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Embryonic and fetal morphology in the lowland paca (*Cuniculus paca*): A precocial hystricomorph rodent

Hani Rocha El Bizri [a, b, *], Frederico Ozanan Barros Monteiro [a],
Rafael dos Santos de Andrade [a], João Valsecchi [b], Diva Anelie de Araújo Guimarães [c],
Pedro Mayor [a, d, e]

[a] *Universidade Federal Rural da Amazônia (UFRA), Postgraduate Program in Animal Health and Production in Amazonia (PPGSPAA), Belém, PA, Brazil*
[b] *Instituto de Desenvolvimento Sustentável Mamirauá (IDSM), Tefé, AM, Brazil*
[c] *Universidade Federal do Pará (UFPA), Postgraduate Program in Animal Science (PPGCAN), Belém, PA, Brazil*
[d] *Universitat Autònoma de Barcelona (UAB), Department of Animal Health and Anatomy, Faculty of Veterinary, Bellaterra, Barcelona, Spain*
[e] *FUNDAMAZONIA, Iquitos, Loreto, Perú*

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ABSTRACT

In mammals, the embryonic and fetal development of a species has evolved to maximize neonatal survival. In this study, we use a sample of 132 embryos/fetuses of wild lowland paca (*Cuniculus paca*), obtained over a period of 15 years through collaborative methods with local hunters in the Amazon to describe the intrauterine development of external and internal morphology of this Neotropical rodent. We also compare the newborn survival strategy in this species with other rodents. The crown-rump length (CRL) ranged between 0.6 and 24.6 cm. External features appeared in the following chronological order: limbs, eyelid buds, fusioned eyelids, genitalia, outer ear, tactile pelage, claws, skin, skin spots, covering pelage, teeth and open eyelids. Fetuses with CRL >19.5 cm presented all external features fully developed. The growth formula of fetal age was calculated as $\sqrt[3]{W} = 0.082\ (t - 37.25)$, and age was accurately associated with CRL. We described the relationship between CRL and external and internal biometry. The liver declined in proportion within the internal cavity, while the relative volume of tubular gastrointestinal organs increased significantly along the embryo/fetal development. All organs, except the heart and the thymus, had similar relative volumes in advanced fetuses and adults. Our comparison of the intrauterine development in several rodent species indicates that the paca's reproductive strategy is comparable to species that are subject to low natural predation. Given that *C. paca* is perhaps the most hunted animal in Latin America, sustainable hunting throughout its range must take into account its relative reproductive performance.

1. Introduction

The embryonic and fetal development in mammals comprises an individual's maturation from fecundation to parturition, in which neonates are prepared for extra-uterine life [1]. The study of intrauterine development allows the understanding and comparison of strategies adopted by different mammal species to maximize their maternal and neonatal survival [2,3]. In addition, these studies are useful for informing *in situ* and *ex situ* reproductive management practices as well as the clinical diagnosis of developmental parameters of mammal species [4].

Mammalian species are divisible into two main neonatal development groups, altricial and precocial. Altricial species have short gestation periods, produce relatively small-sized offspring, and large numbers of young on which they invest little after their birth [5]. Whereas altricial newborn complete their fetal development in the extra-uterine environment, largely depending on an extended maternal care for feeding and moving [6], precocial species deliver well-developed neonates, with greater brain mass and motor and visual capacity, being able to move and forage independently from the mother soon after birth [7].

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* Corresponding author. Estrada do Bexiga, 2584 - Bairro Fonte Boa, Tefé, Amazonas 69553-225, Brazil.

E-mail addresses: hanibiz@gmail.com (H.R. El Bizri), frederico.monteiro@ufra.edu.br (F.O.B. Monteiro), rafael.marca.vet@gmail.com (R.S. de Andrade), joao.valsecchi@mamiraua.org.br (J. Valsecchi), diva@ufpa.br (D.A.A. Guimarães), mayorpedro@hotmail.com (P. Mayor).

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The reproductive strategies of wild species influence their population dynamics as well as their responses and resilience to environmental and anthropogenic disturbance [8]. However, for most mammals particularly those species found in tropical regions, their reproductive biology remains poorly studied [9]. Often this is due to logistic and financial restrictions, resulting in small sample sizes. Alternatively, through the examination of reproductive tracts from animals hunted for subsistence purposes, obtained with the collaboration of local hunting communities, it is possible to collect *in situ* data and biological samples with a higher level of reliability [9]. This sampling strategy permits us to take advantage of materials that would otherwise be discarded, at the same time as obtain larger sample sizes.

The lowland paca (*Cuniculus paca*, Rodentia, Cuniculidae) is a medium-sized Neotropical hystricomorph rodent (average body mass 7.7 kg) found in tropical rainforests from southern Mexico to northern Argentina. Pacas are prized for their meat, being one of the most hunted species in Latin America [10,11], and has led to past interest in their captive breeding for production of meat. Nevertheless, despite some advances, the reproductive biology of this species is still poorly studied, hindering the species' effective management in *situ* and *ex situ* conditions [12].

The majority of extant mammals produce altricial neonates, and this includes most rodent species [13]. Despite the fact that small rodents commonly are very highly productive, pacas in contrast have a relatively long gestation period (around 149 days), have on average 1.37 parturitions in a year, and produce only 1.03 young per birth [14,15]. Moreover, despite a close phylogenetic proximity within rodents, the newborn survival strategies determined by the embryonic and fetal development may be diverse. In this study, we use samples of paca genitalia collected over a period of 15 years using an innovative and collaborative process involving subsistence hunters in the Amazon. Here we describe the development of the external and internal morphology of paca embryos/fetuses and compare the newborn survival strategy adopted by this species to other rodents.

2. Material and methods

2.1. Study sites

We selected two areas in the Amazon rainforest region for our study. The first area, the Yavarí-Mirín River (YMR, S 04°19.53; W 71°57.33) in northeastern Peruvian Amazon, 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 region has a dry (July–October) and a wet/flooded season (November–June). The second site, the Amanã Sustainable Development Reserve (ASDR, S 01°54.00; W 64°22.00) is a reserve of 2,313,000 ha in the Central Brazilian Amazon, between the Negro and Japurá rivers, consisting predominantly of upland forests. Within the ASDR, there is a population of approximately 4000 riverine people, found in 23 communities and some isolated settlements. In the ASDR, there is a dry (August–December) and a wet/flooded season (January–July). 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.

2.2. Collection of samples

From 2002 to 2016, local hunters collected and voluntarily donated genitalia from 127 pregnant pacas, 64 in the YMR and 63 in the ASDR. A total of 132 embryos/fetuses was analyzed including 5 twin gestations (3.9% of all samples). During the study, we trained hunters 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, we avoided any invasive procedure or any additional mortality for the purpose of the study [9].

Our research protocol was approved by the Research Ethics Committee for Experimentation in Wildlife at the Dirección 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 No 29092–1) and by the Committee on Ethics in Research with Animals of the Federal Rural University of the Amazon (UFRA CEUA protocol 007/2016). Samples were sent to UFRA, Belém, Pará, Brazil, under the export license CITES/IBAMA (No 14BR015991/DF).

2.3. Laboratory procedures

We dissected the genital organs to remove all conceptuses. We first inspected each embryo/fetus to describe the following morphological features: 1) differentiated genitalia, 2) differentiated limbs, 3) eyelids, 4) skin, 5) covering and tactile pelage, 6) skin spots, 7) erupted teeth, 8) claws and 9) outer ear. The embryo/fetal stage was determined according to the *International Committee on Veterinary Embryological Nomenclature* [16].

We measured each embryo/fetus' external biometry using: 1) body mass, 2) crown-rump length (CRL), 3) longitudinal length from rostral edge of nose to distal portion of the tail, 4) biparietal diameter, 5) cranial occipito-frontal diameter, 6) cranial circumference, 7) thoracic diameter and thoracic circumference, 8) abdominal diameter and abdominal circumference, 9) femur and humerus length, and 10) length of forelimbs and hindlimbs. Thoracic and abdominal measurements were taken from the last rib and from the insertion of the umbilical cord, respectively. We measured body mass in grams using a digital weighing scale (0.1 g accuracy), and employed a tape measure (0.1 mm accuracy) and a metal caliper (full measurement capability 300 mm) for body measurements.

We eviscerated the thoracic and abdominal organs (heart, lungs, thymus, liver, spleen, kidneys and tubular gastrointestinal organs) from fetuses >5 cm CRL ($n = 109$). By applying the Archimedes Principle, which considers the volume of water displaced by a given organ as a proxy of the organ volume [17], we calculated an organ's volume by submerging it in hypodermic syringes (0.01 ml accuracy) containing water. We summed the volumes of all individual organs to obtain the total visceral volume and to calculate each organ's relative volume (as a percentage) relative to the total visceral volume. After excluding organs showing signs of autolysis, we analyzed 82 samples. For comparison, we measured the volumes of thoracic and abdominal organs and the total visceral volume of 21 adult pacas.

2.4. Data analysis

Since adult pacas from YMR and ASDR did not differ in body mass [18,19], samples from both study areas were pooled. Fetal age was calculated using Huggett and Widdas' formula [20]: $\sqrt[3]{W} = a\ (t\text{-}t_0)$, where W is the fetal weight, a is the specific fetal growth velocity, t is the fetal age in days, and t_0 is the calculated intercept on the age axis; thus, in t_0, the body weight of the individual is so low that it approximates to zero. In species presenting between 100 and 400 days of pregnancy, t_0 is equal to 20% of gestation time [20]. Therefore, an estimated delivery date was used for these calculations, considering 149 days of gestation [15] and a mean weight of 787.79 g at birth, which is the average body weight of fetuses that showed stabilized mass in advanced pregnancy stages (>22.3 cm CRL).

We used logistic regressions to determine the probability of the presence of each morphological character along the growth in CRL, using the software Statistica 8.0. To assess the relationship between age, external biometric measures and relative and absolute organ volumes with CRL, we employed multiple regressions using the software CurveExpert 2.4 to complete the regressions and set the best function to the plots. We used the Akaike Information Criteria (AIC) scores to compare the functions, considering the lowest score as the best fit. For fetal measurements and absolute organ volumes, we forced linear regressions to origin and only considered those functions with starting point on or near to zero, since we expect both CRL and these measures to be at zero in the beginning of the intra-uterine development. We compared the relative volumes of the organs between adults and advanced fetuses using a T-student Test.

We compared the relative fetal length (as a percentage of neonatal length) of occurrence of external morphological characters, the relative neonatal weight (in percentage of adult weight) and the relative litter biomass (sum of the weight of all neonates per pregnancy in relation to the adult weight) between our sample and data for other rodent species from the literature. To examine the association between the relative neonatal weight and litter size, and between relative litter biomass and rate of fetal development, we employed regression analyses. For these, we calculated the rate of fetal development based on the fetal length when each species presents developed tactile pelage and outer ears, since these characteristics were assessed in all studies consulted. The value of $P < 0.05$ was considered significant.

3. Results

3.1. External morphological characters

The average CRL for all embryos/fetuses was 11.9 (±0.56 SE) cm (range 0.6–24.6 cm). Initial differentiation of the limbs was observed at around 1.7 cm of CRL, but these were still paddle-shaped and showed no separation of digits. All embryos/fetuses

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	124	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	$y = \frac{exp^{(-3.38+0.95x)}}{1+exp^{(-3.384+0.95x)}}$
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	$y = \frac{exp^{(-19.47+1.11x)}}{1+exp^{(-19.47+1.11x)}}$

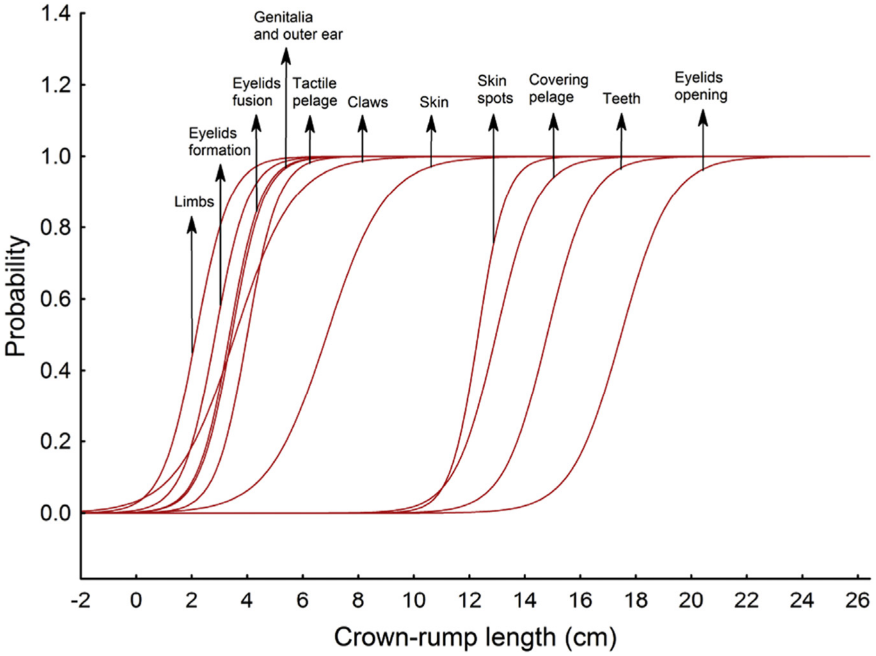

Fig. 1. The occurrence probability of external features in embryos/fetuses of lowland paca (*Cuniculus paca*) according to their crown-rump length (CRL).

of >2.9 cm CRL had well differentiated limbs and digits. The smallest individual with eyelid buds was 2.3 cm; thus, this CRL represents the approximate transitional phase between embryo and fetus. The fusion of eyelid buds occurred in fetuses of >3.1 cm CRL (Table 1; Fig. 1; Fig. 2). The genitalia and the outer ears were also differentiated in fetuses larger than 3.1 cm. The following fetal features appeared in chronological order: tactile pelage (CRL>4.2 cm), the complete formation of claws (CRL > 4.9), skin (CRL > 6.5 cm), skin spots (CRL > 13.6 cm) and covering pelage (CRL > 15.2 cm). Teeth started to erupt sporadically in fetuses of 14.0–15.5 cm CRL, and all fetuses with CRL over 15.9 cm had erupted teeth. All fetuses with CRL of >19.5 cm presented open eyelids (Figs. 1 and 2).

3.2. External biometry

All relationships between biometric measurements and CRL were significant, with $r^2 > 0.92$ (Fig. 3 and Fig. 4). The logistic model was the best fitted to the body weight, while the power was the best adjusted to the humeral length and the logistic power model furnished the best fit to the remaining measures (Fig. 4). The strongest relationships with CRL were for the biparietal diameter,

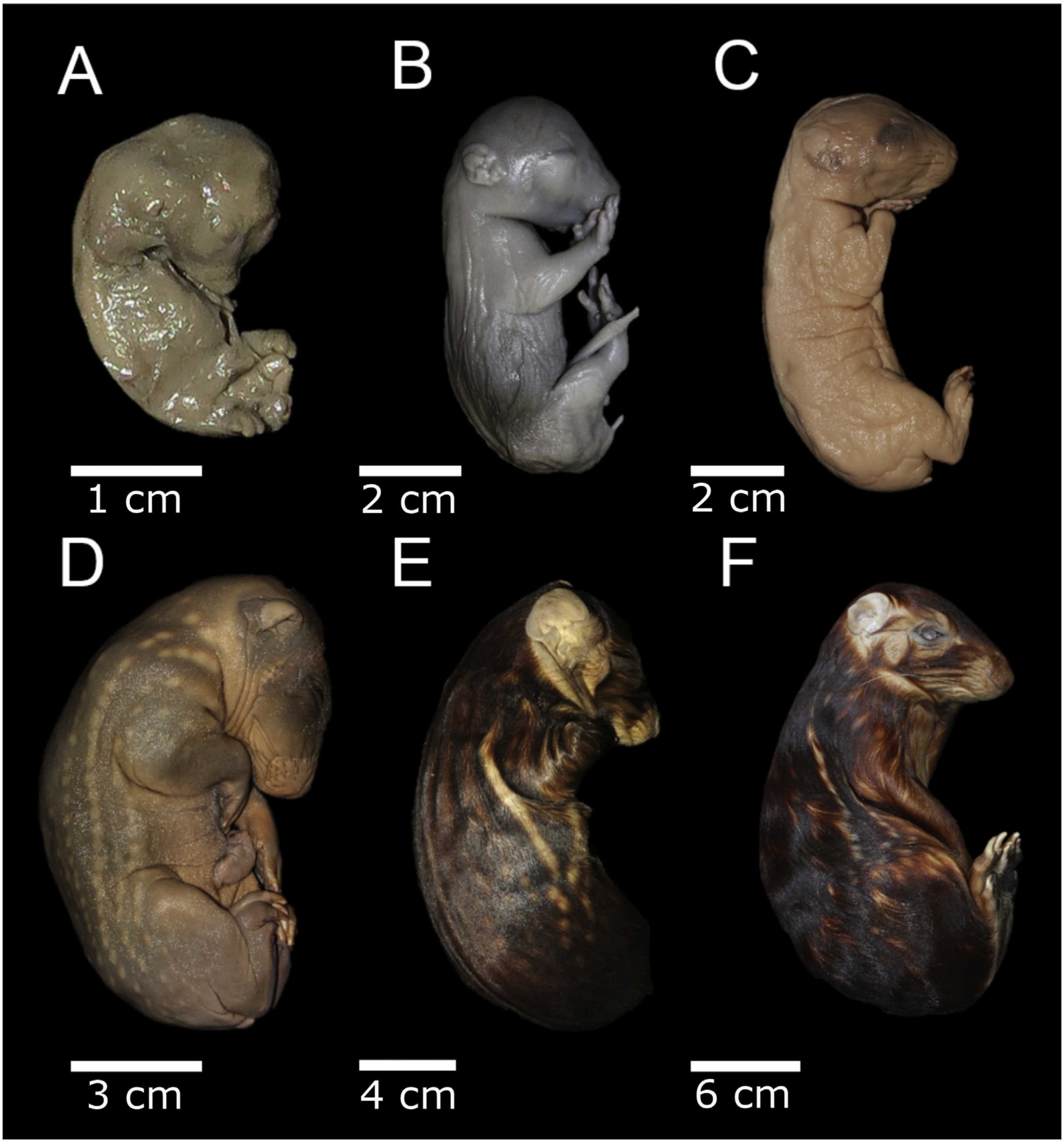

Fig. 2. Fetuses at different stages of intrauterine development, presenting distinct external features according to their crown-rump-length (CRL). A–a fetus with 2.3 cm of CRL presenting initial growth of eyelids but no outer ear or claws. B–a fetus with 6.3 cm of CRL presenting an early fusion of the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

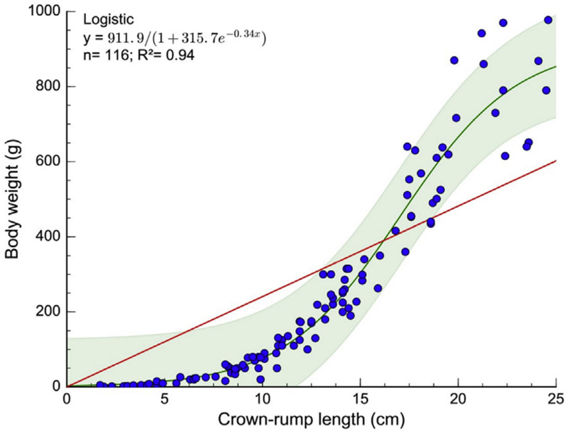

Fig. 3. The relationship between the body weight and the crown-rump length in embryos/fetuses of lowland paca (*Cuniculus paca*). The green line represents the best model fitted to the plots ±95% CI, while the red line represents an expected linear trend with no intercept. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

thoracic circumference, abdominal circumference, femoral length and humeral length (Fig. 4). Fetal body mass ranged from 5.0 g to 977.5 g, and advanced paca fetuses had the 10% (787.79 g) of the adult weight. The growth formula of fetal age was $\sqrt[3]{W} = 0.082$ (t - 37.25), and the age was accurately associated with CRL ($r^2 = 0.95$; $P < 0.01$) (Fig. 5).

3.3. Internal volumetry

Fig. 6 shows the best models for the increase in absolute organ volumes along the fetal development. The strongest associations with CRL were found between the total visceral volume and the tubular gastrointestinal organs. Relative volumes of the heart, lungs, spleen, kidneys and thymus did not vary along the fetal development ($r^2 < 0.1$; $P > 0.05$). On the other hand, relative volumes of the liver and the tubular gastrointestinal organs showed a strong relationship with the fetal CRL. While the liver had a decreasing trend ($r = -0.67$; $r^2 = 0.45$; $P < 0.01$), the relative volume of tubular gastrointestinal organs increased along the fetal development ($r = 0.77$; $r^2 = 0.60$; $P < 0.01$) (Fig. 7). All organs in advanced fetuses, but the heart and the thymus, presented similar relative volumes compared to adult pacas (Table 2).

3.4. Development of the lowland paca in comparison with other rodent species

We observed a strong positive relationship between litter size and relative neonatal weight ($r^2 = 0.71$; $P < 0.01$) (Fig. 8A). Table 3 shows the comparison among the stages in which morphological characters appear in five rodent species during the embryonic/fetal development, including the lowland paca. There is a strong negative association between the rate of fetal development and the relative litter biomass per pregnancy ($r^2 = 0.83$; $P < 0.01$) (Fig. 8B). Rodents with larger litter biomass per pregnancy has a lower rate of fetal development, and thus, present the same fetal morphological characters in later pregnancy stages (Table 4).

4. Discussion

The results presented in this study show that pacas produce precocial neonates with appropriate structures for afterbirth survival of low mother's care dependence. The development of fetal characteristics in paca follows a similar pattern to other large-sized rodents [21,22]. However, the paca shows earlier fetal development and hence produce more developed neonates than other studied members of the order Rodentia.

At 146 days (around 22.0 cm or 83.3% of neonatal length), paca fetuses presented fully developed external characteristics, including complete covering pelage and open eyelids. In newborn pacas, the developed skin, including covering pelage and spots, might be important for independent temperature control and for camouflaging while moving in the forest; while the open eyelids, tactile pelage and outer ears serve for predator detection and food recognition, and claws for handling seeds and digging burrows. This is similar to other rodent species such as the agouti (*Dasyprocta prymnolopha*), the coypu (*Myocastor coypus*), and the Guinea pig (*Cavia porcellus*), which present body covered by pelage and open eyelids at birth [21,22,25]. In contrast, neonates of the hytricomorph coruro (*Spalacopus cyanus*) have naked venter and closed eyelids at birth, which open only around four days after birth [26]. Thus, although the strategy of producing highly precocial neonates is frequent in hystricomorphs, few small-sized species in this group produce less-developed neonates [27]. In contrast, newborns of mice show closed eyelids and first signs of covering pelage only appear after three days of postnatal development, at Theiler's Stage 28 [23,28].

The strategy adopted by species to maximize neonatal survival is influenced by the risk of maternal and newborn mortality. Fetal growth implies a considerable increase in the female's weight that can compromise the mother's response to predators. Therefore, the strategy of having a shorter gestation period and producing a higher number of less developed neonates is usually a characteristic of prey species [29]. In these species, neonates will complete their development in the extra-uterine environment in order to optimize the mother's survival. Contrastingly, precocial species usually spend more energy in longer gestations, delivering fewer and heavier neonates with higher prenatal brain and muscle growth [7,13], which will be important for their early independent locomotion and interpretation of environmental stimulus through better sensory capacity.

Because wild paca females have a relatively long gestation period and produce only one precocial young [14], they face high-energy costs to produce a highly developed and competent neonate. Agoutis, coypus, Guinea pigs and even the world's largest rodent, the capybara (*Hydrochoerus hydrochoeris*), produce more than one young per gestation, dividing the energy of pregnancy among the offspring. Furthermore, distinct from the large hytricomorphs, the behavior of building underground burrows by adult pacas may enhance the survival of the young. However, the results of this study show that the overall energy costs for the mother are better explained by the relative litter biomass, which in turn determines the rate of fetal development, by which the higher the relative weight of the litter, the faster their intrauterine development and, consequently, less developed the neonates will be.

Reproduction requires a large energetic trade off balanced between pregnancy and lactation. Altricial species tend to space reproductive events, increasing the interval between births due to the long lactation period. In precocious species, the early weaning allows the offspring to reach competitiveness enough for autonomous survival. The high level of energy spent during pregnancy is then compensated by the less time spent on lactation and offspring care [30]. This strategy allows the mother to rapidly recover its body condition for the next reproductive event.

This is consistent with the time invested by pacas on lactation. Pacas' offspring, although heavier, start eating solids at 21 days after birth and are weaned at around 42 days [31], while agoutis (*D. prymnolopha*) are weaned at 45 days [32], and coypus at 54 days

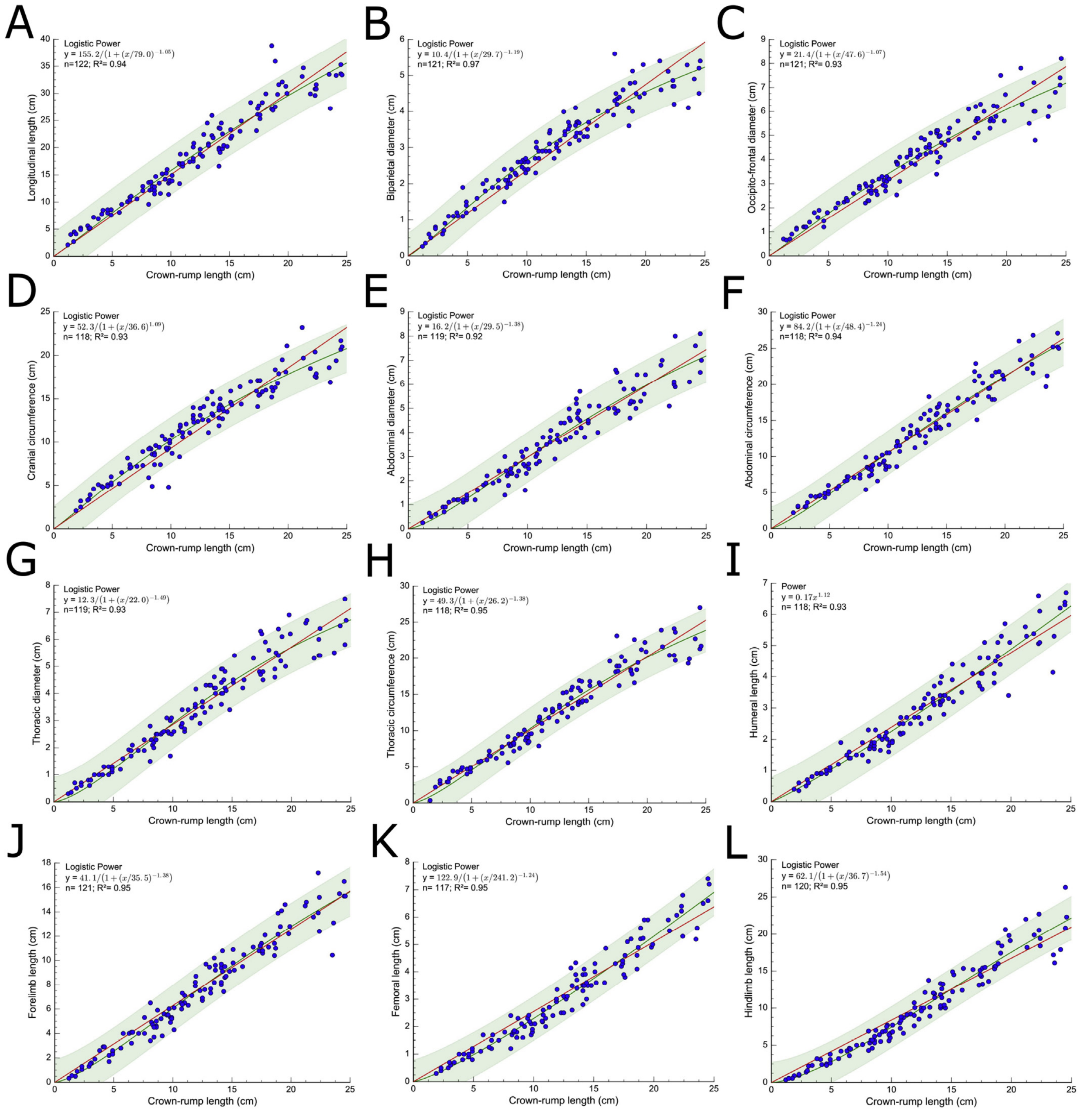

Fig. 4. The relationship between external biometric measures and the crown-rump length in embryos/fetuses of lowland paca (*Cuniculus paca*). The biometric measures taken were: A–Longitudinal length; B–Biparietal diameter; C–Occipito-frontal diameter; D–Cranial circumference; E–Abdominal diameter; F–Abdominal circumference; G–Thoracic diameter; H–Thoracic circumference; I–Humeral length; J–Forelimb length; K–Femoral length; L–Hindlimb length. The green line represents the best model fitted to the plots ±95% CI, while the red line represents an expected linear trend with no intercept. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

[33]. Moreover, the lactation period in hystricomorphs is usually shorter than in small rodents from other groups [34].

All these differences in rodents' reproductive traits will affect the survival of neonates in the wild. The greater litter size with less developed neonates of small rodents and other hystricomorphs might result in lower offspring survival rate. The mortality rate reaches at least 27% in capybara young [35], 30.5% in the mice [36] and 40% in the Richardson's ground squirrel [37]. Conversely, the rate of mortality in non-primiparous pacas is estimated at only 10% [38]. All these paca reproductive traits reinforce our results that the species' newborns are indeed more developed than neonates of small rodents and other Neotropical hystricomorphs.

The increase in the biometric measures in paca embryos/fetuses is similar to those in agouti [22], in which the abdominal diameter

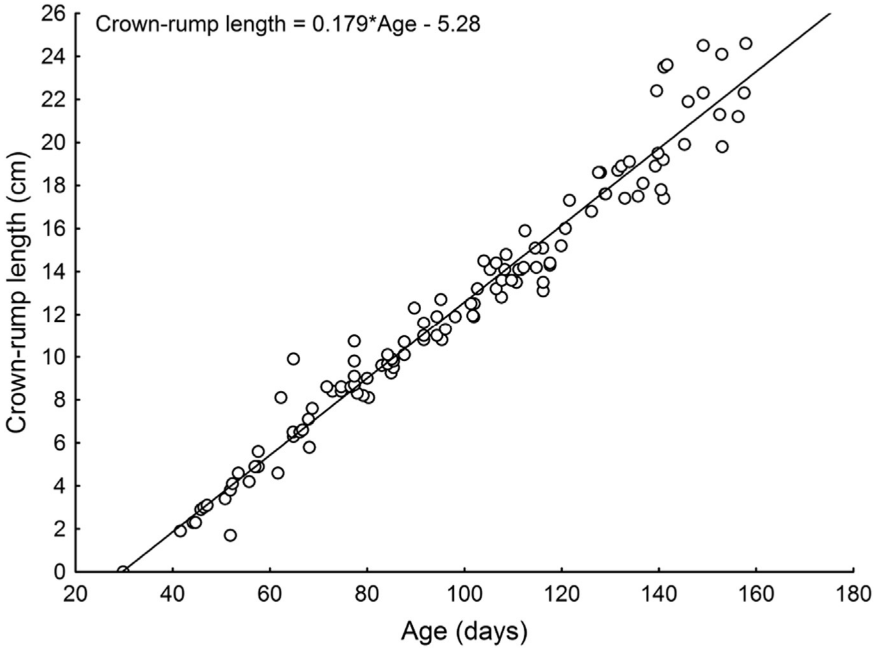

Fig. 5. The relationship between embryo/fetal age and the crown-rump length in the lowland paca (*Cuniculus paca*).

showed a linear-like pattern of increase, while the biparietal diameter and the occipito-frontal diameter showed a discrete tendency to stabilization in the final stage of fetal development. Furthermore, the Guinea pig also presents a linear trend between gestation age and abdominal circumference [39]. On the other hand, while pacas in advanced fetal stages showed very consistent body weight stabilization, the agouti shows a linear increase throughout the intrauterine development [22]. This difference may be because the study conducted on agoutis may have not followed the whole gestation length in this species; in that sense, other studies have detected heavier *D. prymnolopha* neonates at birth [32]. In the mouse, there was also observed a linear increase in body weight during the intrauterine development, with no sign of stabilization [23].

The mean longitudinal length in advanced paca fetuses (around 34 cm) was very similar to that found for the species using B-mode ultrasound (33.46 cm) [40], and by measures of newborns in captivity (33.45 ± 0.62 cm for females and 33.30 ± 0.52 cm for males) [41]. Furthermore, the fetal age curve built is very consistent with previous paca body measurements, since the calculated biparietal values at 60 days (1.51 cm) and 90 days (2.68 cm) using the formula of the present study is very similar to the values found in studies using ultrasound (1.25 cm and 2.34 cm, respectively) [42]. In addition, the mean body weight of advanced paca fetuses, used for age calculations, is within the expected interval of 550–800 g [43] and is similar to the mean weight of newborn pacas found in previous studies: 671.30 g [15], 754.39 g [40] and 741.14 g [41]. This reinforces the point that the measures obtained in this study can be used as accurate standard parameters. By describing and determining the timing of principal events in the fetal development, the results presented here may improve the analysis of diagnostic imaging technologies, i.e. ultrasonography and tomography, allowing the accurate pregnancy monitoring, the teratological diagnosis, and the prediction of gestational age and parturition in pacas.

Except for the heart and the thymus, the organs of advanced fetuses maintain the same volumetric proportion of those in adult pacas. Since the heart keeps increasing in absolute volume until adulthood, the reduction we found in the relative volume in adults is possibly an effect of the overall variation in the relative volumes of larger organs. On the other hand, the absolute volume of the thymus in adults remains the same as in advanced fetuses, causing a sharp decrease in its relative volume along the life. In several mammalian species, the thymus is a very functional organ during the fetal phase, being the main producer of lymphocytes [44,45]. In adults, however, it reduces in size with the maturation of the immune system and eventually disappear [46,47], as we detected in 13 (62%) adult pacas.

The liver and the gastrointestinal organs showed opposite development trends. During the initial phases of the mouse's embryological development, the liver endothelium attracts stem cells responsible for the hematopoiesis; in this period, the liver assumes the role of producing blood [48]. These hematopoietic cells, in turn, secrete oncostatin M, which induces the maturation of the hepatocytes and the early increase in liver size [49]. The liver greatly enlarges in the Theiler's Stage 17 of mouse embryonic development [28], and this may explain the high relative volume (more than 40%) in small paca fetuses. Since the hepatic hematopoietic function is gradually assumed by the bone marrow, the liver reduces its representativeness along the fetal development, reaching in advanced paca fetuses a similar proportion as in adults (28%).

On the other hand, the progressive increase in the proportion of tubular gastrointestinal organs may be directed to assure their vital digestive functions just after birth. In mammals, the fetus starts to swallow amniotic fluids some days after the organogenesis and the gastrointestinal organs undergo rapid growth through the nutritional components provided by this fluid [50]. In the late gestational age, tubular gastrointestinal organs suffer high level of differentiation and the number of cells of their tissues increases greatly, which will be important in the extra-uterine life for the digestion of milk and other food resources [50].

Along with the external morphological features, the consistency in the proportion of the organs between advanced fetuses and adults reveals, once more, that the species' neonates are indeed well developed and prepared for an early independence during the

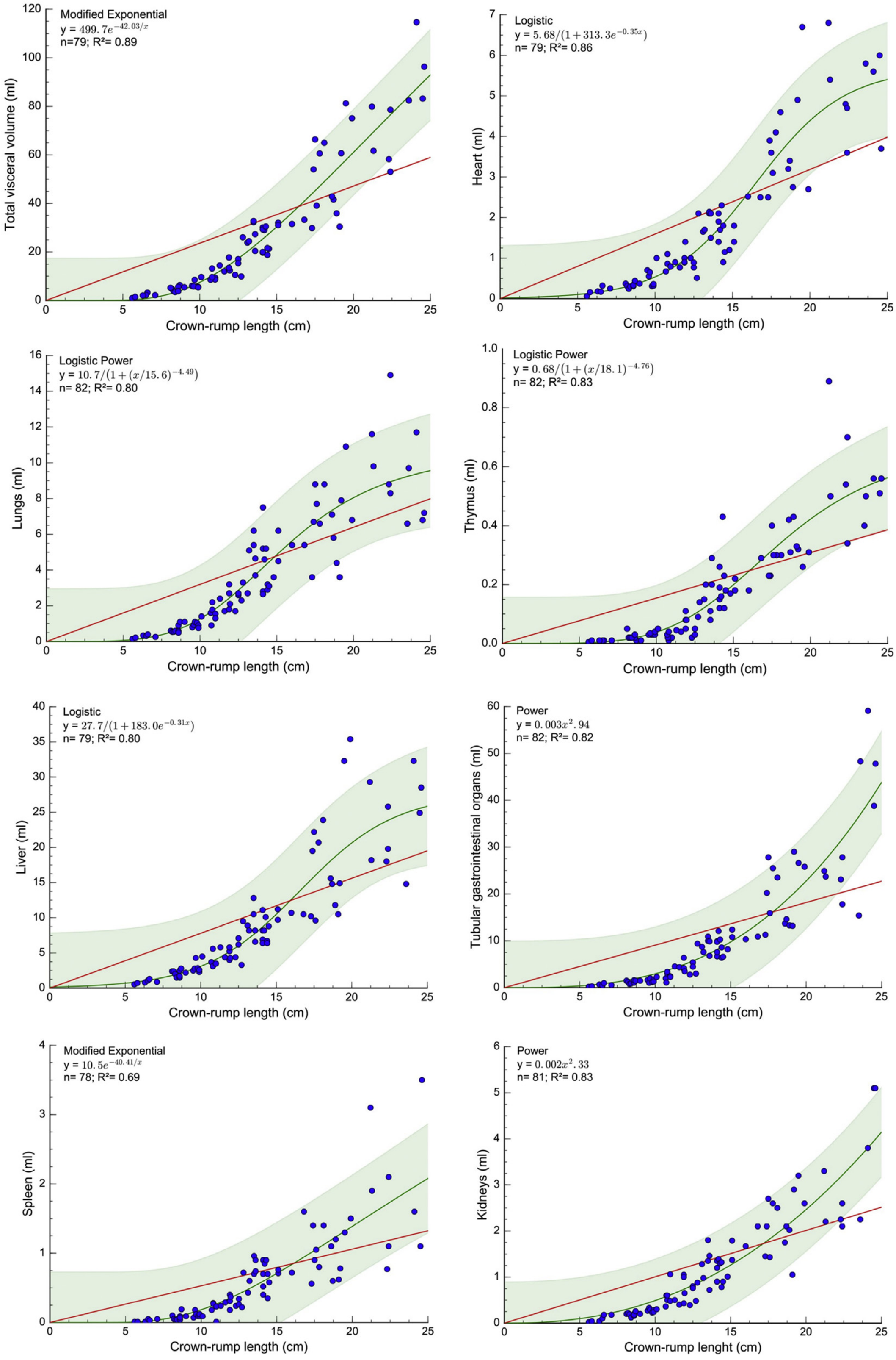

Fig. 6. The relationship between the absolute volumes of the visceral organs and the crown-rump length in fetuses of lowland paca (*Cuniculus paca*). The green line represents the best model fitted to the plots ±95% CI, while the red line represents an expected linear trend with no intercept. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

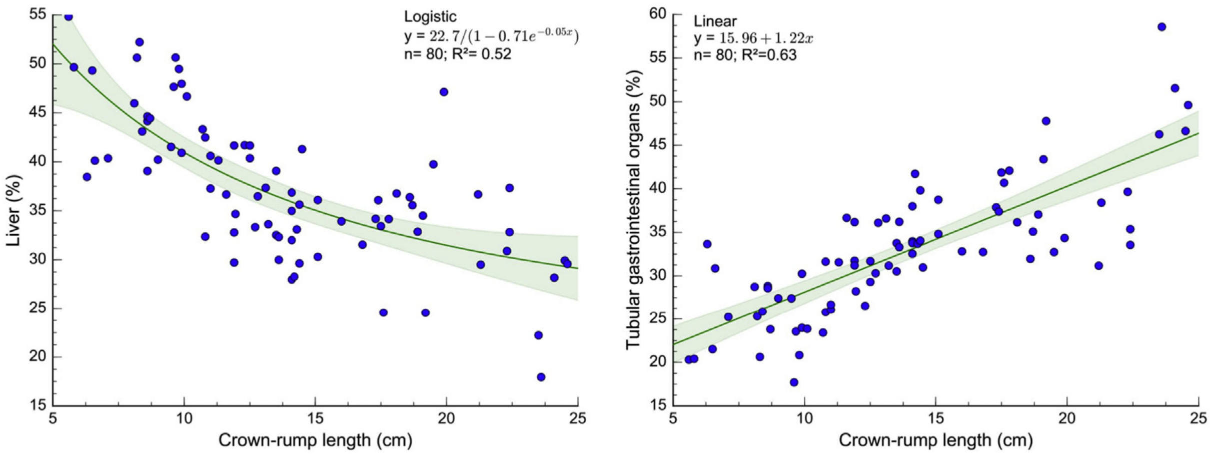

Fig. 7. The relationship between the relative volume of the liver and tubular gastrointestinal organs with the crown-rump length in fetuses of lowland paca (*Cuniculus paca*). The green line represents the best model fitted to the plots ±95% CI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

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

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

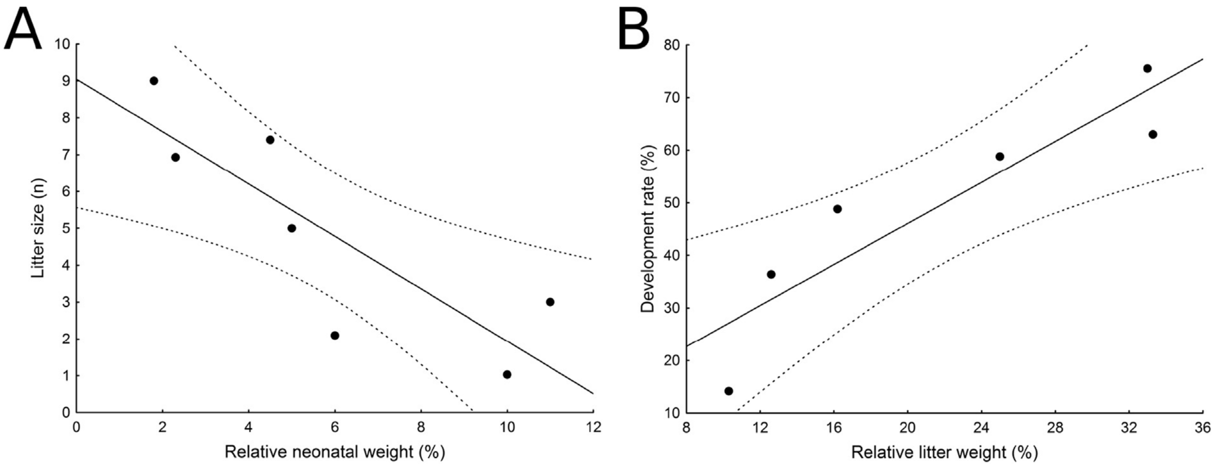

Fig. 8. The relationship between: A–the relative neonatal weight in relation to adults and the litter size in seven rodent species; B–the relative weight of the litter in relation to adults and the fetal development rate of six rodent species. The relative fetal length when each species presents developed 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.

extra-uterine life. This study shows that pacas produce highly precocial neonates. Although rodents are usually potential prey, the reproductive strategy of the paca is compatible to a species with a low natural predation rate, with a low production of young and high neonatal survival rate in nature. Although the paca is possibly the most hunted species in Latin America [10,11], its reproductive strategy suggests that this species is not adapted to respond to high hunting pressures. These findings should motivate further investigations on how best to establish *in situ* management plans for this species. Finally, this study describes the most important morphological events of the fetal development in pacas, which allow the improvement of imaging techniques for the reproductive diagnosis in the species and other histricognath rodents.

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. Species are ranked in increasing order of relative litter weight. The relative fetal length in relation to the newborn 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
Lowland paca (*Cuniculus paca*)	10	1.03	10.3	14.2%	[9], This study
Agouti (*Dasyprocta prymnolopha*)	6	2.1	12.6	36.4%	[22,32,51,52]
Richardson's ground squirrel (*Urocitellus richardsonii*)	2.3	6.93	15.9	–	[53,54]
Rat (*Rattus norvegicus*)	1.8	9	16.2	48.8%	[55]
Coypus (*Myocastor coypus*)	5	5	25.0	58.8%	[21,33,56–58]
Mice (*Mus musculus*)	4.5	7.4	33.3	63.0%	[23,37]
Guinea pig (*Cavia porcellus*)	11	3	33.0	75.5%	[25,59,60]

Table 4
A comparison among the stages in which morphological characters appear during the embryonic/fetal development in five rodent species.

Morphological character	Percentage of neonatal length[a] (individual length/pregnancy days)				
	Paca[b] *Cuniculus paca*	Coypus *Myocastor coypus*	Agouti *Dasyprocta prymnolopha*	Mice *Mus musculus*	Cavy *Galea spixii*
Embryonic and fetal transitional phase	8.1% (2.5 cm/42 days)	16.7% (1.9 cm/60 days)	25.0% (3.5 cm/45 days)	44.0% (0.12 cm/14 days)	–
Outer ear and tactile pelage	14.2% (3.5 cm/49 days)	58.8% (6.7 cm/90 days)	36.4% (5.0 cm/50 days)	63.0% (0.17 cm/16 days)	49.8% (3.3 cm/34 days)
Claws	14.2% (3.5 cm/49 days)	58.8% (6.7 cm/90 days)	72.7% (10.0 cm/75 days)	–	83%% (5.5 cm/44 days)
Erupted teeth	61.4% (15.0 cm/113 days)	–	72.7% (10.0 cm/75 days)	–	–
References	This study	[21]	[22]	[23]	[24]

[a] The percentage of neonatal length was calculated as the length of the embryo/fetus in the given pregnancy stage divided by the average length of the newborn.
[b] An approximation to 75% of probability occurrence (Fig. 1) was used as reference to obtain the individual length, the percentage of neonatal length and the pregnancy days for each morphological character in *Cuniculus paca*.

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