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

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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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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).