MINISTÉRIO DA EDUCAÇÃO
UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA - UFRA
DOUTORADO EM SAÚDE E MEIO AMBIENTE

RAFAEL DOS SANTOS DE ANDRADE

ASPECTOS RELACIONADOS À ALTRICIALIDADE E PRECOCIALIDADE EM MACACOS BARRIGUDO (*Lagothrix poepigii*) E QUEIXADAS (*Tayassu pecari*)

BELÉM
2018

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Tese apresentada à Universidade Federal Rural da Amazônia, como parte das exigências do Curso de Doutorado em Saúde e Produção Animal na Amazõnia: área de concentração: Saúde e Meio Ambiente, para obtenção do título de Doutor.

Orientador: Prof. Dr. Frederico Ozanan Barros Monteiro
Co-Orientador: Prof. Dr. Pedro Mayor Aparício

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Aprovado em___de_________de 2018.

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“Não basta ensinar ao homem uma especialidade. Porque se tornará assim uma máquina utilizável, mas não uma personalidade. É necessário que adquira um sentimento, um senso prático daquilo que vale a pena ser empreendido, daquilo que é belo, do que é moralmente correto.”

Albert Einstein

AGRADECIMENTOS

Agradeço a minha namorada/noiva/esposa/companheira Rianne, por ser meu amparo nos momentos de aflição, pelas palavras de ânimo, pelo consolo e abraço. Agradeço a minha família, meus pais Joaquim e Dinair, e irmãos Aline e Diogo, por sempre me fazer lembrar sobre o valor da educação, da humildade, da paciência e perseverança, absolutamente nada em minha vida seria possível sem os ensinamentos e valores adquiridos com vocês.

Aos meus sogros, seu Ariel e minha sogra dona Rosely, pelas orações e pedidos de iluminação (acho que deram certo), minha cunhada Rielli pelas visitas e conversas.

A equipe que fez parte desse trabalho, na figura de bolsistas ou voluntários, Thyago Habner (agora é mestrando), Sandy Matos, Laryssa Borges e Gessy Silva.

Agradeço especialmente ao Hani El Bizri, Prof Frederico Ozanam e Pedro Mayor, pela oportunidade, pela paciência e por nunca desistir de corrigir meus manuscritos longuíssimos.

Aos comunitários das comunidades de Nueva Esperanza (Peru), Nova Jerusalém, Boa Esperança, Bom Jesus do Baré, São José do Urini e Belo Monte (Brasil), por todo empenho e esforço dedicado na coleta e doação de amostras e dados para as pesquisas.

Agradeço profundamente a todos que ajudaram a tornar este trabalho possível, de forma direta ou indireta, a todos que dedicaram seus esforços em meio ao desamparo estrutural e financeiro pelo qual passa a ciência no Brasil.

Aos meus filhos queridos de quatro patas, companheiros nas madrugadas a fio, Vic, Mozart, Frederico, White, e ao Darwin.

À Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES [protocolo 452908/2016-7], pela bolsa fornecida e apoio financeiro, ao Programa de Pós-graduação em Saúde e Produção Animal na Amazônia – PPGSPAA, pela oportunidade de aprendizado e confiança.

A Deus.

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RESUMO

Macacos-barrigudo (*Lagothrix* sp.) e queixadas (*Tayassu pecari*) sofrem alta pressão de caça na região Amazônica e apresentam desenvolvimento fetal diferenciado, classificando-os como altriciais (maior dependência parental) e precociais (menor dependência parental), respectivamente. Barrigudos organizam-se em grupos heterossexuais coesos, multi-macho/multi-fêmeas com 20-40 indivíduos; são frugívoros, dispersores de sementes, com ceco e cólon desenvolvidos; o ciclo ovariano dura 21 dias e gestação cerca de 225 dias, com média de 1 filhote/parto, intervalo entre partos de 22.3-25.2 meses e amamentação de 12-24 meses. Queixadas formam bandos com 25-130 indivíduos, alimentam-se de raízes, frutas e caules, com ingestão ocasional de artrópodes e anelídeos, possuem estômago multicavitário fermentador; não apresentam sazonalidade reprodutiva em vida livre na Amazônia, mas parem no período chuvoso; gestação entre 156-162 dias, média de 1.60-1.67 filhotes e intervalo entre partos de 250-253 dias. Barrigudos possuem desenvolvimento fetal mais lento quando comparado aos queixadas (velocidade de crescimento fetal a = 0. 042 e a = 0.084, respectivamente), permitindo que os neonatos de queixadas possuam maior autonomia em relação aos cuidados parentais. Os barrigudos, apesar de produzirem neonatos com maior dependência, contam com o alto investimento parental no período pós-natal para garantir a sobrevivência da prole. Fetos de barrigudos apresentam maior desenvolvimento visceral em relação aos queixadas quando comparados os volumes relativos de órgãos em fetos e animais adultos. As informações contidas nesta revisão contribuem para melhorar o entendimento das estratégias reprodutivas adotadas por essas espécies e fornecem dados que auxiliam o manejo reprodutivo destas espécies.

Palavras-chave: Mamíferos, desenvolvimento fetal, região Amazônica

ABSTRACT

Woolly monkeys (*Lagothrix sp.*) and whitte-lipped peccary (*Tayassu pecari*) suffer from high hunting pressure in the Amazon region and present differentiated fetal development, classifying them as altricial (greater parental dependency) and precocial (less parental dependence), respectively. Woolly monkeys are organized into cohesive, multi-male / multi-female heterosexual groups with 20-40 individuals; are frugivorous, seed dispersers, with developed cecum and colon; the ovarian cycle lasts for 21 days and gestation for about 225 days, with a mean of 1 pup / calving, a calving interval of 22.3-25.2 months and a calving of 12-24 months. whitte-lipped peccary form flocks with 25-130 individuals, feeding on roots, fruits and stems, with occasional ingestion of arthropods and annelids, have a multicavity fermenting stomach; do not present reproductive seasonality in the Amazon, but stop in the rainy season; gestation between 156-162 days, mean of 1.60-1.67 pups and interval between births of 250-253 days. Woolly monkeys have slower fetal development when compared to whitte-lipped peccary (fetal growth rate a = 0. 042 and a = 0.084, respectively), allowing the newborns of whitte-lipped peccary to have greater autonomy regarding parental care. The potbellied, despite producing more dependent neonates, rely on the high parental investment in the postnatal period to ensure the survival of the offspring. Bellied fetuses show greater visceral development compared to jaws when comparing the relative volumes of organs in fetuses and adult animals. The information contained in this review contributes to improve the understanding of the reproductive strategies adopted by these species and provides data that support the reproductive management of these species.

Keywords: Mammals, fetal development, Amazon region

INTRODUÇÃO

Os macacos barrigudos (*Lagothrix poepigii*) e queixadas (*Tayassu pecari*) são altamente sofrem grande pressão antrópica dentro do bioma amazônico, e isso tem contribuído para redução das populações de vida livre[1,2]. Dentro desse tema, a caça de subsistência é de extrema importância para as comunidades tradicionais que habitam a região Amazônica, onde é permitida nessa região no Peru e Brasil (no Brasil, somente como recurso alimentar de subsistência, sendo proibida em outras modalidades, como comércio ou criação sem devida legalização)[3]. Os estudos de sustentabilidade de caça são imprescindíveis para avaliar os parâmetros reprodutivos de espécies cinegéticas, e utiliza a taxa intrínseca de incremento natural de populações ($r_{max,}$), como forma de avaliar o desempenho reprodutivo e a capacidade de resposta desses animais a pressão de caça[3,4].

Evolutivamente, barrigudos e queixadas apresentam desenvolvimento gestacional e estratégias reprodutivas diferenciadas, que permitem classificá-los como altriciais e precociais, respectivamente[5,6]. Essa classificação, no entanto consiste em uma forma simplificada de caracterizar o desenvolvimento fetal nos mamíferos, e apresenta diferenças entre ordens, gêneros e espécies que podem se contradizer, de acordo com a espécie estudada[7,8]. Fatores como massa corporal nos adultos, peso ao nascimento, tamanho da ninhada e duração da gestação são as características mais utilizadas para essa classificação, no entanto, observa-se que o grau de independência neonatal e o desenvolvimento de parâmetros fetais são mais eficientes para diferenciação de espécies entre altriciais e precociais[5,6,8]. Os primatas considerados como altriciais produzem crias com alta dependência materna, pouco preparo para a vida extrauterina, com gestações longas que culminam em pequeno número de descendentes e maior gasto energético materno. Em contrapartida os queixadas, quando comparados aos barrigudos, apresentam tempo gestacional mais curto, mais filhotes por gestação e que necessitam de menor cuidado parental[5,6,9,10].

Boa parte da literatura disponível sobre o desenvolvimento gestacional em primatas e pecaris está baseada no monitoramento ultrassonográfico da gestação. Essa técnica tem sido muito útil, principalmente por sua capacidade de gerar informações em tempo real. Entretanto, quando isso não é possível, a biometria realizada em embriões e fetos oriundos de fêmeas que foram caçadas produz informações altamente relevantes para compreensão dos eventos relacionados à gestação. Assim, os trabalhos conduzidos por meio do manejo colaborativo com comunidades, que realizam a caça de subsistência resultam em oportunidades únicas para desenvolver estudos voltados para conservação de diversas espécies selvagens, tais como os realizados por Gottdenker & Bodmer[10], Mayor etal[4,11,12,13,14], El Bizri et al[15] e Andrade et al[5,6]). Esse material tem sido útil para compreender as estratégias evolutivas e adaptativas utilizadas para garantir o sucesso reprodutivo e sobrevivência dessas espécies, aproveitando importantes recursos biológicos que de outro modo seriam descartados, sem a necessidade de abate para fins científicos através do compartilhamento de saberes com comunidades tradicionais que realizam a caça de subsistência. Além disso, fornecem parâmetros importantes para o manejo clínico e reprodutivo de animais silvestres em cativeiro, contribuindo para a conservação *ex situ* e *in situ* desses animais. Sendo assim, a presente revisão objetivou contextualizar aspectos relacionados às características altriciais e precociais do desenvolvimento gestacional de macacos barrigudos e queixadas.

MACACO BARRIGUDO (*Lagothrix* sp.)

O macaco barrigudo pertence à subordem Haplorrhini, família Atelinae e subfamília Atelidae, que compreende os gêneros *Ateles*, *Brachyteles*, *Oreonax* e *Lagothrix*, é um dos maiores primatas neotropicais (adultos pesam aproximadamente 8-10 kg) junto com os primatas do gênero *Alouatta*, *Ateles* e *Brachyteles*[16]. Possui membros torácicos mais longos que os membros pélvicos, cauda preênsil que mede mais que o comprimento do corpo e cabeça juntos, e que possui em sua extremidade modificações anatômicas que facilitam a aderência enquanto de deslocam[17]. O gênero se subdivide em quatro espécies (*L. cana*, *L. lagotricha*, *L. lugens* e *L. poeppigii*), todas endêmicas da região amazônica, distribuídas entre Brasil, Equador, Peru e Colômbia[18] (FIGURA 1).

FIGURA 1: Mapa de distribuição geográfica do barrigudo (*L. poeppigii*), em laranja, área de ocorrência da espécie dentro da região Amazônica.

Adaptado de: © *The IUCN Red List of Threatened Species: Lagothrix poeppigii – published in 2008. http://dx.doi.org/10.2305/IUCN.UK.2008.RLTS.T39927A10290256.en*

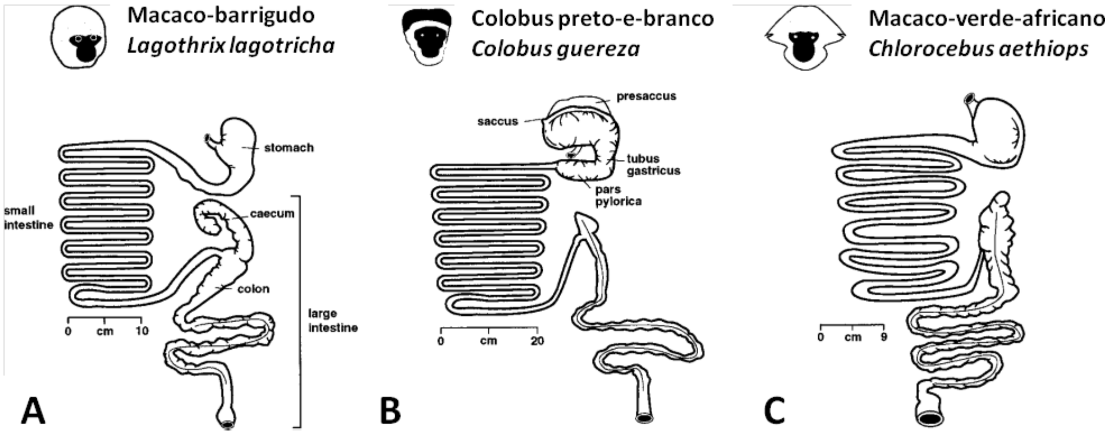
Quando adultos, os barrigudos apresentam pelagem densa como “lã”, com coloração entre as espécies variando de cinza-claro a marrom-escuro, com alguns tons de negro[17]. A espécie *L. poeppigii* apresenta coloração tipicamente avermelhada ou marrom-avermelhada, com cabeça e extremidades apresentando coloração marrom-escuro[17]. No Brasil, os barrigudos ocorrem nos estados do Acre e Amazonas, desde o sul do rio Solimões ao oeste do rio Juruá. Estende-se também ao oeste da Amazônia peruana, Equador até os Andes, a uma altitude máxima de 1800 m. Ocupam apenas áreas de floresta primária, não tolerando presença ou distúrbios antrópicos[18].

De acordo com a IUCN (International Union for Conservation of Nature), o declínio populacional do gênero *Lagothrix* está relacionado principalmente ao aumento da atividade e da presença humana nas áreas de ocupação desses animais (agricultura, mineração, abertura de estradase construções de habitações), considerados ameaçados, com níveis variados de ameaça de acordo com cada espécie[18]. No Brasil, à exceção da espécie *L. cana* que é classificada como “em perigo”, o gênero *Lagothrix* apresenta predição de redução populacional em torno de 30% da população total em três gerações (45 anos). Suspeita-se que a área de ocupação desses animais no Brasil é menor que sua extensão de distribuição, devido a isso foram caracterizados como “vulnerável a extinção”[19].

Os barrigudos possuem hábitos alimentares principalmente frugívoros, mas dependendo da oferta de alimentos podem consumir grandes quantidades de artrópodes[20]. Na natureza, exercem importante função como dispersores de sementes, responsáveis por até 33% da biomassa dispersadas dentro das áreas onde habitam, aumentando consideravelmente a diversidade florestal ao longo de toda a extensão onde estão distribuídos[21].

Os primatas não humanos apresentam grande diversidade de adaptações no trato digestório (estômago, ceco e cólon) que permitem a eles a ingestão de tipos diferentes de dietas, de acordo com o hábito alimentar de cada espécie[22,23]. Durante muitos anos, acreditou-se que os primatas eram ineficientes em realizar a fermentação de carboidratos complexos, no entanto atualmente sabe-se que intensa atividade microbiana ocorre em porções do ceco e cólon na maioria das espécies de primatas, e que essas são responsáveis pela fermentação e produção de ácidos orgânicos essenciais para a digestão de material vegetal[23] (Figura 2).

FIGURA 2: Diferenças entre trato gastrintestinal de (A) macaco-barrigudo – *Lagothrix lagothricha* - apresentam estômago simples e ceco e cólon desenvolvidos; (B) colobus preto-e-branco – *Colobus guereza* – apresentam estômago multicavitário e ceco menos desenvolvido, e (C) macaco-verde-africano – *Chlorocebus aethiops* – apresenta estômago simples e ceco e cólon desenvolvidos.



Adaptado de: Lambert et al 1998.

O bugio-de-manta (*Alouatta palliata)*, primata quase exclusivamente folívoro, e que pertence à mesma família dos barrigudos está entre os mamíferos que apresentam as maiores taxas de fermentação fecal, contribuindo com 31% da energia de manutenção diária desses animais[24]. O tipo de dieta nos primatas influencia diretamente o tempo de trânsito intestinal, diferente de outros mamíferos herbívoros em que a massa corporal é o principal influenciador. Desse modo, existe baixa relação significativa entre massa corporal e tempo de transito intestinal nesses animais, assim, primatas com massa aproximada podem apresentar diferenças entre o tempo de transito intestinal de acordo com a dieta, como nos barrigudos (frugívoro) que apresenta transito intestinal rápido (6.5 horas em média), enquanto bugios (folívoro), apresentam transito lento, com média de 20-35 horas[23].

Em vida livre, os barrigudos se organizam em grupos heterossexuais coesos, multi-machos/multi-fêmeas, com 20-40 indivíduos. Os grupos geralmente encontram-se dispersos ao longo das áreas ocupadas, dessa forma, a vocalização representa importante meio de comunicação entre diferentes grupos desses animais[20].

O dimorfismo sexual dentro do gênero *Lagothrix* é muito pequeno, e está relacionado com as diferenças entre as proporções corporais (machos apresentam crânio e comprimento corporal maiores do que fêmeas, enquanto as fêmeas apresentam caudas mais longas que os machos) e desenvolvimento dos pelos de cobertura (machos apresentam pelos ventrais mais escuros, longos e espessos do que fêmeas)[17]. Os atelídeos, incluindo os barrigudos, apresentam fases pré-reprodutivas longas em vida livre, com os machos iniciando o comportamento sexual aos 3.5 anos, enquanto as fêmeas iniciam a reprodução por volta dos 9 anos[25]. Em cativeiro, ambos machos e fêmeas atingem a maturidade sexual em torno de 4-5 anos[9,26].

Poucos dados sobre a reprodução em vida livre estão disponíveis. Mayor et al[13,14], realizaram a descrição anatômica e histológica do trato reprodutivo de 60 fêmeas de macaco-barrigudo de vida livre em diferentes estágios reprodutivos, oriundas da caça de subsistência na Amazônia peruana, enquanto Bowler et al[27], também em estudo com animais de caça na mesma região, buscaram estabelecer parâmetros reprodutivos e a taxa reprodutiva para fêmeas de vida livre. Sabe-se que nas fêmeas o ciclo ovariano possui duração de 21 dias[20]. Em fêmeas não prenhes durante a fase folicular, observa-se aumento do endométrio, das glândulas endometriais e do miométrio, relacionado ao crescimento folicular. Durante a fase luteal em fêmeas não prenhes, observa-se grande proliferação endometrial, das glândulas e do miométrio quando comparado ao período folicular[13]. Quando ocorre a ovulação, o folículo ovulado sofre processo de luteinização, transformando-se em corpo lúteo funcional, e quando há fecundação do oócito, o corpo lúteo pode alcançar duas vezes o diâmetro de um folículo antral; com o avanço da gestação ocorre a diminuição progressiva do volume luteal [14]. As fêmeas apresentam diferentes características do epitélio vaginal de acordo com o estado reprodutivo, desse modo, a citologia vaginal pode auxiliar a caracterizar o ciclo estral nessa espécie[13]. Estudos recentes realizados em quatro espécies de primatas neotropicais mostraram que o *L. poeppigii*, não apresentou descolamento da camada funcional do endométrio, sugerindo que esta espécie apresenta um ciclo não menstrual[28].

O período gestacional é longo (cerca de 225 dias), com média de um filhote por gestação que nascem com peso proporcional a 10% do peso materno[29]. Durante a gestação ocorre grande produção de muco cervical, que se acumula no canal endocervical e forma barreira contra a entrada de micro-organismos no interior do útero. Ondas foliculares ocorrem até o final da gestação, e os folículos dominantes não alcançam o diâmetro máximo dos folículos pré-ovulatórios. Alguns folículos não ovulados passam por processo de luteinização, transformando-se em corpos lúteos acessórios, que contribuem com até 7% do volume luteal produzido durante a gestação[14]. O intervalo entre partos varia de 22.3-25.2 meses, período de amamentação entre 12-24 meses e taxa reprodutiva de 0.48-0.54 filhote por fêmea, por ano[26,27].

Em animais mantidos em cativeiro, o manejo reprodutivo apresenta obstáculos devido ao longo intervalo entre as gestações, longo tempo de maturação sexual dos indivíduos e das baixas taxas de natalidade[29]. No entanto, apesar das dificuldades, grande parte dos dados reprodutivos disponíveis para a espécie é oriunda de nascimentos em santuários ou parques zoológicos. Sucesso reprodutivo e nascimento de filhotes foram relatados no National Zoological Park, em 1979, no entanto, com poucos animais nascidos[26]. Mooney & Lee[29], analisando dados reprodutivos de zoológicos na Europa e América do Norte também referem poucos episódios de nascimento. Barnes & Cronin[30], relatam o nascimento em cativeiro de 12 indivíduos de *L. lagotricha*, dos quais 3 infantes nascidos entre 2006 e 2008 precisaram ser removidos e criados artificialmente, devido a incapacidade da mãe em manter os filhotes. Os autores relatam ainda a falta de dados publicados de nascimentos em outros zoológicos, o que dificulta mais os estudos reprodutivos sobre esses animais.

Em 1998, o "*European Endangered Species Program"* (EEP), demonstrou que a população de macacos-barrigudos em parques zoológicos na Europa diminuiu em 16% naquele ano, devido às limitações da importação de animais silvestres e aos problemas associados com a reproduçã[31]. Até o ano de 2010, a população desses animais era de 99 indivíduos distribuídos em 20 zoológicos europeus, dos quais apenas 10 integravam programas de reprodução[30]. Além da baixa reprodução, os barrigudos são susceptíveis a muitas enfermidades em cativeiro. As causas mais comuns de óbito incluem complicações na gravidez e condições relacionadas à hipertensão[31], além da ocorrência de endoparasitoses, doenças virais como a hepatite B e doenças oportunistas, como a toxoplasmose[32]. Estimou-se que, até o ano de 2012, para cada indivíduo que veio a óbito em cativeiro houve uma taxa de 0.65 nascimentos, caracterizando a população cativa desses animais como insustentável do ponto de vista reprodutivo[30].

QUEIXADA (*Tayassu pecari*)

O gênero *Tayassu* pertence à ordem Cetartiodactyla, subordem Suiformes e família Tayassuidae, da qual fazem parte outras duas espécies, o caititu (*Pecari tajacu*) e o taguá (*Catagonus wagneri*). São reconhecidas cinco sub-espécies com diferentes distribuições geográficas para o gênero, são elas: *T. p. ringens* do sul do México ao norte da Nicarágua; *T. p. spiradens* (sul da Nicarágua até Colômbia); *T. p. aequitorius* (sudoeste da Colômbia e noroeste do Equador); *T. p. pecari* (da Colômbia ao Brasil, norte do rio Amazonas, Figura 3) e *T. p. albirostris* (sul do Equador)[33].

FIGURA 3: Mapa de distribuição geográfica do queixada (*T. pecari*) no continente americano, em laranja, área de ocorrência da espécie, em amarelo, áreas de provável ocorrência e em vermelho, áreas onde a espécie está extinta.

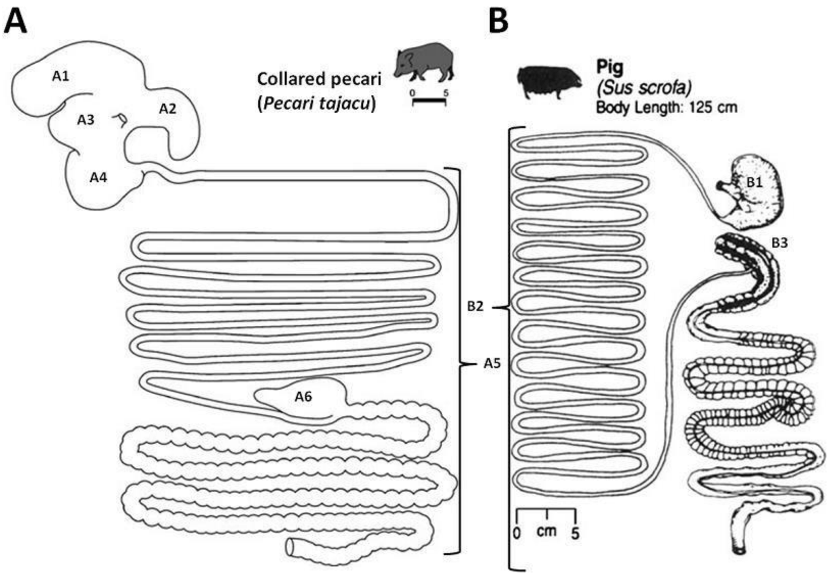
Adaptado de: © *The IUCN Red List of Threatened Species: Tayassu pecari – published in 2013. http://dx.doi.org/10.2305/IUCN.UK.2013-1.RLTS.T41778A44051115.en*

Os queixadas apresentam pouco dimorfismo sexual, massa corporal entre 25-40 kg, comprimento e altura até o ombro entre 90-140 cm e 40-60 cm respectivamente[2]. Como os outros animais do gênero, apresentam forma corporal semelhante aos suídeos do velho mundo, com corpo arredondado, membros torácicos e pélvicos finos e alongados e cascos pequenos[33]**.** Quando adultos, apresentam pelagem rígida de coloração mista, que varia de marrom-avermelhado mais claro na base e escuro a negro nas porções distais do pelo. Na cabeça, os pelos apresentam coloração marrom-escuro, com pelagem de coloração branca em torno da boca e mandíbula que se destacam no animal. Os pelos laterais são mais curtos (3.0-6.0 cm), enquanto que no dorso são mais longos, com até 11.5 cm de comprimento, e nesses animais podem se erigir, dando a impressão de maior tamanho quando ameaçado, além de oferecer proteção, devido sua estrutura rígida que facilita o deslocamento por áreas de vegetação mais densa[33,34].

Por possuir grande massa corporal, queixadas representam importante fonte de proteína de subsistência e, por esse motivo, sofrem grande pressão de caça[2]. Devido à grande distribuição geográfica, o nível de ameaça para esses animais é variável, no entanto, a IUCN considera essa espécie como vulnerável à extinção, e com atual declínio populacional devido principalmente à pressão antrópica (urbanização de áreas rurais, agricultura, caça predatória)[35]. A região amazônica é o habitat onde apresentam maior número populacional e, em teoria, deve ser o bioma onde a espécie está menos ameaçado. No entanto, o incremento da caça e da expansão humana tem aumentando a preocupação sobre a condição de ameaça para a espécie em outros biomas. Por exemplo, na caatinga, essa espécie poderá ser classificada como “criticamente ameaçada” devido ao declínio populacional em um futuro próximo[36]. Na Mata Atlântica os queixadas também se encontram criticamente ameaçados, e foram identificados em apenas 31.3 % dos remanescentes florestais, com área aproximada de 44.000 km² [36].

Em vida livre, os pecarídeos atuam como importantes dispersores de sementes, e apesar da proximidade filogenética com os suínos domésticos que são monogástricos, diferem desses por apresentarem estômago multicavitário[37,38]). No estômago esses animais realizam a fermentação dos elementos de sua dieta que incluem em sua maioria frutas, sementes, caule de diversas plantas, raízes, flores e também artrópodes e anelídeos em menor grau[39]. O estomago é formado então por uma porção gástrica (85% do volume total do estômago), dois sacos cegos (inferior e superior) e uma porção glandular[40] (Figura 4). Queixadas adultos apresentam intestino delgado e grosso com maior massa, e intestino grosso mais longo que nos caititus, enquanto o estômago não apresenta diferenças significativas no comprimento das curvaturas em todos os compartimentos[38]. Apesar de fermentadores, alguns autores consideram que a capacidade de digestão de fibras nos pecaris é intermediária entre mamíferos não ruminantes e verdadeiros ruminantes[41].

Figura 4: Diferenças entre trato gastrintestinal: (A) caititu – *P. tajacu* - pecarídeo pertencente a família dos queixadas - *T. pecari* – ambos apresentam estômago fermentador multicavitário composto por (A1) sac. cego superior; (A2) sac. cego inferior; (A3) porç. gástrica; (A4) porç. glandular; (A5) intestino e (A6) ceco. (B) porco selvagem - *Sus scrofa* – trato digestório tubular composto por (B1) estômago simples; (B2) intestino; (B3) ceco.



Adaptado de: Schwarm et al, 2010.

Queixadas vivem em bandos, com número que pode variar entre as regiões onde a espécie está distribuída. Na Amazônia, grupos entre 50-130 indivíduos são mais frequentes[42], enquanto que no Pantanal mato-grossense esse número varia entre 25-75 indivíduos[43]. Em vida livre, possuem poucos predadores naturais, a exemplo da onça-pintada (*Panthera onca*) e da suçuarana (*Puma concolor*). A organização em grupos auxilia esses animais a se precaver desses predadores, além de facilitar o aprendizado de animais mais jovens pelo contato com animais mais experientes, uma vez que os jovens são os mais susceptíveis à predação[33,37]. Contudo, a formação em bandos também é um facilitador para a caça humana, já que bandos inteiros podem ser abatidos de uma única vez[36]. Além da caça, a ocorrência de epidemias naturais e não naturais também poderiam contribuir para o declínio populacional[42].

A biologia reprodutiva dos queixadas em vida livre ainda é pouco conhecida em comparação com a de caititus (*Pecari tajacu*). No entanto, Mayor et al[12], caracterizou a performance reprodutiva dessa espécie na Amazônia peruana em estudo reprodutivo com fêmeas de vida livre, caçadas para fins de subsistência por comunidades tradicionais. Esses animais não apresentam sazonalidade reprodutiva, mas boa parte das concepções concentram-se em junho e dezembro nessa região. Comparando as taxas reprodutivas de queixadas e caititus de vida livre na mesma região, observou-se menor desempenho reprodutivo dos queixadas em comparação com os caititus, evidenciado pela produção anual de 0.53 fetos/fêmea em 219 fêmeas adultas de queixada avaliadas e 0.89 fetos/fêmea em 89 fêmeas adultas de caititus, o que sugere maior susceptibilidade dos queixadas à pressão de caça[10].

Fêmeas de vida livre não apresentam sazonalidade na dinâmica ovariana, pois apresentam estágios foliculares e luteal durante todo ano[10]. Por outro lado, em animais de vida livre na Guiana Francesa[42], a sazonalidade pode ser observada durante o período chuvoso, onde se observou maior concentração de nascimentos. As gestações nas fêmeas duram entre 156-162 dias[44], com média de 1.60-1.67 filhotes por gestação e intervalo entre partos que variam entre 250 a 253 dias[10, 12].

ALTRICIALIDADE & PRECOCIALIDADE

Estudos recentes descreveram os eventos morfológicos mais importantes do desenvolvimento intrauterino no macaco barrigudo[5,6], em queixadas[6]. Tais estudos mostraram que o macaco barrigudo produz fetos que apresentam crescimento lento e incompleto ao nascimento. Porém, em queixadas observou-se alto preparo morfológico dos fetos para a vida extrauterina e, consequentemente, uma capacidade importante para detectar e escapar de predadores, reduzindo a taxa de predação de animais jovens. A estratégia reprodutiva do macaco barrigudo foi compatível com uma espécie com baixa taxa de predação natural, baixa produção de neonatos e elevada vulnerabilidade, o que requer um longo cuidado parental durante o período pós-natal. Entretanto, o macaco barrigudo é o primata neotropical mais caçado e um dos mamíferos mais abatidos na Amazônia[45]. Portanto, sua estratégia reprodutiva sugere que essa espécie não está adaptada para responder a altas pressões de caça.

Segundo a fórmula para estimar a idade gestacional em diversos mamíferos de Huggett & Widdas[46], a velocidade específica de crescimento fetal no queixada (a = 0,084)[6] foi semelhante à da paca (a = 0,077)[15] e o dobro da velocidade encontrada em primatas altricial, como o macaco barrigudo (a = 0,042)[5]. O maior preparo dos queixadas e pacas também reflete o desempenho reprodutivo dos animais adultos, uma vez que as gestações mais curtas e o reduzido cuidado parental diminuem o gasto energético materno, o que resulta em intervalos entre partos mais curtos quando comparados a espécies altriciais como o barrigudo[5,6,12].

Nos macacos barrigudos durante a gestação, as características fetais não estão totalmente formadas até o nascimento, mas a maioria já está presente. A pele e mucosa pigmentada estão presentes aos 5.2 cm de comprimento dorsal total (CTD), enquanto os pelos de cobertura e tácteis já estão presentes em animais com CTD 8.0 cm. Até o CTD máximo de 17.3 cm, todas as características fetais estarão presentes (com exceção da abertura das pálpebras e da erupção dentaria), no entanto, de forma incompleta[5], uma vez que ainda se desenvolverão durante o período pós-natal, em fase de cuidado materno, que pode variar de entre 12-24 meses (período de desmame), observado em animais nascidos em cativeiro[9, 26].

Nos fetos de queixada, a pele é notável nos animais a partir de 11.5 cm CTD, pelos tácteis e de cobertura a partir de 13.8 cm e 20.9 cm respectivamente, e aos 26.4 cm todas as características estão presentes, incluindo a erupção dentária e as pálpebras abertas, evidenciando maior independência dos neonatos, em oposição ao que se observa nos fetos de barrigudo[6].

Em estudo comparativo sobre altricialidade e precocialidade entre diferentes espécies de mamíferos, Derrikson[8] determina o desenvolvimento neonatal sensorial, termoregulatório, locomotor e nutricional como essenciais para diferenciação entre espécies altriciais e precociais. Apesar do desenvolvimento sensorial e motor incompleto[5], os macacos barrigudos contam com o alto investimento parental no período pós-natal como estratégia reprodutiva para garantir a sobrevivência de sua prole. Além disso, o comportamento arborícola e o pequeno número de predadores (rapinantes, em sua maioria) são fatores favoráveis à sobrevivência do filhote até a fase adulta[20].

O trato digestório tubular é o que apresenta maior aumento de volume relativo em função do CTD nos fetos de macacos barrigudo, apresentando crescimento positivo ao longo de toda a vida intrauterina do animal (de 22 % do volume relativo ao 90° dia de gestação para 41 % ao final da gestação), e nos fetos em idade gestacional mais avançada não há diferenças significativas entre o volume relativo do trato digestório tubular fetal e o mesmo volume nos animais adultos[5]. Apesar disso, a ingestão de alimentos sólidos nos barrigudos é tardia quando comparados ao queixada, uma vez que a observação de ingestão de sólidos em animais mantidos em cativeiro inicia a partir da 11° semana pós-natal, mas o desmame ocorra entre 12-24 meses de vida[9, 26]. Semelhante aos barrigudos, durante a fase fetal os queixadas apresentam crescimento acelerado do volume relativo do trato digestório tubular (15.2 % para 38.5 % do 70° ao 159° dia de gestação), até o volume relativo máximo de 58.8 % na fase adulta[6].

Nos fetos de queixadas observa-se rápido crescimento do sistema digestório no último terço da gestação, além de já nascerem com a dentição eruptada, o que os torna aptos à ingestão e digestão de sólidos logo após o período de lactação, que nesses animais é menor em relação aos barrigudos[6]. Contudo, apesar de maior preparo do trato digestório, o desenvolvimento visceral nos queixadas é menor quando comparado ao macaco-barrigudo. O volume relativo dos órgãos torácicos e abdominais nos fetos de queixada em fase avançada de gestação apresentam diferenças significativas quando comparados aos adultos. Isso indica que nesses animais, o desenvolvimento visceral ocorre ainda durante o período pós-natal, enquanto que nos fetos avançados de barrigudo o desenvolvimento visceral é muito aproximado ao encontrado nos animais adultos [5,6].

CONCLUSÃO

Dados sobre o desenvolvimento gestacional nesses animais fornecem subsídios para manejo reprodutivo e o diagnóstico ultrassonográfico, por exemplo, como ferramenta de detecção de doenças reprodutivas, predição da idade gestacional, identificação de viabilidade ou má formação fetal[5,6]. Dessa forma, as informações contidas nesta revisão contribuem para melhorar o entendimento das estratégias reprodutivas adotadas por essas espécies, além de fornecer dados para o auxílio do manejo *in situ* para esses animais.

AGRADECIMENTOS

Agradecemos ao Programa de Pós-graduação em Saúde e Produção Animal na Amazônia (PPGSPAA) da Universidade Federal Rural da Amazônia (UFRA) e ao Instituto de Desenvolvimento Sustentável Mamirauá (IDSM).

APOIO FINANCEIRO

Coordenação de Aperfeiçoamento de Pessoal de Nível Superior – CAPES e Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), pelo apoio logístico e financeiro.

CONFLITO DE INTERESSE

Este trabalho não apresentou conflitos de interesse.

CONTRIBUIÇÃO DOS AUTORES

Todos os autores contribuíram com a idealização do estudo, e com a redação do manuscrito, aprovando a versão final publicada. Declaram-se responsáveis pelo conteúdo integral do artigo, garantindo sua precisão e integridade.

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Theriogenology 110 (2018) 34–43

Contents lists available at ScienceDirect

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Fetal development of the Poeppig's woolly monkey (*Lagothrix poeppigii*)

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ARTICLE INFO

Article history:
Received 10 September 2017
Received in revised form
2 December 2017
Accepted 7 December 2017
Available online 8 December 2017

Keywords:
Woolly monkey
Reproduction
Fetal features
Gestational development
Amazon

ABSTRACT

The intrauterine development is an evolutionary strategy that prepares the neonate for extra-uterine life, thus providing important information on the life history of species. In this study, we described the external and internal morphology of 25 fetuses of Poeppig's woolly monkeys (*Lagothrix poeppigii*) by taking advantage of a 10-year participatory collection of biological samples originated from animals hunted for subsistence purposes in the Peruvian Amazon. Logistic regressions estimated the probability of occurrence of each external morphological characteristic in relation to the crown-rump length (CRL). The presence of nails, closed eyelids, differentiated genitalia and formed limbs with separation of the digits were observed in all analyzed fetuses (≥4.2 cm CRL). The other characteristics appeared in the following order: skin with epidermal pigmentation, oral and nasal mucosal pigmentation, tactile pelage and covering pelage. Although advanced fetuses (>15.8 cm CRL) showed most fetal external characteristics, they were not fully developed and no specimen showed tooth eruption or opened eyelids. The growth formula used to determine fetal age was $\sqrt[3]{W} = 0.042$ (t - 45), with a high linear relationship between CRL and gestational age. All associations between the external biometry, absolute volume of internal organs and the CRL had a high coefficient of determination. Advanced fetuses and adults showed similar relative volume of thoracic and abdominal organs, except for thymus and the liver with a higher and lower relative volume, respectively. The relative volume of the tubular gastrointestinal tract and the thymus had a constant increase along fetal development, and the liver showed a significant decrease. This study describes important morphological events for understanding the gestational development in the *Lagothrix* genus. In addition, these results may be useful to improve imaging techniques, contributing to the *in situ* and *ex situ* reproductive management of this highly hunted species in the Amazon.

1. Introduction

The Poeppig's woolly monkey (*Lagothrix poeppigii*, Atelidae) is the most frequently hunted Neotropical primate in the Amazon region, especially due to its large body size (7 kg), and is considered vulnerable by the IUCN [1]. Although reproductive information can provide clues for the development of conservation strategies, data on woolly monkey reproduction are still scarce, and most studies have been conducted under captive conditions. The captive woolly monkey has a long gestation period (225 days), late puberty (at 4–5 years), and one young per birth on average [2]. The infants are born with approximately 10% of the mother's weight and show a long weaning period of 12–24 months [3,4]. In the Peruvian Amazon, the Poeppig's woolly monkey in the wild produces 0.48–0.54 offspring per female per year, and has an interbirth interval of 22.3–25.2 months [5].

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https://doi.org/10.1016/j.theriogenology.2017.12.022
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Imaging techniques are vital for developing appropriate reproductive management practices in wild species, improving pregnancy diagnosis, parturition prediction, and the determination of malformations and viability of fetuses [6,7]. Previous studies have reported the successful use of imaging techniques to conduct fetal measurements and characterization in captive primates, such as in the genera *Pan, Gorilla, Mandrillus, Erythrocebus* and *Macaca* [8–11]. However, the reliable standardization of fetal measurements for applying imaging techniques in most Neotropical primates, except for the genus *Sapajus* [12], has been hampered due to insufficient number of samples. To our knowledge, these measurements have never been conducted in the woolly monkey, since the species' high susceptibility to diseases [2] and low reproductive efficiency [13] make it difficult to sustain captive populations.

The use of pregnant reproductive tracts from animals hunted for subsistence purposes, through collaborative methods with local communities, represents an alternative for *in situ* biological collection of a large sample size. This sampling strategy allows for the utilization of embryos and fetuses that would be otherwise discarded by the locals [14]. The present study aimed to describe the fetal development of the wild Poeppig's woolly monkey, by taking advantage of a 10-year participatory collection of biological samples originated from subsistence hunting activity in the Peruvian Amazon.

2. Material and methods

This study was conducted in the Yavari-Mirín River, in the Northeastern Peruvian Amazon, a continuous area of 107,000 ha of predominantly non-flooded upland forest. The temperature in this region ranges from 22° to 36 °C, with an annual precipitation of 1500–3000 mm. Nueva Esperanza (S 04°19.53 W 71°57.33) is the only indigenous community in the study area, with 307 inhabitants. Local residents rely on agriculture, fishing, logging, and hunting for subsistence.

2.1. Biological sample collection and processing

From 2005 to 2014, hunters living in the study area voluntarily donated genital organs from 25 hunted pregnant Poeppig's woolly monkeys. During the study, we trained hunters to remove all abdominal and pelvic organs complete with the perineal region and store these in buffered 4% formaldehyde solution (v/v). Since we collected only unconsumed organs, we assured that no additional mortality of woolly monkeys was incurred for the purpose of this study.

Genital organs were dissected to remove all conceptuses. The 25 *Lagothrix poeppigii* fetuses obtained were analyzed [15]. First, a description of the external morphological features was conducted by searching for the presence and overture of eyelids, tooth eruption, presence of skin, nails, tactile and covering pelage, dermal and oral/nasal mucosal pigmentation, and genitalia and limb buds. The fetal stage was determined and all feature nomenclatures were based on the *International Committee on Veterinary Embryological Nomenclature* [16].

This research was evaluated and approved by the Ethics Committee for Experimentation from the Servicio Nacional Forestal y de Fauna Silvestre of Peru (protocol number 0350-2012-DGFFS-DGEFFS). Samples were sent to Federal Rural University of the Amazon (UFRA), Belém, Pará, Brazil, through the export license CITES/IBAMA (N° 14BR015991/DF). No animal was killed specifically for the research and hunters were not paid for the sample collection.

External biometric measurements were performed in 23 fetuses, since two specimens were flattened and were excluded. The measurements included body mass, crown-rump length (CRL), total dorsal length, biparietal diameter (BPD), occipital-frontal diameter (OFD), cranial circumference (CC), femur and humerus length (FL and HL), length of thoracic and pelvic limbs (TL and PL), thoracic diameter and circumference (TD and TC), as well as abdominal diameter and circumference (AD and AC). Thoracic and abdominal measurements were obtained from the last rib and the insertion of the umbilical cord, respectively. The body mass was measured in grams using a digital weighting scale (0.1 g accuracy), and a tape measure (0.1 mm accuracy) and a metal caliper (full measurement capability 300 mm) were employed for body measurements.

All fetuses were dissected to calculate the volume of thoracic and abdominal organs fetal organs, including the heart, lungs, liver, spleen, kidneys, tubular gastrointestinal tract, and thymus. Seven fetuses with signs of visceral autolysis were discarded from this analysis. Volumetric measurements were conducted by submerging the organs in hypodermic syringes with 0.01 ml accuracy filled with water and applying the Archimedes Principle [15], considering the value of water volume displaced as a proxy of the organ volume. The summative volume of all organs was considered as the total visceral volume. The relative volume of each fetal organ was calculated as a percentage respective to the total visceral volume. In parallel, the volume of the same organs was also measured in 13 adult Poeppig's woolly monkeys hunted in the same study area to compare the relative volume of fetal organs in advanced pregnancy stage with that in adults.

2.2. Statistical analysis

Logistic regressions were applied to estimate the probability of occurrence of each external morphological characteristic in relation to the CRL using the software Statistica 8.0 (StatSoft Inc., Tulsa, USA). Multiple regression modeling between the studied biometric measures, organ volumes and CRL were performed using the software CurveExpert 2.4 (© Copyright 2017, Daniel G. Hyams) to define those functions that were best fitted to the plots. Regressions were also used to assess allometric relationships between the BPD and OFD; TC and AC; HL and FL; TL and PL. For absolute measurements,

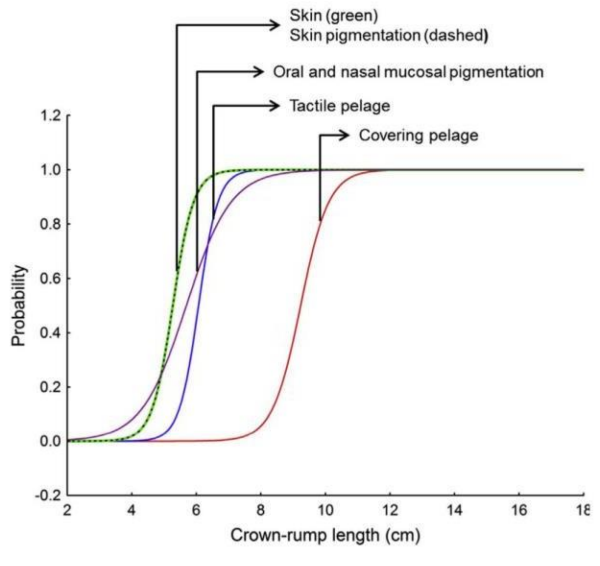

Fig. 1. Probability curves for morphological features along the increase in crown-rump length (CRL) in 25 fetuses of the Poeppig's woolly monkey (Lagothrix poeppigii).

Table 1
Logistic equations of the external morphological features parameters in 25 Poeppig's woolly monkey (*Lagothrix poeppigii*) fetuses.

Morphological features	Equation	Chi-square (Df)	P value
Dermis	$y = \frac{2.72^{(-16.342+(3.10132)*x)}}{1+2.72^{(-16.342+(3.10132)*x)}}$	11.45 (1)	<0.01
Dermal pigmentation	$y = \frac{2.72^{(-16.342+(3.10132)*x)}}{1+2.72^{(-16.342+(3.10132)*x)}}$	11.45 (1)	<0.01
Mucosal pigmentation	$y = \frac{2.72^{(-8.2166+(1.44394)*x)}}{1+2.72^{(-8.2166+(1.44394)*x)}}$	20.09 (1)	<0.01
Tactile pelage	$y = \frac{2.72^{(-20.21+(3.33067)*x)}}{1+2.72^{(-20.21+(3.33067)*x)}}$	18.57 (1)	<0.01
Covering pelage	$y = \frac{2.72^{(-21.126+(2.28893)*x)}}{1+2.72^{(-21.126+(2.28893)*x)}}$	25.29 (1)	<0.01

we forced linear regressions to origin and only considered those functions with a starting point on or near zero, since we expected both internal and external measurements to be zero on day 0 of fetal development.

The fetal age was estimated using the formula proposed by Huggett and Widdas [17], $\sqrt[3]{W} = a\ (t\text{-}t0)$, where W is the fetal weight, a is the specific fetal growth velocity, t is the fetal age in days, and $t0$ is the calculated interception on the age axis. According to those authors, $t0$ is equal to 20% of gestation time in species that present between 100 and 400 days of pregnancy. To use that equation, we considered a gestation length of 225 days [2], and a mean weight of 450 g for pre-partum fetuses, taking into account the weight stabilization in fetuses in more advanced stages of gestation (CRL > 15.8 cm).

We compared the relative volumes of visceral organs of larger fetuses (>15.0 cm CRL) with those of adults by means of T tests. Differences with a probability value of 0.05 or lower ($p < 0.05$) were considered significant. All descriptive values of fetal measurements are expressed as the mean ± standard deviation (SD).

3. Results

In the studied fetuses, the average CRL was 10.1 ± 3.7 SD cm, ranging from 4.2 to 17.3 cm, and the average body mass was 129.1 ± 143.8 SD g, ranging from 7 to 500 g. Fig. 1 shows the probability curves for the occurrence of external morphological features according to CRL, while Table 1 shows the regression models. The presence of nails, closed eyelids, differentiated genitalia (14 females and 11 males) and formed limbs with separation of the digits was observed in all analyzed fetuses (≥4.2 cm CRL). Fetuses with a CRL of 5.2 cm showed the first signs of skin with epidermal pigmentation, and oral and nasal mucosal pigmentation. Tactile pelage was first observed in fetuses with a CRL of 6.5 cm. All fetuses larger than 8.0 cm presented tactile and covering pelage, but no studied fetus presented a fully developed covering pelage (Fig. 2). No studied specimen in advanced pregnancy stages (<15.8 cm CRL) showed tooth eruption or opened eyelids.

All associations between the external biometric measures and the CRL had a high determination coefficient ($r^2 > 0.80$, $P < 0.05$;

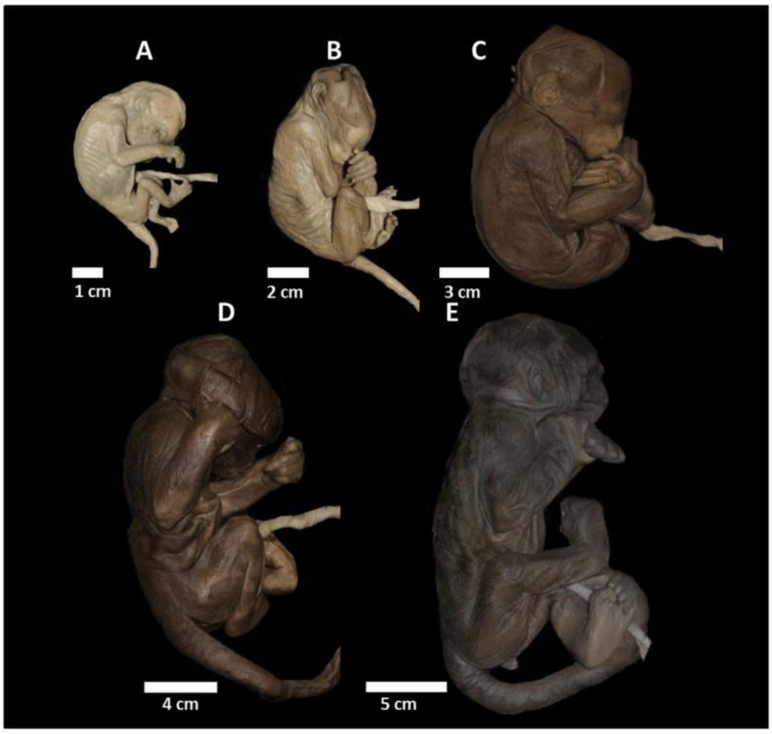

Fig. 2. Fetuses of Poeppig's woolly monkey (*Lagothrix poeppigii*) at different stages of fetal development according to crown-rump length (CRL). (A) Fetus of 4.2 cm and 7 g, presenting closed eyelids and absence of all features (skin, epidermal and mucosal pigmentation, tactile and covering pelage). (B) Fetus of 6.5 cm and 19.3 g, presenting skin and mucosal membranes with pigmentation and tactile pelage in formation. (C) Fetus with 9.6 cm and 80 g, showing covering and tactile pelage. (D) Fetus with 13.8 cm and 250 g, showing all the fetal characteristics but with closed eyelids. (E) Fetus with 17.3 cm and 410 g with all observed fetal characteristics but with closed eyelids.

Figs. 3 and 4). The body mass ($r^2 = 0.98$, $P < 0.01$), OFD ($r^2 = 0.98$, $P < 0.01$), and CC ($r^2 = 0.98$, $P < 0.01$) presented the best determination coefficients related to CRL. The allometric relationships showed strong interaction for all analyzed parameters ($r^2 < 0.90$, $P < 0.01$). Although, the relationship between HL and FL showed a proportion of growth of approximately 1:1 ($r^2 = 0.98$, $P < 0.01$), the TL presented a faster growth than PL during the fetal phase ($r^2 = 0.96$, $P < 0.01$, Fig. 5). The growth formula used to determine

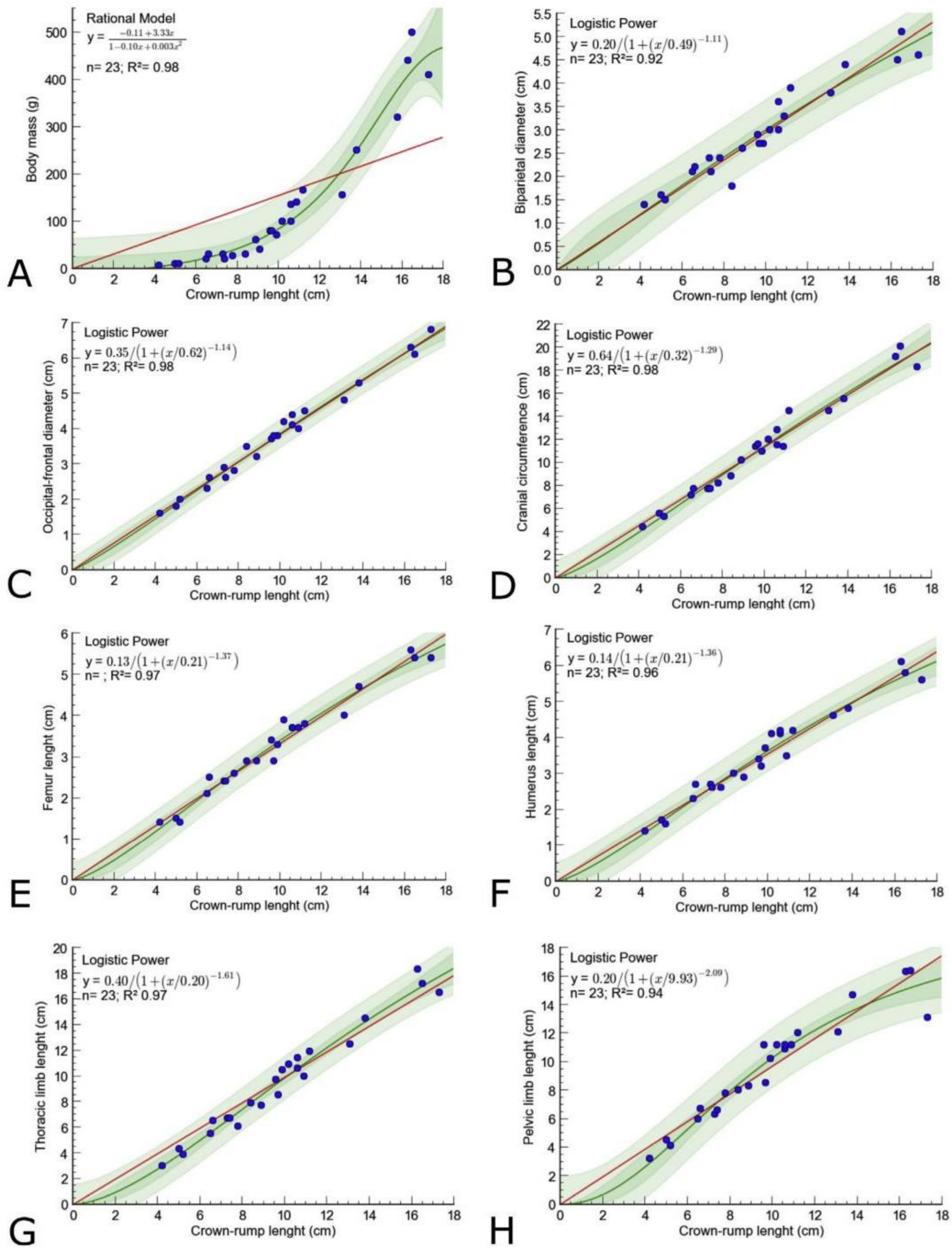

Fig. 3. Relationship between the crown-rump length (CRL) and the body mass (A), biparieral diameter (B), occipital-frontal diameter (C), cranial circumference (D), femur (E) and humerus (F) length, and length of thoracic (G) and pelvic (H) limbs in 23 Poeppig's woolly monkey (Lagothrix poeppigii) fetuses. The green line represents the model best fitted to the plots, while the red line represents an expected linear trend with no intercept. The logistic power model was the best fitted to the measurements of the skull, thoracic limbs, and pelvic limbs, while the rational model was the best fitted to body mass. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

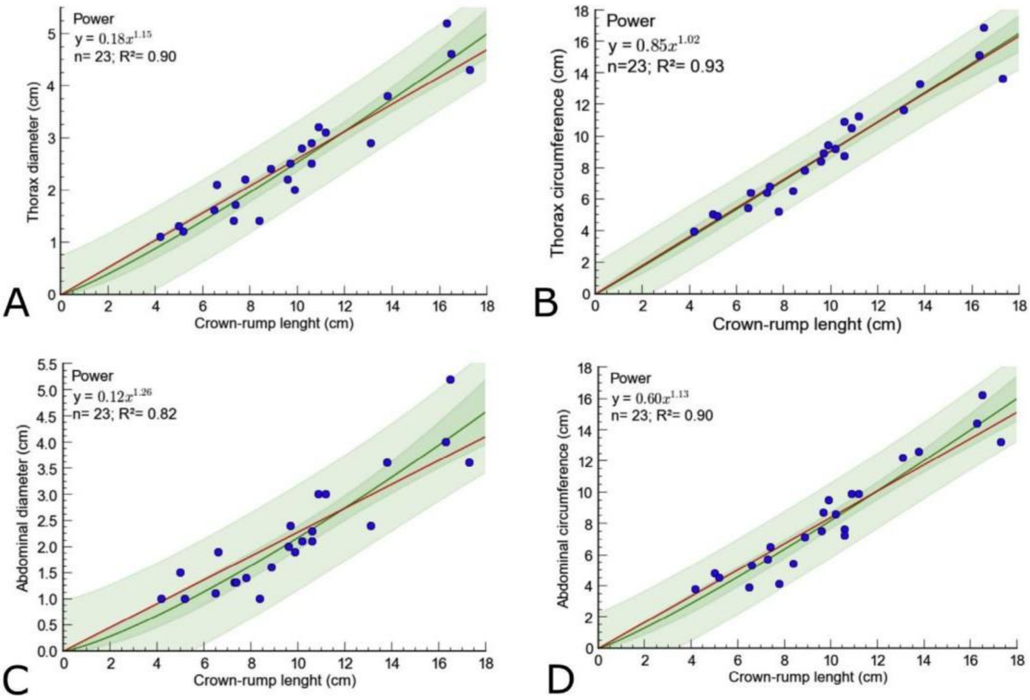

Fig. 4. Relationship between the crown-rump length (CRL) with thorax diameter (A), thorax circumference (B), abdominal diameter (C) and abdominal circumference (D), in 23 Poeppig's woolly monkey (Lagothrix poeppigii) fetuses. The green line represents the model best fitted to the plots, while the red line represents an expected linear trend with no intercept. The power model was the best fit for the thoracic and abdominal parameters. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

fetal age was $\sqrt[3]{W} = 0.042$ (t - 45). There was a high linear relationship between CRL and gestational age ($r^2 = 0.97$, $P < 0.001$; Fig. 6).

All associations between the absolute volume of internal organs and the fetal CRL showed a high determination coefficient ($r^2 \geq 0.78$, $P < 0.05$; Fig. 7). The highest associations with CRL were found for the total volume of viscera ($r^2 = 0.93$, $P < 0.01$), tubular gastrointestinal organs ($r^2 = 0.92$, $P < 0.01$), and thymus ($r^2 = 0.92$, $P < 0.01$). The relative volume of the spleen ($r = 0.07$, $r^2 = 0.005$, $P = 0.76$), the heart ($r = 0.23$, $r^2 = 0.05$, $P = 0.65$), and the lungs ($r = -0.44$, $r^2 = 0.19$, $P = 0.06$) did not show significant association with CRL (Fig. 8). In contrast, the relative volume of the tubular gastrointestinal tract ($r = 0.65$, $r^2 = 0.42$, $P = 0.003$) and the thymus ($r = 0.76$, $r^2 = 0.58$, $P = 0.0004$) had a constant increase along fetal development contrary to the relative volume of the liver ($r = -0.64$, $r^2 = 0.41$, $P = 0.004$), which showed a significant decrease. Most organs showed no differences in their relative volumes between advanced fetuses and adults (Table 2). The only significant differences between advanced fetuses and adults were observed in the thymus and the liver, with a higher and lower relative volume in the studied fetuses, respectively.

4. Discussion

Studies on the reproductive biology of wild species represent a challenge, especially due to the difficulty of keeping specimens in captivity and obtaining information in the wild [14]. In this context, this was the first study that assessed the fetal development of the wild Poeppig's woolly monkey. The results discussed here were based on gestational and postnatal data in humans, NHP, and other species.

The fetal development of external features in the woolly monkey shows that the species presents high levels of altriciality, since the covering pelage was not fully developed and we detected no open eyelids or tooth eruption in the largest fetus assessed (97.3% gestation time). An increase in pelage volume was observed in the woolly monkey between the 4th and 16th postnatal weeks, showing that these structures indeed develop after birth. The development of covering pelage is fundamental for thermal regulation of the progeny of altricial species [4]. In Rhesus monkeys (*Macaca mulatta*), thermal regulation depends on the maternal contact up to the time when the juvenile presents developed covering pelage and an autonomous central nervous system [18]. In domestic dogs and cats, neonates do not perform thermoregulation effectively until the second week after birth [19]. In contrast, in precocial species, the covering pelage is more developed before birth, such as in cattle (230 days and 81% gestational time) [20], horses (220 days and 65% gestational time) [21], and sheep (119 days and 78% gestational time) [22]. In the paca (*Cuniculus paca*), a precocial histricognath rodent, the covering pelage is fully formed on the 114th day of gestation (76.5% of gestation length) and is able to effectively aid in thermoregulation after birth [15].

Since no fetus in this study had open eyelids, we suggest that, similar to humans, this occurs during the fetal period [23], or soon after delivery [24]. Some altricial species, such as domestic dogs and cats, develop postnatal visual capacity in the extra-uterine environment, showing closed eyelids until 14 days postpartum [19,25]. In contrast, precocial species such as ruminants already have open eyelids during the final third of gestation [26].

Dental eruption was not observed in fetuses in the woolly monkey. The appearance of teeth in NHP is an important factor for the independence of the neonate because it is related to the

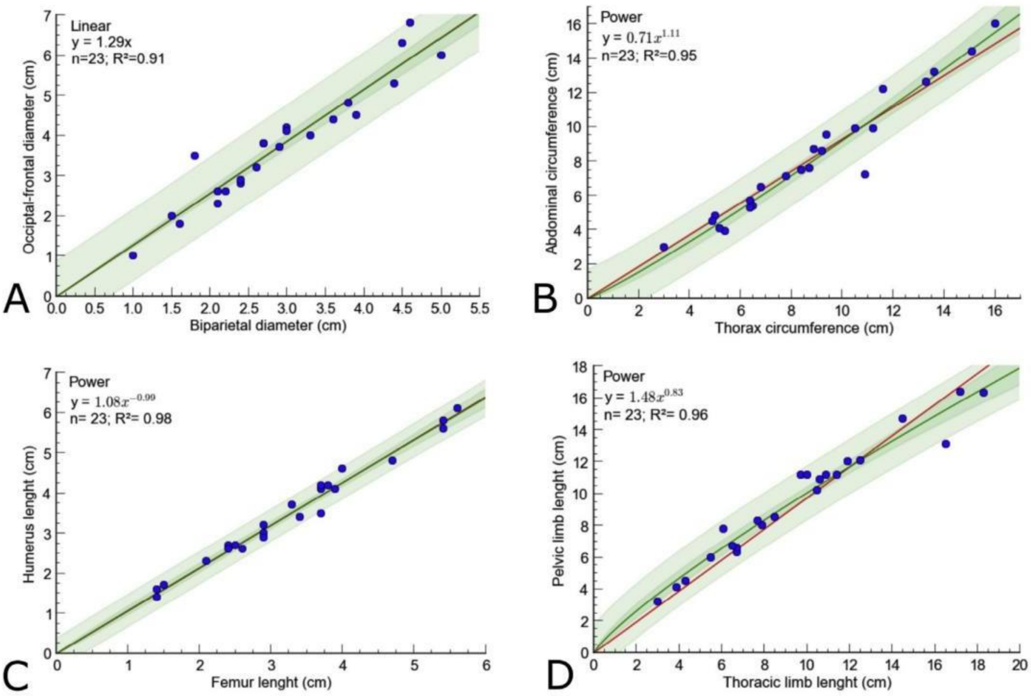

Fig. 5. Allometric relationship between the biparietal diameter versus the occipital-frontal diameter (A), the thorax circumference versus the abdominal circumference (B), the humerus versus the femur length (C), and the length of thoracic versus pelvic limbs (D) in 23 Poeppig's woolly monkey (Lagothrix poeppigii) fetuses. The green line represents the model best fitted to the plots, while the red line represents an expected linear trend with no intercept. The logistic power model was the best fitted to the allometric relationships. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

ingestion of solid foods. In the captive woolly monkey, this process occurs from the 11th postnatal week onwards [4]. In this species, the second molars appear before the canines, whereas in humans the incisors are the first to erupt, at 22–24 weeks gestation (55–60% of gestational time) [27]. An increasing relationship between tooth eruption time, and body and brain mass was observed in primate species [27]. This relationship explains the usual late dental eruption in species with high parental investment and reduced numbers of offspring, which includes the Poeppig's woolly monkey. In NHP species, the late tooth eruption is associated with longer weaning processes [28]. In contrast, dental eruption is

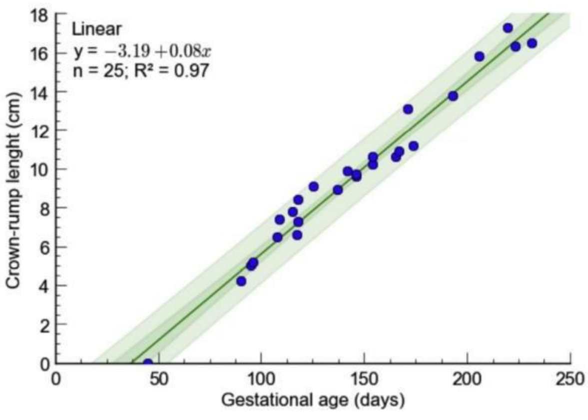

Fig. 6. Relationship between gestational age and crown-rump length (CRL) in 25 Poeppig's woolly monkey (Lagothrix poeppigii) fetuses.

already present in fetuses of bovines (110 days, 38.9% gestational time) and sheep (105 days, 69.1% gestational time), which already have masticatory function shortly after birth [20,22].

Precocial species has a faster development of fetal morphological characteristics in comparison with altricial species [20–22]. This reproductive strategy guarantees well-developed and prepared newborns for an early independence during the extra-uterine life, allowing the reduction of the interbirth interval [15]. In contrast, the parental care in NHP implies a slower postnatal growth and a higher maternal energy [4], increasing the interval between deliveries and decreasing reproductive rates. The strategy adopted by species to maximize neonatal survival is influenced by the risk of maternal and newborn mortality. Thus, predation is an important selective pressure in natural ecosystems and also an important driving force for evolution. The predation of arboreal primates has been widely reported for various cat species and raptors [29,30]. The strategy of having a large gestation period and producing one altricial neonate [13] is usually a characteristic of a non-prey species. Primates usually deliver one neonate with a non-developed prenatal brain and muscle growth, which difficult their interpretation of environmental stimulus and motor coordination since nerve-regulated functions are not yet synchronized with the somatic growth of the individual [24]. The high maternal investment in NHPs gives to the newborn conditions to develop the necessary skills and structures to ensure postnatal survival [31–33].

Fetal biometric analyses are commonly employed in obstetric ultrasonography in NHP and are important for determining gestational age, fetal morphology, and fetal viability [8,9]. Although there was no data in the literature on birth weight of the Poeppig's woolly monkeys, it was possible to estimate gestational age using the formula proposed by Huggett and Widdas [17]. The strong

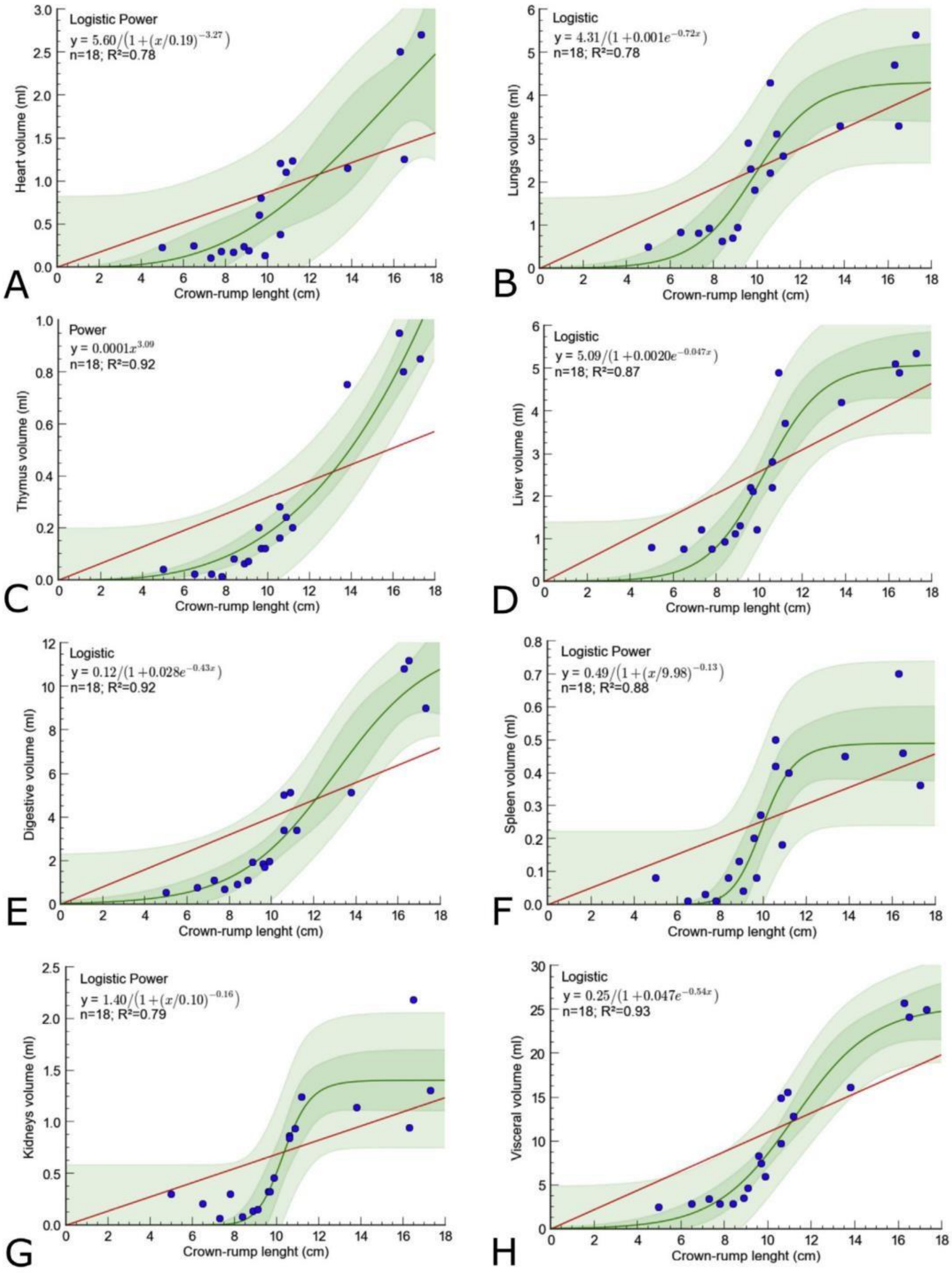

Fig. 7. Relationship between the volume of the heart (A), lungs (B), thymus (C), liver (D), digestive tract (E), spleen (F), kidneys (G), and visceral (H) tissues, with the crown-rump length (CRL) in 18 Poeppig's woolly monkey (Lagothrix poeppigii) fetuses. The green line represents the model best fitted to the plots, while the red line represents an expected linear trend with no intercept. The logistic model was the best fitted to the volume of lungs, liver, tubular digestive, and total viscera; the logistic power model was the best fitted to the volume of heart, kidneys, and spleen; and the power model was best fitted to the thymic volume. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

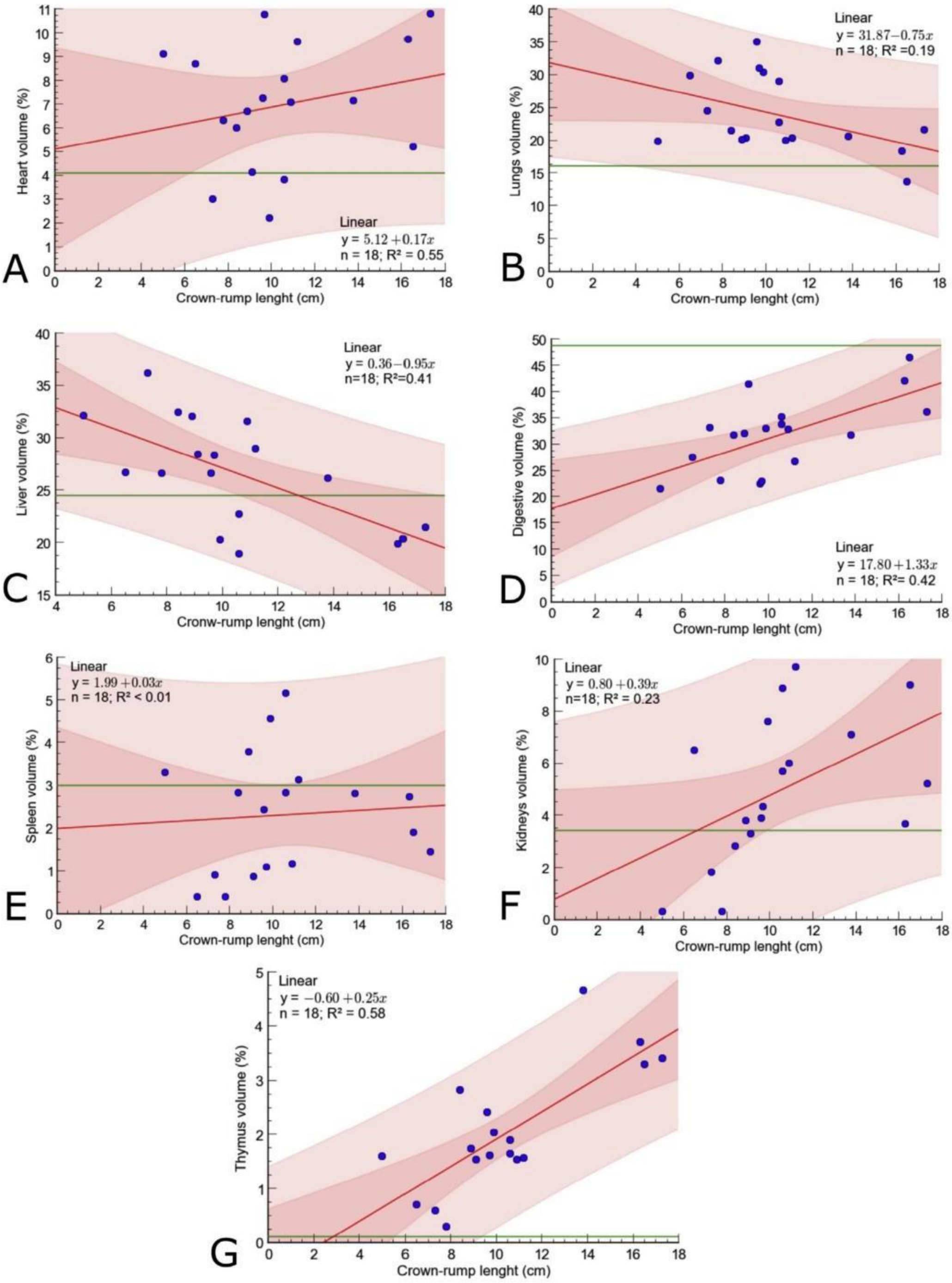

Fig. 8. Relationship between the relative volume of the heart (A), lungs (B), liver (C), tubular digestive organs (D), kidneys (E), spleen (F), and thymus (G) with respect to the crown-rump length (CRL) in 18 Poeppig's woolly monkey (Lagothrix poeppigii) fetuses. The green line represents the relative volume in adult animals, while the red line represents the linear model. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

relationship obtained with the CRL guarantees the reliability of this result. The relationship between CRL and BPD, DOF, CC, and AC variables in this study were similar to those described in *Sapajus apella* [12], *Macaca mulatta* and *M. fascicularis* [8].

The *Lagothrix* genus encompasses quadruped primates that are able to perform jumps and escalations, requiring a similar proportion of limb sizes [34]. The faster growth of the thoracic limbs in relation to the pelvic limbs observed in this study is non-suitable

Table 2
Absolute and relative volume of the visceral organs of Poeppig's woolly monkeys (*Lagothrix poeppigii*) in advanced pregnancy stage (CRL > 15.0 cm; n = 03) and adulthood (n = 13).

Organ	Absolute volume ± SD (ml)		Relative volume ± SD (%)		T value	F value	df	P value
	Fetus	Adult	Fetus	Adult				
Heart	2.15 ± 0.78	25.56 ± 8.4	8.58 ± 2.97	4.11 ± 0.9	4.30	0.09	2	0.12
Thymus	0.86 ± 0.07	0.76 ± 1.2	0.39 ± 0.20	0.10 ± 0.2	2.57	1.16	5	<0.01
Lungs	4.46 ± 1.06	100.00 ± 26.9	17.87 ± 3.98	16.00 ± 2.7	4.30	0.44	2	0.52
Liver	5.11 ± 0.22	152.33 ± 35.7	20.52 ± 0.81	24.53 ± 2.8	2.17	11.57	12	<0.01
Tubular gastrointestinal organs	10.33 ± 1.17	302.22 ± 63.1	41.53 ± 5.23	48.79 ± 4.3	3.18	0.66	3	0.11
Kidneys	1.47 ± 0.63	21.15 ± 4.72	5.95 ± 2.74	3.40 ± 0.4	4.30	0.02	2	0.24
Spleen	0.50 ± 0.17	18.34 ± 7.3	2.02 ± 0.64	3.02 ± 1.2	2.44	3.50	6	0.09
Total visceral volume	24.91 ± 0.80	620.26 ± 121.2	–	–	–		–	–

for their type of locomotion. Since the type of locomotion influences the proportion between limbs, the growth of the limbs will probably be compensated during postnatal development.

In the wooly monkey, there were no significant differences between the relative volume of internal organs in advanced fetuses larger than 15.8 cm CRL and adults, with the exception of the thymus and liver. The relative fetal hepatic volume decreased from 30 to 34% on day 90 (40% of gestational time) to 20% at the end of gestation (97.3% gestational time), and adults had a relative volume of 24%. In parallel, the tubular gastrointestinal tract presented a relative growth from 20-22%–41% during the same period, and adults had a relative volume of 24%. In humans, the liver weight accounts for 10% of fetal weight at the 9th week of gestation (25% gestation time), 5% in the neonate, and 2% in adults [35]. This decrease is explained by the maturation of hepatic tissues and the replacement of hepatic hematopoietic function by splenic and lymphoid tissues throughout fetal development [35,36]. The relative hepatic volume in the fetal period of woolly monkeys apparently occurs simultaneously with the relative growth of other systems, such as the tubular gastrointestinal tract, which grows in the postnatal period up to the proportional volume of 24% in adults.

The heart of the fetuses in the wooly monkey presents an accelerated volume increase between fetuses of 9.6 cm CRL, and 0.5 ml in organ volume (65% of gestational time) to 16 cm CRL, and 2.5 ml in organ volume (91% of gestation time). This is similar to humans, in which the organ grows 4-fold its size from the 17th week (42% of gestation time) until the end of gestation [37].

In the Poeppig's woolly monkey, the absolute increase of lung volume is similar to described for *M. nemestrina*, where a linear increase was observed in pulmonary weight related to CRL, during the 2nd and 3rd trimesters of gestation [38]. In *M. mulata*, a similar increase is also observed in the 3rd trimester [39]. The decrease in the relative volume observed in *L. poeppigii* in this study suggests a still incomplete maturation process, since the organ is non-functional during the fetal period. In humans, prenatal lung morphology undergoes complete remodeling, and in the first three years of adulthood the alveoli undergo a process that increases the area of gas exchange, giving the lung a mature morphology [40].

The spleen of the adult woolly monkey has a constant relative volume, which is maintained in adults. In humans, the spleen presents an initial important hematopoietic and immunological function between 3 and 6 months of gestation, but afterwards, the function decreases [41].

During the fetal development in the woolly monkey, the thymus presents a significant growth, reaching 4% of relative volume in the advanced fetuses, being significantly larger than the 0.1% observed in adults. In humans, the thymus is a rather functional organ and the main producer of lymphocytes in the fetal phase [42], but reduces in size around 11–15 years as the immune system matures [35].

This study describes most important morphological events of the fetal development in the woolly monkey, showing that the species produces highly altricial newborns with a slow and incomplete fetal development at birth. The reproductive strategy of the woolly monkey is compatible to a species with a low natural predation rate, low production of youngs, and low neonatal survival rate in nature, which requires for a long parental care during the postnatal period in order to guarantee the newborn independence. Since the woolly monkey is currently the most hunted Neotropical primate, and one of the most hunted mammals in the Amazon [43], its reproductive strategy suggests that this species is not adapted to respond to high hunting pressures. In addition, the data obtained is also useful to improve imaging techniques, and may contribute to the reproductive management *in situ* and *ex situ* of the most hunted primate in the Amazon.

Acknowledgments

We sincerely thank all the people from the communities in the Yavarí-Mirín River, without whom this work would not be possible. This work was supported by the Foundation for Research Support of Pará (FAPESPA, protocol 005/2015), the National Council of Technological and Scientific Development (CNPq, protocol 452908/2016-7 and 201475/2017-0), the Earthwatch Institute, the IVITA (Instituto Veterinario de Investigaciones de Trópico y de Altura), and the Museo de la Universidad Nacional de la Amazonía Peruana (UNAP). We would like to thank Mr. Walfrido Monteiro (https://www.behance.net/WalfridoMonteiro) for his wonderful video produced with 3D fetal images attached as supplementary material of this paper.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.theriogenology.2017.12.022.

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Theriogenology 119 (2018) 163–174

Contents lists available at ScienceDirect

Theriogenology

journal homepage: www.theriojournal.com

Embryonic and fetal development of the white-lipped peccary (*Tayassu pecari*)

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A R T I C L E I N F O

Article history:
Received 17 February 2018
Received in revised form
4 July 2018
Accepted 7 July 2018
Available online 10 July 2018

Keywords:
Tayassuidae
Reproduction
Fetal features
Gestational development
Amazon

A B S T R A C T

The white-lipped peccary (*Tayassu pecari*) is an endangered large-sized Neotropical ungulate that is one of the most hunted mammals in the Amazon. Here, we used two embryos and 102 white-lipped peccary fetuses originated from animals hunted for subsistence in the Peruvian and Brazilian Amazon to describe the intrauterine development of external and internal morphology of this Neotropical ungulate. Logistic regressions were used to estimate the probability of occurrence of main external characteristics in relation to the total dorsal length (TDL), while multiple linear and non-linear regressions were conducted to assess the relationship between external and visceral biometry with TDL. External characteristics appeared in the following chronological order: limbs, differentiated genitalia and opened eyelids (≥5.1 cm TDL), fused eyelids (≥6.2 cm TDL), hooves and outer ear (≥7.9 cm TDL), dorsal gland (≥9.4 cm TDL), skin (≥11.5 cm TDL); tactile pelage (≥13.8 cm TDL), covering pelage (≥20.9 cm TDL), tooth eruption (≥26.4 cm TDL) and opened eyelids (≥27.8 cm TDL). The formula of fetal age was $\sqrt[3]{W} = 0.084\,(t - 31.80)$, with a high linear relationship between TDL and gestational age. All external biometric parameters and absolute volume of visceral organs showed strong positive relationship with TDL. Except for the liver, we found differences in the relative volume of most visceral organs between advanced fetuses (≥34.2 cm TDL) and adults. The most important events during the intrauterine development in the white-lipped peccary show that, in contrast with the domestic pig, it is a highly precocial species producing newborns with a high fetal growth velocity which allows newborns to achieve an early autonomous functionality. Our results are relevant to improve imaging techniques and assist the reproductive and clinical management for the white-lipped peccary both in captivity and in the wild.

1. Introduction

The white-lipped peccary (*Tayassu pecari*) is a large-sized Neotropical ungulate (30–50 kg) that represents an important source of subsistence protein [1], being one of the most hunted mammals in the Amazon [2]. Since 1970s onwards, a large-scale decline of its population has been observed in different areas in northern South America [1,3–5], probably due to the mixed effects of hunting and non-anthropogenic causes, such as widespread diseases [4,6]. Accordingly, the white-lipped peccary is currently considered "Vulnerable" by the International Union for Conservation of Nature (IUCN) and is predicted to become "Critically Endangered" in the short-term due to predatory hunting and habitat fragmentation by agricultural expansion [7].

Alternatively, commercial breeding of wildlife is increasing as a strategy to concomitantly conserve wildlife and provide food for people. However, while commercial breeding of the collared

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https://doi.org/10.1016/j.theriogenology.2018.07.006

peccary (*Pecari tajacu*) is common, breeding actions for the white-lipped peccary are rare, causing a greater difference in the data available on the social behavior and reproductive biology between both species [8,9]. The few data available in the literature on the species' reproduction shows that white-lipped peccary females present a gestation length of 156–162 days [10], a mean of 1.60–1.67 neonates per gestation, and a farrowing interval ranging from 250 to 253 days [11,12].

Ultrasonography is a minimally invasive and low cost technique, and is a valuable tool for pregnancy diagnosis, prediction of parturition and detection of reproductive pathologies. This technique has already been proved useful to evaluate the stages of embryonic and fetal developments in wild species [13,14], but due to the scarcity of captive breeding actions for and the difficulty in capturing white-lipped peccaries in the wild, it has been barely employed in studies on this species [15]. On the other hand, for those wild species with lacking data, participatory collection of females' reproductive tracts with the aid of subsistence hunters may provide sufficient samples to allow accurate assessments of their reproduction [16–18] and to standardize parameters for application of ultrasonography and other imaging techniques.

Therefore, this study aims to describe the embryonic and fetal development of the white-lipped peccary using embryos/fetuses collected through the collaboration of Amazon dwellers in rural communities whose protein income depends on the subsistence hunting. The information generated here is useful for developing more appropriate reproductive management practices and standard measures for application of imaging techniques, enhancing the species' captive breeding success, as well as answering questions related to the life history of the white-lipped peccary and the ability of ungulate newborns to survive during postnatal life.

2. Material and methods

2.1. Study sites

This study was conducted in two areas in the Amazon rainforest region. The first area, the Yavarí-Mirín River (YMR, S 04°19.53; W 71°57.33) is located in northeastern Peruvian Amazon and is a continuous area of 107,000 ha of predominantly upland forests. A single indigenous community of 307 inhabitants is found in the region. The second site, the Amanã Sustainable Development Reserve (ASDR, S 01°54.00; W 64°22.00), is a protected area of 2,313,000 ha in the Central Brazilian Amazon, between the Negro and Japurá rivers, and consists primarily of upland forests. The ASDR has a population of approximately 4000 riverine people, found in 23 communities and some isolated settlements. In both areas, local communities rely mainly on agriculture for income and on hunting and fishing for subsistence. The climate in both study areas is typically equatorial with annual temperatures ranging from 22 °C to 36 °C, a relative humidity of 80%, and annual rainfall between 1500 and 3000 mm, comprising dry and wet/flooded seasons.

2.2. Biological sample collection and processing

From 2002 to 2015, local hunters collected and voluntarily donated reproductive tracts from 69 pregnant white-lipped peccaries, 54 (78.3%) in the YMR and 15 (21.7%) in the ASDR, including 36 (52.2%) single, 31 (44.9%) double and two (2.9%) triple gestations. Hunters were trained to remove all abdominal and pelvic organs complete with the perineal region and to store these in buffered 4% formaldehyde solution (v/v). Since hunters do not consume these materials, any invasive procedure or any additional mortality for the purpose of the study was avoided [16]. The research protocol was approved by the Research Ethics Committee for Experimentation in Wildlife at the Direccion General de Flora y Fauna Silvestre from Peru (License 0229-2011- DGFFS-DGEFFS), by the Instituto Chico Mendes for Biodiversity Conservation from Brazil (License SISBIO N^{o} 29092) and by the Committee on Ethics in Research with Animals of the Federal Rural University of the Amazon (CEUA/UFRA protocol 008/2016). Samples were sent to UFRA, Belém, Pará, Brazil, under the export license CITES/IBAMA (No 14BR015991/DF).

Uteri were dissected and 2 embryos and 102 fetuses were collected. We externally examined the embryo/fetus of all gestations to describe the presence of the following morphological features: 1) differentiated genitalia, 2) differentiated limbs, 3) eyelids, 4) skin, 5) covering and tactile pelage, 6) erupted teeth, 7) hooves, 8) dorsal gland and 9) outer ear. The embryo/fetal stage was determined according to the *International Committee on Veterinary Embryological Nomenclature* [19].

We performed biometry measurements in 97 embryos/fetuses from 65 gestations to describe the external biometry, since one embryo and six fetuses presented signs of autolysis and were discarded. The external measurements included body mass, total dorsal length (TDL), crown-rump length (CRL), biparietal diameter (BPD), occipital-frontal diameter (OFD), cranial circumference (CC), femur and humerus length (FL and HL), length of thoracic and pelvic limbs (TL and PL), thorax diameter and circumference (TD and TC), as well as abdominal diameter and circumference (AD and AC). Thoracic and abdominal measurements were obtained from the last rib and the insertion of the umbilical cord, respectively. The body mass was measured in grams using a digital weighting scale (0.1 g accuracy), while a tape measure (0.1 mm accuracy) and a metal caliper (full measurement capability 300 mm) were employed for body measurements.

We selected 41 pregnancies with fetuses homogenously distributed according to their TDL, and eviscerated the thoracic and abdominal organs (heart, lungs, thymus, liver, spleen, kidneys and tubular gastrointestinal organs) from one fetus per pregnancy. Volumetric measurements of the organs were conducted by submerging these in hypodermic syringes with 0.01 ml accuracy filled with water and applying the Archimedes Principle [17], considering the value of water volume displaced as a proxy of the organ volume. The summative volume of all organs was considered as the total visceral volume. The relative volume of each fetal organ was calculated as a percentage respective to the total visceral volume. In parallel, the volume of the same organs was also measured in 18 adult white-lipped peccary hunted in the YMR to compare the relative volume of fetal organs in advanced pregnancy stages with that in adults.

2.3. Statistical analysis

The gestational age was estimated using the formula proposed by Huggett and Widdas [20], $\sqrt[3]{W} = a\ (t\text{-}t0)$, where W is the fetal weight, a is the specific fetal growth velocity, t is the fetal age in days, and $t0$ is the calculated interception on the age axis. According to those authors, $t0$ is equal to 20% of gestation length in species that present between 100 and 400 days of pregnancy. To use that equation, we considered a mean gestation length of 159 days [10], and a mean weight of 1245 g at birth, taking into account the weight stabilization in fetuses in more advanced stages of gestation ($\geq$34.2 cm TDL).

Logistic regressions were applied to estimate the probability of occurrence of each external morphological characteristic in relation to TDL using the software Statistica 8.0 (StatSoft Inc., Tulsa, USA). Multiple regression modeling relationships between the TDL and biometric measures and absolute and relative organ volumes were conducted using the software CurveExpert 2.4 (© Copyright 2017, Daniel G. Hyams), which defined those functions that best fitted to the plots. Regressions were also used to assess allometric relationships between BPD and OFD, TC and AC, HL and FL, TL and PL, and to assess the trends in the relative volume of each organ according to the log total visceral volume, considering both fetuses and adults. We evaluated the biometric variation of twins across gestation using linear regressions between the average TDL and the standard deviation (SD) of TDL, body mass, CC and FL. For all biometric statistics, in cases of double and triple gestations, we used the average of each biometric parameter of all fetuses within a single gestation. For absolute measurements, we forced linear regressions to origin and only considered those functions with a starting point on or near zero, since we expected both internal and external measurements to be zero on day 0 of fetal development. We compared the relative volumes of visceral organs of larger fetuses (≥34.2 cm TDL) with those of adults by means of T-student tests.

All descriptive values of fetal measurements are expressed as the mean ± SD. Differences with a probability value of 0.05 or lower ($P < 0.05$) were considered significant.

3. Results

In the studied embryo/fetuses, the average TDL was 19.7 ± 9.5 SD cm (range 2.3–38.6 cm). The body mass was 325.9 ± 355.5 SD g (range 0.2–1288 g). The growth formula used to determine fetal age was $\sqrt[3]{W} = 0.084\,(t - 31.80)$. Both associations between gestational age and TDL ($r^2 = 0.96$, $P < 0.001$) and CRL ($r^2 = 0.96$, $P < 0.001$) presented high positive linear relationships (Fig. 1). The associations between TDL and CRL and body mass presented high coefficients of determination ($r^2 = 0.95$, $r^2 = 0.93$ and $P < 0.05$ respectively; Figs. 2 and 3). Except for femur length ($r^2 = 0.12$, $P = 0.06$), the significant associations between TDL and the standard deviation of TDL, body mass and cranial circumference in twins show that the fetal variability within the same pregnancy increases as the pregnancy advances ($r^2 = 0.16$–0.21, $P < 0.05$, Fig. 4).

The probability curves and the regression models for the occurrence of external morphological features according to TDL are presented on Fig. 5 and Table 1, respectively. Embryos and fetuses with TDL ≤2.9 cm presented genital and limb buds, but no eyelid buds or any other external fetal characteristic. Limbs, differentiated genitalia (52 females and 50 males) and opened eyelids were observed in fetuses with TDL ≥ 5.1 cm and CRL ≥ 3.5 cm. Presence of fused eyelids were observed in fetuses with TDL 6.2 cm and CRL 3.9 cm. Hooves and outer ear were observed in fetuses from 7.9 cm TDL onwards (≥6 cm CRL). The dorsal gland was first observed in fetuses with 9.4 cm TDL (≥7.4 cm CRL), and first signs of skin development from 11.5 cm TDL (≥6.6 cm CRL). Fetuses had tactile pelage from 13.8 cm TDL onwards (≥10.1 cm CRL) and covering pelage from 20.9 cm TDL onwards (≥16.0 cm CRL). Tooth eruption and opened eyelids were the last characteristics observed in advanced fetuses, from 26.4 cm TDL (≥18.4 cm CRL) and 27.8 cm TDL onwards (≥22.8 cm CRL), respectively (Fig. 6).

All associations between TDL and external biometric measures had high and significant coefficients of determination ($r^2 > 0.86$, $P < 0.05$; Fig. 2). The FL ($r^2 = 0.96$, $P < 0.01$) and OFD ($r^2 = 0.96$, $P < 0.01$) presented the best relationships with TDL. The allometric relationships showed strong interactions among all analyzed parameters ($r^2 \geq 0.94$, $P < 0.01$), but while the relationship between HL and FL showed a 1:1 proportion of growth ($r^2 = 0.96$, $P < 0.01$), the PL presented a faster growth than TL during the fetal phase ($r^2 = 0.96$, $P < 0.01$, Fig. 3).

All associations between TDL and the absolute volume of internal organs showed high and significant coefficients of determination ($r^2 \geq 0.59$, $P < 0.05$; Fig. 7). The best associations were found for the thymus ($r^2 = 0.92$, $P < 0.01$), the total visceral volume ($r^2 = 0.90$, $P < 0.01$), and the tubular gastrointestinal organs ($r^2 = 0.89$, $P < 0.01$).

All associations between the log of total visceral volume and the relative volume of internal organs, including fetuses and adults, showed high and significant coefficients of determination ($r^2 = 0.39$–0.90, $P < 0.05$; Fig. 8). The best associations were found for the relative volume of tubular gastrointestinal organs ($r^2 = 0.90$, $r = 0.95$ and $P < 0.01$), kidneys ($r^2 = 0.84$, $r = -0.91$ and $P < 0.01$), and liver ($r^2 = 0.80$, $r = -0.89$ and $P < 0.01$). The relative volume of tubular gastrointestinal organs and spleen increased during both fetal and post-natal development; the relative volume of heart, lungs and thymus presented an increasing growth during the fetal development but diminished during the post-natal development. The relative volume of the liver diminished during the fetal development and maintained the relative volume of the end of gestation during the post-natal development. Finally, the relative volume of kidneys and liver presented a constant decrease during both fetal and post-natal development (Fig. 8). Comparisons between advanced fetuses (≥34.2 cm TDL, n = 4) and adults (n = 18) showed that all organs, except the liver, presented significant differences in their relative volumes (Table 2).

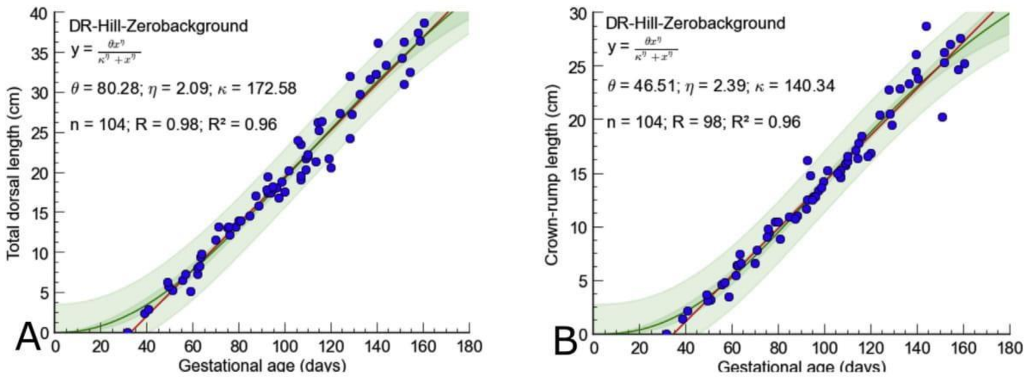

Fig. 1. Relationship between gestational age and total dorsal length (TDL) and crown-rump length (CRL) in 102 white-lipped peccary (*Tayassu pecari*) fetuses. The red line represents an expected linear trend for CRL ($y = -7.45 + 0.21x$) and TDL ($y = -9.45 + 0.29x$). (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

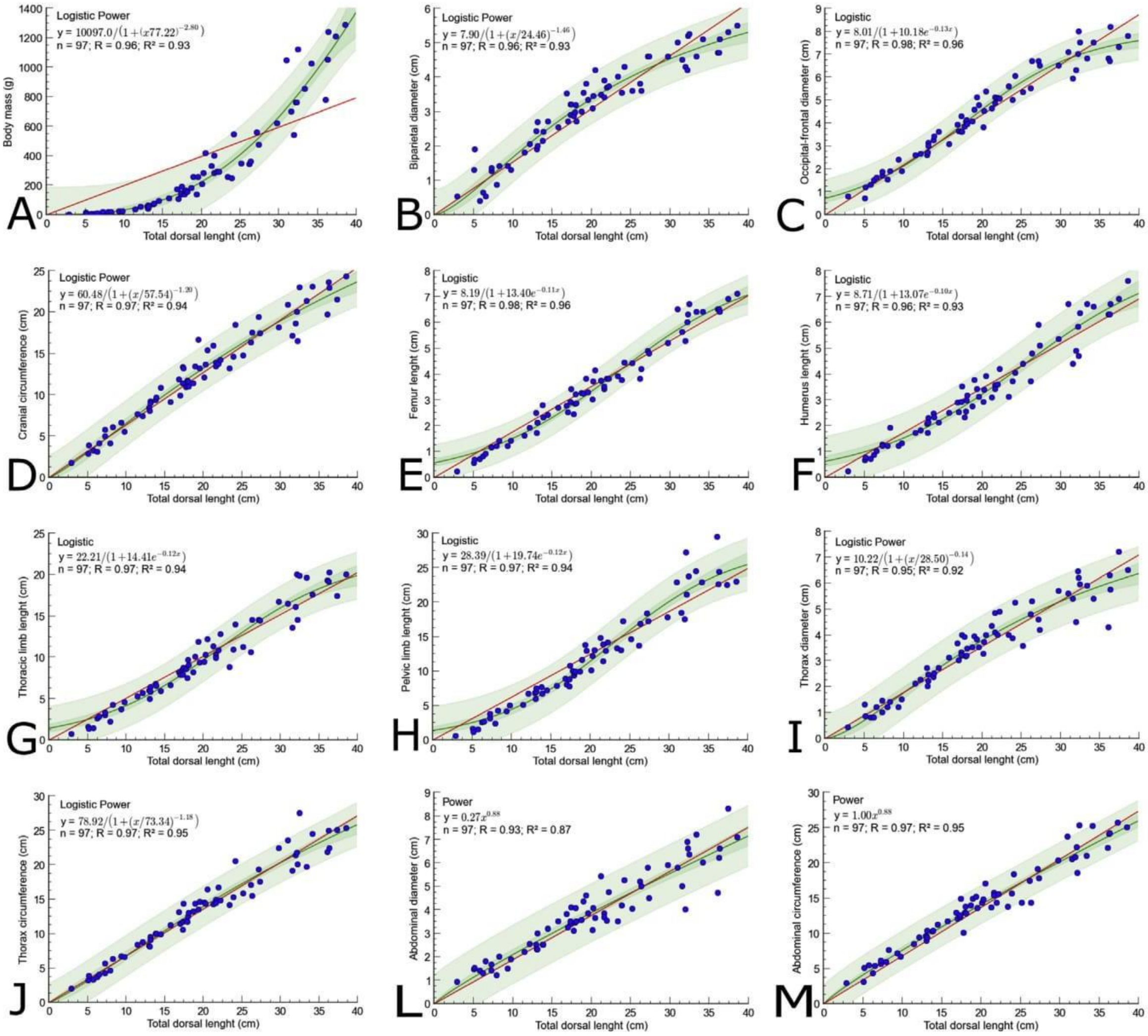

Fig. 2. Relationship between the total dorsal length (TDL) and the body mass (A), biparieral diameter (B), occipital-frontal diameter (C), cranial circumference (D), humerus (E) and femur length (F), length of thoracic (G) and pelvic limbs (H), thorax diameter (I), thorax circumference (J), abdominal diameter (L) and abdominal circumference (M) in 97 white-lipped peccary (*Tayassu pecari*) embryo/fetuses. The green line represents the model best fitted to the plots, while the red line represents an expected linear trend with no intercept. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

4. Discussion

This study describes important features and steps of the fetal development in the white-lipped peccary, which are useful to develop appropriate reproductive management practices for endangered species both in captivity and in the wild. As most ungulates, white-lipped peccary neonates present characteristics of high precociality, with developed structures for the autonomous postnatal survival and the low dependence on parental care. For instance, all external morphological characteristics were already present in fetuses at the end of gestation. In addition, according to Huggett and Widdas [20], the specific fetal growth velocity in the white-lipped peccary ($a = 0.084$) is similar to the paca, another precocial Neotropical mammal ($a = 0.082$ [17]), and two-fold the velocity found in altricial primates such as the woolly monkey ($a = 0.042$) [18].

Eyelids are absent in embryos embryos at 40 days (25.1% gestation length) and are already fused from pregnancy day 49 onwards (30.8% gestation length), whereas eyelid opening occurs around pregnancy day 125 (78.6% gestation length). This result is very similar to that reported for domestic pigs, for which eyelid formation occurs on pregnancy day 28 (24.3% gestation length), fused eyelids on pregnancy day 50 (43.4% gestation length), and opened eyelids on pregnancy day 90 (78.2% gestation length) [21]. The opening of eyelids in the prenatal phase is also frequent in other Neotropical precocious species such as the lowland paca (*Cuniculus paca*), which shows opened eyelids at the final phase of gestation (95% of gestational length) [17]. This phenomenon has direct implications for the independence of the individual during the postnatal phase, determining the level of sensorial processing and the ability to receive stimuli from the environment [22,23]. The evolution of the ability to receive environmental stimuli is also influenced by the early formation of the outer ear on pregnancy day 62 (39.0% gestation length), and the tactile pelage on pregnancy day

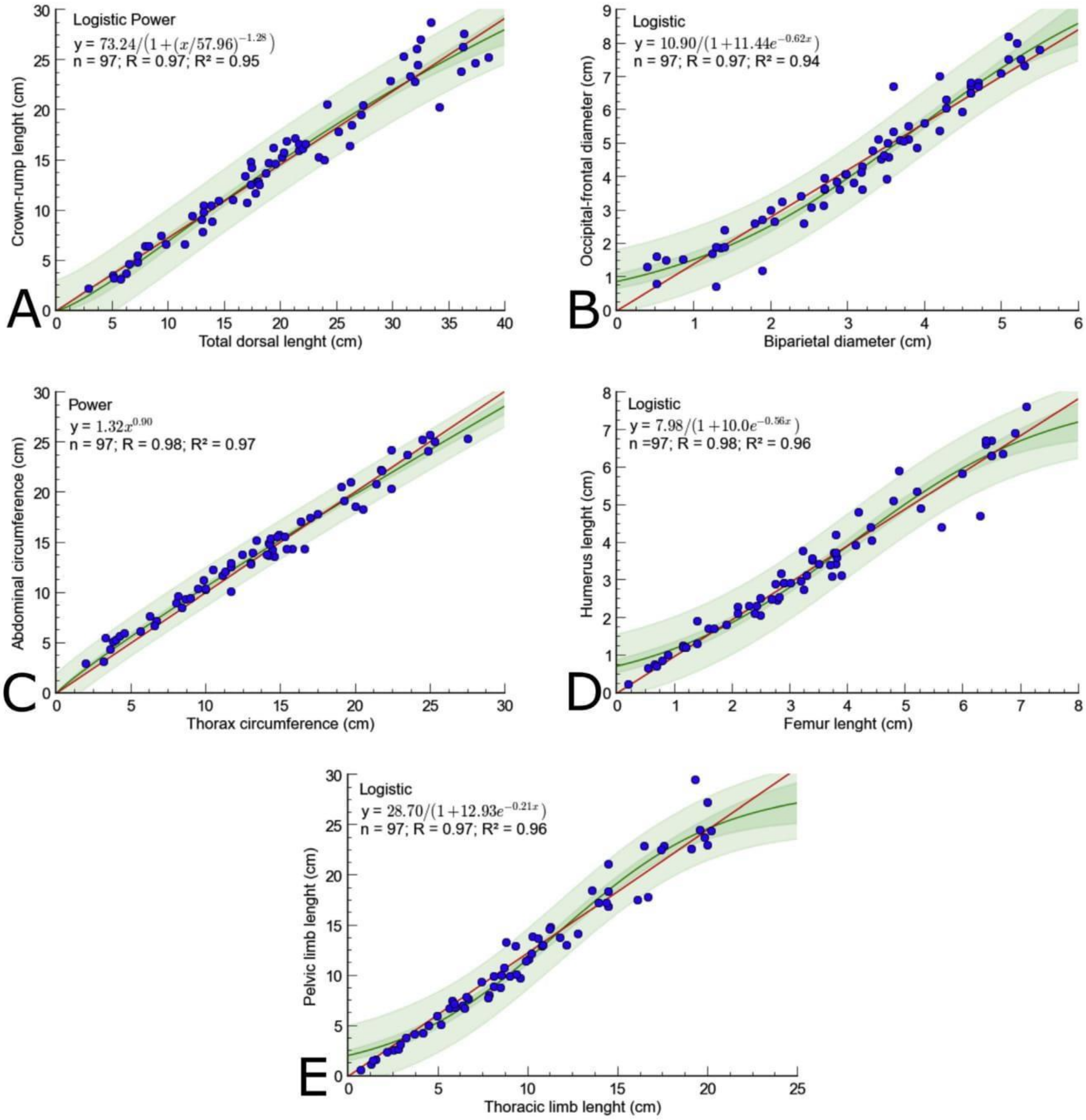

Fig. 3. Allometric relationship of the total dorsal length *versus* crown-rump length (A), biparietal diameter *versus* the occipital-frontal diameter (B), thorax circumference *versus* abdominal circumference (C), humerus length *versus* femur length (D), and length of thoracic limbs *versus* length of pelvic limbs (E) in 97 white-lipped peccary (*Tayassu pecari*) embryo/fetuses. The green line represents the model best fitted to the plots, while the red line represents an expected linear trend with no intercept. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

80 (50.3% gestation length) in this species. The early development of the sensory system is of paramount importance for both prey and predator species to locate imminent dangers, food sources and communicate with individuals from the same social group [24,25].

The dorsal gland is formed before the skin, around pregnancy day 62 (38.9% gestation length), similar to observations in the collared peccary (60th day; 43.5% gestation length) [26]. This structure located between the skin and the subcutaneous lumbar fascia, is related to recognition of the individuals within the group, and used for territory demarcation and signaling the existence of threats to the group [27,28].

In peccaries, the covering pelage acts in thermoregulation and as external protection, because of its rigid structure [29]. In the white-lipped peccary, the initial growth of the covering pelage is around the day 120 of pregnancy (75.5% gestation length), and this characteristic is fully formed at the end of gestation. Similarly, in the collared peccary, first signs of covering pelage were observed on the 100th day of gestation (72.5% gestation length), and it is fully formed at the end of gestation [29]. In domestic pigs, hair follicles are visible in the first third of gestation, from the day 28 of pregnancy (24.3% gestation length) [22]. In contrast, in the woolly monkey (*Lagotrhix poeppigii*), an altricial species, the formation of covering pelage is only completed around the 112th day of the postnatal development, and during the first 4 months of age

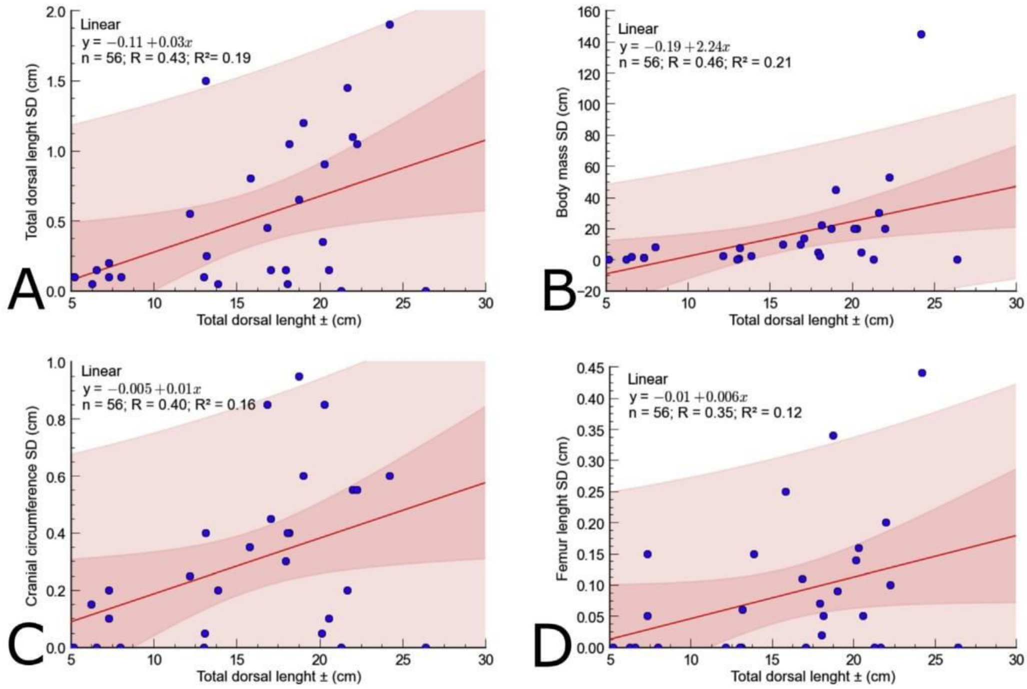

Fig. 4. Relationship between the average total dorsal length (±), and standard deviation (SD) of the total dorsal length (A), body mass (B), cranial circumference (C) and femur length (D) in 56 fetuses of white-lipped peccary (*Tayassu pecari*) from double gestations.

thermoregulation depends on the maternal contact [18].

Dentition in newborns allows the early foraging and the maternal independence [23,26]. In the white-lipped peccary, canines were the first teeth to erupt at around pregnancy day 116 (72.9% gestation length). In the collared peccary, canine eruption begins later, from pregnancy day 120 (86.9% gestation length), the upper and lower canines and lower incisors were observed just after birth, and few days after birth the entire deciduous dentition is formed [26,30]. In both peccary species, solid food intake starts between 4 and 6 weeks postnatal and, after 2 weeks, the young is already prepared to be weaned [31].

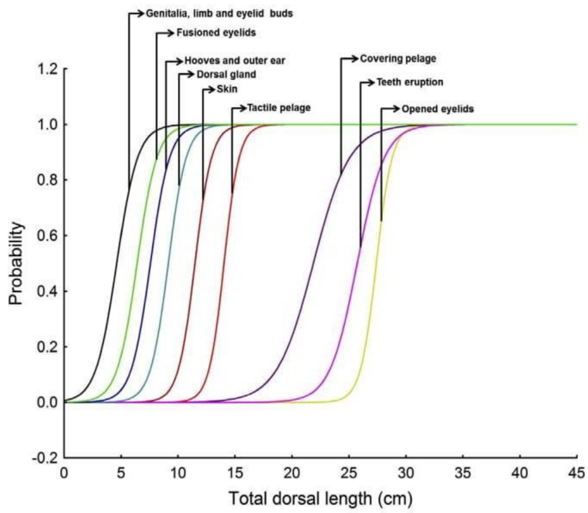

Fig. 5. Probability curves for external morphological features along the increase in total dorsal length (TDL) in 104 embryos/fetuses of white-lipped peccary (*Tayassu pecari*).

White-lipped peccary precociality is related to early autonomous functionality of structures in newborns to offer early after-birth control of thermoregulation, nutrition, locomotion and sensorial processes. The species has few natural predators, such as large felines (*Puma concolor* and *Panthera onca*) [7] and a low predation rate of adult individuals [32]. Since newborns and juveniles are the most vulnerable prey for natural predators, the white-lipped peccary produces precocial newborns with an early autonomous functionality, which allows the detection and the response against predation. Additionally, the early independence of newborns avoids prolonged energetic costs for the mother and results in shorter intervals between births when compared to altricial species [15,18].

Ultrasound examination has been widely used and generated important reproductive knowledge for the detection of pathological processes related to gestation in the collared peccary [15,33,34]. However, reproductive information is still scarce in the studied species, making this critical for the *in situ* and *ex situ* reproductive management of the species. The fetal measures provided here can be used as standard estimates for ultrasound examination and assessment of fetal development. Ultrasonography routinely uses CRL as a standard measure for fetal assessments [15]. However, TDL is not influenced by fetal positioning, and our study shows a very high association between TDL and CRL, and TDL and gestational age, allowing its use for ultrasound purposes. The low standard deviation in measurements between twins shows that fetal development in double pregnancies of the white-lipped peccary is more synchronized in comparison to the domestic pig [35,36], probably because of its lower prolificity.

Table 1
Logistic equations for the external morphological features parameters in 104 white lipped peccary (*Tayassu pecari*) embryo/fetuses.

Morphological features	Equation	Chi-square (Df)	P value
Genitalia, limb and eyelid buds	$y = \frac{2.72^{(-50416+(1.09075)*x)}}{1+2.72^{(-5.0416+(1.0975)*x)}}$	20.10 (1)	<0.01
Fused eyelids	$y = \frac{2.72^{(-7.3166+(1.14252)*x)}}{1+2.72^{(-7.3166+(1.14252)*x)}}$	31.85 (1)	<0.01
Hooves and outer ear	$y = \frac{2.72^{(-8.9679+(1.18409)*x)}}{1+2.72^{(-8.9679+(1.18409)*x)}}$	43.17 (1)	<0.01
Dorsal gland	$y = \frac{2.72^{(-11.595+(1.26777)*x)}}{1+2.72^{(-11.595+(1.26777)*x)}}$	57.37 (1)	<0.01
Skin	$y = \frac{2.72^{(-15.647+(1.36188)*x)}}{1+2.72^{(-15.647+(1.36188)*x)}}$	63.28 (1)	<0.01
Tactile pelage	$y = \frac{2.72^{(-21.194+(1.50892)*x)}}{1+2.72^{(-21.194+(1.50892)*x)}}$	72.90 (1)	<0.01
Covering pelage	$y = \frac{2.72^{(-13.591+(0.621429)*x)}}{1+2.72^{(-13.591+(0.621429)*x)}}$	69.04 (1)	<0.01
Teeth eruption	$y = \frac{2.72^{(-21.464+(0.834519)*x)}}{1+2.72^{(-21.464+(0.834519)*x)}}$	72.24 (1)	<0.01
Opened eyelids	$y = \frac{2.72^{(-39.131+(1.42841)*x)}}{1+2.72^{(-39.131+(1.42841)*x)}}$	72.44 (1)	<0.01

The liver is the first fetal organ responsible for the erythropoietic function [37], which explains the presence of a large liver during the early fetal development. In the white-lipped peccary, the relative volume of the liver decreased from 61.6% (0.11 ml liver/g fetus) to 17% (0.01 ml liver/g fetus) between pregnancy days 70 and 159 (44.0% and 100.0% gestation length), and did not differ to the mean relative volume in adults (18.1%). In domestic pigs, the relative weight also decreases from 4.2% respect to the fetal body at pregnancy day 72 (62.6% gestation length) to 2.9% at 110 day (95.6% gestation length) [38]. The deceleration in liver relative growth is associated with the substitution of hepatic prenatal erythropoiesis by medullary erythropoiesis in the postnatal phase [39].

The gastrointestinal tract in the white-lipped peccary presented a relative increase from 15.2% to 38.5% between pregnancy days 70 and 159 (44.0% and 100.0% gestational length), presenting an accelerated growth between pregnancy days 106 and 159, reaching a 58.8% relative volume in adulthood. In the domestic pig, a similar growth was observed during the last third of gestation (76th –115th day) [38]. In adult peccaries, due to the great ingestion of plant material, the multicavity stomach performs a fermentative process, and its anatomy resembles the stomach of true ruminants with glandular and non-glandular portions and two blind sacks [40,41]. Comparatively, the stomach accounts for most of the weight and volume of the digestive tract, weighing about 1.54% of body weight in the white-lipped peccary and 0.64% in the domestic pig [41]. The development of the digestive tract in final stages of gestation is justified by the need of the neonate to perform the digestion of breast milk and solid food after weaning [42]. Finally, in ruminants and domestic pigs, the gastrointestinal tract develops, and its relative size increases as soon as the animals start ingesting solid matters [43].

The relative heart volume in the white-lipped peccary passes from 3.0% (0.63 ml heart/g fetus) to 8.1% (0.59 ml heart/g fetus) between pregnancy days 70 and 159 (44.0% and 100.0% gestation length, respectively), and the relative volume of the adult heart decreases to 5.2%. Similar results were observed in the domestic pig, since the heart showed a 0.7% and 0.6% weight respect to the fetal body mass between pregnancy days 72 (73.1% gestation length) and 115 (100.0% gestation length) [44]. Due to genetic selection processes in order to maximize meat production, the heart of pigs <45 kg reduced to 0.4% of body mass, while the same organ represents 0.2% in pigs >200 kg, resulting in cardiovascular anomalies that can lead stressed animals to death [45]. The similar relative weight of the heart in newborns of both species suggests that the white-lipped peccary may be also predisposed to cardiovascular overload.

From day 70 of gestation (0.02 ml lungs/g fetus) to day 159 (0.03 ml lungs/g fetus), the relative volume of the respiratory tract in the white lipped peccary remained constant, ranging from 0.02 to 0.03 ml lungs/g fetus. Similarly, in the domestic pig during the fetal phase, the lungs present a constant relative weight, ranging from 3.35% (pregnancy day 75, 44.0% gestational length) to 3.36% (110 day, 98.6% gestational length) in relation to the fetal mean weight [38]. The growth of the lungs in the postnatal phase is related to the onset of respiratory function (absent in the fetal period) and the maturation of the alveolar system in adulthood [46].

The relative volume of the spleen showed a constant growth during the fetal phase, from 0.3% to 70 days of gestation (0.5 ml spleen/kg fetus) to 4.7% at the end of gestation (2 ml spleen/kg fetus), decreasing in adult phase to 2.7%. In contrast, the relative spleen weight in the domestic pig decreases during the second half of pregnancy [44] and first 5 months of the post-natal development [47]. The increase of the splenic volume in the fetal phase in the mammals is related to the erythropoietic function, shared with the liver and bone marrow in this period, while in the postnatal phase the spleen function is related to the control of erythrocyte cell activities and the induction of immune reactions against systemic [48].

The relative volume of kidneys decreased during fetal phase from 9.5% (10 ml kidneys/kg fetus) at pregnancy day 70 (44.0% gestation length) to 4.9% (4 ml kidneys/kg fetus) at the end of gestation (100.0% gestational length); in adult animals, the kidneys represent 2.5% of the mean visceral volume. In the domestic pig, between day 75 (60.8% gestational length) to day 110 (95.6% gestational length) the relative weight remains constant [38]. During the fetal phase, the kidneys perform the excretion of hypotonic urine inside the aminiotic cavity [49]. In the domestic pig, a continuous formation of nephrons is observed up to the 21th day postnatal, after which the kidneys undergo the differentiation process of the existing nephrons [50].

In the white-lipped peccary, the thymus shows a constant growth during the fetal period, and is no longer detected during the adult phase. Similarly, in the domestic pig, the thymus increases during the fetal phase and the first 3 months of life, presents slight weight gain during the pre-pubertal phase (3–6 months), diminishes during the pubertal phase (10–18 months), and disappears in the adulthood [51]. The thymus is involved in the

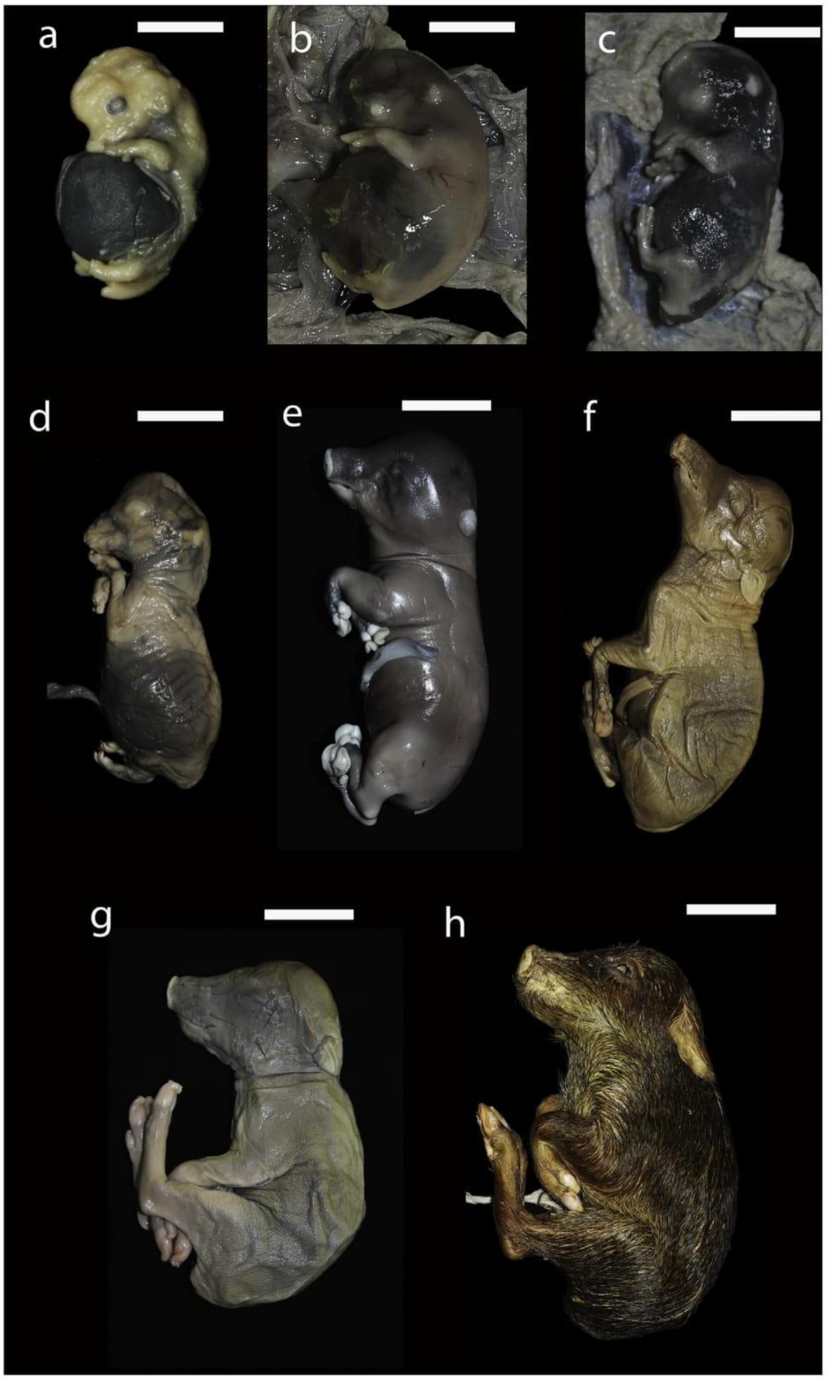

Fig. 6. Embryos and fetuses of white-lipped peccary (*Tayassu pecari*) at different stages of development according to total dorsal length (TDL): (a) Embryo of 2.9 cm TDL and 0.4 g, presenting genital and limb buds, but no eyelid buds and any other external fetal characteristic (bar: 1 cm); (b) Fetus of 5.2 cm TDL and 4.3 g, presenting eyelid buds, differentiated limbs and genitalia and opened eyelids (bar: 1 cm); (c) Fetus with 6.0 cm TDL and 5.3 g, presenting eyelid buds in the final stage of development, and the initial development of hooves and the outer ear (bar: 2 cm); (d) Fetus with 8.0 cm TDL and 21.5 g, presenting fused eyelids, and the initial development of hooves and the outer ear (bar: 2 cm); (e) Fetus with 11.6 cm TDL and 35.8 g, presenting fused eyelids, the initial development of skin and tactile pelage, and developed hooves and the outer ear (bar: 2.5 cm); (f) Fetus of 13.8 cm TDL and 65 g, presenting developing skin and tactile pelage (bar: 3 cm); (g) Fetus of 23.1 cm TDL and 250 g, presenting the initial development of covering pelage (bar: 4 cm); (h) Fetus of 36.8 cm TDL and 1320 g, showing all fetal external characteristics, including opened eyelids (bar: 5 cm).

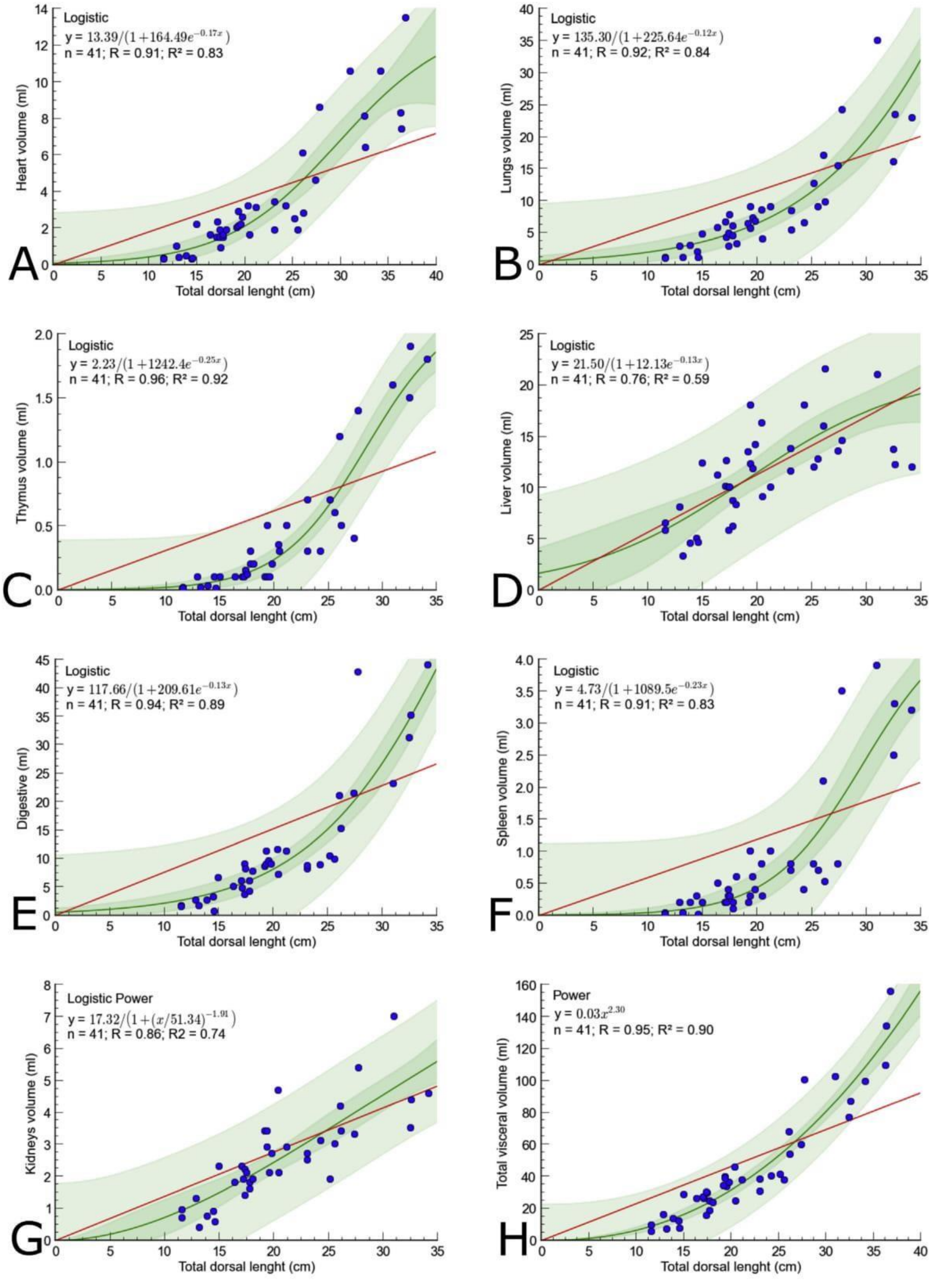

Fig. 7. Relationship between the absolute volume of the heart (A), lungs (B), thymus (C), liver (D), digestive tract (E), spleen (F), kidneys (G), and total visceral tissues (H), with the total dorsal length (TDL) in 41 white-lipped peccary (*Tayassu pecari*) fetuses. The green line represents the model best fitted to the plots, while the red line represents an expected linear trend with no intercept. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

maturation of the immune system, and regresses when the bone marrow becomes the main responsible for the animals' immunity [39].

The present study describes the most important events during the external and internal fetal development in the white-lipped peccary, showing that it is a highly precocial species compared to

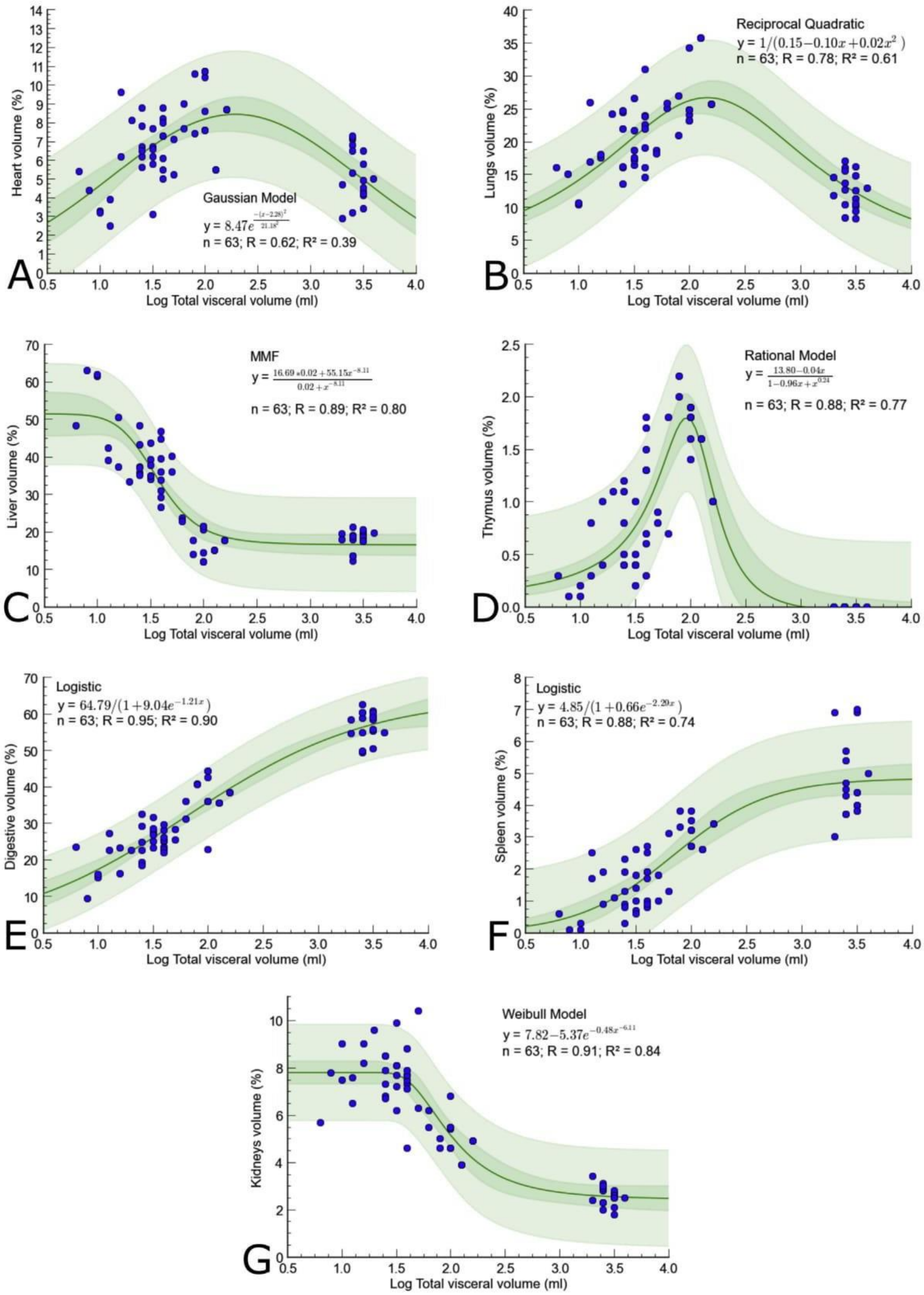

Fig. 8. Relationship between the relative volume of the heart (A), lungs (B), liver (C), thymus (D), tubular digestive organs (E), spleen (F), and kidneys (G) with the log of total visceral volume in 41 fetuses and 18 adults of white-lipped peccary (*Tayassu pecari*).

Table 2
Comparison between the absolute and relative volumes of the visceral organs of white-lipped peccaries (*Tayassu pecari*) in advanced pregnancy stage (total dorsal length ≥ 34.2 cm; n = 04) and adulthood (n = 18).

Organ	Absolute volume ± SD (ml)		Relative volume ± SD (%)		T value	F value	df	P value
	Fetus	Adult	Fetus	Adult				
Heart	9.95 ± 2.36	147.07 ± 41.77	8.11[a] ± 1.87	5.20[b] ± 1.43	−3.37	0.44	20	<0.01
Thymus	1.90 ± 0.21	0.00 ± 0.00	1.58 ± 0.34	0.00 ± 0.00	0.00	0.00	0.00	0.00
Lungs	34.55 ± 9.98	351.93 ± 83.16	27.37[a] ± 4.96	12.55[b] ± 2.74	−7.97	0.22	20	<0.01
Liver	20.88 ± 5.77	517.78 ± 132.99	16.62[a] ± 3.48	18.09[a] ± 2.50	0.96	0.38	20	0.34
Tubular gastrointestinal organs	47.75 ± 7.65	1613.61 ± 307.65	38.58[a] ± 3.52	56.85[b] ± 3.97	8.30	0.95	20	<0.01
Kidneys	5.88 ± 1.16	72.12 ± 13.10	4.73[a] ± 0.58	2.56[b] ± 0.38	−9.02	0.32	20	<0.01
Spleen	3.75 ± 0.91	133.89 ± 38.24	2.99[a] ± 0.33	4.72[b] ± 1.19	2.82	9.85	20	<0.01
Total visceral volume	124.65 ± 21.98	2836.40 ± 491.61	–	–	–		–	–

Values appearing in rows with different letters are significantly different (P < 0.05).

the domestic pig, probably due to the domestication and zootechnical selection process suffered by the domestic pig. Although the white-lipped peccary does not have many natural predators, newborns are usually targeted prey for large felines. In this sense, the white-lipped peccary presents a high morphological preparation for the early autonomous functionality of newborns in terms of thermoregulation, nutrition, locomotion and sensorial process, and consequently, a relative ability to detect and escape from predators, reducing the predation rate of young animals. The information presented in this study will serve to assist the reproductive and clinical management of white-lipped peccaries, both in captivity and in the wild, contributing to the conservation of this endangered species.

Supplementary video related to this article can be found at https://doi.org/10.1016/j.theriogenology.2018.07.006.

Acknowledgments

We sincerely thank all the people from the community of Nueva Esperanza in the Yavarí-Mirín River, and from the communities of Nova Jerusalém, Boa Esperança, Bom Jesus do Baré, São José do Urini and Belo Monte in the Amanã Sustainable Development Reserve, who actively participated in data collection, showing that communal participation is an important step in the development of wildlife management. The authors have no conflict of interest to declare. We are especially thankful for the institutional support provided by the Instituto de Investigaciones de Trópico y de Altura, the Museo de la Universidad Nacional de la Amazonía Peruana and the Dirección General de Flora y Fauna Silvestre from Peru. This work was supported by the National Council of Technological and Scientific Development (CNPq, protocol 452908/2016-7, 201475/2017-0 and Edital nº 016/2014 PPP-CNPq), Fundação de Amparo à Pesquisa do Estado do Amazonas (FAPEAM, Edital nº 016/2014 PPP-CNPq) and the Earthwatch Institute. We would like to thank Mr. Walfrido Monteiro (https://www.behance.net/WalfridoMonteiro) for the video produced with 3D fetal images attached as supplementary materialof this paper.

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3. ANEXOS

Anexo 1. Normas para formatação da revista Pan-Amazônica de Saúde - RPAS, na qual será submetido o primeiro capitulo desse trabalho.

"A RPAS é de acesso aberto e não são cobradas quaisquer taxas de submissão/publicação. Segue orientações do International Committee of Medical Journal Editors (ICMJE) e os princípios da ética na publicação contidos no código de conduta do Committee on Publication Ethics (COPE).

TIPOS DE CONTRIBUIÇÕES ACEITAS

1. Artigo original – trabalho de pesquisa original e inédita, que contribua para o desenvolvimento do conhecimento em uma área específica. Deve ser estruturado, obrigatoriamente, em seis seções principais: Introdução, Materiais e métodos, Resultados, Discussão, Conclusão, Referências (máximo de 4.000 palavras, excluindo resumos, figuras/tabelas e referências).

2. Artigo histórico – descreve um episódio passado ou uma personalidade que representou grande relevância à área da saúde em um campo de pesquisa, uma profissão, uma descoberta e outros. Texto dividido em seções definidas pelo autor, com títulos e subtítulos de acordo com a abordagem do assunto (máximo de 5.000 palavras, excluindo resumos, figuras/tabelas e referências).

3. Artigo de revisão

3.1) Revisão sistemática – revisão planejada com base no resultado de estudos originais, que procura responder, de forma sintetizada, a um objetivo específico. Descreve, criticamente e em detalhes, os procedimentos empregados na busca, seleção, análise e síntese dos dados dos estudos incluídos na revisão e que são os mais significativos ao tema abordado. Sugere-se observar o PRISMA Statement (Preferred Reporting Items for Systematic Reviews and Meta-Analyses), disponível em inglês e português. Deve incluir as seções: Introdução, Métodos, Resultados, Discussão, Conclusão, Referências (máximo de 5.000 palavras, excluindo resumos, figuras/tabelas e referências).

3.2) Revisão narrativa – análise crítica, de caráter descritivo-discursivo sobre tema de interesse científico à área da saúde. Deve ser elaborada somente por pesquisadores com vasta experiência na temática abordada (máximo de 5.000 palavras, excluindo resumos, figuras/tabelas e referências).

4. Comunicação – artigo curto sobre um trabalho científico que ainda está sendo realizado, relatando resultados preliminares de pesquisa. Tem a função de dar conhecimento à comunidade científica sobre o andamento de novas descobertas, de maneira rápida, garantindo prioridade ao autor. Deve incluir as mesmas seções que um artigo original (máximo de 2.000 palavras, excluindo resumos, figuras/tabelas e referências).

5. Relato de caso – descrição de caso clínico importante e bem documentado do ponto de vista clínico e laboratorial. Deve conter Introdução (com breve revisão da literatura), Descrição do Caso, Discussão, Conclusão, Referências (máximo de 3.000 palavras, excluindo resumos, figuras/tabelas e referências).

6. Nota técnica – contempla guias, sínteses de manuais, recomendações institucionais e roteiros (máximo de 3.000 palavras, excluindo resumos, figuras/tabelas e referências).

7. Carta ao Editor – manifestação de opinião de leitores sobre artigos publicados pela Revista. Deve trazer comentário consubstanciado sobre tema publicado e/ou sobre assunto polêmico (máximo de 1.000 palavras, excluindo resumos, figuras/tabelas e referências).

CRITÉRIOS DE AUTORIA E RESPONSABILIDADE DOS AUTORES

Os critérios de autoria baseiam-se nas resoluções do ICMJE. A autoria reconhecida fundamenta-se em contribuição substancial, relacionada aos seguintes aspectos: (i) idealização e desenho do estudo, análise e interpretação dos dados; (ii) redação ou revisão crítica relevante do conteúdo intelectual do manuscrito; (iii) revisão e aprovação final da versão a ser publicada; e (iv) responsabilidade por todos os aspectos do trabalho, incluindo a garantia de sua precisão e integridade. Todos os designados como autores devem atender a esses critérios.

No ato da submissão, o manuscrito deve ser acompanhado da Declaração de Responsabilidade, assinada por todos os autores, na qual assumem participação na elaboração intelectual de seu conteúdo e responsabilizam-se pela veracidade e originalidade do trabalho, além de atestar que o estudo não foi publicado anteriormente, parcial ou integralmente, nem encaminhado para publicação por outro periódico. Para autores de diferentes locais, é possível enviar cópias assinadas em separado.

FONTES DE FINANCIAMENTO

As fontes de financiamento – privado ou institucional – e o fornecimento de equipamentos, materiais e insumos à pesquisa de forma gratuita ou com desconto devem ser declarados pelos autores no tópico “Apoio Financeiro” dentro do manuscrito.

CONFLITO DE INTERESSES

Conflitos de interesses podem surgir quando autores, revisores ou editores possuem interesses – aparentes ou não – capazes de influenciar no processo de elaboração ou avaliação dos manuscritos. Esses conflitos podem ser de natureza pessoal, comercial, política, acadêmica ou financeira, razão pela qual os autores devem reconhecê-los e revelá-los, quando presentes, no tópico “Conflito de Interesses” dentro do manuscrito.

ASPECTOS ÉTICOS

Trabalhos envolvendo seres humanos ou animais

Devem ter a aprovação dos Comitês de Ética em Pesquisa (CEP) da instituição onde a pesquisa foi realizada e cumprir os princípios éticos contidos na Declaração de Helsinki. Para pesquisas realizadas com seres humanos no Brasil, os autores devem observar as normas constantes na Resolução CNS nº 466, de 12 de dezembro de 2012, do Conselho Nacional de Saúde, além de atender à legislação pertinente. Enviar cópia do documento de aprovação no momento da submissão do manuscrito. Informar, na seção “Materiais e Métodos”, o nome do CEP, o número do protocolo e a data da aprovação do projeto. Informar também se os pacientes incluídos nos estudos assinaram um Termo de Consentimento Livre e Esclarecido e se nesse consta o compromisso de preservação da privacidade dos pacientes. Os casos omissos serão analisados pelos Editores.

Proteção dos direitos e privacidade dos pacientes que participam de pesquisas

Informações que possam identificar os participantes da pesquisa ou do relato de caso clínico não serão publicadas, a menos que seja essencial para os propósitos científicos, e o paciente ou seu responsável conceda permissão, por escrito, para a publicação. O consentimento, por escrito, para esses propósitos exige que se mostre ao paciente ou responsável o manuscrito a ser publicado. Na publicação deverá constar que se obteve a autorização. Na busca do anonimato, nunca alterar nem falsificar os dados do paciente. Omitir os detalhes que sirvam para identificar as pessoas, caso não sejam essenciais. Não usar o nome do paciente, suas iniciais ou registro que lhe tiver sido conferido no hospital, especialmente no material ilustrativo.

Padrões para apresentação de resultados de pesquisa clínica

Para manuscritos que apresentarem resultados parciais ou integrais de pesquisas clínicas, recomenda-se a adoção dos seguintes padrões indicados pelo ICMJE e pela Equator Network:

- Ensaio clínico randomizado: CONSORT (checklist e fluxograma)
- Revisões sistemáticas e metanálises: PRISMA (checklist e fluxograma)
- Estudos observacionais em epidemiologia: STROBE (checklist)
- Relatos de casos: CARE (checklist)
- Estudos qualitativos: COREQ (checklist)

IDIOMA DO MANUSCRITO E TRADUÇÕES

Os textos podem ser submetidos em português, inglês ou espanhol. O Núcleo Editorial encarrega-se da tradução para os outros idiomas, sem custo aos autores.

FORMA DE APRESENTAÇÃO DOS ORIGINAIS

Estrutura geral do documento

Os trabalhos deverão ser apresentados: em um arquivo .doc/.docx (padrão Microsoft Word); digitados para papel tamanho A4; com tipo de fonte Times New Roman, tamanho 12 pt; com espaçamento simples entre linhas e 6 pt para parágrafos em todo o texto; e margens superior, inferior, esquerda e direita igual a 3 cm. Cada arquivo (texto, figuras, documentos, etc.) não deve ultrapassar 5 MB.

Primeira página

Área do conhecimento: para facilitar a designação do artigo por tema e Editor, informar em qual área temática o artigo melhor se enquadra. Escolher dentre uma das seguintes:

1) Antropologia Médica
2) Bacteriologia e Micologia
3) Biomarcadores e Bioindicadores
4) Desenvolvimento Tecnológico e Inovação em Saúde
5) Educação em Saúde e Educação Ambiental
6) Entomologia
7) Farmácia
8) Imunologia
9) Odontologia
10) Parasitologia
11) Psiquiatria
12) Saúde e Meio Ambiente
13) Saúde Pública e Epidemiologia
14) Virologia e Arbovirologia

Título: deve ser conciso, informativo e atrativo, de modo que o tema e a área do conhecimento sejam imediatamente reconhecidos. Quando citado o nome de cidade e estado, inserir também o nome do país. Deve ser apresentado centralizado, em negrito e em minúsculo, a exceção de início de frase e nomes próprios.

Autoria: informar os nomes completos de todos os autores, sem abreviação (ao centro e em negrito). Abaixo do nome de cada autor deve conter sua respectiva afiliação completa (ao centro, normal), respeitando-se a hierarquia do órgão, seguida da indicação da cidade, do estado e do país de origem, além do ORCID e do e-mail do referido autor (Ex.: Instituto, Departamento, Laboratório, Cidade, Estado, País – https://orcid.org/0000-0003-3517-2227 – email@mail.com).

Resumo: deve ser apresentado no mesmo idioma do texto, digitado em um único parágrafo, com até 250 palavras. Para manuscritos do tipo Artigo Original, Comunicação e Revisão Sistemática, o resumo deve ser estruturado nas seguintes seções: Objetivo, Materiais e Métodos, Resultados, Conclusão.

Palavras-chave: indicar de três a seis termos que mais representem o conteúdo central da pesquisa. Sugere-se o uso do vocabulário estruturado de Descritores em Ciências da Saúde (DeCS), criado pelo Centro Latino-Americano e do Caribe de Informação em Ciências da Saúde (BIREME) com o objetivo de padronizar uma linguagem única de indexação, facilitando a recuperação de documentos científicos.

Endereço para correspondência: informar o nome e o endereço para correspondência do autor responsável pelo contato sobre o trabalho. Deve conter nome completo, endereço completo (preferencialmente institucional), telefone e e-mail.

Corpo do Texto
A estrutura do texto deverá obedecer às orientações de cada categoria de trabalho já descritas anteriormente, de modo a garantir uma uniformidade e padronização dos textos apresentados pela Revista.

Introdução – apresentação do problema, justificativa e objetivo do estudo, nessa ordem, em texto corrido, sem inserir subtópicos.

Materiais e Métodos – deve conter o detalhamento dos materiais utilizados, dos métodos aplicados e, quando pertinente, a descrição e o cálculo do tamanho da amostragem, os procedimentos de coleta de dados, entre outros, de modo que outro pesquisador possa repetir o estudo com os dados fornecidos. Técnicas padronizadas bastam ser referenciadas. No caso de estudo envolvendo seres humanos ou animais, observar o item Aspectos Éticos destas Instruções.

Resultados – apresentação dos dados obtidos com a pesquisa, sem interpretá-los ou discuti-los. Podem ser incluídas tabelas e figuras, as quais devem ser autoexplicativas e possuir chamada inserida no corpo do manuscrito, além de oferecer uma leitura direta, simples e clara (ver o item Ilustrações destas Instruções).

Discussão – deve apresentar a análise crítica dos resultados, suas implicações e limitações, confrontando-os com os resultados de outras publicações de relevância para o tema.

Conclusão – deve evidenciar o que foi alcançado com o estudo, relacionando os resultados obtidos com as hipóteses levantadas e sugerindo, quando necessário, outros estudos que complementem a pesquisa ou recomendações de ordem prática.

Agradecimentos – (opcional) contribuição de pessoas e/ou organismos que prestaram colaboração técnica e/ou intelectual à pesquisa.

Apoio Financeiro – indicação da existência de financiamento ao desenvolvimento da pesquisa por órgão ou instituição de fomento. Não abreviar nomes de instituições.

Conflito de Interesses – informar a existência ou não de possíveis formas de conflitos de interesse. Essa informação será publicada, caso o trabalho seja aceito.

Referências

Para citação das referências no texto, deve ser utilizado o sistema de chamada numérico sequencial (ordem de aparecimento no texto), que corresponde ao número sobrescrito, sem parêntese, disposto imediatamente após o trecho a que se refere. No caso de mais de uma citação para o mesmo trecho, os números devem ser separados entre si por vírgulas. Evitar o excesso de citação em alguns trechos, elegendo sempre as mais relevantes (máx. 6). As citações diretas (transcrições) serão aceitas apenas em artigos históricos e de antropologia médica; em outros tipos de artigos, só serão aceitas até três linhas, desde que inseridas no texto (aspadas e sem utilização de recuo).

As referências devem ser listadas ao final do manuscrito, em ordem sequencial numérica, conforme ordem de aparecimento no texto; e normalizadas segundo os Requisitos Uniformes para Manuscritos Apresentados a Periódicos Biomédicos. Em referências com mais de seis autores, deve-se listar até os seis primeiros, seguidos da expressão “et al" para os demais; títulos de periódicos deverão ser abreviados de acordo com o estilo utilizado no Index Medicus. Observar os exemplos abaixo:

a) Artigos de periódicos:

Schofield CJ, Jannin J, Salvatella R. The future of Chagas disease control. Trends Parasitol. 2006 Dec;22(12):583-8.

Carvalho RGC, Carneiro ICRS, Pinheiro MS, Pinheiro SC, Azevedo PSR, Santos SD, et al. Caracterização fenotípica e genotípica de Serratia marcescens provenientes de Unidade Neonatal de Referência em Belém, Pará, Brasil. Rev Pan-Amaz Saude. 2010 mar;1(1):101-6.

- Volume com parte:

Marcus FI. Drug interaction with amiodarone. Am Heart J. 1983;106(4 Pt 2):924-30.

- Volume com suplemento:

Mirra SS, Gearing M, Nash F. Neuropathologic assessment of Alzheimer’s disease. Neurology. 1997;49 Suppl 3:S14-6.

Vinhaes MC, Dias JCP. Doença de Chagas no Brasil. Cad Saude Publica. 2000 jan;16 supl 2:7-12.

- Número com suplemento:

Wise MS. Childhood nacolepsy. Neurology. 1998 Feb;50(2 Suppl 1):S37-42.

Mello Jorge MHP, Gawryszewski VP, Latorre MR. Análise dos dados de mortalidade. Rev Saude Publica. 1997 ago;31(4 supl):5-25.

- Em fase de impressão:

Oliveira SV, Gurgel-Gonçalves R. Análise preditiva da distribuição geográfica de hantavírus

no Brasil. Rev Pan-Amaz Saude. No prelo 2013.
Stewart WC, Geiger AC, Jenkins JN. The benefit of repeated intraocular pressure measurements in clinical trials. Arch Ophthalmol. In Press 2014.
b) Livros:

Leão RNQ, coordenador. Medicina tropical e infectologia na Amazônia. Vol. 1. Belém: Samauma; 2013.
Fletcher RH, Fletcher SW, Fletcher GS. Clinical epidemiology. 5th ed. Baltimore: Lippincott Williams & Wilkins; 2012.
- Autoria institucional:
World Health Organization. Communicable disease alert and response for mass gatherings: key considerations. Geneva: WHO; 2008.
Ministério da Saúde (BR). Secretaria de Vigilância em Saúde. Departamento de Vigilância Epidemiológica. Doenças infecciosas e parasitárias: guia de bolso. 8. ed. rev. Brasília: Ministério da Saúde; 2010.
Secretaria de Estado de Planejamento e Coordenação Geral (Mato Grosso). Informativo populacional e econômico de Mato Grosso: 2008. Cuiabá: Secretaria de Estado de Planejamento e Coordenação Geral; 2008.
- Capítulos de livro:
• Quando o autor do capítulo não é o mesmo do livro
Kapikian AZ, Hoshino Y, Chanock RM. Rotaviruses. In: Knipe DM, Howley PM, editors. Fields virology. 4th ed. Philadelphia: Lippincott Williams & Wilkins; 2001. p. 1787-833.
Dewey WL, Martin BR. Abuso de drogas. In: Craig CR, Stitzel RE. Farmacologia moderna: com aplicações clínicas. 6. ed. Rio de Janeiro: Guanabara Kroogan; 2011. p. 380-94.
• Quando o autor do capítulo é o mesmo do livro
Cefrey H. Yellow fever. New York: Rosen; 2002. Prevention and control; p. 48-56. Contanzo LS. Fisiologia. 4. ed. Rio de Janeiro: Elsevier; 2011. Neurofisiologia; p. 65-110.
c) Teses, Dissertações e Monografias:

Bührer-Sékula S. Simple dipstick assay for the detection of antibodies to phenolic glycolipid-1 of Mycobacterium leprae [thesis]. Amsterdam (NL): Royal Tropical Institute; 1998.
Queiroz ML. A hanseníase no Estado de Mato Grosso [dissertação]. Cuiabá (MT): Universidade Federal de Mato Grosso, Instituto de Saúde Coletiva; 2009. 137 p.
d) Portarias e Leis:

Brasil. Ministério da Saúde. Portaria nº 533, de 28 de março de 2012. Estabelece o elenco de medicamentos e insumos da Relação Nacional de Medicamentos Essenciais (RENAME) no âmbito do SUS. Diário Oficial da União, Brasília (DF), 2012 mar 29; Seção 1:95.
Brasil. Lei n° 9.431, de 6 de janeiro de 1997. Decreta a obrigatoriedade do Programa de Controle de Infecção Hospitalar em todos os hospitais brasileiros. Diário Oficial da União, Brasília (DF), 1997 jan 7; Seção 1:165.
e) Anais de Congresso:

Dittmar A, Beebe D, editors. 1st Annual International IEEE-EMBS Special Topic Conference on Microtechnologies in Medicine & Biology; 2000 Oct 12-14; Lyon, France. Piscataway (NJ): IEEE; 2000. 643 p.
Anais do 51º Congresso da Sociedade Brasileira de Medicina Tropical; 2015 jun 14-17; Fortaleza, Brasil. Brasília (DF): Sociedade Brasileira de Medicina Tropical; 2015. 2078 p.
f) Trabalho de evento publicado em periódico:

Ohnishi MDO, Ventura AMRS, Libonati RF, Souza JM. O pulmão na malária vivax: relato de caso. In: 44º Congresso da Sociedade Brasileira de Medicina Tropical, 2º Encontro de Medicina Tropical do Cone Sul e 3º Encontro de Medicina Tropical dos Países de Língua Portuguesa; 2008 mar 4-7; Porto Alegre, RS. Brasília (DF): Sociedade Brasileira de Medicina Tropical; 2008. p. 215. (Revista da Sociedade Brasileira de Medicina Tropical; vol. 41; supl. 1).

g) Documentos eletrônicos:

- Livros e periódicos na Internet

World Health Organization. International health regulations: 2005 [Internet]. 2nd ed. Geneva: World Health Organization; 2008 [cited 2013 Dec 11]. Available from: http://www.who.int/ihr/publications/9789241596664/en/.

Lucena AR, Velasco e Cruz AA, Cavalcante R. Estudo epidemiológico do tracoma em comunidade da Chapada do Araripe - Pernambuco Brasil. Arq Bras Oftalmol [Internet]. 2004 mar-abr [citado 2004 jul 12];67(2):197-200. Disponível em: http://www.scielo.br/pdf/abo/v67n2/19740.pdf.

- Websites e base de dados na Internet

PubMed Central [Internet]. Bethesda (MD): NCBI/NLM; 2015 [updated 2014 Aug 15; cited 2015 Feb 10]. Available from: http://www.ncbi.nlm.nih.gov/pmc/.

Instituto Brasileiro de Geografia e Estatística. Cidades@: Pará [Internet]. Rio de Janeiro: IBGE; 2015 [citado 2015 mar 23]. Disponível em: http://www.cidades.ibge.gov.br/xtras/uf.php?lang=&coduf=15&search=para.

Ilustrações

São aceitas, no máximo, seis ilustrações, que podem ser do tipo tabela, quadro, figura, gráfico ou mapa (cada arquivo não deve ultrapassar 5 MB). Caso os autores julguem essencial que uma determinada ilustração permaneça, se extrapolado o número máximo permitido, solicita-se um contato especial com o Núcleo Editorial. Cada ilustração deve ser apresentada em folha separada ao final do artigo, numerada na ordem de aparecimento no texto e conter um título sucinto, porém explicativo, bem como suas respectivas legendas (quando houver). Ilustrações que não sejam de autoria dos mesmos autores do manuscrito só serão publicadas caso o criador/produtor/autor da ilustração conceda autorização para publicação nesta Revista.

- Tabelas e quadros:

Devem complementar, e não duplicar, o texto. Recomenda-se seguir Normas de Apresentação Tabular da Fundação Instituto Brasileiro de Geografia e Estatística (IBGE). Todas as explicações devem ser apresentadas nas legendas (rodapé) da tabela/quadro e não no título, identificadas por símbolos nesta ordem: * , † , ‡ , § , ‖ , ¶ , ** , †† , ‡‡ . Não sublinhar ou desenhar linhas dentro das tabelas/quadros e não usar espaços para separar colunas. Para as células que apresentarem dado com valor igual a zero, substituir o "0" por "–" e inserir no rodapé a seguinte nota: "Sinal convencional utilizado: – Dado numérico igual a zero não resultante de arredondamento".

- Figuras:

Podem ser submetidas em preto ou em cores. Fotografias e mapas devem ser enviados sob a forma de arquivos nos formatos JPG ou TIFF, com resolução mínima de 300 dpi, para possibilitar uma impressão nítida; na versão eletrônica, a resolução será ajustada para 72 dpi. Imagens geradas em computador, como gráficos e esquemas, podem ser anexadas sob os formatos DOC, XLS, PSD, ou CDR. O Núcleo Editorial reserva-se o direito de configurar o material ilustrativo de modo mais econômico, desde que não prejudique sua apresentação. Os autores são convidados a submeter, para consideração da Revista, ilustrações de seus manuscritos que poderão vir a ilustrar a capa.

Unidades de medida
As unidades de medida devem seguir os padrões do Sistema Internacional de Unidades.

Siglas e Acrônimos
Siglas são palavras formadas a partir da redução de um grupo de palavras, onde cada letra é pronunciada separadamente (Ex.: OMS – Organização Mundial da Saúde, AVC – acidente vascular cerebral); escritas em maiúsculas, a exceção das originalmente grafadas de forma diferenciada (Ex.: CNPq – Conselho Nacional de Desenvolvimento Científico e Tecnológico).

Acrônimos são palavras formadas a partir da junção de letras ou sílabas iniciais de um grupo de palavras, formando vocábulos. São escritos apenas com a inicial maiúscula (Ex.: Fiocruz – Fundação Oswaldo Cruz, Funasa – Fundação Nacional de Saúde), a exceção das que possuem menos de quatro letras que devem ser grafadas todas em maiúsculas (Ex.: ONU – Organização das Nações Unidas).

Para siglas e acrônimos estrangeiros, recomenda-se a designação correspondente em português, se a forma traduzida for largamente aceita; ou sua utilização na forma original se não houver correspondência em português, ainda que o nome por extenso em português não corresponda à sigla. (Ex: UNESCO – Organização das Nações Unidas para a Educação, a Ciência e a Cultura, CDC – Centers for Disease Control and Prevention).

Em sua primeira aparição no texto, siglas e acrônimos deverão ser escritos por extenso e acompanhados da respectiva redução entre parênteses. A partir daí, utilizar apenas a sigla ou acrônimo no restante do texto.

Um caso excepcional é o da palavra "aids" (síndrome da imunodeficiência adquirida), que o Conselho Nacional de Aids, do Ministério da Saúde, recomenda, dada a popularização do acrônimo original "Aids – acquired immunodeficiency syndrome", que seja grafada em minúsculas, representando o nome da doença.

ENVIO DE MANUSCRITOS
Os trabalhos devem ser enviados por meio do Sistema de Submissão da Revista. Juntamente com o arquivo do manuscrito, os autores devem enviar a "Declaração de Responsabilidade", assinada por cada um deles, digitalizada em formato PDF.

Os autores podem enviar um documento à parte com a indicação de até três possíveis revisores, também especialistas no assunto abordado em seu manuscrito. É possível ainda indicar até três revisores especialistas para os quais não gostariam que seu manuscrito fosse submetido. Caberá aos Editores a decisão de acatar ou não as sugestões dos autores.

AVALIAÇÃO E PUBLICAÇÃO
Depois de submetido, o trabalho passa por 11 etapas no trâmite editorial. Ressalta-se que ele só passará para a fase seguinte se aprovado na anterior. São elas:

1) Análise inicial: realizada pelos Editores Chefes e Científicos,

2) Análise técnica: realizada pelo Núcleo Editorial, que verifica se o manuscrito foi formatado segundo essas normas e se o assunto se enquadra no escopo da Revista; além de realizar a busca por possíveis transcrições sem o devido crédito à fonte original ou qualquer outro ponto que possa atestar contra sua originalidade.

3) Análise de Editor Associado: realizada por um Editor Associado, designado com base na área do manuscrito, que analisa seu potencial para publicação e seu interesse para os leitores da Revista, além de verificar se os preceitos éticos foram obedecidos. Trabalhos que não atenderem a essas exigências serão recusados.

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Anexo 2. Normas para formatação da revista Theriogenology, na qual foram publicados o segundo e terceiro capitulos desse trabalho.

THERIOGENOLOGY
An International Journal of Animal Reproduction

AUTHOR INFORMATION PACK

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Additional Style Notes

Please use the following words, phrases, abbreviations, and stylistic conventions

• Avoid the word "injected," (e.g., "Cows were injected with cloprostenol") but include the generic name, proprietary name, dosage and route of administration (e.g., "Cows were treated with cloprostenol [Estrumate 500 μg im]").
• Either cite a P value (recommended for Abstract and for Results) or use the term 'significant' (recommended for Discussion), but generally avoid doing both.
• Terms with a specific statistical meaning (i.e. significant, tended and correlated), should only be used in a strict statistical context.
• Numbers less than 10 are written as a word, unless followed by an abbreviation for unit of measure, e.g. five embryos, 5 min

Use the following expressions

• transrectal palpation, not rectal palpation
• nucleus transfer, not nuclear transplant
• estrus (noun) synchronization, but, estrous (adjective) behavior
• sperm can be used as both noun and adjective
• 120 to 125, not 120-125
• treatment by period, not treatment X period
• gravity: 100 X g (in lieu of speed for centrifugation)
• magnification: X 100
• identification number of an animal: No. 10, but 30 animals: n = 30
• 3 d, Day 3 (define Day 0)

Standard definitions

• oogonium: female gamete before meiosis
• oocyte, primary: female gamete from onset of the first maturation division (meiosis) to extrusion of the first polar body
• oocyte secondary: female gamete from onset of second meiosis to extrusion of the second polar body
• ovum: female gamete from the end of both meiotic divisions until the union of the male and female pronuclei (differs from the common use of ovum as a general term for any female gamete)
• germinal vesicle: nucleus of the ovum
• zygote: a fertilized ovum, from fusion of the male and female gamete to completion of first cleavage
• embryo: a conceptus from the 2-cell stage to the stage when cell migration and differentiation are largely complete
• fetus: a conceptus after organogenesis is mostly complete (primarily increasing in size)
• conceptus: an embryo or fetus with all its membranes and accessory structures
• abortion: expulsion of a conceptus incapable of independent life
• premature parturition: expulsion (before full term) of a conceptus capable of independent life
• stillbirth: avoid this term (use fetal death or abortion)

Abbreviations

Never use an abbreviation to start a sentence. Some abbreviations may be used anywhere else, including the manuscript's title and in figures, table titles and legends, without definition; others may not be used in the title, but may be used in the text without definition. In general, abbreviations must be defined when used for the first time (this may be avoided in the ABSTRACT if necessary to conserve space). To make reading the paper more pleasant, avoid using excessive abbreviations and acronyms; instead use short synonyms, for instance: for "Cesarean section" instead of "CS" use "section" or "hysterotomy."
The following abbreviations may be used in the text without definition (note that abbreviations exclude periods):
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Units of Measure
cpm - counts per min
dpm - disintegrations per min
g - gram
ga - gauge of hypodermic needle
h - hour
kg - kilogram
L - liter
mL - milliliter
μL - microliter
m - meter
min - minute
mo - month
s - second
v:v - volume ratio
wk - week
wt/vol - weight per volume
y - year
Routes of treatment
id - intradermal
im - intramuscular
iu - intrauterine
iv - intravenous
sc - subcutaneous
po - oral
Statistical expressions
ANOVA - analysis of variance
CV - coefficient of variation
df - degrees of freedom
F - variance ratio
NS - not significant
P - probability
SD - standard deviation
SEM - standard error of the mean
r - correlation coefficient
r2 - coefficient of regression
Additional information
• For issues of style and format not addressed here, please consult Scientific Style and Format: The CBE Manual for Authors, Editors, and Publishers, Sixth Edition.
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AFTER ACCEPTANCE

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Anexo 2. Certificado de aprovação do experimento pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA/Ufra).

UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA
COMISSÃO DE ÉTICA NO USO DE ANIMAIS

CERTIFICADO

Certificamos que a proposta intitulada "**Fisiologia reprodutiva de animais selvagens e domésticos na Amazônia**", registrada com o nº de **Protocolo 008/2016 (CEUA) 23084.00268/2016-94 (UFRA),** sob a responsabilidade do Professor Dr. **Frederico Ozanan Barros Monteiro,** que envolve a produção, manutenção ou utilização de animais pertencentes ao Filo Chordata, Subfilo Vertebrata (exceto humanos), para fins de **pesquisa científica** - encontra-se de acordo com os preceitos da Lei nº 11.794, de 8 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle de Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA) da Universidade Federal Rural da Amazônia, em reunião de **28/04/2016, com inclusão de espécies aprovada em reunião realizada em 01/06/2017, sob protocolo 029/2017 (CEUA).**

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	01/06/2016 a 01/08/2018

Espécie/linhagem/raça	Espécies silvestres não brasileiras: *Lagrothrix poeppigii* (n= 103); *Cacajao calvus* (n= 21); *Sapajus apela* (n= 20); *Cebus albifrons* (n= 17); *Tayassu pecari* (n= 246); *Pecari tajacu* (n= 227); *Mazama* sp. (n= 103); *Cacajao melanocephalus* (n= 22); *Cuniculus paca* (n= 180); *Dasyprocta fuliginosa* (n= 120); *Alouatta seniculus* (n= 20); *Bradypus variegatus* (n= 180); *Hydrochoerus hydrochaeris* (n= 3); *Panthera onca* (n= 2); *Tamandua tetradactyla* (n= 1); *Myoprocta accouchy* (n= 2).
Nº de animais	1267 (mil duzentos e sessenta e sete) animais
Peso/Idade	20 a 40kg / Indeterminada (fetos a adultos)
Sexo	Machos e fêmeas
Origem	Bacia do Rio Yavarí Mirín – Amazônia Peruana

Ufra

COMISSÃO DE ÉTICA NO USO DE ANIMAIS – CEUA
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COMISSÃO DE ÉTICA NO USO DE ANIMAIS

Espécie/linhagem/raça	*Ovis aries* (ovinos Santa Inês – mestiço) *Capra aegagrus* (caprinos Anglo-nubiano – mestiço)
Nº de animais	20 (10+10)
Peso/Idade	30 a 40kg / 24 a 48 meses
Sexo	Fêmeas
Origem	Centro de Pesquisas em Caprinos e Ovinos do Pará – CPCOP/ISPA – UFRA

Belém, 2 de Outubro de 2017.

Profª Maria Cristina Manno
Coordenadora CEUA UFRA