Phenotypical characterization of aortic rupture in Friesian horses
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Phenotypical characterization of aortic rupture in Friesian horses

Veronique Saey

Dissertation submitted in fulfillment of the requirements for the degree of Doctor in Veterinary Sciences (PhD)

Promoters
Prof. Dr. Koen Chiers
Prof. Dr. Gunther van Loon
Prof. Dr. Catherine Delesalle

Department of Pathology, Bacteriology and Avian Diseases
Faculty of Veterinary Medicine
Ghent University
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Promoters

Prof. Dr. K. Chiers
Faculteit Diergeneeskunde, UGent

Prof. Dr. G van Loon
Faculteit Diergeneeskunde, UGent

Prof. Dr. C. Delesalle
Faculteit Diergeneeskunde, UGent

Members of the Examination Committee

Prof. Dr. P. Deprez
Voorzitter van de examencommissie

Prof. Dr. T. De Backer
Faculteit Geneeskunde en Gezondheidswetenschappen, UGent

Prof. Dr. R. Ducatelle
Faculteit Diergeneeskunde, UGent

Prof. Dr. A. Gröne
Faculteit Diergeneeskunde, Utrecht

Prof. Dr. P Segers
Faculteit Ingenieurswetenschappen en Architectuur, UGent

Prof. Dr. S. Sys
Faculteit Diergeneeskunde, UGent

Dr. L. Young
Specialist Equine Cardiol Serv, Suffolk
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<tr>
<td>A/AF</td>
<td>Friesian horse with aortic rupture (affected)</td>
</tr>
<tr>
<td>ADD</td>
<td>aortic Aneurysm and Dissection disorders</td>
</tr>
<tr>
<td>APF</td>
<td>aortopulmonary fistulation</td>
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<tr>
<td>B/LA</td>
<td>thoracic aorta at the ligamentum arteriosum</td>
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<td>BMFS</td>
<td>Bovine Marfan Syndrome</td>
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<td>BTAI</td>
<td>blunt traumatic aortic injury</td>
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<tr>
<td>CT</td>
<td>computed tomography</td>
</tr>
<tr>
<td>ECM</td>
<td>extracellular matrix</td>
</tr>
<tr>
<td>EMILIN-1</td>
<td>elastin microfibril interface-located protein 1</td>
</tr>
<tr>
<td>FAO</td>
<td>Food and Agriculture Organization of the United Nations</td>
</tr>
<tr>
<td>FBN</td>
<td>fibrillin</td>
</tr>
<tr>
<td>GWAS</td>
<td>genome-wide association studies</td>
</tr>
<tr>
<td>HERDA</td>
<td>hereditary equine regional dermal asthenia</td>
</tr>
<tr>
<td>HP</td>
<td>pyridinoline/hydroxylsylpyridinoline</td>
</tr>
<tr>
<td>LTBP</td>
<td>latent transforming growth factor binding protein</td>
</tr>
<tr>
<td>I-PA</td>
<td>left branch of the pulmonary artery</td>
</tr>
<tr>
<td>LOX</td>
<td>lysyl oxidase enzymes</td>
</tr>
<tr>
<td>LP</td>
<td>deoxypyridinoline/lysylpyridinoline</td>
</tr>
<tr>
<td>MAPG</td>
<td>microfibril-associated glycoproteins</td>
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<tr>
<td>MEFs</td>
<td>musculo-elastic fascicles</td>
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<td>MFS</td>
<td>Marfan syndrome</td>
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<tr>
<td>MMPs</td>
<td>matrix metalloproteinases</td>
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<td>MRI</td>
<td>magnetic resonance imaging</td>
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<tr>
<td>NAF/NA</td>
<td>nonaffected Friesian horses</td>
</tr>
<tr>
<td>PA</td>
<td>pulmonary artery</td>
</tr>
<tr>
<td>PAU</td>
<td>penetrating atherosclerotic ulcer</td>
</tr>
<tr>
<td>PBS</td>
<td>phosphate buffered saline</td>
</tr>
<tr>
<td>PSA</td>
<td>pseudoaneurysm</td>
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<tr>
<td>r-PA</td>
<td>right branch of the pulmonary artery</td>
</tr>
<tr>
<td>SMC</td>
<td>smooth muscle cells</td>
</tr>
<tr>
<td>TAAD</td>
<td>thoracic aortic aneurysms and type A dissections</td>
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<td>T1</td>
<td>mid-thoracic aorta</td>
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<td>T2</td>
<td>distal end of the thoracic aorta</td>
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<tr>
<td>TAA</td>
<td>thoracic aortic aneurysm</td>
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<tr>
<td>TGF-β</td>
<td>transforming growth factor-β</td>
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<td>WB</td>
<td>warmblood horse</td>
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CHAPTER 1: INTRODUCTION
Chapter 1: Introduction

The Friesian horse breed originates from a region in the Netherlands, called Friesland. It is an old and pure horse breed characterized by a very high inbreeding rate. Several presumed genetic disorders (including aortic rupture) have been noticed at a higher frequency in this horse breed compared to other breeds. This introduction will start by providing general information regarding the aorta, followed by a short overview of aortic pathology in humans and other animal species. Finally, the breed characteristics and several genetic disorders occurring in Friesian horses are discussed.

1.1. Aorta: development, structure and function

1.1.A Function and properties
The aorta is the main artery in the body directing oxygenated blood to the entire body through the systemic circulation (Maton et al., 1995). Generally, arteries are classified into three types based on size and properties: elastic arteries, muscular arteries and arterioles (Moore and Dalley, 1999). Both the aorta and pulmonary artery are elastic arteries. The elastic properties of the aorta are crucial as the pulsating blood pressure waves originating from the heart expose them to high circumferential stresses (Labrosse, 2007). Most of the energy of the left ventricular contraction is stored briefly in the stretch of the aortic wall. During the diastole, the elastic recoil of the wall of the aorta maintains the blood flow in forward direction against a closed aortic valve (Martyn and Greenwald, 1997).

1.1.B Anatomy
The aorta emerges from the left ventricle (Figure 1). The aortic valve prevents backflow from the aorta into the left ventricle when the ventricle relaxes (Frandson et al., 2009). At the root of the aorta, there are three little pockets between the cusps of the aortic valve and the wall of the aorta, the aortic sinuses or sinuses of Valsalva. The left and right sinus give rise to the left and right coronary artery (Drake et al., 2010).
The aortic arch is the first part of the aorta. In the horse, the brachiocephalic trunk is the only artery originating from the aortic arch (Figure 2). It gives rise to the subclavian arteries and the bicarotid trunk and is thus responsible for the blood flow to the head, neck and thoracic limbs in horses (Vitums, 1970). In contrast to humans, the curvature of the aortic arch is nearly absent in the horse (Casteleyn et al., 2010).
The aorta then runs dorsal and caudal just below the thoracic vertebrae to form the thoracic aorta. The dorsal side of the thoracic aorta gives rise to the paired intercostal arteries, which supply the thoracic wall and epaxial muscles. Each intercostal artery enters an intercostal space and gives rise to a spinal branch. The latter supplies the spinal cord and spinal nerve roots. The thoracic aorta also maintains the blood flow to the oesophagus, lungs and diaphragm (Frandson et al., 2009).

After passing through the hiatus aorticus of the diaphragm, the aorta is called the abdominal aorta. The abdominal aorta gives rise to paired lumbar arteries, which feature spinal branches. Paired visceral branches from the abdominal aorta include the renal arteries and the testicular/ovarian arteries. Three unpaired visceral branches maintain the blood supply of the other abdominal viscera: the celiac, cranial and caudal mesenteric artery. The abdominal aorta ends at the last lumbar vertebra, splitting into two external and two internal iliac arteries (Frandson et al., 2009).

**Ductus arteriosus**

In utero, approximately 2/3 of the foetal blood bypasses the pulmonary artery, as it is shunted directly to the aorta via the ductus arteriosus (Bankl, 1977). During the late stages of gestation, the ductus begins to narrow. The lungs expand during ventilation postnatally and the pulmonary resistance falls. The pulmonary arterial pressure will drop below the systemic arterial pressure, leading to a reversal in the blood flow within the duct until the latter closes (Bankl, 1977; Machida et al., 1988). The exact mechanism is still unknown, but contraction of the muscular layers of the ductus is involved and results in functional closure. The ductus arteriosus is then gradually replaced by a fibrous tissue band called the ligamentum arteriosum, leading to anatomical closure (Glazier et al., 1974). In horses, the ductus arteriosus closes physiologically within three days postpartum (Machida et al., 1988). The incidence of a patent ductus arteriosus in horses is very low (Guarda et al., 2005).

### 1.1.C Embryologic development

The dorsal aortae fuse with the endocardial tubes. Caudal to the heart, the dorsal aortae fuse to form a single caudal aorta. Cranial to the heart, they remain paired (McGeady et al., 2006). The aortic sac forms by fusion of the two ventral aortae (Kau et al., 2007) (Figure 3).

The aortic arches are a series of six paired embryological vascular structures, which give rise to several major arteries. They develop between the dorsal and ventral aortae and arise from the aortic sac. The first and second pairs regress very early (Vitums, 1969). The third pair, the carotid arch, constitutes the commencement of the internal carotid artery in humans and in horses (Rüsse et al., 1998). The cranial portions of the dorsal aortae form the remainder of the internal carotid arteries (McGeady et al., 2006). The right arch of the fourth pair gives rise to the right subclavian artery while the left plays a role in the formation of the definitive aortic arch (Rüsse et al., 1998). The fifth pair appears relatively late when the truncus arteriosus divides into the aortic and pulmonary channels, is
rudimentary and regresses (Vitums, 1969). The left arch of the sixth pair is involved in the development of the main and left pulmonary arteries and the ductus arteriosus. The right sixth arch contributes to the formation of the right pulmonary artery (Kadir, 1991). The part of the dorsal aortae between the third and fourth arch arteries atrophies. The left fourth aortic arch plays a role in the development of the final aortic arch. The aortic sac and the left dorsal aorta form the remainder of the aortic arch. The part of the right dorsal aorta between the origin of the right subclavian artery and the common caudal aorta atrophies. The brachiocephalic trunk develops from remodelling of the aortic sac, which fuses with parts of the left and right third and fourth aortic arches (McGeady et al., 2006). In horses, the arch of the aorta and the common brachiocephalic trunk appear at approximately day 42 of gestation (Vitums, 1969).

Figure 2.

Vascular smooth muscle cells (SMC) seem to have a wide range of origin. In humans, both the ascending aorta and aortic arch, the ductus arteriosus, the innominate and right
subclavian artery, as well as the right and left common carotid arteries originate from the neural crest (Majesky, 2007). The arterial pole of the heart is the region where the ventricular myocardium joins the vascular smooth muscle cells of the tunica media of the aorta or pulmonary trunk. Two “seams” are present in the arterial pole: 1) the junction between the myocardium with the secondary heart field-derived SMC and 2) the junction between the secondary heart field derived SMC with the neural crest derived SMC (Figure 4). Both of these seams are predisposed areas for aortic dissection in Marfan’s Syndrome and other syndromes (Waldo et al., 2005). The patterns of smooth muscle cell lineage diversity may be important in understanding the vascular bed-specific patterns of disease, for example atherosclerosis (Majesky, 2007).

Figure 3.
Developmental basis for vascular SMC. A mosaic distribution of SMC subtypes is present in the aorta and its major branch arteries (Majesky, 2007).
1.1. D Aortic wall structure

The aorta is composed of a tri-laminar wall: the tunica intima, tunica media and tunica adventitia (Figure 5).

The tunica intima's main function is to provide a non-clotting interface between the blood stream in the aortic lumen and the vessel wall. Simultaneously, it provides a transport gateway for nutrients from and towards the blood stream (Labrosse, 2007). The tunica intima consists of an innermost endothelium lying on a basement membrane and a thin connective tissue layer. An internal elastic membrane forms the outer lining of the intima (Glagov and Wolinsky, 1968).

The tunica media consists of a cellular component, the vascular smooth muscle cells (SMC) and the extracellular matrix (ECM). Three groups of macromolecules are associated to form the ECM: a) fibrous structural proteins (the collagens and elastins), b) adhesive glycoproteins, e.g. fibronectin and laminin and c) a gel of proteoglycans and hyaluronan. They assemble into the ‘interstitial matrix’ and ‘basement membrane’ that together forms the complex network of the ECM (Barbour et al., 2007).

Collagen fibre bundles, elastin fibres and SMC are organized in medial lamellar units to form a complex three-dimensional network (Sommer et al., 2008). Medial lamellar units are composed of overlapping, musculo-elastic fascicles (MEFs) of which size, orientation and composition vary depending on the distribution of tensile stresses (Figure 6). The main distribution of the MEFs is circumferential. Each muscular fascicle is surrounded by an elastic fibre system. Wavy collagen bundles run between these successive MEFs (Clark and Glagov, 1985). The wavy appearance of the elastic fibres alternating with rows of
smooth muscle cells in paraffin-embedded, light microscopic preparations of undistended (“stress-free”) aorta is due to the recoil of the elastic fibres. This results in an overlap of facing elastic fibre systems of adjacent layers of fascicles (Clark and Glagov, 1985). The lamina elastica externa forms the transitional layer between the tunica media and adventitia (Krstic, 1994).

Figure 5.
Simplified, schematic representation of two smooth muscle cells (SMC) and two elastic lamellae (EL) with their interconnections. Both elastic lamellae represented have large, round fenestrations. Thick collagen fibres (Coll) are closely associated with elastic lamellae. Longitudinal surface ridges (but not main cell body) of left smooth muscle cell is connected to both lamellae via long, thin elastin protrusions. The right smooth muscle cell is connected to the lower elastic lamella via oxytalan fibre (Ox). A thin, fibronectin-positive basal lamina-like layer covers most of the cell surface and, in addition, bridges gaps between cells. Next to the basal lamina-like layers, larger deposits (D) containing type IV collagen and heparan sulphate proteoglycan are found especially at indentations of cell surfaces. (Dingemans et al., 2000)

The tunica adventitia is a thin connective tissue layer consisting of mainly collagen type I and collagen type III fibres (von der Mark, 1981; Howards and Macarak, 1989), fibroblasts, fibrocytes, ground matrix, elastin fibres, nerves and vasa vasorum (Labrosse, 2007).
1.1.E Aortic Components

a) Elastin

Elastin is an essential protein of various tissues that depend on elasticity. It is able to deform under physiological forces and to release stored energy directing passive recoil (Sherratt, 2009). Elastin has a rather uncommon amino acid composition and is insoluble due to the presence of interchain cross-links (Ayad et al., 1994). This protein is found in substantial amounts in the walls of large blood vessels (such as the aorta), skin, ligaments, the lungs and the uterus (Barbour et al., 2007; Almine et al., 2010). It contributes to up to 50% the dry weight of large arteries (Parks et al., 1993). The number of elastic lamellae is greatest in the proximal part of the aorta (Davis, 1995). Elastin is the major protein of the aortic extracellular matrix and is not only essential for the elastic properties but also for vascular morphogenesis (Karnik et al., 2003) as it plays a role in the direction of vascular branching (Wendel et al., 2002). Elastin also plays a role in modulating the proliferation of SMC, as evidenced by uncontrolled proliferation of SMC when the interactions between elastin and the other components are disrupted (Wagenseil et al., 2005). Elastogenesis in the aorta of mammals starts subendothelially and then continues from the lumen to the adventitia (Jensen and Bertelsen, 1961). It begins at midgestation and proceeds until completion of postnatal growth (Kielty et al., 2002). From a young age on, the production of elastin decreases and by adulthood, its biosynthesis is substantially reduced. Later in life, the elastic tissues must thus rely on the elastin that was deposited in utero and during the first few years of life (Cleary et al., 1967; Wirtschafter et al., 1967). Elastin is the longest lasting protein in the body and has an estimated half-life of 40 to 74 years (Rucker and Tinker, 1977; Shapiro et al., 1991; Lefebvre and Rucker, 1980).

The smooth muscle cells are responsible for the synthesis of these elastin fibres that are arranged into concentric rings around the aortic lumen in the tunica media (Barbour et al., 2007) and this synthesis is influenced by local hemodynamic conditions (Leung et al., 1977). In elastic arteries, elastin fibres are concentrated in the internal and external elastic laminae and in the tunica media where elastic lamellae alternate with SMC (Kielty et al., 2007). The structure of the elastin in the elastic lamellae of the media is oriented in such a manner that it can sustain the circumferential mechanical stress of pulsation (Farand et al., 2007).

Elastin fibres are composed of a cross-linked elastin core and an outer layer of microfibrils. The key step in the synthesis of insoluble elastin is the association and cross-linking of tropoelastin molecules (Karnik et al., 2003) (Figure 7). Strong similarity exists between tropoelastins of different species, more than 70% on average. However, some variations exist as exon 26A is only found in humans (Otsuni et al., 2002). Tropoelastin is first secreted into the extracellular matrix. Lysyl oxidase enzymes (LOX) conduct the cross-linking of tropoelastin monomers (Karnik et al., 2003). The forming elastin is then introduced to microfibrils in the extracellular matrix. Fibrillin is the major component of the microfibrillar scaffolding upon which elastin is deposited, combined with several microfibril-associated proteins, such as latent transforming growth factor (TGF-β), binding proteins (LTBP 1-4) (Isogai et al., 2003), elastin microfibril interface-located protein 1 (EMILIN-1), microfibril-associated glycoproteins (MAPG-1 and -2) and fibulins (Yurchenco et al., 1994). Fibrillins are produced prior to the tropoelastin deposition and the
polymerization occurs in a typical ‘beads-on-a-string’ structure (Kielty et al., 2002). Fibrillin-2 (FBN-2) is predominantly expressed during early development while fibrillin-1 is mainly expressed in mature tissues (Cain et al., 2006). The fibulins are important for aortic wall homeostasis and consist of a group of 7 ECM proteins. Fibulin-1 is located within the elastin core (Roark et al., 1995). Fibulin-2 and -4 are found between the core and the microfibrils and fibulin-5 is associated with microfibrils (Wagenseil and Mecham, 2009). Fibulin-5 plays key features in the elastin fibre assembly. Fibulin-3 is thought to be a negative regulator of matrix metalloproteinases MMP-2 and MMP-9 in arteries in association with hypertension (Zhongwei et al., 2016). Fibulin-6 and -7 are not found in the aorta (Wu et al., 2013). The resulting elastin is very stable with an impressive ability to confer recoil to tissues (Muiznieks et al., 2010).

Figure 6.
Schematic representation of Elastin fibre formation.
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Microfibrils and elastin fibres are organized into tissue-specific architecture depending on the mechanical demands of the individual organ systems (Kielty et al., 2002).

b) Collagen

Collagen is the most abundant structural protein in the body. It plays an essential role in the functional properties of the aorta. The arterial collagen content and distribution is species-dependent (Grant, 1967). Collagen fibres consist of a group of collagen fibrils that are connected by a matrix rich in proteoglycans. The latter also play a role in the mechanical properties of collagen fibres (Kannus, 2000). Most collagens in the intima and media are produced by SMC while in the adventitia by fibroblasts (Fitzsimmons and Shanahan, 2002). The collagens are composed of a triple helix of 3 polypeptide alpha chains. The triple-helical structure of collagen is obtained from an abundance of three amino acids (glycine, proline, and hydroxyproline), which make up the characteristic repeating motif Gly-Pro-X (X can be any amino acid). First of all, glycosylation of procollagen occurs in the rough ER and Golgi complex. Then, galactose and glucose
residues are added to the hydroxylsine residues. Long oligosaccharides are added to asparagine residues in the C-terminal propeptide. Next, there is hydroxylation of proline and lysine residues in the middle of the chains. Finally, disulfide bonds between the N- and C-terminal propeptides align the three chains before the triple helix is formed. Secretion into the extracellular space follows. During or following exocytosis, there is cleavage by procollagen peptidases and tropocollagen is the resulting protein. After excision of both propeptides, there is polymerization of the collagen molecules into normal fibrils in the extracellular space. Post-translational modification of procollagen leads to the formation of mature collagen molecules and their assembly into fibrils (Lodish et al., 2000) (Figure 8).

Figure 7.
Schematic representation of the major events during fibrous collagen formation (Lodish et al., 2000)

Collagen types I, II and III are ‘interstitial’ or ‘fibrillar’ collagens. Types IV, V and VI are ‘nonfibrillar’ or ‘amorphous’ collagens (Barbour et al., 2007). Collagen type I (about 30%) and type III (about 70%) are the most abundant collagen fibres in the aortic wall and are responsible for the tensile strength (Shekhonin et al., 1985; Pozzi et al., 1998). Type III collagen is essential for normal collagen type I fibrillogenesis (Liu et al., 1997). Collagen is also important for the sequestration of cytokines and the mediation of cell proliferation by binding to integrins (Pozzi et al., 1998) (Figure 9). Intact elastic fibres are necessary for organized collagen deposition (Wu et al., 2013).
c) Smooth muscle cells

Vascular smooth muscle cells can perform both contractile and synthetic functions (Rensen et al., 2007). SMC are also responsible for the synthesis of collagen, elastin, laminin and proteoglycans. They play a crucial role in arterial remodelling by their ability to synthesize collagen within minutes (Huang et al., 1993). Different vessels and even different segments of the same vessel are composed of smooth muscle cells that originate from distinct embryologic sources of progenitors (Nakamura et al., 2006) (Figure 4). At homeostasis, SMC of adult animals are of a quiescent, differentiated contractile phenotype (Thyberg et al., 1990). Under pathological conditions however, vascular SMC can undergo profound changes in response to changes in the extracellular matrix. These changes include enhanced proliferation and migration and a switch from the contractile to the synthetic phenotype (Owens et al., 2004). This phenotypic switch is associated with a repression of smooth muscle cell marker genes and the production of matrix metalloproteinases (Ailawadi et al., 2009). The phenotypic alterations are suggested to play a key role in many disorders, including atherosclerosis, hypertension and aortic aneurysm (Owens et al., 2004; Ailawadi et al., 2009).

d) Proteoglycans

Proteoglycans are the most abundant glycoproteins present in the aortic wall. There are large proteoglycans, for example versican and aggrecan and small, leucine-rich proteoglycans, such as decorin, biglycan, fibromodulin, osteoglycin, and lumican. The small proteoglycans bind to the ECM molecules such as collagen, tropoelastin and microfibrils. Versican is necessary for the migration and proliferation of vascular SMC.
case of injury to the vessel wall, the elastin levels are downregulated and versican degradation is increased (Wight, 2008).

1.1 F Biomechanical characteristics of the aorta

Three major microstructural components of the aortic wall underly its biomechanical characteristics: smooth muscle cells, collagen and elastin. Elastin and collagen are the main determinants of the passive mechanical properties by their intrinsic properties (Glagov and Wolinsky, 1963; Cox, 1978). The smooth muscle cells are responsible for the active mechanical properties and the synthesis of the ECM (Dobrin and Rovick, 1969). Biomechanically, the aorta exhibits a nonlinear stress-strain response with a typical exponential stiffening at higher pressures (Holzapfel et al., 2000) that is based on the recruitment of the load-carrying wavy collagen fibrils. This phenomenon leads to the typical anisotropic mechanical behaviour of arteries (Roach and Burton, 1957). The composition of the arterial wall and thus also the shape of the stress-strain curve varies along the arterial tree (Cox, 1978).

Studies of the biomechanical characteristics of the aorta have yielded important physiological information related to pathologies but are limited by practical considerations. Even though in vivo tests are preferred, they are limited by many factors, for example hormonal influences (Humphrey, 1995). Ex vivo “uniaxial tests” can provide basic information, however, they can not completely quantify the anisotropic behaviour of the aortic wall (Hayashi, 1993). Ring tests, meaning uniaxial testing on intact circumferential aortic segments, also do not suffice (Cox, 1983).

1.2 Pathology of the aorta

1.2 A Basic concepts

Arterial vascular diseases are the leading cause of human mortality in the modern world. The following section includes some important definitions of regularly used terms in cardiovascular medicine.

*Arteriosclerosis:*
Currently, arteriosclerosis is classified into 3 forms: atherosclerosis, Mönckeberg’s medial calcific sclerosis and arteriolosclerosis (Kumar et al., 2014).

Atherosclerosis:
Different terms such as atheromas, protruding atheromas, atherosclerotic debris and plaques have been used in medical literature to describe aortic atherosclerosis (Kronzon and Tunick, 2006). This condition implies thickening of the arterial walls, in general, but includes the aortic wall. Fatty streaks are the first detectable lesions and contain macrophage foam cells that are derived from recruited monocytes. More advanced atherosclerotic lesions, called fibrofatty plaques, are composed of monocytes and proliferating smooth muscle cells (Lucas and Greaves, 2001).
Mönckeberg’s medial calcific sclerosis: implies a mineralization process of the tunica media (McCullough et al., 2008).

Arteriosclerosis: is not further discussed as it includes thickening of only small arteries.

*An aortic aneurysm is defined as a focal dilation of the aorta with a cross-sectional diameter greater than 1.5 times its normal size (Santilli and Santilli, 1997). There are two types of aneurysms (Figure 10):

A true aneurysm is an expansion of the entire vessel wall, lined by an intact tunica intima and caused by weakening of the tunica media (Maxie and Robinson, 2007). A true aneurysm can have a saccular or a fusiform shape (Figure 10).

A false/pseudo aneurysm (PSA) denotes a ruptured aortic wall with encapsulation of the extravasated blood by fibrous tissue (Maxie and Robinson, 2007) (Figure 10).

![Figure 9](www.flandershealth.us)
Left: true aneurysm 1: saccular – 2: fusiform
Right: pseudoaneurysm

*An aortic dissection starts with a laceration of the tunica intima and the inner layer of the tunica media. An entrance tear is formed and blood can enter the media and split this layer in two with the formation of an intramural haematoma. This splitting of the tunica media eventually leads to the formation of a double-channelled aorta (Coady et al., 1999) (Figure 11). A dissection typically occurs at the junction of the middle and outer third of the media due to weakening of the region by vascular ischemia (Woerner, 1959).
A periaortic haematoma reflects a contained aortic leak within the periadventitial tissues (Gleason and Bavaria, 2003) (Figure 12).

*An aortic intramural haematoma (Figure 13) occurs when there is: a spontaneous haemorrhage from vasa vasorum into the media; a penetrating atherosclerotic ulcer; or
blunt chest trauma. This phenomenon can progress either to outward rupture of the aortic wall or to aortic dissection (Macura et al., 2003).

Figure 12.
Aortic intramural haematoma
(Jani et al., 2007)

1.2.B Pathology of the aorta in humans

Aortic Aneurysm and Dissection Disorders (ADD) are a leading cause of morbidity and mortality in the modern world despite advances in diagnostic and surgical treatments (Lilienfeld et al., 1987). This condition can be triggered by several factors including age, hypertension, inflammation and connective tissue anomalies. Thoracic ADD mainly occurs in association with specific genetic disorders, for example Marfan syndrome (De Backer et al., 2009).

a) Aortic aneurysm

Aortic aneurysms are caused by segmental weakening of the wall due to a primary or secondary defect in the matrix structures and can eventually lead to rupture of the aortic wall (Figure 14). Loss of elastin has long been considered the hallmark of aneurysm formation, but it is now accepted that impaired collagen homeostasis is the main cause (Thompson et al., 2002). Thoracic aortic aneurysms (TAA) can involve the ascending aorta (60%), the aortic arch (10%) or the descending aorta (40%). These numbers do not add up to 100% as patients with TAA can develop aneurysms at multiple locations. The ascending aorta is predisposed most likely because of the highest biomechanical forces at this location (Wang et al., 2010).
Figure 14.
Schematic representation of the cascade of events leading to aneurysm formation and rupture (Martufi et al., 2016).
A: recirculation and flow disturbances create low and oscillatory wall shear stress patterns that may promote wall damage and further dilatation
B: changes in mechanical environment cause elevated stress in the aortic wall, which in turn may lead to histologic changes and weakening of the wall
C: when the stress acting on the aortic wall exceeds the strength of the wall, rupture ensues

The most striking histologic features of aneurysmatic aorta are found in the tunica media and intima. Lesions include accumulation of lipids in foam cells, extracellular free cholesterol crystals, calcifications, adventitial inflammatory infiltration, thrombosis and ulcerations and rupture of the layers (Treska et al., 1999).

TAA are mainly seen in young people and are typically associated with an inherited connective tissue disorder (Reed et al., 1992; Jain et al., 2011). Apart from genetic disorders of collagen and elastin, a familial predisposition has been shown in non-Marfan patients (familial clustering) (Elefteriades, 2002). Marfan syndrome (MFS) is the most well-known connective tissue disorder in humans associated with elastolysis resulting in aneurysm formation (Figure 15). MFS was first described in 1896 and is an autosomal dominant disorder caused by mutations in the fibrillin-1 (FBN1) gene (major constituent of extracellular microfibrils). These mutations cause cardiovascular, ocular and skeletal abnormalities (Dietz et al., 1991). The incidence is approximately 2-3 per 10,000 individuals (Judge and Dietz, 2005). The decrease in FBN1-containing microfibrils results in inappropriate release of bioactive TGF-β. The latter activates the Smad cascade at the level of the smooth muscle cell nucleus, increasing transcription of messenger RNA for matrix metalloproteinases and proteoglycans (McLoughlin, 2012).
Aortic aneurysms in Marfan patients are due to the loss of tensile strength from lack of fibrillin connections together with inflammation and a diminished aortic medial layer (Pereira et al., 1999). Patients are predisposed to develop a potentially fatal aortic dissection (van Karnebeek et al., 2001). Cystic medial degeneration is a typical histological finding. The severity of this lesion varies greatly from one patient to another (Jain et al., 2011). Smooth elastic laminae, defective type I collagen synthesis, disorganized medial collagen fibres, increased collagen deposition, smooth muscle cell apoptosis and decreased elastin concentrations are other typical hallmarks (Nienaber and Eagle, 2003). Other genetic conditions that are associated with thoracic aortic aneurysm and sometimes dissection include: Loeys-Dietz syndrome, Arterial Tortuosity syndrome, Bicuspid aortic valve syndrome, Turner syndrome, vascular Ehler’s Danlos syndrome, homozygous familial hypercholesterolemia, autosomal polycystic kidney disease, Noonan syndrome, Tetralogy of Fallot, familial thoracic aortic aneurysm and dissection, coarctation of the aorta, autosomal recessive cutis laxa and Shprintzen-Goldberg syndrome (Jain et al., 2011).

b) Aortic dissection

Population based studies estimate the incidence of acute aortic dissections (AAD) to be 3 cases per 100,000 people annually (Meszaros et al., 2000; Olsson et al., 2006). Thoracic aortic dissections rank among the most lethal vascular diseases as 21% of patients die
before reaching the hospital (Meszaros et al., 2000). There is a variety of ADD in the populations, depending on the prevalence of risk factors (Khan and Nair, 2002). Aortic dissections are classified according to the origin of the initial intimal tear. Type A dissection initiates in the ascending aorta while type B starts in the descending aorta. Without surgical intervention, progressive dilation (aneurysms) of the ascending aorta can result in a Type A dissection, and thus, are associated conditions (Pannu et al., 2005). Type A dissections usually occur in young patients with connective tissue disorders characterized by elastic tissue degeneration and fragmentation. Descending thoracic aortic dissections (Type B), on the other hand, occur in older people who often have a history of hypertension (Wu et al., 2013). A circadian and even a seasonal variation have been mentioned (Bilfinger, 2010). Cystic medial necrosis, which is associated with connective tissue disorders, was once believed to contribute to the development of atraumatic aortic dissection. Only a minority of patients however present medial degeneration (Larson and Edwards, 1984).

Thoracic aortic dissections are characterized by fragmentation of elastic fibres (Borst et al., 1996) and reduction in overall elastin content (Wang et al., 2005). It was recently shown that inflammatory pathways underlie aortic dissections. Apart from vascular SMC apoptosis and ECM destruction, an increased degree of inflammation can be noticed. Macrophages release matrix metalloproteinases (MMPs) and pro-inflammatory cytokines, resulting in matrix degradation and neoangiogenesis (del Porto et al., 2010; Cifani et al., 2015). ECM fragments could possibly function as chemoattractants for immune cells (Weathington et al., 2006), as there is an increased infiltration of T lymphocytes, macrophages, mast cells and neutrophils (He et al., 2006). Increased expression of mainly MMP-1, -2, -7, -9 and to a lesser extent MMP-11, -14 and -19 have been measured (Wang et al., 2006).

Several predisposing conditions for aortic aneurysm and/or dissection are described:

- **Chronic hypertension** causes intimal thickening, fibrosis, calcification and extracellular fatty acid deposition. Intimal thickening, as well as adventitial fibrosis and fibrosis of vasa vasorum, lead to a decreased oxygen supply resulting in necrotic smooth muscle cells and fibrosis. The latter implies increased stiffness of the vascular wall and vulnerability to pulsatile forces, which predisposes the formation of aneurysms and dissections (Larson and Edwards, 1984; Reed et al., 1992). Hypertension-related spontaneous rupture of vasa vasorum can lead to an intramural haematoma (IMH), which weakens the aortic media. This can lead to an intimal tear, subsequently resulting in blood from the lumen entering the media (Coady et al., 1999). Hypertension can also lead to increased expression of MMPs altering the ECM structure (Ishii and Asuwa, 2000).
Smoking, dyslipidaemia, weightlifting, severe physical exercise and cocaine abuse are known risk factors (Jain et al., 2011). Smoking is a major risk factor in the development of cardiovascular disease as cigarette smoke can induce vascular damage and dysfunction (Pini et al., 2016). Chronic cocaine abuse can lead to a decrease in elastic properties of the aorta and, in rare cases, predispose the aorta to rupture (Bigi et al., 2008). Weightlifting and severe physical exercise are other predisposing factors because of the rapid and dramatic elevation of blood pressure during the intense exercise (Hatzaras et al., 2007).

Atherosclerosis is a major cause of mortality worldwide as the formed plaque can possibly rupture, erode or mineralize (Fleg et al., 2012). However, in only a small number of cases, an association between the atheroma and the location of the dissection can be noticed (Coady et al., 1999). The calcification of the plaque can possibly be due to improper vitamin K-dependent γ-carboxylation (Li et al., 1998).

Inflammatory diseases lead to destruction of the tunica media resulting into weakening and possible dissection or aneurysm formation. Autoimmune diseases may affect the vasa vasorum and lead to nutrient deficiency of the vascular wall (Nienaber and Eagle, 2003). Takayasu arteritis (TA) (typically associated with transmural fibrosis), giant cell arteritis (GCA) and isolated arteritis are all reported inflammatory diseases of the aorta (Jain et al., 2011).

The male gender correlates with aortic dissection, as well as advanced age (Nienaber and Eagle, 2003). The thoracic aortic diameter and wall thickness are higher in old mice and there is an increase in collagen content with age (Wheeler et al., 2015). The latter results in a reduced compliance (Kovacic et al., 2011). Aortic aneurysms also occur more frequently in men, but the aortic aneurysms in women rupture at a smaller diameter compared to men. Affected women are also generally older compared to the male patients and suffer from more cardiovascular defects (Skibba et al., 2015). Gender differences in aortic compliance have been reported as the decreased abdominal aortic wall distensibility with ageing occurs at an earlier age in men. This suggests that the abdominal aorta in men is more prone to degenerative changes (Sonesson et al., 1994).

For patients who are at risk of aortic dissection, aortic root movement during the heart cycle is an important risk factor. The aortic root is displaced downward during systole and returns to its previous position in diastole. This downward displacement of the aortic root may cause tears in the aortic wall (Beller et al., 2004). The degree of aortic curvature has also been mentioned as a possible cause (Pouillis et al., 2008).

Pregnancy-related aortic dissection is very rare unless the patient is affected by a connective tissue disorder. Pregnant women are also predisposed to develop hypertension, another risk factor for aortic dissection (Nienaber and Eagle, 2003).

Patients with congenital vascular diseases (e.g. bicuspid aortic valve disease) are at higher risk of developing thoracic aortic dissection (Larson and Edwards, 1984).
c) Traumatic aortic injury

*Blunt traumatic aortic injury (BTAI) is part of a spectrum of diseases termed acute aortic syndrome and accounts for 20% of road traffic accident related deaths (Pearson et al., 2008). BTAI includes: intimal flap, sub-intimal haematoma, intra-mural haematoma, localized dissection and pseudoaneurysm (Ahmad et al., 2006). Traumatic aortic transection (complete transverse tearing of the aorta) from BTAI (specifically, motor vehicle accidents), occurs in 15% of cases. Eleven percent of BTAI patients also develop a periaortic haematoma with the blood maintained in the aorta (Gleason and Bavaria, 2003).

Transverse laceration of the aorta occurs, most commonly at the isthmus region (between the left subclavian and the third intercostal artery) where the aorta is fixed by the ligamentum arteriosum (Gammie et al., 1998; Shkrum et al., 1999; Macura et al., 2003, Pearson et al., 2008). The aortic isthmus appears to be an inherently weaker spot as was proven by the tensile tests on aortic samples conducted by Lundevall (1964). The aortic isthmus could be more susceptible due to a higher density of tributary vessels at this point. The avulsion of these tributary vessels during trauma could be a contributing factor (Field et al., 2007).

The rupture is typically circumferential, on the inner side of the aorta and appears to initiate on the inner wall of the aorta extending towards the outer wall (Richens et al., 2002).

All the hypotheses of BTAI are based on: a) mechanical deformation, b) intraluminal hypertension and c) mechanical characteristics of the aortic wall (Richens et al., 2002). The mechanism most likely corresponds to a complex combination of motion of the structures within the thorax and local loading of the tissues, as a result of their anatomy or the impact (Pearson et al., 2008). A pressure spike secondary to chest compression can contribute but is unlikely the primary cause of the aortic injury (Pearson et al., 2008).

*Iatrogenic aortic dissection is usually associated with catheter invasion or valve/aortic surgery (Larson and Edwards, 1984).

*Penetrating aortic rupture (eg shotgun or stab wound): has been associated with mortality rates greater than 90% as the majority of patients die at the scene (Demetriades et al., 1996).

d) Aortopulmonary fistulation

Aortopulmonary fistulation is a very rare condition and is usually found in association with a chronic ascending thoracic aortic (pseudo)aneurysm following trauma, inflammation, atherosclerosis or aortic surgery (Dossche et al., 1999; Belkin et al., 2003). A study of 4,000 cases of thoracic aortic aneurysms revealed that only 3.7% ruptured into the pulmonary artery (Kort et al., 2001). In very rare cases, an aortopulmonary fistula can evolve from an acute aortic dissection (Piciche et al., 1999). Erosion of the aneurysm or pseudoaneurysm into the pulmonary artery is most likely due to continuous pulsatile friction between the aneurysmatic wall and the right pulmonary artery. The left-to-right shunting results into pulmonary hypertension and cardiac insufficiency (Bol et al., 2006).
The aortopulmonary fistulas however may sometimes be asymptomatic and pain is an unusual feature (Da Valle et al., 1985).

e) Aortobronchial fistulation

Aortobronchial fistulas are rare complications following post cardiac surgery and are associated with the formation of a pseudoaneurysm. Haemoptysis, intermittent or massive, is usually the main symptom of an aortobronchial fistula (Piciche et al., 2003). More than two thirds of the aortobronchial fistulas occur in male patients and the incidence generally increases with age (MacIntosh et al., 1991).

f) Aorto-cardiac fistulation

Aorto-cardiac fistulation is mostly reported in men (Eagle and De Sanctis, 1992). This condition can be caused by an uncommon congenital sinus aortic aneurysm, consisting of a separation or lack of fusion between the media of the aorta and the annulus fibrosis of the aortic valve (Friedman, 1988). Acquired sinus aortic aneurysms can also fistulate into the heart and are associated with several aortic diseases (syphilis, bacterial endocarditis) (Jones and Langley, 1949).

1.2.C Pathology of the aorta in other animal species

Aortic rupture is generally rare in animals. In dogs, a few cases of aortic rupture have been described (Boulineau et al., 2005; Sanger et al., 1980). In cats, only isolated cases have been reported (Scollan and Sisson, 2014). In turkeys, swine and cattle, aortic rupture is more regularly encountered compared to other animal species.

a) Poultry

Aortic rupture is typically seen in young, male, fast-growing, heavy turkeys. It has also been described in chickens, waterfowl and ostriches (Van Veen, 1999). Losses usually reach 1-2% (Van Veen, 1999; Crespo et al., 2003). Known predisposing factors are high blood pressure and fast growth rates (Peckham, 1984). Sudden stress, anxiety and nutrition rich in proteins and fat could also play a role in the disease (Mitchinson and Keymer, 1977). Deficiencies in copper and zinc are mentioned as important contributing factors (Carlton and Henderson, 1963; Minor, 1980). In the case of copper, it is a cofactor of lysyl oxidase, which is essential in the cross-linking of elastin and collagen (Carlton and Henderson, 1963). Normal cross-linking is, in turn, essential for providing resistance to elastolysis and collagenolysis by proteinases (Romero et al., 1989). Intoxication by the ingestion of Beta-aminoproprionitrile (lathyrism) can also lead to aortic rupture by negative impact on collagen cross-linking (Simpson et al., 1970). In poultry, lesions usually present as ruptured aortic aneurysms resulting in a fatal intracoelomic haemorrhage (Van Veen, 1999). The rupture is usually found in the outer third of the media. Ruptures of the thoracic aorta are less frequent and are located in the midzone of the media (Neumann and Ungar, 1973). Intimal plaques, composed of fibrous tissue, fibro-cellular tissue or mainly smooth muscle cells, are typically seen near the aortic rupture in poultry (Neumann and Ungar, 1973; Crespo and Shivaprasad, 2003). Intimal deposition of fat tissue and even mineralized cartilage have also been described (Neumann and Ungar,
1973; Pritchard et al., 1958). Intimal plaques are a common phenomenon in young turkeys, as it is seen in 88.2% of turkeys aged two months (Neumann, 1968). Histologically, the ruptured aneurysms in poultry are characterized by medial changes: loss of elastic laminae, an increase in ground substance, proliferating fibroblasts and oedema with laceration of degenerated smooth muscle cells (Crespo and Shivaprasad, 2003). In copper deficient poultry and in lathyrism, the fragmentation and separation of the elastic laminae is prominent (Carlton and Henderson, 1963; Simpson et al., 1971). Transmural medial necrosis and increased collagen deposition have also been reported in turkeys dying from aortic rupture (Shipravasad et al., 2004). The lesions are mostly located in the abdominal aorta, which lacks vasa vasorum, and is associated with intimal plaques. Thus, the ruptures are most likely due to ischemia of the wall that leads to decreased elasticity (Neumann and Ungar, 1973).

b) Bovines

*Bovine Marfan Syndrome
Aortic rupture in bovines is mainly associated with the Bovine Marfan Syndrome (BMFS). This is an autosomal dominant inherited disease that is very similar to the human counterpart and is most likely due to a defective microfibrillar metabolism. Affected calves show skeletal, ocular and cardiovascular defects (Besser et al., 1990). These animals typically die between the age of 13 and 33 months (Potter and Besser, 1994). The aortic rupture is located in the ascending aorta and the aortic arch. Four categories of gross cardiovascular lesions can be differentiated (Potter and Besser, 1994):

a) moderate dilation of the aorta without the presence of an aneurysm or rupture
b) aortic rupture resulting in cardiac tamponade
c) ruptured aneurysm of the aorta and pulmonary artery
d) rupture of the pulmonary artery

Morphologically, there is prominent degeneration and fragmentation of abnormal short and thick elastic laminae in the tunica media of affected animals (Potter and Besser, 1994). Elastin concentration and elastin-associated cross-links are not significantly decreased, while there is an increase in collagen, suggesting a diminished aortic compliance (Parsons et al., 1992). In contrast to the human disease, cystic medial necrosis is absent in the affected calves, correlating with the normal elastin concentration (Parsons et al., 1992). This absence of cystic medial necrosis in BMFS could be caused by species-related differences in response to injury (Potter and Besser, 1994).

*Idiopathic arterial aneurysm in dairy cattle:
Holstein Friesians are predisposed for arterial aneurysms. A retrospective study by Lamm et al. (2007) described 33 cases, with ages varying from 2.5 to 5.5 years. The intense management and high production might be causative factors. There is usually dilation and rupture of the abdominal aorta or one of its branches (mesenteric, left gastric, celiac artery,...) leading to a fatal haemobdomen. The tunica media shows a decreased amount of disrupted and fragmented elastic fibres. Intimal and smooth muscle cell hyperplasia, medial mineralization and increased deposition of mucinous substance in the media are also reported. Granulation tissue and haemorrhage can be found at the site of the rupture (Lamm et al., 2007). A recent study by Wessels et al. (2014) described 75 cases of idiopathic arterial aneurysms in Holstein-Friesian cattle. Aneurysms are typically
found in the cranial mesenteric or coeliac arteries and in the abdominal aorta. Histological hallmarks are medial degeneration and disorganization, intimal hyperplasia and vasa vasorum perivascular infiltrations. Ruptures of abdominal arteries have been reported in certain genetic lines (Schuiringa-Sybesma, 1961). A deficiency of iron has more recently been suggested as a possible causative factor (Lamm et al., 2007).

c) Swine

Copper-deficient diets can cause death by rupture of the aorta or heart (Shields et al., 1962). Aortic ruptures in swine are typically found in the aorta ascendens resulting in cardiac tamponade (Maxie and Robinson, 2007). Macroscopic lesions consisting of cracks and fissures can also be noticed in the abdominal aorta, however not resulting in rupture. A prominent increase in mucopolysaccharides and ruptured elastic fibres are associated lesions. Medial necrosis is sometimes reported and a causative role of the vasa vasorum has been suggested. A decrease in tensile strength of affected aortas was demonstrated (Shields et al., 1962).

1.2.D Aortic rupture in horse breeds other than Friesian horses

a) Aortic rupture

Rupture of the aorta is generally very uncommon in horse breeds other than Friesian horses and is most commonly reported in stallions, sometimes during coitus or shortly thereafter (Rooney et al., 1967). The majority of the reports on aortic ruptures in non-Friesian horses are old and are possibly subjected by reporting bias as stallions, especially those used for breeding, are more likely to undergo veterinary or post-mortem examination because of their high financial value. Several sport horses acutely died of aortic rupture during an international event, for example Cypriano, Hickstead and Admire Ratki. The massive negative international attention that is associated with such an abrupt tragedy is of great concern to the horse industry. In an international multicentre study of post-mortem findings in 268 Thoroughbred racehorses dying acutely during exercise, only 2 cases of aortic rupture were diagnosed (Lyle et al., 2011). Two other studies reported a combined incidence of only 3 of 984 dead young racehorses attributable to aortic rupture (Johnson et al., 1994; Baker et al., 1981). A recent review by Briceno et al. (2015) mentioned a total of 137 cases of aortic rupture in horses during a time period of 28 years (1986-2014). Twenty-five of these horses were Friesian horses (Briceno et al., 2015).

Tears can occur in the ascending aorta at any level from the aortic valvular ring to the branching of the branchiocephalic trunk (Maxie and Robinson, 2007). In most cases, the aortic tear can be found close to the semilunar valve (Rooney et al., 1967). Terms such as “aortic ring rupture” and “aortic root disease” have also been used to describe the same rare condition (Rooney et al., 1967; Sleeper et al., 2001). Rooney et al. (1967) described 8 cases of aortic rupture in stallions of which 4 died suddenly post copulation. Other reports (Marr et al., 1998; Lester et al., 1992) presented cases which survived for months or even years after the initial critical period. As haemorrhage can start at different sites, consequences can vary. A haemopericardium with sudden death occurs in most cases (Van Vleet and Ferrans, 2007). Haemorrhage into or disruption of the atrioventricular node or bundle of His can also cause acute death (Maxie and Robinson, 2007). An aorto-
cardiac fistulation is often found in cases of aortic rupture penetrating into the heart. The fistula most commonly arises from the right aortic sinus and then dissects into the right ventricle (Figure 16). Less often, the rupture penetrates into the right atrium, tricuspid valve, septum or left ventricle (Marr et al., 1998). An aneurysmal dilation of the aortic sinus sometimes precedes the thoracic aortic rupture. Aorto-cardiac fistulation is primarily reported in middle-aged stallions in acute distress with exercise intolerance and tachycardia (Marr et al., 1998). Both intact (Reef et al., 1990) and ruptured (Marr et al., 1998; Sleeper et al., 2001) cases of aneurysms of the sinuses of Valsalva have been reported. In humans as well, the right coronary sinus is the most common site to tear or dilate with subsequent rupture into the right ventricle or right atrium. The coronary sinus aneurysms are considered to be congenital in humans and males are predisposed (male to female ratio = 3:1) (Yilmaz et al., 1997). They are attributed to a congenital lack of continuity between the aortic media and the aortic annulus, resulting in aneurysmal dilation (Van Son et al., 1995).

Aortic root rupture in horses has been attributed to hypertension at the time of copulation or exercise combined with pre-existing degeneration of the aortic media (Rooney et al., 1967). In some cases however, there is no histological evidence of medial degeneration (Marr et al., 1998). Briceno et al. (2015) suggested degenerative changes to discrete elastic fibres in the intima of the aortic root to be a predisposing cause for aortic rupture (Briceno et al., 2015). Intense exercise seems to be predisposing. It is estimated that impulsive forces of more than 100 kPa are applied to the chest wall by each scapula when a 500 kg horse is galloping (Schroter et al., 1998).

Lester et al. (1992) suggested that the aorta would be at its thinnest and thus weakest just dorsal to the semilunar valve (Lester et al., 1992). As the tear is typically located in the chondroid fibrous tissue of the aortic root, it is possible that this is an anatomical-mechanical weak spot. At the end of systole, the heart is pulled down. Meanwhile the thoracic aorta lies strongly fixated against the vertebrae. This could lead to high tension at the base of the aorta resulting in rupture during exercise (Shirai et al., 1999). An elevated blood pressure could even increase the tensile force by the recoiling column of the blood (Rooney et al., 1967).

Others believe that tearing of the aorta in horses is often caused by falling of the horse on the chest and is thus traumatic. This sudden trauma could bend the aorta during the early diastole when the valve closes and elastic recoil begins (John King, personal communication).
Figure 16.
Upper: Aortic rupture (arrow) in a Friesian horse, typically further away from the aortic valve.
Under: Aortic rupture (arrow) in the right aortic sinus in a warmblood horse.
b) Aortic aneurysm

With weakening of the aortic tunica media, a progressive dilation (aneurysm) can occur (Eagle and Sanctis, 1992). Aortic aneurysms in the horse are often located at the sinuses, as described above (Marr et al., 1998). Aneurysms of the abdominal aorta have been associated with infection of the umbilical artery in foals (Archer et al., 2012). Verminous aneurysms have also been reported (Greatorex, 1977) and are usually located at the cranial mesenteric artery in association with Strongylus vulgaris infestations (Rooney and Robertson1, 1996). In rare cases, these parasites are associated with aneurysms of the thoracic aorta (Simoens et al., 1999) (Figure 17). Mycotic aneurysms have seldom been described in horses (Okamoto et al., 2007). Extensive intimal thickening of the vasa vasorum has been mentioned as a predisposing factor in dissecting aortic aneurysms (Shirai et al., 1999).

![Figure 17. Equine thoracic aortic aneurysm associated with Strongylus vulgaris larvae. Arrow: larva.](image)

Rupture of the pulmonary artery is less common than aortic rupture and is a possible complication of mitral regurgitation (Reef et al., 1998). It has also been reported in association with a patent ductus arteriosus (Buergelt et al., 1970).
d) Aortopulmonary fistulation

Only 2 cases of aortopulmonary fistulation in non-Friesian horses have been described so far (Holmes et al., 1973; van der Linde-Sipman et al., 1985). A true aortic aneurysm rupturing into the left pulmonary artery was reported in a 4-year-old hunter gelding. The ligamentum arteriosum was not involved in the aneurysm and a possible cause of the lesions was not identified (Holmes et al., 1973). The other horse was a three-year old Dutch halfbred suffering from a true aneurysm between the carotid trunk and the insertion of the ligamentum arteriosum (van der Linde-Sipma et al., 1985).

e) Aortitis

Aortitis is a very rare condition in horses (Diaz et al., 2000). It is most commonly associated with bacterial aortic valve endocarditis (Reef, 1998). *Strongylus vulgaris* infection has also been described as a possible cause (Cranley and McCullagh, 1981).

f) Aortic calcification

Atherosclerosis does not seem to occur in horses (Rooney and Robertson, 1996). Cases of aortic arteriosclerosis in horses are very rare (Rothenbacher and Tufts, 1964; Pauli, 1973). In a morphological study of the aorta and pulmonary artery of thoroughbred racehorses (1-5 years old), degenerative and sclerotic changes were regularly noticed. The severity of the lesions seems to be associated with the racing career of the horse rather than with age (Imaizumi et al., 1989). Mainly the tunica media was affected, but also the tunica intima and tunica adventitia were sometimes involved (Arroyo et al., 2008). The calcification seems to be a bio-mineralizing phenomenon rather than a degenerative process. It is associated with damaged extracellular matrix components (Arroyo et al., 2008). Increased wall stress due to arterial geometry and exercise-induced hypertension are predisposing for arterial wall mineralization (Teeter et al., 2010). It is suggested that these medial changes could be due to ischemia as concurrent lesions of the vasa vasorum and vascular nerves in the wall of these vessels are regularly encountered (Imaizumi et al., 1989).

Thurman et al. (1984) described a case of a Quarter foal with dirofilariasis and arteriosclerosis. Histologically, the lesions in the pulmonary vasculature resembled those described as ‘proliferative endarteritis’ in dogs infected by *Dirofilaria immitis*.

g) Aortico-pulmonary window

An incomplete development of the spiral septum, normally dividing the pulmonary trunk and aorta, results in a communication between the two great vessels, called an aortico-pulmonary septal defect or aortopulmonary window (Noden and de Lahunta, 1985). This very rare defect has been reported in a 4-day-old thoroughbred foal (Valdes-Martinez et al., 2006).
1.3 The Friesian horse

1.3.A History of the breed

The Friesian horse breed is native to and the oldest breed in the Netherlands. In 1879, the studbook, “Het Koninklijk Friesch Paarden-Stamboek”, was founded. Friesian horses are called the “Black Pearls” because of their complete black colour, majestic front, elevated gaits and generous mane and feathering. Reports of these black horses go back to the medieval times when they were used by knights (Douma, 1994). In the following time period, Friesian horses were mainly used as farm horses. They were more short-legged and compact with a broad chest compared to their ancestors. In the early 1900s, the increased agricultural mechanization caused a decline in the horse population. By the sixties, the population had declined to historically low numbers. Thanks to its friendly character and the support of the studbook, the Friesian horse breed was kept alive. Today, more than 70,000 Friesian horses are registered worldwide. An estimated 1,000 of them are in Belgium. Friesian horses are particularly used under saddle and in front of the carriage. The “uphill” built with elevated knee action is typical for the Friesian horse. The angled and long shoulder provides a far extension of the forelegs. Friesian horses also typically have a more vertical neckline compared to the regular riding and driving horses, for example the Belgian Warmblood horses. Sloping hindquarters, low-set tails and rather short limbs are other breed characteristics of the Friesian horse (Figure 18).
Figure 18.
Typical conformation of a Belgian warmblood horse (above) versus a Friesian horse (below).
1.3.B Genetic traits in the Friesian horse

As mentioned higher, the Friesian horse breed almost became extinct (Osin ga, 2000). In 1913, only 3 older stallions were left for breeding and in 1965, 500 mares were registered. Approximately 2.5% of the registered stallions were used for breeding and the “popular sire’s effect” had a severe influence (Sevinga et al., 2004). Luís et al. (2007), studied the genetic diversity in 33 horse breeds. Friesian horses showed the lowest heterozygosity (0.454) and gene diversity (0.466) for the microsatellite loci. An inbreeding rate of the total population of 1.9% per generation (mean generation interval of 9.6 yrs) was noted from 1979 to 2000 (Sevinga et al., 2004) (Figure 19). The computed inbreeding rate of the Friesian horse breed thus exceeds the Food and Agriculture Organization of the United Nations (FAO) limit of 1% per generation (FAO, 1998). This limit is set to maintain the remaining genetic variation in a breed and restrict the potential negative impact of inbreeding. In a study of 35 horse populations, the Friesian horse breed was the most inbred population due to genetic isolation (van de Goor et al., 2011). In the Netherlands, approximately 7% of the horse population is Friesian. Some clinical problems seem to have a higher incidence compared to other horse breeds (van Vliet and Back, 2004). A genetic basis has been confirmed for some diseases and is suspected for others.

![Inbreeding coefficient in the Friesian horse population from 1863 to 2000 (Sevinga et al., 2004)](image)

Figure 19.

Inbreeding coefficient in the Friesian horse population from 1863 to 2000 (Sevinga et al., 2004)
*Hydrocephalus*

Hydrocephalus is an uncommon condition in horses. However, Friesian horses have shown a higher incidence compared to other horse breeds (Sipma et al., 2013) (Figure 20). The presence of a distorted, non-functional jugular foramen appears to lead to a communicative hydrocephalus with a reduced absorption of cerebrospinal fluid into the systemic circulation (Sipma et al., 2013). A nonsense mutation in B3GALNT2 has recently been shown to be associated with hydrocephalus in Friesians. The mode of inheritance is autosomal recessive (Ducro et al., 2015).

![Figure 20. Friesian foal with hydrocephalus (Sipma et al., 2013)](image)

*Dwarfism*

Dwarfism is a congenital defect with an estimated incidence of 0.25% in Friesian horses (Osinga, 2000). Friesian dwarf foals have a characteristic disproportional growth disturbance with physeal growth retardation in limbs and ribs. Their body-weight is 50% lower compared to age-matched normal Friesian foals. Dwarfs typically have hyperextension of the fetlock joints (Figure 21). Histological examinations of the growth plates at the costochondral junction show similar findings as seen in osteochondrodysplasia (Back et al., 2008). Tendon laxity of control Friesians is intermediate between those of ponies and dwarf Friesians (Gussekloo et al., 2011). Using high-density single nucleotide polymorphism (SNP)-based genotyping arrays, a region on chromosome 14 that is strongly associated with the dwarf phenotype has been identified (Orr et al., 2010).
*Orthopaedic problems*
A breed-specific variation in tendon properties has been demonstrated in Friesian horses (Gussekloo et al., 2011). This increased tendon and ligament laxity in Friesian horses could lead to a more horizontal position of the fetlock, which could ease ossification of the hoof cartilages (Dakin et al., 2010; Boerma et al., 2012). Bilateral hip dysplasia has been encountered in Friesian foals as well and a genetic background is suspected (Hermans et al., 2010). Friesian horses also suffer from an increased prevalence of axial osteitis and intersesamoidean ligament desmitis (Voermans et al., 2009). This condition carries a poor prognosis (Brommer et al., 2014).

*Decreased performance of the reproductive system:*
A high inbreeding rate has been associated with a decreased performance of the reproductive system in Friesian horses, such as underdeveloped ovaries (van der Mey, 1979), cryptorchidism (van der Mey GJW, 1979), low sperm quality (Colenbrander et al., 1992) and retained placenta (Sevinga et al., 2004). In the general horse population, the incidence of retained placenta is about 2-10% (Provencher et al., 1988), while in Friesian mares it reaches 54% (Sevinga et al., 2003). This higher incidence could at least partly be due to the high inbreeding coefficient (Sevinga et al., 2004).

*Megaoesophagus/Oesophageal dysfunction:*
Megaoesophagus is characterized by chronic dilatation and atony of the oesophagus (Figure 22). Friesian horses seem to be predisposed for this condition and a hereditary basis is suspected (Broekman and Kuiper, 2002). Affected horses develop oesophageal obstruction or milder signs such as loss of appetite, wasting, salivating and mild colic. It is mostly seen in young horses and even foals of only one week old can be affected (Boerma and Van Oldruitenborgh-Oosterbaan, 2008). Muscular hypertrophy of the oesophagus has
been demonstrated (Komine et al., 2014), but is not necessarily present in affected cases (Ploeg et al., 2015). In a recent study by Ploeg et al. (2015), only 12 animals of the 18 horses with clinically observed megaesophagus suffered from oesophageal dilation at necropsy. An underlying genetic neuromuscular disorder has been suggested (Van der Kolk et al., 2011) while others suspect a causative role for collagen (Komine et al., 2014; Ploeg et al., 2015).

![Laterolateral radiograph of a megaoesophagus after barium ingestion in a Friesian foal.](image)

*Insect bite hypersensitivity*
A study performed by van Grevenhof et al. (2007) showed that the prevalence of insect bite hypersensitivity is about 18% in the Friesian horse population in the Netherlands, compared to 8% in Shetland ponies (van Grevenhof et al., 2007). A large study in Dutch Friesian broodmares showed that genetic and permanent environmental factors affect insect bite hypersensitivity in Dutch Friesian horses. The dam affects the development of the disease in her offspring through an additive genetic influence but also by being part of the foal’s rearing environment (Schurink et al., 2011).

*Neonatal isoerythrolysis*
An unusual presentation of neonatal isoerythrolysis has been reported in 3 Friesian foals. In all cases, the mares had haemolytic alloantibodies that were not attributable to a specific antigenetic group. Further research is needed to find out if a genetic component was present in these patients (De Graaf-Roelfsema et al., 2007).

*Figure 22.*
Laterolateral radiograph of a megaoesophagus after barium ingestion in a Friesian foal.
*Chronic progressive lymphedema

Chronic progressive lymphoedema (CPL) is a well-known condition in draught horses and is characterised by an altered elastin metabolism (van Brantegem et al., 2007). It is also a very common problem in the Friesian horses (Boerma et al., 2012) (Figure 23). A genetic background of this condition is suspected (De Cock et al., 2009).

![Image of severe chronic progressive lymphedema in a 15-year-old Friesian gelding](image)

*Corneal dystrophy and distichiasis

Corneal dystrophy in Friesian horses is characterized by bilateral symmetric stromal loss and responds well to surgical repair. It is more frequently diagnosed in males and may be a variant of pellucid marginal degeneration. A genetic component is suspected (Lassaline-Utter et al., 2014). Friesian horses also show a breed predisposition for distichiasis, a condition in which the cilia emerge from the eyelid margin (Hermans and Ensink, 2014).

1.3.C Aortic rupture in Friesian horses

Aortic rupture has more frequently been encountered in Friesian horses compared to other horse breeds (Van Vliet and Back, 2006; van Loon et al., 2011; Ploeg et al., 2013). The first multiple-case study of aortic rupture in Friesian horses was described in 1985
(Van Der Linde-Sipman et al., 1985) and encompassed 3 Friesian horses with an aortopulmonary fistula. These horses were descendants of the same sire, suggesting a genetic cause. Histological examination of these three Friesians revealed medial necrosis in both the aorta and pulmonary trunk. This necrosis was attributed to intimal thickening and/or medial fibrosis of the vasa vasorum. A larger case study of aortic rupture and aortopulmonary fistulation in Friesians was recently published by our research group (Ploeg et al., 2013). The clinical and gross post-mortem findings were described in 24 Friesian horses affected by aortic rupture. In all Friesians, a transverse rupture of the aorta was noticed near the ligamentum arteriosum. In the majority of cases, this thoracic aortic tear was associated with a circumferential cuff of perivascular haemorrhage (n=8) and aorto-pulmonary fistulation (n=13). The mean age of the horses was 4 years and there was no gender predisposition. Some cases acutely collapsed (n=3) but the majority of horses showed signs of recurrent (“false”) colic, increased rectal temperature, persistent tachycardia, increased respiratory rate and presence of jugular pulsation. In cases with long-standing heart failure, peripheral oedema and coughing was noticed. Ploeg et al. (2013) also reported that pleural effusion, pulmonary and peripheral oedema, and congestion of the liver were observed on post-mortem examination in such patients with long-standing heart failure. An aortopulmonary fistula can be diagnosed on ultrasound from a left cranial parasternal approach or using transoesophageal ultrasound (de Bruijn et al., 2013). Right heart catheterisation in these cases shows hypertension and an upward oxygen shift in the pulmonary artery, compatible with a left-to-right shunt (van Loon et al., 2011).
References


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CHAPTER 2: SCIENTIFIC AIMS
Chapter 2: Scientific aims

This thesis describes the observations on a naturally occurring disease that specifically affects the aorta in Friesian horses. Friesian horses seem to be the only animal breed in which spontaneous thoracic aortic rupture regularly occurs without infection, inflammation, prior trauma or true aneurysm formation. Clinical and gross lesions have been described in affected Friesians, but large-case morphological studies are missing. The disease is 100% fatal once clinical signs develop and is often not noticed until animals have reached maturity and, possibly, given rise to progeny. Thus, gaining more information concerning the pathogenesis is important. The literature overview of aortic rupture in humans shows that this is a very complex condition. As aortic rupture is a major topic in human medicine, possible causative gene abnormalities discovered in the Friesians could also be useful to better understand human aortic diseases.

The working hypothesis was that aortic rupture in Friesians is due to a genetic defect. The general aim of the present thesis was to thoroughly investigate the pathological lesions underlying aortic rupture in Friesian horses, in order to formulate a hypothesis regarding which underlying genetic defect is present. This should be a valuable support to the “gene hunting” approach in future genetic studies. The ultimate goal is to provide a reliable diagnostic test.

- The first hypothesis was: a local defect is present in a specific structural component which underlies the specific location of the aortic rupture. In order to investigate this first hypothesis, the studies described in chapter 3 and 4 were performed.

- The second hypothesis was: a generalized defect is present in a specific structural component throughout the entire aortic wall, which leads to weakening of the wall and/or is (temporarily) compensated by an increase in the other structural components. In order to investigate this hypothesis, the studies described in chapter 5, 6 and 7 were performed.

The specific aims of this study were:

- obtaining a three-dimensional characterization of the aortopulmonary fistula in Friesians via vascular casting as this information is difficult to gather via ultrasound or standard post-mortem techniques (chapter 3)
- describing the histological changes at the level of the aortic rupture in affected Friesians (chapter 4)
- gaining insight into the compositional changes at the mid thoracic aorta in Friesian and Warmblood horses to elucidate the possible role of elastin, collagen or smooth muscle cells in aortic rupture in Friesian horses (chapter 5)
- evaluating ultrastructural changes in smooth muscle cells at the mid thoracic aorta in Friesians with aortic rupture and control Friesian and Warmblood horses to study the possible role of smooth muscle cells in this disease (chapter 6)
- comparing the biomechanical and biochemical characteristics of the thoracic aorta in affected Friesians against control warmblood and Friesian horses to determine if differences in tensile strength or stiffness in Friesian horses correlate with aortic rupture in Friesians (chapter 7)
CHAPTER 3: THREE-DIMENSIONAL REPLICATION OF AORTOPULMONARY FISTULATION IN FRIESIAN HORSES USING VASCULAR CASTING


V Saey, T Vandecasteele, G van Loon, P Cornillie, M Ploeg, C Delesalle, A Gröne, I Gielen, R Ducatelle and K Chiers
Chapter 3: Three-dimensional replication of aortopulmonary fistulation in Friesian horses using vascular casting

Abstract

Background: Acquired aortopulmonary fistulation is a rare condition in humans. It usually results as a late complication of a true or pseudoaneurysm of the thoracic aorta. It is most commonly associated with trauma or surgery, less commonly with atherosclerosis, inflammation or Marfan’s syndrome. Aortopulmonary fistulation is also seen as a rare complication of acute aortic dissection. On rare occasions, acquired aortopulmonary fistulation is reported in aged patients without any of the above mentioned triggering factors. Thus, these cases should be considered as idiopathic aortopulmonary fistulation. Clearly, the pathogenesis of this condition is not yet completely understood.

Friesian horses are highly inbred and are affected by several genetic conditions. Rupture of the thoracic aorta has a relatively high prevalence in Friesian horses and is often characterized by the formation of a pseudoaneurysm with subsequent fistulation into the pulmonary artery. Affected animals may survive for several weeks to months. Both in vivo and post-mortem visualizations of the lesion are restricted because of the large posture of these animals. An exact topographical description of the lesions could possibly aid in the search for causative genes.

Findings: We performed vascular casting in three affected Friesian horses. In all three cases, an aortic rupture at the caudoventral side of the aorta was connected with a rupture of the main pulmonary artery just proximal to its bifurcation.

Conclusions: Vascular casting showed a consistent location and configuration of the aortic rupture in all three Friesian horses, which is consistent with a genetic factor underlying this disease. As the lesions in Friesian horses are very similar to the human condition, they may represent an important spontaneous model of the pathogenic mechanism of this disease in humans.

Keywords: aortic rupture—pseudoaneurysm—aortopulmonary fistulation—Friesian horses

Introduction

In spite of the relative frequency of thoracic aortic (pseudo)aneurysms and the close anatomical relation between aorta and truncus pulmonalis, aortopulmonary fistulation is very rare in humans. In a review by Boyd (1924), 4000 cases of thoracic aortic aneurysms were studied and only in 4% there was an aortopulmonary fistulation. The diagnosis is made primarily by echocardiography and aortography (Razzouk et al., 1993), followed by further imaging, such as computed tomography (CT) and magnetic resonance imaging (MRI) (Kort et al., 2001). Reports of successful surgical management of aortopulmonary fistulas are scarce due to the magnitude of operative problems encountered (Lahey, 1993; Coselli et al., 1995; Atay et al., 1998).
Aortic rupture is a rare condition in the general horse population. It can occasionally be seen in older breeding stallions (Rooney et al., 1967) and sport horses in full exercise at the level of the aortic root (Brown et al., 1988). In Friesian horses, however, aortic rupture is relatively common. We have demonstrated that it typically occurs as a transverse tear located immediately proximal to the ligamentum arteriosum (Ploeg et al., 2013; Ploeg et al., 2015). Between 2007 and 2013, we have diagnosed 37 Friesian horses with aortic rupture during post-mortem examination at the Faculty of Veterinary Medicine of Ghent University, Belgium, the Faculty of Veterinary Medicine of Utrecht University, and Wolvega Equine Clinic, the Netherlands. Of these 37 affected Friesians, 27 were diagnosed with an aortopulmonary fistula with survival for several weeks to months (Ploeg et al., 2015). Three-dimensional visualization of the fistulation between the aorta and pulmonary artery at necropsy is difficult due to extensive pseudoaneurysm (PsA) formation, sometimes combined with dissections and periaortic haematomas (Ploeg et al., 2013). Also in vivo visualization of this structure is hard to obtain since medical imaging is restricted to cardiac ultrasound due the large posture of horses (van Loon et al., 2011). Recently we reported on the potential benefits of performance of transoesophageal ultrasound in attained cases to gain a better view on this region (De Bruijn et al., 2013).

The purpose of this study was to obtain a three-dimensional characterization of the aortopulmonary connection in Friesian horses via vascular casting.

**Material and methods**

A standard transthoracic echocardiographic examination was performed in the standing horse. Echographic findings of one affected Friesian horse (gelding, 9 years) were included. Images were optimized to visualize the relation between aorta, pseudoaneurysm and pulmonary artery.

To obtain a better insight into the 3D conformation of the site of rupture, post-mortem vascular casting of the aortopulmonary fistulation was performed in three affected Friesian horses (horse 1: mare, 4 years; horse 2: gelding, 11 years; horse 3: mare, 6 years) using the technique described by Vandecasteele et al. (2015). Silicone casting of the proximal thoracic aorta and pulmonary artery was done in the first two horses. In the third horse, a Technovit® 7143 cast was made. The complete cardiopulmonary set with the intact vessels was dissected from the thorax. The heart was then positioned in an upside down position. The left and right ventricle were opened and the aorta and truncus pulmonalis were flushed with a hosepipe. The silicone (base and catalyst, ratio 1:1) or Technovit® 7143 was then infused with the aid of a funnel through the left and right ventricles into the aorta and truncus pulmonalis. After hardening overnight at room temperature, the casts were dissected.

**Results**

The rupture site and pseudo-aneurysm could be visualized on ultrasound but interpretation of the exact 3-D morphology was difficult (Figures 24 and 25).
Vascular casting in all three samples revealed an aortic rupture at the caudoventral side of the aorta and a rupture of the main pulmonary artery just proximal to its bifurcation (Figure 26). The fistula between both arteries consisted of a pseudoaneurysm containing several (1-3) pocket-like spaces. These findings were consistent with the post-mortem findings described earlier (Ploeg et al., 2013).

**Discussion**

In humans, in more than 90% of the cases, pseudoaneurysms occur at the aortic isthmus (between the left subclavian and the third intercostal artery) near the ligamentum arteriosum (Nzewi et al., 2006; Gleason and Bavaria, 2003). Pseudoaneurysms are mainly associated with accidents involving pronounced deceleration or torsional trauma (Gleason and Bavaria, 2003). Aortopulmonary fistulation in humans can develop even decades after the traumatic event (Giglioli et al., 2013). The exact pathophysiology of aortic pseudoaneurysm formation induced by blunt traumatic injury is still unknown, but it is most likely the result of a complex interaction between both motion of anatomical structures and local force loading (Pearson et al., 2008). The periadventitial aortic isthmus tissue at that site seems to offer protection against complete aortic transection with subsequent acute exsanguination (Gleason and Bavaria, 2003).

It is striking, that pseudoaneurysms occur at the same location in Friesian horses as in humans suffering from traumatic injury. This could indicate a common pathogenic mechanism predisposing certain humans to aortapulmonary fistulisation after traumatic aortic injury. However, in contrast to humans, none of the affected Friesian horses had a history or showed signs of traumatic injury. Furthermore, inflammation and atherosclerosis, two other predisposing factors for pseudoaneurysm formation in humans (Raijah, 2013), were not reported in Friesians (Ploeg et al., 2015).

Aortopulmonary fistulation most likely develops by continuous pulsatile friction between the wall of the aneurysm or pseudoaneurysm and the pulmonary artery (Bol et al., 2006). The aortic arch loops over the left pulmonary artery and the bifurcation of the main pulmonary trunk, to which it remains connected by the ligamentum arteriosum (Drake et al., 2010). Also in horses, the thoracic aorta is positioned closely to the left pulmonary artery at the level of the bifurcation (Sisson et al., 1938). However, the geometry of the equine aorta differs from humans as the typical deviation of the aortic arch in humans is almost absent in horses (Casteleyn et al., 2010). In Friesian horses with an aortopulmonary fistulation, the pseudoaneurysm typically merges just proximal to the bifurcation of the pulmonary artery. In humans, fistulation into the pulmonary artery is mainly observed when the aortic intimal tear is present at the left side (Massetti et al., 1995). The latter is however rare, since aortic intimal tear formation usually occurs at the right anterolateral side of the ascending aortic wall which is the area that endures the highest stress (Bauer et al., 2006). This could explain the discrepancy between the relative high frequency of chronic aortic (pseudo)aneurysms and the rare incidence of APF.

This technique of vascular casting has some limitations as the casting was performed post-mortem and not in situ. The situation thus not completely corresponds to the situation in vivo. The use of Technovit® requires multiple precautions for safe handling.
compared to silicone, which is considered rather safe in use. The silicone casts endured manipulation well and showed good flexibility. The silicone casting provided the ability to fill the entire arterial supply of the lungs in a simple manner. The Technovit® cast was more brittle and did not endure handling as well as the silicone cats. This Technovit® cast was more rigid, enabling us to obtain a rotating portable document format (pdf) of the specimen.

In summary, aortic rupture and aortopulmonary fistulization formation in Friesian horses occur without any history of trauma or signs of inflammation. Considering the similar location of the lesions in Friesian horses and humans, the chronic aspect of this disease, the fatal outcome once clinical signs develop and the possibility to obtain vascular casts, the Friesian horse could be a valuable spontaneous model for this condition in humans.
Figure legends

Figure 24. Ultrasound image showing the blood flow (arrow) from the ruptured aorta (Ao) into the pseudoaneurysm (PsA). The pulmonary artery (PA) is severely dilated due to pulmonary hypertension. (RV: right ventricle).

Figure 25: Ultrasound image: from the ruptured aorta (Ao) blood flows (dotted arrow) into the pseudoaneurysm (PsA) and subsequently through the fistula (arrow) that enters the pulmonary artery (PA) near the bifurcation towards the left (l-PA) and right (r-PA) branch of the PA.
Figure 26. Dorsal view of a silicone cast from a Friesian horse with caudoventral aortic rupture (Ao) and aortic pseudoaneurysm (PsA) fistulating into the dorsal side of the pulmonary artery (PA). (l-PA: left branch of the pulmonary artery, r-PA: right branch of the pulmonary artery).


CHAPTER 4:

THORACIC AORTIC RUPTURE AND AORTOPULMONARY FISTULATION IN THE FRIESIAN HORSE: HISTOMORPHOLOGIC CHARACTERIZATION

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V Saey*/M Ploeg*, C Delesalle, A Gröne, R Ducatelle, CM de Bruijn, PR van Weeren, G van Loon and K Chiers

*both authors contributed equally
Chapter 4: Thoracic aortic rupture and aortopulmonary fistulation in the Friesian horse: histomorphologic characterization

Abstract

Aortic rupture in horses is a rare condition. Although it is relatively common in the Friesian horse breed, only limited histopathologic information is available. Twenty Friesian horses (1–10 years old) were diagnosed with aortic rupture by post-mortem examination. Ruptured aortic walls were analysed with histology and immunohistochemistry. Based on the histological and immunohistochemical findings, these cases were divided into 3 groups: acute (n=4, 20%), subacute (n=8, 40%), and chronic (n=8, 40%). Features common to samples from horses in all groups included accumulation of mucoid material; disorganization and fragmentation of the elastic laminae; aortic medial smooth muscle hypertrophy and medial necrosis of varying degrees, ranging from mild and patchy in the acute cases to severe midzonal necrosis in the chronic cases. Inflammation, most likely secondary to medial necrosis, varied from predominantly neutrophilic infiltrates in the media and periadventitial tissue in the acute group to the presence of mainly haemosiderophages in the periadventitial tissue in the chronic group. Medial fibrosis with aberrant collagen morphology was seen in the subacute group and, more commonly, in the chronic group. Only minimal changes were seen in the aortic vasa vasorum. Smooth muscle hypertrophy and accumulation of mucoid material were not related to the age of the lesions. The findings of this study suggest that a connective tissue disorder affecting elastin or collagen in the aortic media is potentially the underlying cause of aortic rupture in Friesian horses.

Keywords: Friesian horses, aorta, histology, immunohistochemistry, laminar medial necrosis, aortopulmonary fistulation, pseudoaneurysm, vasa vasorum

Introduction

More than 40 cases of aortic rupture in Friesian horses have been reported to date (Ploeg et al., 2013; Van der Linde-Sipman et al., 1985). These ruptures occurred spontaneously, in the absence of trauma, infectious disease, or iatrogenic injury. There was no sex predilection, and the median age of affected horses was 4 years, ranging from 1 to 20 years. Ruptures occurred consistently in the thoracic aorta near the ligamentum arteriosum. Frequent findings at post-mortem examination included a concurrent circumferential cuff of perivascular haemorrhage (periaortic haematoma) and aortopulmonary fistulation, which is a well-known condition in the Friesian breed (Ploeg et al., 2013). Aortic rupture in non-Friesian horse breeds is rare and mainly reported in stallions, in which it often manifests as acute death or results in aorto-cardiac fistulation (Rooney et al., 1967; Marr et al., 1998). In these cases, the rupture appears near the
sinuses of Valsalva, and the macroscopic lesions and clinical signs differ from those seen in Friesian horses with aortic rupture.

An aneurysm is a localized abnormal dilation of any vessel. True aneurysms are composed of all or most layers of the intact vessel wall. False aneurysms, also called pseudoaneurysms, result from rupture of an artery or aneurysm, with disruption of all 3 layers of the arterial wall and communication with the arterial lumen (Maxie et al., 2007; Webber et al., 2007). Thoracic aortic aneurysm is rare in horses and is often located at the sinuses (Marr et al., 1998). It has also been reported near the aortic arch (Reef et al., 1990). In some cases, Strongylus vulgaris arteritis can cause aneurysm formation in the abdominal or thoracic aorta (Simoens et al., 1999). Spontaneous aortic rupture in humans is a multifactorial disease and a relatively uncommon cause of death (Grootenboer et al., 2010; Hoel, 2013; Merlo et al., 2001). Histopathologic findings include abnormalities of the cellular and matrix constituents of the aortic media. Aortic rupture in humans occurs mostly in the abdominal portion of the vessel (Ernst, 1993) and is frequently associated with atherosclerosis (Grange et al., 1997). Thoracic aortic rupture is uncommon and predominantly linked to hereditary diseases (Heegaard et al., 2007; Kontusaari et al., 1990; Milewicz et al., 1996). In abdominal aortic aneurysms, the structure and amount of the matrix proteins are abnormal, as elastin is decreased and collagen increased (Baxter et al., 1994; Minion et al., 1994). Both the vasa vasora and the increased synthesis of matrix metalloproteinases by smooth muscle cells have been implicated in the progression of abdominal aortic aneurysms (Herron et al., 1994; Patel et al., 1996). Additionally, inflammatory processes have been shown to play an important role in the development of this disease. These can be divided into non-infectious causes, such as atherosclerosis (Abdul-Hussien et al., 2007; Tilson, 1995), and infectious causes, such as mycotic aneurysms of the aorta (Pasic et al., 1993). In thoracic aortic rupture, alterations in fibrillin (Marfan syndrome), collagen (Ehlers-Danlos syndrome type IV), and proteoglycans (biglycan gene deficiency) have been determined (Heegaard et al., 2007; Kontusaari et al., 1990; Milewicz et al., 1996). In rare cases, a functional connection occurs between the ascending aorta and the pulmonary artery. Such an aortopulmonary fistula is a diagnostically challenging condition (Ishizaki et al., 1990). It occurs most frequently as a result of erosion and/or rupture of a chronic process or pseudoaneurysm of the aorta (MacIntosh et al., 1991; Sobral et al., 2004). Histologic examination of the ruptured aortic wall has proved to be a useful tool for investigation of the underlying causes of aortic rupture in humans (Aoyagi et al., 1991; Schmitto et al., 2010). To date, only 3 cases of aortic rupture with aortopulmonary fistulation in Friesians have been histologically examined and described in the literature (Van der Linde-Sipman et al., 1985). In 2 horses, the lesion was described as scattered medial necrosis throughout the wall, surrounded by neutrophils and granulation tissue; in the third horse, no inflammatory reaction was observed. Many vasa vasora with intimal thickening and/or medial fibrosis were seen in the aortic adventitia, and this may predispose to aortic medial necrosis and rupture (Van der Linde-Sipman et al., 1985). The purpose of the present study was to describe the histologic lesions in a larger series of Friesian horses with aortic rupture to gain better insight into the underlying mechanisms of this unique disease.
Methods

Animals
Twenty Friesian horses were included in this study (1-10 years old; 14 mares, 3 geldings, 2 stallions, and 1 animal of unknown sex). The gross lesions in 7 of these animals have been described previously (Ploeg et al., 2013). All horses were diagnosed with aortic rupture by means of post-mortem examination over a period of 14 years (1997-2011) at Wolvega Equine Hospital (n=6); the Faculty of Veterinary Medicine, Ghent University, Belgium (n=5); or the Faculty of Veterinary Medicine of Utrecht University, The Netherlands (n=9). Six horses were found dead or showed acute clinical signs in the few hours preceding death. However, the majority (n=14) showed signs such as fever, tachycardia, and colic in the days, weeks, or even months before euthanasia.

Histopathology
Tissue samples were taken at the level of the aortic rupture, fixed in phosphate-buffered formalin, routinely embedded in paraffin wax, and cut into 4-μm-thick sections. Periaortic haematomas, pseudoaneurysms, and arterial dissections were also analyzed. Sections were stained with hematoxylin and eosin, Van Gieson’s stain (Klinipath 64089, Duiven, Netherlands) to visualize possible collagen deposition of the media, Alcian Blue (Sigma A4045-25G, Zwijndrecht, The Netherlands) for detection of accumulation of mucoid material, and Von Kossa stain for demonstration of mineralization.

Immunohistochemistry
The immunohistochemical antibodies were used according to manufacturers’ instructions. Presence of smooth muscle was demonstrated with mouse anti-smooth muscle actin (Biogenex, Fremont, CA) as the primary antibody and horse anti-mouse/biotin (Vector Laboratories, Peterborough, UK) as the secondary antibody. Vasal vasora were identified by visualization of factor VIII–related antigen with rabbit anti-factor VIII (Dako, Glostrup, Denmark) as primary antibody and goat anti-rabbit IgG (Vector Laboratories) as secondary antibody. Additionally, factor VIII–related antigen was used to determine the nature of the lining of the pseudoaneurysms and dissections. Lymphocytes were analysed with a polyclonal rabbit anti-CD3 antibody (Dako) that labels human T lymphocytes (both helper and cytotoxic T lymphocytes), a polyclonal rabbit anti-CD20 antibody that labels human B-lymphocytes (Thermo Fisher Scientific, Erembodegem, Belgium), and a monoclonal mouse antibody that labels reactive and tissue macrophages (MAC387, Abcam, Cambridge, UK). Aortic elastin was visualized with a monoclonal anti-elastin antibody BA-4 (Leica Biosystems, Diegem, Belgium). A standard avidin–biotin complex method with diaminobenzidine as chromogen was used for visualization (Envision, Dako). Negative controls were prepared from serial sections in which the primary antibody was omitted and replaced by dilution buffer. The specificity of the primary antibodies was validated with recognized positive control tissues.

Scoring of Lesions
Histologic scoring of necrosis of the media, accumulation of mucoid material, altered smooth muscle orientation, elastin fragmentation, and fibrosis was based on protocols
described previously (Schlatmann and Becker, 1977). This semiquantitative grading system uses a scale from 0 (no changes) to 3 (severe changes). The smooth muscle cells in the media were also assessed for the presence of hypertrophy. The presence or absence of fibrin and mineralization was assessed. The degree of medial and adventitial inflammation (density of inflammatory cells) was scored with grade 0 representing no inflammation to grade 3, severe inflammation (Koch et al., 1990). Finally, the numbers of vessels in the media and adventitia were assessed by counting the number of vasa vasora in 5 randomly chosen fields irrespective of the size of the vessel. The division of the lesions into the three groups was based on the fact that pathological lesions might be sooner present compared to clinical signs. Indeed, some of the horses that presented with acute signs actually showed chronic histological lesions.

The patients were divided into 3 groups based on the presence of following histological criteria:

1. only fibrin and neutrophils: acute
2. endothelial hypertrophy, hemosiderin-laden macrophages, immature fibroblasts: subacute
3. granulation tissue, fibrosis, endothelization of the pseudoaneurysm or dissection: chronic

Results

Major findings are summarized in supplementary table 1.

Macroscopic Findings

Macroscopic lesions in the present series (including those in the horses included in the previously mentioned study) were similar to those previously described. In summary, all Friesians had an aortic rupture just proximal to the ligamentum arteriosum, and 6 horses showed a hemothorax. A periaortic haematoma was present in 4 cases. Aortopulmonary fistulation was present in 13 animals and was in most cases associated with 1 or more pseudoaneurysms (extensive encapsulated perivascular haematoma, retaining communication with the aortic lumen) (Figure 27). Pulmonary artery ruptures were transverse, located at the level of the ligamentum arteriosum, and varied from 2 to 7 cm in length. In 4 cases, a dissection of the aorta was present, and in 1 case, there was an additional dissection of the pulmonary artery wall. Dissection took the form of a longitudinal split in the mid-media, creating a “false” lumen running distally over a length of up to 15 cm.

Histological Findings of Ruptured Aortas

In 4 animals, aortic lesions were histologically classified as acute based on the presence of fibrin clots in the haematoma and neutrophils as the dominant infiltrative cell type (acute group, Nos. 1–4). Two of these horses collapsed because of a hemothorax; the other 2 had periaortic haematomas, and 1 of these suffered an aortopulmonary fistula. All horses in the acute group had mild (n=3) to moderate (n=1) (grades 1 and 2) necrosis of the media, which was mainly patchy. Small numbers (grade 1) of B and T lymphocytes, plasma
cells, and macrophages were seen both in the media and in the periadventitial tissue, but neutrophils were the predominant inflammatory cell type. Accumulation of mucoid material (grades 1–3) and disorganization and fragmentation (grades 1 and 2) of the elastic laminae (Figure 28 and 29) were seen in the aortic media of all horses in the acute group. Medial fibrosis was not present. Mild mineralization of the midzone of the media was present in a single case and occurred in association with mild medial necrosis. Smooth muscle hypertrophy was present in all affected Friesians, but no animals in this group showed altered smooth muscle orientation. The number of vasa vasora in the media ranged from 26 to 78 (mean, 45), and 2 of these showed moderate intimal thickening by subendothelial smooth muscle cells without medial fibrosis. The number of vasa vasora in the adventitia was determined in only a single case (17 vasa vasora) but could not be detected in others due to the extensive haemorrhage or the absence of adventitia. Subacute stages of the lesions were identified in 8 horses on the basis of the presence of immature fibroblasts in the adventitia and periadventitial tissue and infiltration of mononuclear cells and hemosiderin-laden macrophages (subacute group, Nos. 5–12). Five of these horses demonstrated a periaortic haematoma, which was associated with hemothorax in 4 cases and with an aortopulmonary fistula in 1 case. The other 3 horses showed an aortopulmonary fistula combined with an aortic dissection. Fibrin clots were observed in the haematoma in 6 cases. Medial necrosis was seen in all cases with subacute lesions and varied from mild in 3 cases to severe (grades 1–3) in 5 cases. In all cases, there was infiltration of the adventitia and periadventitial tissue predominantly by hemosiderin-laden macrophages (grades 1–3) was present in all cases. In 4 cases, these macrophages were accompanied by neutrophils, and in 2 cases, they were admixed with several eosinophils. Accumulation of mucoid material (grades 1–3) and disorganization and fragmentation of the elastic laminae (grades 1–3) were seen in the aortic media of all horses in the subacute group. In 2 cases, mild to moderate (grades 1 and 2) medial fibrosis was observed. Increased fibrosis was typically characterized by disorganization, fragmentation, or clumping of fibres (Figure 30). In 1 horse, there was multifocal mineralization of well-defined areas of medial smooth muscle cell necrosis and extensive elastin fragmentation. Smooth muscle hypertrophy was present in all affected Friesians, but no animals in the subacute group showed changes in smooth muscle orientation. The number of vasa vasora in the media ranged from 21 to 159 (mean, 70), and a single case showed medial fibrosis of vasa vasorum in the aortic media. In the adventitia, the number of vasa vasora ranged from 37 to 276 (mean, 149) and a single case also showed moderate intimal thickening with subendothelial smooth muscle cells and proteoglycans. The remaining 8 animals showed chronic lesions in the aorta (chronic group, Nos. 13–20). These were similar to the lesions seen in the subacute cases but additionally showed fibrosis in the adventitia and periadventitial tissue. All cases with chronic lesions showed aortopulmonary fistulation combined with a pseudoaneurysm, and all cases had moderate (n=3) to severe (n=5) necrosis (grades 2 and 3) of the midzone of the media. In all cases, a mild infiltration (grade 1) of B and T lymphocytes and plasma cells was found in the media and/or adventitia. In 5 cases, there was infiltration of the periadventitial tissue, mainly by hemosiderin-laden macrophages, sometimes admixed with neutrophils. Accumulation of mucoid material (grades 2 and 3) and disorganization and fragmentation (grades 1–3) of the elastic laminae were seen in the aortic media of all horses in the chronic group. Mild to moderate (grades 1 and 2) medial fibrosis was
observed in 7 cases and was similar to the morphologically abnormal fibrosis described in the subacute cases. In 5 cases, there was multifocal mineralization of well-defined areas of medial smooth muscle cell necrosis with extensive elastin fragmentation. Smooth muscle hypertrophy was present in all affected Friesians, but no animals in the chronic group showed altered smooth muscle orientation. Moderate to marked intimal thickening of the vasa vasorum of 2 to 5 vessels was seen in 3 of the chronic cases, and 2 of these were located in the adventitia. Mild medial fibrosis of the vasa vasorum was present in the media (n=2) and/or adventitia (n=3). The number of vasa vasora in the media ranged from 26 to 133 (mean, 59) and in the adventitia from 63 to 434 (mean, 163).

Pseudoaneurysms
Pseudoaneurysms were present in 1 of the acute cases, 4 of the subacute cases, and all of the chronic cases. The adventitial side of the wall of the pseudoaneurysm was characterized by a thick layer of spindle-shaped cells (fibroblasts) that formed streams and bundles in various directions in a myxomatous matrix. Whorls were frequently formed, and multifocal small- to medium-sized blood vessels were present. The centre of the wall was composed of a layer of smooth muscle cells that were longitudinally oriented. The smooth muscle cells were multifocally mixed with a moderate amount of collagen fibres that showed various orientations. Close to the intimal side of the wall of the pseudoaneurysm, these smooth muscle cells were replaced in large areas by moderate amounts of collagen, high numbers of small- to large-sized blood vessels with occasionally marked intimal proliferation, high numbers of B and T lymphocytes, multinucleated giant cells, a moderate amount of fat, low numbers of viable and degenerate neutrophils, plasma cells, and a few cholesterol clefts (Figure 31). The luminal side was lined by well-differentiated endothelial cells, confirmed by staining with factor VIII–related antigen. Pseudoaneurysms contained large blood clots with high numbers of degenerated and viable neutrophils and low numbers of eosinophils and multinucleated giant cells.

Periaortic Haematomas
Periaortic haematomas were observed in 2, 5, and 1 animals demonstrating acute, subacute, and chronic histologic lesions, respectively. The periphery was bordered by a moderately thick layer of longitudinally arranged thick collagen fibres. In some cases, the adventitia showed large areas of fragmented (degenerate) collagen, haemorrhage, and an inflammatory infiltrate consisting of large numbers of lymphocytes and plasma cells. In a few cases, the adventitia showed multifocal proliferation of randomly aligned bundles of fibroblasts admixed with fat tissue. Multifocal areas with large numbers of neutrophils and a moderate amount of necrotic debris admixed with small deposits of hematoidin were also present.

Aortic Dissections
In 4 cases (3 subacute and 1 chronic), the aortic wall was dissected within the media close to the adventitia with the formation of a pseudolumen, which was walled off by large numbers of smooth muscle cells, a large amount of collagen, and moderate numbers of medium- to large-sized vessels. The smooth muscle cells were longitudinally aligned parallel to the lumen, but this alignment became less organized further from the lumen.
The variable arrangement of collagen fibres became increasingly loose and disorganized toward the periphery. Both sides of the dissection were lined by endothelial cells (positive factor VIII–related antigen immunostaining).
Figure 27. Friesian horse, case No.19. Aortic rupture with the aortic lumen (#) on the left and a typical pseudoaneurysm (*) in the centre characterized by finger-like sacculations in its wall.

Figure 28. Friesian horse, case No. 8. Accumulation of mucoid material in the aortic mid-media adjacent to the rupture site. Alcian Blue.
Figure 29. Friesian horse, case No. 14. Note marked disorganization and fragmentation in the elastin within the aortic media. Immunohistochemistry for elastin, hematoxylin counterstain.

Figure 30. Friesian horse, case No. 13. Midregion of the aortic media demonstrating marked disorganization of collagen fibres. Von Gieson.
Figure 31.
Friesian horse, case No. 14. Wall of pseudoaneurysm demonstrating fibrosis, fat tissue and inflammatory infiltrate consisting mainly of macrophages. HE.
Discussion

The histologic lesions described in the present study correlated well with the gross lesions and clinical signs. All horses in the acute group suffered from acute death or presented with acute signs, whereas all horses of the chronic group had shown clinical signs over a prolonged period due to aortopulmonary fistulation.

To date, there is only a single report in the literature describing the histologic features of Friesian horses (n=3) with aortic rupture and aortopulmonary fistulation. These animals had severe progressive symptoms of cardiac distress, died within a few hours to 8 days after referral, and are comparable to the chronic group in the present study. These authors reported the histologically detected alterations in the vasa vasorum as a possible cause of aortic medial necrosis. Furthermore, the scar of the former site of the ductus arteriosus was considered to be predisposed to rupture and fistulation (Van der Linde-Sipman et al., 1985).

In this study, aortic medial necrosis was observed in all horses. By weakening the aortic wall, this may predispose affected vessels to dissection and spontaneous rupture (Murray et al., 1973). Medial necrosis has also been suggested as a predisposing lesion in non-Friesian horses dying from acute rupture of the aorta at the sinuses of Valsalva (Rooney et al., 1967). However, chronic histologic lesions as seen in the Friesian horses, such as fibrosis of the adventitia and perivascular tissue with infiltration of hemosiderin-laden macrophages, have not been described. Nearly all affected Friesian horses (85%) showed laminar medial necrosis as a histologically evident laminar pink band with loss of nuclei in the mid-media. The middle part of the media is situated between the area supplied by the vasa vasorum and the area nourished by the intraluminal blood and is therefore most prone to ischemic damage (Wilens et al., 1965). For the same reason, aortic dissection usually occurs at the junction of the middle and outer third of the media, as seen in the Friesian horses in this study (Woerner, 1959). The laminar medial necrosis of the aorta in the Friesian horse could be attributed to ischemia. A high number of vasa vasora with intimal thickening and/or medial fibrosis in the media and adventitia of the aorta was described in 3 Friesian horses with aortic rupture (Van der Linde-Sipman et al., 1985). The lumen of the vasa vasorum was completely obliterated, and this was suggested to cause hypoxia or anoxia of the aortic wall, resulting in local circulatory compromise, necrosis, and, finally, wall rupture. In the present study, only 5 of the 20 cases demonstrated mild to moderate intimal thickening of the vasa vasorum without complete occlusion. Medial fibrosis in 2 to 5 vessels of the vasa vasorum was infrequently present in subacute and chronic cases. There is a high discrepancy between our findings and those previously reported, therefore making ischemic damage as an underlying cause unlikely. The alterations observed in the vasa vasorum in the present study are believed to be a secondary phenomenon related to blood flow changes, such as severe circulatory disturbances caused by ruptured aortas or pseudoaneurysms.

Another cause of medial necrosis is connective tissue abnormalities that, in humans, are often related to gene dysfunctions. In such cases, cystic medial necrosis is often observed
(degeneration of elastic fibres and collagen in the media of the aorta and subsequent accumulation of mucoid material) (Yuan and Jing, 2011). Accumulation of mucoid material in the tunica media resembling cystic medial necrosis was present in all cases reported here, suggesting that a primary connective tissue disorder may contribute to aortic rupture in Friesian horses. There is controversy about the significance of cystic medial necrosis in human aortic rupture. Some authors have suggested that it is primarily an aging process (Schlatmann and Becker, 1977), but other studies have indicated that cystic medial necrosis is an expression of metabolic activity rather than the result of a degenerative process (Erdheim, 1930; Schlatmann and Becker, 1977). In this study, the role of aging is questionable, as the majority of animals were less than 7 years old. Furthermore, cystic medial necrosis has been observed in nondiseased horses (Robinson and Maxie, 1993). Therefore, it is possible that cystic medial necrosis is not a primary feature of the disease.

Some degree of medial necrosis is related to aging and is typically observed as small focal defects in the human aorta (Braunstein, 1970). In a minority of affected Friesian horses, a patchy distribution of necrotic medial foci was observed and could be attributed to age. However, 1 of these horses was only 4 years old, suggesting that age may not be a factor in the development of this lesion. In addition, this patchy medial necrosis was seen only in acute and subacute cases, indicating that this lesion may represent an early stage in the disease.

Finally, medial necrosis can be initiated by mediators from structural elements of the media. This mechanism is seen in abdominal aortic aneurysms in humans, where vascular smooth muscle cells promote matrix metalloproteinase release resulting in medial damage (Koole et al., 2012). In such cases, inflammation is a prominent and consistent secondary finding characterized by extensive infiltrates of B and T lymphocytes, plasma cells, and dispersed macrophages in the adventitia (Hellenthal et al., 2009). Since smooth muscle hypertrophy, fibrosis, and the infiltration of lymphocytes and macrophages were observed in the affected Friesian horses, involvement of proteinases cannot be excluded.

Smooth muscle cell hypertrophy of the human aorta, often accompanied with protein deposition, is associated with hypertension and aberrant blood flow, creating cyclic stretch and shear stresses (Assoian and Marcantonio, 1997; Haga et al., 2007, Owens et al., 1981). In bovine Marfan syndrome, smooth muscle hypertrophy is considered to be a secondary reaction, replacing damaged elastic fibres of the aortic wall (Potter and Besser, 1994). The extracellular matrix can play a pivotal role in the regulation of arterial vascular smooth muscle cell differentiation and proliferation (Assoian and Marcantonio, 1997; Koyama et al., 1996; Mercurius and Morla, 1998). In the present study, all Friesians showed aortic medial smooth muscle cell hypertrophy, accumulation of mucoid material, and disorganization of the elastin. It is therefore possible that primary changes in the extracellular matrix with subsequent aberrant stretching may have caused smooth muscle hypertrophy in these cases. In about half the chronically affected Friesian horses, abnormal collagen deposition was seen predominantly in the midzone of the media. As seen in humans, this collagen deposition may reflect a reactive strengthening of the vessel wall as a reaction to the abnormal hemodynamic changes caused by the
pseudoaneurysm and/or aortopulmonary fistula (Abdul-Hussien et al., 2007; Carlson et al., 1970). Additionally, as fibrosis was most obvious in the chronic group, the collagen deposition may be a secondary reaction to chronic injury. Possible triggers for repair in vascular tissues are necrosis of smooth muscle cells, disruption of elastic laminae, or loss of endothelial continuity (Paik and Lalich, 1973). However, the collagen deposition observed in the affected Friesian horses was clumped and disorganized, which is not a common characteristic of fibrosis.

Disorganization and fragmentation of elastic laminae in the media were seen in all horses and could be due to proteolytic activity caused by the damaged extracellular matrix at the site of the rupture. In the past, elastin fragmentation was interpreted as being secondary to smooth muscle cell necrosis (Gsell, 1928). However, elastin degeneration was present in both chronic and acute cases, which may suggest an underlying primary connective tissue disorder in the Friesians. Well-known causes of elastin defects include Williams syndrome (Francke, 1999), “cutis laxa” (Tassabehji et al., 1998) and human and bovine Marfan syndromes (Abraham et al., 1982; Potter and Besser, 1994). In these syndromes, vascular anomalies are accompanied by alterations in other organs. Since no other abnormalities were observed, a syndromal elastin defect seems unlikely in the Friesian horses.

In humans, aortopulmonary fistulation is rare and occurs most frequently as a complication of aortic pseudoaneurysm. The latter has been associated with a true aneurysm, aortitis, atherosclerosis, arteriosclerosis, aortic dissections, or traumatic aortic tear or as a postsurgical event (Bol et al., 2006). Occasionally, a foreign body has been the inciting factor (Ishizaki et al., 1990). In the present study, there were no indications for infectious agents, atherosclerosis, or trauma as the primary cause. In one human case, a small aortic aneurysm extended into the pulmonary artery, and this was believed to be due to pressure and the close proximity of the pulmonary artery and the aorta (Jennings, 1953). In Friesian horses, the location of the aortopulmonary fistulation was assumed to be due to tension created by the ligamentum arteriosum on the previously damaged walls of aorta and pulmonary trunk (Van der Linde-Sipman et al., 1985). However, in this study, an ongoing chronic process with weakening of the aortic wall and pseudoaneurysm formation toward the pulmonary artery is believed to be the primary cause in at least some of the cases. Nevertheless, as there are several cases with a fistula in the absence of a pseudoaneurysm, rupture of the pulmonary artery may be caused by an intrinsic defect.

Histologic lesions seen in affected Friesian horses differ from non-Friesian horses suffering from abdominal aneurysms. In 45% of the Strongylus vulgaris–mediated equine arteritis, one or more larvae can be found, and this finding is consistently associated with thrombosis (Morgan et al., 1991). The current study showed no true aneurysms, and there were no macroscopic or histologic indications for parasite migration in the affected Friesian horses. Rupture of the uterine artery is a common condition in predominantly aged multiparous mares, and copper deficiency is presumed to be a predisposing factor (Stowe et al., 1968). Peripartum haemorrhage in these mares is characterized by atrophy
of smooth muscle cells with fibrosis of the tunica media and disruption and/or calcification of the internal elastic lamina (Ueno et al., 2010). In this study, the median age of the affected Friesians was only 4 years old, suggesting a different pathogenesis. The finding of a shared set of clinical signs and gross and histologic findings in all cases of aortic rupture reported here supports the recognition of this syndrome as a distinct clinical entity unique to this breed. It is conceivable that an underlying genetic defect of the connective tissue in the aortic media predisposes these animals to aortic rupture, dissection, and aortopulmonary fistulation at an anatomically and haemodynamically predisposed site. The Friesian horse population appears to be the only animal species in which aortopulmonary fistulation is regularly encountered. In light of these findings, biochemical and ultrastructural examination of the extracellular matrix in the aorta of affected Friesians may be useful to further extend our understanding of the pathogenesis of this syndrome.
Supplementary table 1. Animals, clinical signs, and main gross and histological findings of Friesian horses with aortic ruptures that were classified as acute (Nos. 1-4), subacute (Nos. 5-12) and chronic cases (Nos. 13-20).

<table>
<thead>
<tr>
<th>Animal No.</th>
<th>Gender, age</th>
<th>Clinical signs</th>
<th>Gross lesions</th>
<th>Fibrin&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Medial necrosis&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Medial fibrosis&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mucoid material accumulation&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Mineralization&lt;sup&gt;b&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Mare, 4 years</td>
<td>Acute colic</td>
<td>AP fistula&lt;sup&gt;c&lt;/sup&gt;, pseudoaneurysm, periaortic hematoma</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>2</td>
<td>Mare, unknown</td>
<td>Sudden death</td>
<td>Periaortic hematoma</td>
<td>+</td>
<td>2</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>3</td>
<td>Unknown, unknown</td>
<td>Sudden death</td>
<td>Hemotorax</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Stallion, 4 years</td>
<td>Sudden death</td>
<td>Hemotorax</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>5</td>
<td>Mare, 1 year</td>
<td>Colic, tachycardia</td>
<td>AP fistula&lt;sup&gt;c&lt;/sup&gt;, pseudoaneurysm, periaortic hematoma</td>
<td>+</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Mare, 3 years</td>
<td>Sudden death</td>
<td>Hemotorax, periaortic hematoma</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Mare, 10 years</td>
<td>Acute discomfort</td>
<td>Hemotorax, periaortic hematoma</td>
<td>+</td>
<td>3</td>
<td>0</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Mare, 3 years</td>
<td>Acute discomfort</td>
<td>Hemotorax, periaortic hematoma</td>
<td>+</td>
<td>1</td>
<td>0</td>
<td>1</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Mare, 7 years</td>
<td>Coughing, tachycardia, edema, lameness</td>
<td>AP fistula, aortic dissection, pseudoaneurysm</td>
<td>-</td>
<td>1</td>
<td>2</td>
<td>2</td>
<td>-</td>
</tr>
<tr>
<td>10</td>
<td>Mare, 6 years</td>
<td>Colic, fever</td>
<td>Hemotorax, periaortic hematoma</td>
<td>+</td>
<td>3</td>
<td>0</td>
<td>3</td>
<td>-</td>
</tr>
<tr>
<td>11</td>
<td>Mare, 4 years</td>
<td>Colic, tachycardia</td>
<td>AP fistula, aortic dissection</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>+</td>
</tr>
<tr>
<td>No.</td>
<td>Animal</td>
<td>Gender, age</td>
<td>Clinical signs</td>
<td>Gross lesions</td>
<td>Fibrin(^a)</td>
<td>Medial necrosis(^b)</td>
<td>Medial fibrosis(^b)</td>
<td>Mucoid material accumulation(^b)</td>
</tr>
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</tr>
<tr>
<td>12</td>
<td>Mare, 3 years</td>
<td>Colic, tachycardia, fever</td>
<td>pseudoaneurysm</td>
<td>AP fistula, aortic dissection, pulmonary dissection, pseudoaneurysm</td>
<td>+</td>
<td>3</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>13</td>
<td>Mare, 2 years</td>
<td>Colic, tachycardia</td>
<td>pseudoaneurysm</td>
<td>AP fistula(^*), aortic and pseudoaneurysm</td>
<td>-</td>
<td>3</td>
<td>0</td>
<td>3</td>
</tr>
<tr>
<td>14</td>
<td>Gelding, 5 years</td>
<td>Fever, tachycardia, exercise intolerance</td>
<td>pseudoaneurysm</td>
<td>AP fistula, periaortic hematoma, pseudoaneurysm</td>
<td>-</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>15</td>
<td>Mare, 6 years</td>
<td>Tachycardia, edema</td>
<td>pseudoaneurysm</td>
<td>AP fistula, pseudoaneurysm</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>16</td>
<td>Gelding, 7 years</td>
<td>Diarrhea, edema</td>
<td>pseudoaneurysm</td>
<td>AP fistula, pseudoaneurysm</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>17</td>
<td>Stallion, 10 years</td>
<td>Tachycardia, coughing</td>
<td>pseudoaneurysm</td>
<td>AP fistula, pseudoaneurysm</td>
<td>-</td>
<td>3</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>18</td>
<td>Gelding, 4 years</td>
<td>Tachycardia, exercise intolerance</td>
<td>pseudoaneurysm</td>
<td>AP fistula, pseudoaneurysm</td>
<td>-</td>
<td>3</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>Mare, 4 years</td>
<td>Tachycardia, edema, lameness</td>
<td>pseudoaneurysm</td>
<td>AP fistula, pseudoaneurysm</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>Mare, 2 years</td>
<td>Tachycardia, edema, fever</td>
<td>pseudoaneurysm</td>
<td>AP fistula, pseudoaneurysm</td>
<td>-</td>
<td>2</td>
<td>1</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^a\)Symbols represent: + = present and - = absent. \(^b\)Numeric values represent: 0 = no changes, 1 = mild changes, 2 = moderate changes and 3 = marked changes observed. \(^*\)AP fistula: aorto-pulmonary.
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CHAPTER 5: MORPHOMETRIC PROPERTIES OF THE MID-THORACIC AORTA OF WARMBLOOD HORSES AND FRIESIAN HORSES WITH AND WITHOUT AORTIC RUPTURE

Adapted from Journal of Comparative Pathology 154(2-3): 225-230 (2016)

Chapter 5: Morphometric properties of the mid-thoracic aorta of warmblood horses and Friesian horses with and without aortic rupture

Abstract

Rupture of the aorta is much more common in Friesians compared to other horse breeds. It always occurs adjacent to the scar of the ligamentum arteriosum. Previous histologic examination of aortic walls at the site of the rupture suggested the presence of an underlying disorder of the collagen or elastin. In the present study, the structural components of the tunica media of the mid thoracic aorta, away from the lesion, were compared in warmblood horses, and Friesian horses with and without thoracic aortic rupture.

In unaffected Friesian horses, the thickness of the tunica media, as well as the collagen type I area percentage were significantly higher compared to the warmblood horses, supporting the hypothesis of a primary collagen disorder in the Friesian horse breed. In the tunica media of the affected Friesian horses, however, there was no significant wall thickening. Moreover, the area percentage of elastin was significantly lower while the smooth muscle area percentage was higher compared to unaffected Friesian and warmblood horses. These lesions are suggestive of an additional mild elastin deficiency with compensatory smooth muscle cell hypertrophy in affected Friesians.

Keywords: morphometric, Friesian, aorta, rupture, media, collagen, elastin, medial thickness

Introduction

The aorta is a crucial dynamic functional unit in the cardiovascular system (Watanabe et al., 1993). It is composed of multiple constituents that ensure the proper structure and function of the wall. The tunica media plays a major role in the aortic stability (Dingemans et al., 2000) and is characterized by lamellar units, consisting of elastin, smooth muscle cells and collagen (Clark and Glagov, 1985). Elastin and collagen impart the aortic elastic properties and tensile strength, respectively (Holzapfel, 2000). Elastin is the main component of the thoracic aorta (McCloskey and Gleary, 1974). The aortic collagen consists predominantly of types I and III that account for 80-90% of the total collagen in the aortic media (Dingemans et al., 2000; Silver et al., 2001). Whereas type I collagen is the major structural component of the vessel wall, type III collagen is mainly a reparative component (Raman et al., 2011).

Rupture of the aorta is rare in horses. When it occurs, it is typically located near the junction with the heart (Sleeper et al., 2001). In Friesian horses, however, aortic rupture is
much more common and occurs as a transverse tear near the ligamentum arteriosum. The rupture is often associated with a dissection, periaortic haematoma or pseudoaneurysm that ruptures into the pulmonary artery (Ploeg et al., 2013). Abnormalities in both elastin and collagen amount and structure could result in a weakening of the aortic wall resulting in rupture (Tsamis et al., 2013). The detailed architectural features of the aortic wall have not been defined in horses yet. The aim of the present study was to analyse the morphological characteristics of the equine thoracic aorta tunica media in order to gain insight into the possible role of the different structural medial components in the pathogenesis of aortic rupture in Friesian horses. Therefore, the tunica media structure of the mid thoracic aorta was compared in Friesian horses with aortic rupture, healthy Friesian horses and warmblood horses.

**Materials and methods**

**Animals**

*Group WB* comprised 17 warmblood horses (WB) (0-10 years old, median age: 4 years). Twelve of these horses were presented for post-mortem examination for reasons unrelated to the cardiovascular system at Utrecht University (n=5) and Ghent University (n=7). The other horses were obtained from a Belgian slaughterhouse (n=5).

*Group NAF* consisted of 18 non-affected Friesian horses (NAF) (0-10 years old, median age: 4 years). All but one of the horses of this group were presented for post-mortem examination for reasons unrelated to the cardiovascular system at Utrecht University (n=3) and Ghent University (n=14). A single horse of this group was obtained from a Dutch slaughterhouse.

*Group AF* consisted of 20 Friesian horses with an aortic rupture (affected Friesian horses, AF) (1-10 years old, median age: 5 years). All horses were diagnosed with aortic rupture during post-mortem examination at Utrecht University (n=8) and Ghent University (n=12). All of the patients that were admitted alive to the University hospital, were treated following the institutional guidelines. A formal ethical approval was waived by the chairperson of the ethical committee, based on Belgian and European legislation (EU directive 2010/63/EU), as all tissues were derived post-mortem from the necropsy room or from a commercial abattoir.

**Sampling and preparation**

The complete thoracic aorta was removed from the heart base to the diaphragm. The surrounding connective tissue was removed. Samples were taken from the middle of the thoracic aorta and fixed in a buffered 4% formaldehyde solution for at least 24h and routinely embedded in paraffin. Sections were stained with haematoxylin and eosin. For demonstration of elastin and smooth muscle cells, 5 μm thick sections were stained with a monoclonal mouse anti-human anti-elastin antibody BA-4 (1/600, Leica Bio-systems, Diegem, Belgium) and a monoclonal mouse anti-human smooth muscle actin (1/200, DakoCytomation, Brussels, Belgium), respectively. Immunolabeling was achieved with a
high-sensitive horseradish peroxidase diaminobenzidine kit (Envision DAB+ kit, Dako) in an immunostainer (Cytomation S/N S38-7410-01, Dako).
For collagen I and III staining, sections of 3 μm thick were treated with 1/10 normal horse serum and incubated with a monoclonal mouse anti-collagen type I (SIGMA C2456-2ML) or a monoclonal mouse anti-collagen type III (ABCAM 6310). Visualization was obtained using biotinylated horse anti-mouse antiserum IgG (VECTOR LABS BA-2000), ABC/PO complex solution elite (VECTOR LABS, PK-6100) and diaminobenzidine chromogen.

**Morphometric analyses**
All tissue sections were randomized and blindly examined. HE stained sections were used to evaluate medial aortic lesions.
The medial thickness (defined as the perpendicular distance between the innermost and outermost elastic lamella of the aortic media) was measured on sections stained with anti-elastin antibody under low-power magnification (25x). Three image frames were taken on each specimen. Of each image, one measurement was performed and the averages on the 3 images were calculated.
The area percentage of elastin, collagen types I and III and smooth muscle actin was determined by image analysis. The measurements were made with a light microscope to visualize the tunica media at a magnification of 400x using a Leica camera DFC320 (Leica Microsystems Ltd, Wetzlar, Germany) coupled to a computer-based image analysis system LAS v.3.8. (Leica Microsystems Ltd). Three (collagen) or four (elastin-smooth muscle actin) image frames were taken per slide.
Fragmentation of the elastic fibres in the tunica media was scored from 0 (no fragmentation) to 4 (severe) according to Carr-White et al. (2000). Fragmentation of the collagen type I and III fibres was scored using a scale from 0 (no fragmentation) to 3 (severe fragmentation).

**Statistical analysis**
The differences between groups were analysed using a mixed model with horse as random effect and group and their interaction as categorical fixed effects. Testing was done based on F-tests at the 5% global significance level, but for the pairwise comparisons the P-value was adjusted by Tukey’s multiple comparisons technique.
Results

HE
The tunica media showed a regular lamellar organization of smooth muscle cells, elastin and collagen fibres in all three groups. Prominent smooth muscle cell hypertrophy was seen in fourteen AF. Patchy medial necrosis was present in one AF (gelding, 5 years old), two NAF (mare 5 years old; mare, 0 years old) and two WB (mare, 6 years old; gelding, 3 years old). Multifocal medial mineralization was observed in one WB (mare, 6 years old). Inflammation of the tunica media was absent in all horses.

Medial thickness
The tunica media of the mid thoracic aorta of non-affected Friesian horses was significantly thicker compared to warmblood horses (p<0.001) (NAF: 4773μm ± 369; WB: 3546μm ± 319) (Figure 32).

Elastin
The elastin fibres occupied a significantly smaller area percentage of the aortic media in affected Friesian horses compared to nonaffected Friesian horses and warmblood horses (Figure 33 A and B) (AF vs NAF: p=0.0004; AF vs WB: p=0.0054) (AF: 42% ± 2; NAF: 51% ± 2; WB: 49% ± 2) (Figure 34). The median score for elastin fragmentation was not significantly different between the groups (AF: 3; NAF: 2.75; WB: 3). The range of the scores was smaller in the affected Friesians (AF: 2-4) compared to the control horses (NAF: 1-4; WB: 0-3.5).
Figure 33.
(A) Mid-thoracic aorta. Loss of elastin in an affected Friesian horse shown by elastin immunohistochemistry. (B) Mid-thoracic aorta. Lamellar pattern of elastin in an unaffected warmblood horse using elastin immunohistochemistry.
Collagen
The collagen type I occupied a significantly higher percentage of the aortic media in the Friesian horses compared to the warmblood horses (AF: 37% ± 4; NAF: 46% ± 4; WB: 26% ± 4) (AF vs NAF: p=0.0974; AF vs WB: p=0.0486; NAF vs WB: p<0.0001) (Figure 35). There were no differences found for collagen type III area percentage between the different groups (AF: 33% ± 3; NAF: 30% ± 3; WB: 35% ± 3).

The median score for collagen types I and III fragmentation was not different between the groups and was the same for both collagen types (AF: 1; NAF: 1; WB: 1).
Smooth muscle cells
The area percentage of the smooth muscle cells was higher in affected Friesian horses compared to unaffected Friesian horses and warmblood horses (Fig. 36 A and B) (AF vs NAF: p=0.0024; AF vs WB: p=0.0025) (AF: 52% ± 2; NAF: 41% ± 2; WB: 41% ± 2) (Figure 37).

Figure 36.
(A) Mid-thoracic aorta. Smooth muscle hypertrophy in an affected Friesian horse using smooth muscle actin (SMA) immunohistochemistry. (B) Mid-thoracic aorta. Typical morphology of smooth muscle cells in an unaffected warmblood horse using SMA immunohistochemistry.
Figure 37.
Percentage area of smooth muscle cells in the tunica media of the mid-thoracic aorta of Friesian horses with aortic rupture (AF), non-affected Friesians (NAF) and warmblood horses (WB). ∗p <0.005.
**Discussion**

Aortic rupture in Friesian horses can result in sudden death or lesions can be present for several weeks to months with many horses developing an aortopulmonary fistula (Ploeg et al., 2013). Recently, samples taken at the aortic rupture (which is located 1-2 cm proximal to the ligamentum arteriosum) of Friesian horses have been examined microscopically (Ploeg et al., 2015). Lesions included accumulation of mucoid material, disorganization and fragmentation of the elastic laminae, aortic smooth muscle cell hypertrophy and medial necrosis. Inflammation varied from predominantly neutrophilic infiltrates in the media and periadventitial tissue in the acute cases to the presence of mainly hemosiderophages in the periadventitial tissue in the chronic cases. Medial fibrosis with aberrant collagen morphology was present in the subacute and chronic cases. A primary connective tissue disorder was suggested based on the presence of mucoid accumulation combined with elastin fragmentation (Ploeg et al., 2015).

In order to demonstrate a possible underlying connective tissue defect, we examined samples of mid-thoracic aortic media. Since this location is distant from the predilection site, secondary changes as observed adjacent to the aortic rupture will be absent or minimal. Since the warmblood horse is not considered a breed at risk for aortic rupture, comparison of aortic wall samples of Friesian horses (affected and non-affected) with warmblood horses could therefore be a first step to elucidate a possible underlying mechanism of aortic rupture in Friesian horses.

In the present study, two important differences were observed in the thoracic aortic wall tunica media between warmbloods and Friesians. First of all, nonaffected and affected Friesians had a higher collagen type I area percentage compared to the warmblood horses. This phenomenon could be a secondary fibrosis caused by hypertension (Repova-Bednarova et al., 2013). In hypertension induced aortic fibrotic remodelling however, both collagen type I and III are expected to increase, while only collagen type I was increased in the Friesian horses in this study (Repova-Bednarova et al., 2013). Alternatively, the higher collagen type I area percentage may indicate a primary collagen defect. The existence of a general collagen disorder in the Friesian horse breed has been suggested before, as tendon stretch properties of Friesians differ from that of other breeds (Gusseklo et al., 2002). Collagen I is indeed a primary component of tendons (Wang et al., 2006).

The second breed-specific difference was a larger mean aortic medial wall thickness in Friesians compared to warmbloods. Racial/ethnic differences in mean aortic wall thickness have also been described in humans (Rosero et al., 2010). Increased aortic medial thickness together with the above mentioned increased density of collagen I fibres may compensate for any reductions in tensile strength. This may explain why in in vitro tensile tests, no significant differences were observed between Friesians and warmbloods (Saey et al., 2015).

Friesian horses with aortic rupture, however, for some unknown reason, did not present a significant increase in aortic medial thickness when compared to the warmbloods. This may be one of the reasons why they were prone to develop aortic rupture. Decreased
aortic wall thickness indeed has been mentioned as a potential risk factor for aortic dissection in humans (Shiran et al., 2014).

In the affected Friesians, the elastin area percentage of the tunica media of the mid thoracic aorta was significantly lower. Since elastin is deposited mostly early in life and has poor regenerating potential (Pezet et al., 2008), reduced elastin area percentage may indicate either a primary deficiency in elastin synthesis or an increased susceptibility of elastin to elastolysis. In either case, a mild primary elastin defect can be suspected. Indeed, in case of severe elastin defects as for example observed in Marfan syndrome, aneurysm formation is typical (Milewicz et al., 2005), which is however not observed in Friesians. A mild defect in elastin could result in an increased load-bearing on the smooth muscle cells leading to hypertrophy. This has also been demonstrated in bovine and human Marfan syndrome (Scheck et al., 1979; Potter and Besser, 1994). In the affected Friesian horses, indeed, the smooth muscle actin area percentage in the mid thoracic aorta was increased compared to the unaffected Friesian and Warmblood horses. This can be explained by the smooth muscle cell hypertrophy, as observed in the HE sections. This phenomenon could be induced by hemodynamic changes. Smooth muscle cell hypertrophy of the aorta has been associated with cyclic mechanical stress (Chiu et al., 2013), as for example seen in induced hypertension (Owens, 1987). Interestingly, blood pressure values in horses vary between breeds whereby for example Thoroughbreds generally have higher values compared to Standardbreds (Parry et al., 1984). Therefore, the smooth muscle hypertrophy observed in the aorta of the affected Friesian horses could be secondary to increased blood pressure levels. Medial collagen deposits, as observed in the present study in nonaffected and affected Friesians, have also been associated with hypertension (Rossi et al., 2002). A sustained increase in blood pressure can lead to arterial wall thickening (Ghiadoni et al., 1998). Affected Friesians, however, lack the typical collagen type III increase as well as the aortic wall thickening.

To conclude, the present study points to the following mechanism underlying the pathogenesis of aortic rupture in Friesian horses: a breed-specific collagen defect with a mild elastin defect superimposed. These results will help to focus further genetic studies to unravel the pathogenesis of this intriguing disease.
References


CHAPTER 6: ULTRASTRUCTURE OF THE SMOOTH MUSCLE CELLS IN THE MID- THORACIC AORTIC MEDIA IN FRIESIAN HORSES WITH AORTOPULMONARY FISTULATION

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V Saey, M Ploeg, K Chiers, C Delesalle, A Gröne, W Back, PR van Weeren, CM de Bruijn, K D’Herde, G van Loon and R Ducatelle
Chapter 6: Ultrastructure of the smooth muscle cells in the thoracic aortic media in Friesian horses with aortopulmonary fistulation

Introduction

Aortic rupture is a known condition in Friesian horses and most likely has a genetic component. An underlying general collagen disorder has been suggested based on histological observations and morphometric measurements (Ploeg et al., 2015; Saey et al., 2016). Changes in smooth muscle cells ultrastructural characteristics can be expected in association with a connective tissue disorder in the aortic wall (Bunton et al., 2001; Ichii et al., 2001; Takaluoma et al., 2006). Smooth muscle cell hyperplasia has been observed not only at the level of the lesion but also further away, in the mid thoracic aorta (Ploeg et al., 2015; Saey et al., 2016). Ultrastructural examination of the aortic media focussing on the smooth muscle cells could be useful to get insight into the specific changes occurring in these cells.

Material and methods

Three Friesian horses (mare, 6 years old; gelding, 4 years old; mare, 4 years old) diagnosed with an aortic rupture and aortopulmonary fistulation at the Faculty of Veterinary Medicine of Ghent University, Belgium were included. They underwent a complete necropsy. Horse number 1 foaled 3 weeks earlier. The mare was apathetic and ventral oedema was noticed a few days before. In horse number 2, lethargy and ventral oedema was first noticed 10 days earlier. Horse number 3 showed ventral oedema and a jugular pulse that persisted for months.

The group of control animals consisted of 6 horses, 3 nonaffected Friesian and 3 warmblood horses. These 3 nonaffected Friesian horses (mare, 21 years old; stallion; 11 years old; mare, 9 years old) underwent a complete necropsy at the Faculty of Veterinary Medicine for reasons unrelated to the cardiovascular system. The three control Warmblood horses (mare, 15 years old; mare, 9 years old; mare, 18 years old) were healthy slaughter horses.

Samples for electron microscopic examination were taken from the mid-thoracic aorta and fixed in cacodylate buffered glutaraldehyde/paraformaldehyde solution and processed for transmission electron microscopy as described by De Spiegelaere et al. (2008).
**Results**

The smooth muscle cells in all affected horses showed signs of hypertrophy compared to the control animals (Figure 38). The smooth muscle cells of the affected Friesians also showed more pronounced organelles (mitochondria, rough endoplasmic reticulum, Golgi) in the perinuclear cytoplasm with loss of contractile myofilaments (Figure 39). Moreover, the smooth muscle cells contained subplasmalemmal vacuoles.

![Figure 38. Hypertrophy of smooth muscle cells.](image)
An important limitation of this study was the fact that the control horses were older compared to the affected Friesian horses. However, degenerative lesions in the aortic wall are generally considered to progress with aging. Indeed, smooth muscle cell necrosis has been observed more frequent with ageing in rats (Kojimahara, 1985).

In all affected horses, it was evident that SMC distant from the rupture demonstrated several changes such as hypertrophy, loss of myofibres, vacuolization and increased number of organelles. It is likely that the observed changes in the smooth muscle cells are a compensatory phenomenon to altered mechanical stresses, for example primary
hypertension. Indeed, hypertrophy, hyperplasia and an increased metabolic activity of vascular smooth muscle cells have typically been reported in man and animal models of hypertension (Tajsic and Morrell, 2011). In such conditions, a phenotypic switch from contractile (prominent myofilaments) to synthetic (prominent organelles involved in protein synthesis) phenotype, as seen in this study, is also typically associated with altered wall stresses (Rensen et al., 2007). In our study, samples of the aorta were taken distally from the rupture. It is possible that shunting of blood through the aortopulmonary fistula can cause alterations in wall stresses leading to a phenotypic switch. However, as pressure post fistula formation in the aorta is expected to decrease instead of increase, hypertrophy of the smooth muscle cells seems rather unlikely.

Vacuolation of smooth muscle cells has also been observed in bovine Marfan syndrome at the level of the aortic aneurysm (Potter and Besser, 1994). Similar lesions have also been observed in aortic medial smooth muscle cells in human Marfan syndrome where these lesions precede elastolysis, suggesting that the loss of cell attachments is the triggering factor (Bunton et al., 2001). The existence of an underlying general connective tissue disorder in Friesians, as suggested in several reports (Ploeg et al., 2015 and Saey et al., 2016) could imply a weakening of the aortic wall resulting in changed wall stresses and associated smooth muscle cell alterations. Therefore the observed changes in the SMC could be a primary or secondary phenomenon or a combined effect.

**Conclusion**

In conclusion, smooth muscle cells in the mid thoracic aorta of Friesian horses with aortic rupture demonstrated hypertrophy and morphological changes suggestive of a synthetic phenotype. Such changes could be related to a general connective tissue disorder and/or secondary to altered mechanical wall stresses.
References


CHAPTER 7: BIOMECHANICAL AND BIOCHEMICAL PROPERTIES OF THE THORACIC AORTA IN WARMBLOOD HORSES, FRIESIAN HORSES, AND FRIESIANS WITH AORTIC RUPTURE

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V Saey, N Famaey, M Smoljkic, E Claeys, G van Loon, R Ducatelle, M Ploeg, C Delesalle, A Gröne, L Duchateau and K Chiers
Chapter 7: Biomechanical and biochemical properties of the thoracic aorta in warmblood horses, Friesian horses, and Friesians with aortic rupture

Abstract
Thoracic aortic rupture and aortopulmonary fistulation mainly affect Friesian horses. An underlying general connective tissue disorder has been suggested. This connective tissue disorder could result in intrinsic differences in biomechanical properties of the aortic wall predisposing this horse breed to aortic rupture. The biomechanical and biochemical properties of the thoracic aorta were characterized in warmblood horses, unaffected Friesian horses and Friesians with aortic rupture. Samples of the thoracic aorta at the ligamentum arteriosum (LA), mid thoracic aorta (T1) and distal thoracic aorta (T2) were obtained from Friesian horses with aortic rupture (AF), nonaffected Friesian (NAF) and warmblood horses (WB). The biomechanical properties of these samples were determined using uniaxial tensile and rupture assays. The percentages of collagen and elastin (mg/mg dry weight) were quantified. Data revealed no significant biomechanical or biochemical differences among the different groups of horses. The distal thoracic aorta displayed an increased stiffness associated with a higher collagen percentage in this area and a higher load-bearing capacity compared to the more proximal segments. Our findings match reported findings in other animal species. Study results did not provide evidence that the predisposition of the Friesian horse breed for aortic rupture can be attributed to altered biomechanical properties of the aortic wall.

Keywords: horse, aorta, rupture, tensile test, collagen, elastin

Background
The aorta not only serves as a conduit but also plays a major role in regulating the entire cardiovascular system thanks to its biomechanical properties. Vascular biomechanical properties play a major role in cardiovascular function. Nevertheless, information about the biomechanical and biochemical composition of the equine thoracic aorta is very sparse. Biomechanical properties of the aortic wall are influenced by its composition and organization of its main structural components (Holzapfel et al., 2000). The walls of large arteries are composed primarily of elastin, collagen and vascular smooth muscle cells. Elastin supports passive wall forces at low values of wall strain. At high pressure, the resistance to stretch is mainly attributed to collagen (Wolinsky and Glagov, 1967). Characterizing the biomechanical properties of soft biological materials is challenging as they are non-homogeneous and anisotropic (Holzapfel et al., 2000). Biomechanical properties of the aorta are usually determined by tensile testing. A method commonly
applied is uniaxial tensile testing in which tissue is stretched in a controlled way from a
quasi zero-stress state to rupture. During the subjection to extension, the force (or
displacement) is recorded. Force-extension data can then easily be converted into stress
and strain measurements (Avanzini et al., 2014).
Atraumatic, spontaneous aortic ruptures are uncommon and life-threatening in humans.
In most cases, the aortic rupture occurs in the abdominal part and is associated with an
aneurysm (Grange et al., 1997). In non-Friesian horse breeds, aortic rupture is rare. It
typically occurs at the level of the sinuses of Valsalva. In most cases it leads to acute
death (Rooney et al., 1967). In Friesian horses, however, thoracic aortic ruptures are
much more frequently encountered compared to other horse breeds (Van Vliet and Back,
2006). The rupture is typically transverse near the ligamentum arteriosum. It often leads
to the formation of a pseudoaneurysm and fistulation into the pulmonary artery. Survival
up to several months has been reported (Ploeg et al., 2013).
To the best of the authors’ knowledge, tensile properties of the thoracic aorta have only
been measured in hereditary equine regional dermal asthenia (HERDA) in affected and
healthy Quarter Horses (Bowser et al., 2014). The purpose of the present study was to
obtain reference data concerning the strength of the equine thoracic aorta and to
investigate the possible role of tensile strength in aortic rupture in Friesian horses.

Methods

Animals and aorta preparation

*Group AF consisted of 8 affected Friesian horses (4–10 years old, median age 5.4 years, 1
stallion, 3 mares and 4 geldings) that were diagnosed with aortic rupture by post-mortem
examination at the Faculty of Veterinary Medicine of Ghent University, Belgium. In the
group of affected Friesian horses, time between death and tissue harvesting varied
between 1 and 24 h.

*Group NAF comprised 10 nonaffected Friesian horses (1–10 years old, median age 5.5
years, 2 geldings and 8 mares). The animals were presented for post-mortem examination
at the Faculty of Veterinary Medicine of Ghent University, Belgium, for reasons unrelated
to the cardiovascular system.

*Group WB consisted of 10 warmblood horses (1–10 years old, median age 5.4 years, 1
gelding and 9 mares). The horses of group 3 were presented for post-mortem
examination at the Faculty of Veterinary Medicine of Ghent University, Belgium, for
reasons unrelated to the cardiovascular system (n = 5) or were collected at the
slaughterhouse (n = 5).

All of the patients that were admitted alive to the University hospital, were treated
following the institutional guidelines. A formal ethical approval was waived by the
chairperson of the ethical committee, based on Belgian and European legislation (EU
directive 2010/63/EU), as all tissues were derived post-mortem from the necropsy room
or from a commercial abattoir.
The complete thoracic aorta, from semilunar valve to the diaphragm, was dissected and
the surrounding connective/fat tissue was removed. Each thoracic aorta was cooled (4–5
°C) for maximum 8 h in isotonic physiological solution (phosphate buffered saline (PBS,
pH: 7.4). The time between sample harvesting and testing varied between 4 weeks and 6
months.

**Tensile and rupture test**

A rectangular and a dumbbell-shaped sample were cut in axial direction at 3 different
locations: adjacent to the ligamentum arteriosum (avoiding the area of rupture and the
fibrotic scar of the ligamentum) (LA), at the mid region of the thoracic aorta (T1) and at
distal end of the thoracic aorta (T2).

Rectangular specimens (1 × 6 cm) were cut using a custom made cutting knife with razor
blades. Dumbbell-shaped specimens (3.5 × 5.5 cm) were cut using a template and surgical
scissors. Original thickness \(t_0\) and width \(w_0\) of each strip were measured at the zero-
stress state using a digital calliper. The strips were kept frozen in PBS (−20 °C) until
testing, at which point they were thawed slowly and tested at room temperature. In 3 of
the 5 affected Friesian horses used for tensile testing, sampling at the level of the
ligamentum arteriosum (LA) was impossible due to extensive rupture at this site. T2
samples from the 5 slaughtered warmblood horses could not be collected.

For the cyclic tensile assay, rectangular aortic samples were fixed in a set of grips and
mounted on an Instron® 5567 uniaxial tensile testing bench. To avoid slipping, sandpaper
was placed between the tissue and the grips. The load cell had a capacity of maximum
1kN with a resolution of 0.05N. The grip-to-grip length of each sample was manually
entered based on the calculation of this length at 0 % strain by the chosen program. Both
the original width and thickness, as well as the grip-to-grip length, were manually entered
in the program and were thus all taken into account for calculation of the aortic
properties.

The cyclic tensile test was performed at a crosshead speed of 1mm/s (10 cycles at 10 %,
10 cycles at 25 % and 10 cycles at 50 % strain). Then the samples were left to relax for 30
s at 50 % extension, followed by stretching until rupture. At each time point \(i\), the tensile
force \(F_i\) and gauge length \(l_i\) were recorded at a sampling rate of 10 Hz. Assuming
incompressibility of the tissue, the true stress \(\sigma_i\) and true strain \(\varepsilon_i\) response of the
tissue were derived from the force-displacement measurements as follows:

\[
\sigma_i = \frac{F_i}{A_i},
\]

\[
\varepsilon_i = \log \left( \frac{l_i}{l_0} \right) + 1
\]

\[
A_i = \frac{w_0 \cdot t_0 \cdot l_0}{l_i}
\]
With the cross-sectional area of the sample at a certain time point $i$. Data analysis was performed using Matlab® and following parameters were calculated from the stress-strain curves:

$E_{10}$, $E_{20}$, $E_{30}$ and $E_{40}$ (MPa): the tangent stiffness moduli or Young moduli at 10, 20, 30 and 40 % strain, which are defined as the slopes of the stress-strain curve at the respective strain level. As the aorta is a nonlinear material, a single value of elastic Young’s modulus would not represent the stiffness of the material. A typical curve obtained when determining the tangent stiffness is depicted in Figure 40.

![Sample stress strain curve. $E_{10}$, $E_{20}$ and $E_{30}$ are the tangent stiffness moduli. The maximum engineering stress at failure is presented as $\sigma_{\text{fail}}$, $\sigma_b$ and $\sigma$ are the tensile stresses at the beginning and end of the relaxation phase.](image)

$\tau_r$ (s): the relaxation time during the relaxation phase of the cyclic tensile test. This is a measure of the viscoelasticity of the material and is the ratio of the viscosity $\eta$ to the tangent elasticity $E$:

$$\tau_r = \frac{\eta}{E} = -\frac{t^{\text{hold}}}{\log \left( \frac{\sigma_e}{\sigma_b} \right)}$$
With $t^{\text{hold}} = 30\text{s}$, i.e., the time at which the crosshead remained in place, and $\sigma^b$ and $\sigma^e$ the tensile stress at the beginning and at the end of the relaxation phase, respectively. Note that a higher relaxation time corresponds to a lower viscoelastic response.

For the rupture test, dumbbell shaped aortic samples were mounted on the same tensile machine. At the same crosshead speed of 1mm/s, the sample was elongated until rupture. Again, tensile force and gauge length were recorded. Two specimen modes of failure were used as data exclusion criteria: edge slipping out of the grips or when failure did not occur at the necked region of the dumbbell-shaped specimens. From these tests, the following parameter was extracted using Matlab®:

$$\sigma_{\text{fail}} = \frac{F_{\text{max}}}{A_0} \text{(MPa)}$$

the engineering stress value at which failure occurred, a measure of the strength of the material. In the equation, $F_{\text{max}}$ is the maximum force recorded during the tensile experiment and $A_0 = w_0 \cdot t_0$ is the initial cross-sectional area of the sample at the necked region.

For a more detailed explanation of the variables defined here, see Dill, 2006: chapters 2.5 and 5.1.

Collagen and elastin quantification

Quadratic samples of the aorta (5 × 5 cm) were cut using surgical scissors at the three levels as mentioned above and stored frozen (−20 °C).

For collagen analysis, one gram of frozen aortic tissue was oven dried for 2 h at 100 °C. Dry weight was determined. The collagen concentration was then quantified using the ISO/DIS 3496.2 method (Narine et al., 2006) and was expressed as a percentage of dry weight.

For elastin analysis, one gram of frozen aortic tissue was first pulverized in liquid nitrogen using a B-Braun Biotech International mikro-disembrator. Elastin was then prepared by the hot alkali method (Mecham, 2008), a modification of the original method of Lansing et al. (1952). This method is based on the degradation of the peptide bonds in elastin (Serafini-Fracassini, 1975). The remaining pellet was dried by lyophilisation for 24 h to yield elastin weight fractions. Elastin concentration was expressed as percentage of dry weight. The purity of the elastin was confirmed by histology and immunohistochemistry. From each group of horses, an elastin pellet was embedded in paraffin wax, and cut into 4-µm-thick sections. Sections were stained with hematoxylin and eosin which showed fragmented eosinophilic fibrillary material without nuclei (compatible with elastin fibres). All fibres present on the slides stained positive with a monoclonal anti-elastin Leica Bio-systems antibody BA-4. A standard Envision avidin-biotin complex method with diaminobenzidine as chromogen was used for visualization.

Collagen and elastin quantification was not performed in the 5 slaughtered warmblood horses.
Statistical analysis

Statistical analyses were based on a mixed model with horse nested in group as random effect and location (LA/T1/T2), group of horse (AF/NAF/WB) and their interaction as fixed effects. For the analysis of the modulus, the strain level was added to the model as a fixed effects factor. The normality assumption was tested by the Shapiro-Wilk test and was not rejected. Overall F-tests and pairwise comparisons were performed. A Bonferroni correction was applied for the pairwise comparisons between horse groups with comparisonwise significance level set at 0.0166 (=0.050/3).

Results

Tensile and rupture test

No significant differences between the three groups of horses were observed for $\sigma_{\text{fail}}$, $\tau_r$ and $E_i$ and also no significant interactions between the group of horse and location were found ($p > 0.05$). There was however a significant overall effect (over the horse groups) of the location on these parameters. The $\sigma_{\text{fail}}$ (MPa), the tissue strength, was significantly ($p < 0.001$) higher at location T2 compared to LA and T1 (B: 0.22 ± 0.06; T1: 0.31 ± 0.06; T2: 0.73 ± 0.07) (Figure 41). The tangent moduli $E_i$ of the aorta at location LA and T1 were lower ($p < 0.001$) compared to location T2 (LA: 0.19 ± 0.05; T1: 0.29 ± 0.05; T2: 0.70 ± 0.05). The relaxation time $\tau_r$ (s) was higher at location LA compared to T1 and T2 (LA: 508 ± 29; T1: 381 ± 26; T2: 343 ± 30) ($p < 0.002$) (Figure 42).
Figure 41. Maximum engineering stress at failure of the equine thoracic aorta at 3 sample locations (LA-T1-T2) in 3 groups of horses (red: Friesian horses with aortic rupture; green: nonaffected Friesian horses; blue: nonaffected warmblood horses) (*: p < 0.001)

Figure 42. Relaxation time, determined during cyclic testing, of the equine thoracic aorta at 3 sample locations (LA-T1-T2) in 3 groups of horses (red: Friesian horses with aortic rupture; green: nonaffected Friesian horses; blue: nonaffected warmblood horses) (*: p < 0.002)
Collagen and elastin concentration

No significant effect of horse group or interaction between horse group and location was found for the collagen and elastin concentration \( (p > 0.05) \). The collagen concentration was significantly higher \( (p < 0.001) \) at T2 compared to LA and T1 (LA: 17 % ± 1; T1: 16 % ± 1; T2: 31 % ± 1). The aorta of the affected Friesians at the rupture site (LA) tended to contain a numerically higher collagen percentage compared to the non-affected Friesian and warmblood horses, however this difference was not statistically significant \( (p > 0.05) \) (Figure 43). The elastin percentage showed no significant regional differences \( (p > 0.05) \) (Figure 44).
Discussion

The present study demonstrates that the biomechanical and biochemical characteristics of the equine aortic wall show regional differences. The distal site of the thoracic aorta (T2) could withstand a higher maximum stress and was stiffer compared to the more proximal sites (LA and T1). The aorta at the ligamentum arteriosum (LA) showed a lower visco-elastic response compared to T1 and T2.

These regional differences are in accordance with the majority of reports in the literature confirming the regional variation of the biomechanical properties of the aorta in humans and several animal species (Cohen et al., 1972; Purslow, 1983; Guo and Kassab, 2003). In canine aortas, the force needed to rupture longitudinal strips is minimal in the ascending aorta and in general increases with distance from the semilunar valve (Cohen et al., 1972). In our study, the equine distal thoracic aorta (T2) was also able to withstand higher maximum stresses before rupture compared to the more proximal thoracic aorta (LA and T1).

In the study by Bowser et al. (2014), samples were collected from the thoracic aorta of Quarter Horses (6 HERDA affected - 6 control) with a similar median age as in our study. The tensile strength of the thoracic aorta in the HERDA study of the control Quarter Horses (0.45 MPa) was comparable to the average failure stress $\sigma_{\text{fail}}$ of our samples (0.42 MPa). To compare the tangent moduli to the elastic modulus, the method used in the present study is preferred for two reasons. First, we report the tangent modulus related to the true stress-strain curve, whereas Bowser et al. (2014) reported the slope of the engineering stress-strain curve. The latter is based on the initial cross-sectional area of the sample and underestimates the stiffness for large deformations. Secondly, we report a number of tangent moduli along the nonlinear stress-strain curve, thereby accounting for the material nonlinearity, whereas Bowser et al. reported one linearized (or averaged) value. Other explanations for differences in stiffness can be attributed to breed differences and the difference in location of sampling.

In Friesian horses, an underlying genetic defect of the connective tissue in the aortic media has been suggested based on the morphological characteristics of the lesions (Ploeg et al., 2015). The higher percentage of collagen near the ruptured site of the affected Friesian horses, although not significant, can be explained by the medial fibrosis seen at the level of the aortic rupture histologically. This was suggested to be due to chronic injury or abnormal hemodynamic changes (Ploeg et al., 2015). As the aortic rupture in Friesian horses typically occurs near the scar of the ligamentum arteriosum (van der Linde-Sipman et al., 1985), samples at this site (LA) were included and biomechanical properties were determined. Unfortunately, as mentioned above, due to the small number of samples of affected Friesians and because the aortic rupture is relatively large, it is likely that the exact predisposed site in susceptible animals was not included. Friesians were not different from warmblood horses in their biomechanical or biochemical properties. Our results suggest that aortic rupture in Friesians is not associated with a generalized aortic
wall condition. However, the relative small number of samples and the data exclusion criteria used for rupture testing, were substantial limiting factors in this study.

The average sum of the collagen and elastin content in all horses ranged from 55 % at location LA to 56 % at T1 and 67 % at T2. This is in accordance with the reported content of about 60 % of collagen plus elastin in the aortic media, regardless of species (Harkness et al., 1957).

The distal thoracic aorta (T2) was stiffer compared to the proximal thoracic aorta (LA and T1) and contained a higher percentage of collagen. It is generally accepted that, due to the predominance of collagen fibres, the distal aorta becomes stiffer (Lee and Kamm, 1994). In contrast, elastin content is reported to be equally distributed along the thoracic aorta (Zou and Zhang, 2009), as was also found in our study. Zou and Zhang (2009) also suggested that the biomechanical properties of elastin can vary along the thoracic aorta. Indeed, solely the arteries’ collagen and elastin content is not sufficient to explain the differences in elastic moduli at different sites, but depends on their in vivo biomechanical properties and architectural arrangement (Cox, 1978).

In human Marfan syndrome, a hereditary disorder of the fibrillar metabolism, cystic medial necrosis is a typical finding and associated loss of elastin has been mentioned (Halme et al., 1985). Perejda et al. (1985) were able to demonstrate a decreased tensile strength of the aorta in affected patients. In the histological study of aortic lesions in Friesians with aortic rupture, cystic medial necrosis was reported (Ploeg et al., 2015). In the affected Friesians in this study, there was no decrease in elastin concentration and mechanical properties were not altered near the area of the rupture. In bovine Marfan syndrome however, cystic medial necrosis is absent and elastin concentrations are reported to be normal (Parsons et al., 1992). True aneurysms are not observed in aortic rupture in Friesian horses (Ploeg et al., 2013) and there are no indications pointing towards a Marfan-like syndrome in these horses.

Our method of testing shows some limitations as only uniaxial testing on aortic strips was performed. Excised aortic strips retract leading to thickening of the wall and reorientation of intramural structures (Dobrin, 1978). As this test method ignores the multiaxial loading state under physiological conditions, it gives no information on the anisotropy or the relation between pressure and radius in the intact vessel (Holzapfel, 2006). All measurements were performed in the axial direction, while under physiological conditions, the aorta is subjected to cyclic strains in both circumferential and longitudinal direction. In Friesian horses, aortic rupture typically occurs transversely (Ploeg et al., 2013), indicating a possible weakening of the aorta and thus a decreased tensile strength. Therefore, axial testing of the aorta in longitudinal direction was preferred in this study as we wanted to compare the tensile strength of the aorta between horse breeds. Due to practical reasons, it was impossible to create a standardized time between tissue harvesting and testing. However, we believe that this did not affect our results. After all, samples that were stored for a longer period of time were present in all three groups.
Note that the variables discussed here pertain to the material properties of the artery and hence make abstraction of the arterial geometry. This is a conscious choice, as it allows a fair comparison of the intrinsic material without having to take into account the complex geometry and loading state in each animal.

Conclusions

The equine thoracic aorta is stiffer more distally. This can be explained at least partially by a higher collagen content. No significant biomechanical and compositional differences were found between warmblood horses, Friesian horses with aortic rupture and nonaffected Friesians. Our results suggest the existence of a local, hereditary defect, rather than a generalized aortic wall condition, predisposing Friesian horses for aortic rupture. At this point, one cannot exclude a difference in organization of collagen and elastin being responsible for the rupture, however this would most probably reflect on the general biomechanical properties.
References


### APPENDIX

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CHAPTER 8: GENERAL DISCUSSION

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Chapter 8: General Discussion

Possible hypotheses for aortic rupture in the Friesian horse breed that were advanced:

1) aortic rupture in Friesians is a **breed-related** pathology, as differences are observed between Friesian and Warmblood horses

2) aortic rupture in Friesian horses is part of a **generalized** connective tissue disorder, as lesions are also observed outside the rupture site. The location of the rupture is possibly due to anatomical and/or haemodynamic factors

3) aortic rupture is due to a **local**, hereditary defect at the ligamentum arteriosum

4) aortic rupture in Friesians is a **case-related** pathology, as differences are seen between affected and nonaffected Friesian horses

5) aortic rupture is a **poly-genetic** condition

6) **environmental** factors, such as nourishment, might play a role in the pathogenesis
1. Aortic rupture in Friesian horses

Since the underlying cause of the rupture is so far not elucidated, identification of factors involved in development of aortic rupture and/or associated lesions/symptoms could contribute to create preventive measures and/or aid in obtaining a definitive diagnosis. From 1997-2015, our research group has performed necropsy on 42 Friesian horses suffering from an aortic rupture. Of these 42 horses, 23 were mares. This is in agreement with the suggestion by Ploeg et al. (2013) that there is probably no gender predisposition for aortic rupture in Friesian horses.

The affected Friesian horses were mostly young horses (approximately 70% of the horses were 1-5 years old) that had just started training. It is possible that physical exercise could be a contributing factor since this results in an increased heart rate and blood pressure (Evans, 1985). As mentioned above, severe physical exercise in humans predisposes to aortic dissection due to the dramatic rise of the blood pressure (Hatzaras et al., 2007). Moreover, training is associated with increased cortisol levels in horses as it is considered a stressful event (Kedziersky et al., 2014) and could possibly also increase blood pressure. Four of the 23 Friesian mares with an aortic rupture were in foal and a single affected mare was admitted to the hospital shortly after parturition. Pregnancy is an influential predisposing factor for aortic pathology in humans, as half of the acute aortic dissections in women under the age of 40 occur during pregnancy or in the peripartum period (Coulon, 2015). Pregnancy is associated with an increased maternal blood volume, blood pressure, stroke volume and cardiac output (Morris et al., 2014). Moreover, the alterations in oestrogen and progesterone plasma concentrations change the vascular structure (remodelling), rendering the aortic wall more susceptible to injury (Jovanovic and Jovanovic, 1997).

It is thus tempting to speculate that both physical exercise and pregnancy could contribute to aortic rupture in Friesians.

It is possible that environmental factors such as feeding, management etc. play a role in the development of this disease in Friesians.

1.1. Probably an underestimated prevalence

The prevalence of aortic rupture in Friesians is estimated to be 2% (Van Vliet and Back, 2006). However, this percentage is possibly an underestimation. This prevalence is based on necropsy reports. However, Friesian horses that suddenly collapse due to exsanguination or cardiac tamponade are less likely to arrive in hospital and/or are not always admitted for necropsy. Furthermore, a large number of horses develop an aortopulmonary fistula. Clinical symptoms are not always obvious in those patients and the lesions can easily be missed during routine post-mortem examination as modified incision techniques of the heart are required for diagnosis (Ploeg et al., 2013). It is thus
possible that affected cases were missed in the past because of the obscenity of this disease at that time. Moreover, Friesian horse breeders may not always be keen to have a necropsy done on their deceased animals, fearing negative publicity (personal experience). All of this suggests that the number of reported cases could be much lower than the true number of cases occurring in the field. Currently, genetic research is being performed in an attempt to localize the genetic defect. If a genetic test would become available, this would enable the studbook to obtain a true vision on the number of affected and carrier animals.

1.2. Aortic rupture in Friesian horses: unique site

In all affected Friesians monitored by our research group, the rupture was located near the ligamentum arteriosum. This site is very unique to Friesian horses compared to other horse breeds. The reason for this predisposition site is yet not clear, but several factors (embryonic, anatomic and physiologic) could be involved.

The site of aortic rupture in Friesians is also the most common site of aortic dilation and/or rupture in humans. This is for instance observed in Marfan syndrome (De Backer et al., 2009) and blunt traumatic aortic injury (Pearson et al., 2008). During embryologic development, there is fusion between the aorta derived from the neural crest (proximal part) and mesoderm (distal part) at this particular location (Majesky, 2007). The composition of the aorta derived from these germ layers is also different. The neural crest derived aortic arch shows thin elastic fibres in both circular and longitudinal directions, while the mesoderm derived descending aorta shows neatly circularly arranged thick elastic lamellae. The transition between those two types of elastin organization is gradual (Bergwerff et al., 1998). Additionally, the ligamentum arteriosum has a high collagen content compared to the remainder of the thoracic aorta and is thus relatively inelastic. A transition zone, characterized by incomplete integration of the elastic fibres, is present between the ligament and the aorta’s adventitia/media (Pearson et al., 2008), which can explain the predisposition to rupture at this site. Indeed, a detailed heart-aorta model by Richens et al. (2004) also predicted maximum stresses at the aortic isthmus, thus at the site most commonly affected by traumatic injury. Elastin fibres are also present in a higher concentration in the proximal part of the aorta. This means that connective tissue disorders such as MFS affecting elastic fibres will manifest more frequently in this region (De Backer, 2009). Finally, in physiological conditions, the proximal aorta is continuously stressed by repetitive ejection of blood during ventricular systole (Roman et al., 1993). The position of the scapula anatomically (only linked to the spine by ligaments in the horse), coupled with the direction of force at the shoulder, creates a high impulsive force on the rib cage during exercise. It is estimated that a force of more than 100kPa is applied on the chest wall by each scapula when a horse of 500kg starts galloping. These forces have already been associated with exercise induced pulmonary haemorrhages (Schroter et al., 1998), but could also be important in aortic rupture.
Thanks to its typical conformational characteristics and its gentle nature, the Friesian horse is a unique and popular horse breed. Anatomically, Friesians have a unique conformation compared to warmblood horses: a long, arched neck and sloping shoulders. It is possible that the position of the aorta in the thoracic cavity is also different in affected or all Friesian horses. This could lead to different blood flow and wall stresses, predisposing the thoracic aorta to rupture. This hypothesis needs further investigation.

1.3 Aortopulmonary fistula: a unique lesion in Friesians

From 2010-2015, twenty Friesian horses affected by aortic rupture were necropsied at the Faculty of Veterinary Medicine in Ghent. All of these horses showed an aortopulmonary fistulation. This lesion is extremely rare in animals. In horses, only 2 other cases of aortopulmonary fistulation in non-Friesian horses have been described (van der Linde-Sipman et al., 1985 and Holmes et al., 1973). The reason for this high prevalence of aortopulmonary fistulation in Friesians is not clear. A possible explanation could be that the ductus of botalli persists after birth (patent ductus). However, involvement of the ductus arteriosus was excluded in all affected Friesians as a local fibrotic scar was noticed at the level of the former ductus arteriosus. Intriguingly, despite the peculiarity of patent ductus arteriosus in horses (Guarda et al., 2005), 2 of the 5 horses presented with this disease at the Faculty of Veterinary Medicine of Ghent University (2008-2016) were Friesians (Gunther van Loon, personal communication) (Saey et al., 2016).

In humans, aortopulmonary fistulas typically form at the same location as in Friesians (Gleason and Bavaria, 2003). Considering the protective effect of the periaortic tissue against exsanguination at the level of aortic rupture, it is likely that the formation of pseudoaneurysms in Friesians can be attributed to the anatomical location of the aortic rupture (Gleason and Bavaria, 2003). As the pseudoaneurysm(s) expand, they exert pressure on and can subsequently evoke inflammation in adjacent structures with tissue necrosis and eventually breakdown of the vascular wall. This will then finally result in direct communication between the aorta and pulmonary artery.

1.4 Variations in clinical course and lesions

Clinically: acute, subacute and chronic forms of aortic rupture have been described (Ploeg et al., 2013). Patients with a very acute clinical history can present with a haemothorax and/or periaortic haematoma, while the more chronic patients suffer from aortopulmonary fistulation. However, the aorta always ruptures at the same typical location in affected animals. These 3 clinical forms are thus variants of the same disease. It is worth mentioning that the aorta of many of the acute clinical cases actually show chronic gross and histological lesions. As the clinical signs associated with aortopulmonary fistulation are quite vague (Ploeg et al., 2013), it is possible that these are first missed by owners/veterinarians.

An atypical location of aortic rupture has been described in a 24-days-old Friesian foal (Diel de Amorin et al., 2016). This foal developed an aortic rupture more proximally, at the level of the aortic root, resulting in cardiac tamponade and acute death. A different
aetiology, immaturity of the connective tissue and possible thoracic trauma were all suggested as possible reasons for this atypical location (Diel de Amorim et al., 2016). The very young age of this animal is atypical. The youngest affected Friesian horse reported by our research group was a yearling (Ploeg et al., 2015). Very young Friesian foals succumbing to thoracic aortic rupture have, however, been mentioned in meetings with veterinarians and owners, but have never been confirmed by post-mortem examination.
2. Towards a unifying hypothesis for the aetiology and pathogenesis of aortic rupture in Friesian horses

2.1. Statement 1: Aortic rupture in Friesian horses is a hereditary disease

Considering the unique and consistent characteristics of the lesion (a transverse rupture near the ligamentum arteriosum) and the fact that these lesions are typically seen in purebred Friesians, a hereditary genetic cause seems most plausible. This is further underlined by the results of our vascular casting study as the three sampled affected Friesians showed exactly the same location of the aortic rupture. A genealogical analysis of 35 affected Friesians, going back 5 generations, also showed a close relatedness among these animals compared to other horses of the same generation (1999-2009; 60,000 horses in total). It was also shown that some founders that were not that important in the control group, had a high importance in the affected group (unpublished data). Finally, histological examination of aortic specimens showed no indications for an infectious agent. This all supports our hypothesis of a hereditary genetic problem underlying this disease. Genomic studies are ongoing to detect possible genes involved.

2.2. Statement 2: A generalized connective tissue disorder underlies aortic rupture in Friesian horses.

In affected Friesians, an aberrant deposition of collagen was noticed at the site of the aortic rupture (chapter 4). This could be due to a disorder in collagen synthesis but could also be the consequence of local inflammation with activation of proteolytic pathways in the aortic wall. This could also explain why especially Friesians belonging to the group of horses with chronic histological lesions showed the most prominent medial fibrosis at the rupture site (chapter 4). Therefore, this phenomenon could be regarded as a local reactive (repair) process, rather than a local collagen defect. Indeed, pathologic conditions of the vascular wall can result in a reversal of the collagen components from structural collagen type I to reparative collagen type III (Raman-Purushothaman et al., 2011). An increase of collagen type III instead of type I would thus be expected in the case of pathologic reparative medial fibrosis in affected Friesians. However, this presumed increase in collagen type III could not be demonstrated in affected horses (chapter 5). In contrast, we have demonstrated an increase in the area percentage of collagen type I in affected and non-affected Friesians compared to warmblood horses in the mid thoracic aorta (chapter 5). A primary genetic disorder affecting collagen type I in Friesian horses could result in a (mildly) deficient function of this collagen type. This could explain the higher percentage of this collagen type in all Friesian horses throughout the aorta as a compensatory phenomenon and further supports our hypothesis that the aortic rupture is not due to a site-specific anomaly but rather to a generalized connective tissue disorder.

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Collagen mainly determines the strength of the tissue (Gosline et al., 2002) while elastin is held responsible for the recoil (Rauscher et al., 2012). One would thus expect to find a difference in maximum failure stress caused by this presumed collagen disorder. A difference in aortic strength was however not detected by our rupture tests (chapter 7). These rupture tests of the thoracic aorta were however less reliable compared to the tensile tests as slipping out of the grips or rupturing adjacent to the grips resulted into inconclusive data. Furthermore, the aortic wall stiffness is not only determined by collagen and elastin, but also by smooth muscle cells (Bank et al., 1996). Therefore, other mechanisms (increased collagen density and overall aortic wall thickening) may compensate in the non-affected Friesians.

2.3. Statement 3: We should monitor blood pressure in Friesians

An increase in collagen type I, as seen in all Friesians, is likely to stiffen the aortic wall resulting in an increased blood pressure and possibly predisposing them to aortic rupture (Figure 45). Studies in humans confirmed that alterations favouring collagen type I synthesis are associated with an augmented aortic stiffness in arterial hypertension (Stakos et al., 2010). This presumed aortic stiffening in Friesian horses could explain the monitored higher heart rate compared to Dutch warmblood stallions of similar age (Munsters et al., 2013). However, biomechanical differences, including changes in elasticity, could not be detected in the thoracic aorta between Friesian and warmblood horses. Moreover, aortic adventitial fibrosis, which is typically striking in hypertension (Wu et al., 2016), was not detected outside the lesion in affected Friesians nor in nonaffected Friesians. Prominent left ventricular wall hypertrophy, which one would expect to find with long-term hypertension (Kahan and Bergfeld, 2005), was also not conspicuous in Friesian horses during necropsy.

Figure 45.
Schematic presentation of inter-relations of arterial compliance, pulse pressure, and synthetic activity of vascular smooth muscle cells in large conduit arteries (Martyn and Greenwald, 1997).
Smooth muscle cells are able to sense changes in mechanical strains applied to them via cell-surface receptors. In response to these changes, their expression and synthesis of ECM molecules is altered and an increased collagen deposition can take place (Gupta and Grande-Allen, 2006). Primary hypertension in affected Friesians could explain the smooth muscle cell hypertrophy and the increased collagen deposition, which were found in affected Friesians (chapter 5). Vascular smooth muscle cell hypertrophy could also explain the larger medial thickness in Friesian horses. However, vascular smooth muscle cell hypertrophy was only observed in the affected Friesians, making it unlikely that nonaffected Friesians suffer from hypertension.

Apart from hypertrophy, smooth muscle cells are also expected to undergo a phenotypic switch from a contractile to a synthetic phenotype in reaction to altered wall stresses (Figure 46). Both of these alterations in SMC were observed in affected Friesians (chapter 6). This phenotypic switch of smooth muscle cells can result in a higher synthesis of matrix proteins, including collagens (Kolpakov et al., 1995). The marker alpha-smooth muscle actin is specific to both contractile and synthetic smooth muscle cell phenotypes (Tukaj, 2008). This phenotypic switch of the SMC to a synthetic phenotype (containing less myofilaments with α-smooth muscle actin) seems contrary to the increased area percentage of SMC in affected Friesians measured by image analysis. However, image analysis informs us merely about the area surface, not about the density of this marker in the SMC. It should be noted that collagen type I promotes the modulation of arterial SMC from a contractile to a synthetic phenotype (chapter 5) (Yamamoto et al., 1993). In our study, collagen type I area percentage was increased and the synthetic phenotype of SMC was most prominent (chapter 6).
Figure 46. Phenotypic switching by vascular smooth muscle cells. Left: synthetic phenotype. Right: contractile phenotype. (Milewicz et al., 2010)

2.4. Unifying hypothesis

Since in Friesians the rupture always occurs at the same location and in only a limited number of closely related horses, a hereditary basis of disease seems most likely. It is possible that the intense selection on the typical “elastic gait” and appearance of Friesian horses lead to several breed-related conditions, including aortic rupture. A general different collagen type I metabolism in Friesian horses as suggested by our results (chapter 5) and by Ploeg et al. (2016) could predispose this horse breed to aortic rupture. It is likely that compensating, remodelling phenomena, for example the thicker tunica media in nonaffected Friesian horses, are responsible for the absence of biomechanical differences in Friesian compared to warmblood horses. The thinner tunica media in affected compared to nonaffected Friesians and the reduced elastin area percentage in affected horses are possible responsible for the occurrence of aortic rupture in those horses. It is possible that local hemodynamic factors result in rupture at a consistent location in Friesian horses. After all, in blunt traumatic aortic injury in humans, aortic rupture is observed at a similar site (Pearson et al, 2008).
2.5. Alternative hypotheses of the pathogenesis of aortic rupture

Before the start of our research, the only histological study on aortic rupture in Friesian horses was a small-scale study of 4 horses affected by aortopulmonary fistulation. Three of these horses were purebred, related Friesians. Two Friesians with aortopulmonary fistulation were described as having a true thoracic aortic aneurysm. As this is a rather old manuscript and the term pseudoaneurysm is rarely used in veterinary medicine, it is possible that the monitored dilations in fact represented pseudoaneurysms. Medial necrosis, as also seen in our case series, was the main histological finding and was attributed to altered vasa vasorum with intimal thickening and fibrosis (van der Linde-Sipman et al., 1985). Interruption of vasa vasorum flow can indeed cause ischemic necrosis of the media with loss of smooth muscle cells and elastin and collagen fibres (Angouras et al., 2000). Only minimal changes of vasa vasorum were found in our study (chapter 4). As medial necrosis was only prominent in affected Friesians at the site of the rupture, and not further down the thoracic aorta, it is likely that the rupture itself with ensuing disruption of the vasa vasorum at that site caused the necrosis of the smooth muscle cells.

A deficiency in copper has been mentioned as an important cause of aortic rupture in turkeys (Graham, 1977) and has also been associated with uterine artery rupture in peripartum mares (Stowe, 1968). Copper concentrations in the liver of 7 affected Friesian horses were within normal limits (unpublished data).

The possible existence of a primary truncus pulmonalis instead of aortic rupture was suggested during debates based on the sometimes ‘chronic’ appearance of the pulmonary rupture. Indeed, intimal thickening and sometimes even dissection with reendothelization was noticed in the pulmonary artery in several cases. However, this hypothesis seems unlikely for several reasons:

The highest pressure is measured in the aorta, thus a primary aortic rupture with eventually fistulation into the pulmonary artery seems unlikely.

Pure aortic ruptures without aortopulmonary connections have been reported. However, ruptures of the pulmonary artery without an aortic rupture have never been described.

The vascular casts in the three Friesian horses with an aortopulmonary fistulation showed an identical rupture site in all aortas. However, the site where the aortic pseudoaneurysm(s) connected with the pulmonary artery showed some minor variations.
General conclusions

Our results suggest the existence of a generalized collagen disorder throughout the Friesian horse population. Considering the differences observed between the affected and nonaffected Friesians, it is likely that a superimposed genetic defect and/or an anatomical variation of the aortic arch is present in the affected Friesians. This presumed supplemental genetic defect in affected Friesians could perhaps affect the elastin fibres, which could explain the decrease of elastin in affected Friesians.

Intensive selection on breed characteristics in Friesians have possibly lead to the rise of several unwelcome congenital diseases. Considering the risk of fatal outcome of aortic rupture once clinical signs are observed and the dangerous situation of affected animals collapsing during full action, the development of a genetic test to identify carrier animals is important. DNA tests for unwelcome characteristics should become progressively more important in the selection process of Friesian horses for breeding. Selecting breeding animals based on DNA tests implies that a possible liberalization of the selection rules regarding other characteristics might be necessary to remain or possibly widen the genetic variety within the Friesian horse breed. As spontaneous rupture of the thoracic aorta in the absence of trauma or inflammation is very rare in humans, revealing the causative gene of aortic rupture in Friesians could help understanding the pathogenesis of aortic disease and more specific aortopulmonary fistulation in humans as well.
References


CHAPTER 9: FUTURE PROSPECTS
3D-image modelling based on vascular casts

Considering the typical conformation of the Friesian horse (long, arched neck and sloping shoulder), it is possible that differences in heart-aorta geometry are present as well. This could explain the differences in location of aortic rupture in Friesian compared to warmblood horses. In a human mathematical model, aortic curvature has been shown to be relatively more important than aortic diameter, blood pressure, cardiac output, and patient size with regard to the force acting on the aortic wall (Poullis et al., 2008).

As the casting of the thoracic aorta would be performed in situ, a geometrical comparison of the thoracic aorta in warmblood and Friesian horses should be possible. Casting of the thoracic aorta in affected Friesian horses and control warmblood and nonaffected Friesian horses could be performed in situ post-mortem. Casting prevents collapse of the aortic lumen and allows computed Tomography (CT)-scans that can be analysed by medical software (Mimics; Materialise, Leuven) in order to obtain 3D reconstructions. These 3D reconstructions can then be used as input for computational representations.

Genetic study

The draft genome sequence of the horse is available (Wade et al., 2009). Equine high-density single nucleotide polymorphism (SNP)-based genotyping arrays can be used for genome-wide association studies (GWAS) for disease gene mapping (Orr and Chanock, 2008). Recent successes using this technique in Friesian horses were obtained for dwarfism (Orr et al., 2010) and hydrocephalus in Friesian horses (Ducro et al., 2015).

Currently, genetic testing is being performed at Wageningen University (Bart Ducro, WU Animal Sciences, Wageningen, the Netherlands) on tissues from Friesians that died from aortic rupture and control Friesians in an attempt to localize strategic base pairs.

Determination of collagen cross-links in equine urine

The pyridinium derivatives hydroxylsylpyridinoline/pyridinoline (HP) and lysylpyridinoline/ deoxypyridinoline (LP) are intermolecular crosslinking compounds of collagen that are excreted in urine (Gunja-Smith and Boucek, 1981). Hydroxypyridinium crosslinks are abundantly present in the aorta. Deoxypyridinoline is mainly released from bone and dentin and, because of its low turnover, in insignificant amounts from tendons and aorta. Pyridinoline is the major crosslink of cartilage and is present in the collagen of bone and other tissues (Eyre et al., 1984). Reduced concentrations of collagen cross-links are associated with diminished stability of bone tissue (Oxlund et al., 1995).

Recently, it was shown that deep flexor tendons of warmbloods contain more HP cross-links compared to healthy Friesians, suggesting a breed-related difference in collagen metabolism. This difference was not found in aortic tissue. The latter is possibly due to
the lower amount of collagen in aortic tissue (Ploeg et al., 2016). If a different collagen metabolism is present in the Friesian horse, a breed-related difference in HP and LP urinary excretion urine is expected.

Non-invasive measurement of blood pressure in Friesian and warmblood horses

Some lesions observed in affected Friesians, including smooth muscle cell hypertrophy and increased collagen deposition, are typical of hypertension. It is thus possible that affected Friesians suffer from primary hypertension or that hypertension at least plays a role. Blood pressure can be measured noninvasively in standing, conscious horses using a cuff placed over the ventral coccygeal artery. Recently, it was shown that this technique is accurate and precise in the adult horse across a range of blood pressures, with higher variability at subnormal blood pressure (Olsen et al., 2016). As this technique is noninvasive, it can be performed in a large number of Friesian and warmblood horses to monitor possible breed-related differences.
References


SUMMARY – NEDERLANDSE SAMENVATTING
The Friesian horse suffers from a very high inbreeding rate. In several of the diseases that are typically diagnosed in Friesians, a genetic component has been proven. Aortic rupture is generally rare in horses and in-depth information regarding this condition is missing. Aortic rupture occurs, however, more frequently in Friesians compared to other horse breeds, suggesting the existence of a hereditary basis in this disease as well. Large-case studies describing the histological lesions or biochemical or biomechanical characteristics of the thoracic aorta in Friesian horses were however missing.

Precise topographical descriptions of the lesions may help in the search for underlying candidate genes as certain genes involved in aortic morphogenesis are expressed during particular embryonic stages. Therefore, Chapter 3 describes a novel technique, vascular casting, which was performed in three Friesian horses suffering from an aortopulmonary fistulation in order to obtain an exact three-dimensional reconstruction of the lesions. In all Friesian horses, the aortic rupture was located at the same location, underlining the possible hereditary basis of this disease.

The histomorphological abnormalities in 20 affected Friesian horses at the site of rupture are described in Chapter 4. Characteristic lesions described in all affected animals included medial necrosis, elastin fragmentation and accumulation of mucoid material. Inflammatory changes were minimal. The observed alterations were suggestive of a general connective tissue disorder.

The architecture of the aortic wall, distant from the site of the rupture, was described in Chapter 5. Again, warmblood horses were used as a control population. In affected Friesian horses, the smooth muscle cell area percentage was increased and the elastin area percentage was decreased. Image analysis demonstrated a higher collagen type I percentage in the general Friesian horse population, suggesting a primary general collagen disorder in the Friesian horse breed.

Considering the smooth muscle cell alterations observed in chapter 4 and 5, in-depth structural examination of the smooth muscle cells was performed in Chapter 6. Prominent hypertrophy, vacuolisation and a phenotypic transition to the synthetic type were observed and suggestive of altered arterial wall stresses.

Finally, in order to distinguish between a local phenomenon and a generalized condition and to gain information about the biomechanical and biochemical characteristics of the equine aorta, uniaxial tensile tensile and biochemical measurements were performed at three locations in both Friesian and warmblood horses as reported in Chapter 7. Biomechanical and biochemical differences were not noticed between the difference
horse groups. Regional biomechanical differences were found in all horses and were similar as observed in other animal species.

In conclusion, this thesis offers insights into the pathogenesis of aortic rupture in Friesian horses. A general collagen disorder in the Friesian horse breed is very likely and probably plays a role into typical breed characteristics such as increased tendon laxity and aortic rupture. Moreover, a possible extra genetic defect or geometric variation in affected Friesians could predispose them to aortic rupture. The future development of a genetic test in order to identify possible carrier animals is important for the preservation of the Friesian horse breed.
Nederlandse samenvatting

Er zijn meerdere specifieke aandoeningen bij het Friese paarden ras beschreven die niet of nauwelijks voorkomen bij andere paarden rassen. De zeer hoge inteelt graad binnen dit ras is hiervoor vermoedelijk verantwoordelijk. Bij meerdere van deze ziekten werd er intussen een genetische component aangetoond. Aorta ruptuur is een zeldzame en weinig onderzochte aandoening bij paarden. Het is een vrij frequent voorkomende ziekte bij Friese paarden en is hoogstwaarschijnlijk eveneens een erfelijke ziekte. Grootschalige histologische studies noch biochemische of biomechanische studies omtrent deze aandoening bij het Friese paard ontbraken echter.

Een exacte topografische beschrijving van de letsels wordt gegeven in hoofdstuk 3 aan de hand van 3-D reconstructies. Hiervoor werd er een nieuwe techniek, vasculaire casting, aangewend in 3 Friezen met een aortopulmonale fistel. In deze 3 dieren was de aorta ruptuur telkens gelokaliseerd op dezelfde locatie. Bijgevolg is een genetische basis van de ziekte zeer waarschijnlijk.

In hoofdstuk 4 werd er een grootschalige beschrijvende studie uitgevoerd om de histologische afwijkingen in 20 aangetaste Friese paarden te beschrijven. Karakteristieke histologische letsels in alle aangetaste dieren waren mediale necrose, fragmentatie van de elastine vezels en acummulatie van mucoid materiaal. Inflammatoire veranderingen waren over het algemeen slechts minimaal. De waargenomen letsels waren suggestief voor een mogelijke algemene bindweefsel aandoening.

In hoofdstuk 5 werd de architectuur van de aorta wand distaal van het letsel onderzocht in zowel Friezen als warmbloeden. In de aangetaste Friezen was het oppervlakte percentage van de gladde spiercellen duidelijk gestegen en dat van het elastine gedaald. Via beeld analyse werd er een hoger collageen type I percentage gemeten in de media van de algemene Friese paarden populatie in vergelijking met de controle warmbloeden. Dit laatste suggereert een algemene collageen aandoening binnen het Friese paarden ras.

Aangezien er veranderingen in de gladde spiercellen werden opgemerkt, zoals beschreven in hoofdstuk 4 en 5, werd er besloten de gladde spiercellen van de aorta media ultrastructureel te bekijken. In hoofdstuk 6 wordt er zowel hypertrofie, vacuolisatie als een fenotypische transitie van de gladde spiercellen beschreven. Deze veranderingen zijn suggestief voor verandering in de arteriële bloed druk in aangetaste Friezen.

Tenslotte werden er uniaxiale trekrek testen en biochemische metingen uitgevoerd op 3 verschillende lokaties in de thoracale aorta, dit bij zowel warmbloedens als Friese paarden. Het doel was om te achterhalen of de aandoening eerder lokaal dan algemeen is en om algemene informatie omtrent de biomechanische en biochemische kenmerken van de aorta bij paarden te verzamelen. Deze resultaten werden gerapporteerd in hoofdstuk 7.
Biomechanische noch biochemische verschillen werden waargenomen tussen de verschillende groepen paarden. Regionale biomechanische en biochemische veranderingen stemden overeen met de literatuur in andere diersoorten.

Deze thesis levert nieuwe inzichten in de pathogenese van aorta ruptuur in Friese paarden. Alle waarnemingen samengenomen, is een algemene collageen aandoening in Friezen zeer waarschijnlijk, hetgeen mogelijk verantwoordelijk is voor meerdere raskenmerken zoals een toegenomen laxiteit van de pezen.

Bovendien is het bestaan van een bijkomende genetische of geometrische variatie in de aangetaste Friezen mogelijk. Het ontwikkelen van een genetische test voor het opsporen van dragers van deze fatale ziekte is belangrijk voor het behoud van dit paardenras.
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Dankwoord

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Veel liefs,

Vero
CURRICULUM VITAE

In 2011 begon ze als assistente aan haar doctoraatsonderzoek aan de vakgroep Pathologie, Bacteriologie en Pluimveeziekten onder leiding van Prof Dr Koen Chiers, Prof Dr Gunther van Loon en Prof Dr Catherine Delesalle. Daarnaast gaf ze praktisch onderricht in de pathologie en histopathologie en verzorgde ze mee de dienstverlening.

In 2012 voltooide ze aan de Universiteit Gent de opleiding “Master in Laboratory Animal Science” volgens FELASA (Federation of European Laboratory Animal Science Associations). Datzelfde jaar volgde ze eveneens met goed gevolg de permanente vorming: “Inleiding tot het recht voor gerechtelijke experts” aan de Faculteit Rechtsgeleerdheid van de Universiteit Gent.

Veronique Saey is auteur en co-auteur van meerdere wetenschappelijke publicaties in internationale tijdschriften. Ze nam reeds deel aan verschillende congressen en symposia met actieve inbreng. In september 2013 behaalde ze een award for excellent oral presentation voor haar deelname aan het ESVP congres te London.
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