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HANI ROCHA EL BIZRI

**DESENVOLVIMENTO INTRAUTERINO DA PACA (*Cuniculus paca*), UM ROEDOR
HISTRICOMORFO PRECOICIAL**

BELÉM
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Dissertação apresentada à Universidade Federal Rural da
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Mestrado em Saúde e Produção Animal na Amazônia:
área de concentração Relação Saúde e Meio Ambiente na
Amazônia, para obtenção do título de Mestre.

Orientador: Prof. Dr. Pedro Ginés Mayor Aparício

Co-orientador: Prof. Dr. Frederico Ozanan Barros
Monteiro

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Orientador: Prof. Dr. Pedro Ginés Mayor Aparício

Co-orientador: Prof. Dr. Frederico Ozanan Barros Monteiro

BANCA EXAMINADORA

Dr. Pedro Ginés Mayor Aparício - Orientador
UNIVERSIDAD AUTÓNOMA DE BARCELONA - UAB

Dra. Diva Anelie Guimarães – 1º Examinador
UNIVERSIDADE FEDERAL DO PARÁ – UFPA

Dr. Wilter Ricardo Russiano Vicente – 2º Examinador
UNIVERSIDADE ESTADUAL PAULISTA – UNESP

Dr. Washington Luiz Assunção Pereira – 3º Examinador
UNIVERSIDADE FEDERAL RURAL DA AMAZÔNIA – UFRA

El Bizri, Hani Rocha

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CDD:

*“Quando os ventos de mudança sopram,
umas pessoas levantam barreiras, outras
constroem moinhos de vento”*

Érico Veríssimo

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RESUMO

O desenvolvimento intrauterino consiste em uma preparação dos recém-nascidos para a vida aérea. Além das relações filogenéticas, esta fase reflete as estratégias adotadas pelos mamíferos para maximizar a sobrevivência materna e neonatal, o que influencia fortemente na dinâmica de suas populações silvestres e na sua resiliência a distúrbios. Neste estudo, nós utilizamos amostras de 137 pacas (*Cuniculus paca*) gestantes, obtidas ao longo de 15 anos através de métodos colaborativos com caçadores locais na Amazônia, para descrever o desenvolvimento da morfologia externa e interna da espécie durante a fase intrauterina, discutindo a estratégia adotada pela espécie para sobrevivência do recém-nascido em comparação com outros roedores. Nos embriões/fetos adquiridos, foram realizados (i) a avaliação da probabilidade de presença de características externas ao longo da gestação; (ii) a biometria externa e uma estimativa da idade fetal e; (iii) medições do volume de órgãos torácicos e abdominais, comparando suas proporções com os órgãos de adultos. O comprimento fetal total (CRL) variou de 0,6 cm a 24,6 cm. As características fetais apareceram na seguinte ordem cronológica: membros, pálpebras, pálpebras fusionadas, genitália, orelha externa, pelagem tátil, garras, pele, pintas, pelagem de cobertura, dentes e pálpebras abertas. Fetos com CRL > 19,5 cm apresentaram todas as características externas avaliadas. A fórmula de crescimento da idade fetal foi $\sqrt[3]{W} = 0,082 (t - 37,25)$, e a idade foi fortemente associada com CRL. O fígado mostrou uma tendência decrescente na sua proporção ao longo do desenvolvimento fetal, enquanto o volume relativo de órgãos gastrointestinais tubulares aumentou significativamente. Todos os órgãos, exceto o coração e o timo, apresentaram volumes relativos semelhantes entre fetos avançados e adultos. Este estudo revela que pacas produzem recém-nascidos precociais com estruturas adequadas para a sobrevivência com baixa dependência do cuidado parental. Além disso, os neonatos da espécie são mais desenvolvidos do que os de outros roedores estudados. Embora a paca seja atualmente a espécie mais caçada na Amazônia, sua estratégia reprodutiva não é compatível com uma condição de presa, sugerindo que a espécie não é reprodutivamente preparada para responder a altas pressões de caça. Portanto, nossos resultados têm uma importância crítica para o diagnóstico clínico e o manejo reprodutivo *in situ* e *ex situ* da espécie.

ABSTRACT

The intrauterine development consists of a preparation of the neonates for the aerial life. Beyond the phylogenetic relationships, this phase reflects the strategies adopted by mammals to maximize the maternal and neonatal survival, which strongly influences the dynamics of their wild populations and their resilience to disturbances. Here, we used samples of 137 pregnant pacas (*Cuniculus paca*), obtained along 15 years through collaborative methods with local hunters in the Amazon, to describe the development of the external and internal morphology of the species during the intrauterine phase, discussing the newborn survival strategy adopted in comparison to other rodents. In the embryos/fetuses acquired, we performed (i) an assessment of the presence probability of external features along the gestation; (ii) an estimation of fetal age and an external biometry; and (iii) a measurement of the volume of thoracic and abdominal organs, comparing their relative volumes with those of adults. The crown-rump length (CRL) ranged from 0.6 cm to 24.6 cm. The fetal features appeared in the following chronological order: limbs, eyelid buds, fused eyelids, genitalia, outer ear, tactile pelage, claws, skin, skin spots, covering pelage, teeth and open eyelids. Fetuses with CRL > 19.5 cm presented all features assessed. The growth formula of fetal age was $\sqrt[3]{W} = 0.082 (t - 37.25)$, and the age was accurately associated with CRL. The liver showed a decreasing trend in its proportion in the internal cavity, while the relative volume of tubular gastrointestinal organs increased significantly. All organs but the heart and the thymus presented similar relative volumes between advanced fetuses and adults. This study reveals that pacas produce precocial neonates with appropriate structures for survival with low dependence on the mother's care. In addition, the species' neonates are more developed than other studied rodents. Although the paca is currently the most hunted species in the Amazon, its reproductive strategy is compatible to a low prey condition, suggesting that the species is not reproductively prepared to respond to high hunting pressures. Therefore, our results have a critical importance for clinical diagnosis and *in situ* and *ex situ* reproductive management of the species.

1 CONTEXTUALIZAÇÃO

Produto de um longo processo evolutivo, a história de vida dos organismos pode ser entendida como o conjunto de atributos que visam garantir a sobrevivência da espécie (VAN NOORDWIJK; DE JONG, 1986), dentre os quais a reprodução desponta como um dos elementos mais importantes. O sucesso reprodutivo de uma espécie concerne na capacidade de produzir descendentes viáveis e garantir a sobrevivência da progênie (WAUTERS; DHONDT, 1995), sendo, portanto, influenciado tanto por fatores biológicos intrínsecos quanto por sua interação com o habitat (FESTA-BIANCHET; GAILLARD; JORGENSON, 1998).

Nos mamíferos, os processos reprodutivos respondem ao ciclo ovárico, que culmina com a liberação de um ou mais ovócitos passíveis de fecundação pelo espermatozoide. Na natureza, um ciclo ovário que não resulte em fecundação pode ser considerado um evento raro (CONAWAY, 1971). Em espécies de ovulação espontânea, a cópula se dá no período conhecido como cio, no qual a probabilidade de concepção é otimizada.

O tempo despendido entre a fecundação e o parto, período denominado gestação, é quando ocorre o desenvolvimento intrauterino do indivíduo, consistindo em uma preparação do filhote para a vida aérea (GLUCKMAN; SIZONENKO; BASSETT, 1999). Esta etapa é intimamente influenciada pelo risco de predação da progenitora e das suas crias e, por isso, espécies utilizam estratégias reprodutivas para maximizar a sobrevivência do neonato.

De acordo com o nível de desenvolvimento neonatal, as espécies são classificadas em dois grandes grupos, altriciais e precociais. As altriciais apresentam gestação mais curta, produzindo maior número de descendentes com investimento energético relativamente pequeno em cada um, resultando em recém-nascidos de menor massa corporal (MARTIN; MACLARNON, 1985). Estes neonatos completam seu desenvolvimento no ambiente extrauterino também de forma lenta, no qual o cuidado parental prolongado é crucial para sua sobrevivência (FLOWERDEW, 1987). Por outro lado, as espécies precociais gastam mais energia em gestações mais longas, dando à luz a um menor número de filhotes mais pesados e bem desenvolvidos, com maior massa cerebral e capacidade motora e visual, permitindo uma considerável independência da mãe logo após ao nascimento (GRAND, 1992). Estas diferentes estratégias reprodutivas influenciam profundamente a dinâmica das populações silvestres, sendo importantes, principalmente, na determinação de suas respostas e sua capacidade de resiliência frente aos distúrbios ambientais e humanos (GOTELLI, 1995).

Neste sentido, o estudo do desenvolvimento intrauterino e das estratégias adotadas para maximizar a sobrevivência de recém-nascidos representa uma importante ferramenta para esclarecer as relações filogenéticas entre espécies (e.g. CLANCY et al., 2001; FRANCIOLLI et al., 2011a) e tem relevância crítica para melhoria do diagnóstico clínico de parâmetros reprodutivos de mamíferos e o seu manejo *ex situ* e *in situ* (FRANCIOLLI et al., 2011b). Entretanto, estes processos permanecem ainda pouco conhecidos para a maioria das espécies silvestres, particularmente as tropicais, devido à dificuldade de obtenção de dados na natureza (EL BIZRI et al., 2016; MAYOR et al., 2016). Enquanto a fisiologia reprodutiva de animais em cativeiro pode ser entendida por meio de medições de hormônios sexuais extraídos de sangue ou amostras fecais e imagens ultrassonográficas periódicas, estes mesmos procedimentos normalmente não são viáveis para animais de vida livre.

Portanto, é necessário que se aprimorem as metodologias e se obtenham melhores amostragens sobre a reprodução de mamíferos silvestres, visando compreender os diversos fatores internos e externos que podem afetar a função reprodutiva e, especialmente, elucidar cada passo do desenvolvimento intrauterino para as espécies de interesse.

1.1 A paca (*Cuniculus paca*)

A paca (*Cuniculus paca*) é um roedor da subordem Hystricomorpha com ocorrência em toda a América Latina, desde o sudeste do México até o sul do Brasil (EMMONS, 1990). A espécie é o maior roedor neotropical depois da capivara (*Hydrochoerus hydrochaeris*), apresentando indivíduos adultos que pesam, em média, 8 kg, embora possam atingir até 14 kg (MATAMOROS, 1982). A paca é um animal solitário e de comportamento noturno e elusivo (GÓMEZ et al., 2005) que, para proteção, constrói elaborados abrigos sob o solo, prioritariamente sob raízes e troncos caídos (BEISIEGEL, 2006; AQUINO et al. 2012). A atividade da paca está intimamente relacionada com o ciclo lunar. Harmsen et al. (2011), utilizando armadilhas fotográficas, demonstraram uma relação inversa entre iluminação da lua e o número de registros fotográficos de paca em Belize, costa nordeste da América Central. Da mesma forma, Valsecchi, El Bizri e Cortes (2014), utilizando lanternas em trilhas noturnas, detectaram menor número de pacas em noites muito iluminadas pela lua na Amazônia Central.

A paca possui uma área de vida de cerca de 2,5 ha (BECK-KING; VON HELVERSEN; BECK-KING, 1999) e a distribuição de suas populações na paisagem está relacionada a locais com alta densidade do dossel e presença de corpos d'água, perto dos quais os indivíduos

constroem seus abrigos (GOULART et al., 2009; FIGUEROA-DE-LEÓN et al., 2016). Quando ameaçadas por predadores, as pacas imergem nos corpos d'água, como frequentemente observado por El Bizri et al. (2016) ao capturar com redes as pacas submergidas nos rios, após afugentá-las com cães treinados para expulsá-las de seus abrigos.

A dieta da paca é majoritariamente composta por frutos (DUBOST; HENRY, 2006). Neste sentido, a espécie desempenha um serviço ecológico primordial como dispersora de sementes em florestas tropicais (BECK- KING; VON HELVERSEN; BECK-KING, 1999). No entanto, a paca pode também se alimentar de folhas, sementes e insetos, itens que se tornam especialmente importantes na dieta da espécie durante períodos de escassez de frutos (DUBOST; HENRY, 2006).

A paca é um dos animais preferidos para consumo humano ao longo de sua distribuição (EL BIZRI et al., 2016). Sua carne é altamente apreciada pelas populações rurais de florestas neotropicais (DEUSTSCH; PUGLIA, 1990), sendo classificada por caçadores de subsistência como a segunda e a terceira mais saborosa na Amazônia brasileira e peruana, respectivamente (BODMER, 1995; VALSECCHI; AMARAL, 2009). Além disso, também possui grande valor nutricional e alta produtividade líquida, possuindo mais proteína e menos gordura do que a carne bovina e ovina e consideravelmente maior proporção de conteúdo comestível por indivíduo do que as carnes de catitu (*Pecari tajacu*), veado (*Mazama americana*) e jabuti-amarelo (*Chelonoidis denticulatus*) (GÁLVEZ et al., 1999)

Devido a esses atributos, a paca é, possivelmente, a espécie mais caçada na América Latina na atualidade. El Bizri et al. (2015) demonstraram que a paca é a principal espécie alvo da caça esportiva ilegal no Brasil, sendo duas vezes mais abatida que outros roedores de maior porte, como as cutias (*Dasyprocta* spp.) e a capivara. Além disso, foi considerada a espécie mais caçada nas florestas tropicais mexicanas, totalizando 49% de todos os espécimes abatidos (GUTIÉRREZ-GRANADOS, 2015).

O consumo de paca é notavelmente alto na bacia Amazônica, onde a espécie está constantemente presente nas refeições de populações rurais e indígenas. Estudos revelaram que a carne de paca está presente em mais de 50% de todas as refeições à base de carne silvestre consumidas por crianças na Amazônia colombiana (VAN VLIET et al., 2015). Read et al. (2010) demonstraram que a paca é a espécie mais caçada pelas comunidades indígenas da Amazônia guianense, enquanto Peres (2000) indicou que a paca é a segunda espécie mais consumida na Amazônia brasileira, correspondendo a 730.886 de indivíduos extraídos da natureza e 11,35% do número total de mamíferos caçados anualmente. Da mesma forma, é

também a segunda espécie mais consumida na Amazônia nicaraguense (KOSTER, 2008) e peruana (BODMER; LOZANO, 2001).

Se por um lado a caça da espécie contribui para a segurança alimentar de moradores locais, por outro a atividade tem sido atestada como insustentável na maioria das avaliações realizadas, o que vem causando uma drástica redução de suas populações naturais (e.g., KOSTER, 2008; ZAPATA-RÍOS; URGILÉS; SUÁREZ, 2009; VALSECCHI; EL BIZRI; CORTES, 2014). Além disso, Morcatty et al. (2013) relataram que a paca é uma espécie sensível à fragmentação e à redução de habitat, não tolerando fragmentos florestais degradados e de pequena extensão. O esgotamento das populações levou à classificação da espécie como ameaçada de extinção em cinco listas vermelhas estaduais no Brasil (CHIARELLO et al., 2008) e à ocorrência de inúmeras extinções locais.

Além do preocupante cenário conservacionista, observa-se que o conhecimento sobre diversos aspectos da biologia da paca ainda é incipiente (EL BIZRI et al., 2016), e nenhum plano de manejo *in situ* foi, até o momento, definido para a espécie. Nesse contexto, a criação de animais silvestres em cativeiro pode ser uma alternativa para a caça no Brasil, favorecendo a criação de estratégias para conservação de espécies cinegéticas (e.g. GOVONI; FIELDING; PAOLETTI, 2001; MAYOR; FITA; BÉJAR, 2007; NOGUEIRA; NOGUEIRA-FILHO, 2011). Porém, inversamente ao sucesso obtido com capivaras, cutias e catetos (*Pecari tajacu*), a criação e o rendimento da paca em cativeiro vem provando ser um desafio (EMMONS, 1987), especialmente devido ao baixo conhecimento científico sobre o manejo reprodutivo e nutricional da espécie (SMYTHE, 1987). Assim, estudos detalhados acerca da biologia reprodutiva de paca ainda são bastante demandados, especialmente relacionados às técnicas de diagnóstico de imagem e de reprodução aplicadas ao manejo - visando a melhoria da sua produtividade *ex situ* - e aos modelos de sustentabilidade e viabilidade do uso das populações silvestres – visando sua conservação *in situ*.

1.2 O estado da arte sobre a reprodução da paca

O conhecimento atual sobre a morfologia reprodutiva da paca ainda é básico. A fêmea possui um útero bicórneo, composto por dois cornos completamente independentes, medindo cerca 12 cm de comprimento cada. Seus ovários apresentam coloração amarelada, formato ovalado e textura lisa, localizando-se em posição caudal em relação aos rins, apoiados numa bolsa ovárica incompleta (MAYOR et al., 2013). Os machos possuem um par de testículos

ovalados e alongadas crânio-caudalmente, que se mantêm envoltos pelo escroto no interior da cavidade abdominal (BORGES et al., 2013). Os testículos somente são evidenciados no saco escrotal na época reprodutiva, quando se posicionam ventralmente ao ânus (BORGES et al., 2013). Isso pode ser explicado pela ausência do anel inguinal, que possibilita maior mobilidade testicular em roedores silvestres (LANGE; SCHMIDT, 2014).

Em vida livre, fêmeas e machos atingem a maturidade reprodutiva com cerca de um ano (COLLET, 1981). Similarmente, Guimarães et al. (2008) observaram duas pacas com primeiro cio aos 12 meses de idade, embora outro indivíduo tenha apresentado o primeiro cio com oito meses. Os mesmos autores concluíram que o ciclo estral da espécie tem duração média de 32,5 ($\pm 3,69$) dias, sendo 10,3 ($\pm 4,21$) dias para o proestro, 1,05 ($\pm 0,22$) dias para o estro, 5,6 ($\pm 3,87$) dias para o metaestro e 14,7 ($\pm 4,57$) dias para o diestro. Um novo ciclo estral pode ocorrer logo após o nascimento da cria ou seguido ao desmame (NOGUEIRA; GIANNONI; TONIOLLO, 2006; HOSKEN, 2001).

Em cativeiro, os primeiros partos foram observados em fêmeas com idade de 575 e 635 dias por Nogueira, Giannoni e Toniollo (2006) e 612, 695 e 703 dias por Merrit (1989), gerando uma média de 644 ($\pm 54,65$) dias. Partos mais precoces foram relatados aos 390 dias (SMYTHE, 1991) e aos 395 dias (GUIMARÃES et al., 2008). Esta grande variação encontrada entre os estudos pode estar relacionada às diferenças climáticas e de manejo nutricional e reprodutivo, além de possíveis alterações fisiológicas dos indivíduos.

A duração média da gestação obtida em diferentes estudos também é bastante variável. Essas diferenças encontradas provavelmente se devem à dificuldade de detecção da ocorrência da monta efetiva. Entretanto, o período gestacional foi superior a 100 dias de duração, com maioria das fêmeas apresentando um intervalo entre 135 e 155 dias de gestação (Tabela 1).

Mayor et al. (2013) afirmaram que, na natureza, a porcentagem de fêmeas prenhes em relação ao total de fêmeas adultas (taxa de gestação) variou entre 57.1% e 61.4%, e o número de partos anuais por fêmea foi estimado entre 1.37 e 1.48 partos por ano.

Tabela 1. Duração média da gestação em pacas (*Cuniculus paca*) obtida em diversos estudos.

Duração média da gestação (dias)	Referência
114	Matamoros e Pashov (1984)
118	Kleiman et al. (1979)
137.5 (135 – 139)	Oliveira, Machado e Canola (2003)
148	Guimarães et al. (2008)
150	Nogueira, Giannoni e Toniollo (2006)
150 (145 – 155)	Rengifo et al. (1996)
152	Ribeiro et al. (2012)
155	Smythe (1991)

Guimarães et al. (2008) afirmam que as pacas em cativeiro possuem reprodução poliestral contínua, não apresentando sinais de sazonalidade dos nascimentos. Collet (1981) também não detectou picos sazonais de nascimento dos filhotes em pacas de vida livre na Colômbia. Em contraste, Mayor et al. (2013) e El Bizri, Mayor e Valsecchi (2015), demonstraram que, em pacas de vida livre na Amazônia, as concepções e os partos são fortemente sazonais, havendo maior concentração de ambos os eventos durante o período chuvoso. Portanto, a paca poderia ser considerada como uma espécie de estacional oportunista, pois ainda que possua potencial de parição ao longo de todo o ano, fatores ambientais como escassez de alimento ou competição por espaço – como em épocas alagadas na Amazônia – podem influenciar na fertilidade das fêmeas e, conseqüentemente, no número de filhotes paridos por mês.

A paca é uma espécie unípara, apresentando raros casos de gestações gemelares (MONDOLFI, 1972; PÉREZ & HERNANDES, 1979). A uniparidade da espécie também foi constatada por meio de estudos ultrassonográficos (OLIVEIRA, MACHADO & CANOLA, 2003; OLIVEIRA et al., 2007). Meritt (1989) não observou nenhuma parição gemelar nas 21 gestações acompanhadas em zoológico, e o mesmo foi relatado por Guimarães et al. (2008) ao observarem 51 pacas prenhes em cativeiro. Entretanto, Matamoros (1982) observou apenas um parto gemelar em 22 nascimentos acompanhados. Mayor et al. (2013), analisando o útero de 78 fêmeas prenhes caçadas na Amazônia peruana, encontrou apenas duas fêmeas com gestação gemelar, gerando uma média de 1.03 filhotes por gestação. Desta forma, estima-se que uma fêmea adulta de vida livre produza anualmente entre 1.41 e 1.51 filhotes.

Aparentemente, a paca demonstra uma leve tendência à maior produção de filhotes machos, uma vez que a razão sexual dos filhotes encontrada foi estimada em 2 machos/fêmea (OLIVEIRA et al., 2003), 1,21 machos/fêmea (GUIMARÃES et al., 2008) e 1,29 machos/fêmea (MAYOR et al., 2013). Por outro lado, El Bizri, Mayor e Valsecchi (2015) encontraram uma proporção de 2,2 fêmeas/macho em uma zona caçada da Amazônia Central. Em vista disso, estas diferenças podem ter relação com respostas denso-dependentes das populações frente ao uso da espécie. Os neonatos de pacas se assemelham profundamente aos adultos, apresentando pelos amarronzados e pintas brancas ao longo dos flancos, características diagnósticas da espécie (MERITT, 1989). Os neonatos não apresentam dimorfismo sexual, o qual aparecerá em pacas adultas, especialmente em relação à largura do arco zigomático, a qual é maior em indivíduos machos (MARCUS, 1984).

Enquanto o desenvolvimento intrauterino de alguns roedores histricomorfos foi bastante elucidado (e.g. FORTES et al., 2013; FELIPE; MASSON, 2008), poucos estudos foram realizados até o momento para pacas, sendo esta uma das maiores lacunas de informação sobre a reprodução da espécie. O conhecimento atual para pacas está restrito a características morfométricas e fisiológicas da placenta (MIGLINO et al., 2002) e poucos órgãos fetais, geralmente obtido por meio de ultrassonografia de fêmeas prenhes em cativeiro (e.g. BONATELLI et al., 2005). Oliveira et al. (2003) realizaram ultrassonografia de nove pacas prenhes, obtendo medições de área da placenta e do coração dos fetos, enquanto Ribeiro et al. (2012) realizaram medidas do diâmetro bi-parietal de 27 fetos aos 60 e 90 dias de gestação. Adicionalmente, Francioli et al. (2011) descreveram o desenvolvimento embriológico da paca, comparando-o com outros três roedores histricomorfos – a cutia (*Dasyprocta leporina*), a preá (*Galea spixii*) e a capivara (*Hydrocherus hydrochaeris*) –, porém obtendo resultados pouco concludentes por utilizarem apenas dois embriões de cada espécie.

Assim sendo, é notável que o conhecimento sobre desenvolvimento intrauterino da paca é ainda bastante escasso, sobretudo aquele proveniente de condições naturais e de estudos que executem medições e descrições diretas em um abundante número de amostras. Desta forma, este estudo vem preencher uma importante lacuna científica, descrevendo a morfologia externa e interna da paca ao longo de sua fase intrauterina à luz da estratégia evolutiva adotada pela espécie para a sobrevivência de seus recém-nascidos. Além disso, a partir de uma abordagem comparativa entre o desenvolvimento fetal de roedores, este estudo permite entender este processo não somente na paca, mas para os demais membros da ordem Rodentia.

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**2. ARTIGO: Embryological and fetal morphology in the lowland paca (*Cuniculus paca*):
a precocial Hystricomorph rodent**

Hani Rocha El Bizri^{1,2}, ...

¹Programa de Pós-Graduação em Saúde e Produção Animal na Amazônia, Universidade Federal Rural da Amazônia, Belém, Brasil.

²Grupo de Pesquisa em Ecologia de Vertebrados Terrestres, Instituto de Desenvolvimento Sustentável Mamirauá

*Corresponding author: Hani Rocha El Bizri.

E-mail: hanibiz@gmail.com

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1 **Abstract**

2 The intrauterine development consists of a preparation of the neonates for the
3 aerial life. Beyond the phylogenetic relationships, this phase reflects the strategies
4 adopted by mammals to maximize the maternal and neonatal survival, which strongly
5 influences the dynamics of their wild populations and their resilience to disturbances.
6 Here, we used samples of 137 pregnant pacas (*Cuniculus paca*), obtained along 15 years
7 through collaborative methods with local hunters in the Amazon, to describe the
8 development of the external and internal morphology of the species during the
9 intrauterine phase, discussing the newborn survival strategy adopted in comparison to
10 other rodents. In the embryos/fetuses acquired, there were performed (i) an assessment of
11 the presence probability of external features along the gestation; (ii) an estimation of fetal
12 age and an external biometry; and (iii) a measurement of the volume of thoracic and
13 abdominal organs, comparing their relative volumes with those of adults. The crown-
14 rump length (CRL) ranged from 0.6 cm to 24.6 cm. The fetal features appeared in the
15 following chronological order: limbs, eyelid buds, fused eyelids, genitalia, outer ear,
16 tactile pelage, claws, skin, skin spots, covering pelage, teeth and open eyelids. Fetuses
17 with CRL > 19.5 cm presented all features assessed. The growth formula of fetal age was
18 $\sqrt[3]{W} = 0.082(t - 37.25)$, and the age was accurately associated with CRL. The liver showed
19 a decreasing trend in its proportion in the internal cavity, while the relative volume of
20 tubular gastrointestinal organs increased significantly. All organs but the heart and the
21 thymus presented similar relative volumes between advanced fetuses and adults. This
22 study reveals that pacas produce precocial neonates with appropriate structures for
23 survival with low dependence on the mother's care. In addition, the species' neonates are
24 more developed than other studied rodents. Although the paca is currently the most
25 hunted species in the Amazon, its reproductive strategy is compatible to a low prey

26 condition, suggesting that the species is not reproductively prepared to respond to high
27 hunting pressures. Therefore, our results have a critical importance for clinical diagnosis
28 and *in situ* and *ex situ* reproductive management of the species.

29 *Keywords:* reproduction, fetal development, mammals, survival, Rodentia, Amazon,

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51 **1. Introduction**

52 In mammal species, the intrauterine development is the phase of individual
53 maturation between fecundation and parturition, consisting of a preparation of the
54 neonates for the aerial life [1]. The study of the intrauterine development is primordial to
55 understand the strategies adopted by mammals to maximize the maternal and neonatal
56 survival and allows comparisons among species, enlightening their phylogenetic
57 relationships [2, 3]. In addition, these studies have critical importance for *in situ* and *ex*
58 *situ* reproductive management and clinical diagnosis of developmental parameters of
59 mammal species [4].

60 According to the level of neonatal development, species are divided in two major
61 groups, altricial and precocial species. Altricial species present faster gestation period,
62 producing large number of offspring with a relatively small energetic investment in each
63 one, resulting in a small newborn weight [5]. Whereas altricial newborns complete their
64 fetal development in the extra-uterine environment, largely depending on an extended
65 maternal care for feeding and moving [6], precocial species deliver well-developed
66 neonates, with greater brain mass and motor and visual capacity, moving and foraging
67 independently from the mother soon after birth [7].

68 Reproductive strategies of species influence the dynamics of their wild
69 populations and their responses and resilience to disturbances [8]. However, the
70 intrauterine development of most mammal wild species remains poorly studied,
71 particularly in tropical species due to logistical and financial restrictions [9]. Most studies
72 relied on very few embryological and fetal samples or imaging techniques of captive
73 pregnant females, limiting the results obtained. In this sense, the use of reproductive tracts
74 from hunted animals for subsistence purpose, through collaborative methods with local
75 communities, represents an alternative for *in situ* data collection and the recycle of

76 biological samples with a high level of reliability [9]. This sampling strategy permit us to
77 take advantage of materials that would be discarded by the locals and facilitate the
78 obtaining of a large sample size, increasing our knowledge on the reproductive patterns
79 of wild species.

80 The lowland paca (*Cuniculus paca*, Cuniculidae, Rodentia) is a medium-sized
81 hystricomorph rodent (7.7 kg) with occurrence in the South and Central America. Pacas
82 are very prized for their meat, being one of the most hunted species in Latin America [10,
83 11] and arousing highly demand for its captive production. Nevertheless, the biology of
84 this species is still poorly studied, hindering its effective management both *in situ* and *ex*
85 *situ* [12].

86 The majority of extant mammals produce altricial neonates, and this includes most
87 rodent species [13]. Despite small rodents are characterized by their high reproductive
88 performance, pacas have an average of 1.37 parturitions per year, produce only 1.03
89 young per birth [14] and have a relatively longer gestation length of 149 days [15].
90 Consequently, notwithstanding the close phylogenetic proximity, the newborn survival
91 strategies determined by the embryological and fetal development in rodents may be
92 diverse. In this study, we used samples obtained along 15 years from subsistence hunting
93 in the Amazon to describe the development of the external and internal morphology of
94 paca during the intrauterine phase, discussing the newborn survival strategy adopted by
95 the species in comparison to other rodents.

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97 **2. Material and methods**

98 *2.1. Study sites*

99 Two areas in the Amazon rainforest were selected. The Yavarí-Mirín River
100 (YMR, S 04°19.53; W 71°57.33), northeastern Peruvian Amazon, is a continuous area of

101 107,000 ha composed predominantly by upland forests (“terra firme”). A single
102 indigenous community of 307 inhabitants is found in the region. The region has a dry
103 (July-October) and a wet/flooded season (November-June). The Amanã Sustainable
104 Development Reserve (ASDR, S 01°54.00; W 64°22.00) is a reserve of 2,313,000 ha in
105 the Central Brazilian Amazon, between the Negro and Japurá rivers, consisting
106 predominantly of upland forests. Within the ASDR, there is a population of
107 approximately 4,000 riverine people, found in 23 communities and some isolated
108 settlements. In the ASDR, there is a dry (August-December) and a wet/flooded season
109 (January-July). In both areas, local communities rely mainly on agriculture for income
110 and on hunting and fishing for subsistence. The climate in both study areas is typically
111 equatorial with annual temperatures ranging from 22°C to 36°C, a relative humidity of
112 80%, and annual rainfall between 1500 and 3000 mm.

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114 2.2. *Collection of samples*

115 During 2002 and 2014, local hunters collected and voluntarily donated genital
116 organs from 137 pregnant pacas, 74 in the YMR and 68 in the ASDR, totalizing 142
117 embryos/fetuses. Thus, twin gestations represented only 3% (n=5) of our samples of the
118 total of pregnancies. Hunters were trained to remove all the abdominal and pelvic organs
119 complete with the perineal region and store the samples in buffered 4% formaldehyde
120 solution (v/v). Since these materials are not consumed by hunters, this methodology
121 assured the absence of any invasive procedure or additional mortality for the purpose of
122 the study [9].

123 The research protocol was approved by the Research Ethics Committee for
124 Experimentation in Wildlife at the Dirección General de Flora y Fauna Silvestre
125 from Peru (License 0229-2011-DGFFS-DGEFFS), by the Instituto Chico Mendes

126 Institute for Biodiversity Conservation from Brazil (License SISBIO No 29092-1) and
127 by the Committee on Ethics in Research with Animals of the Federal Rural University of
128 the Amazon (UFRA CEUA protocol 007/2016). Samples were sent UFRA, Belém, Pará,
129 Brazil, through the export license CITES/IBAMA (No 14BR015991/DF).

130

131 *2.3. Laboratory procedures*

132 Genital organs were dissected to remove all conceptuses. An external physical
133 inspection was performed to describe the following morphological features: genitalia,
134 differentiated limbs, eyelids, skin, covering and tactile pelage, skin spots, erupted teeth,
135 claws and outer ear. The embryo/fetal stage was determined according to the
136 *International Committee on Veterinary Embryological Nomenclature* [16].

137 Afterwards, an external biometry of each embryo/fetus was performed by
138 measuring the values of body weight, crown-rump length (CRL), longitudinal length from
139 rostral edge of nose to distal portion of tail, bi-parietal diameter, cranial occipital-frontal
140 diameter, cranial circumference, thoracic diameter and thoracic circumference,
141 abdominal diameter and abdominal circumference, femur and humerus length, and the
142 length of forelimbs and hindlimbs. Thoracic and abdomen measurements were taken from
143 the last rib and from the insertion of the umbilical cord, respectively. To perform the
144 biometry, we used a scale, a tape measure with accuracy of 0.1 cm and a metal caliper
145 with full measurement capability of 300 mm.

146 Thoracic and abdominal organs (heart, lungs, liver, spleen, kidneys, tubular
147 gastrointestinal organs and thymus) from fetuses larger than 5 cm CRL (n= 109) were
148 eviscerated, and their volumes measured by submerging them in hypodermic syringes
149 with accuracy of 0.01 ml containing water. The Archimedes Principle was applied,
150 considering the volume of the water displaced by a given organ as a proxy of the organ

151 volume [17]. All volumes of individual organs were summed to obtain the total visceral
152 volume and calculated the relative volume, in percentage, of each organ within the total
153 visceral volume. Since organs showing signs of autolysis were excluded, 82 samples were
154 analyzed. In addition, we measured the volumes of the organs and the total visceral
155 volume of 21 adult pacas hunted for subsistence and donated by local people.

156

157 *2.4. Data analysis*

158 Since adult pacas from YMR and ASDR do not present differences in their body
159 weight [18, 19], the samples from both study areas were pooled. Fetal age was calculated
160 using Huggett and Widdas' formula [20]:

161

$$162 \quad \text{Equation 1: } \sqrt[3]{W} = a(t-t_0)$$

163

164 where W is the fetal weight, a is the specific fetal growth velocity, t is the fetal
165 age in days, and t_0 is the calculated intercept on the age axis; thus, in t_0 , the body weight
166 of the individual is so low that it approximates to zero. In species presenting between 100
167 and 400 days of pregnancy, t_0 is equal to 20% of gestation time [20]. Therefore, an
168 estimated delivery date was used for these calculations, considering 149 days of gestation
169 [15] and a mean weight of 787.79 g at birth, which is the average body weight of fetuses
170 that showed stabilized mass in advanced pregnancy stages (CRL > 22.3 cm).

171 Logistic regressions were applied to estimate the presence probability of each
172 morphological character along the growth in CRL, using the software Statistica 8.0.
173 Multiple regressions were performed to assess the relationship between the age, external
174 biometric measures and relative and absolute organ volumes with CRL, using the
175 software CurveExpert 2.4 to complete the regressions and set the best function to the

176 plots. The Akaike Information Criteria (AIC) scores were used to compare the functions,
177 considering the lowest score as the best fit. For fetal measurements and absolute organ
178 volumes, we forced linear regressions to origin and only considered those functions with
179 starting point on or near to zero, since we expect both CRL and these measures to be at
180 zero in the beginning of the fetal development. We compared the relative volumes of the
181 organs between adults and advanced fetuses using a T-student Test. The value of $P < 0.05$
182 was considered significant.

183

184 **3. Results**

185 *3.1. External morphological characters*

186 The fetuses presented a CRL ranging from 0.6 cm to 24.6 cm. The embryos
187 presented initial differentiation of the limbs at around 1.7 cm of CRL, but these were still
188 paddle-shaped and showed no separation of the digits. All individuals with CRL over 2.9
189 cm had well differentiated limbs and digits. The first fetus presenting formation of the
190 eyelids had 2.3 cm, and their fusion occurred in fetuses larger than 3.1 cm (Table 1; Figure
191 1; Figure 2). The genitalia and the outer ears were also differentiated in fetuses larger than
192 3.1 cm. The following fetal features appeared in chronological order: the tactile (CRL > 4.2
193 cm), complete formation of claws (CRL > 4.9), skin (CRL > 6.5 cm), skin spots (CRL >
194 14.1 cm) and covering pelage (CRL > 14.2 cm). Finally, the teeth eruption started to
195 appear sporadically in fetuses between around 14.0 cm and 15.5 cm. All fetuses with CRL
196 over 15.9 cm had erupted teeth cm. All fetuses with CRL higher than 19.5 cm presented
197 open eyelids (Figure 1).

198

199

200

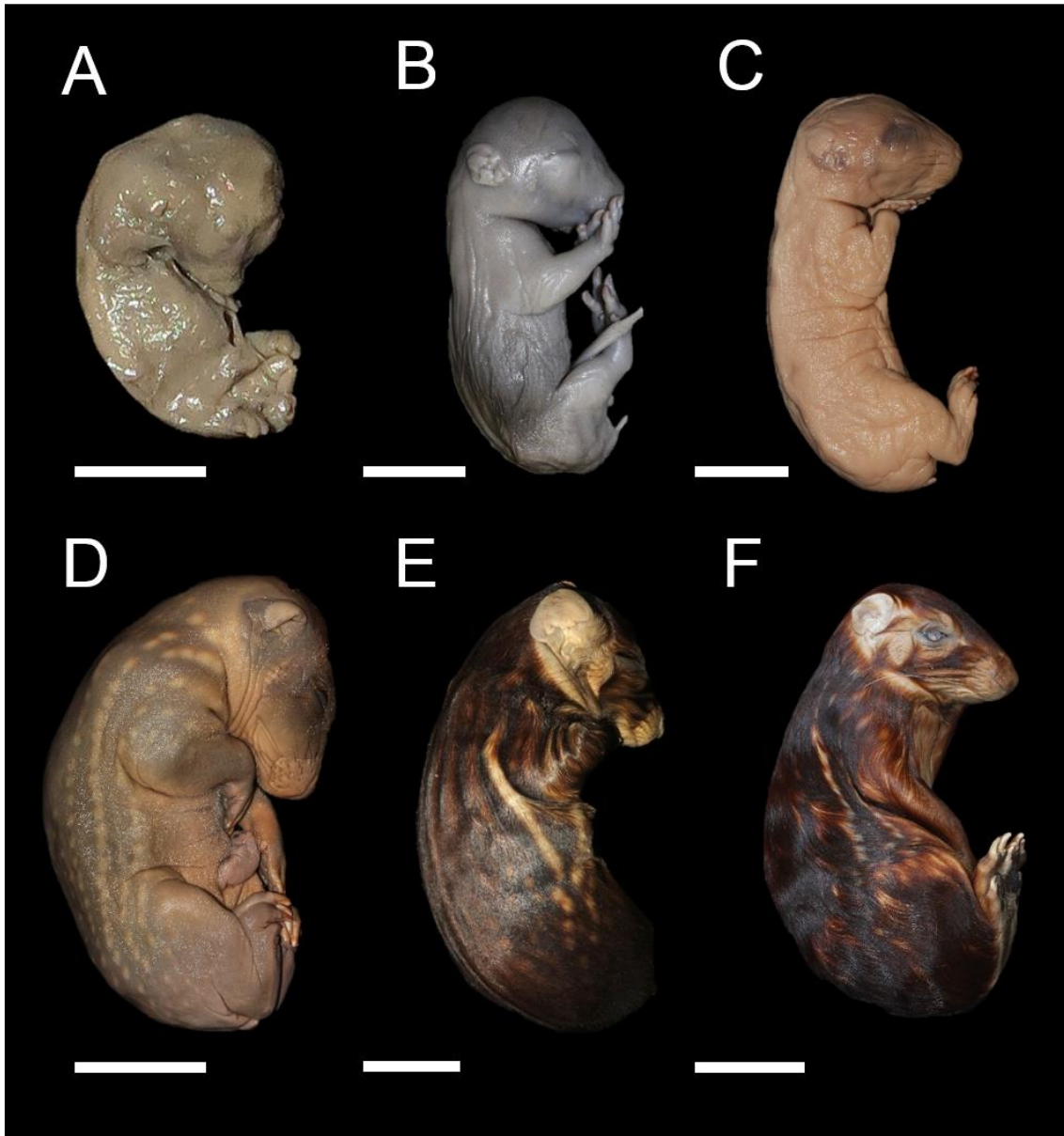


Figure 1. Fetuses at different stages of intrauterine development, presenting distinct external features according to their crown-rump-length (CRL). A – a fetus with 2.3 cm of CRL presenting initial growth of eyelids but no outer ear or claws. Bar: 1 cm. B – a fetus with 6.3 cm of CRL presenting an early fusion of the eyelid buds, outer ear, claws and initial growth of tactile pelage. Bar: 2 cm. C – a fetus with 8.1 cm of CRL presenting a developing skin. Bar: 2 cm. D – a fetus with 13.1 cm pf CRL presenting complete skin, initial growth of covering pelage, and the species’ characteristic dermic spots. Bar: 3 cm. E – a fetus with 17.8 cm of CRL presenting covering pelage completely developed but eyelids still fusioned. Bar: 4 cm. F – a fetus with 22.3 cm presenting open eyelids. Bar: 6 cm.

Table 1. Statistics and formulas for the presence probability of external features in lowland paca (*Cuniculus paca*) embryos/fetuses along the increase in crown-rump length (CRL).

External feature	n	χ^2	P	Formula
Limbs	123	36.73	<0.01	$y = \frac{\exp^{(-3.55+1.64x)}}{1 + \exp^{(-3.55+1.64x)}}$
Eyelid buds	124	53.28	<0.01	$y = \frac{\exp^{(-4.83+1.70x)}}{1 + \exp^{(-4.83+1.70x)}}$
Fusioned eyelids	109	48.52	<0.01	$y = \frac{\exp^{(-5.88+1.75x)}}{1 + \exp^{(-5.88+1.75x)}}$
Differentiated genitalia	124	64.83	<0.01	$y = \frac{\exp^{(-5.98+1.74x)}}{1 + \exp^{(-5.98+1.74x)}}$
Outer ear	124	64.83	<0.01	$y = \frac{\exp^{(-5.98+1.74x)}}{1 + \exp^{(-5.98+1.74x)}}$
Tactile pelage	124	76.57	<0.01	$y = \frac{\exp^{(-7.14+1.79x)}}{1 + \exp^{(-7.14+1.79x)}}$
Claws	124	54.73	<0.01	$y = \frac{\exp^{(-3.38+0.95x)}}{1 + \exp^{(-3.38+0.95x)}}$
Skin	124	95.27	<0.01	$y = \frac{\exp^{(-6.48+0.94x)}}{1 + \exp^{(-6.48+0.94x)}}$
Skin spots	124	148.20	<0.01	$y = \frac{\exp^{(-24.79+2.01x)}}{1 + \exp^{(-24.79+2.01x)}}$
Covering pelage	124	133.17	<0.01	$y = \frac{\exp^{(-17.35+1.34x)}}{1 + \exp^{(-17.35+1.34x)}}$
Erupted teeth	121	121.77	<0.01	$y = \frac{\exp^{(-19.04+1.29x)}}{1 + \exp^{(-19.04+1.29x)}}$
Open eyelids	113	95.86	<0.01	$y = \frac{\exp^{(-19.47+1.11x)}}{1 + \exp^{(-19.47+1.11x)}}$

201 3.2. External biometry

202 The logistic function better represented the trend for the body weight, while the
 203 power model better fitted to the hindlimb and the logistic power model furnished the best
 204 fit to the plots for the remaining measurements (Figure 3). The strongest relationships
 205 with CRL were found for the bi-parietal diameter, thoracic circumference, abdominal
 206 circumference, femoral length and humeral length. The body weight ranged from 5.0 g to
 207 977.5 g. The growth formula of fetal age was $\sqrt[3]{W} = 0.082 (t - 37.25)$, and the age was
 208 accurately associated with CRL ($r^2=0.95$) (Figure 4).

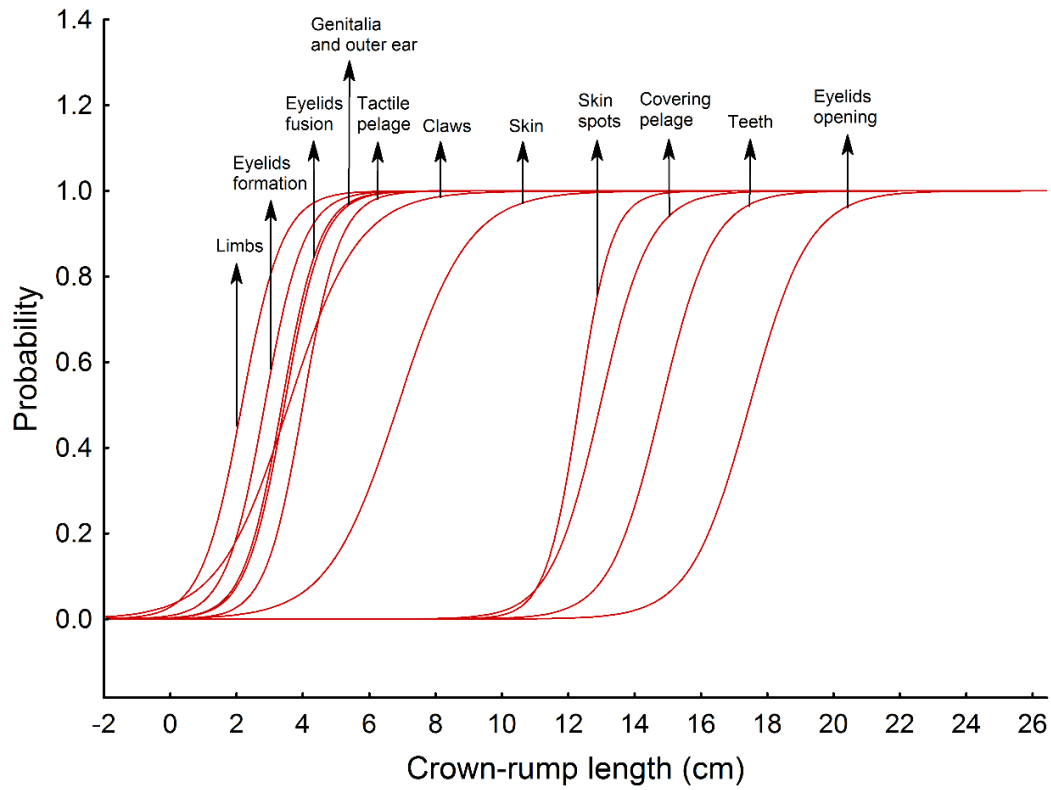
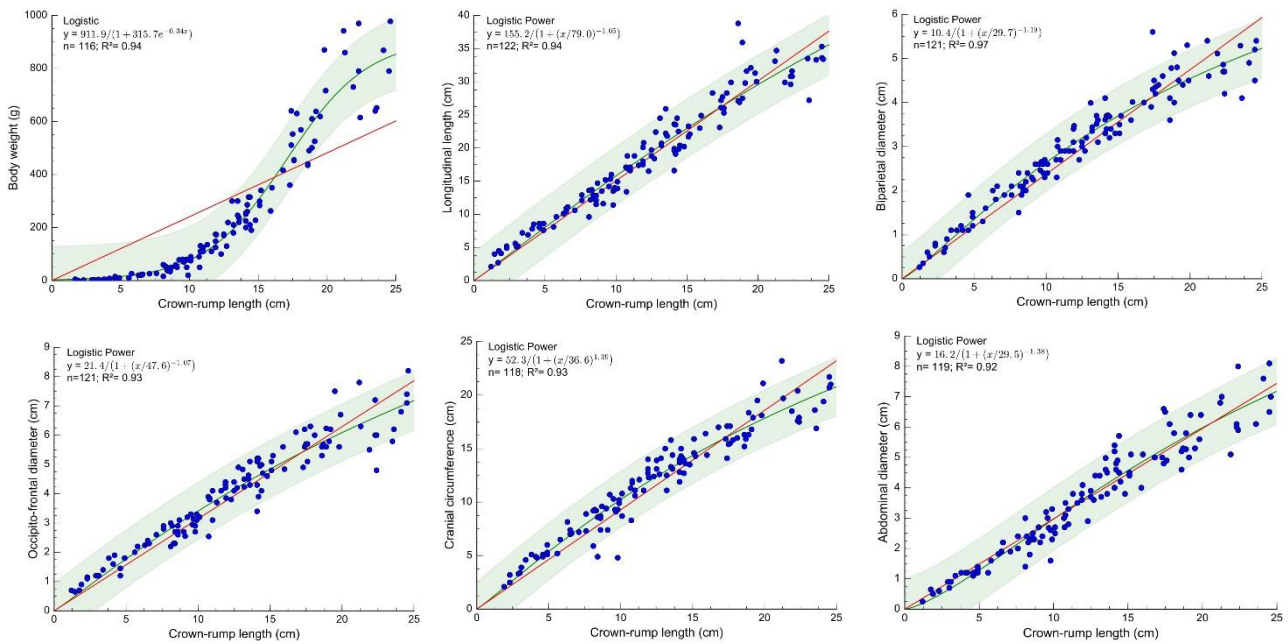


Figure 2. The occurrence probability of external features in embryos/fetuses of lowland paca (*Cuniculus paca*) according to their crown-rump length (CRL).



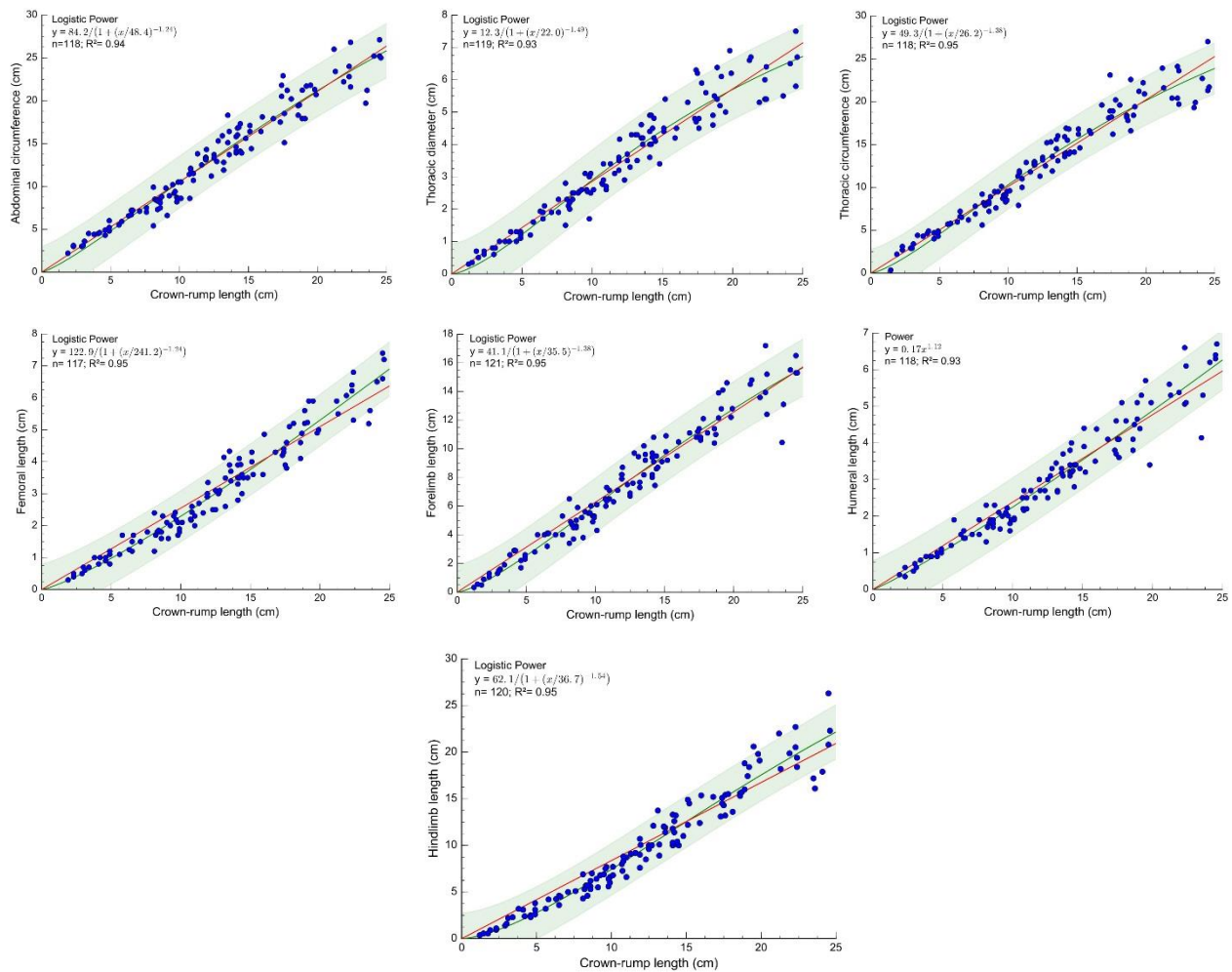


Figure 3. The relationship between external biometric measures and the crown-rump length in fetuses of lowland paca (*Cuniculus paca*). The green line represents the best model fitted to the plots \pm 95% CI, while the red line represents an expected linear trend with no intercept.

209 3.3. Internal volumetry

210 Figure 5 shows the best models for the increase in absolute organ volumes along
 211 the fetal development. The strongest associations with CRL were found for the total
 212 visceral volume and the tubular gastrointestinal organs. The relative volume of heart,
 213 lungs, spleen, kidneys and thymus did not vary along the fetal development ($r^2 < 0.1$;
 214 $p > 0.05$). On the other hand, the relative volumes of the liver and the tubular
 215 gastrointestinal organs showed a strong relationship with the fetal CRL. While the liver

216 had a decreasing trend ($r=-0.67$; $r^2=0.45$; $p<0.01$), the relative volume of tubular
 217 gastrointestinal organs increased along the fetal development ($r=0.77$; $r^2=0.60$; $p<0.01$)
 218 (Figure 6). Comparing the volumes of organs of advanced fetuses and adults, all organs
 219 but the heart and the thymus presented similar proportion between the groups (Table 2).

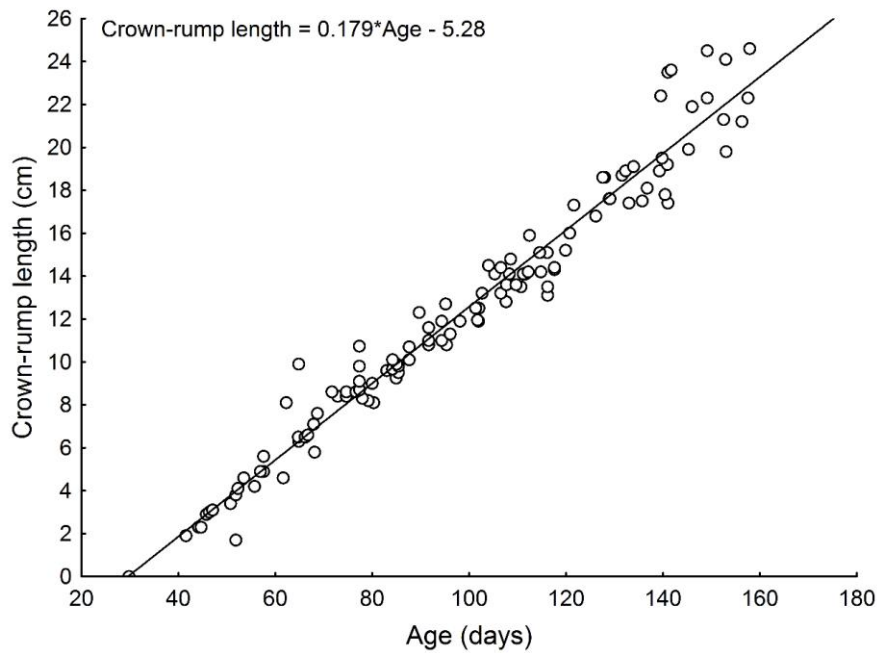
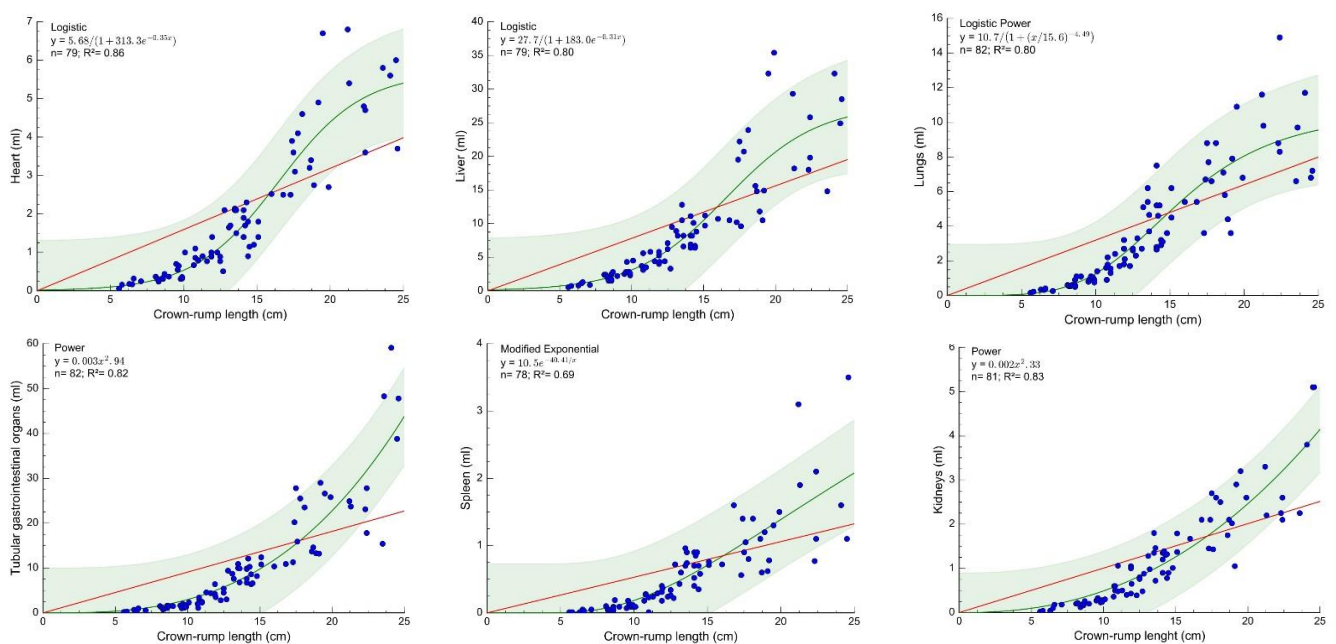


Figure 4. The relationship between embryo/fetal age and the crown-rump length in the lowland paca (*Cuniculus paca*).



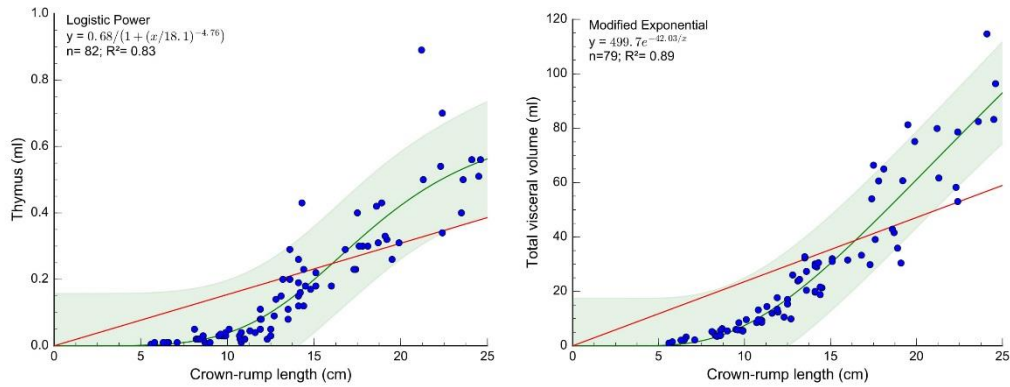


Figure 5. The relationship between the absolute volumes of the visceral organs and the crown-rump length (CRL) in fetuses of lowland paca (*Cuniculus paca*). The green line represents the best model fitted to the plots \pm 95% CI, while the red line represents an expected linear trend with no intercept.

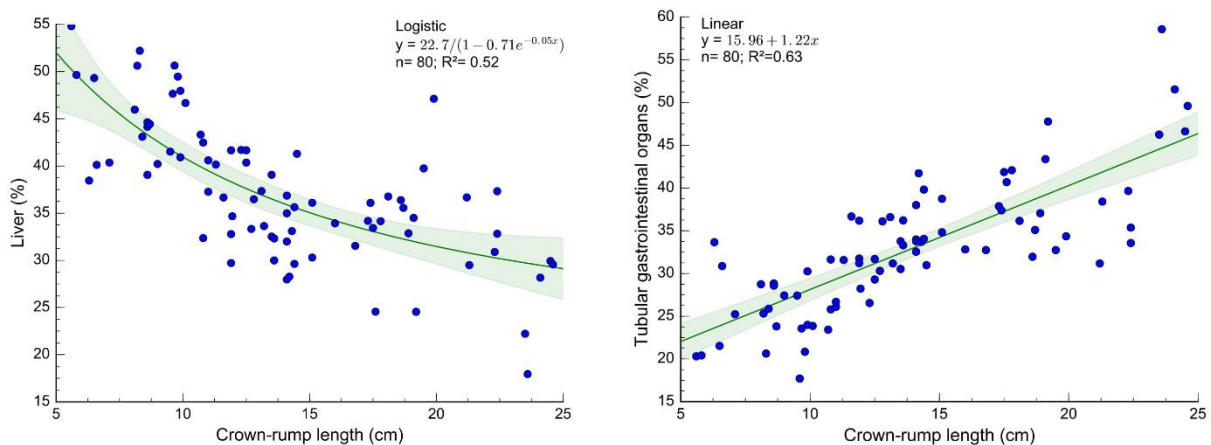


Figure 6. The relationship between the relative volume of the liver and tubular gastrointestinal organs with the crown-rump length in fetuses of lowland paca (*Cuniculus paca*). The green line represents the best model fitted to the plots \pm 95% CI.

220 4. Discussion

221 The results presented in this study supports the hypothesis that pacas produce
 222 precocial neonates with appropriate structures for afterbirth survival with low dependence
 223 on the mother's care. The development of fetal characteristics in paca follows an expected
 224 pattern of other large-sized rodents [21, 22]. However, the paca shows an earlier fetal

225 development and, thus, produces more developed neonates than other studied members
 226 of the Order Rodentia.

Table 2. Absolute and relative volumes, in percentage, of the visceral organs of pacas in advanced fetal development (crown-rump length > 23.2 cm) and adulthood.

Organ	Absolute volume \pm SD (ml)		Relative volume \pm SD (%)		T value	df	P
	Fetus	Adult	Fetus	Adult			
Heart	4.56 \pm 1.30	29.27 \pm 8.44	6.34 \pm 1.40	4.50 \pm 1.01	3.95	27	<0.01
Thymus	0.51 \pm 0.11	0.64 \pm 0.99	0.74 \pm 0.24	0.09 \pm 0.15	8.90	27	<0.01
Lungs	9.25 \pm 2.84	80.00 \pm 26.52	13.39 \pm 4.71	12.31 \pm 3.38	0.69	27	0.49
Liver	21.44 \pm 8.06	190.42 \pm 45.67	28.61 \pm 6.05	29.14 \pm 4.10	-0.27	27	0.79
Tubular gastrointestinal organs	34.76 \pm 16.08	315.59 \pm 94.53	45.15 \pm 8.49	47.46 \pm 6.68	-0.77	27	0.45
Kidneys	3.01 \pm 1.52	40.14 \pm 20.70	3.89 \pm 1.24	4.44 \pm 1.00	-1.23	27	0.23
Spleen	1.46 \pm 0.97	13.13 \pm 4.11	1.87 \pm 0.87	1.70 \pm 3.7	-0.60	27	0.55
Total visceral volume	74.99 \pm 25.82	669.20 \pm 145.12	-	-	-	-	-

df = degrees of freedom.

227 Comparing the proportion of the maximum CRL when the morphological
 228 characters appear in each species, the level of development of a paca individual at 42 days
 229 (2.0 cm length or 8.1% neonatal length) is convergent to a coypus (*Myocastor coypus*)
 230 individual at 60 days (1.9 cm or 16.7% neonatal length) [21], and an agouti (*Dasyprocta*
 231 *prymnolopha*) fetus at 45 days (3.5 cm or 25% neonatal length) [22]. At this stage, all
 232 three species present eyelid buds and are still completing the differentiation of the pelvic
 233 and thoracic limbs. This stage is convergent to the Theiler's Stage 22 in the development
 234 of the mice (*Mus musculus*), with 14 days (0.12 cm or 44% of neonatal length) [23].

235 Paca fetuses at 53 days (3.5 cm or 14.2% of neonatal length), have initiated the
 236 development of the outer ear, tactile pelage and claws. Agoutis, on the other hand, present
 237 the initial development of the outer ear and tactile pelage at 50 days of development (5.0
 238 cm or 36.4% of neonatal length), and claws only at 75 pregnancy days (10 cm or 72.7%

239 of neonatal length) [22]. Teeth started their eruption earlier in paca fetuses, at 110 days
240 (14 cm or 57.0% of neonatal length) compared to agouti (10 cm or 72.7% of neonatal
241 length) [22]. Similarly to agoutis, coypus at 90 days of gestation (6.7 cm or 58.8% of
242 neonatal length) presented developed outer ear, tactile pelage and claws [21]. In the cavy
243 (*Galea spixii*), these structures are formed only during the advanced fetal development,
244 outer ears at 33-35 pregnancy days (75%), and claws at 43-45 pregnancy days (98%) [24].
245 The outer ear is developed in the mice at Theiler's Stage 24, at 16 pregnancy days (17
246 mm or 63% of development) [23].

247 At 144 days (19.5 cm or 79.2% of neonatal length), paca fetuses presented all
248 external characteristics fully developed, including complete covering pelage and open
249 eyelids. In newborn pacas, the developed skin, including spots and covering pelage, might
250 be important for the independent temperature control and for camouflaging while moving
251 in the forest, while the open eyelids, the tactile pelage and outer ears serve for detecting
252 predators and food, and the claws for handling seeds and making burrows. Similarly, the
253 agouti, the coypus, and the Guinea pig (*Cavia porcellus*) present body covered by pelage
254 and open eyelids at birth [21, 22, 25]. On the other hand, neonates of the hytricomorph
255 coruro (*Spalacopus cyanus*) have naked venter and closed eyelids at birth, which open
256 only around four days after the individuals are born [26]. Thus, although the strategy of
257 producing precocial neonates is frequent in hystricomorphs, some few small-sized species
258 of the group produce less-developed neonates [27]. In contrast, newborns of mice show
259 closed eyelids and first signs of covering pelage will only appear after three days of
260 postnatal development, at Theiler's Stage 28 [23, 28].

261 The strategy adopted by species to maximize neonatal survival is influenced by
262 the risk of maternal and newborn mortality. Fetal growth implies a considerable increase
263 in the female's weight that can compromise the mother's response to predators. Therefore,

264 the strategy of having a shorter gestation periods and producing a higher number of less
265 developed neonates is usually a characteristic of prey species [29]. In these species, the
266 neonates will complete their development in the extra-uterine environment before
267 endangering the mother's survival.

268 Contrary, precocial species usually spend more energy in longer gestations,
269 delivering fewer and heavier neonates with higher prenatal growth of brain and muscle
270 [7, 13], which will be important for their early independent locomotion and interpretation
271 of environmental stimulus through better sensory capacity.

272 The wild paca female has a long gestation delivering only one precocial young
273 [14], and faces high energy costs to produce a highly developed and competent neonate.
274 Agoutis, coypus, Guinea pigs and even the world's largest rodent, the capybara
275 (*Hydrochoerus hydrochoeris*), produce more than one litter per gestation, dividing the
276 energy of pregnancy among the offspring. Furthermore, in contrast to those large
277 hytricomorphs, the behavior of building underground burrows guaranteeing the adult
278 paca protection may supplementary enhance the survival of the young.

279 Considering a mean adult weight of 7.78 kg [19], advanced paca fetuses had
280 around the 10% (787.79 g) of the adult body weight. The relative neonatal weight in other
281 studied rodents ranges from 1.8% in the brown rat and 6% in the agouti (Table 3).
282 Although the strong relationship between litter size and individual relative neonatal
283 weight ($r^2=0.71$; $p<0.01$) (Figure 7A), we observed a better association between the fetal
284 development and the relative litter biomass per pregnancy ($r^2=0.83$; $p<0.01$) (Figure 7B).
285 For this calculation, we considered the relative fetal length when each species presents
286 developed tactile pelage and outer ears as a measure of the rate of fetal development.
287 Thus, the higher the relative weight of the litter, the faster their intrauterine development
288 and, consequently, less developed will be the neonates. Thus, the overall energy costs for

289 the mother is better explained by the total fetal biomass and determines the fetal
 290 development.

Table 3. The litter size, the relative neonate and litter weight in relation to adults, and the fetal development rate in the lowland paca (*Cuniculus paca*) and six other rodent species. The relative fetal length when each species presents developed tactile pelage and outer ears was used as a measure of the fetal development rate.

Species	Relative neonate weight (%)	Litter size (n)	Relative litter weight (%)	Fetal development rate (%)	Reference
Agouti (<i>Dasyprocta prymnolopha</i>)	6	2.1	12.6	36.40%	[22, 31, 50, 51]
Coypus (<i>Myocastor coypus</i>)	5	5	25.0	58.80%	[21, 32, 51-53]
Guinea pig (<i>Cavia porcellus</i>)	11	3	33.0	75.50%	[25, 55, 56]
Lowland paca (<i>Cuniculus paca</i>)	10	1.03	10.3	14.20%	[9], This study
Mice (<i>Mus musculus</i>)	4.5	7.4	33.3	63%	[23, 36]
Rat (<i>Rattus norvegicus</i>)	1.8	9	16.2	48.80%	[57]
Richardson's ground squirrel (<i>Urocitellus richardsonii</i>)	2.3	6.93	15.9	-	[59, 59]

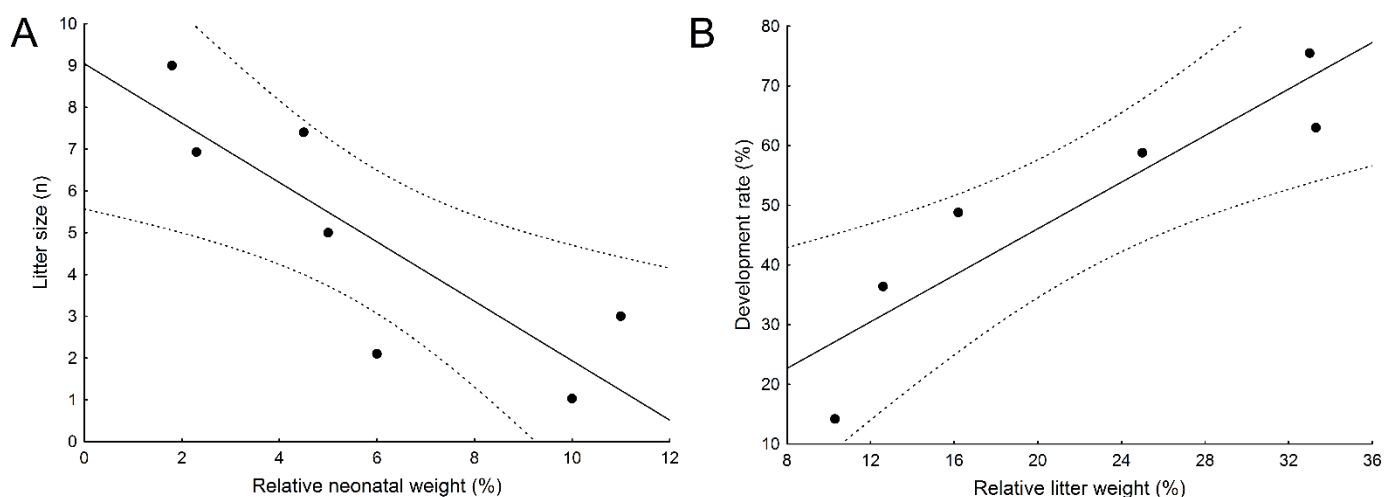


Figure 7. The relationship between: A – the relative neonatal weight in relation to adults and the litter size in seven rodent species; B – the relative weight of the litter in relation to adults and the fetal development rate of six rodent species. The relative fetal length when each species presents developed tactile pelage and

outer ears was used as a measure of the fetal development rate. Dashed lines represent the 95% CI. For species names, see Table 3.

291 Reproduction requires to the female a great energy cost balanced between
292 pregnancy and lactation energy costs needed to produce competent young. Altricial
293 species tend to space reproductive events, increasing the interval between births due to
294 the long lactation period. In precocious species, the early weaning allows the offspring to
295 reach competitiveness enough for autonomous survival. The high level of energy spent
296 during pregnancy is then compensated by the less time spent on lactation and offspring
297 care [30]. This strategy allows the mother to rapidly recover its body condition for the
298 next reproductive event.

299 This is consistent with the time invested by pacas on lactation. Pacas' offspring,
300 although heavier, start eating solids at 21 days after birth and are weaned at around 42
301 days, while agoutis (*D. prymnolopha*) are weaned at 45 days [31], and coypus at 54 days
302 [32]. Moreover, the lactation period in hystricomorphs is usually shorter than in small
303 rodents from other groups [33].

304 All these differences in rodents' reproductive traits will affect the survival of
305 neonates. The greater litter size with less developed neonates of small rodents and other
306 hystricomorphs results in lower offspring survival rate. The mortality rate in capybara
307 young reaches at least 27% [34] and only 40% of the young of Richardson's ground
308 squirrel survive until adulthood [35], while this proportion is 30.5% in the mice [36].
309 Conversely, the rate of mortality in non-primiparous pacas is estimated at only 10% [37].
310 All these paca reproductive traits reinforce our results that the species' newborns are
311 indeed more developed than neonates of small rodents and other hystricomorphs. Thus,
312 the reproductive strategy of pacas is compatible to a species in a low prey condition and

313 suggests that the paca is not reproductively prepared to response to high hunting
314 pressures.

315 The increase in the biometric measures in paca embryos/fetuses is similar to those
316 in agouti [22], in which the abdominal diameter showed a linear-like pattern of increase,
317 while the bi-parietal diameter and the cranial length showed a discrete tendency to
318 stabilization in the final stage of fetal development. Furthermore, the Guinea pig also
319 present a linear trend between gestation age and abdominal circumference [38]. Pacas in
320 advanced fetal stages showed a very consistent body weight stabilization, while the agouti
321 showed a linear increase throughout the intrauterine development [22]. This difference
322 may be because the study conducted on agoutis may have not followed the whole
323 gestation length in this species, since other studies have detected heavier *D. prymnolopha*
324 neonates at birth [31]. In the mouse, there was also observed a linear increase in body
325 weight during the intrauterine development, with no sign of stabilization [23].

326 The mean longitudinal length in advanced paca fetuses (around 34 cm) was very
327 similar to that found using B-mode ultrasound (33.46 cm) [39], and by measures of
328 newborns in captivity (33.45 ± 0.62 for females and 33.30 ± 0.52 for males) [40].
329 Furthermore, the fetal age curve we built is very consistent with previous paca body
330 measurements, since the calculated bi-parietal values at 60 days (1.51 cm) and 90 days
331 (2.68 cm) using our formula is very similar to the values found in studies through
332 ultrasound (1.25 cm and 2.34 cm, respectively) [41]. In addition, the mean body weight
333 of advanced paca fetuses, which was used for age calculations, is within the expected
334 interval of 550 to 800g [42] and is similar to the mean weight of newborn pacas found in
335 previous studies: 671.30 g [15], 754.39g [39] and 741.14 g [40]. This reinforces that the
336 measures obtained here could be used as trustful standard parameters. By describing and
337 determining the timing of principal events in the fetal development, our results may

338 improve the analysis of diagnostic imaging technologies, i.e. ultrasonography and
339 tomography, allowing the accurate pregnancy monitoring, the teratological diagnosis, and
340 the prediction of gestational age and parturition in pacas.

341 Except for the heart and the thymus, the organs of advanced fetuses maintain the
342 same volumetric proportion that those in adult pacas. The absolute volume of the thymus
343 in adults remains the same as in advanced fetuses, causing a sharp decrease in its relative
344 volume along the life. In several mammalian species, the thymus is a very functional
345 organ during the fetal phase, being the main producer of lymphocytes [43, 44]. In adults,
346 however, it reduce its size with the maturation of the immune system and eventually
347 disappear [45, 46], as we detected in 13 (62%) adult pacas.

348 The liver and tubular gastrointestinal organs showed opposite intrauterine
349 development trends. During the initial phases of the mouse's embryological development,
350 the liver endothelium attracts stem cells responsible for the hematopoiesis; in this period,
351 the liver is assumes the role of producing blood [47]. These hematopoietic cells, in turn,
352 secrete oncostatin M, which induces the maturation of the hepatocytes and the increase
353 in liver size [48]. The liver greatly enlarge in the Theiler's Stage 17 of mouse embryonic
354 development [28], and this may explain the high relative volume in small paca fetuses
355 (more than 40%). Since the hematopoietic function is gradually assumed by the bone
356 marrow, the liver reduces its representativeness along the fetal development, reaching in
357 advanced paca fetuses the same proportion as in adults (28%).

358 On the other hand, the increase in the proportion of tubular gastrointestinal organs
359 may be related to the long preparation of these organs along the fetal development to
360 assure their important digestive functions after birth. In mammals, the entrance and exit
361 sites of future mouth and anus are formed some days after the organogenesis. During this
362 stage, the fetus starts swallowing amniotic fluids and the gastrointestinal organs undergo

363 rapid growth through the nutritional components provided by this fluid [49]. In the late
364 gestational age, tubular gastrointestinal organs suffer high level of differentiation and the
365 number of cells of their tissues increases greatly, which will be important in the extra-
366 uterine life for the digestion of milk and other food resources [49].

367 Along with the external morphological features, the consistency in the proportion
368 of the organs between advanced fetuses and adults reveals, once more, that the species'
369 neonates are indeed well-developed and prepared for an early independence during the
370 extra-uterine life.

371

372 **5. Conclusions**

373 This study shows that pacas produce neonates with high precocity level. Although
374 rodents are usually potential prey, the strategy for offspring survival adopted by pacas is
375 similar to a non-prey condition, suggesting a high survival rate of neonates in nature.
376 Although the paca is currently the most hunted species in the Amazon, its reproductive
377 strategy suggests that this species is not reproductively prepared to response to high
378 hunting pressures. These findings should trigger urgent action focused on the
379 establishment of *in situ* management plans involving this species. This study also
380 contribute important knowledge for the improvement of imaging techniques for the
381 reproductive diagnosis in the captive paca.

382

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392

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3. ANEXOS

Anexo 1. Regras para formação de artigos da revista *Theriogenology*, na qual este trabalho será submetido.



THERIOGENOLOGY
An International Journal of Animal Reproduction

AUTHOR INFORMATION PACK

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DESCRIPTION

Theriogenology provides an international forum for researchers, clinicians, and industry professionals in **animal reproductive biology**. This acclaimed journal publishes articles on a wide range of topics in **reproductive and developmental biology**, of domestic mammal, avian, and aquatic species as well as wild species which are the object of veterinary care in research or conservation programs.

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- 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."

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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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Anexo 2. Declaração da Comissão de Ética no Uso de Animais (CEUA) da Universidade Federal Rural da Amazônia autorizando a presente pesquisa.



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

DECLARAÇÃO

Declaramos para fins de comprovação juto ao Edital 005/2015 – Pesquisador Visitante Sênior (PVS) e Taxa de Bancada da Fundação de Amparo a Pesquisa do Estado do Pará - FAPESPA que o projeto intitulado “FISIOLOGIA REPRODUTIVA DE ANIMAIS SELVAGENS E DOMÉSTICOS NA AMAZÔNIA” coordenador o prof. Dr. Frederico Ozanan Barros Monteiro foi devidamente cadastrado neste CEUA sob número 007/2016.

Belém, 19 de dezembro de 2016.

Andréa Bezerra de Castro

Profª Msc. Andréa Bezerra de Castro
Coordenadora em exercício CEUA UFRA



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Av. Tancredo Neves, nº 2501, Bairro Montese, Belém – PA. CEP: 66.077-901
Contatos: (91) 3210-5165 ceua@ufra.edu.br www.comissao.ufra.edu.br/ceua

