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Histomorphometry of the placental vasculature and microcotyledons in Thoroughbred mares with chronic laminitis

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ABSTRACT

The objective of this study was to assess the placental vasculature and microcotyledons in pregnant mares with chronic laminitis. Twenty-six pregnant mares were enrolled in the study, 13 had chronic laminitis (Laminitis Group) and 13 were healthy mares (Healthy Group). Arterial systolic pressure and heart rate were measured in the last 30 days of gestation. After foaling, the fetal membranes were grossly evaluated and samples were harvested for histopathologic examination. All mares had digitalized images taken from chorioallantois for histomorphometry analyses (software-NIH ImageJ). Images were assessed for: (i) arterioles from the allantoic region: total and lumen vascular diameter and vascular wall thickness; (ii) microcotiledonary and capillary area/field. Mares in the Laminitis Group showed hypertension, shorter gestational length, lower placental weight and lower birthweight ($p < 0.05$) foal in comparison with mares in the Healthy Group. Laminitis mares had a reduction of vascular lumen diameters in the uterine body and pregnant horn ($p < 0.05$), vascular wall thickening in the pregnant horn ($p < 0.05$) and smaller capillary area/field in the microcotyledons of uterine body and pregnant horn ($p < 0.05$). In conclusion, pregnant mares with chronic laminitis presented signs of hypertension syndrome, and vascular abnormalitities in placental vessels such as reduction in the vascular lumen and capillary area in the microcotyledones, and thickening of the vascular wall. Foals born from mares with chronic laminitis showed lower birth weight and shorter gestation lengths.

1. Introduction

The placenta is the organ that provides essential communication between the mother and the fetus, and regulates the amount and composition of the nutrients and oxygen transported between maternal and fetal circulations [1]. During gestation the vascular network of the placenta becomes more extensive and the vessels enlarge to accommodate the increasing demands of the growing fetus [2–4]. In concert, the blood flow in both the uterus, via the uterine arteries, and in the chorioallantois, via the umbilical cord, increase exponentially during gestation to support fetal growth [2]. However, the uteroplacental unit can be compromised by systemic disease in the mother, leading to deficits in fetal development and growth [5].

Gestational hypertension is a common medical disorder in women, which has been reported to affect 1 in 10 pregnancies [6]. In humans, hypertension, and other conditions such as diabetes, can result in remodeling of the vascular network in the placenta which, in turn, results in alterations in placental perfusion and, hence, fetal compromise [6]. Likewise, in the horse, it is possible that metabolic and cardiovascular abnormalities during gestation may lead to changes in the development and morphology of the vascular network in the placenta [7].

The human placenta is haemochorial in structure, which means that the maternal blood comes in direct contact with the fetal chorion, this is in contrast to the horse with its epitheliochorial placenta where six tissue layers separate the fetal and maternal blood supplies. Therefore, gestational hypertension in these two species may have different consequences on vascular remodeling within the placenta [8]. Chronic laminitis in horses frequently leads to a hypertensive syndrome, resulting from adrenergic activation and subsequent changes in the vascular endothelium which

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increase peripheral resistance [9,10]. Moreover, laminitis promotes the release of increasing levels of pro-inflammatory factors, including vascular mediators [11]. In mares, chronic laminitis during pregnancy may result in abortion, stillbirth, premature foaling and delivery of compromised foals with low birthweight [7]. However, there is limited information of placental features of mares suffering with chronic laminitis.

In the present study we hypothesised that mares with chronic laminitis experience hypertension during gestation that leads to changes in the vascular network of the placental vessels. Therefore, the objective of this study was to assess the placental vasculature and microcotyledons in pregnant mares with chronic laminitis with the use of computer-assisted histomorphometry.

2. Material and methods

A prospective observational study of a population of Thoroughbred mares from three farms in Aceguá, Rio Grande do Sul, Brazil (31°51′55″ S; 54°10′02″ W) was performed during the 2011 foaling season. In the farms all the mares were maintained in a semi-extensive system and received a balanced concentrated diet with 12% protein and 27.5 mCal of digestible energy. Free access to water was provided. All procedures in the animals were approved by the Ethical Committee on Animal Experimentation of the College of Veterinary Medicine, UPel under protocol number 5810. Also, these procedures have been carried out according to European Union Directive 2010/63/EU for animal experimentations.

2.1. Case selection

The study was performed with 26 Thoroughbred mares carrying normal pregnancies [12], aged 8.9 ± 0.9 years old and with 3 ± 0.5 parturition number. All the horses had eutocic parturition and healthy neonatal foals. Of these, 13 mares had chronic laminitis during gestation (Laminitis Group) and 13 comtemporaneous mares served as healthy controls (Healthy Group). All mares in the Healthy Group did not present signs of chronic laminitis or major clinical abnormalities during the current and previous pregnancies. All mares were spread into three farms as follows: farm 1 n = 6 mares/group, farm 2 n = 4 mares/group, and fam n = 3 mares/group.

Starting at 5 months of gestation, monthly, all mares underwent transrectal ultrasonography to assess the fetus and placenta. The combined thickness of the uterus and placenta (CTUP) was measured at the placenta-cervical junction using a 5-MHz linear transducer (SonoVet600©, Medison, Seul, South Korea), starting at the fifth month of pregnancy until delivery.

The mares had chronic laminitis throughout gestation. Those mares showed lameness greater than two on a scale of 1–5, as described by Obel [13] as the presence of chronic pain, foot deformities, foot stress lines and rotation of the distal phalanx more than five degrees in radiological evaluation. The diagnosis and treatment of laminitis were performed by the same authors (CA Santos). Other than, supportive therapy of the hoof mainstay, no medical treatment was offered during pregnancy [14].

Daily, during the 30 days before parturition, mares had the arterial systolic pressure and heart rate assessed. On each day, the measurements were performed in triplicate and averaged [15]. The tail cuff method with an oscillometric sphygmomanometer (Medeqco easy control®) was used to measure the arterial systolic pressure. The heart rate was performed by auscultation of the apex beat in the proximity of the 5th intercostal space dorsal to the point of the olecranon by stethoscope evaluation [16]. Arterial systolic pressure and heart rate were evaluated daily in both groups of mares. Due to animal husbandry restriction imposed by farm 3, we were unable to perform daily assessment of heart rate and arterial systolic pressure in this farm.

2.2. Foaling management and placental evaluation

The mares were maintained in paddocks near the foaling barn until the moment of delivery. After the chorioallantois ruptured, the mares were brought into the stalls for assisted foaling. After delivery, all foals had physical examination performed and data recorded. At the time of foaling data were recorded for each mare: gestational length, placental release time, placental weight, time form foaling to rupture os the umbilical cord, umbilical cord length and attachment to the chorioallantois. The umbilical cord vascular patterns were classified in 3 patterns as previously reported [17].

The fetal membranes weighed was recorded following release. Thereafter, each placenta was placed in an “F” shape for gross evaluation, as described by Schlafer [18]. The chorionic and allantoic surfaces of the chorioallantois were examined for abnormalities in color, areas devoid of villi, thickened areas and presence of exudate. Samples of 3 × 3 cm dimension were obtained from nine points from each placenta as follows: the cervical star area, the pregnant horn, the nonpregnant horn, the uterine bifurcation, the uterine body, the amnion and three segments of the umbilical cord. All samples were fixed using formalin 10% before processing for histology. Histological sections (3- to 5-μm thick) were mounted on glass slides and stained with hematoxylin and eosin in stardand fashion. The slides from all sampled portions of each mare's fetal membranes were evaluated using optical microscopy to rule out histological abnormalities.

2.3. Computer-assisted histomorphometry of placental vascular network and microcotyledons

Histomorphometry evaluation of the vascular network and microcotyledons of the chorioallantois were carried out for all mares. Digitalized images of the chorioallantois were taken from the segments of the uterine body, pregnant and nonpregnant horns with the aid of a high-resolution Olympus DP72 camera in the Olympus BX51 (Olympus America, Center Valley, PA) microscope. Then, the digital images were processed using the open source software NIH ImageJ 1.48r software (US National Institute of Health, available at http://rsb.info.nih.gov/il/).

Vascular network measurement was performed. The arterioles in the basal lamina between the allantoic and chorionic surface of the chorioallantois were evaluated. Initially, each image had total pixels calibrated to μm. Then, the measures corresponding to the total vascular diameter, vessel lumen diameter (S-Fig. 1), and vascular wall thickness were made using the plug-in “Straight” (S-Table 1). All measurement was performed as the smallest transversal diameter.

Histomorphometry was performed on the microcotyledonary area. The capillary area of microcotyledons was performed on the chorionic epithelium of the chorioallantois. The images were evaluated in RGB color with the total pixels calibrated to μm. Quantifications of the microcotyledonary and capillary area/field (S-Fig. 1) were performed with the “Color Threshold” macro (S-Table 1), and the color range was adjusted by the RGB scale. Differences in color intensity were analyzed through differential absorption method [19]. If there are not erythrocytes into capillaries, the correction of capillary area/field measurements was performed by the plug-in “Freehand” (S-Table 1). The field area (100× magnification) to acquire digitalized images was 146.673 μm^2.

The procedures were carried out randomly ten times on each section on three separate samples for each mare, corresponding to the uterine body, pregnant horn and nonpregnant horn, giving a