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Apoptosis and cell proliferation in porcine placental vascularization

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ABSTRACT

The placenta is a highly vascularized organ, indispensable tothe transfer of nutrients to the growing fetuses. During gestation, there exists an expansion of the placental vascular network through active angiogenesis. The aim of this research was to study cell proliferation and apoptosis through high resolution light microscopy (HRLM) and transmission electron microscopy (TEM) ultrastructure, immunohistochemistry for Ki67and caspase-3, determination of placental vascular area,and TUNEL assay. Crossbred sows placental tissues from approximately 30 ± 2(n = 5), 40 ± 2(n = 5), 60 ± 2 (n = 5), 80 ± 2(n = 5), 90 ± 2(n = 5) and 114 ± 2(n = 5) days of gestation were used. The evaluation of cell proliferation showed the highest%Ki67 values on days 30 ± 2 and 80 ± 2 of pregnancy. Caspase-3 expressed the highest value on day 30 ± 2, while the highest apoptotic indexes were found on days30 ± 2 and 90 ± 2. The placental vascular area was higher on day 80 ± 2 of pregnancy. According to our results, an active vascular cell remodeling by a caspase-3 dependent apoptosis seems to be present in early pregnancy. The increase in the vascular area on day 80 ± 2 would be the result of the intense vascular cell proliferation detected with Ki67. Further studies are needed to understand the complex processes of angiogenesis, cell proliferation and apoptosis that interact in the placenta during porcine gestation.

1. Introduction

Gestation in pigs extends forapproximately 114 days (Hafez and Hafez, 2002). During this period, the placenta plays a vital role in the maintenance of the embryos and fetuses (Wooding and Burton, 2008). The pig placenta is diffuse, rough and epitheliochorial,althoughimportant morphogenetic changes occur in this organ during gestation (Dantzer and Winther, 2001; da Anunciação et al., 2017; Croy et al., 2009)

Placentation includes extensive angiogenesis in maternal and extraembryonic tissues, accompanied by a marked increase in uterine and umbilical blood flow (Goldstein et al., 1980; Biensen et al., 1998; Reynolds and Redmer, 2001; Merkis et al., 2006; Reynolds et al., 2006; Gourvas et al., 2012). The dense networks of blood vessels within the placenta are responsible for exchanging respiratory gases, nutrients and wastes between the mother and the fetus throughout pregnancy, which is essential for proper fetal

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growth (Mayhew, 2002; Burton et al., 2009; Sanchis et al., 2015; Pereira et al., 2015). In porcine placenta a remodelation of the placental tissue associated to vascular changes was described by Dantzer and Leiseronly in the initial gestation using corrosion cast technique (Dantzer and Leiser, 1994; Leiser and Dantzer, 1994).

During placental angiogenesis, proliferation of endothelial cells is pivotal to the development of a vascular bed suitable for nutrition of the fetuses. However, the apoptosis, a process that is antagonistic to proliferation, is also necessary for angiogenesis (Troyanovsky et al., 2001).

Together with mitosis, apoptosis controls the number of tissue cells (Heazell et al., 2006; Heazell and Crocker, 2008). Programmed cell death by apoptosis has been linked to the angiogenic process (Troyanovsky et al., 2001). In humans, it has been demonstrated that the endothelial cell apoptosis plays a regulatory role in adult neovascularization (Dimmeler and Zeiler, 2000). Placental angiogenesis has also been indicated as an apoptotic-dependent process, since human placental vessels form and expand due to the intervention of apoptosis (Tertemiz et al., 2005). Moreover, inhibition of endothelial cell apoptosis providing endothelial cell survival has also been indicated as an essential issue during angiogenesis (Zhang et al., 2000; Chavakis and Dimmeler, 2002). Our research group hasmade an attempt to characterize the apoptotic process during placentation in pigs (Cristofolini et al., 2013; Merkis et al., 2010). However, the role of apoptosis and its relation to a proliferativeprocess in porcine placental vascularization is still not completely clear.

Our objective was to evaluate the processes of cell proliferation and apoptosis during the development of the porcine placental vascular network throughout gestation.

2. Materials and methods

2.1. Animals and tissue collection

The study protocol was approved by the Research Ethics Committee of the National University of Río Cuarto. Reproductive tracts ofcrossbred healthy sows from different slaughterhouses from Río Cuarto city, Argentine (33.11° S; 64.3° O) were used. The reproductive tract was obtained immediately after slaughter (approximately on days 30, 40, 60, 80, 90 of gestation) or after delivery (approximately 114 days of gestation), washed with saline solution of Hanks (SSH) containing sodic penicillin G, streptomycine sulphate and fungizone (Gibco, Grand Island, NY, USA), and maintained at 4 °C until processingwithin 30 min. The uterine horns were opened longitudinally with an incision inthe anti-mesometrial edge. Embryos or fetuses were removed and their gestational age was determined according to the crown-rump length of the litter (Marrable 1971). A total of 30 placentas were selected for the study, including periods from early to term gestation: 30 ± 2 days of gestation (n = 5), 40 ± 2 days of gestation (n = 5), 60 ± 2 days (n = 5),80 ± 2 days(n = 5), 90 ± 2 days (n = 5) and 114 ± 2 days of gestation (n = 5).Every period of gestation was selected considering a variation range of approximately 2 days.

Tissue samples were taken from five placentas at every gestational period (one placenta was randomly chosen from each animal). Samples were gathered from the feto-maternal interface and used to determine placental architecture through histological techniques, Ki67 and caspase-3 through immunohistochemistry, and apoptosis through TUNEL.

2.2. Conventional histological technique

Portions of approximately 6 mm^3 of placental tissue were fixed by immersion in 10 per cent (v/v) buffered-saline formaldehyde pH 7.2–7.4 at 4 °C, dehydrated with alcohol and embedded in paraffin. Then, they were cut in ± 4 μm histological sections with a microtome (Micron, Germany) and mounted on slides. Paraffin embedded sections were used for immunohistochemistry, TUNEL assay and vessel determination. Previous to analyses, the sections were rehydrated with alcohol and washed with distilled water and PBS.

2.3. High resolution light microscopy

For high resolution light microscopy (HRLM), portions of approximately 1 mm^3 of placental tissue were processed by conventional transmission electron microscopy technique. Placental samples were fixed in 2.5% glutaraldehyde in 0.2 M S-collidine pH 7.4, post-fixed in 1% osmium tetroxide in 0.2 M S-collidine pH 7.4, dehydrated in increasing concentration acetone, embedded in EMbed 812 resin and sectioned with an ultramicrotome to obtain semi-thin sections (± 0.25 μm). These sections were counterstained with toluidinés blue and were cover-slipped in DPX (Merk, Germany) embedding agent. They were then observed in a light microscope Axiophot (Carl Zeiss, Germany) fitted with a high resolution digital camera Powershot G6 7.1 megapixels (Canon INC, Japan). Digital images were captured with Axiovision 4.6.3 software (Carl Zeiss, Germany).

2.4. Transmission electron microscopy

Ultra-thin sections (± 60 nm) were cut with an ultramicrotome from resin embedded tissues. They were placed on cupper grids, counterstained with saturated uranyl acetate and aqueous lead citrate. The sections were examined with a transmission electron microscope Elmiskop 101 (Siemens, Germany) .Acquisition, digital analysis and morphometric measurements were performed with transmission electron microscope JEM 1200 ExII (JEOL, Japan), using aDigital MicrographTM (Gatan, Inc., Japan) software. The reagents employed were from Electron Microscopy Science.