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Endothelial cells and angiogenesis in the horse in health and disease—A review

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Abstract

The cardiovascular system is the first functional organ in the embryo, and its blood vessels form a widespread conductive network within the organism. Blood vessels develop de novo, by the differentiation of endothelial progenitor cells (vasculogenesis) or by angiogenesis, which is the formation of new blood vessels from existing ones. This review presents an overview of the current knowledge on physiological and pathological angiogenesis in the horse including studies on equine endothelial cells. Principal study fields in equine angiogenesis research were identified: equine endothelial progenitor cells; equine endothelial cells and angiogenesis (heterogeneity, markers and assessment); endothelial regulatory molecules in equine angiogenesis; angiogenesis research in equine reproduction (ovary, uterus, placenta and conceptus, testis); angiogenesis research in pathological conditions (tumours, ocular pathologies, equine wound healing, musculoskeletal system and laminitis). The review also includes a table that summarizes *in vitro* studies on equine endothelial cells, either describing the isolation procedure or using previously isolated endothelial cells. A particular challenge of the review was that results published are fragmentary and sometimes even contradictory, raising more questions than they answer. In conclusion, angiogenesis is a major factor in several diseases frequently occurring in horses, but relatively few studies focus on angiogenesis in the horse. The challenge for the future is therefore to continue exploring new therapeutic angiogenesis strategies for horses to fill in the missing pieces of the puzzle.

Abbreviations: Ac-LDL, Acetylated low-density lipoprotein; aMSC, Adipose tissue-derived mesenchymal stem cells; Ang, Angiopoietin(s); BDNF, Brain-derived neurotrophic factor; CD31, Cluster of differentiation 31; CGRP, Calcitonin gene-related peptide; CL, Corpus luteum, Corpora lutea; COX, Cyclooxygenase; DiI-Ac-LDL, DiI-acetylated Low-Density Lipoproteins; ECFC, Endothelial colony forming cells; ET-1, -2, -3, Endothelin-1, -2, -3; FGF-2, Fibroblast growth factor 2 (synonym bFGF, HBGF-2); HIF-1α, Hypoxia-inducible factor 1α; ICAM-1, Intercellular adhesion molecule 1; IGF, Insulin-like growth factor; IL, Interleukin; LPS, Lipopolysaccharide; MAPKs, Mitogen-activated protein kinases; MFGE8, Milk-fat globule epidermal growth factor 8 (synonym lactadherin); MMP, Matrix metalloproteinase; MSC, Mesenchymal stem cells; NO, Nitric oxide; NOS, Nitric oxide synthase; NSAIDs, Non-steroidal anti-inflammatory drugs; Ntrk2, Neurotrophic receptor tyrosine kinase 2; pDNA, Plasmid DNA; PECAM1, Platelet endothelial cell adhesion molecule 1 (synonym CD31); PEDF, Pigment epithelium-derived factor; PGC-1α, Peroxisome proliferator-activated receptor-γ coactivator 1α; PlGF, Placenta growth factor; PRP, Platelet-rich plasma; ROS, Reactive oxygen species; SPARC, Secreted protein acidic and cysteine-rich; TGF, Transforming growth factor; THBS2, Thrombospondin II; Tie1, 2, Tyrosin kinase-receptor 1, 2; TNF, Tumour necrosis factor; VE-cadherin, Vascular endothelial cadherin (synonym Cadherin-5); VEGF, Vascular endothelial growth factor; VEGFR-1, Vascular endothelial growth factor receptor-1 (synonym flt-1); VEGFR-2, Vascular endothelial growth factor receptor-2 (synonym KDR, flk-1); VEGFR-3, Vascular endothelial growth factor receptor-3; vWF, Von Willebrand factor.

KEYWORDS
angiogenesis, endothelium, vascular, horses, neovascularization, pathologic, neovascularization, physiologic

1 | INTRODUCTION

Blood vessels transport vital oxygen and nutrients through the body, remove metabolic waste products and facilitate communication between tissues and organs via the blood stream. Development, maintenance and remodelling of the circulatory system in health and disease require a plethora of regulatory mechanisms. The growth of blood vessels, for example in response to tissue hypoxia, is in general often referred to as angiogenesis. In sensu stricto, angiogenesis is defined as the formation of new vessels from pre-existing ones. The term "neovascularization" is used for different types of vessel formation, that is, vasculogenesis, angiogenesis as well as arterio- and venogenesis. Here, the prefix "neo" (from ancient Greek νέος néos "new," "fresh," "young") emphasizes the new development of blood vessels. The term "neoangiogenesis" is often applied to pathological angiogenesis in tumours and other diseases (Chopra, Hung, Kwong, Zhang, & Pow, 2018; Ribatti, Vacca, Nico, Roncali, & Dammacco, 2001; Sharma, Sharma, & Sarkar, 2005; Simons, 2005).

The cardiovascular system is the first functional organ in the embryo, and its blood vessels form a widespread conductive network within the organism. Blood vessels develop de novo (Figure 1a) in the embryo but also in the postnatal period and the adult through differentiation from mesoderm-derived endothelial precursor cells. This process is termed vasculogenesis or de novo vascularization and, depending on the location and size of the vessel, results in a multicellular tube, comprising endothelial cells, smooth muscle cells, pericytes (or mural cells) and basement membrane components (Emanueli, Schratzberger, Kirchmair, & Madeddu, 2003; Potente & Carmeliet, 2017; Risau, 1997; Tang & Conti, 2004).

Angiogenesis expands existing vessel networks via sprouting or splitting (Figure 1b). Sprouting angiogenesis *in vivo* takes place in several successive stages or steps, the angiogenic cascade. Briefly, angiogenic stimuli cause increased endothelial cell permeability, invasiveness and proliferation; basement membrane components are proteolysed to facilitate invasion of endothelial cells into the stroma of the neighbouring tissue; lumen formation occurs, as the sprout forms a multicellular structure and meets up with an adjacent sprout; the new capillary is stabilized with a basement membrane and pericytes are recruited (Potente & Carmeliet, 2017; Yoo & Kwon, 2013). The angiogenic cascade of endothelial cells *in vitro* has been classified and quantified for several species, including the horse (Dietze et al., 2014). In splitting or intussusceptive angiogenesis, a single vessel is split in two. This type of angiogenesis requires reorganization of resident endothelial cells and is not based on immediate proliferation or migration of the endothelium (Adair & Montani, 2010; De Spiegelaere et al., 2012).

Arterio- and venogenesis are the result of modification and expansion of existing arteries and veins (Nagy & Dvorak, 2012). Interestingly, little information is available on venogenesis and the transformation of collateral vessels (collateralization, the development of an alternate circulation around a blocked artery or vein) after thrombosis, although many diseases such as thrombosis are associated with the venous system (Palmer, Braybrooks, Cao, Diaz, & Greve, 2019).

The cells that line the lumen of blood vessels—the endothelial cells—are a highly heterogeneous population. While endothelial cells are usually quiescent as "phalanx" cells throughout adult life, they can quickly become highly active migratory "tip" cells or proliferative "stalk" cells and sprout into avascular tissue to form new blood vessels (Zecchin, Kalucka, Dubois, & Carmeliet, 2017). Since the change from quiescence to growth during angiogenesis is metabolically demanding, not only the coordination of the morphogenetic behaviour, but also an adjustment of the metabolic activities is necessary (Potente & Carmeliet, 2017).

These processes must be tightly regulated, and dysregulated angiogenesis as well as endothelial dysfunction has severe consequences for the system. Endothelial dysfunction leads to a decrease in the bioavailability of vasodilators, in particular nitric oxide (NO),

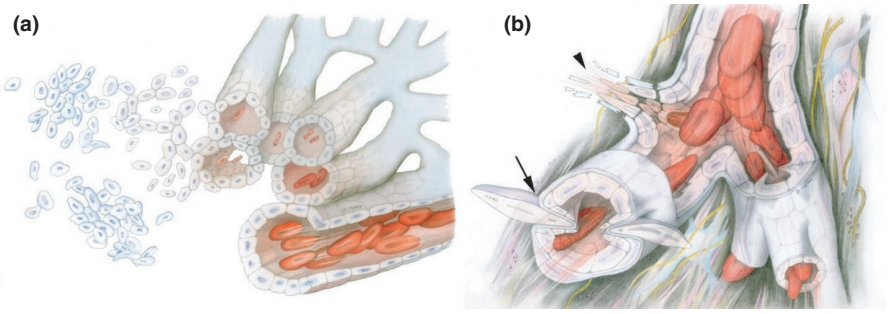

FIGURE 1 Vasculogenesis and angiogenesis. (a) Vasculogenesis is the differentiation of endothelial progenitor cells and their formation into a simple blood capillary network; (b) Angiogenesis is the formation of new vessels from existing ones. There are two mechanisms of angiogenesis: sprouting (arrowhead) and intussusception (arrow). Published in: Kassmeyer, Plendl, Custodis, and Bahramsoltani (2009). New insights in vascular development: vasculogenesis and endothelial progenitor cells. Anat Histol Embryol 38(1): 1–11

and/ or to an increase in contraction factors derived from the endothelium, which leads to an impairment of endothelium-dependent vasodilation. Another aspect of endothelial dysfunction can be endothelial activation, which is characterized by an inflammatory, proliferative and procoagulant state favouring atherogenesis and the induction of aberrant angiogenesis (Hadi, Carr, & Al Suwaidi, 2005; Rajashekhar et al., 2006). Numerous forms of cardiovascular disease are associated with endothelial dysfunction. Free radicals can disrupt the NO balance, damage the endothelium and make it excessively permeable so that toxins can get into the body tissues (Rajendran et al., 2013). Dysregulated angiogenesis contributes to impaired wound healing, ocular pathologies, problems of the musculoskeletal system, laminitis and many forms of cancer. Angiogenesis and its inhibition, anti-angiogenesis, are principal factors in these diseases that frequently occur in horses. Taken into account that the regulation of angiogenesis in horses is, at least to some extent, horse-specific (Morgan et al., 2018), it is crucial to understand more about angiogenesis in equidae.

In order to increase our knowledge on equine angiogenesis, and to work towards new dependable therapeutic pro- and anti-angiogenic strategies for horses, we undertook this review. It provides an overview on the current knowledge on equine angiogenesis and endothelial cells in both physiological and pathological conditions. Compared to the vast amount of research in many other species, relatively few studies focus on equine angiogenesis and only a small number of research groups worldwide focuses on species-specific research of equine endothelial cells, the most important cells in angiogenesis.

To identify topics in equine angiogenesis research for this review, the software VOSviewer was used to produce a graphical abstract (Figure 2). Therefore, in May 2019, "web of science" was searched for the terms "angiogen*" AND "horse OR equine OR mare OR stallion" in "topic". No search limitations for the range of years were applied. Web of science was chosen due to the availability of author key words in the metadata, from which the graphical abstract was compiled. Out of the resulting 260 publications, approximately 50% were used for the graphical abstract and included research on/ for the horse, research with cells from the horse, reviews in which the term "horse" was found or at least studies referring to the horse. The other publications were not included in this manuscript, because they were only peripherally related to the horse, meaning that in the broadest sense, materials or viruses relating to the horse were used (e.g. equine chorionic gonadotropin or equine infectious anaemia virus). Publications that had nothing to do with horses but included the term "horse" in words like horse chestnut, horse mint, Trojan horse or horse-radish peroxidase, were excluded, too. The remaining 139 publications were used to create the graphical abstract with the author key words. The graphic was created with VOSviewer

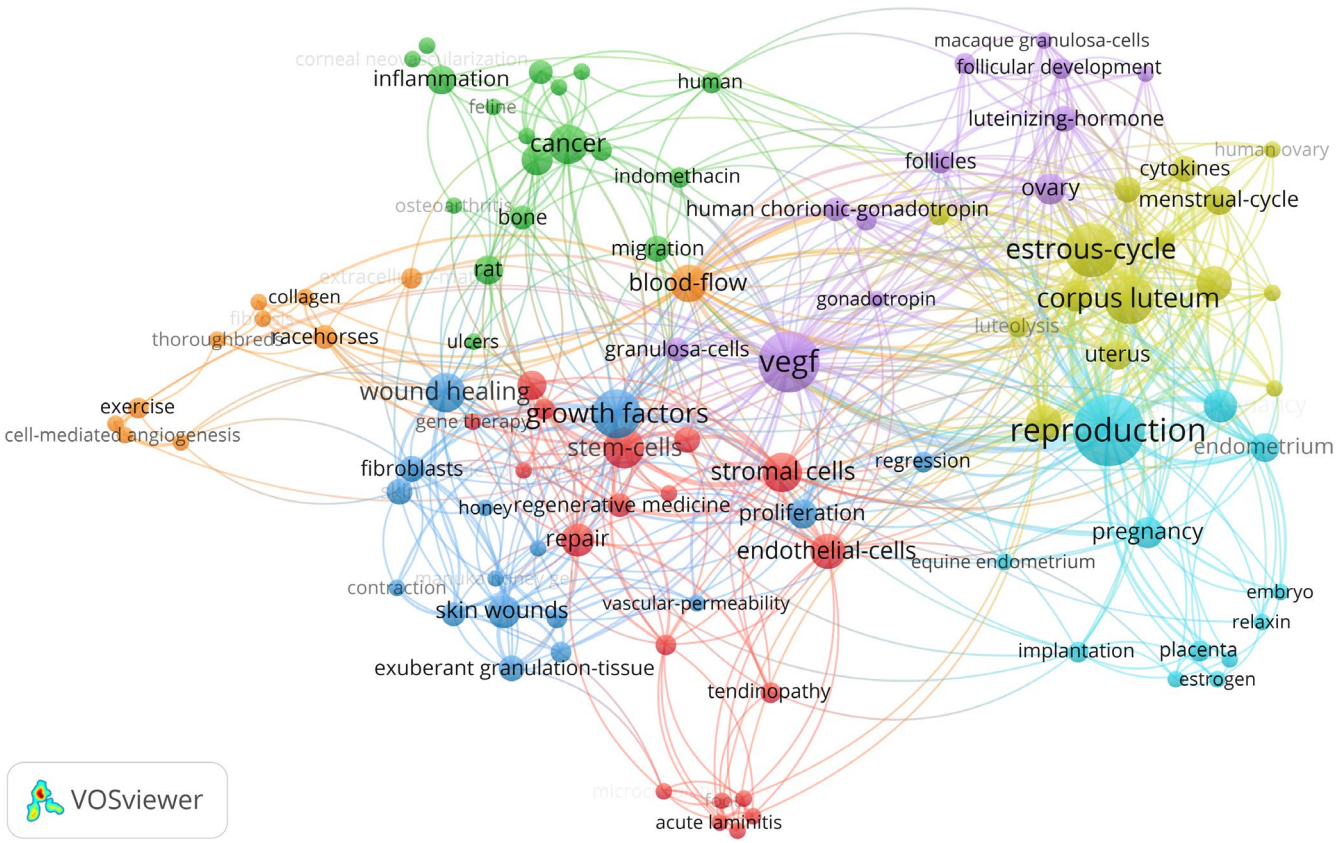

FIGURE 2 Principal study fields in equine research associated with angiogenesis. The graphic shows an author key word analysis for literature sources referring to angiogenesis in horses. A full version of this figure can be opened with the freely available VOSviewer software (Centre for Science and Technology Studies, Leiden University, Leiden, The Netherlands), using the supplementary files

software version 1.6.10 (Centre for Science and Technology Studies, Leiden University, Leiden, The Netherlands; http://www.vosviewer.com; Copyright (c) 2009–2019 Nees Jan van Eck and Ludo Waltman) according to instructions of the supplier. A thesaurus file was used to delete general terms (like animals or tissue), merge matching terms (like tumour and cancer) and delete terms plausibly present but not necessary (like horse or angiogenesis). From the plethora of principal study fields identified in equine angiogenesis research (Figure 2), we picked the ones presented in this review. We had to skip several topics and were only able to briefly address a few others. We encourage the interested reader to take a closer look at Figure 2, using the Data S1 and S2, if other topics of angiogenesis research in the horse are of interest. Other databases such as Medline or PubMed were additionally used for the actual review and the search terms were adapted to the respective topics.

2 | EQUINE ENDOTHELIAL PROGENITOR CELLS

Until the 1990s, it was assumed in mammals that new blood vessels were mainly formed prenatally by vasculogenesis of endothelial progenitor cells and that new vessels only grew out of existing vessels postnatally, via angiogenesis. Today, there is a consensus that endothelial progenitor cells play a significant role not only prenatally but also in the adult in the formation of blood vessels, both in the course of regenerative and pathological processes such as tumour growth (Shi et al., 2017; Wang et al., 2010). Especially, in regenerative medicine, endothelial progenitor cells have gained great importance due to their ability to regenerate and revasculate tissues, for example ischaemic tissues, as well as due to their potential for the vascularization of engineered tissues, for example reviewed by Chong, Ng, and Chan (2016); Hristov, Erl, and Weber (2003); Peters (2018).

In recent years, it has become increasingly clear that the effectiveness of cell-based tissue regeneration and tissue engineering therapy approaches depends on the vascularization of these newly formed tissues. Without the establishment of a new vascular bed, their regeneration potential remains poor, because of the lack of nutrients and oxygen (Tasev, Koolwijk, & van Hinsbergh, 2016). Consequently, endothelial progenitors are used for the therapy of equine diseases associated with ischaemia or to enhance vascularization within tissue-engineered models or cell-based therapeutic concepts (Koch, Berg, & Betts, 2009; Winter et al., 2018).

Within veterinary medicine, equine medicine is at the forefront when it comes to innovative cell-based therapies to regenerate or replace damaged tissues. Beginning around the year 2000, stem cell-based therapies for horses have been used for diseases of the musculoskeletal system affecting tendons, ligaments, articular cartilage and bone (Barboni et al., 2018). Equine mesenchymal stem cells (MSC), derived from bone marrow or fatty tissue, are mainly used for this purpose (Arnhold, Absenger, Klein, Addicks, & Schraermeyer, 2007; Arnhold, Elashry, Klymiuk, & Wenisch, 2019). MSC are ideal for tissue engineering and cell therapy for the musculoskeletal structures because they can differentiate into different mesenchymal cell lines like for example chondrocytes or osteocytes and are easy to obtain (Bara, McCarthy, Humphrey, Johnson, & Roberts, 2014; Park et al., 2018).

Recently, endothelial colony forming cells (ECFC) have been characterized and described in the equine medical literature. ECFC are endothelial progenitor cells with robust proliferative and clonal abilities; therefore, they are also called outgrowth endothelial cells or "late" endothelial progenitor cells. These cells are useful candidates in the initiation and facilitation of neovascularization (Banno & Yoder, 2018). In adult horses, they can be used therapeutically for diseases associated with ischaemia or delayed vascularization (Salter et al., 2015; Seeto et al., 2017; Sharpe et al., 2016; Winter et al., 2018). While in human medicine, ECFC are isolated primarily from peripheral blood or from umbilical cord blood (Tasev et al., 2016), those used in equine medicine are usually isolated from the peripheral blood of horses that was collected from the jugular or the cephalic veins (Salter et al., 2015; Sharpe et al., 2016).

Research and application of ECFC in equine medicine are still in its early stages and the majority is basic and describes the isolation and characterization of these cells. Recently, Seeto et al. (2017) studied the application of autologous ECFCs in equine wound healing. For this purpose, the cells were encapsulated into microspheres made from polyethylene glycol-fibrinogen hydrogel and injected locally into deep skin wounds of the horses distal limb, affecting all skin layers. First results showed that the injected ECFCs proliferated and migrated into the injured tissue. In addition, the ECFCs maintained their viability, proliferation and phenotype within the injured tissue (Seeto et al., 2017). In a follow-up study, the procedure was found to be well-tolerated and deemed practical for clinical application. The treatment had positive effects on blood vessel density and wound inflammation (Winter et al., 2020).

3 | EQUINE ENDOTHELIAL CELLS AND ANGIOGENESIS (HETEROGENEITY, MARKERS AND ASSESSMENT)

In order to analyse angiogenesis *in vivo* or *in vitro*, it is essential to specifically identify the cells that perform angiogenesis, that is, endothelial cells. However, there is no marker for all endothelial cells because, by nature, these cells are not a homogenous population but rather a heterogenous one. Endothelial cells form a single layer that lines the interior surfaces of all blood vessels and the heart. Endothelial cells are not a homogenous "wallpaper" of the circulatory system, but rather a highly heterogeneous cell population with distinct functions in different vessels and organs. Depending on the organ, capillary endothelium comes in continuous, fenestrated or discontinuous form. In addition, endothelial heterogeneity is linked to gene and antigen expression resulting in differences in morphology and function. Beyond that, endothelial cells vary between different species, sexes, developmental stages and organs and even

neighbouring endothelial cells of the same organ and blood vessel may be different in antigen expression (Aird, 2007a, 2007b, 2012). For identification of the endothelial nature of cells, several different markers are available for the horse.

Amongst the first markers published for equine endothelial cells *in situ* were lectins, that is proteins or glycoproteins recognizing specific carbohydrates. Alroy, Goyal, and Skutelsky (1987) found endothelial cells in tissue specimens of myocardium, uterus and adipose tissue from horses to be positive for the lectins *Bandeiraea simplicifolia* agglutinin I, *Ricinus communis* agglutinin I, soybean agglutinin and wheat germ agglutinin. Moreover, the lectins *Evonymus europaeus* agglutinin and *Cytisus sessilifolius* agglutinin react with equine heart endothelium (Roussel & Dalion, 1988). In both aforementioned studies (Alroy et al., 1987; Roussel & Dalion, 1988) *Ulex europaeus* agglutinin I was reported to be negative for equine endothelium. Contrary to this, Johnson, Miller, Floss, and Turk (1996) used positive labelling by *Ulex europeus* I to confirm the endothelial origin of vascular structures in benign vascular neoplasms (haemangiomas) of young horses.

For horses, as in many other species, immunohistochemical staining of the coagulation co-factor von Willebrand factor (vWF) (Figure 3) has been found to be a useful marker of endothelial cells (Bochsler, Slauson, Chandler, & Suyemoto, 1989; Bosch, Moleman, Barneveld, van Weeren, & van Schie, 2011; Chiam et al., 2006; Dietze et al., 2014; Johnson et al., 1996; MacEachern, Smith, & Nolan, 1997; Rieger et al., 2018; Rosenberg, Greengard, & Montgomery, 2000). The quantitative expression of this marker seems to vary in the horse. Benazzi, Torre, Sarli, and Marcheselli (2000), who used vWF to identify endothelial cells of blood vessels in the equine fetlock annular ligament, found strong capillary immunostaining, especially in cases with chronic inflammation.

Another *in vitro* marker used for endothelial cells in different species including the horse is acetylated low-density lipoprotein (Ac-LDL). Caution has to be taken in interpreting results as Ac-LDL is not only internalized and metabolized by endothelial but also by other cells like macrophages (Li, Hu, Jorgenson, & Slayton, 2009). Using DiI-Ac-LDL, equine endothelial cells have been labelled for example by Bochsler et al. (1989); Laval, Favoreel, Poelaert, Van Cleemput, and Nauwynck (2015); Wunn, Wardrop, Meyers, Kramer, and Ragle (1999).

An additional endothelial marker that works very well is CD31 (Platelet endothelial cell adhesion molecule 1, PECAM1). Equine PECAM1 was cloned and sequenced by Gregg and Schenkel (2008), who describe it to be highly conserved amongst several species resulting in a broad species cross-reactivity for the marker. This molecule, a transmembrane receptor that is expressed by endothelial and other cells, is involved in angiogenesis and was found to be up-regulated in samples of pregnant mare's endometrium (Merkl et al., 2010). PECAM1 also labels equine endothelium within granulation tissue during wound repair, where a high density of blood vessels was documented using immunostaining of this antigen (Miragliotta, Ipina, Lefebvre-Lavoie, Lussier, & Theoret, 2008; Wise et al., 2018).

In angiogenesis, capillary endothelial cells have the capability to make the first steps in angiogenesis, that is degradation of the basal membrane by matrix metalloproteinases (MMPs) and subsequent migration (Rundhaug, 2005). Accordingly, Ellenberger, Muller, Schoon, Wilsher, and Allen (2009) found heterogeneity in immunostaining for matrix metalloproteinase-2 in the equine ovary: only endothelial cells of capillaries but not of other vessel types were positive for this enzyme that degrades components of the extracellular matrix allowing endothelial cells to migrate into the surrounding tissue.

Another striking example of endothelial heterogeneity is their morphological and functional heterogeneity observed during the angiogenesis cascade, where endothelial tip and stalk cells can be distinguished. The tip cells lead the new sprout, stalk cells migrate and proliferate behind them and build up the new sprout (Aird, 2012). Tip and stalk cells (Figure 4) were documented in equine endothelial cell cultures by Dietze et al. (2014) and Rieger et al. (2018). Tip cells are non-proliferative, display numerous filopodia (Gerhardt et al., 2003) and express CD 34 (Siemerink et al., 2012).

The assessment of proliferation, for example in stalk cells, is possible with Ki67, a marker of proliferating cells. It has been performed successfully in equine endothelial cells by several groups (Al-zi'abil, Watson, & Fraser, 2003; Martano et al., 2018; Watson &

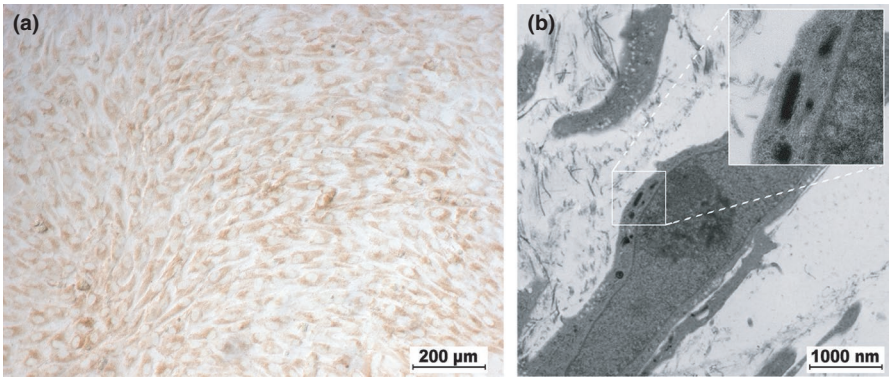

FIGURE 3 Von Willebrand factor and Weibel–Palade bodies in cultured equine arterial endothelial cells. (a) Immunohistochemical staining of von Willebrand factor (b) Transmission electron microscopic image of endothelial cells with Weibel–Palade bodies, the storage granules for the von Willebrand factor. Images are unpublished results from Rieger et al. (2018). Detailed methods of immunolabelling and electron microscopy are published in Dietze et al. (2014)

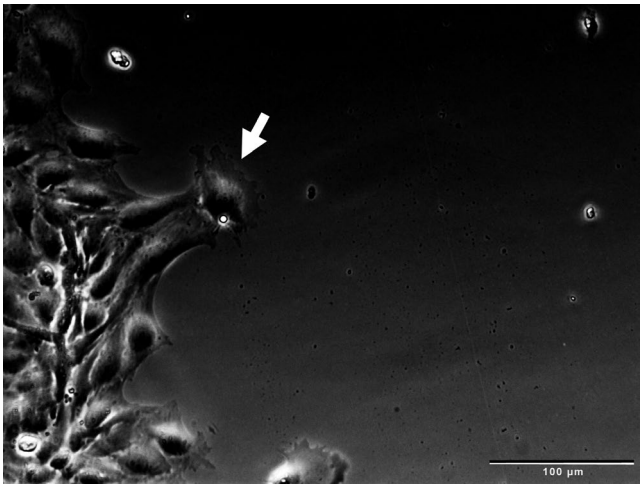

FIGURE 4 Tip cell (arrow) followed by stalk cells. Cultured equine endothelial cells in a scratch assay. Migration fringe of endothelial cells into denuded area to the right. The image is an unpublished result from Rieger et al. (2018)

Ai-zi'abi, 2002). Moreover, Histone-3 has been shown to label equine endothelium at certain stages of mitosis (Hendzel et al., 1997).

The opposite of mitosis and cell proliferation, that is, programmed cell death (apoptosis), is also important in angiogenesis. The complex development of a vascular network from a primitive endothelial sprout involves apoptotic death of endothelial cells during lumen formation and vascular remodelling (Lienau et al., 2005; Watson, Grant, & Coultas, 2017). Analysis of equine endothelial apoptosis can be successfully done via TUNEL test detecting DNA fragmentation (Al-zi'abi, Fraser, & Watson, 2002) or via activated caspase-3, another marker for apoptosis (Aguilar et al., 2006).

It can be noted that there is no "marker of angiogenesis" but only markers of vascular endothelial cells. The search for markers of angiogenesis implies the identification of angiogenic and non-angiogenic endothelial cells. New technologies, including proteomics, are promising tools; however, so far, there is still a lack of reliable markers for the identification of angiogenic endothelial cells that perform all stages of the angiogenic cascade (Bahramsoltani, Harms, Drewes, & Plendl, 2013; Nowak-Sliwinska et al., 2018). For example, Matsuda, Hagio, and Ishiwata (2013) found that nestin, an intermediate filament protein, is an angiogenesis and tumour angiogenesis marker, but, the authors also state that, "nestin is expressed at the early stages of normal development, under pathological conditions, it is re-expressed in repair processes, various neoplasms and proliferating vascular endothelial cells."

For the assessment of angiogenesis, different assays are available (Bahramsoltani, Plendl, Janczyk, Custodis, & Kaessmeyer, 2009). The aortic ring model has been used to assess the effects of cortisol in equine vessels (Morgan et al., 2018). The development of capillary-like structures was observed in isolated equine endothelial cells *in vitro* (Dietze et al., 2014). *In vitro* tubule formation on basement membrane matrix has been done on both equine endothelial progenitor cells (Salter et al., 2015; Seeto et al., 2017; Sharpe et al., 2016) and equine endothelial cells (Bussche & Van de Walle, 2014). A wound healing or scratch assay, an *in vitro* assay, has been used in horse endothelial cells (Rieger et al., 2018).

In order to study *in vitro* angiogenesis in the horse, equine endothelial cells are needed for culture. Worldwide, there are relatively few laboratories isolating and/or working on equine endothelial cells. Table 1 lists *in vitro* studies that either describe the isolation procedure of equine endothelial cells or use endothelial cells that have previously been isolated from horses.

4 | ENDOTHELIAL REGULATORY MOLECULES IN EQUINE ANGIOGENESIS

Angiogenesis is regulated by a variety of soluble pro- and anti-angiogenic growth factors (Schroder, 2019). The two most important ones are the vascular endothelial growth factors (VEGF) and the angiopoietins (Ang) (Fagiani & Christofori, 2013; Melincovici et al., 2018). The regulatory role of these molecules in the horse will be pointed out in different chapters of this review, and therefore, they are only generally introduced in the following:

VEGF is known to trigger endothelial proliferation, migration and angiogenesis. As recently reviewed by Melincovici et al. (2018), the VEGF family includes VEGF-A, B, C, D, E, F, the placenta growth factor (PlGF) and the endocrine gland-derived vascular endothelial growth factor. Bussche and Van de Walle (2014) examined the presence of angiogenic factors in conditioned medium of equine peripheral blood-derived MSC and examined their influence on equine endothelial cells. They confirmed that peripheral blood-derived MSC induce angiogenesis and that endothelial VEGF-A is important in autocrine and paracrine signalling in the horse (Bussche & Van de Walle, 2014).

VEGF binds to three receptors, that is, VEGFR-1, VEGFR-2 and VEGFR-3. VEGFR-1 (or Flt-1) and VEGFR-2 (also known as KDR or flk1) are primarily found on the vascular endothelium; in contrast, VEGFR-3 is found on lymphatic endothelium (Melincovici et al., 2018).

Angiopoietins (Ang1, Ang2, Ang3, Ang4) are secreted vascular growth factors required for vasculogenesis and angiogenesis. Tyrosine kinase receptor 1 and 2 (Tie1 and Tie2) are the cell surface receptors that bind the angiopoietins (Fagiani & Christofori, 2013).

Other molecules involved in angiogenesis and investigated in the horse include endothelins, nitric oxide synthases (NOS), reactive oxygen species (ROS) and glucocorticoids.

The endothelins comprise three structurally similar peptides, that is, endothelin-1, -2 and -3 (ET-1, -2, -3), which act as potent vasoconstrictors and pro-angiogenic triggers. They are secreted by endothelial as well as other cells, for example smooth muscle cells, and their receptors are located on both endothelial and vascular smooth muscle cells (reviewed by Davenport et al., 2016 and Bek & McMillen, 2000). Endothelins are the focus of many studies in the horse not so much because of their angiogenic but rather because of their vasoconstrictive effect. For example, Stokes,

TABLE 1 *In vitro* studies on equine endothelial cells, either describing the isolation procedure or using endothelial cells previously isolated

Topic/aim of study	Organ/vessel of origin	Authors and title of study
Isolation of endothelial cells and life cell angiogenesis assay with primary cultures	Jugular veins and carotid arteries	Dietze et al. (2014) Isolation of equine endothelial cells and life cell angiogenesis assay.
Comparison of the angiogenic response of human and equine endothelial cells to lesions in an *in vitro* scratch assay	Jugular veins and carotid arteries	Rieger et al. (2018) Human and equine endothelial cells in a live cell imaging scratch assay *in vitro*.
Equine herpesvirus 1 transfer between peripheral blood mononuclear cells and endothelial cells	Carotid arteries	Spiesschaert et al. (2015) Role of gB and pUS3 in Equine Herpesvirus 1 Transfer between Peripheral Blood Mononuclear Cells and Endothelial Cells: a Dynamic In Vitro Model.
In vitro responses of vessel rings from 8 non-laminitic horses to endothelin-1	Palmar digital arterial and venous rings	Stokes et al. (2006) Comparison of 2 endothelin-receptor antagonists on *in vitro* responses of equine palmar digital arterial and venous rings to endothelin-1.
In vitro thrombosis model for characterisation of EHV-1 strain variation. Polarised equine endothelial cell cultures on microcarrier beads	Umbilical veins	Chiam et al. (2006) Use of polarised equine endothelial cell cultures and an *in vitro* thrombosis model for potential characterisation of EHV-1 strain variation.
Adherence of equine eosinophils to endothelial cells	Digital vein	Bailey and Cunningham (2001a, 2001b) 2001a: Adherence of eosinophils from allergic and normal ponies to cultured equine endothelial cells. 2001b: Inflammatory mediators induce endothelium-dependent adherence of equine eosinophils to cultured endothelial cells.
Isolation and characterisation of endothelial cells including staining for nitric oxide synthase	Pulmonary artery	MacEachern et al. (1997) Methods for the isolation, culture and characterisation of equine pulmonary artery endothelial cells.
Modulation of inflammatory host immune response genes in equine endothelial cells through equine herpesvirus type 1	Cardiac artery	Johnstone, Barsova, Campos, and Frampton (2016) Equine herpesvirus type 1 modulates inflammatory host immune response genes in equine endothelial cell.
Characterization of equine E-selectin on activated endothelium for extravasation of leucocytes during inflammation	Pulmonary artery	Hedges et al. (2001) Characterization of equine E-selectin.
Pharmacologic effect of endothelin-1 antagonists on endothelial cells	Colonic vessels (arterial and venous rings)	Venugopal et al. (2001) *In vitro* pharmacologic effect of two endothelin-1 antagonists on equine colonic arteries and veins.
Cytotoxicity of stimulated equine neutrophils during interaction with endothelial cells	Carotid arteries	Benbarek et al. (2000) Cytotoxicity of stimulated equine neutrophils on equine endothelial cells in culture.
Culture and characterization of terminal arch endothelial cells	Palmar digital terminal arch arteries	Wunn et al. (1999) Culture and characterization of equine terminal arch endothelial cells and hoof keratinocytes.
Role of endothelium and nitric oxide	Colonic venous rings	Moore, Venugopalan, Sedrish, and Holmes (1997) Role of endothelium and nitric oxide in the *in vitro* response of equine colonic venous rings to vasoconstrictor agents.
Growth characteristics of equine arteritis virus in primary equine endothelial cell	Pulmonary artery	Moore, Balasuriya, Hedges, and MacLachlan (2002) Growth characteristics of a highly virulent, a moderately virulent, and an avirulent strain of equine arteritis virus in primary equine endothelial cells are predictive of their virulence to horses.
Expression of E-selectin and vascular endothelial growth factor	Pulmonary artery	Huang, Lavoie-Lamoureux, and Lavoie (2009) Cholinergic stimulation attenuates the IL-4 induced expression of E-selectin and vascular endothelial growth factor by equine pulmonary artery endothelial cells.

(Continues)

TABLE 1 (Continued)

Topic/aim of study	Organ/vessel of origin	Authors and title of study
Expression of CXCL-8, E-selectin, VEGF, inducible nitric oxide synthase mRNA, IL-4 of endothelial cells	Pulmonary artery	Huang, Lavoie-Lamoureux, Moran, and Lavoie (2007) IL-4 stimulates the expression of CXCL-8, E-selectin, VEGF, and inducible nitric oxide synthase mRNA by equine pulmonary artery endothelial cells.
Isolation and identification of endothelial cells	Aorta and pulmonary arteries	Lamar, Turek, Bottoms, and Fessler (1986) Equine endothelial cells *in vitro*.
Mechanism by which equine herpesvirus type 1 is transferred from CD172a + cells to endothelial cells	Vena cava	Laval et al. (2015) Equine Herpesvirus Type 1 Enhances Viral Replication in CD172a(+) Monocytic Cells upon Adhesion to Endothelial Cells.
Production of free radicals and oxygen consumption by endothelial cells	Carotid arteries	de Rebiere de Pouyade et al. (2011) Equine Herpesvirus Type 1 Enhances Viral Replication in CD172a(+) Monocytic Cells upon Adhesion to Endothelial Cells.
Establishment of a model for endotoxin exposure in the horse	Pulmonary veins and aorta	Turek et al. (1987) Ultrastructure of equine endothelial cells exposed to endotoxin and flunixin meglumine and equine neutrophils.
LPS-induced digital hypoperfusion; vasoactive mediators, endotoxin, ibuprofen, cycloheximide, or L-nitroarginine methyl ester, prostacyclin, cyclic guanosine monophosphate, endothelin-1, thromboxane A(2), inducible nitric oxide synthase, cyclooxygenase-2	Digital veins	Menzies-Gow et al. (2008) Evaluation of the induction of vasoactive mediators from equine digital vein endothelial cells by endotoxin.
Horses and ponies; factor VIII-related antigen, metabolized acetylated low-density lipoprotein	Fresh omental tissue (microvascular endothelial cells)	Bochsler et al. (1989) Isolation and characterization of equine microvascular endothelial cells *in vitro*.
Angiogenic stimulating factors in conditioned medium of peripheral blood-derived equine mesenchymal stromal cells (PB-MSCs) and investigation of their *in vitro* effect on angiogenic-related behaviour of endothelial cells	Carotid arteries	Bussche and Van de Walle (2014) Peripheral Blood-Derived Mesenchymal Stromal Cells Promote Angiogenesis via Paracrine Stimulation of Vascular Endothelial Growth Factor Secretion in the Equine Model.

Venugopal, Hosgood, Eades, and Moore (2006) found ET-1 to be a powerful vasoconstrictor of equine palmar digital arteries and veins. Comparing endothelin-receptor antagonists, they revealed the antagonist PD145065 to be more effective than PD142893 in reducing the contractile effects of endothelins. ET-1 antagonists have also been tested in equine arterial and venous rings of colonic vessels, where they effectively block ET-1-induced contractions (Venugopal et al., 2001).

It is known that NOS and ROS are involved in angiogenesis (Schroder, 2019), and it has been shown that this is also true for equine angiogenesis. NOS are a family of enzymes inducing the production of endothelial NO, a free radical regulating vascular resistance and blood flow and enhancing angiogenesis (reviewed by Tejero, Shiva, & Gladwin, 2019). NOS expression was evaluated in equine endothelial cells of the myometrium (da Costa et al., 2007).

ROS production is activated by hypoxia, ischaemia, anoxia–reoxygenation, VEGF and Ang1 (Zhou et al., 2013). Equine endothelial cells have been shown to produce ROS *in vitro* when subjected to anoxia–reoxygenation (Anderson & Singh, 2018; de Rebiere de Pouyade et al., 2011).

Glucocorticoids are known to act as anti-inflammatory and anti-angiogenic agent in rodents and humans (Yano, Fujii, Iwai, Kageyama, & Kihara, 2006). Cortisol has been found to down-regulate inflammation in both, murine and equine species, but, in contrast to the mouse, it up-regulates angiogenesis in the horse. The reason and detailed pathways of this species-specific regulation of angiogenesis have not been studied (Morgan et al., 2018).

Last, not least, there are several cell surface molecules and receptors relevant to angiogenesis that have been identified in equine endothelial cells—although not necessarily in studies on angiogenesis but in a different context. Examples are the immunolocalization of leptin and ghrelin in equine luteal endothelial cells *in vitro* (Galvao et al., 2014).

5 | ANGIOGENESIS RESEARCH IN EQUINE REPRODUCTION

Angiogenesis research in equine reproduction has been primarily focused on the female genital system and pregnancy. Almost all published studies emphasize the clinical relevance of the subject.

The principal aims of this research include manipulation of the oestrous cycle in mares, therapeutic strategies for anovulatory follicles, haemorrhagic corpora lutea and premature luteolysis, therapy of endometrial disorders and prevention of abortion. Further studies aimed to compare the physiological mechanisms and events between humans and domestic/ laboratory animals. In addition, the regulation of angiogenesis in the equine ovary under physiological conditions has been compared to the conditions in tumour growth (equine granulosa cell tumour—see section about tumours).

5.1 | Ovary

In the ovary, the development of follicles and corpora lutea (CL) as well as their regression is accompanied by rapid changes in the vasculature by angiogenesis and anti-angiogenesis (Fraser, 2006; Klagsbrun & D'Amore, 1991; Plendl, 2000; Redmer & Reynolds, 1996). Follicle growth, ovulation and CL development and regression are regulated by signalling pathways that act on the vasculature of the ovary (Galvao, Ferreira-Dias, & Skarzynski, 2013; Sayasith & Sirois, 2014). Angiogenic activity was monitored by different methods. Measurements of vascular perfusion (Abdelnaby & El-Maaty, 2017), capillary density (Ferreira-Dias, Bravo, Mateus, Redmer, & Medeiros, 2006; Ferreira-Dias & Mateus, 2003; Watson & Ai-zi'abi, 2002), proliferation and apoptosis *in situ* (Aguilar et al., 2006) as well as evaluation of angiogenesis assays *in vitro* (Ferreira-Dias, Costa, et al., 2006) show an increase in angiogenesis before ovulation and in the early and mid-luteal phase in the equine ovary.

Regulation of angiogenesis in the equine ovary is mediated by opposite actions of pro- and anti-angiogenic factors (Galvao et al., 2013). Several studies confirmed the role of VEGFs and angiopoietins and their receptors in the ovary of the mare. Watson and Ai-zi'abi (2002) found high VEGF levels together with a high proliferative activity of endothelial cells in the theca of preovulatory follicles (Watson & Ai-zi'abi, 2002). In the mare, anovulatory haemorrhagic follicles showed physiological levels of VEGF-A. A relative lack of VEGFR-2 affected the pro-angiogenic activity of VEGF leading to ovulation failure (Ellenberger et al., 2009). Muller, Ellenberger, and Schoon (2009) did a histomorphological and immunohistochemical study of angiogenesis on cyclic ovaries of mares. The most intensive co-expression of the factors VEGF-A, VEGF-B, Ang1 and Ang2 and the receptors VEGFR-1, VEGFR-2 and Tie2 was detected during the periovulatory period, when strong ovarian angiogenesis takes place. The authors propose VEGF-A, Ang2, VEGFR-2 and Tie2 to be important for angiogenesis during follicular and luteal development, while Ang1 stabilizes vessels. In the absence of VEGF-A, Ang2 and Tie2 contributed significantly to vascular regression and thus to luteolysis and follicular atresia (Muller et al., 2009).

A high expression of VEGF in follicles stimulated by intrafollicular injection of insulin-like growth factor (IGF)-1 was detected during follicle selection by Ginther, Gastal, Gastal, Checura, and Beg (2004). The role of cytokines in the control of luteal angiogenesis was investigated in an *in vitro* study with equine ovaries by Galvao et al. (2012). The results suggest an auto/ paracrine effect, especially of tumour necrosis factor (TNF), on the up-regulation of VEGF to stimulate angiogenesis in early CL, while the cytokines examined down-regulated angiogenesis during luteolysis. TNF increased the angiogenic activity of VEGF and decreased the expression of CD36, the receptor for thrombospondin (Galvao et al., 2012). Comparing natural and deslorelin (GnRH analogue)-induced ovulation, Maia et al. (2016) found a positive correlation between the expression of VEGF and the LH-receptor in mares with natural ovulation and an increased expression of the LH-receptor in induced ovulation.

The action of hormones, cytokines and angiogenic factors changes depending on the luteal phase. Maturation and ovulation of the dominant follicle in mares were accompanied by increasing levels of estradiol and leptin and a decreasing level of IGF-1. On day 3 before ovulation, NO peaked and thereafter decreased until ovulation (Abdelnaby & El-Maaty, 2017). The effect of TNF and NO on the production of angiogenic factors and prostaglandin E2 was studied in cultured equine explants of corpora lutea (Ferreira-Dias & Skarzynski, 2008). The secretory function and the release of angiogenic factors of equine corpora haemorrhagica were found to be stimulated by TNF and NO (Ferreira-Dias et al., 2011). During the late luteal phase, NO and progesterone 4 inhibit angiogenesis, preparing the CL for functional and structural regression (Ferreira-Dias & Skarzynski, 2008). Leptin and ghrelin play opposing roles during development and regression of the equine CL, with leptin promoting angiogenesis and ghrelin enhancing luteolysis (Galvao et al., 2014).

5.2 | Uterus, placenta and conceptus

Vascular density in histological sections of the equine endometrium was found to be similar in the follicular and luteal phase (da Costa et al., 2007; Ferreira-Dias & Mateus, 2003; Ferreira-Dias, Serrao, Durao, & Silva, 2001; Otzen et al., 2016), indicating that vascular and stromal growth are coordinated. Similar results were presented by Merkl et al. (2010) in early equine pregnancy. Using narrow band imaging hysteroscopy, Otzen et al. (2016) found an increase in vascular density from oestrus towards diestrus, while results from histomorphology and immunohistochemistry suggested that the higher vascularity measured *in vivo* is not due to angiogenesis, but caused by a higher perfusion of the existing capillaries (Otzen et al., 2016).

Throughout the oestrous cycle, VEGF and its receptor VEGFR-1 were localized in lumenal and glandular epithelia of the endometrium, in the invasive trophoblast cells of the endometrial cups and in the non-invasive trophoblast of the allantochorion. VEGFR-2 was found in maternal epithelium during the oestrous cycle as well as in the foetal epithelium in pregnancy. Likewise, anti-VEGFR-2 stained the maternal and foetal endothelia of the placenta (Allen, Gower, & Wilsher, 2007). Silva, Klein, Ealy, and Sharp (2011) examined endometrial tissue during different cyclic stages and throughout pregnancy. They found no differences in VEGF and VEGFR-1 protein localization between pregnant and cycling mares, whereas a differential staining

pattern was detected for VEGFR-2 with higher levels in luminal and glandular epithelium of pregnant mares. Additionally, VEGFR1 staining in the equine endometrial tissue was described to be essentially absent during the oestrus, while the opposite was true during diestrus (Silva et al., 2011).

Angiogenic activity in the equine endometrium during the follicular and luteal phases was found to be related to an increase of NO and was stimulated by treatment with TNF, oestradiol and oxytocin. TNF and NO increased prostaglandin secretion of the endometrium in the early and mid-luteal phases (da Costa et al., 2008; Galvao et al., 2013).

A systematic analysis by Merkl et al. (2010) of transcriptome changes in equine endometrial biopsy samples at day 8 and 12 of pregnancy revealed 374 differentially expressed genes at day 12. The expression of genes seemed to be regulated by oestrogen, progesterone and prostaglandins. Changes in the expression of genes related to angiogenesis and vascular remodelling were identified. These genes are involved in the dynamics of the VEGF and angiopoietin families, endothelial cell regulators and hypoxia-induced genes as well as genes acting on inhibition of endothelial cells and angiogenesis (Merkl et al., 2010).

Milk-fat globule epidermal growth factor 8 (MFGE8) seems to be involved in regulating angiogenesis at the embryo– and foetal–maternal interface during early pregnancy, persisting at the foetal–maternal interface, where it is located, inter alia, in the pericytes of endometrial blood vessels. Expression of MFGE8 was found to be up-regulated by oestrogen in non-pregnant endometria (Barua, Macedo, Kolb, Wynne-Edwards, & Klein, 2018).

Brain-derived neurotrophic factor (BDNF) and its receptor (Ntrk2) were found in the endometrium, myometrium and vascular smooth muscle cells in many species including the horse. The factor is capable of activating angiogenesis as well as cell adhesion, apoptosis and proliferation pathways (Wessels, Wu, Leyland, Wang, & Foster, 2014).

The role of relaxin in mare reproduction was reviewed by Klein (2016). During pregnancy, relaxin promotes endometrial angiogenesis through up-regulation of VEGF. Relaxin messenger RNA was also found as early as 8 days after ovulation in the conceptus, possibly driving endometrial angiogenesis and promoting the embryo´s own development (Klein, 2016). Thus, conceptus-derived relaxin could be involved in the control of angiogenesis in the endometrium and in the embryo itself.

Equine microRNA cluster C24MC, related to the human chromosome 14 microRNA cluster C14MC, was demonstrated during early stages of pregnancy in the vascular endothelium, chorion and allantoic epithelium. Functional analysis indicated a relation to endothelial cell migration and angiogenesis (Dini et al., 2018).

Recently, the proteomic profile of uterine secretions (histotrophs) in pregnant and cyclic mares was reported by Bastos et al. (2019). They found an abundance of proteins related to angiogenesis, uterine motility and maternal immunological tolerance. These alterations were related to the presence of the conceptus and may be important for maternal recognition of pregnancy and the development of the embryo (Bastos et al., 2019).

5.3 | Testis

Treatment of prepuberal stallions with the anabolic steroid testosterone had a temporary effect on vascular density and the expression of angiogenic factors and their receptors. The highest volume density and numerical density of capillaries and the smallest vessel sizes were measured morphometrically at 4 weeks after treatment with the testosterone derivative Durateston. This effect was related to the highest expression of transforming growth factor (TGF), Ang2 and VEGFR-2 and considerably reduced after 12 weeks, suggesting an only temporary stimulation of the blood vessels by the anabolic steroid Durateston (Teubner et al., 2015).

Expression of constitutive endothelial NOS was found in the equine testis and epididymis (Ha, Kim, & Shin, 2004).

5.4 | ANGIOGENESIS RESEARCH IN PATHOLOGICAL CONDITIONS

Angiogenesis in the healthy organism is tightly regulated and—with a few exceptions—down-regulated in the adult. However, up-regulation of angiogenesis is involved in many diseases, the best known examples are solid tumours. Their growth and metastasis are dependent on angiogenesis (Folkman, 1971). The fragmentary knowledge on the role of angiogenesis in equine pathological conditions is presented in the following chapter.

5.5 | Tumours

Increased angiogenesis plays a crucial role in the equine sarcoid, a frequent neoplasm of horse skin without effective treatment option (Knottenbelt, 2019). In this tumour, VEGF is highly expressed due to hypoxia, leading to inadequate, immature vascularization. VEGF was found in endothelial cells as well as in keratinocytes and fibroblasts of sarcoid tumour samples by Martano et al. (2018). The authors conclude that VEGF may stimulate tumour growth by activation of angiogenesis.

Müller et al. investigated angiogenesis in the equine ovary (Muller et al., 2009) and ovarian tumours (Muller, Ellenberger, Hopper, & Schoon, 2012). They reported that the equine granulosa cell tumour, which is the most common benign ovarian neoplasia in the mare, was characterized by the patterns of expression of VEGF-A, VEGF-B, Ang1, Ang2, VEGFR-1, VEGFR-2 and Tie2 similar to expression patterns in the normal ovaries. In this tumour, the angiogenic factors are expected to contribute significantly to angiogenesis and vascularization (Muller et al., 2012).

Grey horses have a genetic predisposition to spontaneously develop melanomas. Many of these tumours are well-vascularized, reflecting enhanced angiogenesis (Moore et al., 2013). Preclinical studies with grey horses have demonstrated that immunotherapy with a plasmid encoding the cytokine IL-12, known to be a potent anti-angiogenic agent, leads to a pronounced reduction in tumour growth (Heinzerling et al., 2001).

Finally, Bartonella species are known to be potential causes of proliferative vascular diseases in humans and different animals. It has been reported that some Bartonella species can induce proliferation of endothelial cells and are associated with *in vivo* formation of vasoproliferative tumours in horses (Beerlage et al., 2012; Cerimele et al., 2003). The exact mechanism of action of the bacteria is not known; however, it has been shown that they are able to induce angiogenesis in pro-angiogenic circulating progenitor cells (O'Rourke et al., 2015).

5.6 | Ocular pathologies

Physiologically, the cornea is an avascular tissue that maintains its transparency by regulating its optimal consistency based on an equilibrium of pro- and anti-angiogenic factors including endostatin, angiostatin and MMPs. Any disequilibrium of factors, which may be the case in hypoxia or inflammatory diseases, initiates angiogenesis in the cornea (Shank, Teixeria, & Dubielzig, 2019).

Equine recurrent uveitis (synonym moon blindness) is a frequent cause of blindness in horses that is accompanied with angiogenesis in the cornea (Figure 5). Amongst others, leptospirosis has been implicated as an infectious cause (review see Allbaugh, 2017). The disease serves as a model for human uveitis, where, in a similar fashion, T lymphocytes target retinal proteins (Zipplies et al., 2010). Hauck and colleagues examined proteins of lymphocytes at different stages of equine recurrent uveitis and identified considerable differences in angiogenesis-related proteins including integrins (Hauck, Lepper, Hertl, Sekundo, & Deeg, 2017). Moreover, quantitative changes in MMPs, important angiogenic factors, have been found in equine recurrent uveitis (Hofmaier, Hauck, Amann, Degroote, & Deeg, 2011). Another study revealed that high-molecular-weight kininogen, a plasma protein enhancing angiogenesis, was significantly up-regulated in the retina of horses with equine recurrent uveitis (Zipplies et al., 2010).

Angiogenesis of the cornea can also be a consequence of post-traumatic keratouveitis. Moore and colleagues studied this condition in horses and revealed the presence of leucocytes as well as growth factors and cytokines such as; IL-1, IL-2 fibroblast growth factor 2 (FGF-2) and VEGF with a stimulating effect on corneal angiogenesis (Moore, Halenda, Grevan, & Collins, 1998).

In human patients suffering from corneal neovascularization, promising results have been described for antibodies binding VEGF. Muellerleile, Buxbaum, Nell, and Fux (2019) examined binding of the anti-human VEGF antibodies bevacizumab (Avastin®) and aflibercept (Zaltrap®) for equine VEGF. Unfortunately, the veterinary patients did not benefit from this therapeutic approach because the antibody that binds VEGF is species-specific.

Pearce, Janardhan, Caldwell, and Singh (2007) examined the presence of the anti-angiogenic molecule angiostatin and the pro-angiogenic integrin $\alpha_V\beta_3$ in the retina and cornea of different mammalian species including the horse. Whereas angiostatin was present, integrin $\alpha_V\beta_3$ was absent in the normal equine cornea and retina (Pearce et al., 2007).

5.7 | Equine wound healing

Wound repair is a process dependent on intense but strictly controlled angiogenesis (DiPietro, 2016). In horses, it is known to be subject to numerous complications (Theoret, 2004). In physiological wound healing, endothelial cells migrate into the wound space together with fibroblasts and macrophages. Macrophages are a continuous source of cytokines and growth factors necessary to stimulate angiogenesis. Fibroblasts form new extracellular matrix and new blood vessels carry oxygen and nutrients needed for cell metabolism. Interestingly, wounds heal faster in ponies than in horses. This seems to be due to differences in the local inflammatory response and leucocyte function (Wilmink & van Weeren, 2005). In terms of wound healing models, rodent models have been criticized because wound healing occurs primarily by contraction rather than by epithelialization, contrary to what is observed in humans. The horse's wound healing behaviour is more like that of humans (Wise et al., 2018).

Wound healing in horses is studied in several ways. A fundamental approach is the use of *in vitro* wound healing assays such as the scratch assay, done by scratching an endothelial monolayer followed

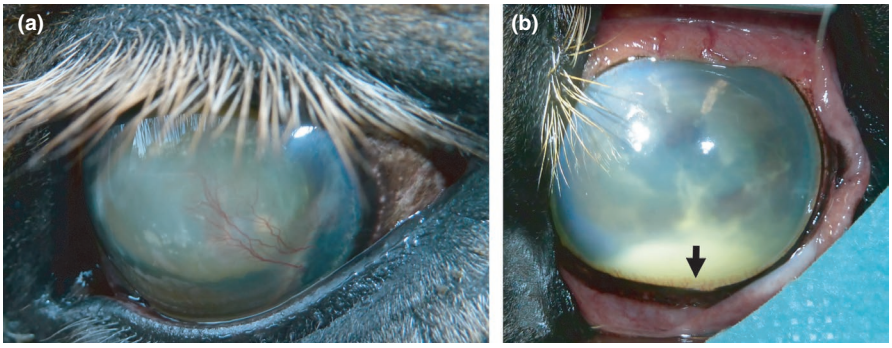

FIGURE 5 Corneal angiogenesis is evident in keratouveitis due to an intraocular response to leptospiral infection in two Haflinger horses. In image (a), elongate arborizing vessels are clearly evident. In image (b), numerous short vessels (arrow) occur circumferentially starting at the corneoscleral junction. Pictures provided by Univ.-Prof. Dr. Corinna Eule, Dipl. ECVO, Small Animal Clinic, Department of Veterinary Medicine, Freie Universität Berlin

by studying endothelial migration and cell–cell interaction during closure of the "wound gap". Human and equine endothelial cells revealed differences in this wound healing assay. In a scratch assay, horses' arterial endothelial cells, compared to equine or human venous endothelium, showed a delay in closing the gap and re-endothelialization, possibly as a result of disoriented and uncoordinated migration of endothelial tip cells (Rieger et al., 2018). In an aortic ring model, where aortic explants were cultured in a three-dimensional biomatrix, and in scratch assays, Pascucci et al. (2014) examined the response of endothelial cells to membrane vesicles. The vesicles, which were produced by equine adipose-derived mesenchymal stromal cells, were found to be involved in intercellular communication with endothelial cells from rats and to stimulate angiogenesis, which is a crucial step in tissue regeneration (Pascucci et al., 2014).

Basic wound healing principles are also studied *in vivo* in the horse. In horses, injuries, especially on the limbs, often develop a persistent inflammation. They have an inefficient blood supply, leading to excessive fibrosis and clinical complications such as exuberant granulation tissue (Wakelin et al., 2016). This complication of wound healing in horses is characterized by highly vascularized tissue that clinically behaves like a benign tumour. The developing scar remains chronically in the proliferative repair phase, resulting in fibrosis. Extensive scars are the result that can affect form and function (Miragliotta, Raphael, Ipina, Lussier, & Theoret, 2009). Wakelin (2015) applied VEGF-E to improve wound closure and resolution in chronic wounds in horses. Although inflammatory mediators and innate immune cell infiltration decreased and angiogenesis increased, she concluded that the dosage and timing for VEGF-E treatments had to be optimized to achieve therapeutic benefits (Wakelin, 2015).

The distribution and reaction of microvessels are heterogenous in different anatomical regions of the body. Over most of the body surface the skin is supplied by small capillaries, that is functional nutrient vessels; while in the bodies' extremities, arterioles and venules predominate with their inherent lower resistances and higher flow rates. Accordingly, the response of these areas to lesions—vasodilation in the skin and vasoconstriction in the distal limbs—varies (Celeste, Deschesne, Riley, & Theoret, 2013). Celeste et al. (2013) investigated blood flow during cutaneous wound healing with a focus on exuberant granulation tissue formation in the horse: interestingly, although more angiogenesis occurs in exuberant granulation tissue, functional perfusion was found to be poorer because the microvessels were occluded (Celeste et al., 2013). Occlusion of granulation tissue microvessels is seen frequently and seems to be caused by endothelial cell hypertrophy (Dubuc, Lepault, & Theoret, 2006). It was concluded that it is not the number of blood vessels that is crucial but their functionality (Celeste et al., 2013). Microvascular occlusion followed by hypoxia is likely to result in excessive production of extracellular matrix components by fibroblasts through up-regulation of angiogenic and fibrogenic factors such as TGF-β (Wilmink & van Weeren, 2005).

Ipina, Lussier, and Theoret (2009) investigated wound healing in experimentally induced wounds on the trunk and limbs of horses. They investigated the temporal expression of equine pigment epithelium-derived factor (PEDF) that is known to have potent anti-angiogenic properties. Equine PEDF shares 87% sequence and 88% peptide homology with human PEDF. A relative overexpression of PEDF in the trunk's wounds compared to those of the limbs was reported. The authors concluded that a higher expression of PEDF in trunk wounds may contribute to better healing by protecting against overgrowth of vascular granulation tissue (Ipina et al., 2009).

According to Miragliotta et al. (2009), equine thrombospondin II (THBS2) and secreted protein acidic and cysteine-rich (SPARC) are up-regulated during wound healing in horses and may modulate angiogenesis. The glycoproteins THBS2 and SPARC are matricellular proteins that modulate cell–matrix interactions and participate in tissue repair by simultaneously binding to other matrix proteins, cell surface receptors and molecules such as proteinases and cytokines. Both exhibit potent anti-angiogenic activities. SPARC inhibits the proliferation and migration of endothelial cells stimulated by FGF-2 and VEGF and is reported to disrupt the VEGF/ VEGFR-1 signalling pathway. It was found to be significantly less expressed in the equine limb. The absence of THBS2 is associated with persistence of blood vessels through the later stages of wound repair (Miragliotta et al., 2009).

Due to the often unsatisfactory wound healing in horses, research focusses on different treatment strategies.

The working group of Christine Theoret (https://www.researchgate.net/profile/Christine_Theoret/research) performed several wound healing experiments in the horse focussing on the treatment of dermal fibroproliferative disorders. They found that the treatment of wounds in horses using the Orf virus IL-10 (a homolog of interleukin-10 encoded by the parapoxvirus orf virus) and VEGF-E had an anti-inflammatory and repair-promoting effect without accelerating wound closure. Enhanced angiogenic gene expression was found mainly in body wounds in which the blood vessel density and persistence of myofibroblasts were altered. Prolonged healing of horse leg wounds appears to be associated with increased and prolonged proinflammatory gene expression, which is associated with an enhanced immune response. The inflammatory cells that remain in the wounds of the limbs excrete molecules (e.g. TNFα) that potentiate angiogenic stimuli. Furthermore, there seems to be a lack of the anti-inflammatory effect of equine IL-10. In addition, initially blood vessels are more permeable and may mature later due to the delayed production of angiogenic factors following injury. This may be due to a delayed production of platelet-derived growth factor-b (PDGF), necessary to coordinate the pericyte coverage of vascular sprouts for vascular stabilization during angiogenesis. Although in the horse VEGF-A has been shown to promote epithelialization by up-regulating keratinocyte proliferation, VEGF interaction with the VEGFR-1 may result in the formation of immature blood vessels, prolonged vascular leakage and inflammation of the tissue (Bodaan et al., 2016; Wise et al., 2018). Although corticosteroids may be used initially in the treatment of exuberant granulation tissue, continued use is not recommended because it may interfere with angiogenesis, wound contraction and epithelialization (Wilmink & van Weeren, 2005).

The application of dressings to wounds promotes the formation of exuberant granulation tissue. Dressings increase the oxygen gradient between the damaged tissue and the surface, which can stimulate angiogenesis, reduce tissue oxygen tension and stimulate fibroblastic proliferation (Wilmink & van Weeren, 2005).

Silver nanoparticles enhanced equid distal limb wound healing in donkeys. Following topical treatment, extensive neovascularization and the presence of few fibroblasts and collagen fibres perpendicular to the newly formed blood vessels and parallel to the wound surface were observed (Khafaga, Abu-Ahmed, El-Khamary, Elmehasseb, & Shaheen, 2018).

Protein C (autoprothrombin IIA/coagulation factor XIV) is a zymogen whose activated form plays an important role in the regulation of anticoagulation, inflammation, cell death and the maintenance of the permeability of blood vessel walls. Activated protein C supposedly improves angiogenesis and may have potential to reduce inflammation and promote physiological angiogenesis, furthering healing of equine distal limb wounds (Bischofberger et al., 2015). According to Carmona, Lopez, and Giraldo (2011), platelets are crucial in wound healing. They contain numerous growth factors in their α-granules and can release them specifically. They cause chemotaxis, cell proliferation as well as differentiation, angiogenesis and extracellular matrix deposition. The use of autologous platelet concentrates to accelerate wound healing, reduce inflammation, stimulate the regeneration capability of injured tissues, reduce fibroblastic activity and prevent the production of non-functioning scar tissue has been suggested for the horse. Some growth factors and chemokines contained in platelet α-granules stimulate equine angiogenesis (PDGF, TGF-β, VEGF, FGF-2, hepatocyte growth factor) while others have anti-angiogenic properties (Platelet factor 4) (Carmona et al., 2011).

Insect products, particularly those of the European honey bee (*Apis mellifera*), have been reported to promote wound healing by stimulating angiogenesis, granulation tissue formation, epithelialization, reduction of wound hyperaemia and their broadband antibacterial properties (Bischofberger et al., 2016; Hananehl, Ismail, Alshehabat, & Ali, 2015).

A clinical study using essential oils from the aromatic medicinal plant pepper-rosmarin *(Lippia sidoides*) as a mouthwash in oral wounds of horses elicited an intensive stimulation of angiogenesis. This could be because of its high levels of thymol and carvacrol but also may be due to some, as yet unknown, substance (de Alencar-Araripe et al., 2014).

Low oxygen content in tissues is one of the problems in wound healing. Therefore, in human medicine, topical oxygen therapy is used to promote healing of chronic wounds. In contrast, in horses, this experimental approach had little effect on the healing of wounds, although angiogenesis showed a trend towards increased blood vessel density (Tracey et al., 2014).

Finally, non-steroidal anti-inflammatory drugs (NSAIDs) are used regularly to suppress inflammation and pain. Administration of phenylbutazone has been shown to reduce the expression of pleiotropin, a cytokine and growth factor known to be involved in angiogenesis and bone growth. Pleiotropin is an important mediator of cell migration and thus a potential target by which NSAIDs can inhibit wound healing (Silver, Desormaux, Freeman, & Lillich, 2012).

5.8 | Musculoskeletal system

The musculoskeletal system is of eminent importance for the horse as an athlete. Eivers et al. (2012) investigated gene expression in response to exercise of the skeletal muscles in Thoroughbred horses. Peroxisome proliferator-activated receptor-γ coactivator 1α (PGC-1α) was found to be an important control factor in adaptation of the skeletal muscle to exercise, acting via transcriptional control of genes amongst others responsible for angiogenesis. Expression of *PPARGC1A*, the gene encoding PGC-1α, was found to be significantly correlated with genes involved in angiogenesis (*ANGP2* and *VEGF-A*) (Eivers et al., 2012).

Mechanical properties and functional anatomy of tendons and ligaments of the equine distal limb are of critical significance in this species (Denoix, 1994). These bradytrophic anatomic structures are crucially dependent on blood supply and appropriate vascularization. Degenerative lesions in draught horses are particularly obvious at the endothelial level of the distal deep digital flexor tendon. Here, endothelial proliferation and angiogenesis, endothelial cell oedema, metaplasia and finally apoptosis and necrosis lead to endothelial desquamation and clinical problems (Crisan et al., 2013).

Platelet-rich plasma (PRP) is an autologous platelet concentrate and a potential treatment commonly used in orthopaedic medicine for tendon and ligament injuries. It is rich in growth factors, has been proven to improve the repair process of injured tendons and can be injected directly into the tendon. Vascularization is important during the healing process and an increase during the proliferative phase of healing appears to be beneficial. The potent stimulator of angiogenesis VEGF is abundant in PRP, suggesting that the enhancement of neovascularization may be one of its working mechanisms. It should be noted that this treatment is recommended for acute lesions and not for chronic ones. After the healing of a tendon lesion is completed, the vascular system usually recedes and it has been suggested that the prolonged presence of blood vessels may play a role in prolonged pain and healing of tendon injuries (Bosch et al., 2011). Autologous platelet concentrate is also used in intra-articular therapy for horses with refractory fetlock osteoarthritis. This approach takes advantage of the growth factors it contains that are known to have anabolic and angiogenic properties and have a positive effect on synovial epithelium, cartilage and pain (Pichereau, Decory, & Ramos, 2014).

Gene therapy using plasmid DNA encoding *VEGF164* and *FGF2* genes was proposed as a novel treatment of naturally occurring tendinitis and desmitis in horses. The authors report positive effects after treatment and rely this effect to stimulation of angiogenesis (Kovac et al., 2018). Litvin, Zakirova, Zhuravleva, and Rizvanov (2016) generated a dual expression cassette plasmid DNA (pDNA) construct containing the species-specific horse (*Equus caballus*) sequence encoding the pro-angiogenic factors VEGF-A and FGF-2

under eukaryotic promoters (EF-1 alpha and CMV promoters, respectively). They showed recombinant protein expression *in vitro* and stated that the resulting pDNA is suitable for potential gene therapy applications in horses (Litvin et al., 2016). In a follow-up clinical study, this direct gene therapy was used to restore severe injuries of the suspensory ligament branch and superficial digital flexor tendon in horses (Kovac et al., 2017). The pDNA (VEGF164 and FGF-2) was injected at the site of injury with the expectation of a positive influence on the regeneration of the vessels and connective tissue. It was postulated that VEGF164 increased vascular permeability at the site of injury, promoting the formation of granulation tissues, attracting endothelial progenitor cells, stimulating angiogenesis and increasing the activity of pericytes that stabilize newly formed vessels. FGF-2 stimulated cell proliferation, regeneration of nerve, muscle and connective tissue. The treatment led to a complete recovery within a period of 2–3 months (Kovac et al., 2017).

Placental stem cells are reported to be one of the most promising stem cell sources for the regeneration of tendons. They control the healing of the tendons by stimulating an immediate restoration of tissue function without prior transfection. It is believed that the release of pro-angiogenic factors enhances the endogenous regenerative environment (Barboni et al., 2018).

Side effects of NSAIDs (e.g. phenylbutazone) have already been mentioned in the context of equine wound healing. These substances also inhibit equine bone healing. The inhibition of angiogenesis has been suggested as a mechanism of NSAID negative effects on bone healing, since the metabolic products (mainly prostaglandins) of cyclooxygenase-2, a biological target of NSAIDs, are stimulators of angiogenesis and thus essential for fracture healing (Barry, 2010).

5.9 | Laminitis

Laminitis is a disease that occurs in horses and other ungulates. Inflammation of the laminae of the hoof can cause the hoof capsule to detach from the dermis. Acute laminitis is a common condition in horses that often becomes chronic and can even lead to death (Katz & Bailey, 2012). Acute laminitis often develops following a variety of primary diseases; therefore, there are numerous mechanisms involved in its pathogenesis (Eades, 2010). Huntington, Pollitt, and McGowan (2008) consider five hypotheses for how laminitis initially damages the hoof's lamellae. These are the enzymatic, inflammatory, vascular, metabolic/endocrine and mechanical/traumatic theories. Katz and Bailey (2012) subdivide the various forms of naturally acquired laminitis into two categories, that is endocrinopathic and sepsis-related (nonendocrine) forms of laminitis, where endocrinopathic laminitis arises from hormonal rather than inflammatory conditions and is associated with obesity and insulin resistance.

The vascular endothelium plays a major role in the early stages of equine laminitis, consequently, the equine digital vascular endothelium and blood vessels became a subject of numerous studies for a better understanding of the pathogenesis mechanisms and for the development of novel therapeutic approaches.

In both, insulin resistance and sepsis-related conditions, the digital vascular endothelium dysfunction is likely to play a major role in the pathogenesis mechanisms in acute laminitis. Sepsis-related laminitis seems to be the result of two local events. The first is a systemic inflammatory syndrome inducing severe endothelial alteration (Angelone et al., 2017) and the second is the change in digital blood vessel tone and haemodynamics resulting in decreased lamellar blood flow as result of the increase of the post-capillary resistance. (Allen, Clark, Moore, & Prasse, 1990). The inflammatory events, which lead to laminar failure in septic horses, seem to be similar to the organ failure occurring in human sepsis. These events include leucocyte activation, endothelial activation and leucocyte adhesion, extravasation and emigration as well as activation of degradative enzymes (MMPs) and the presence of oxidative stress (Belknap et al., 2007; Belknap, Moore, & Crouser, 2009; Loftus, Black, Pettigrew, Abrahamsen, & Belknap, 2007). The migration of leucocytes through the activated vascular wall to the epithelial–dermal interface plays a significant role in initiating laminar proinflammatory cytokine expression leading to the development of laminitis (Black et al., 2006; Faleiros et al., 2009). The dysadhesion of laminar basal epidermal epithelial cells from their basement membrane seems to be the main cause of structural failure in equine laminitis (Black et al., 2006; Faleiros et al., 2009).

The role of endothelial cells in equine laminitis has been analysed and discussed for decades. As early as 1987, Turek, Lamar, Fessler, and Bottoms exposed primary equine cell lines from pulmonary vessels and aortae to varying combinations of endotoxin and the cyclooxygenase inhibitor flunixin meglumine and equine neutrophils. Then they evaluated the endothelial cells by transmission electron microscopy. Endotoxin alone did not cause cell damage, but endotoxin plus neutrophils caused pathological changes in the endothelial cells. The cells became round and had cytoplasmic vacuolization, mitochondria were swollen and distorted (Turek, Lamar, Fessler, & Bottoms, 1987).

In the early stage of acute laminitis, vascular endothelial dysfunction led to the up-regulation of adhesion molecules that promote adherence of platelets and leucocytes (Katz & Bailey, 2012). Loftus et al. (2007) found that endothelial activation and laminar inflammation are early events in equine laminitis. Laminar concentrations of endothelial activating cytokines such as IL-1β, IL-6, IL-8, COX-2, endothelial adhesion molecules like intercellular adhesion molecule 1 (ICAM-1), and E-selectin were significantly increased in horses with laminitis, compared with that of control horses. Furthermore, concentrations of IL-1β, IL-8, ICAM-1 and E-selectin mRNA peaked early at 1.5 hr after the application of Black walnut extract, which induced laminitis in horse (Loftus et al., 2007). Loftus et al. (2007) reported that the activation of endothelial pattern recognition receptors (toll-like receptors) by circulating toxins induced IL-8 gene expression. Subsequently, Xing et al. (2012) found that IL-8 enables the binding of leucocytes to the endothelium, which is essential for ensuing leucocyte emigration (Xing et al., 2012).

E-selectin and ICAM-1 are endothelial adhesion molecules, which are important in IL-8-mediated neutrophil adhesion and

extravasation (Collins et al., 2000; Loftus et al., 2007). Loftus et al. (2007) found that E-selectin and ICAM-1 mRNA concentrations peaked early in the condition and then they decreased noticeably thereafter. Thus, they concluded that endothelial inflammation and activation could be the initiating event leading to laminar failure.

Brooks et al. (2009) investigated the role of p38 mitogen-activated protein kinases (MAPKs) in endotoxin-induced activation of equine digital vein endothelial cells. P38 MAPK, which is triggered by VEGFR2, is important for shear stress-induced angiogenesis (Gee, Milkiewicz, & Haas, 2010). Brooks et al. (2009) exposed cultured equine digital vein endothelial cells to endotoxin (lipopolysaccharide [LPS]) and assessed the phosphorylation of p38 MAPK by Western blotting. Furthermore, they quantified the neutrophil adhesion and studied the effects of p38 MAPK inhibitors on neutrophil adhesion. They found that the binding of endotoxin to endothelium initiated activation of the p38 MAPK, which was involved in the initiation of inflammatory responses. Furthermore, the presence of p38 MAPK inhibitors significantly inhibited neutrophil adhesion to endotoxin-stimulated endothelial cells. The authors suggested that inhibition of p38 MAPK might reduce inflammatory events in the endotoxemic horse (Brooks et al., 2009).

Moreover, Brooks, Menzies-Gow, Bailey, Cunningham, and Elliott (2010) described the effect of endotoxin (LPS) and hypoxia on cultured equine digital vein endothelial cells. They revealed that the two stimuli significantly increased hypoxia-inducible factor 1α (HIF-1α), neutrophil adhesion and endothelial permeability, all enhancing tissue damage. The endothelial permeability in response to both stimuli was dependent on p38 MAPK and HIF-1α. It was concluded that inhibitors of HIF-1α could reduce unwanted effects in the endotoxemic horse (Brooks et al., 2010).

In laminitis, digital vasoconstriction associated with oxidative injury caused by ischaemia–reperfusion injury occurs. Endotoxin can modify the local qualitative–quantitative balance of vasoactive substances or vessel responsiveness resulting in haemodynamic alternation (Zizzadoro et al., 2011).

The most notable vasoconstrictors acting on the equine digital blood vessels are norepinephrine, 5-hydroxytryptamine (serotonin) and endothelin-1 (ET-1) (Baxter, Laskey, Tackett, Moore, & Allen, 1989; Eades et al., 2007; Menzies-Gow, Bailey, Katz, Marr, & Elliott, 2004). ET-1 is the most potent endothelium-derived vasoconstrictor, it interacts with endothelial-derived relaxing factors such as NO and prostacyclin to control the local vascular flow (Masaki & Sawamura, 2006). Eades et al. (2007) assessed ET-1 immunoreactivity in digital and jugular venous blood from horses with carbohydrate overload-induced laminitis. After 11 hr of carbohydrate overload, they found a significant increase from baseline in digital blood ET-like immunoreactivity. Moreover, the ET-like immunoreactivity of digital blood was significantly greater than that of the jugular vein (Eades et al., 2007).

Menzies-Gow, Bailey, Berhane, Brooks, and Elliott (2008) incubated equine digital vein endothelial cells with or without endotoxin (LPS) and determined concentrations of vasoactive mediators at different time intervals. The production of the vasoactive mediators prostacyclin, cyclic guanosine monophosphate, ET-1 and thromboxane A2 was found to be stimulated by endotoxin.

Furthermore, in relation to vasoactive properties, the equine digital arteries and veins are unique (Bailey & Elliott, 1998; Katz, Marr, & Elliott, 2003; Peroni et al., 2006). It has been confirmed that several vasoconstrictors (including serotonin and ET-1) are more potent vasoconstrictor of equine digital veins than arteries (Peroni et al., 2006).

Katz and Bailey (2012) described how the endothelial dysfunction could lead to the development of laminitis in some animals due to selective venoconstriction. They assumed that dysfunctional endothelium impairs the vasodilatory action of the important vasodilatory neuropeptides P and calcitonin gene-related peptide (CGRP) that are stored in endothelial cells. Consequently, the digital blood vessels failed to relax adequately to maintain normal vascular blood flow (Katz & Bailey, 2012). Moreover, according to Katz, Marr, and Elliott (2011) equine digital arteries relax more than the digital veins in response to CGRP and that even in the absence of the endothelium, the relaxant effect of CGRP on blood vessels was still evident.

Recently, the establishment of a pro-angiogenic environment became a novel therapeutic target in the treatment of laminitis. Angelone et al. (2017) described application of adipose tissue-derived MSCs in the therapy of equine laminitis. An experimental protocol based on the local intravenous administration of adipose tissue-derived MSCs in combination with platelet-rich plasma was developed for the treatment of horses affected by chronic laminitis. In the long term, the venograms showed a progressive amelioration of the vascularization of the foot, which led to an improvement in the structure and function of the hoof and thus greatly enhanced the quality of life of all treated horses. Angelone et al. (2017) proposed that both adipose tissue-derived MSCs and PRP could have the potential to contribute to restore a functional vascular network by controlling inflammation, recruiting local cells to restore tissue, lowering ROS and MMP damage, stabilizing vascular walls and promoting neoangiogenesis. The results of Angelone et al. (2017) should be treated with caution, since the study was restricted to only nine horses, which were previously unsuccessfully treated with conventional therapies. Also, there was no control group. Further investigations are needed to clarify the possible role of regenerative therapies including the establishment of a pro-angiogenic environment in the treatment of chronic laminitis.

6 | PERSPECTIVE

It has become evident that excessive, deficient or dysfunctional angiogenesis contributes to the pathogenesis of many equine disorders. To create a solid foundation for future research towards new therapeutic angiogenesis-related strategies for horses, many parts of the puzzle are still missing.

Relatively little ongoing research is concerned with the importance of endothelial progenitor cells for horses and with pro- and anti-angiogenic treatments with endothelial regulatory molecules for

pathological conditions such as tumours, eye diseases, wound healing disorders, musculoskeletal system diseases or laminitis. In addition, the published results are unfortunately fragmentary and sometimes even contradictory and raise more questions than they answer. For example, the specific role of the capillary bed in diseases like equine laminitis remains unexplained (Morgan et al., 2018; Morgan, Keen, Walker, & Hadoke, 2016). Vascular endothelial cells may be a target for the diagnosis or therapy of laminitis and other diseases.

Another interesting topic could be the role of pericytes in angiogenesis and their potential in regenerative medicine in different species including the horse. An exciting review has been published recently by Esteves and Donadeu (2018).

The jugular vein in horses is very sensitive to mechanical or chemical lesions. Intravenous injections frequently result in thrombophlebitis, which often has fatal consequences (Dias & Neto, 2013). One potential cause for this could be endothelial dysfunction, since activated endothelial cells have an important role in the pathogenesis of thrombosis (Poredos & Jezovnik, 2018). Moreover, dysfunctional angiogenesis is known to result in ineffective thrombolysis (Alias et al., 2014).

In order to learn more about the huge population of heterogeneous endothelial cells, a potential future focus may be the investigation of interendothelial communication. In the last decade, a new way of intercellular communication came into the focus of research, involving extracellular vesicles like exosomes and ectosomes (Meldolesi, 2018). First studies revealed that membrane vesicles produced by equine mesenchymal stem cells are involved in intercellular communication with endothelial cells, stimulating angiogenesis in the horse (Pascucci et al., 2014).

The challenge for the future is to continue research into the involvement of disproportionate angiogenesis in horse diseases. There is also a need to describe the molecular basis of angiogenesis-associated horse diseases in a more detailed and integrative manner so that the results of science can be better transferred to the development of new therapeutic treatments.

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CONFLICT OF INTEREST

The authors state that there is no conflict of interest that could affect the impartiality of the reported research results.

DATA AVAILABILITY STATEMENT

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

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