provenientes do Zoológico de Cleveland (Ohio, USA). As amostras foram colhidas pelos funcionários do Zoológico, congeladas e enviadas à Kent State University (KSU). As amostras foram processadas (secagem, pesagem) e dosadas por enzimaimunoensaio no Laboratório da Dra Rafaela Takeshita. Além disso, aprendi como solucionar problemas de ensaio, otimizando um dos ensaios internos da Dra Takeshita e validei dois ensaios para medição de hormônios esteróides em saliva humana

4.2. Atividades no Departamento de Ciências Biológicas (Kent State University, USA)

No laboratório do Dr. Chung, aprendi técnicas de biologia molecular, incluindo extração de RNA, design de primers, análises de PCR e qPCT da glândula adrenal e tecido cerebral congelados. A partir disso, compararei a expressão de enzimas esteroidogênicas em cinco regiões cerebrais e nas adrenais de três espécies de primatas (sagui, macaco rhesus e humanos). Os resultados parciais sugerem diferentes padrões de expressão dessas enzimas nas diferentes espécies avaliadas, que podem estar relacionados às variações das concentrações de DHEAS e o papel desse hormônio na evolução e adaptação dessas espécies às suas respectivas sociedades e ambiente.

4.3. Análises estatísticas

Os resultados obtidos dos exames clínicos, laboratoriais e ultrassonográficos foram inseridos em planilhas Microsoft Excel, levando-se em consideração a idade, o sexo, e o estado reprodutivo (no caso das fêmeas) e saúde e correlacionados com os valores hormonais. Serão construídos modelos lineares (Generalized Linear Mixed-Effect Model), para correlacionar os níveis hormonais com fatores fixos, como a idade, estado reprodutivo, estado de saúde (nível de parasitas e exames clínicos e ultrassonográficos) e morfologia das adrenais (volume/massa corporal). Para evitar pseudo-replicação devido à múltiplas amostras de um mesmo indivíduo, o modelo permite a inclusão de sujeito ID como fator aleatório. Os fatores fixos foram removidos sistematicamente de forma a comparar qual modelo representa melhor os fatores que influenciam os níveis hormonais, através do Akaike Index Criterion (AIC). O modelo final será selecionado com base no menor valor de AIC (Burham and Anderson 2002).

5. Produtos gerados

Os resultados preliminares das atividades da pesquisa de na KSU foram apresentados no Simpósio de Neurociências (The 2022 Neuroscience Symposium) promovido pelo Brain Health Research Institute (BHRI) (Kent State University, Ohio), com apresentação do pôster "Comparative expression of steroidogenic genes in primate brain", e foi premiado como 2º melhor pôster na categoria Pós Graduação (certificado em anexo). Além disso, participei na conferência Midwest Primate Interest Group (MPIG 2022, Ann Arbor University, Michigan), com apresentação oral do trabalho "Effect of age and sex in renal function by ultrasound and serum chemistry in two primate species (*Alouatta caraya* and *Sapajus apella*)".

Outros resultados desta parceria foram artigos os científicos, sendo quatro capítulos da minha tese, publicados em periódicos internacionais: 1) Female squirrel monkeys as models

for research on women's pelvic floor disorders (https://doi.org/10.1177/00236772211032506). 2) "Validation of a Dehydroepiandrosterone-Sulfate assay in three platyrrhine primates (*Alouatta caraya, Aotus azarae infulatus,* and *Sapajus apella*)" (https://doi.org/10.1007/s10764-021-00239-x). 3) "Effect of age and sex in renal function by ultrasound and serum chemistry in two primate species (*Alouatta caraya* and *Sapajus* apella)" (https://doi.org/10.1111/jmp.12599). 3) Hematological and serum biochemistry evaluation in howler monkeys (*Alouatta caraya)* and capuchin monkeys (*Sapajus apella*): a comparative study (https://doi.org/10.1111/jmp.12644). Em parceria com a Dra Takeshita e sua orientada de doutorado na KSU, Emilee Hart submetemos o artigo 5) "Dehydroepiandrosterone and dehydroepiandrosterone-sulfate: biomarkers of pregnancy and of fetal health" no periódico Theriogenoly wild (encontra-se em processo de revisão pela revista).

5. Cronograma

Abaixo, segue a atualização do cronograma apresentado à Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) por ocasião da candidatura ao Doutorado Sanduíche no exterior a ser desenvolvido na Kent State University, Ohio, Estados Unidos, por 12 meses (✓ para atividades já realizadas).

	2019		**2020**		**2021**		**2022**		**2023**
País de estadia	**Brasil**	**Brasil**	**Brasil**	**Brasil**	**Brasil**	**Brasil**	**Estados Unidos**	**Estados Unidos**	**Brasil**
Atividades	**Mar-Jun**	**Jul-Dez**	**Jan-Jun**	**Jul-Dez**	**Jan-Mar**	**Abr-Nov**	**Jan-Jun**	**Jul-Nov**	**Jan-Ago**
Curso dos créditos do programa	✓	✓	✓						
Seleção e manejo dos animais		✓	✓						
Coleta das amostras e exames clínicos/ultrassonográficos			✓						
Análise dos resultados parciais				✓	✓				
Redação e submissão de artigos/resumos iniciais						✓			
Exame de Qualificação						✓			
Treinamento em desenvolvimento de ensaios imunoenzimáticos para quantificação de hormônios esteroidais							✓		
Participação em projetos locais (treinamento com dosagem hormonal e de marcadores genéticos em tecido nervoso de primatas, que permite a comparação entre níveis hormonais periféricos e centrais; e expansão da técnica para							✓		

avaliação do tecido das glândulas adrenais									
Participação nas aulas da disciplina de antropologia de forma a auxiliar a supervisora e aprimorar os conhecimentos e desenvolver habilidades docentes, bem como aprimorar a fala e escrita na língua inglesa							✓	✓	
Redação e submissão de artigos/resumos referentes aos dados gerados durante a viagem, em periódicos internacionais									✓
Defesa da tese									✓

7. Considerações finais

O projeto desenvolvido durante o Doutorado Sanduíche foi de suma importância para o aprofundamento de conhecimentos relacionados à morfofisiologia de primatas, especialmente a neuroendocrinologia. A supervisora no exterior, e pesquisadora e atual Professora do Departamento de Antropologia da Kent State University (Ohio/EUA), Dra. Rafaela Takeshita, com sua ampla experiência com a temática proposta e teve papel fundamental na supervisão e realização do projeto. O laboratório da Dra. Takeshita desenvolve os próprios kits para ensaios hormonais, o que reduz os custos das análises em 90%, O departamento de Antropologia ainda dispõe de equipamentos de ponta para estudos nas áreas de genética e neurobiológica, voltados para o estudo da evolução humana. Essa parceria além de garantir que as análises hormonais fossem realizadas adequadamente, contribuíram, para a expansão da capacidade de pesquisa nacional, visto que as técnicas de confecção de kits para ensaios hormonais poderão ser posteriormente desenvolvidas no CENP, fatores que contribuem para abrir novas oportunidades para futuros projetos de pesquisas no Brasil.

Além disso, essa oportunidade do doutorado sanduíche contribuiu grandemente para o meu desenvolvimento profissional, uma vez que participei de outros projetos de pesquisa em andamento na instituição, incluindo processamento e dosagens hormonais em tecido nervoso de primatas, no qual interagi com diversos discentes e docentes da KSU e de outras instituições. A participação em conferências permitiu a apresentação dos trabalhos que vêm sendo desenvolvidos e os resultados frutos dessa parceria Brasil-Estados Unidos, fortalecendo pesquisas já desenvolvidas e abriu novas possibilidades com possíveis propostas para o Pós Doutorado. Esse período contribuiu ainda para o aprimoramento de habilidades de comunicação em outro idioma, ampliando a visão da pesquisa e cultura.

8. Agradecimentos

A Dra. Rafaela Takeshita pela orientação, supervisão e incentivo durante todo esse período. Meus especiais agradecimentos ao Dr. Wilson Chung pela supervisão e ensino durante as atividades no Departamento de Ciências Biológicas. Aos graduandos e Pós graduandos da KSU. Ao orientador no Brasil Dr. Frederico Monteiro por todo auxílio pré, durante e após o estágio. A toda a equipe da CAPES pela concessão da oportunidade do doutorado sanduíche no exterior e auxílio durante esse processo.

9. Referência

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KENT STATE
UNIVERSITY

April 6, 2023

To whom it may concern,

It is with great pleasure that I write this letter to report Gessiane Pereira da Silva's accomplishments during her visit at Kent State university. Gessi worked in two laboratories: The Behavioral Endocrinology laboratory at the Department of Anthropology, under my supervision, and the Reproductive Physiology laboratory at the Department of Biology, under Dr. Wilson Chung's supervision. In my laboratory, she enhanced her techniques on ELISA by learning how to prepare our in-house assays to measure steroid hormones from biological samples. As part of her PhD dissertation, she measured DHEAS levels from fecal samples in howler monkeys from local zoos. Moreover, she learned how to troubleshoot assay problems, optimized one of our in-house assays, and validated two assays for measurement of steroid hormones in human saliva.

At Dr. Chung's laboratory, Gessi learned molecular biology techniques, including RNA extraction, PCR and qPCT analyses from adrenal gland and brain tissue. She compared the expression of steroidogenic enzymes in five brain regions from three primate species (marmoset, rhesus macaque, and humans). She presented the preliminary results at the Neuroscience symposium organized by the Brain Health Research Institute and was awarded 2nd place in her category! In addition, Gessi presented her published work Midwest Primate Interest Group (Ann Arbor, MI) and was praised by the judges by her confidence and ability to present an academic study in a non-native language.

Gessiane demonstrated that she works well in a team. She collaborated on a study about the effect of cyanotoxins in the HPA axis in mice, and taught other students with the techniques that she learned. While being involved in multiple research projects, Gessi continued working on manuscripts for publication and audited in several classes, including *Neuroendocrinology*, and a few classes from my course *Principles of Biological Anthropology*. She always stood out in class by asking interesting questions. Despite being involved in numerous activities, Gessi managed her time extremely well. She always kept her research responsibilities in first place, treated everyone very respectfully, and treated laboratory and office spaces as if it were her own. By the end of her visit, she did not hesitate in train other students with the techniques that she learned, so that we will be able to expand this project to other species.

Soon after she left the U.S, she resumed our collaborative activities in Brazil by carrying out hormonal analyses, data collection, and manuscript writing. We have

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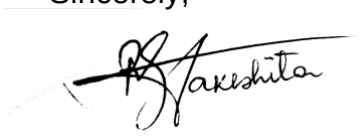
submitted two manuscripts for publication since she concluded her visit, and one of them has already been accepted!

In summary, Gessiane Pereira da Silva exceeded my expectations during her visit. She demonstrated to be a bright, hardworking scientist, and she is well-liked among her peers and mentors for her dedication, compassion towards helping others, and for her good sense of humor! She was featured in the Anthropology department's news due to her impressive accomplishments. I am thankful to CAPES for the opportunity given to Gessi, and I am certain that she will continue to succeed in the scientific community.

Sincerely,



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MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA
PROGRAMA DE PÓS-GRADUAÇÃO EM SAÚDE E PRODUÇÃO ANIMAL NA AMAZÔNIA

PARECER DO ORIENTADOR SOBRE OS TRABALHOS DESENVOLVIDOS NO EXTERIOR

Na qualidade de orientador do projeto intitulado **"MONITORAMENTO DA SENESCÊNCIA E DE NÍVEIS DE ESTRESSE EM PRIMATAS NEOTROPICAIS"**, eu, Frederico Ozanan Barros Monteiro, docente permanente do Programa de Pós-graduação em Saúde e Produção Animal na Amazônia da Universidade Federal Rural da Amazônia (PPGSPAA - UFRA), venho por meio deste apresentar o parecer referente às atividades de pesquisa realizadas pela doutoranda Gessiane Pereira da Silva (matrícula 2019100274), que resultou da concessão de bolsa de doutorado Sanduíche no Exterior por meio do Projeto Procad Amazônia/CAPES processo nº 21/2018), pelo período de vigência total de 10 meses (março 2022/ dezembro 2022).

Desde o mestrado Gessiane demonstrou a capacidade de ser uma líder natural, compartilhando responsabilidades com membros do nosso grupo de pesquisa. Ao fazer isso, ela alcançou seus objetivos e recebeu importantes elogios dos professores de nossa instituição. Ainda durante o mestrado publicou artigo referente a sua dissertação intitulado "Fetal bone development in the lowland paca (*Cuniculus paca*, Rodentia, Cuniculidae) determined using ultrasonography" no renomado periódico Journal of Anatomy (https://onlinelibrary.wiley.com/doi/10.1111/joa.13184).

A bolsista tem desenvolvido, de forma excepcional, todas as atividades planejadas desde o início de seu doutorado, em março de 2019. Gessiane tem se dedicado arduamente a responder questões relacionadas à área da primatologia. Durante as atividades no Brasil e no exterior, a discente tem apresentado excelentes habilidades como pesquisadora: capacidade intelectual, motivação para estudos avançados, curiosidade, proatividade, responsabilidade e espírito de trabalho em equipe. Dessa forma, isso a qualificou para ser selecionada para bolsa de doutorado sanduíche na "Kent State University - KSU" Ohio, EUA, sob a supervisão da professora Rafaela S. C. Takeshita. Em 49 meses de doutorado, incluídos aproximadamente 18 meses de Pandemia de COVID19, a discente produziu 3 artigos científicos e possui um artigo em submissão, sendo todos parte integrante da sua tese de doutorado, conforme listado as seguir:

1. Female squirrel monkeys as models for research on women's pelvic floor disorders. https://journals.sagepub.com/doi/10.1177/00236772211032506. (2021);
2. Effect of age and sex in renal function by ultrasound and serum chemistry in two primate species (Alouatta caraya and Sapajus apella). https://onlinelibrary.wiley.com/doi/10.1111/jmp.12599; (2022);
3. Validation of a Dehydroepiandrosterone-Sulfate Assay in Three Platyrrhine Primates (*Alouatta caraya*, *Aotus azarae infulatus*, and *Sapajus apella*). https://link.springer.com/article/10.1007/s10764-021-00239-x. (2022);
4. Hematological and serum biochemistry evaluation in howler monkeys (*Alouatta caraya*) and capuchin monkeys (*Sapajus apella*): a comparative study. (2023, em submissão ao Periódico Journal of Medical Primatology).

As atividades desenvolvidas no exterior incluíram treinamento laboratorial com discentes (graduandos e pós-graduandos) e docentes da KSU. Foi realizado o aperfeiçoamento e treinamento em técnicas de ensaio imunoenzimáticos, RT-PCR, preparação de kits imunoenzimáticos para análises hormonais e coleta/processamento de amostras de primatas não humanos em zoológicos Norte Americanos. As análises dessas ações permitirão compreender melhor os aspectos abordados na tese de doutorado da discente. Além disso, a discente participou de simpósios e congressos no exterior, apresentado de forma oral os resultados do projeto de Doutorado "Effect of age and sex in renal function by ultrasound and serum chemistry in two primate species (*Alouatta caraya* and *Sapajus apella*)" no evento internacional do Midwest Primate Interest Group (MPIG 2022, Ann Arbor University, Michigan) e recebido premiação de segunda melhor apresentação com o pôster "Comparative expression of steroidogenic genes in primate brain" no evento

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The 2022 Neuroscience Symposium promovido pelo Brain Health Research Institute (BHRI) (Kent State University, Ohio) na categoria Pós Graduação, com os resultados preliminares do projeto realizado na KSU. Portanto, acreditamos que a concessão da bolsa DS foi de grande valia para o implemento das ações de pesquisa desenvolvidas pela discente e para o implemento da produção científica discente em nosso PPG. Além disso, a bolsista, juntamente com a sua supervisora no exterior têm se dedicado a responder questões relacionadas à temática da endocrinologia em primatas não humanos. Isso se reflete na busca por recursos financeiros no exterior e no Brasil para estender o projeto para outras espécies de mamíferos. Tais ações têm sido realizadas em parceria com instituições internacionais tais como as universidades norte-americanas (Kent State University e University of Wisconsin-Madison, EUA). Vale ressaltar que essas iniciativas se encontram vigentes por meio de colaborações do PPGSPAA / UFRA e outras instituições de pesquisa, a exemplo do Centro Nacional de Primatas (CENP, Ananindeua, Pará) e diversos zoológicos brasileiros nos estados de São Paulo, Paraná, Rio de Janeiro e Pará. Sendo assim, agradecemos antecipadamente à CAPES a aprovação do nosso pleito e nos colocamos à disposição para eventuais esclarecimentos.

Atenciosamente,

Frederico Ozanan Barros Monteiro
Orientador da discente no Brasil
Bolsista de produtividade em pesquisa (PQ-1D)
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BRAIN HEALTH
RESEARCH INSTITUTE
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Graduate Student Poster Presentation

Honorable Mention

10th Annual Neuroscience Symposium

Kent State's Contributions to Neuroscience: Past, Present & Future

Gessiane Pereira Silva

October 27-28, 2022

Dr. Michael Lehman
Director, Brain Health Research Institute