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CELLULAR AND MOLECULAR BIOLOGY

# Stereological analysis of the New Zealand rabbits (*Oryctolagus cuniculus*) placenta

CARLA M.F. DE CARVALHO, LUCIANO C.P.C. LEONEL, LUCIANA S. SIMÕES, TAIS H.C. SASAHARA, DANIELE S. MARTINS, PHELIPE O. FAVARON & MARIA A. MIGLINO

**Abstract:** The onset of gestation is characterized by growth, morphological and functional changes of the placenta. We aim to evaluate the placental compartments in New Zealand rabbits by means of stereological methods. The fetal and maternal portion of placenta (12, 14, 18 and 20 gestational days) was randomly sampled for the stereological analysis. Histological sections were scanned to estimate fetal (labyrinth and junctional) and maternal (decidua) compartment volumes. The total volume of the placenta for the ages of 12, 14, 18 and 20 days was, respectively, 320 $mm^3$, 340 $mm^3$, 940 $mm^3$ and 1300 $mm^3$. The volume of the labyrinth was 56 $mm^3$, 119 $mm^3$, 231 $mm^3$ and 481 $mm^3$, respectively. The volume of junctional zone was 75 $mm^3$, 76 $cm^3$, 238 $mm^3$ and 314 $mm^3$, respectively. The volume of decidua was 174 $mm^3$, 143 $mm^3$, 469 $mm^3$ and 504 $mm^3$, respectively. We concluded that the rabbit´s placenta compartments varied according to the gestational period, increasing continuously over the 20 gestational days. However, on the onset of the development of the placenta the decidua presented faster growth, whereas after the 20 days of development, the labyrinth developed more quickly. This study represents an aid to the understanding of placentation in humans.

**Key words:** decidua, junctional zone, labyrinth, placentation, stereology.

## INTRODUCTION

Rabbits represent favorable experimental models for pharmacological and toxicity researches, the placenta is an important organ to understand vascular maternal-fetal components and toxic effects through placental barrier, as well as during the growth of embryo/fetus (Hayashi & Takeshi 2014). Similar with human placenta, rabbits have a discoidal and hemochorial placenta that is why they are chosen to in vitro fertilization, embryological, organogenesis and reproductive pathologies researches (e.g. congenital malformations and intrauterine growth restriction) (Hayashi & Takeshi 2014, Püschel et al. 2010).

The placenta is responsible for maintain the gestation, as well as fetus development, it is composed by a maternal portion (basal plate) and a fetal portion (labyrinth zone), which include large blood vessels responsible for gas exchanges between mother and fetus, with elimination of fetal metabolites during gestation (Flynn et al. 2006). Besides that, placenta has immunological properties, protect the embryo/fetus against xenobiotics and mechanical traumas and release hormones and cytokines (Furukawa et al. 2014).

In the beginning of gestation placenta grows rapidly so that can promote adequate nutritional support contributing to fetus development; in humans, this growth is evidenced mainly in the

first weeks with dynamic changes in its structure and function (Knöfler et al. 2019). Therefore, the weight of placenta is of paramount importance do determinate the organ functionality during gestation, its size is obtained from surface dimension (lateral and expansive growth of chorionic plate related with the area of maternal-fetal interaction) and thickness (related with vascularization) (Cardosoa et al. 2012). Any alteration in placental development leads to dysfunction of its structures and functionality, affecting the communication maternal-fetal, consequently impairing nutrition and growth fetal that can be related with chronical diseases such as hypertension and diabetes (Tarrade et al. 2013) and pregnancy complications such as miscarriage, stillbirth, pre-term labour, intrauterine growth restriction and preeclampsia (Knöfler et al. 2019).

Normal and abnormal morphology of placenta can be evaluated with quantitative methods measuring tridimensional data from two-dimensional structures (e.g. size, length, thickness, surface area and volume). Stereology is one of those quantitative techniques that uses specimen fragments to determinate the size of whole organ (Gundersen et al. 1999, Heidari et al. 2015) and this method has been used to estimate surface area, density and absolute volumes of the placenta in some species, such as human (Heidari et al. 2015), rat (Šerman et al. 2015) and cow (Adeyinka et al. 2016), however few reports have been carried out in rabbit's placenta. As elucidated, rabbits are suitable animal models for reproductive researches and with similarity with human species, the aim of this researcher was evaluated the modifications of placental tissues of New Zealand White rabbits (*Oryctolagus cuniculus*) using stereological analysis.

## MATERIALS AND METHODS

### Samples collection

This study was approved by the Ethical Committee of the Faculty of Animal Science and Food Engineering from the University of São Paulo (USP), Pirassununga Campus– SP (N° 13.1.1910.74.9). Placentas of 10 New Zealand rabbits were used in different phases of the gestational period: 12, 14, 18 and 20 days. The gestational age was estimated by Crown-Rump length and by the external fetal characteristics, as described for the species (Evans & Sack 1973).

Placentas were fixed in paraformaldehyde 4% (PFA 4%) for 48 hours. The samples were then stored in ethanol 70% until stereological analysis.

### Quantitative study

#### *Placenta sampling*

The placental lobes were chosen randomly. Thus, the same probability of choice between right and left lobe was maintained.

#### *Stereological analysis*

Placentas were weighed (g) and the total volume of placenta (Vref) was calculated dividing the weight by the density (1.05g/cm$^3$) (Mayhew 2006). They were then sectioned, perpendicular to the horizontal plane to generate vertical sections, approximately 1.5 mm thick. These sections were sampled following the systematic uniform randomly (SUR) sampling (Gundersen et al. 1999), resulting in a total of 8 to 12 sections. From these sections were generated 15 to 20 histological sections SUR sampled and used to estimate the volume fraction and the total volume of each placental zones.

### *Histological procedures*

The samples were fixed in paraformaldehyde (PFA) 4% for 48 hours and histological standard procedures were done to embedded the samples in paraffin. The paraffin blocks were exhaustively cut into 5µm sections and stained with hematoxylin and eosin (H&E). The slides were scanned by Pannoramic Scan System (3D Histec) to quantitative and qualitative placental zones analysis.

### *Volume fractions of the placental zones (Vv)*

Volume fractions of the placental zones were estimated by point-counting method. The point spacing was 0.4mm. The points hitting the labyrinth zone were counted and divided by the total points hitting the entire placenta: Vv (labyrinth)= Σp (labyrinth)/ Σp (placenta). The same procedure was done to decidua and junctional zones: Vv (junctional zone)= Σp (junctional zone)/ Σp (placenta) and Vv (decidua)= Σp (decidua)/ Σp (placenta).

### *Total volume of the placental zones (V)*

The total volume of each zone was estimated multiplying the volume fraction of each zone by the total volume of placenta (Vref): V (labyrinth)= Vv (labyrinth) x Vref; V (junctional zone)= Vv (junctional zone) x Vref; V (decidua)= Vv (decidua) x Vref. The quantitative results were shown as mean value followed by the coefficient of variation (CV).

## RESULTS

### Qualitative study

In the macroscopic analysis, the New Zealand rabbit placenta was characterized as discoidal and bilobular, maintaining the morphology during the all the gestational period analyzed (Figure 1a). In addition, the placenta of this species is characterized, in terms of maternal-fetal interaction, in hemodicorial, choroalanoid and labyrinth, allowing the nutrient exchanges.

The placental disc was characterized histologically by the labyrinth zone composed by the trophoblastic cells, separating the maternal-fetal circulations, distinguishing itself from the rest of the cellular components due to the large size of the nucleus and the globose format (Figure 1c, d). The junctional zone, composed of spongiotrophoblasts and glycogen-rich cells, was visualized between the labyrinth and decidua (Figure 1b, c). The decidua was characterized by the presence of deciduous cells, originating from stromal cells of the endometrium, and maternal arteries in close contact with the endometrium (Figure 1b).

### Total volume of placenta (Vref)

The mean total volume of the placenta (fetal and maternal portions) presented a gradual increase according to the development of gestation: 12 days (320 $mm^3$) 14 days (340 $mm^3$), 18 days (940 $mm^3$) and 20 days (1300 $mm^3$) (Table I; Figure 2).

### Total volume of placental compartments

The total volume of labyrinth ranged from 320 $mm^3$ on the 12th day of gestation to 1300 $mm^3$ on the 20th day of gestation. The total volume of the junctional zone ranged from 75 $mm^3$ at 12th to 314 $mm^3$ at the 20th day of gestation and the total volume of the decidua ranged from 174 $mm^3$ on the 12th day to 504 $mm^3$ at the 20th day of gestation (Figure 3).

## DISCUSSION

The rabbits present a placenta with hemodichorial structure, presenting two layers of chorion between the maternal and

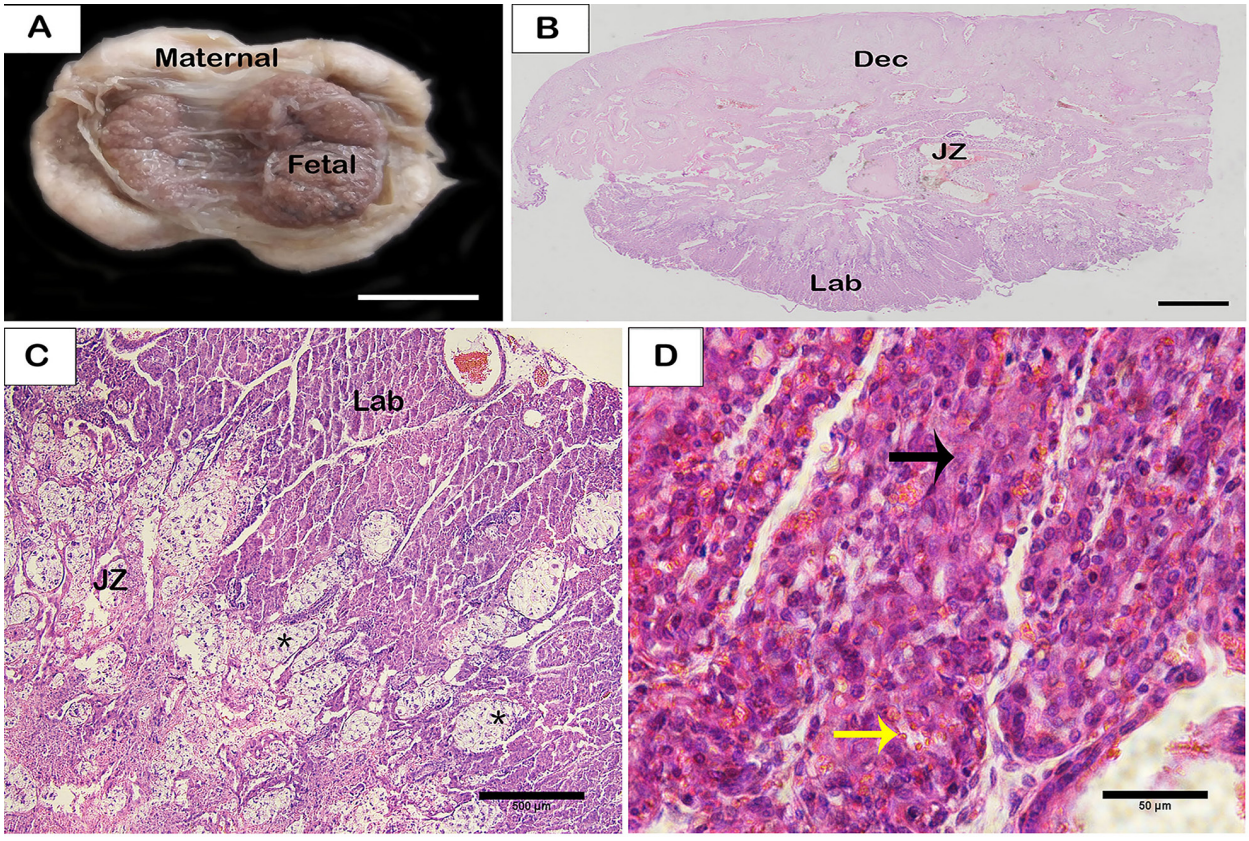


**Figure 1. a)** Fetal and maternal portion of discoidal and bilobular placenta of New Zealand rabbit on the 16th day of gestation (Bar scale: 1 cm). **b)** Photomicrography of the placental compartments (labyrinth, junctional and decidua zones on the 16th day; scale bar: 1mm). **c)** Trophoblastic cells of the labyrinth (Lab) and spongiotrophoblasts (asterisk) of the junctional zone (JZ) (20th day; Scale bar: 500 µm). **d)** Trophoblasts (black arrow) in contact with maternal blood (yellow arrow, 20th day; Scale bar: 50 µm). **Dec:** decidua; **JZ:** junctional zone; **Lab:** labyrinth. Hematoxylin & Eosin staining.

**Table I.** Mean value of total placental volume and placental compartments ($mm^3$) followed by coefficient of variation (CV) for 12 to 20 days of gestation in New Zealand rabbits.

| Days of Gestation | Placenta Total volume | Labyrinth volume | Junctional volume | Decidua volume |
|---|---|---|---|---|
| 12 | 320 (0,14) | 56 (0,30) | 75 (0,17) | 174 (0,16) |
| 14 | 340 (0,42) | 119 (0,48) | 76 (0,14) | 143 (0,51) |
| 18 | 940 (0,07) | 231 (0,06) | 238 (0,05) | 469 (0,01) |
| 20 | 1300 (0,07) | 481 (0,15) | 314 (0,22) | 504 (0,18) |

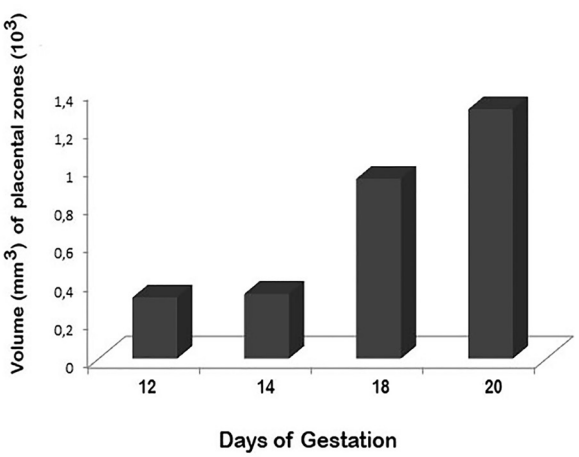


**Figure 2. Total volume of the rabbit placenta ($mm^3$) from the 12 days to the 20 days of gestation.**

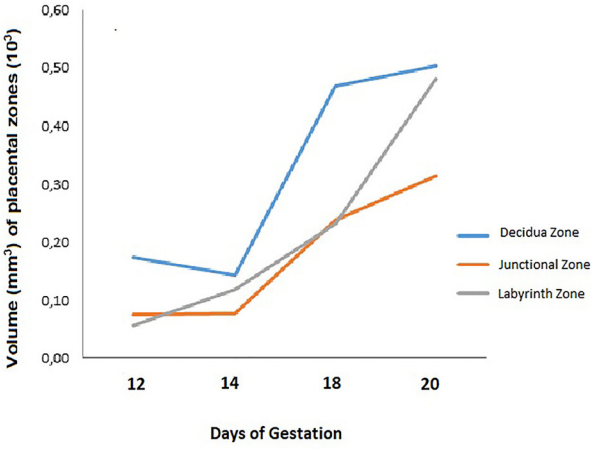


**Figure 3. Total volume of the rabbit placental zones from day 12 to day 20 of gestation.**

fetal blood, similar to the structure of human placenta comparing to other rodents that present hemotrichorial placenta. Furthermore, the rabbit's placenta is more similar about the hemodynamic changes and development of diseases during placentation, comparable to the humans (Skoda et al. 2017).

In this study the total volume of New Zealand rabbit placenta was evaluated, as well as the volume of the placental compartments, using the stereology technique, which estimates the three-dimensional structure of an organ from two-dimensional data obtained from smaller fragments of this tissue, improving the interpretation of results regarding the growth, morphogenesis and functionality of an organ as a whole (Shemer et al. 2012).

In general, there was an increase in the total volume of the placenta and the placental compartments (labyrinth, junctional zone/JZ and decidua) during the 20 days of gestation analyzed, mainly between the 14th and the 18th days. In rodent species, such as *Necromys lasiurus*, in which gestation lasts on average 23 days, there was an increase in the total volume of placenta and placental compartments from the beginning to the middle of the gestational period (10 to 16 days), followed by a near-birth reduction (Favaron et al. 2013); similar results were also described for mice (Coan et al. 2004) and rat (Šerman et al. 2015); while in humans, the placenta shows continuous growth throughout the gestation (Higgins et al. 2011).

In the labyrinth zone, composed of two layers of trophoblasts (syncytiotrophoblast and cytotrophoblast) separating the space of maternal and fetal blood, the duplication of trophoblast layer was observed in all the phases of gestation analyzed, possibly due to a greater requirement of blood and nutrient supply during fetal growth; thus, proliferation and trophoblastic differentiation contributes to the diffusion of $O_2$ into the labyrinth space (Mayhew 2006). In humans, the fetal part of the placenta (chorionic villi) represents the greater part of the placental compartment (Heidari et al. 2015). In mice, the volume of the labyrinth zone was similar to the junctional zone during late gestation in this species (Coan et al. 2004). Similar data were found in this study in the evaluation of rabbit placenta, which on the 18$^{th}$ day of pregnancy presented the volume of the

labyrinth zone similar to that of JZ; presenting an increase of the labyrinth, in relation to the JZ, in the 20th day of development.

The junctional zone is located at the maternal-fetal interface and is composed of different cell types: spongiotrophoblasts, glycogen rich cells and giant trophoblastic cells (Furukawa et al. 2014); these cells originate from trophoblasts on the 10th day of development of the rabbit placenta, but are not present in the human placenta (Fischer et al. 2012); in mice, giant trophoblastic cells are responsible for the invasion of the blastocyst in the maternal uterus, as well as modulate the biological activity of hormones and growth factors (Coan et al. 2004). In this study, the volume of the JZ was increased during gestational days, mainly between the 14th and 18th days, but less significant in relation to the labyrinth and decidua zone.

The decidua originates from stromal cells of the endometrium, which is composed of endometrial glands that synthesize and secrete substances essential for embryo/ fetal development (Santos et al. 2012). In this study, we observed a slight reduction in decidua volume on the 14th day, which increased again in the subsequent days.

Although the stereological study provides quantitative information on a structure and functionality of tissues and organs, other methods can also be applied for the better comprehension about cellular interactions between placental compartments, which directly influence fetal development and growth.

## CONCLUSIONS

The three-dimensional quantification, based on stereological methods, allowed to estimate the volume of the placental compartments during the 20 days of gestation in New Zealand rabbits, allowing a better understanding of the modifications that occur during the development of the placenta in this specie, which interfere directly in maternal-fetal exchanges and, consequently, on the growth of the fetus, serving as a subsidy for understanding the functioning of placentation in humans.

## REFERENCES

ADEYINKA FD, LAVEN RA, NICOLO G, LAWRENCE KE & PARKINSON TJ. 2016. The Use of Stereology Method to Estimate the Volume of Feto-Maternal Exchange Area of the Bovine Placentome during Gestation. Anat Rec 299: 1571-1577.

CARDOSOA V, MAZZITELLIB N, VEIGAC MA, FURLÁNA R & GRANDID C. 2012. Medidas del crecimiento placentário y su relación con el peso de nacimiento y la edad gestacional. Rev Hosp Mat Inf Ramón Sardá 31(2): 69-74.

COAN PM, FERGUSON-SMITH AC & BURTON GJ. 2004. Developmental Dynamics of the Definitive Mouse Placenta Assessed by Stereology 1. Biol Reprod 1813: 1806-1813.

EVANS HE & SACK WO. 1973. Prenatal development of domestic and laboratory mammals: growth curves, external features and selected references. Anat Histol Embryol 2(1): 11-45.

FAVARON PO, MESS AM, OLIVEIRA MF DE, GABORY A, MIGLINO MA, CHAVATTE-PALMER P & TARRADE A. 2013. Morphometric analysis of the placenta in the New World mouse Necromys lasiurus (Rodentia, Cricetidae): a comparison of placental development in cricetids and murids. Reprod Biol Endocrinol 11(10): 1-8.

FISCHER B, CHAVATTE-PALMER P, VIEBAHN C, SANTOS N & DURANTHON V. 2012. Rabbit as a reproductive model for human health. Reprod 144(1): 1-10.

FLYNN L, SEMPLE JL & WOODHOUSE KA. 2006. Decellularized placental matrices for adipose tissue engineering. J Biomed Mater Res A 79(2): 359-369.

FURUKAWA S, KURODA Y & SUGIYAMA A. 2014. A comparison of the histological structure of the placenta in experimental animals. J Toxicol Path 27(1): 11-18.

GUNDERSEN HJ, JENSEN EB, KIÊU K & NIELSEN J. 1999. The Efficiency Of Systematic Sampling In Stereology-Reconsidered. J Microsc 193: 199-211.

HAYASHI K & TAKESHI K. 2014. Characteristic patterns of maternal and fetal arterial construction in the rabbit placenta. Med Mol Morphol 47: 76-82.

HEIDARI Z, SAKHAVAR N, MAHMOUDZADEH-SAGHEB H & EZAZI-BOJNOURDI T. 2015. Stereological Analysis of Human Placenta in Cases of Placenta Previa in Comparison with Normally Implanted Controls. J Reprod Infertil 16(2): 90-95.

HIGGINS M, FELLE P, MOONEY EE & BANNIGAN J. 2011. McAuliffe Fm: stereology of the placenta in type 1 and type 2 diabetes. Placenta 32: 564-569.

KNÖFLER M, HAIDER S, SALEH L, POLLHEIMER J, GAMAGE TKJB & JAMES J. 2019. Human placenta and trophoblast development: key molecular mechanisms and model systems. Cell Mol Life Sci 76(18): 3479-3496.

MAYHEW TM. 2006. Stereology and the Placenta: Where's the Point? – A Review. Placenta 27: 17-25.

PÜSCHEL B ET AL. 2010. The Rabbit *(Oryctolagus cuniculus)*: A Model for Mammalian Reproduction and Early Embryology. Cold Spring Harb Protoc 2010(1): pdb. emo139.

SANTOS TC, TONARELLI C, TEIXEIRA FA, OLIVEIRA MF, MARIA D, REGINATO P, KFOURY JR, OLIVEIRA CAL, LOURENÇO DAL & MIGLINO MA. 2012. Histomorphometrical and proliferative aspects of placenta and uterus of the collared peccary (Tayassu tajacu). Histol Histopathol 27: 793-806.

ŠERMAN L, ŽUNIĆ I, VRSALJKO N, GRBEŠA D, GJURČEVIĆ E, MATAŠIN Z, MARTIĆ TN, JAKUŠ FB, GAJGER IT & ŠERMAN A. 2015. Structural changes in the rat placenta during the last thirdof gestation discovered by stereology. Bosn J Basic Med Sci 15(1): 21-25.

SHEMER EW, THORSELL M, ÖSTLUND E, BLOMGREN B & MARSCHALL HU. 2012. Stereological assessment of placental morphology in intrahepatic cholestasis of pregnancy. Placenta 33: 914-918.

SKODA G, HOFFMANN OI, GÓCZA E, BODROGI L, KEREKES A, BÖSZE Z & HIRIPI L. 2017. Placenta-specific gene manipulation in rabbits. J Biotechnol 259: 86-90.

TARRADE A ET AL. 2013. Sexual dimorphism of the feto-placental phenotype in response to a high fat and control maternal diets in a rabbit model. PloS ONE 8 (12): e83458.

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**CARLA M.F. DE CARVALHO**[1]
https://orcid.org/0000-0002-3282-1095

**LUCIANO C.P.C. LEONEL**[1]
https://orcid.org/0000-0002-8066-4055

**LUCIANA S. SIMÕES**[1]
https://orcid.org/0000-0003-4547-7454

**TAIS H.C. SASAHARA**[1]
https://orcid.org/0000-0002-4871-5625

**DANIELE S. MARTINS**[2]
https://orcid.org/0000-0002-6277-4664

**PHELIPE O. FAVARON**[1]
https://orcid.org/0000-0002-6431-6886

**MARIA A. MIGLINO**[1]
https://orcid.org/0000-0003-4979-115X

[1]Universidade de São Paulo/ USP, Departamento de Cirurgia, Faculdade de Medicina Veterinária e Zootecnia, Av. Prof. Dr. Orlando de Marques Paiva, 87, Vila Universitária, 05508-270 São Paulo, SP, Brazil

[2] Faculdade de Zootecnia e Engenharia de Alimentos, Universidade de São Paulo/USP, São Paulo Av. Duque de Caxias Norte, 225, Zona Rural, 13635-900 Pirassununga, SP, Brazil

Correspondence to: **Carla M. F. de Carvalho**
*E-mail: carlacarvalhovet@gmail.com*

## Author contributions

Carla M.F. de Carvalho: concept/design; collect samples; tissue preparation for analysis; image acquisition; data interpretation; drafting article and critical revision of article. Luciano C.P.C. Leonel: helped collect samples; tissue preparation for analysis and image acquisition. Luciana S. Simões: helped tissue preparation for stereological analysis and image acquisition; data interpretation; drafting article. Tais H.C. Sasahara: experimental design, data interpretation of stereological analysis; drafting article. Daniele S. Martins: experimental design, reproductive management of animals. Phelipe O. Favaron: experimental design, study supervision. Maria A. Miglino: study supervision; critical revision of article; approval of article.