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Morphometric study of the porcine placental vascularization

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Contents

The early development in mammals is characterized by the contribution of nutrients from the maternal tissues through the placenta, which is in apposition with foetal membranes and the endometrium, allowing the physiological interchange between the embryos/foetuses and the mother. The aim of this work was to study the number of placental blood vessels and their vascular area through morphometric analyses and the haemotrophic diffusion distance in porcine placental tissues from early gestations, intermediates gestations, advanced gestations and term gestations. For those purposes, morphometric measurements, blood vessel quantification, high-resolution light microscopy and transmission electron microscopy were performed. The implementation of the high-resolution light microscopy allowed studying the placental vascular and tissue histoarchitecture with higher definition and resolution than using a conventional light microscopy. We highlight the close location of the subepithelial capillaries to the maternal/foetal interface as pregnancy progresses. We found statistically significant evidence to state that the area of blood vessels is dependent on the gestation period. In advanced gestations, the presence of numerous small blood vessels and its near location to foetal/maternal interface agree with the great remodelling reported in our previous studies. In conclusion, in gilts, given the type of non-invasive epithelial placentation, the new blood vessels generation and of haemotrophic diffusion distance reduction, determined in this report, assure the maternal/foetal haemotrophic exchange efficiency during gestation.

1 | INTRODUCTION

Porcine placenta is diffuse, folded, without decidualization, non-invasive and epitheliochorial, accomplished with interdigitations of the trophectoderm microvilli and surface uterine epithelium (Bazer, Spencer, Johnson, Burghardt, & Wu, 2009; Wooding & Burton, 2008). The placental microvilli are interconnected through an adherent glucocalix covering completely the maternal/foetal interface (Ender & Carter, 2004; Merkis, Cristofolini, Sanchis, & Koncurat, 2010; Miglino, Pereira, Santos, & Carvalho, 2001). Such cellular contact is essential for nutrient exchange, and for placental fixation, as each porcine embryo must achieve its place of implantation and subsequent development in the uterine horns, given the epitheliochorial placentation and polytocous character of the species (Hafez & Hafez, 2002; Merkis, Cristofolini, Franchino, Moschetti, & Koncurat, 2005; Merkis et al., 2010).

The placenta is a highly vascularized organ (Burton, Charnock-Jones, & Jauniaux, 2009; Wooding & Burton, 2008) that transports substances between foetal and maternal circulations (Barrio et al., 2003; Greenwood, Slepetis, & Bell, 2000; Mayhew, 2002). In every mammalian placenta, extensive foetal and maternal capillary networks develop parallel to their interface to maximize the efficiency of haemotrophic exchange (Abd-Elnaeim, Leiser, Wilsher, & Allen, 2006; Sanchis, Cristofolini, & Merkis, 2015) by minimizing the diffusion

distance for gases and solutes (Cristofolini, Merkis, & Koncurat, 2012; Sanchis, Cristofolini, & Merkis, 2012). We established earlier the importance of extracellular matrix and apoptosis for the formation, development and support of the placental vascular system (Cristofolini, 2010; Merkis et al., 2010; Sanchis, Cristofolini, Taglialegna, & Merkis, 2011; Cristofolini, Sanchis et al., 2012; Sanchis et al., 2012; Cristofolini et al., 2013).

To achieve a successful pregnancy and a normal gestation, a placental vascularization a correct one must occur allowing a suitable establishment and development of the foetus/embryos (Cristofolini, Merkis et al., 2012; Sanchis et al., 2015). Several angiogenic and non-angiogenic factors are involved in placental vascular development. Best characterized factors include vascular endothelial growth factor (VEGF) and placental growth factor (PlGF) (Sanchis et al., 2011; Tjwa, Luttun, Autiero, & Carmeliet, 2003; Vonnahme, Wilson, & Ford, 2001); these factors act by binding to specific receptors (Roskoski, 2008; Zachary & Gliki, 2001).

We investigated the expression profiles of the angiogenic factors, VEGF and PlGF, and their receptors, Flt-1 and Flk-1. We demonstrated the localization of both VEGF and PlGF and their receptors during several gestational periods (Sanchis et al., 2015).

The aim of this work was to study the number of placental blood vessels and their vascular area through morphometric analyses and the haemotrophic diffusion distance (HDD) in porcine placental tissues from early gestations (25, 30 and 40 days), intermediates gestation (60 and 70 days), advanced gestation (90 days) and term gestation (114 days). The advances in the study of the vascular morphometric throughout the placentation in pregnant gilts will allow determining the importance of neovascularization in this type of placentation, an essential process for the correct foetal/maternal exchange during porcine gestation.

2 | MATERIALS AND METHODS

2.1 | Animals and tissue collection

Our protocol was approved by the National University of Río Cuarto Ethical Committee of Research (CoEdI), Res. 186/2016. We used reproductive tracts from cross-bred healthy gilts from slaughterhouses located in Río Cuarto City, Argentina (33.11°S; 64.3°O). A total of 35 animals were processed: early gestations (25 days: $n = 5$; 30 days: $n = 5$ and 40 days: $n = 5$), intermediates gestations (60 days: $n = 5$ and 70 days: $n = 5$), advanced gestations (90 days: $n = 5$) and term gestations (114 days: $n = 5$). In every case, the reproductive tract was removed immediately after slaughter, washed with saline solution of Hank's (SSH) prepared as instructed by the manufacturer with the addition of penicillin G, streptomycin sulphate and fungizone (Gibco, Grand Island, NY USA) and maintained at 4°C until processing. Palpation was made to detect the location of the embryos or foetuses. The uterine horns were opened carefully and longitudinally with an incision on the antimesometrial edge to observe the implantation site. Embryos or foetuses were removed from each pregnant gilt and the gestational age was determined by crown-rump length (Marrable, 1971). Tissue samples were taken from five placentas of every gestational period (one placenta was randomly chosen from each animal). Samples were taken from mesometrial, endometrial and foetal placental tissues and used for differential staining, morphometric measurements, high-resolution light microscopy and by transmission electron microscopy.

2.2 | Conventional histological technique

Portions of approximately 6 mm^2 of placental tissue were fixed in 4% (v/v) buffered saline formaldehyde pH 7.2–7.4 at 4°C and dehydrated in a graded series of ethanol 65%, 75%, 80%, 90%, 95% and 100% and xylene. Paraffin-embedded placental tissue (Paraplast Plus®) was sectioned at ±4 μm and was stained with Masson's trichromic and Gallegos′s trichromic. The differential staining was used to identify easily the presence the blood vessel of calibre different. The placental sections were observed in a light microscope Axiophot (Carl Zeiss, Thornwood, NY, USA) fitted with a high-resolution digital camera PowerShot G6 7.1 megapixels (Canon INC, Tokyo, Japan). Digital images were captured using AxioVision 4.6.3 software (Carl Zeiss, Göttingen, Germany).

2.3 | High-resolution light microscopy (HRLM)

Portions of approximately 1 mm^3 of placental tissue 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 and embedded in EMbed 812 resin. An ultramicrotome was used to obtain the semi-thin sections (±0.25 μm). These sections were counterstained with toluidina's blue and were coverslipped in DPX (Merck, Alemania) embedding agent. They were then observed in a light microscope Axiophot (Carl Zeiss, Thornwood, NY, USA) fitted with a high-resolution digital camera PowerShot G6 7.1 megapixels (Canon INC, Tokyo, Japan). Digital images were captured using AxioVision 4.6.3 software (Carl Zeiss, Göttingen, Germany).

2.4 | Transmission electron microscopy

Placental tissue embedded in EMbed 812 resin to HRLM was sectioned with an ultramicrotome Sorvall MT1A (Microtome Service Company, New York, USA). The obtained ultra-thin sections (±60 nm) were cut and placed on copper grids, counterstained with saturated uranyl acetate and aqueous lead citrate. The ultra-thin placental sections were observed in 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 a Digital Micrograph TM (Gatan, Inc., Japan) software.

2.5 | Morphometric measurements and blood vessel quantification

Blood vessel quantification and morphometric measurements, vascular area and haemotrophic diffusion distance (HDD) between the

maternal and foetal subepithelial capillaries and foeto/placental interface were performed from digital images of Masson's trichromic and Gallegos's trichromic using AxioVision 4.6.3 software (Carl Zeiss, Göttingen, Germany). The morphometric analysis was carried out by a single operator on two slides per animal/placenta, two sections per slide and five fields per section.

2.6 | Statistical analysis

Data of HDD were analysed by one-way ANOVA with post hoc comparison of means by Tukey's multiple comparison statistics. When a parametric ANOVA test could not be performed, a non-parametric ANOVA by ranks (Kruskal–Wallis test) was used. The independence of data of vascular area respect to the gestational period was performed using Pearson's Chi-squared test. Data were analysed using InfoStat Version 2009 software (Di Rienzo et al., 2009).

3 | RESULTS

3.1 | Morphometric analysis

In Figures 1a and 2a, are identified placental villi characteristic of a gestation at term with tertiary chorioallatoic projections. In addition in the mesenchyme fetal blood vessels of different calibre and numerous capillaries close to the trophectoderm cells are observed, lining the placental villi. In Figures 1b and 2b, the measurements of vascular area are shown; the blood vessels are clearly observed with Masson and Gallego's trichomic dyes (Figures 1 and 2).

A total of 5291 blood vessel was analysed morphometric, which were evaluated in six intervals (*Int*) agree to measurements of vascular area, as follows: *Int* 1 = (1–100 μm^2) *Int* 2 = (100–500 μm^2), *Int* 3 = (500–2,000 μm^2), *Int* 4 = (2,000–10,000 μm^2), *Int* 5 = (10,000–50,000 μm^2), *Int* 6 = (50,000–300,000 μm^2) (Figure 3). In Figure 3, the quantification of blood vessels (vascular area intervals) with respect to pregnancy period was observed. We determine in the gestational periods a clear decrease in the number of blood vessels as the vascular area increase. In early gestations (Days 30 and 40), intermediates gestations (Days 60 and 70) and at term gestations (Day 114), we have determined a greater number of blood vessel of vascular area-*Int 2* (10 a 500 μm^2); while in advanced gestations (Day 90) predominate of blood vessels of vascular area-*Int 1* (1 a 100 μm^2) (Figure 4). Effect of day of gestation on vascular area was found ($p = 2.2^{e-16}$). Statistical differences were found between the following periods: 30 and 90 ($p = .084$), 40 and 90 ($p = .016$), 60 and 90 ($p = .0003$), 60 and 114 ($p = .038$).

The haemotrophic diffusion distance (HDD) between the maternal and foetal subepithelial capillaries to the placental interface was determined by morphometry (Figure 5a,b). The analysis showed a notable decrease in HDD as progresses gestation in the gilts. We highlight that at early gestations (Days 25) the mean-HDD was of 23 μm between the maternal subepithelial capillaries to de interface, and the mean-HDD was of 22 μm the foetal subepithelial capillaries to the zone of fetomaternal contact intimate. In intermediates gestations (Day 70), the mean-HDD decreases to 9.8 μm on the maternal side and of 6.3 μm on the foetal side. The mean-HDD continues to decline at advanced gestations (Day 90) being of 8.6 μm from maternal subepithelial capillaries to the interface and de 3.2 μm of foetal side. Finally, at term gestation (Day 114), the mean-HDD was of 1.4 μm; however, we highlight that were found zones with a HDD as short as 0.18 μm. In the Figure 6a,b, we observed in early gestations of Day 25 and Day 40, respectively, measurements of HDD in interareolar zones of maternal/foetal interface. We highlight the proximity of subepithelial

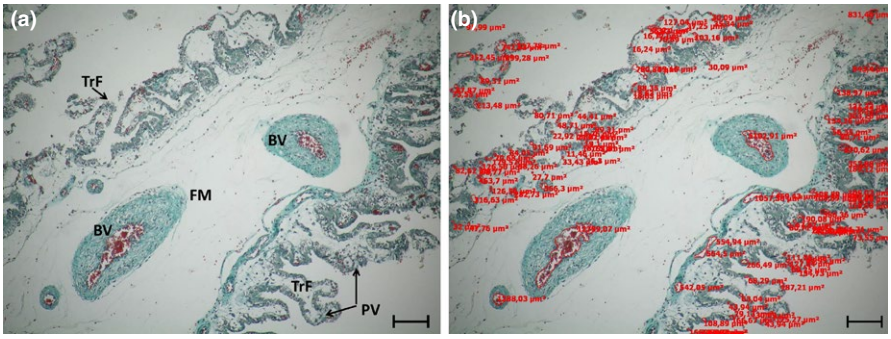

FIGURE 1 Light microscopy of at term porcine placenta stained with Masson's trichomic dyes. In (a) is observed placental villi (PV) characteristic of a gestation of Day 114, blood vessels (BV) of different calibre in the mesenchyme foetal (FM). In (b) the measurements of vascular area were shown. Tr E, trophoblastic epithelium; PV, placental villi. (a,b) scale bar: 100 μm

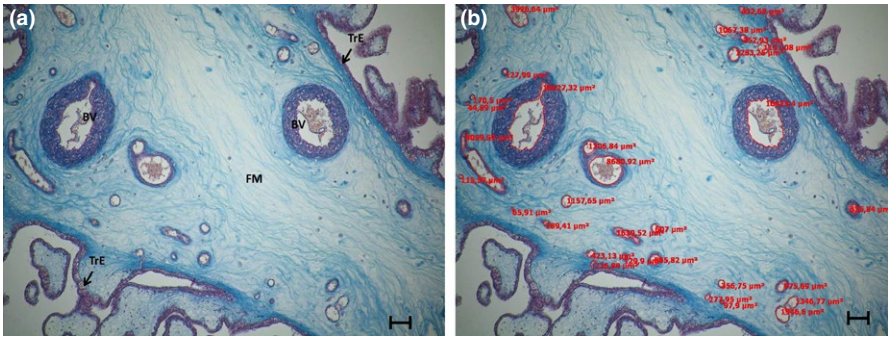

FIGURE 2 Light microscopy of porcine placenta Day 114 of gestation stained with Gallego's trichomic dyes. In (a) are observed numerous blood vessels (BV) very close to trophoblastic epithelium (Tr E) characteristic of a gestation at term and a lax mesenchyme foetal (FM). In (b) the measurements of vascular area are shown. Scale bar: 50 μm

Gestation (days) / Interval (int)	30	40	60	80	90	114	Total
Int 1	387	115	379	86	479	389	1,749
Int 2	647	233	454	252	420	576	2,330
Int 3	221	151	206	122	94	195	867
Int 4	85	42	70	45	13	44	254
Int 5	32	10	14	13	2	13	71
Int 6	4	3	10	5	0	3	20
Total	1,376	554	1,133	523	1,008	1,220	5,291

FIGURE 3 Morphometric analysis of blood vessel, their classification according to six intervals (*Int*) of vascular area at Day 30, 40, 60, 90 and 114 of porcine gestation. *Int* 1 = (1–100 μm^2), *Int* 2 = (100–500 μm^2), *Int* 3 = (500–2,000 μm^2), *Int* 4 = (2,000–10,000 μm^2), *Int* 5 = (10,000–50,000 μm^2) and *Int* 6 = (50,000–300,000 μm^2)

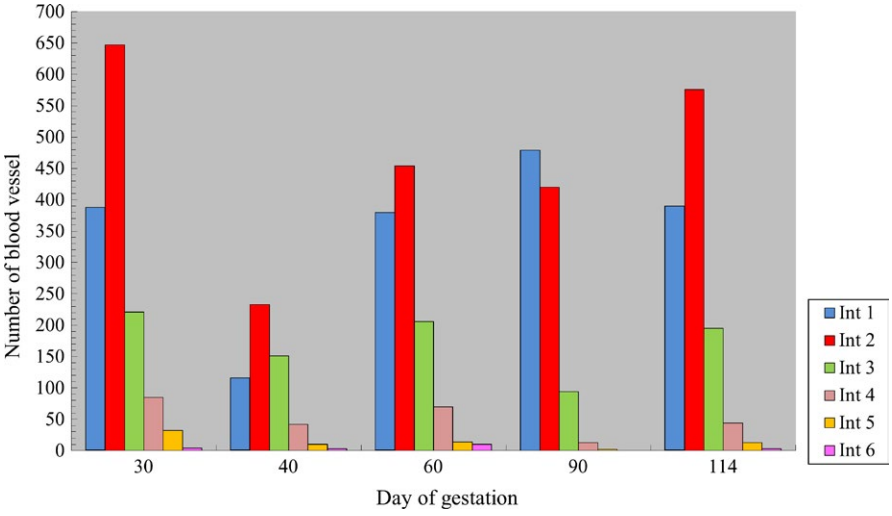

FIGURE 4 The graph shows the behaviour of the blood vessel vascular area according to their classification in intervals (*Int*) with respect to the periods of pregnancy. Effect of gestational period on vascular area was found ($p = 2.2^{e-16}$). 30 and 90 ($p = .084$), 40 and 90 ($p = .016$), 60 and 90 ($p = .0003$), 60 and 114 ($p = .038$)

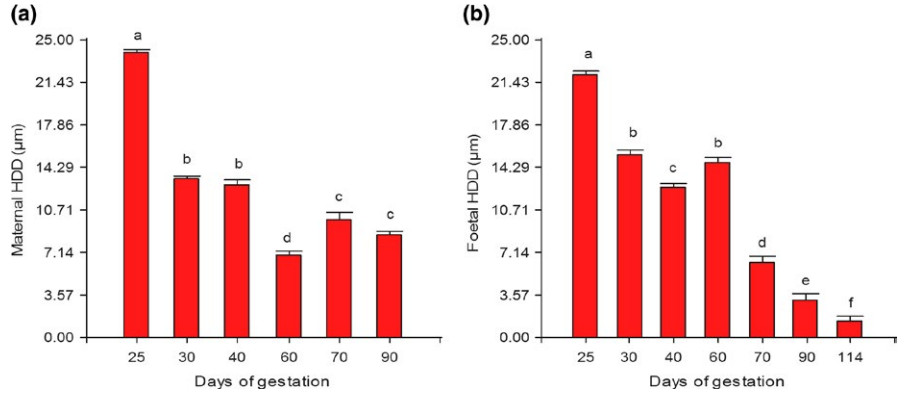

FIGURE 5 Distribution of the haemotrophic diffusion distance in maternal side (a) and in foetal side (b) according to the pregnancy period

capillaries to the fetomaternal haemotrophic exchange zone even in early pregnancy.

3.2 | High-resolution light microscopy (HRLM)

Through HRLM, the placental porcine tissue with greater resolution and definition we observed. The HRLM allowed appreciate in more detail the cellular histoarchitecture. In Figure 7, the cellular morphology of the epithelia to form the fetomaternal interface is observed in the studied periods. During early gestation, we highlight the presence of numerous cytoplasmatic granules of different size in uterine epithelium, while the chorionic epithelium has elongated cells in division (Figure 7a). Addition in the figure highlights the proximity of the subepithelial capillaries to the fetomaternal interface, and the HDD shown

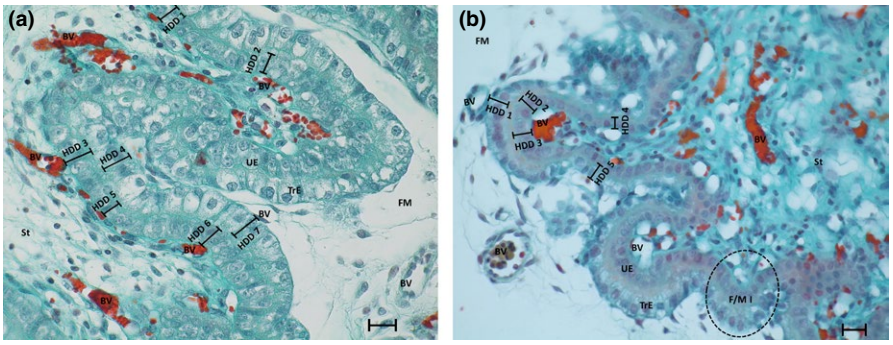

FIGURE 6 Light microscopy of porcine placenta Day 25 of gestation stained with Masson's trichomic dyes (a) and of porcine placenta Day 40 stained with Gallego's trichomic dyes (b). Measurements of haemotrophic diffusion distance (HDD) in interareolaer zones of maternal/foetal interface (F/M I) were observed. *In* (a): HDD 1: 16.48 μm, HDD 2: 18.82 μm, HDD 3: 22.07 μm, HDD 4: 22.46 μm, HDD 5: 14.66 μm, HDD 6: 16.94 μm, HDD 7: 22.53 μm. *In* (b): HDD 1: 16.43 μm, HDD 2: 17.53 μm, HDD 3: 15.93 μm, HDD 4: 9.48 μm, HDD 5: 15.96 μm. In the figure, is highlighting the proximity of subepithelial capillaries to the fetomaternal haemotrophic exchange zone during the early pregnancy. BV, blood vessel; FM, foetal mesenchyme; St, strome; TrE, trophoblastic epithelium; UE, uterine epithelium. Scale bar: 20 μm

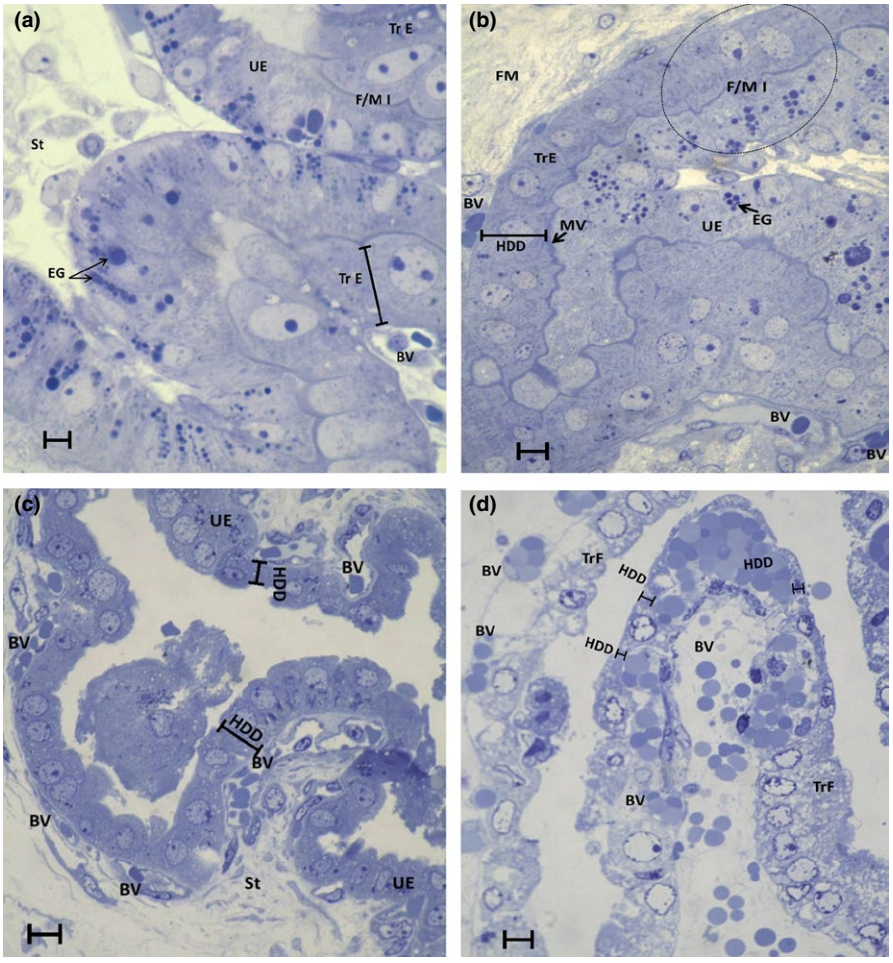

FIGURE 7 Semi-thin sections high-resolution light microscopy (HRLM) of porcine placenta collected during porcine gestation. (a) Day 25; (b) Day 40; (c) Day 60; (d): Day 114. The figures showed the cellular histoarchitecture with more detail and great resolution. It shows the haemotrophic diffusion distance (HDD) in the different periods studied. In (a) HDD 11.4 μm. In (b) HDD 15.94 μm. In (c) HDD 7.42 μm and 3.72 μm. In (d) HDD 2.46 μm, 1.62 μm and 1.93 μm. BV, blood vessel; EG, citoplasmatic electrodense granules; FM, foetal mesenchyme; F/M I, fetomaternal interface; MV, microvilli; St, strome; TrE, trophoblastic epithelial; UE, uterine epithelium. In all panels, scale bar: 5 μm

is 15.94 μm to Day 25 (Figure 7a) and 11.4 μm to Day 40 (Figure 7b). In intermediate gestations (Figure 7c), it shows a uterine epithelium with cubic cells in intimate contact with the maternal blood vessels, the HDD shown in the figure is of 7.42 μm and 3.74 μm.

At term of pregnancy (Figure 7d), we observe in placental villi foetal subepithelial capillaries project between epithelial cells "almost invading" the trophoblastic epithelium, the HDD observed was 2.46–1.62 μm.

The haemotrophic diffusion distance described by HRLM in the analyses gestations periods are consistent with the morphometric measurements performed by light microscopy.

3.3 | Transmission electron microscopy

Through transmission electron microscopy (TEM), we describe the placental cellular and vascular ultrastructure along porcine pregnancy.

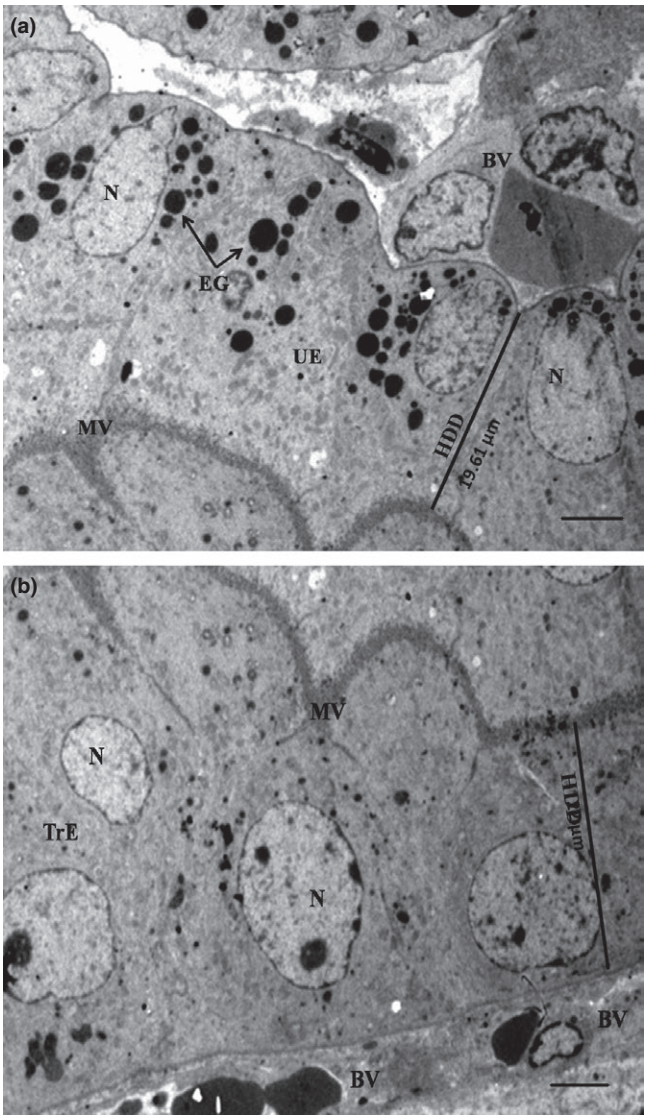

FIGURE 8 Microphotograph of transmission electron microscopy (TEM) of porcine placenta at early gestation. The figures showed the cellular ultrastructure in epitheliochorial fetomaternal interface. In (a) are observed the characteristic uterine epithelium (UE), and in (b) the ultrastructure of trophoblastic epithelium (TrE). Haemotrophic diffusion distance (HDD) determinate in (a) was of 19.61 µm, and the HDD in (b) was of 22 µm. MV, microvilli; BV, blood vessel; N, nucleus; EG, electrodense granules. In all panels, scale bar: 5 µm

At Day 25 (Figure 8a,b), it show the cellular ultramorphology which characterizes to an epitheliochorial fetomaternal interface, highlight the interconnection through the microvillar interdigitation between the uterine and trophectoderm epithelia. The uterine epithelium presents cubic cells with spherical nucleus, small nucleolus, little mitochondria and numerous electrodense body of different size, a HDD of 19.61 µm can also clearly be seen (Figure 8a). Ultrastructurally, the trophectoderm has epithelial cells with rounded nucleus, clear nucleolus and larger mitochondria, and the observed HDD is 22 µm (Figure 8b). As the gestation progresses (Figure 9a,b), a more complex microvillar interdigitation, in addition different size mitochondria, cytoplasmic vacuoles and extensive Golgi cisternae in the epithelia at Day 60, can be seen. In the microphotographs, it showed a HDD of 5.2 µm from maternal subepithelial capillaries at the placental interface (Figure 9a) and a HDD of 5.9 µm in the foetal side (Figure 9b).

At Day 90 and at Day 114, we highlight through TEM a very small HDD, which is characteristic of a term gestation (3.47 and 2.31 µm, respectively) (Figure 10a,b). In the image, endothelial cells very close to trophoblastic cell can be seen; there the blood vessels and the epithelial cells are separated almost exclusively by the basal membrane. As we have previously determinate at term of pregnancy an increase the number of blood vessels of Interval 2 (10 -500 μm^2), which are located very close to fetomaternal interface.

4 | DISCUSSION

Our finding previously indicates that during the gestational development in the gilts, the nutritional demands increase due to continuous growth of the *concepti*. The placenta undergoes physiological remodelling processes necessary to regulate the foetal/maternal exchange (Cristofolini, Merkis et al., 2012; Cristofolini, Sanchis, et al., 2012; Cristofolini et al., 2013; Merkis, Cristofolini, & Koncurat, 2007; Merkis et al., 2005, 2006; Sanchis et al., 2015).

In numerous species, the importance of vascular development for placental function has been extensively studied (Hafez, Borowicz, Reynolds, & Redmer, 2010; Reynolds & Redmer, 2001; Reynolds et al., 2006), because it affects foetal growth, in addition, it is responsible for healthy productivity and sustained over time (Breier et al., 2002; Greenwood et al., 2000; Oken & Gillman, 2003). Thus, the formation of new blood vessels that occurs during porcine placental morphogenesis, through the angiogenic process, is of vital importance (Merkis et al., 2006). Through of the angiogenesis the networks vascular are necessary for a correct exchange between the mother and the fetuses in growth (Demir et al., 2004; Ferrara, Gerber, & Le Couter, 2003; Kaczmarek, Kiewisz, Schams, & Ziecik, 2009).

In the present study, we found in placental tissues of cross-bred gilts that the vascular area depends on the gestational period. In early gestations, intermediate gestations and at term gestations, blood vessels between 100 and 500 μm^2 were found predominantly, whereas in advanced gestations, numerous blood vessels of smaller area, between 1 and 100 μm^2 were found. The present analysis showed an inverse relationship between the size and quantity of placental blood vessels corresponding to each interval, coinciding with other vascular morphometric studies our group performed in gilts (Merkis et al., 2006). In other studies of our research group, we have determined in goat placentas by histomorphometric analysis a significant increase in the capillary number density in the cotyledonary tissue at day 100 of gestation (advanced gestation), whereas no significant differences in the average capillary perimeter (Diaz et al., 2015).

The neovascularization observed in advanced gestations would be related to that in the pregnant female the placental growth achieves its maximum between Days 60 and 70 (Soraci, 2012), since Day 90 does not increase the placental size (Biensen, Wilson, & Ford, 1998, 1999; Merkis et al., 2005), the foetuses develop exponentially from

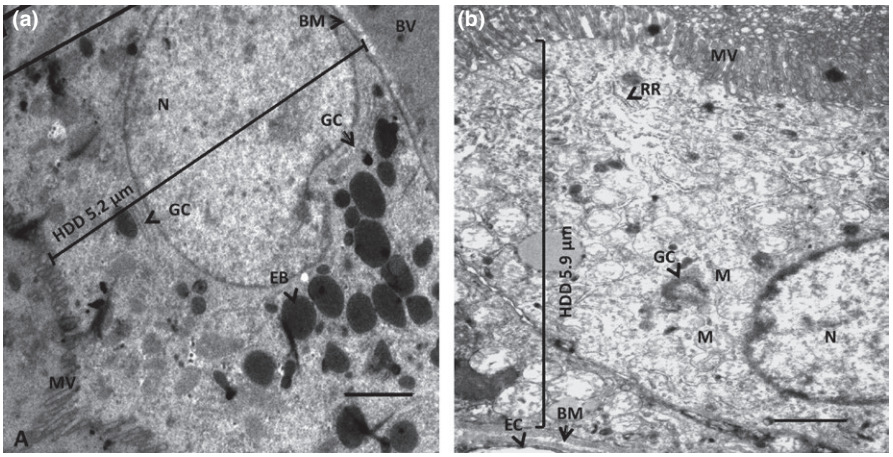

FIGURE 9 Microphotograph of transmission electron microscopy (TEM) of porcine placenta at intermediates gestations. (a) Day 60 and (b) Day 70. In the figures, is observed the interconnection through microvillar interdigitation (MV) in fetomaternal interface and the ultrastructurals characteristic of placenta at intermediates pregnancy. BM, basal membrane; BV, blood vessel; EB, electrodense bodies; EC, endothelial cell; GC, Golgi cisternae; M, mitochondria; N, nucleus; RR, rough endoplasmic reticulum. Haemotrophic diffusion distance (HDD) determinate in (a) was of 5.2 µm, and the HDD in (b) was of 5.9 µm. Scale bar: 1 µm

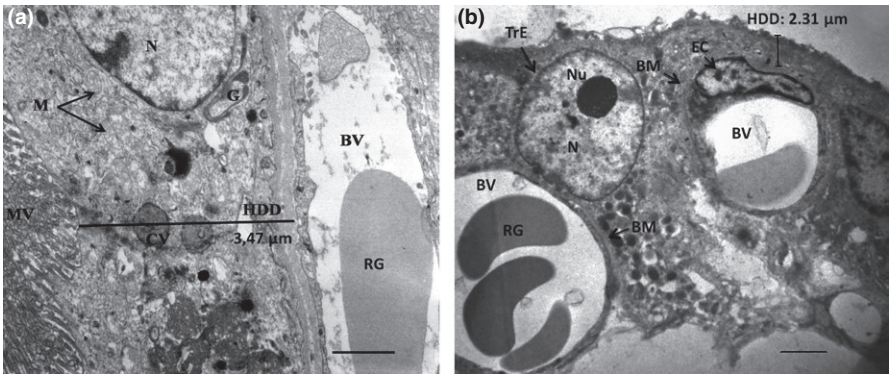

FIGURE 10 Microphotograph of transmission electron microscopy (TEM) of porcine placenta of Day 90 of gestation (a) and at term gestation (b). It observed a very small haemotrophic diffusion distance (HDD) (In (a) HDD: 3.47 µm and in (b) HDD: 2.31 µm) and endothelial cells (EC) very close to trophoblastic epithelium (TrE) can be seen. CV, cytoplasmic vesicle; BM, basal membrane; BV, blood vessel; G, Golgi; M, mitochondria; MV, microvilli; N, nucleus; Nu, nucleolus; RG, red globule. (a) scale bar: 1 µm. (b) scale bar: 3 µm

this gestational periods. Merkis et al. (2005) postulated that from Day 90 and to end of pregnancy, there is a development of the epithelial portion of the placental villi. Those data and the finding the neovascularization to present study indicate that these events would allow an increase in exchange surface and of maternal/foetal blood flow, factors necessary to sustain the foetal great metabolic demand from this gestational period (Burton & Fowden, 2012).

Moreover, Sanchis et al. (2012) report that in gestation of Day 90, the maximum value to programmed cell death by apoptosis observed in porcine placenta become more evident in the placental villi and in maternal blood vessels, favouring cellular remodelling in this zone of intimate contact maternal/foetal and allowing the generation of new blood vessels.

Through the morphometric study, we have determined that the haemotrophic diffusion distance (HDD) decreases markedly as the gestational period progresses in the cross-bred swine. Atkinson, Boyd, and Sibley (2006) describe that nutrient transfer through the placenta can occur through simple diffusion, facilitated diffusion, active transport and receptor-mediated endocytosis. Burton and Fowden (2012) postulated that during placental normal development the simple diffusion rate will be inversely proportional to thickness interhemal barrier separating the maternal and foetal components. Thus, the great decrease in the HDD as the pregnancy progresses that we report in this work, together with the expansion of the contact surface in advanced gestations, allows near maternal and foetal blood currents, optimizing haemotrophic transport, as the nutritional requirements of the embryos/foetuses increase, allowing optimal placental development (Burton & Fowden, 2012; Friess, Sinowatz, Skolek-Winnisch, & Trautner, 1980; Santos, Oliveira, Papa, & Dantzer, 2014).

Friess et al. (1980) postulated that the exchange of nutrients transported by blood occurs in the base of placental villi. Ours reported the presence of high columnar trophoblastic and uterine cells located at the base of placental villi in interareolar zone. These cellular morphological characteristics allow the exchange of nutrients.

On the other hand, gaseous exchange would occur on sides and top of the chorionic ridges (Friess et al., 1980) where Robert, Green,

and Schulz (2016) postulated that at day 110 of pregnancy maternal capillaries project between uterine epithelial cells, bringing the maternal and foetal capillaries within 3–5 μm. Our morphometric study was based on the measurement of HDD, distance between the subepithelial capillaries to the fetomaternal interface, and we highlight the finding of distance as short as 0.18 μm in placentas at term.

During the angiogenic process, by the demand of oxygen of embryos/foetuses in the tissues, a state of hypoxia is generated, which causes an increase in vascular endothelial growth factor (VEGF) (Cristofolini et al., 2008; Hausman & Richardson, 2004; Lee, Lee, Yang, & Park, 2017; Merkis et al., 2006; Reynolds & Redmer, 2001; Vonnahme et al., 2001; Wong, Navarro, Contreras, Fernández Britto, & Llombart, 2000).

In previous studies, we investigated the expression profiles of the angiogenic factors, VEGF and PlGF and their Flt-1 and Flk-1 in porcine placenta. We demonstrated the localization of both VEGF and PlGF during several gestational periods and localized Flt-1 and Flk-1 receptors at Day 30 and Day 114 of pregnancy. We determined during advanced gestations that angiogenesis is due to angiogenic molecules other than members of VEGF family (Sanchis et al., 2015). In the present report, the formation of new blood vessels at Day 90 would occur through angiogenic pathways stimulated by other members of VEGF family.

In other studies performed in advanced gestations of goats, we have found that the immunolocalization of VEGF in trophoblast near small capillaries may indicate the role of VEGF in the vascular development in the goat placenta (Diaz et al., 2015).

Given the type of placentation gilts, non-invasive epithelial, the new blood vessels generation and of haemotrophic diffusion distance reduction, both determined in this report during porcine gestation, assure the maternal/foetal haemotrophic exchange efficiency. This exchange is necessary to cover the increasing physiological demands of foetuses in continuous development. The swine is specie of high productive value characteristic of our geographical livestock activity, and the relevance of our findings is based on the deepening of knowledge about the process of neovascularization throughout the porcine pregnancy.

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CONFLICT OF INTEREST

None of the authors have any conflict of interest to declare.

AUTHOR CONTRIBUTIONS

Cristofolini A involved in acquisition of data, analysed and interpreted the data, drafted the article, revised it critically for important intellectual content and approved the final version to be submitted. Fiorimanti, M involved in acquisition of data, analysed and interpreted the data, drafted the article, revised it critically for important intellectual content and approved the final version to be submitted. Campos, M involved in acquisition of data, analysed and interpreted the data, revised the article critically for important intellectual content and approved the final version to be submitted. Sanchis, E involved in acquisition of data, analysed and interpreted the data, revised the article critically for important intellectual content and approved the final version to be submitted. Diaz, T involved in acquisition of data, analysed and interpreted the data, revised the article critically for important intellectual content and approved the final version to be submitted. Moschetti, E involved in acquisition of data, analysed and interpreted the data, revised the article critically for important intellectual content and approved the final version to be submitted. Merkis, C contributed to the conception and design of the study, participated in acquisition of data, analysed and interpreted the data, revised the article critically for important intellectual content and approved the final version to be submitted. Río Cuarto, Argentina; Area of Electron Microscopy, School of Agronomy and Veterinary, National University of Río Cuarto and National Scientific and Technical Research Council (CONICET); Morphometric study of the porcine placental vascularization; A. Cristofolini, M. Fiorimanti, M. Campos, E. Sanchis, T. Diaz, E. Moschetti and C. Merkis.

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