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Morphometric analysis of fetal development of *Cavia porcellus* (Linnaeus, 1758) by ultrasonography—Pilot study

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ABSTRACT

Measurements on the growth process and placental development of the embryo and fetuses of *Cavia porcellus* were carried out using ultrasonography. Embryo, fetus, and placenta were monitored from Day 15 after mating day to the end of gestation. Based on linear and quadratic regressions, the following morphometric analysis showed a good indicator of the gestational age: placental diameter, biparietal diameter, renal length, and crown rump. The embryonic cardiac beat was first detected at an average of 22.5 days. The placental diameter showed constant increase from beginning of gestation then remained to term and presented a quadratic correlation with gestational age ($r^2 = 0.89$). Mean placental diameter at the end of pregnancy was 3.5 ± 0.23 cm. By Day 30, it was possible to measure biparietal diameter, which followed a linear pattern of increase up to the end of gestation ($r^2 = 0.95$). Mean biparietal diameter in the end of pregnancy was 1.94 ± 0.03 cm. Kidneys were firstly observed on Day 35 as hyperechoic structures without the distinction of medullar and cortical layers, thus the regression model equation between kidney length and gestational age presents a quadratic relationship ($r^2 = 0.7$). The crown rump presented a simple linear growth, starting from 15 days of gestation, displaying a high correlation with the gestational age ($r^2 = 0.9$). The offspring were born after an average gestation of 61.3 days. In this study, we conclude that biparietal diameter, placental diameter, and crown rump are adequate predictive parameters of gestational age in guinea pigs because they present high correlation index.

1. Introduction

Guinea pigs (*Cavia porcellus* [Linnaeus, 1758]) have long been recognized as representatives of laboratory animals [1]. The first experimental use of this species reports to Lavoisier, in 1790, in investigations of the effects of heating [2]. Thereafter, these animals have been largely used as experimental models in several areas of biology [3–5]. In particular, guinea pigs have been used to study diverse aspects of gestation of mammals [6,7]. The relatively long gestational period, which allows for surgical access to fetuses followed by an adequate time for postsurgical monitoring, and the structural similarity of guinea pig and human placenta are among the characteristics making this species suitable models for gestational and prenatal studies [8].

Ultrasonography has been largely employed in many fields of medicine, and its usefulness has been recognized in a variety of clinical situations [9,10]. It has been shown to be a noninvasive and reliable method for pregnancy diagnosis and for monitoring fetal development [5,11]. The procedure does not offer risks to the conceptus once used under previously tested and recommended levels and intensity [10].

The establishment of metric parameters for gestational age and fetal development using ultrasonography may have

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important applications, because it would allow for the establishment of relationships between the chronology of fetal organs and structure development [12]. Measurements as crown rump (distance between the head and caudal poles), diameter of gestational sac, biparietal diameter of the head, femur and kidney lengths, and head and abdominal circumferences are morphometric analyses commonly used in rabbits [13], rats [10], mice [9], large animals, and humans [11]. In guinea pigs, ultrasonographic examinations have been used to measure the biparietal diameter of fetal head [8], to evaluate the morphology and blood flow of the placenta [7,14], and to estimate the number of the offspring between Days 25 and 40 of pregnancy [6].

Early diagnosis of pregnancy and a precise knowledge concerning the gestational age may be important information in a variety of studies using pregnant females. Depending on the animal species, monitoring of mating day is not always possible, and these uncertainties may compromise experimental planning and execution.

Because of scarce information on the fetal development of guinea pigs, the aim of this study was to evaluate the relationship between gestational age and fetal development and morphometric measurements of fetal structures using ultrasound in pregnant guinea pigs between Day 15 of gestation and the proximity of the parturition, and then elaborate regression models that estimate gestational age.

2. Material and methods

All experimental procedures were performed under veterinary supervision, according to recommendations by the National Council for Control of Animal Experimentation from Brazil, under license number 1999/2010 from the University of São Paulo Ethics Committee on Animal Experimentation.

Five female guinea pigs privately owned, 3- to 4-month old, were donated to University of São Paulo, and used for this study. These animals were housed in cages, one guinea pig per cage, at 21 °C, on a 12:12 hour light-to-dark cycle. Diet and water were consumed *ad libitum*.

In this study, Day 0 of gestation was assumed to be the day of mating. Pregnant females were first examined at Day 15 of gestation, then at 5-day intervals up to Day 30. Afterward, ultrasonographic examinations were performed every 3 days until the parturition.

The same operator performed all studies, without anesthesia or sedation. The animals usually stayed calm during the whole examination (about 20 minutes).

The abdomen was shaved, and the guinea pig was fixed in dorsal recumbency, and a water soluble gel Aquasonic 100 (Parker) was applied over the surface of the abdomen. Three consecutive measurements were undertaken from each embryonic, fetal structures, and extra fetal structures, as follows: placental diameter (PD), biparietal fetal diameter (BFP), fetal kidney length (FKL), thoracic diameter, fetal abdominal diameter (FAD) and crown rump. All examinations used the color Doppler function to detect blood flow and pulsed Doppler to determine the fetal cardiac rate. The angle of insonation between the Doppler stream and vascular segment direction was less than 60°, the high-pass filter was set at 313 Hz, and the gain was set at 21.

Abdominal diameter was measured when two echogenic lines were visible at both abdominal sides. Biparietal diameter measures were taken from the highest distance between the two cranial limits.

Ultrasonographic examinations were performed using the model (GE Logiq 3, Wauwatosa, WI, USA) equipped with a convex transducer of 3 to 7 MHz and a linear transducer of 5 to 12 MHz.

Statistical analyses were done by Akaike information criterion (AIC). We selected between quadratic or simple linear regression models using AIC.

3. Results

Fetuses were born between Days 59 and 64. Fetuses from two females presented peristalsis at Day 55, and were born at Days 61 and 64, respectively. Fetuses in which no peristalsis was detected were born at Day 59. Raw data are showed in Table 1.

On Day 15 of gestation, the embryos were identified in the uterine cavity as a hyperechoic structure displaying cardiac pulse at the Color Doppler in the gestational sac, which appeared as circular anechoic structures. One of the females had four gestational sac, and the others had only two.

The crown rump presented a simple linear growth (AIC = 210.427), displaying a high correlation with gestational age ($r^2 = 0.9173$). Crown-rump measurements were performed between Days 15 and 35, after that, images of the full fetuses could not be taken because of their dimensions.

The beginning of skull ossification was observed on Day 30, allowing measurement of the biparietal diameter. This parameter followed a linear pattern of growth up to the end of gestation. Regression models adjusted between biparietal diameter and gestational age presented high determination coefficients, mainly for quadratic models ($r^2 = 0.955$) (Table 2).

The placenta was characterized as hyperechoic structure at the first stages of gestation, with the echogenicity changing as the gestation proceeded. For example, between Days 35 and 40, they were characterized as hypoechoic structures intertwined by anechoic areas.

The PD increased in the first stages of gestation, remaining fairly unaltered in later gestational stages. The PD showed a positive linear relationship with gestation age (Table 2).

The embryonic cardiac beat was first detected at Day 20 of gestation. During the gestation, the heart rates ranged from 147.3 to 259.3 bpm (median = 213.2 bpm). However, at the end of the gestation, between Days 59 and 65, the mean of fetal heartbeat was 205 bpm. Table 2 shows that the heart frequency shows a quadratic relationship with gestational age, reaching a peak at Days 45 to 55, but has a poor explanation power ($r^2 = 0.56$).

Thoracic and abdominal diameters were well characterized only in fetuses beyond Day 35 of gestation. Table 2 presents the equation models for the relationship of thoracic and abdominal diameters with gestational age.