

External Morphological Characterization of 60-Days Gestation *Myocastor coypus* (Coypu) Fetuses

Caracterización de la Morfología Externa de Fetos
de 60 Días de Gestación de *Myocastor coypus* (Coipo)

Antonio Eduardo Felipe; Patricia Gabriela Masson; Julio Armando Rodríguez & Ricardo Horacio Alzola

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SUMMARY: The main objective of this work was to carry out the external morphology characterization of 60-days gestation *Myocastor coypus* (coypu) fetuses since this stage could be considered a crucial point in the prenatal development in this species. Fourteen fetuses out of three litter were analysed. Gestational sacs weighing 3.81 ± 0.27 g showed an elliptical shape with the larger axis along the direction of the uterus. The average weight of fetuses was 1.13 ± 0.19 g, the head-to-tail length was 1.99 ± 0.87 cm, the cephalic length was 1.01 ± 0.37 cm, and the biparietal diameter was 0.63 ± 0.06 cm. Specimens showed differentiation in corporeal, limbs and tail regions; also showed interdigital membranes, well defined optical vesicles and lateral ear lobe sprouts. Comparative analysis of coypu fetuses and equivalent stages of other mammal species will allow to establish similitudes and differences that would permit to mark particularities in the ontogeny of *Myocastor coypus*.

KEY WORDS: Fetal morphology; Coypu; *Myocastor coypus*.

INTRODUCTION

Coypu (*Myocastor coypus*) is a neotropical rodent, Suborder Hystricognathi, it is among forty species recommended to be included in international economical and in research and developmental programs. In Latin America coypu, *Hydrochaeris hydrochaeris* (capibara or carpincho), *Agouti paca* (paca) and *Dasyprocta aguti* (agouti) are hystricognaths species of high economical value. Coypu is an annual polyoestrus animal; its oestral cycles have a characteristic great variability, it has been shown (Felipe *et al.*, 2001a) that their average duration is 35.5 ± 10.8 days ranging from 20 to 60 days. The number of offsprings was 3.82, with a range of 1 to 12 (Colantoni, 1993).

Currently, the knowledge about the developmental biology of coypu is scarce (Felipe *et al.*, 1999 y 2002); in the case of other hystricognaths, it has been shown a few general data (Roberts & Perry, 1974). Since it has been reported (Felipe *et al.*, 2001a,b y 2004) information related with diet, mating and general care for captured coypuses, this species could become a new model for the morphological studies of prenatal development. Knowledge

of coypu embryology would contribute to make more precise the developmental stages of the samples from wild captures, and also to determine the environmental consequences over gestation. Newson (1966) studied the fetal development time course of coypu and found that between 32-35 to 55 poscoitus (pc) days a gradual increase in the fetal vesicle very likely due to the development of the membranes of the fetus and the surrounding liquids. From 55-60 pc days there was a significant increase in the fetal weight. This seemed to correspond to an inflection or crucial point in the prenatal development since the slope of the increase remained constant until birth. This fact was the base of the objective of the present research oriented to morphologically characterize *M. coypus* of sixty days of gestation.

MATERIAL AND METHOD

Three sexually mature females of *M. coypus bonariensis* weighing 4.10 ± 0.33 kg and 7.2 ± 0.18 month

old, were located in a corral with walls of concrete plates and wired, the floor was of concrete and the corral was roofed. The only male of 4.9 kg and 9 month old was lodged in a corral next to the one of females. Animals were fed daily with 0.3 kg of commercial food and had access to water *ad libitum*. Daily colpocytology was performed according to Felipe *et al.* (2001a). Once oestrus was detected in a female by colpocytology it was transferred to the male corral. Mating was followed by direct observation and colpocytological sampling 1 h pc; direct watching and microscopical observation after staining to check the presence of sperms into the vagina (Felipe *et al.*, 2001a). The time of mating was recorded and was taken at the zero time of coitus; the age of fetuses was expressed in postcoitus days (pc days). Pregnant females were sacrificed according the Animal Welfare Acta of Veterinary School of the Center University (Tandil, 2002). Medial laparotomy was the technique used to obtaining complete genital tractus. Each uterine horn was examined to detect fetal or embryonic resorption or fetal death. Fourteen 60-pc days fetuses were collected.

Some parameters were measured in the gestational sacs, placenta and fetuses; those were, weight and size (antero-posterior length, dorso-ventral diameter and width). After opening gestational sacs, placental weight, length and thickness, external morphology qualitative characteristic (color and appearance) were measured and recorded. The umbilical cord was also measured in length and medium diameter. Each fetus was examined in order to get the following: 1) body shape (head, chest, abdomen, pelvis and limbs subdivisions); 2) presence and location of cephalic structures (ears, eyes, nose, nasal fossas, lips); 3) limbs (position, length and presence and number of fingers); 4) macroscopical aspects of genital and perineal areas (penis or vagina and anal opening); 5) organ sprout relief, hair and nails, mammary line or mammary glands; 6) tail length, and 7) weight. Regarding body sizes: cephalo-caudal length (CCL); crown-rump length (CRL); cephalic length (from occipital bone to the nouse anterior limit, CL) and biparietal diameter. To evaluate fetal membranes its total weight, as well as the weight of the liquid contained inside, and its qualitative characteristics (color and appearance) were recorded (Wintour *et al.*, 1986; Cross, 1998).

The observations were done with stereoscopic lens. Measurements were carried out with a Vernier type caliper. Weighings were done in an electronic scale Kern 770 ® (±0.1 mg.). All values was expressed as mean ± standard deviation of the mean (x ± sd.) Fetal structures were named according to *Nomina Embryologica Veterinaria* (1994) and to *Nomina Anatomica Veterinaria* (1992).

RESULTS

Gestational sacs. Gestational sacs showed an elliptic shape (Fig. 1A), with the major axis along the uterus. The average weight was 3.81 ± 0.27 g. Between two gestational sacs, septa or incomplete uterine partitions were observed (Fig. 1B). Morphometrical data of sacs are presented in Table I. A great part of total weight of a gestational sac was the liquid content; fetus and placenta account for the difference (Fig. 2).

Placenta and umbilical cord morphology. Discoidal placentae weighed 0.60 ± 0.14 g, they have an external unilobular appearance and were red-wine colored. Subplacenta showed an irregular conical shape and had a pink color. Morphometrical data of placenta are shown in Table I. Umbilical cord was rolled up around the body of the fetus; it has a smooth and brilliant surface, it was not so much spiraled, it has a proximal expansion in the insertion with fetus abdomen and ramifications in the zone of insertion with the placenta. The length of the cord was 0.99 ± 0.14 cm and it had a diameter of 0.18 ± 0.03 cm.

Table I. Morphometric data of gestational sacs, fetuses and placenta of 60 pc days *Myocastor coypus*. Values are expressed as $x \pm s.d.$

	Parameter	Values (cm)
Gestational sacs	Antero-posterior length	4.76 ± 0.3
	Dorso-ventral diameter	2.89 ± 0.41
	Width	1.91 ± 0.16
Placenta	Length	1.26 ± 0.12
	Width	1.06 ± 0.13
	Thickness	0.41 ± 0.05
Fetal lengths	Cephalic length	1.01 ± 0.37
	Cephalo-caudal length	1.99 ± 0.87
	Crown-rump length	1.89 ± 0.09
	Biparietal diameter	0.63 ± 0.06

Characteristics of the fetal membranes. Fetal envelopes showed as thin and transparent membranes, its weight was 0.52 ± 0.11 g. The liquid inside was clear and light amber colored; it weight was 1.51 ± 0.22 g.

Characteristics of the fetuses. Fetuses weighed 1.13 ± 0.19 g average and were white colored. They body sizes are shown in Table I. Head, chest, abdomen and limbs sprout differentiation were observed at this stage of development (Fig. 3). The head had a 45° flexure which allowed to visualize in a prominent way the telencephalum and mesencephalum vesicles. Embryonic earlobes (*tubercula*

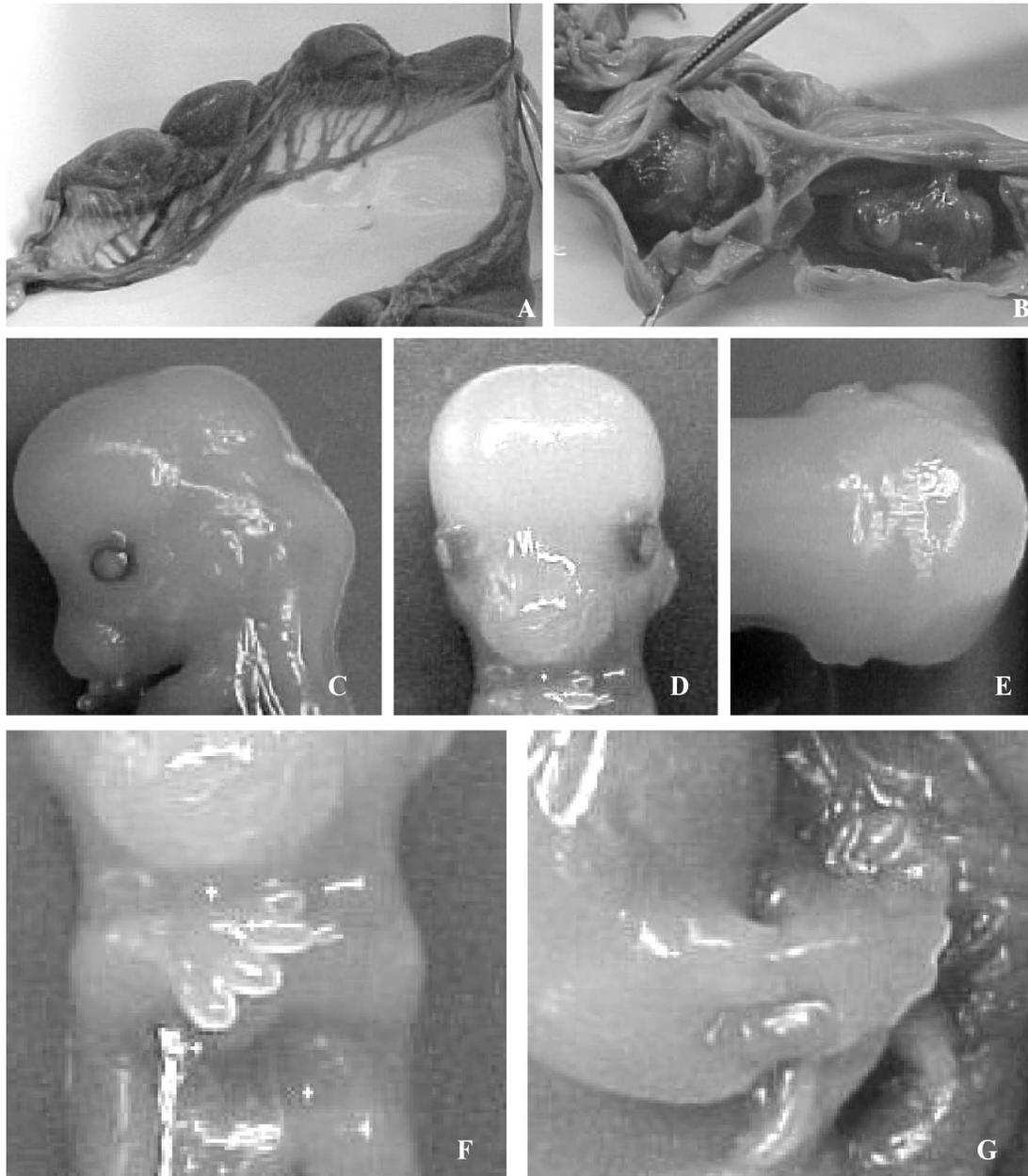


Fig. 1. (A) Uterus horn with four gestational 60 pc days sacs; (B) Opening in uterine wall showing gestational sacs and uterine septum (2); (C), (D) and (E) Lateral, frontal and dorsal views, respectively, of the cephalic region; (F) Overlaying anterior limbs showing finger separation (3); (G) Posterior limb with an interdigital membrane binding fingers (4). Bars: 1 cm.

auricularia) had a low level of development and located at both cranial sides; they were just at the border of cervical area and in line with optical vesicles (Figs. 1C, 1D and 1E). These showed up clearly on the rostral surface, they had a prominent lens placode and are surrounded by palpebral protuberances (*plicae palpebrales*). The lips medium area showed a commissure at its dorsal contour. Nasal edges were

not prominent. At the dorsal region of chest as well as at the abdominal region, the line of spinal cord was distinguished with a rim at both sides; no clear vestige of vertebra were observed. Anterior and posterior limbs were present in such an arrangement that formed right angles with the body axis. Anterior limb sprouts have a length of 0.55 ± 0.04 cm; at this stage of development they showed differentiation into

forearm, arm and hand with separate fingers (*digiti definitivi*) (Fig. 1F). Regarding posterior members, they had a length of 0.64 ± 0.04 cm; the foot (*pes primitivus*) presented an interdigital membrane binding the fingers throughout (*digiti primordialis nonseparati con membrana interdigitalis*) (Fig. 1G). Skin was transparent without hairy follicles (except in the *prominentia nasalis lateralis*). Neither heart elevation nor liver prominence were present in the corresponding location, i.e., chest and abdominal areas. Rim of the mammary line were not observed. Genital tubercle and anal rim were observed in the perineal area. Tail was present and its length was 0.81 ± 0.07 cm.

DISCUSSION

Embryogenesis of a given specimen in any species correlates with a series of continuous changes in a similar manner that prenatal development occurs in the different species (Knospe, 2002; Beaudoin *et al.*, 2003). In spite of accepted similarities, there is a number of embryo classifying systems. For instance, the *Nomina Embryologica Veterinaria* (1994) establishes 15 stages; the Carnegie system names 23 stages (O'Rahilly, 1972); the Theiler system (1972) considers 27 stages for mice, and Dyban *et al.* (1975) system establishes 24 stages for laboratory murine rodents and rabbit. In general, embryo and fetal development description in other species were based on stages built over age, corporeal

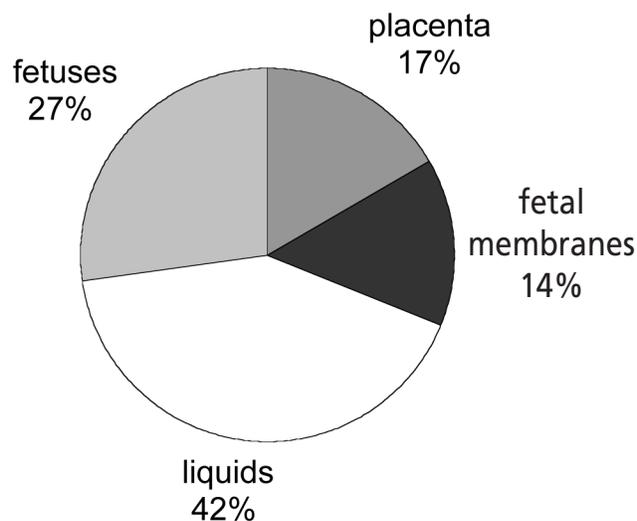


Fig. 2. Percentage of total weight of each component of 60 pc days *Myocastor coypus* gestational sacs.

changes by gradual differentiation, increase of body size and weight, and overall development of organ systems (Theiler; Knospe; Beaudoin *et al.*).

Based on the external morphological characteristics of developmental degree, 60 pc days coypu fetuses are alike human fetuses of Streeter's Horizon XIX (LCG 18 mm, 39 ± 1 days) (Genis Gálvez, 1970) and 19 and 20 Carnegie's stages (Butler & Juurlink, 1987); this is suggested on the

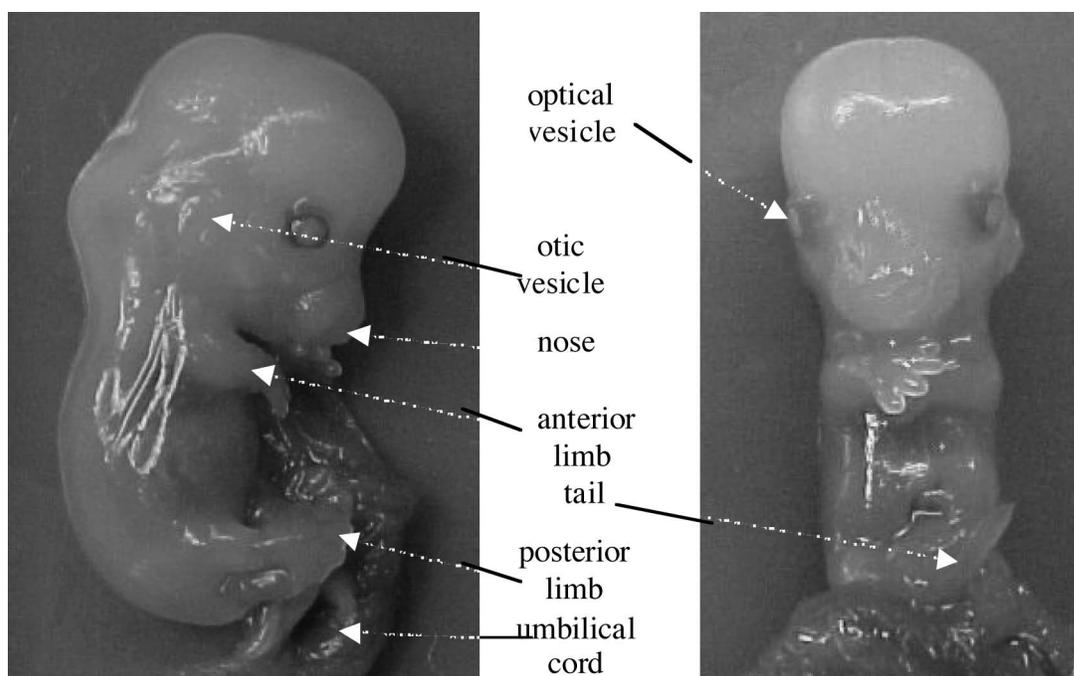


Fig. 3. Lateral and frontal views of 60 pc days fetuses of *Myocastor coypus*. Bar: 1 cm.

base of similarities in the development of limbs (presence of fingers on the anterior limbs) and their position (towards the ventromedial line and with a little separation between them); marked relief of eyelids and early presence of earlobe sprouts. Making comparison of coypu fetuses with those of primates like baboon (*Papio cynocephalus*), are according the observations of Hendrickx (1971) in Horizons XXI (LCG 18-21 mm, 43 ± 1 days) and XXII (LCG 22.5 mm. 45 ± 1 days), such as limbs position, cephalic structures development degree, presence of fisiologic umbilical hernia, thick eyelids, *tragus* and *antitragus*, and superposition toward frontal of the fingers of one hand with the other. In mice, separation of fingers of hands, umbilical hernia, eyes development stage, earlobe and tail correspond to Theiler stage 22 (14 pc days); but differ from that stage because the absence of hairy follicles at pectoral, pelvic and trunk (Davidson & Baldock, 1998). Taking into account limbs development, coypu fetuses analyzed would correspond to stage 11 of mice limbs development (15 pc days) according to Wanek et al. (1989); there was a complete finger separation of anterior limbs but no differentiation of elbows and knees nor hair follicles over the members were observed. According to Wanek *et al.* stage 11 of mice is equivalent to stage 23 of Theiler. However, the presence of hairy follicles in coypu fetuses corresponding to the whiskers makes them more alike to the 13 pc days mice and the remaining external morphological traits to the 14.5 pc days described by Rugh (1969).

Considering the nine levels of development system of Stêrba (1976 and 1995), 60 pc days coypu fetuses would be at level 5. In this level of development fetuses have well defined face, prominence of the mesencephalum, fingers separate in the anterior limb, sprout of earlobes and whiskers follicles at the upper lip making prominence in the epidermal surface. These traits are shared by rodents like *Pitymys subterraneus*, *Pitymys tatricus* and *Microtus nirvalis mirhanreini* at 14 pc day (67 to 68% of gestation) (Stêrba, 1976), *Clethrionomys glareolus* of 14.5 pc days (Mísêk, 2003) and rabbit fetuses of 15.5 and 16.5 pc days (Beaudoin *et al.*).

According to *Nomina Embryologica Veterinaria* (1994), 60 pc days coypu would correspond to the *periodus labii fissi* do to cephalic structures distinction, hand finger differentiation and presence of interdigital membrane at feet (*pes primitivus*).

Comparisons done of coypu fetuses with equivalent stages of other mammals species would allow us to ascertain the notion, suggested by Mísêk, that it is possible to predict the prenatal development stage of a species knowing the traits of other, regardless the length of the period of gestation. Care should be taken with regard of the differences observed and take into account the particularities of the spotted species.

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RESUMEN: El objetivo de este trabajo fue efectuar la caracterización de la morfología externa de fetos de 60 días de gestación en *Myocastor coypus* (coipo), ya que éstos pueden ser considerados como un punto de inflexión en el desarrollo prenatal de la especie. Se analizaron 14 fetos provenientes de tres camadas. Los sacos gestacionales, cuyo peso fue de 3.81 ± 0.27 g, mostraron forma elíptica, con su diámetro mayor orientado en paralelo con la dirección de los hemióteros. El peso medio de los fetos fue de 1.13 ± 0.19 grs, con una longitud céfalo-caudal de 1.99 ± 0.87 cm, longitud céfalica de 1.01 ± 0.37 cm y un diámetro biparietal de 0.63 ± 0.06 cm. Los especímenes mostraron diferenciación de regiones corporales, extremidades y cola, membranas interdigitales en los miembros posteriores, vesículas ópticas bien definidas y esbozos de pabellones auriculares en posición lateral. El análisis comparativo de los fetos de coipo con estadios equivalentes de otros mamíferos, permitió determinar similitudes y también diferencias que indicarían particularidades de la especie.

PALABRAS CLAVE: Morfología fetal; Coipo; *Myocastor coypus*.

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Correspondence to:
 Dr. Antonio E. Felipe
 Grupo de Investigaciones Biológicas (GIB),
 Area de Cs. Morfológicas, Depto. Cs. Biológicas,
 Fac. Cs. Veterinarias, UNCPBA,
 Tandil
 ARGENTINA

E-mail: aefelipe@vet.unicen.edu.ar

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