



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

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### ABSTRACT

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

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### 1. Introduction

The white-lipped peccary (*Tayassu pecari*) is a large-sized Neotropical ungulate (30–50 kg) that represents an important source of subsistence protein [1], being one of the most hunted mammals in the Amazon [2]. Since 1970s onwards, a large-scale

decline of its population has been observed in different areas in northern South America [1,3–5], probably due to the mixed effects of hunting and non-anthropogenic causes, such as widespread diseases [4,6]. Accordingly, the white-lipped peccary is currently considered “Vulnerable” by the International Union for Conservation of Nature (IUCN) and is predicted to become “Critically Endangered” in the short-term due to predatory hunting and habitat fragmentation by agricultural expansion [7].

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

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

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

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

## 2. Material and methods

### 2.1. Study sites

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

### 2.2. Biological sample collection and processing

From 2002 to 2015, local hunters collected and voluntarily donated reproductive tracts from 69 pregnant white-lipped peccaries, 54 (78.3%) in the YMR and 15 (21.7%) in the ASDR, including 36 (52.2%) single, 31 (44.9%) double and two (2.9%) triple gestations. Hunters were trained to remove all abdominal and pelvic organs complete with the perineal region and to store these in buffered 4% formaldehyde solution (v/v). Since hunters do not consume these materials, any invasive procedure or any additional mortality for the purpose of the study was avoided [16]. The

research protocol was approved by the Research Ethics Committee for Experimentation in Wildlife at the 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 N° 29092) and by the Committee on Ethics in Research with Animals of the Federal Rural University of the Amazon (CEUA/UFRA protocol 008/2016). Samples were sent to UFRA, Belém, Pará, Brazil, under the export license CITES/IBAMA (No 14BR015991/DF).

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

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

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

### 2.3. Statistical analysis

The gestational age was estimated using the formula proposed by Huggett and Widdas [20],  $\ln W = a(t-t_0)$ , 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 interception on the age axis. According to those authors,  $t_0$  is equal to 20% of gestation length in species that present between 100 and 400 days of pregnancy. To use that equation, we considered a mean gestation length of 159 days [10], and a mean weight of 1245 g at birth, taking into account the weight stabilization in fetuses in more advanced stages of gestation ( $\geq 34.2$  cm TDL).

Logistic regressions were applied to estimate the probability of occurrence of each external morphological characteristic in relation to TDL using the software Statistica 8.0 (StatSoft Inc., Tulsa, USA). Multiple regression modeling relationships between the TDL and biometric measures and absolute and relative organ volumes were

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

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

### 3. Results

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

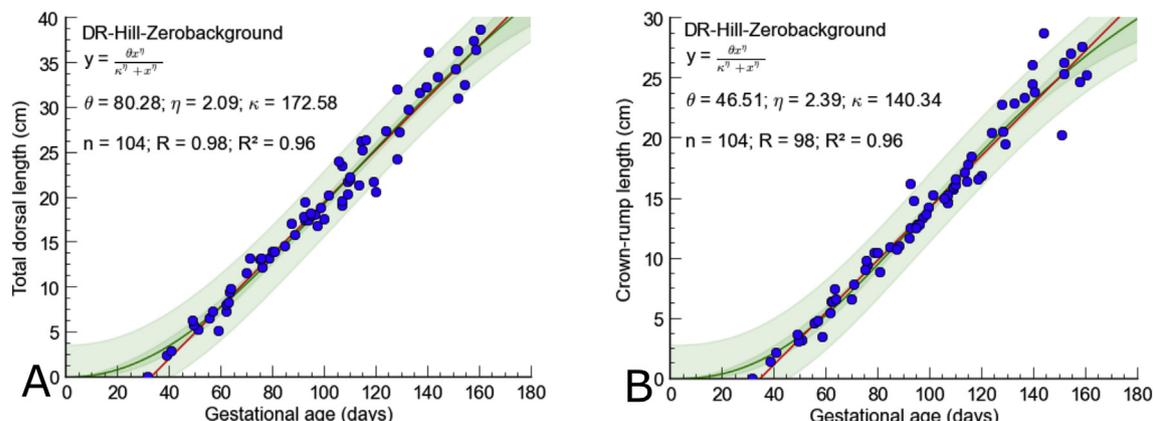
The probability curves and the regression models for the occurrence of external morphological features according to TDL are presented on Fig. 5 and Table 1, respectively. Embryos and fetuses with TDL  $\leq 2.9$  cm presented genital and limb buds, but no eyelid buds or any other external fetal characteristic. Limbs, differentiated genitalia (52 females and 50 males) and opened eyelids were observed in fetuses with TDL  $\geq 5.1$  cm and CRL  $\geq 3.5$  cm. Presence

of fused eyelids were observed in fetuses with TDL 6.2 cm and CRL 3.9 cm. Hooves and outer ear were observed in fetuses from 7.9 cm TDL onwards ( $\geq 6$  cm CRL). The dorsal gland was first observed in fetuses with 9.4 cm TDL ( $\geq 7.4$  cm CRL), and first signs of skin development from 11.5 cm TDL ( $\geq 6.6$  cm CRL). Fetuses had tactile pelage from 13.8 cm TDL onwards ( $\geq 10.1$  cm CRL) and covering pelage from 20.9 cm TDL onwards ( $\geq 16.0$  cm CRL). Tooth eruption and opened eyelids were the last characteristics observed in advanced fetuses, from 26.4 cm TDL ( $\geq 18.4$  cm CRL) and 27.8 cm TDL onwards ( $\geq 22.8$  cm CRL), respectively (Fig. 6).

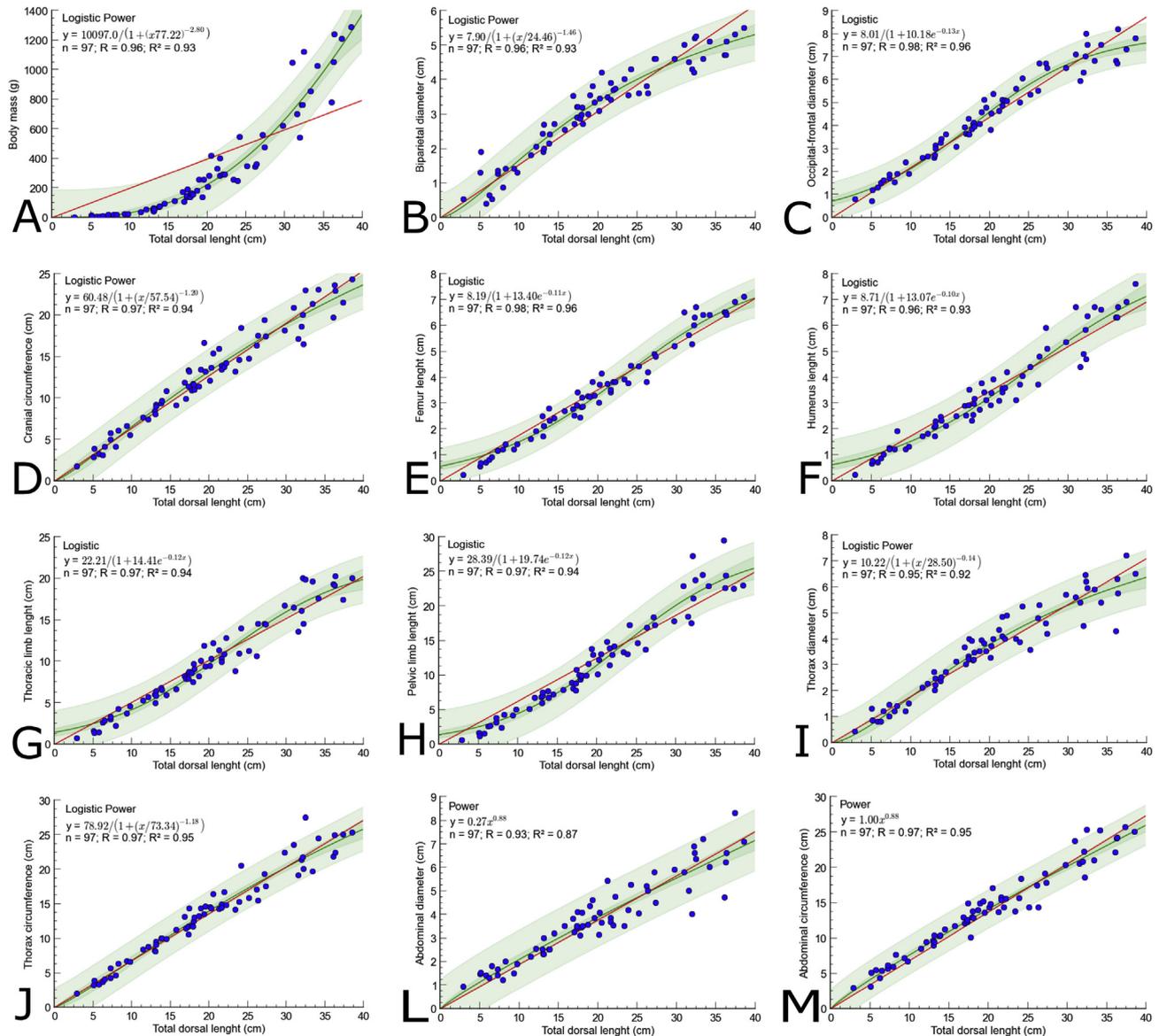
All associations between TDL and external biometric measures had high and significant coefficients of determination ( $r^2 > 0.86$ ,  $P < 0.05$ ; Fig. 2). The FL ( $r^2 = 0.96$ ,  $P < 0.01$ ) and OFD ( $r^2 = 0.96$ ,  $P < 0.01$ ) presented the best relationships with TDL. The allometric relationships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

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

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



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



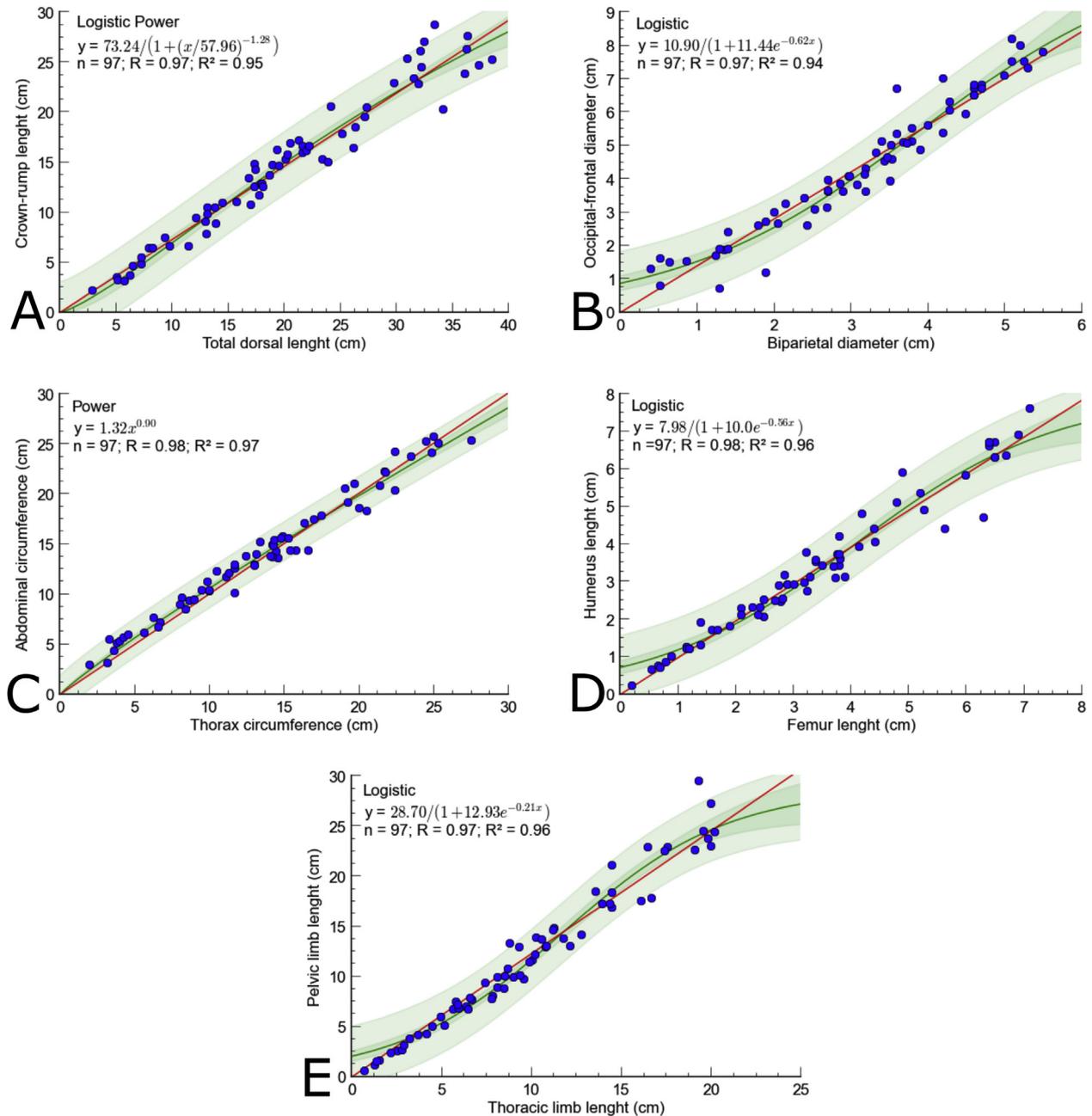
**Fig. 2.** Relationship between the total dorsal length (TDL) and the body mass (A), biparietal diameter (B), occipital-frontal diameter (C), cranial circumference (D), humerus (E) and femur length (F), length of thoracic (G) and pelvic limbs (H), thorax diameter (I), thorax circumference (J), abdominal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

#### 4. Discussion

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

Eyelids are absent in embryos embryos at 40 days (25.1%

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



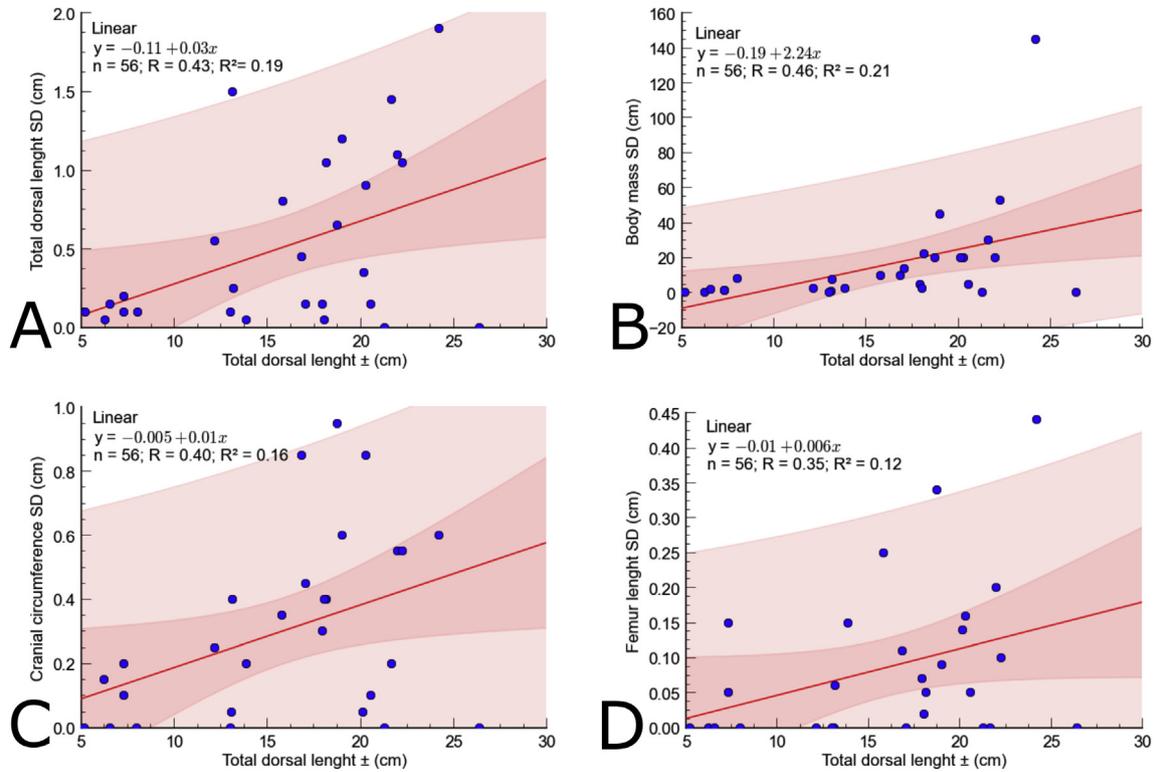
**Fig. 3.** Allometric relationship of the total dorsal length versus crown-rump length (A), biparietal diameter versus the occipital-frontal diameter (B), thorax circumference versus abdominal circumference (C), humerus length versus femur length (D), and length of thoracic limbs versus length of pelvic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

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

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

In peccaries, the covering pelage acts in thermoregulation and

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



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

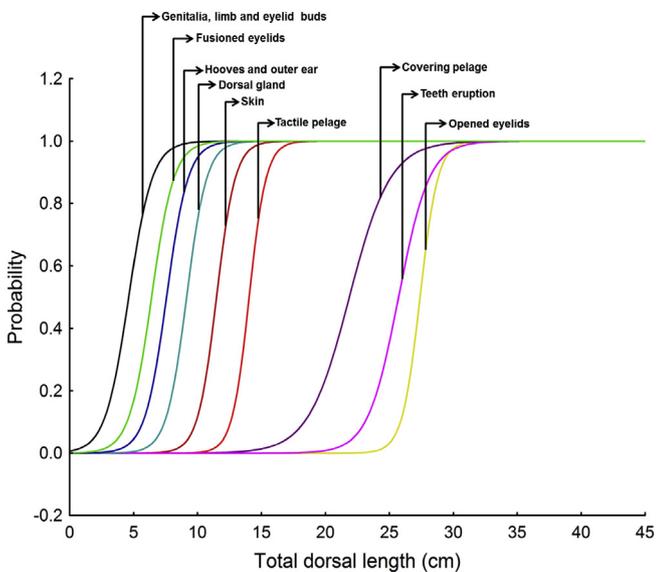
thermoregulation depends on the maternal contact [18].

Dentition in newborns allows the early foraging and the maternal independence [23,26]. In the white-lipped peccary, canines were the first teeth to erupt at around pregnancy day 116 (72.9% gestation length). In the collared peccary, canine eruption begins later, from pregnancy day 120 (86.9% gestation length), the upper and lower canines and lower incisors were observed just

after birth, and few days after birth the entire deciduous dentition is formed [26,30]. In both peccary species, solid food intake starts between 4 and 6 weeks postnatal and, after 2 weeks, the young is already prepared to be weaned [31].

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

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



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

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

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

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

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

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

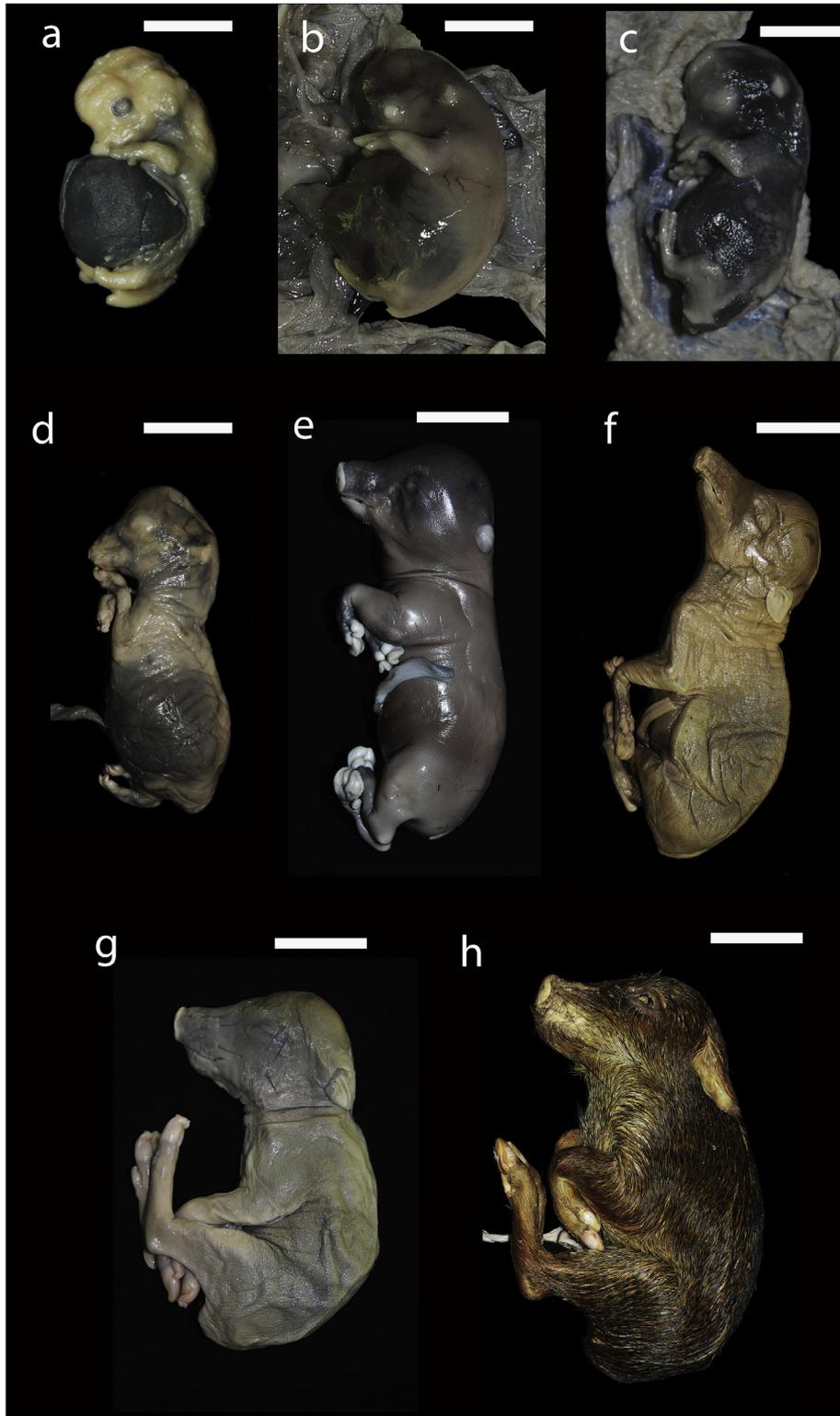
that the white-lipped peccary may be also predisposed to cardiovascular overload.

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

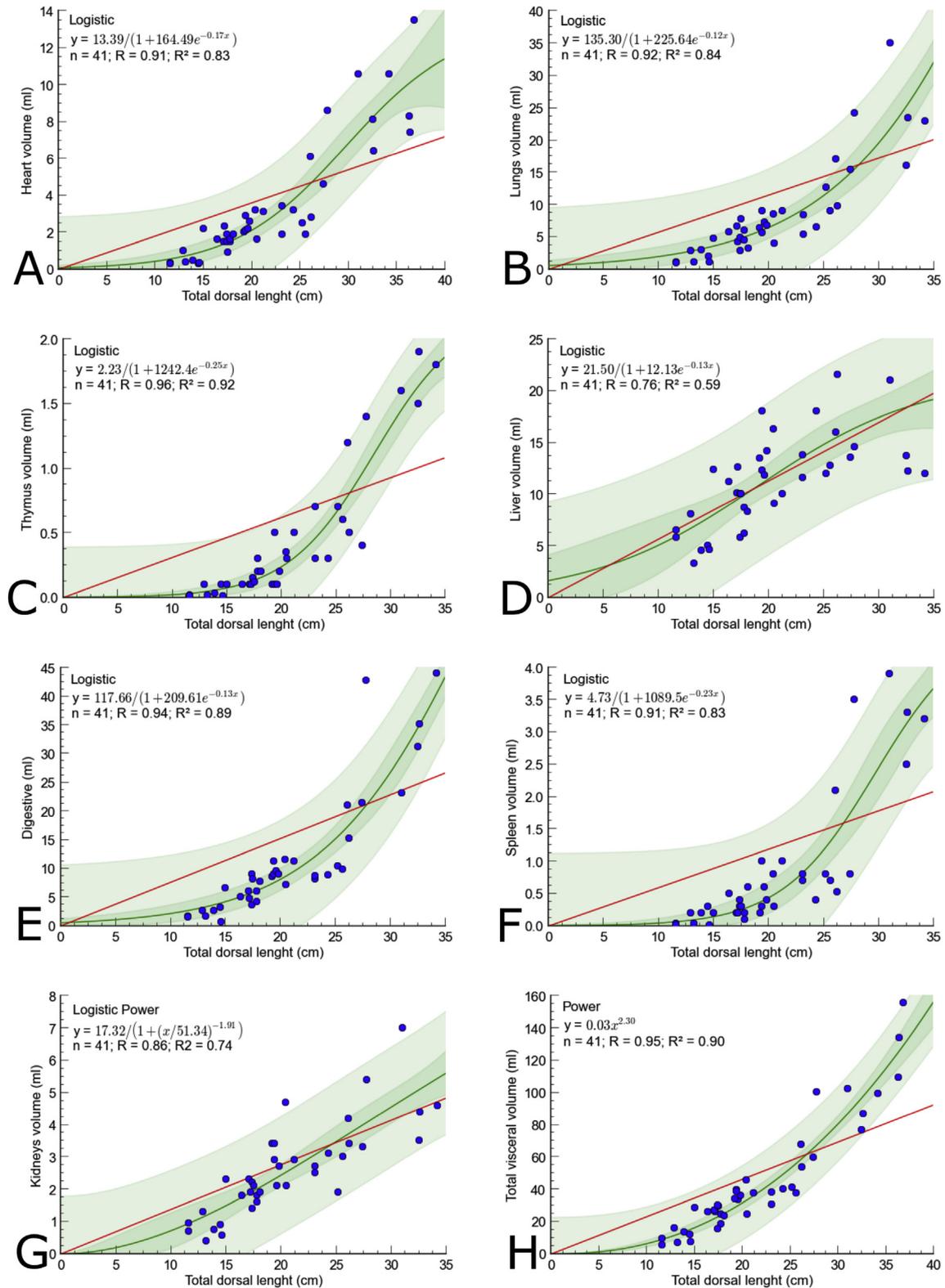
The relative volume of the spleen showed a constant growth during the fetal phase, from 0.3% to 70 days of gestation (0.5 ml spleen/kg fetus) to 4.7% at the end of gestation (2 ml spleen/kg fetus), decreasing in adult phase to 2.7%. In contrast, the relative spleen weight in the domestic pig decreases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

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

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



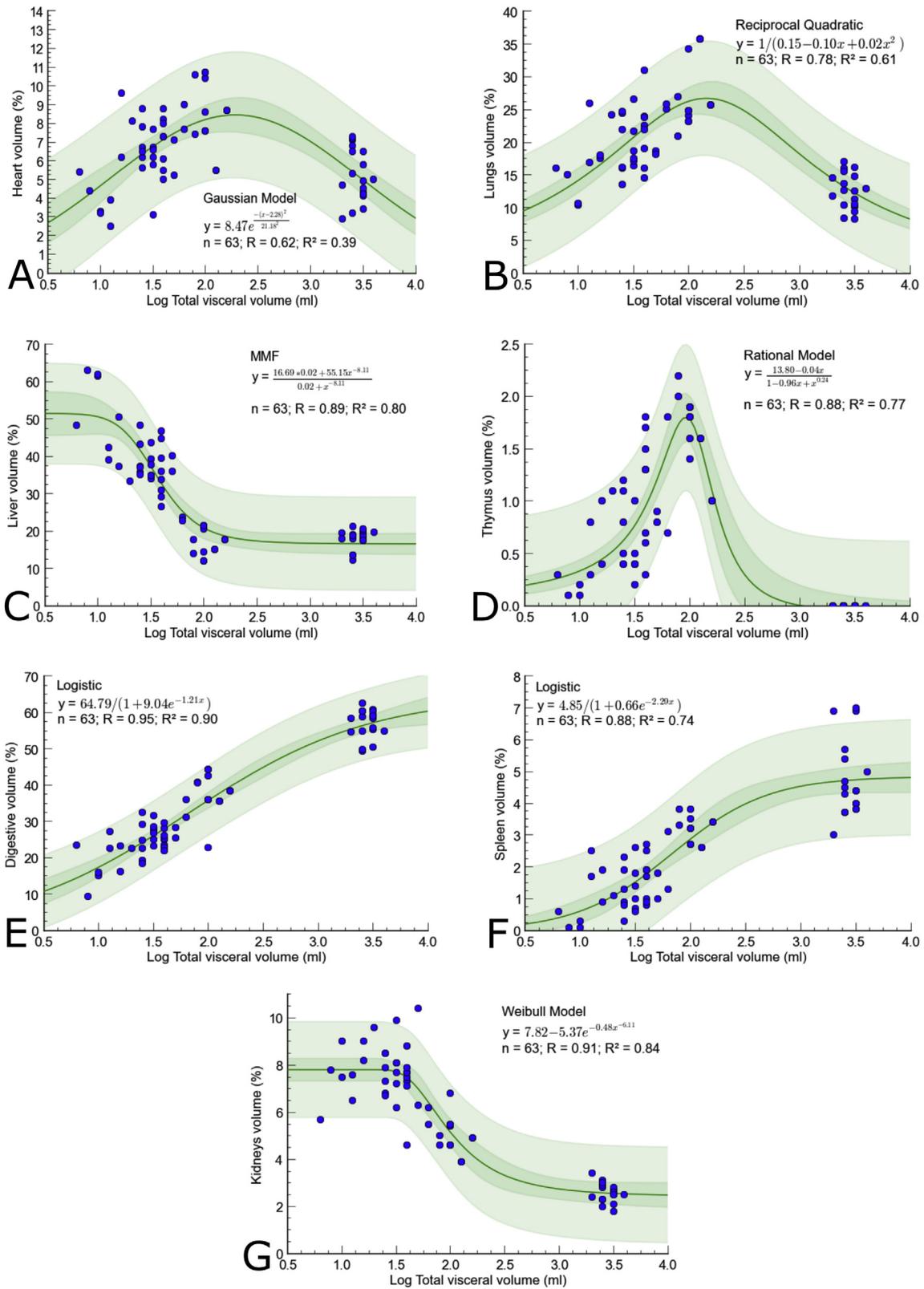
**Fig. 6.** Embryos and fetuses of white-lipped peccary (*Tayassu pecari*) at different stages of development according to total dorsal length (TDL): (a) Embryo of 2.9 cm TDL and 0.4 g, presenting genital and limb buds, but no eyelid buds and any other external fetal characteristic (bar: 1 cm); (b) Fetus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



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

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

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



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

**Table 2**

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

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

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

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

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

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