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AORTIC STENT IMPLANTATION IN THE ISTHMIC REGION IN AN ANIMAL MODEL TO INVESTIGATE PATHOPHYSIOLOGY AND TREATMENT OF HUMAN AORTIC COARCTATION

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to my mentor.

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Summary

BACKGROUND: Balloon dilation with stent implantation is a novel technique for non surgical treatment of aortic coarctation. Residual arterial hypertension is, however, a frequent finding (50% of treated patients), leading to major cardiovascular events and reducing life expectancy, even in the absence of residual restenosis.

AIM OF THIS STUDY: To determine feasibility of stent implantation in the aortic isthmus of an ovine model, to study the pressor, hemodynamic and echocardiographic changes induced by stenting in a growing animal.

MATERIALS AND METHODS: Platinum-iridium stent was implanted in the aortic isthmus of 8 female sheep through vascular catheterization (STENT group). Vascular catheterization and angiographic study was performed in 4 control sheep (SHAM). Pressure was measured in the ascending and descending aorta. All subjects had echocardiographic examination, non invasive anterior and posterior limb pressure and invasive blood pressure measurements (through auricular artery catheterization) every 90 days. Twelve months after intervention the animals were sacrificed after dobutamine stress test.

RESULTS: Stent implantation did not affect growth nor animal wellness. Diastolic blood pressure was significantly lower in the posterior limbs than in the anterior both in SHAM and STENT animals with no difference between groups. Auricular blood pressure did not differ among groups. Echocardiography indices did not differ between groups. MMP9 expression was greater in the ascending and lower in the descending aorta in STENT animals than in SHAM ($p=0.06$), while CASP3 expression was similar.

CONCLUSIONS: Stent implantation in the sheep is feasible and well tolerated. This animal model is useful to study the hemodynamic impact and the aortic stiffness induced by stent implantation and their consequences on the left ventricle.

1. Coarctation of the Aorta

1.1 Definition

Coarctation of the aorta (CoA) is a congenital cardiovascular anomaly consisting of a constricted aortic segment comprising localized medial thickening with some infolding of the media and superimposed neointimal tissue (Rao, 2005).

1.2 Types of coarctation

A number of different anatomical variations with extremely variable features .coexist within the term Coarctation of the Aorta.

Lumen stenosis can be discrete or less frequently can be tunnel-like, involving a longer aortic tract (Rao, 2005). Pseudocoarctation is kinking of the aorta near the ligamentum arteriosus due to elongation of distal aortic arch and proximal descending aorta. This is a rare although benign condition, without evidence of pressure gradient across the kinking. It is rarely associated to bicuspid aortic valve and dissection of the ascending aorta. Reverse coarctation is called improperly called the clinical condition in which lower limb pulses are well palpable but upper limb pulses are not. This is present in a form of Takayasu's arteritis in which there is diffuse aortic involvement including renal arteries but femoral arteries are spared. Functional coarctation occurs to external pressure on aortic wall either by mediastinal mass or tumor.

Multiple coarctation is when different aortic tracts are involved: isthmic and descendent aorta present coarctation.

Abdominal coarctation, the narrowing of a distal abdominal tract of the descending aorta, may be congenital, due to hypoplasia of the abdominal aortic intima, or acquired, secondary to either Takayasu's arteritis or fibromuscular displasia. Although rare, hemodynamically significant narrowing of the

descending thoracic or abdominal aorta often produces life-threatening hypertension that is surgically correctable. Although it often appears to be a congenital lesion, it may result from healed aortitis. For this reason, some Authors avoid the term coarctation and label it middle aortic syndrome. The narrowed aortic segment typically is focal, but diffuse hypoplasia of the abdominal aorta involving the branch arteries may occur. The renal arteries + be stenosed, hypoplastic, or thrombosed, resulting in severe hypertension (Lindsay, 2000)

Recurrent coarctation is a restenosis that develops after a successful repair (Sudhayakumar et al., 2008).

Tubular hypoplasia (TH) is a uniformly narrowed tract of the proximal ascending aorta (Anderson, 1987). Incidence of TH varies from 65-81% of all coarctation forms (Vouhè et al., 1988). Histologically, hypoplastic aortic arch shows more collagen deposition and less elastin fibers in the *tunica media.* Descendent aorta below the coarctation frequently presents post stenotic aneurysm (Johnson, 2001).

In the past, numerous classifications had been proposed.

Initially Bonnet (1903) classified coarctation in *infantile type,* proximal to aorto-ductal junction with patency of the duct, and *adult type,* distal to insertion of the arterial ligament (*ligamentum arteriosus*) associated with collaterals.

Lev (1953) classified into two other groups: *fetal CoA,* elongated, narrow and preductal, and *adult CoA,* localized postductal.

More recently, CoA was classified by its relation to the ductus arteriosus in *preductal* or *juvenile,* if located proximally to the ductus and frequently associated to other vascular defects, or *postductal* if located distally to the aortic insertion of the ductus. Coarctation located exactly above the ductus was defined *juxtaductal.*

Another classification was proposed to combine the anatomical characteristics with the presence of concomitant congenital defects (Amato et al, 1991):

- Type I (CoA with or without PDA).
    IA: CoA associated to ventricular septal defect (VSD)
    IB: CoA associated to other congenital defects

- Type II (istmus hypoplasia with or without PDA).
    IIA: associated to VSD
    IIB: associated to other congenital defects

- Type III (coarctation with tubular hypoplasia involving isthmus and the segment between left carotid and left subclavian arteries with or without PDA).
    IIIA: associated to VSD
    IIIB: associated to other congenital defects

1.3 Embriology

Thoracic coarctation is the manifestation of an abnormal development of the embryologic left fourth and sixth aortic arches. The underlying cause of the abnormal arch development is not well understood. Two concepts have been advanced, neither of which is entirely satisfactory: the ductus tissue theory and the hemodynamic theory (Beekman, 2008).

The *ductus tissue theory* was proposed by Ho and Anderson (1979). Studying 35 histologic specimens of resected coarctation, they found that in all cases with coarctation or tubular hypoplasia a sling of ductal tissue was located around the aortic isthmal orifice. In 6 hearts a diaphragm of ductal tissue was seen to form the coarctation lesion. They proposed that coarctation develops as the result of migration of ductal smooth muscle cells into the periductal aorta, with subsequent constriction and narrowing of the aortic lumen. This concept is concordant with the clinical observation that coarctation often becomes manifest after ductus closure; however, the ductal tissue theory does not adequately

explain aortic coarctation that occurs distant from the insertion of the ductus arteriosus, such as in the transverse arch or abdominal aorta (Beekman, 2008).

In 1971 Hutchins observed that coarctation never appeared associated to anomalities determining aortic arch overflow (for example Tetralogy of Fallot).

The *hemodynamic theory* explains how intracardiac lesions that diminish the volume of left ventricular outflow promote development of coarctation in the fetus by reducing flow volume through the aortic isthmus. This theory does help to explain the common association of coarctation with ventricular septal defect, left ventricular outflow obstruction, and hypoplasia of the transverse aortic arch (Rudolph et al., 1972), but it does not explain the presence of aortic coarctation as the only anomaly present in some patients.

Other Authors hypothesize an embriogenetic cause in the determination of the coarctation, implying that coarctation is the result of a partial involution of the left fourth aortic arch during fetal life (Davies and Guest, 2003)

1.4 Pathology

Coarctation of the aorta is usually a discrete stenosis of the upper thoracic aorta at the point of insertion of the ductus arteriosus. It is thought to be caused by a malformation of the aortic media that creates a prominent posterior infolding (the *posterior shelf*), which, in some cases, extends around the entire circumference of the aorta. In fact, the gross pathology of coarctation varies considerably. The lesion is most often discrete, but it may be long segment or tortuous in nature. In infants, particularly those with associated left ventricular outflow obstruction or a ventricular septal defect, there may be diffuse hypoplasia of the transverse aortic arch and isthmus proximal to the discrete coarctation. Less commonly, coarctation of the aorta occurs in other locations, such as the ascending aorta or the abdominal aorta. Coarctation of the abdominal aorta is a complex lesion that often is associated with renal artery stenosis (Beekman, 2008).

Histological examination of the coarcted aortic segment discloses thick

intimal and medial ridges that protrude posteriorly and laterally into the aortic lumen. The ductus or ligamentum arteriosus insert at the same level anteromedially. Intimal thickening and hyperplasia are observed in the area and are particularly prominent in older patients. Intimal proliferation and disruption of elastic tissue may occur distal to the coarctation (the *jet lesion*) at a site where high-velocity flow impacts the arterial wall. It is this distal site where infective endarteritis, intimal dissections, or aneurysms may occur. Cystic medial necrosis, consisting of depletion and disarray of medial elastic tissue, occur commonly in the aorta adjacent to the coarctation site and has been described in the proximal ascending aorta as well. In some patients, cystic medial necrosis may provide a histological substrate for late aneurysm formation or aortic dissection (Beekman, 2008).

In a study (Niwa et al., 2001), all biopsies from specimens above and below a resected coarctation had medial abnormalities, the degree of which was identical in proximal and distal segments (complete loss of elastic fibers involving large areas of media), implying that the abnormalities were not hemodynamically determined. An inherent pathogenesis is also in accord with observations that in fetuses with a widely patent ductus arteriosus, mechanical forces do not impinge on what is destined to become the postnatal paracoarctation aorta. The same medial abnormalities were present in the paracoarctation aorta in the third week of life and have been reported within 24 hours postpartum (Isner et al, 1987).

1.5 Pathophysiology and Hemodynamics

During fetal life hemodynamics are poorly disturbed because only 10% of cardiac output flows into the aortic isthmus: systemic flow is provided principally through the ductus arteriosus right to left shunting. After birth, with closure of foramen ovale and ductus arteriosus, the entire cardiac output must cross the stenotic aortic segment. Therefore, important hemodynamic disturbances may occur postnatally.

Depending on coarctation severity, left ventricular systolic function and collateral circulation development, pressure gradient through the narrowing can reach up to 60 mmHg. Upper to lower limb pressure differential can be measured non invasively: a pressure difference of more than 20 mm Hg in favor of the arms may be considered evidence for CoA (Rao, 2005).

Hemodynamic changes may range from mild arterial hypertension to congestive heart failure and shock, depending on the severity of coarctation and the mutual presence of other cardiovascular anomalies. CoA increases impedance to left ventricular outflow, thereby elevating systolic pressure in the left ventricle, the ascending aorta, and its branches (Beekman, 2008).

Severe afterbirth stenosis leads to left ventricular systolic dysfunction, diminished stroke volume and increased left ventricular end-diastolic pressure, retrogradely determining elevated left atrial pressure, pulmonary venous congestion and pulmonary artery hypertension. Congestive heart failure can occur in the first week of life.

Early compensatory mechanisms include activation of the sympathetic nervous system (to increase heart rate and enhance myocardial contractility) and Frank-Starling mechanism (to increase left ventricular end-diastolic volume and help maintain a normal stroke volume). The immature myocardium, however, is relatively ineffective in using these compensatory responses (Friedman, 1973). The neonatal myocardium lacks normal sympathetic innervation as a result, in part, of a decrease in sympathetic beta-receptor density. Furthermore, compared with the adult myocardium, the neonatal left ventricular myocardium is poorly compliant and less able to preserve ventricular stroke volume via the Frank-Starling mechanism.

Lesions associated with a reduced systemic cardiac output, such as severe coarctation of the aorta, often present as cardiac failure complicated by a severe metabolic acidemia and relatively high values of arterial oxygen tension. The latter finding, even in the presence of right-to-left shunting across a patent ductus arteriosus, is the result of diminished systemic perfusion and an elevated pulmonary-systemic blood flow ratio (Friedman and Silverman, 2001).

Impedance of ventricular outflow increases afterload that induces late

compensatory response, through different mechanisms: ventricular hypertrophy tending to normalize myocardial wall stress is certainly the most important (Graham et al., 1970).

Interestingly, children with severe LV outflow obstruction have an abnormal pattern of LV diastolic filling detectable by mitral valve Doppler examination: increased wall thickness or mass has been implicated as the cause of impaired LV relaxation, but also myocardial fibrosis, and, in some patients, an increased in the inotropic state of the myocardium. Left atrial hypertension and pulmonary venous congestion occur particularly in patients with an increased left ventricular end-diastolic volume (Meliones et al., 1989).

Frequently, gradient through CoA at rest is 30-40 mmHg, and this is possible thanks to the development of a collateral circulation that permits blood flow to bypass the stenotic isthmic region (fig. 1).

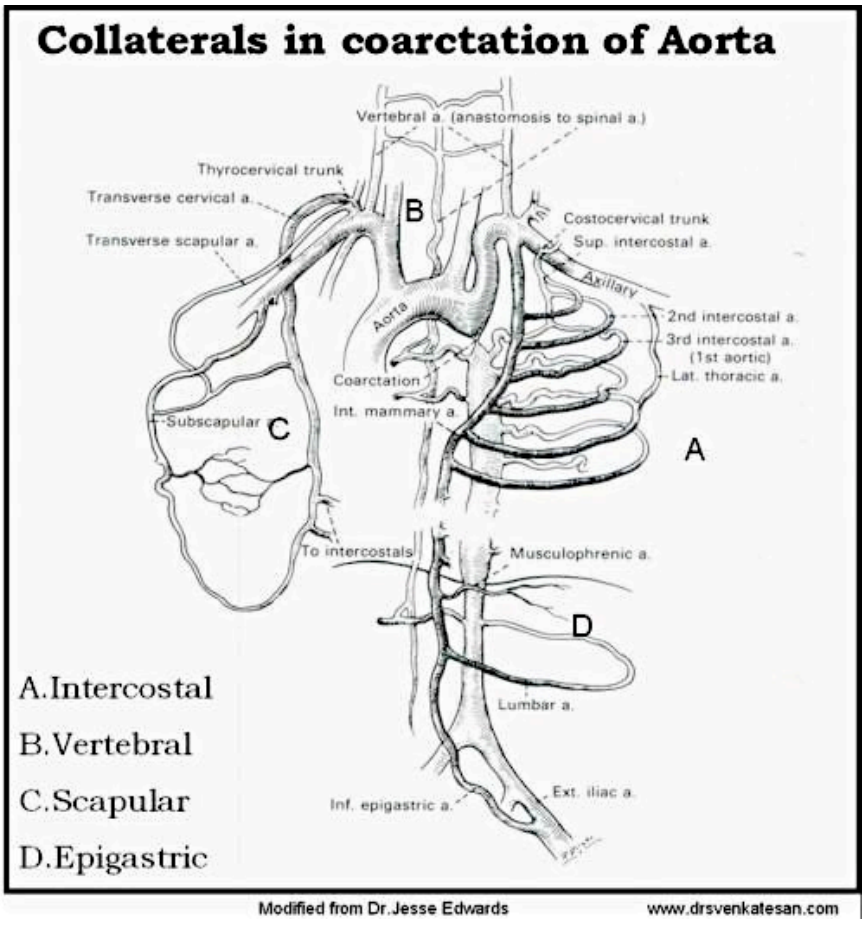

Fig. 1: collaterals in coarctation of the aorta.

Collateral circulation has been studied with novel imaging techniques: using multislice computed tomography it was possible to display collateral circulation of a 21 years old patient with high grade post ductal aortic coarctation. Chronic narrowing of the aortic lumen provokes development of collateral vessels to allow the flow of blood from high pressure to low pressure areas: from the internal thoracic arteries both to epigastric vessels and intercostal arteries, from the subclavian arteries via thoracoacromial and subscapular arteries to intercostal arteries, from the thyrocervical trunks via descending scapular arteries to intercostal arteries, and via vertebral and anterior spinal arteries (fig. 2) (Leschka et al., 2005).

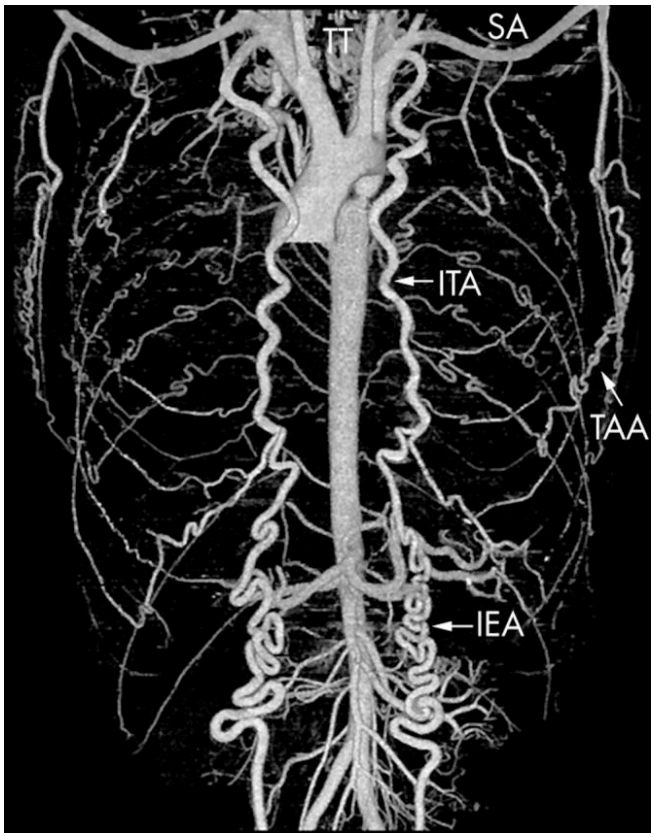

Fig. 2: Anterior volume rendered 64 channel multislice CT angiography image after deleting bones, heart, and pulmonary vessels. IEA, inferior epigastric artery; ITA, internal thoracic artery; SA, subclavian artery; TAA, thoracoacromial artery; TT, thyrocervical trunk (Leschka et al., 2005).

Abnormalities in vascular physiology also occur in patients with coarctation of the aorta. Systolic arterial hypertension is a manifestation of the aortic constriction, but it probably also reflects changes in vascular reactivity, arterial wall compliance, and baroreceptor reflex function that will be discussed later.

1.6 Epidemiology

Aortic coarctation occurs in 6-8% of patients with cardiovascular anomalies (Beekman, 2008). CoA is the sixth most common cardiovascular malformation, with an incidence of 1 affected newborn in 3000-4000 births (Tanous et al., 2009).

This anomaly is sporadic, but authors reported that 4% of children born from affected mothers inherit coarctation (Beauchesne et al., 2001).

As in other systemic circulation obstructive diseases, CoA is more represented in males, with a male : female ratio of 1,27-1,74:1. In a study, 82% of patients of affected patients had CoA as an isolated anomaly (Abruzzese and Fidala, 2007). In a retrospective study evaluating 216 newborns (age < 12 months) referred to a single center with a diagnosis of CoA, this anomaly was isolated in 52% of cases. In 103 patients, CoA was accompanied with other anomalies, ventricular septal defect (VSD) was the most frequent associated anomaly (36 cases), followed by aortic valvular stenosis (13), subvalvular aortic stenosis (SAS) (10), coexisting associated anomalies such as SAS and VSD (9), atrioventricular canal (9), dextroposed aorta and VSD (8) and double chambered right ventricle (6) (Beekman, 2008).

Other less frequent anomalies were atrial septal defect (ASD) (both ostium primum and ostium secundum ASD), double chambered left ventricle, tricuspid atresia associated with great vessels transposition, pulmonary atresia with VSD.

Becker and colleagues (1970) reported a higher incidence of associated anomalies: studying 100 cases of post-mortem coarcted cases they found a coexisting anomaly in 87%. In 76% coexisted more than 2 associated

anomalies; CoA and bicuspid aorta was the most common association (46%), followed by aortic arch hypoplasia. Left to right shunts were present in 50%, with a higher prevalence of persistent patent ductus arteriosus (PDA) and VSD in patients died before 6 months of age

CoA can be also associated to extracardiac abnormalities such as cerebral vascular aneurism, present in 5% of patients (Prisant et al., 2004).

Other anomalies had been reported: Turner syndrome, musculoskeletal, gastrointestinal, respiratory and genitourinary diseases are present in 25% of patients (Beekman, 2008).

1.7 Clinical characteristics

1.7.1 Presentation

The clinical presentation of the coarctation of the aorta generally follows one of three patterns: an infant with congestive heart failure, a child or adolescent with systemic arterial hypertension, or a child with a heart murmur (Beekman, 2008).

Isolated, severe aortic coarctation may cause congestive heart failure as early as the neonatal period when the ductus closes (Warnes and Deanfield, 2000).

A decompensated neonate is frequently pale, anxious and with respiratory distress. Tachycardia, dyspnoea, hepatomegaly and rarely peripheral edema can be signs of low output cardiac failure. Differential cyanosis (lower limbs cyanosis) is present if left-to-right shunting is present, more frequently through the patent ductus. Generalized cyanosis indicates severe intracardiac associated lesions, such as tricuspid atresia or great vessels transposition (Anderson, 1987).

More frequently, however, coarctation producing symptoms during early infancy is associated with other congenital cardiovascular abnormalities, such as ventricular septal defect, left ventricular outflow tract obstruction, or mitral valve abnormality.

Coarctation of the aorta often presents later in childhood as systolic hypertension or as a heart murmur. Delayed diagnosis beyond infancy is common because the physical findings may be subtle and most of these children are asymptomatic. On careful investigation, some children will report lower-extremity claudication with exercise or frequent headaches (Beekman, 2008).

In a review of children (older than 1 year) presenting with coarctation at the Columbia University between 1969 and 1978, the median age at diagnosis was 10 years (Strafford et al., 1982): many patients with undetected coarctation will remain symptom-free until adolescence or early adulthood, when symptoms such as headaches related to hypertension, leg fatigue, or leg cramps may develop. Occasionally, a major catastrophic event, such as a cerebrovascular accident, infective endocarditis, or even rupture of the aorta, is the first recognized symptom (Warnes and Deanfield, 2000).

1.7.2 Physical exam

In an infant with congestive heart failure, one encounters a pale, irritable child in respiratory distress. Tachycardia, dyspnea, diaphoresis, hepatomegaly, and, rarely, extremity edema may signal the presence of congestive heart failure and low cardiac output. Differential cyanosis may be observed (cyanosis confined to the lower extremities) if a right-to-left ductal shunt is present.

In contrast, the appearance of an older child with coarctation may be entirely benign: complaints of headache, cold extremities, and claudication with exercise may be noted, although attention is usually directed to the cardiovascular system by detection of a heart murmur of upper extremity hypertension on routine physical examination (Friedman and Silverman, 2001).

The hallmark physical findings in coarctation consist of discrepant arterial pulses and systolic blood pressures in the upper and lower extremities. Arterial pulses below the coarctation are diminished in amplitude and delayed in timing compared with the proximal pulses. Systolic blood pressure is elevated proximal to the coarctation, and a systolic pressure gradient is present between the arm and leg.

1.7.3 Chest Radiography

The chest X-ray of an infant with coarctation who presents with congestive heart failure is nonspecific. Moderate to severe cardiomegaly is evident and the pulmonary vascular markings are increased. Pulmonary vascular congestion may be indistinct and passive, related to left ventricular failure or mitral stenosis with pulmonary venous hypertension, or it may be active and related to increased pulmonary blood flow resulting from a large left to-right shunt.

Rib notching is not present in infants because the collateral circulation is not yet well developed.

In older children prestenotic and poststenotic dilation of the aorta gives the "3 sign" appearance on a chest radiograph.

The most specific chest radiograph findings in aortic coarctation are inferior rib notching associated to the aortic "figure 3" sign. *Rib notching* is due to pressure erosion by the enlarged and tortuous intercostal arteries in the costal

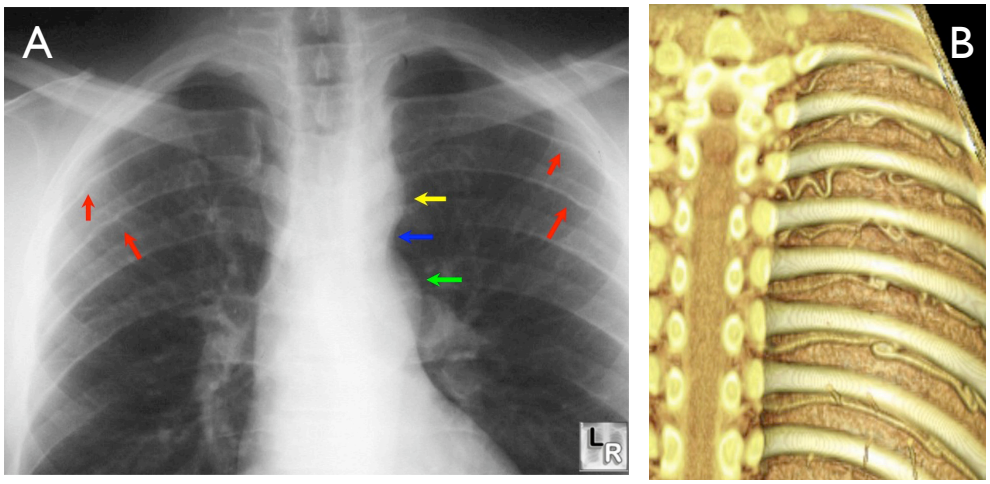

Fig. 3: (A) thoracic x-rays of a patient with CoA; the characteristic “3 sign” is evident (blue yellow and green arrows). Rib notching is evident (red arrow). (B) Chest CTA shows marked enlargement of the intercostal arteries. Available at URL: http://radiopaedia.org/images/25059

sulcus of the ribs (Fig. 3). The intercostal arteries provide collateral blood flow to circumvent the stenotic aortic segment. However, the first two intercostal arteries are supplied by the costocervical trunk rather than the descending aorta below the level of coartation. Therefore, the first two intercostal arteries are not collateral pathways and so ribs one and two do not show notching. Rib notching

implies long-standing obstruction and is not seen before the age of 10. This radiographic sign appearing as sclerotic scalloping on the inferior surface of ribs number 3 through 8 from dilated intercostal arteries that may be present, usually bilaterally, unless the left or right subclavian artery arises aberrantly below the coarctation, giving rise to unilateral right-sided or left-sided rib notching, respectively (Friedman and Silverman, 2001).

A barium swallow may assist in localizing a discrete coarctation if the classic 3 sign is not evident on the plain chest film. With a barium-filled esophagus, an E sign may be seen on the frontal or left anterior oblique film. This finding, also referred to as the *reverse 3 sign*, is due to indentation on the esophagus by the dilated aorta immediately proximal and distal to the coarctation (Beekman, 2008).

1.7.4 Echocardiography.

The coarctation site can be visualized from the suprasternal long axis view and its severity assessed by Doppler mode.

The posterior shelf appears as a thin, fibrous membrane protruding from the posterior aspect of the aorta and oriented toward the ductus arteriosus. Associated findings such as isthmus hypoplasia, poststenotic dilation, and diminished systolic pulsations in the descending aorta serve to confirm the presence of a significant coarctation (Beekman, 2008). A peak gradient greater than 20 mm Hg, especially if accompanied by continuous forward flow during diastole in the descending or abdominal aorta, suggests significant aortic coarctation. A pattern of diastolic runoff is present particularly in patients with a tight stenosis or with a robust collateral circulation.

In addition, the echocardiographer should evaluate other cardiac lesions, notably aortic, mitral, or subaortic abnormality and the status of left ventricular function (Friedman and Silverman, 2001).

Intravascular echography has been used to assess regional aortic stiffness, distensibility, and compliance in patients with CoA (Xu et al., 1997).

Prenatal echocardiography is an important tool for early detection of CoA.

In a retrospective study antenatal diagnosis of coarctation of the aorta was associated with improved survival and preoperative clinical condition (Franklin et al., 2002): the appearance of the aortic arch in the horizontal projection is helpful in distinguishing cases of coarctation (Fig. 4), but difficulties in prenatal diagnosis exist. The most severe forms of coarctation are associated with relative hypoplasia of the left heart structures compared with the right and a correct diagnosis can be made in early pregnancy. A large right ventricle, with a ratio over the left ventricle greater than 1.3 and right ventricular hypertrophy has been reported as suggestive of CoA (Allan et al., 1988).

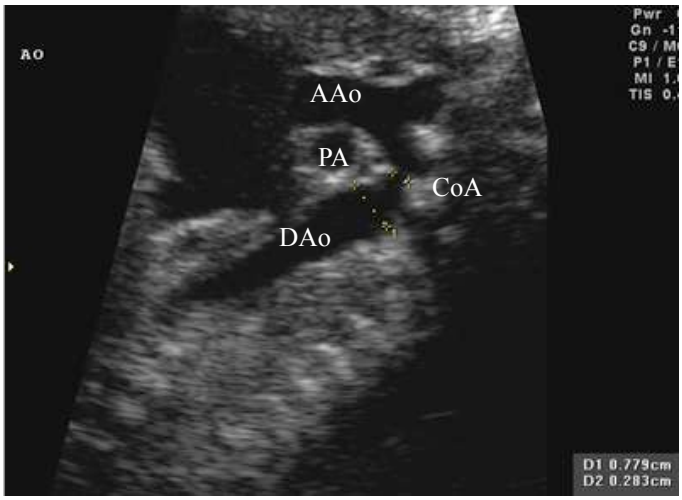

Fig. 4: fetal ultrasoud exhamination. Sagittal plane showing isthmic coarctation of the aorta (CoA). AAo *ascending aorta;* DAo *descending aorta;* PA pulmonary artery. Availeble at URL: http://www.fetalultrasound.com/online/text/7-040.htm (modified).

Small LV is a useful sign (Benacerraf et al., 1989), however some fetuses do not have ventricular size discrepancy and some fetuses with ventricular discrepancy do not have a cardiac lesion: its sensitivity for the diagnosis of coarctation of the aorta is 50-60% (Kirk et al., 1997).

The actual area of narrowing may be difficult to identify antenatally. This may be due to normal patency of the ductus arteriosus in utero. The milder forms of coarctation, however, are consistent with a normal early fetal echocardiogram.

In late pregnancy it may be impossible to exclude coarctation categorically as the right heart structures may appear larger than the left in the

normal fetus (Sharland et al., 1994).

Color Doppler appearance is variable: it may demonstrate normal aortic flow with normal, increased or decreased velocities distal to the coarctation (Allan et al., 1988). Retrograde flow proximal to the coarctation can be seen (Hornberger et al., 1994). Turbulent flow or high velocity jet can be present within the narrowed segment (Snider et al, 1980). Left to right flow through the foramen ovale (Sharland et al., 1994) and increased flow across the tricuspid valve when compared to the mitral valve can also be seen (Allan et al., 1988).

1.7.5 Magnetic Resonance Imaging

High-quality images of coarctation of the aorta can be obtained by magnetic resonance imaging (MRI). MRI images in the sagittal and parasagittal projections can clearly define the location and severity of coarctation and the anatomy of the aortic arch (Beekman, 2008), and may obviate the need for angiography, unless coronary artery disease needs to be excluded (Friedman and Silverman 2001).

The combination of anatomic and flow data obtained by MRI provides a sensitive and specific test for non invasively predicting catheterization gradient more than 20 mmHg (Nielsen et al., 2005) during routine examination to avoid delay in the diagnosis. If the clinical and noninvasive evaluation indicates the need for intervention, some authors prefer to omit MR and perform directly catheterization, angiography and interventional procedures (Rao, 2005). Recently, 4D Flow-Sensitive Magnetic Resonance Imaging was used to characterize hemodynamic alterations and flow-derived vessel wall parameters patients with and without operative repair (Frydrychowicz et al., 2011) (fig. 5 and 6).

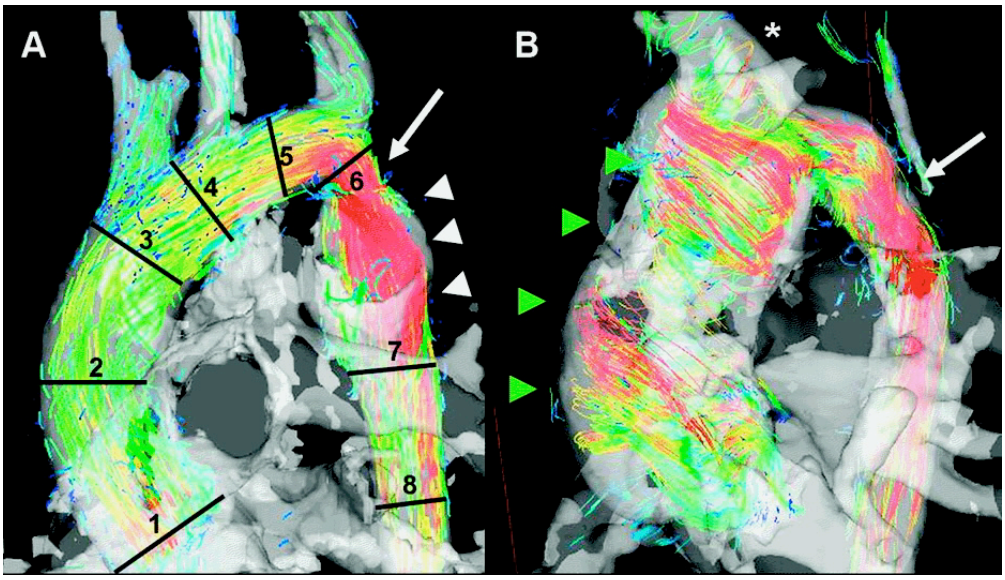

Fig. 5: analysis plane placement for blood flow visualization. In CoA patients, a ninth plane for comparison of velocity data to echocardiography was placed in the region of the CoA/ repair (white arrows). A, Flow patterns in a 35-year-old man with restenosis after treatment. B, 15-year-old girl, 15 years after end-to-end resection of coarctation. Findings include marked flow acceleration at the location of the coarctation (white arrow), increased helicity (green arrowheads) and a poststenotic aneurysm (white arrowheads). Of note, in B a vortical flow pattern can be seen in the proximal descending aorta and the subclavian artery (Frydrychowicz, et al., 2011).

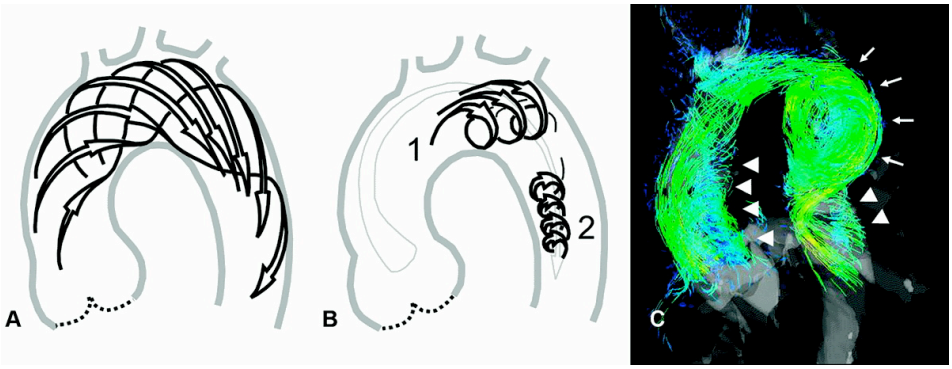

Fig. 6: blood flow patterns. A, the typical right-handed overall helical blood flow in the aorta. In (B) and (C), typically observed flow patterns are displayed. Recirculating blood (1) was termed a vortex (white arrows), helically revolving flow predominantly in plane or flow spiraling as a helical flow smaller than half of the aortic diameter with a core in longitudinal direction (2) was considered as additional regional helix flow (arrowheads) (Frydrychowicz, et al., 2011).

1.8 Treatment

1.8.1 Neonatal presentation

In patients with congestive heart failure, initial treatment, consisting of anticongestive measures including digitalis preparations and diuretics, should be promptly instituted (Rao, 2005).

Perinatal administration of prostaglandin E_1 can be crucial to maintain ductus arteriosus patency in order to increase descending aortic perfusion. Liberman and colleagues (2004) reported three cases of infants presenting with critical coarctation who responded to prostaglandin E_1 infusion without opening the ductus arteriosus. They argued that the administration of prostaglandin E_1 can relax ductal tissue present at the coarctation site (according to the *ductus theory*), relieving obstruction and improving symptoms, even when fibrosis has already formed within the ductus arteriosus, documenting that ectopic ductal tissue constriction can cause coarctation of the aorta in the absence of a patent ductus arteriosus.

Metabolic disorders such as hypoglicemia, acidosis, hypotermia and anemia have to be identified and treated.

If hypertension is considered the major problem, surgical relief of obstruction should be performed as soon as possible, rather than attempt to lower blood pressure pharmacologically (Rao, 2005).

A decrease in the lumen diameter at the site of coarctation greater than 50% and/or a pressure gradient at rest of more than 20 mmHg are usually considered indications for surgery or balloon angioplasty (Castaneda, et al., 1994)

Surgical correction of concomitant associated anomalies is still controversial, frequently coartation resolution permits to ameliorate hemodynamic condition (Puchalski et al., 2004) of associated mitral insufficiency or interventricular defect.

According to existing literature, surgery seems to be the treatment of

choice of neonatal coarctation repair. Balloon angioplasty is eligible for recurrent obstruction after coarctation repair in infancy (Burch et al., 2009).

1.8.2 Presentation during infancy

Correction in asymptomatic children presenting with a heart murmur or upper limb hypertension must be performed as soon as possible. The younger the child, the greater the possibility of residual or recurrent coarctation and the lesser the probability of systemic hypertension. When surgery is performed at older ages, the chances of success at late follow-up decrease and the rate of survival is lower due to the development of cardiovascular complications and higher incidence of systemic hypertension (19.9% to 68%) when compared with that in the general population (Maia et al., 2000).

Surgical repair remains the conventional treatment for most children with coarctation of the aorta (Beekman, 2008).

1.8.3 Adult coarctation correction

Aortic coarctation presenting during adult life most frequently results from recoarctation: surgical or percutaneous balloon angioplasty with or without stent placement and medical therapy are different methods for treatment. The 2008 American College of Cardiology/American Heart Association (ACC/ AHA) guidelines for adults with congenital heart disease (ACHD), recommend intervention for coarctation in the following circumstances: peak to peak coarctation gradient greater than or equal to 20 mmHg or peak to peak coarctation gradient derived from catheterization less than 20 mmHg, in the presence of anatomic imaging evidence of significant coarctation with radiologic evidence of significant collateral flow (Warnes et al., 2008).

The European Society of Cardiology 2010 guidelines for management of grown-up congenital heart disease claim that all patients with a non-invasive pressure difference >20 mmHg between upper and lower limbs, regardless of symptoms but with upper limb hypertension (>140/90 mmHg in adults), pathological blood pressure response during exercise, or significant LVH,

should have intervention (Baumgartner et al., 2010).

Balloon angioplasty has been recommended as the preferred treatment for children and adults with recoarctation after surgery. The major drawback of angioplasty alone is recoil of the vessel wall with recurrence of stenosis. Stent implantation overcomes some of the shortcomings of balloon dilatation, because the metal scaffolding may reduce the incidence of acute elastic recoil as well as late restenosis, due to a more complete elimination of gradient in the high-velocity arterial system flow. In addition, if the patient remains hypertensive with echo-Doppler findings that show substantial residual gradient, repeated dilatation of the stent is a feasible option.

Stent implantation carries the lowest morbidity whereas repeat interventions are more common following endovascular treatment compared to surgery (Anagnaostopoulos-Tzifa , 2007).

1.8.4 Surgical treatment

Surgical treatment of CoA has been performed since mid 20th century: Crafoord and Nyhlin performed the first resection with end-to-end reanastomosis in 1944 (Crafoord and Nyhlin, 1945).

Surgical repair of coarctation can be achieved by several techniques: resection with end-to-end anastomosis, subclavian flap aortoplasty in infants with long-segment coarctation, a bypass graft across the area of coarctation when the distance to be bridged is too long for an end-to-end repair or prosthetic patch aortoplasty.

The technique of subclavian turndown and end-to-end anastomosis described in 1944 and 1945 led to modifications including prosthetic patch grafts, subclavian patch aortoplasty, and prosthetic tube grafts.

Regardless of the technique, surgical coarctation repair generally is performed through a left thoracotomy. If necessary, as when combined with repair of an intracardiac lesion, coarctation repair can be performed from an anterior sternotomy approach.

Scoliosis is a side effect due to posterolateral thoracotomy: in a study the prevalence of scoliosis after standard posterolateral thoracotomy was

significantly higher than after nonsurgical treatment methods, as well as than in the general population (Roclawski et al., 2009).

Resection and end-to-end anastomosis in most centers remains the surgical treatment of choice for patients with a discrete coarctation. With resection and end-to-end anastomosis, the aorta is isolated and the aortic isthmus and ductal tissue are resected. The distal aortic arch is incised along its inferior side, the lower aorta is incised along its lateral side, and the two are sewn together. The advantages of end-to-end technique are that the subclavian artery is not sacrificed, and complete relief of obstruction is easily obtained. Disadvantages of resection relate primarily to the presence of a circumferential suture line, which ledad to high incidence of restenosis in early studies (Beekman, 2008).

In the variant **extended end-to-end anastomosis,** a longitudinal incision is made in the posterior wall of the descending aorta and a second generous incision is made in the arch concavity up to the base of the left carotid artery. An equal circumference of the two aortic ends is obtained. The anastomosis is then completed with a running suture (Beekman, 2008). This procedure improves the effectiveness of stenosis relief in infants with isthmus or transverse arch hypoplasia (van Heurn et al., 1994) and has decreased the risk of late restenosis (Wright et al., 2005) (Fig. 7).

Early complications such as residual obstruction (pressure gradient greater than 20 mmHg) can occur. Postoperative systemic hypertension known as paradoxical hypertension can occur in up to 1/3 of the patients for imbalance in sympathetic discharge, increased sensitivity of aortic and carotid baroreceptors and increase levels of circulating renin and angiotensin. Postoperative paraplegia is rare.

Recurrent coarctation is the most frequent late complication and it is reported to occur in a variable number of patients between 6% and 60% and after a variable time interval following primary repair. Low body weight at the time of surgery, residual ductal tissue or persistence of the isthmic shelf have all

been suggested as risk factors for recoarctation (Gargiulo et al., 2007).

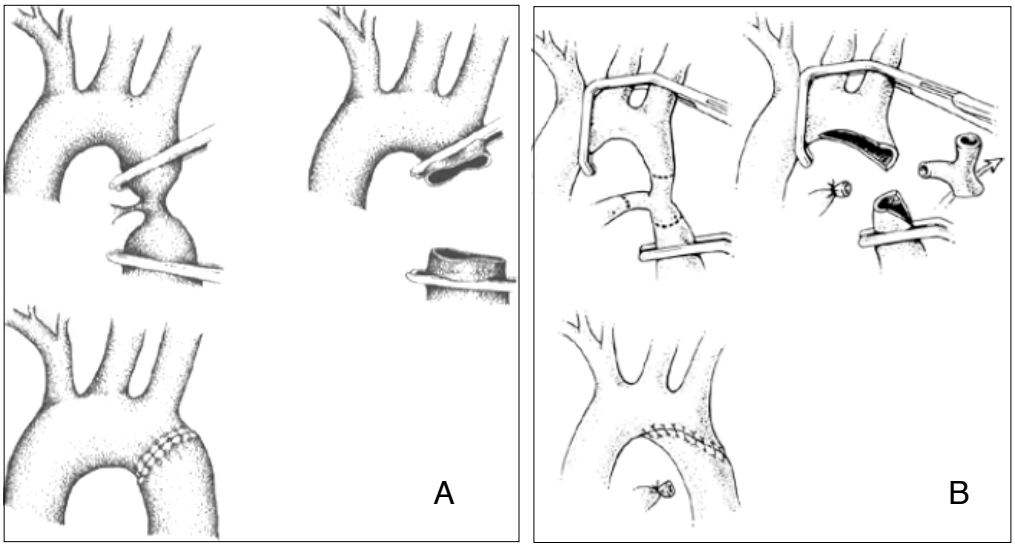

Fig.7: comparison between **end to end anastomosis** and **extended end to end anastomosis. (A)** Coarctation resection and end-to-end anastomosis. The circumferential anastomosis is completed with interrupted sutures anteriorly. **(B)** Resection with an extended end-to-end anastomosis. The anastomosis extends onto the transverse arch under the left common carotid. (Beekman, 2008 *modified*).

In 1961, Vosschulte described the **prosthetic patch aortoplasty** technique. A longitudinal incision is made across the coarctation, if necessary extending onto the proximal left subclavian artery, and the area is enlarged with a patch of Dacron or Gore-Tex. The posterior shelf of the coarctation may or may not be resected. Obvious advantages of this technique is less extensive aortic mobilization, preservation of intercostal arteries and avoidance of circumferential suture. Several reports described a relatively higher risk of development of aneurism formation (Del Nido et al., 1986; Bromberg et al., 1989).

Without the use of prosthetic materials, the subclavian flap aortoplasty described by Waldhausen and Nahrwold (1962) lowers the rate of restenosis: Dehaki and colleagues (2010) showed less recurrence rate of restenosis with this procedure as compared to patch aortoplasty or end to end anastomosis. It requires less extensive aortic mobilization using living subclavian tissue as a patch with theoretical growth potential and avoiding a circumferential anastomosis. The left subclavian artery is ligated and divided, and a longitudinal incision is extended through the proximal subclavian artery and beyond the

coarctation. The proximal subclavian stump is then turned down on the coarctation and used as a patch of autologous tissue. Obvious disadvantage is the subclavian artery sacrifice.

Extra anatomical aortic bypass can be considered in adults with CoA. A prosthetic bypass is positioned connecting ascending aorta to distal thoracic aorta. This technique permits to avoid resection of sclerotic and inelastic segments of adult aortic arch or a long tubular hypoplastic segment. This procedure had been used following iatrogenic rupture of CoA during balloon aortoplasty (Ogasawara et al., 2011)

Recently, ascending sliding arch aortoplasty has been described to repair aortic arch hypoplasia (McKenzie et al., 2011): the distal ascending aorta is transected at the level of the proximal arch and incised longitudinally on the right anterolateral aspect, creating a tongue or flap. The inner curvature of the entire arch and proximal DTA is then incised, and the ascending aorta tongue is then advanced distally to augment that incision.

1.8.5 Percutaneous Balloon Angioplasty and Stenting.

In patients with discrete aortic coarctation, endovascular procedures has been described. Balloon dilation of CoA permits sudden gradient reduction. This procedure has been initially introduced since 1983 to treat restenosis after surgical excision (Lock et al., 1983). Angioplasty enlarges the coarctation lumen by expanding the diameter of the lesion and by producing linear intimal and medial tears at the coarctation site (Sos et al., 1979)

Although this less invasive technique has been universally accepted as effective for a recurrent postoperative coarctation, it remains controversial as an effective primary strategy: surgical repair of an isolated native coarctation carries relatively low risks and a high expectation of success. In contrast, reoperation for recurrent postoperative coarctation is technically more difficult and is associated with increased morbidity and mortality. Balloon angioplasty alone can be ineffective to completely reduce pressure gradient and restenosis can occur. Restenosis following balloon angioplasty also appears to be age dependent; the younger the child, the greater is the probability of recoarctation

(Rao et al., 1996). Stent placement after balloon dilation has been introduced to avoid the risk of restenosis, providing support to the dilated aortic segment.

Balloon angioplasty is performed with fluoroscopic guidance (fig. 8). A single balloon technique is more frequently used. Appropriate sedation and analgesia is used as required by the individual patient. Catheters are placed in the artery via an arterial sheath. An exchange J guide wire is positioned in the ascending aorta, and the appropriate balloon is advanced over this wire and

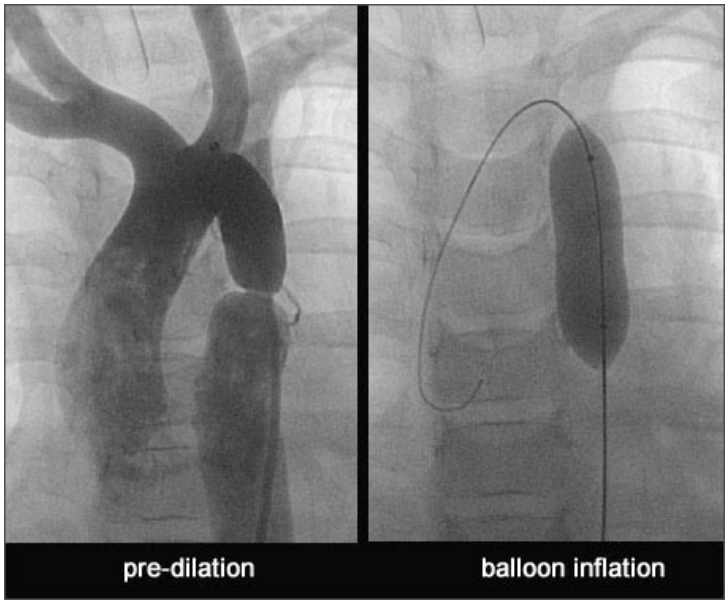

Fig. 8: balloon angioplasty for coarctation of the aorta.

positioned in the center of the coarctation. The diameters of the aortic isthmus (the largest diameter distal to the last great artery branch from the aortic arch and proximal to the coarctation site), the coarctation, and the largest diameter in the descending aorta distal to the coarctation are measured. The diameter of the dilation balloon chosen for the angioplasty is usually equal to or slightly less than the largest diameter of the descending aorta distal to the coarctation including the area of poststenotic dilatation. The balloon is inflated until the waist disappear and held for 5-10 seconds. (Hijazi et al., 1991).

Intimal and medial vessel wall tears, aneurysm formation, residual CoA and Re-CoA are well known complications encountered at follow-up in patients

with residual or native CoA treated with balloon dilation. The incidence of early and late aneurysms after balloon angioplasty has been reported to be between 5 and 12% (Peters et al., 2009).

Endovascular aortic stenting is performed using an endovascular guide advancing a stent-balloon assembly through the sheath to the coarctation site (fig. 9). Balloon-expandable stents (BES) are mounted on balloons, positioned across the site of obstruction and are implanted by inflating the balloon.

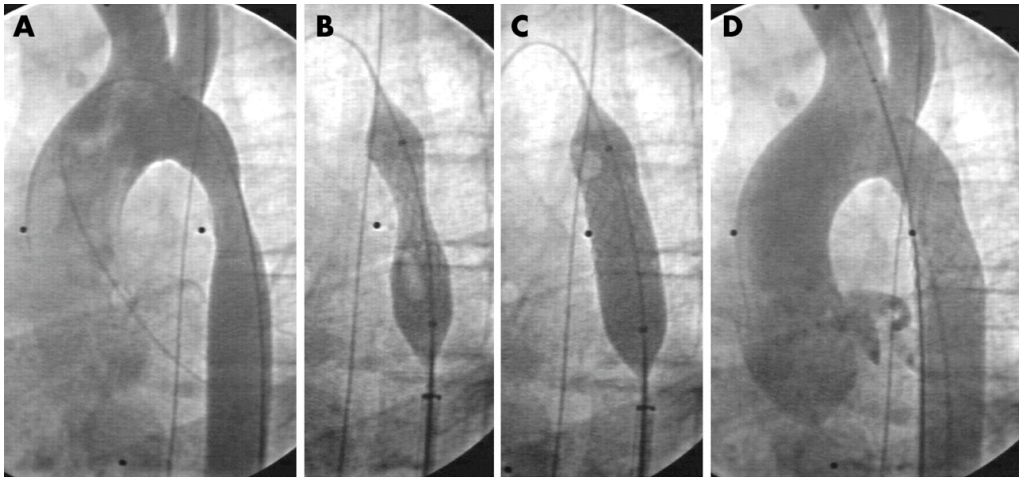

Fig. 9: angiographic pictures of stent insertion for (A) pre-procedure, (B) stent insertion, (C) stent expansion by inflation of internal balloon, (D) final result. (Andrews and Tulloh, 2004).

The size of the inflated balloon determines the expanded diameter of the stent. BES was introduced for the treatment of congenital heart lesions in 1987 (Peters et al., 2009).

The inflation of the balloon permits simultaneous decoarctation and stent positioning (Magee et al., 1999). Stents decrease coarctation restenosis related to vessel recoil and may also diminish the late incidence of aneurysm formation. Several clinical series have documented the effectiveness of coarctation stenting for native or recurrent coarctation. Stenting has been shown to be effective in some patients with transverse arch hypoplasia (Pihkala et al., 2000). This procedure is indicated for patients weighting more than 25 kg, as femoral artery diameter permits safe introduction of an 8-9 french sheath. Stents implanted in

growing children may need to be dilated further when the child has grown up (Beekman, 2008). Forbes et al, (2007) recommend stenting for patients older than 10 years or weighing more than 35 kg.

The perceived advantages of stenting compared with simple ballooning are the ability to expand a tubular long-segment coarctation or an hypoplastic isthmus and distal transverse aortic arch, to increase the diameter of the coarcted segment independent of the intimal tear, to decrease the prevalence of restenosis and to prevent aneurysm formation because of support to the weakened aortic wall segment by the stent.

Large stents are needed for stenting CoA. Traditionally, the large Palmaz stents are the most commonly used stents in large multicenter studies (Forbes et al., 2007).

Covered stents, especially Cheatham-Platinum (CP) covered stents, are useful in adults with coarctation, especially with complex lesions, such as concomitant patent ductus arteriosus, or near aortic arch interruptions and significant arch tortuosity (Pedra et al., 2005). Newer stent designs that are more flexible and thus show better adaptation to the vascular wall might induce less neointimal layer formation: the CP stent or the IntraStent Mega and Maxi LD (EV 3) are more flexible and can be easily dilated up to adult vessel diameters (20–25 mm).

CP STENTS are made from 90% platinum and 10% iridium. These devices are manufactured from a wire, which is bent and welded to a cylindrical meshwork, forming the stent. This leads to more adjustable flexibility and a wide range of size and length, but radial strength is usually less than the ones with slotted tubes.

In a multicenter study including over 500 patients, acute complications have been reported in up to 14% of the patients. The patients above 40 years represent a high-risk group with 31% incidence of complication. Common complications include vessel disruption and displacement of the stent: stent migration is the most frequently encountered technical problem, with an incidence of up to 5%. Balloon rupture with inadequate stent expansion and

injury to access vessels with bleeding from the puncture site (2.3%) has been described (Forbes et al., 2007). Loss of pulse following femoral vessel closure is of course more likely to occur in patients under 6 years of age. Cerebrovascular accidents have been observed in up to 1%. (Golden and Hellenbrand, 2007).

Aneurysm formation after stent implantation or aortic dissection after stent dilatation have been encountered in up to 5% of the patients: these complications are inevitable consequences of the mechanism of dilation of the CoA site. Aggressive pre-stent angioplasty could also increase the likelihood of later aneurysm formation (Golden and Hellenbrand, 2007).

Recurrence of stenosis has been reported in around 11% of all patients. In general, there are two conditions leading to restenosis and late failure after primary successful stent implantation. One is “full-growth” stenosis, depending on the initial age and size of the patient. The other is hyperplasia of the endothelial intima of the vessel. Narrowing of the lumen by 1–2 mm is seen in all cases, but true intimal hyperplasia in less than 25% of patients. Risk factors promoting neointimal proliferation include younger age, lower weight and recurrent CoA. Newer stent designs that are more flexible and thus show better adaptation to the vascular wall might induce less neointimal layer formation (Suárez de Lezo et al., 1999).

CP stents are available in a covered version, with an outer polytetrafluoroethylene (PTFE) membrane. The covering is initially approximately 7 mm in diameter and will stretch over the range of diameters of expansion (usually from 12 to 24 mm diameter) and will always be taut over the stent when expanded. To date, the covered CP stent is the most widely used covered stent in patients with CHD (De Giovanni, 2001).

In 1999, the first covered stent was used to treat coexistent CoA and aneurysm of the aorta in a young man (Gunn et al., 1999). Covered stents or endoluminal stent grafts have been successfully used in the management of aortic aneurysm in an effort to reduce the need for repeat surgery. The main disadvantage of stent grafts is that they require large (22–25 F) vascular sheaths.

Aneurysm formation may occur even after redilatation of a previously implanted uncovered stent (Zanjani et al., 2008). Another major complication of

aortic stenting is aortic disruption, increased in elderly patients (Forbes et al., 2007).

Stent fracture is a well described but uncommon complication after CoA stenting, affecting even the CP stent with reinforced welded sites (Ewert et al., 2005).

One limitation of covered stents is their potential to cover side branch vessels during deployment, usually the subclavian artery ostium (Magee et al., 1999). Uni- or even bilateral surgical reimplantation of the subclavian arteries end-to-side into the carotid arteries should be considered before stenting as this creates a stable “landing zone” for subsequent stent deployment (Kenny et al., 2008). Another option is stent recanalization by perforating the cover (Tsai et al., 2009).

2. Coarctation of the aorta and hypertension

Aortic coarctation represents 0,2% of all cases of hypertension (Katira et al., 1997). If untreated, coarctation determines a typical pressure gradient through the narrowing and pressure differential between upper to lower limbs. Upper vascular system hypertension predisposes patients to major cardiovascular complications such as heart failure, aortic rupture or stroke (Rosenthal, 2005). Furthermore, isthmic narrowing determines systolic hypertension in some patients (Beekman, 2008).

Some adolescents appear normotensive at rest, thanks to the presence of a significant collateral circulatory system that provides blood flow to the lower parts of the body. Frequently these patients develop forms of inappropriate hypertension during physical exercise (Warnes and Deanfield, 2000).

Right after surgical removal of the isthmic defect, upper vascular system hypertension resolves. In some patients with severe coarctation surgical intervention is followed by a transient *paradoxical hypertension,* which can last for days or months (Rosenthal, 2005). This surgical post-operative complication is characterized by elevation of systo-diastolic values to higher levels than preinterventional parameters. In rare cases this condition leads to mesenteric vasculitis and visceral ischemia. The physiopathologic mechanism of *paradoxical hypertension* is unknown, but renin-angiotensin system and sympathetic nervous system seem to be involved (Beekman et al., 1983). This transient condition can be prevented by beta-blocker administration (propanolol) and aggressive antihypertensive treatment after intervention (Gidding et al., 1985). Interestingly, *paradoxycal hypertension* seems to be a peculiar complication of surgery: several authors reported no incidence of this phenomenon in endovascular balloon-treated patients (Choy et al., 1987; Hassan et al., 2007; Fawzy et al., 1999).

Prognosis of effectively treated patients can be negatively affected by development of hypertension later in life. This complication has a higher incidence in patients treated later in life: Nanton and Olley reported higher

incidence of juvenile hypertension in effectively corrected patients aged more than 4 years old at the time at intervention when compared to patients operated earlier (1976). The earlier is time at intervention, the lesser is the incidence of hypertension: Shinebourne and colleagues reported a higher incidence of hypertension in patients treated after 1 year of age (1976).

Different conditions can determine different hypertensive statuses after coarctation repair: severe residual gradient after repair does not reduce pressure differential between upper and lower vasculature. Such condition can be found years after intervention in patients effectively treated due to *recoarctation,* and these patients need to undergo reintervention or endovascular balloon dilatation with stent implantation. In a study focusing on MRI evaluation of postcoarctectomy aortic arch morphology, a high rate of restenosis (67,9%) was found in treated patients (Frydrychowicz et al., 2011).

Besides complete resolution of the isthmic gradient, treated patients are at risk of development of persistent hypertension. This form of juvenile hypertension occurs in 10-20% of effectively treated patients, even if surgery has been performed early in life (Beekam et al., 2008). Other authors reported a higher prevalence of hypertension, occurring in 30% of effectively treated individuals (Presbitero et al., 1987).

In addition, in 20-35% of cases, normotensive patients at rest develop exaggerated pressure rise during exercise (*exercise-induced hypertension,* EIH) (Vriend et al., 2004). EIH can be present right after intervention or develop later in life (Luijendijk et al., 2011) and it is diagnosed revealing abnormal rise of pressure values in the upper limb during treadmill exercise. The physiopathological basis of EHI is associated to the sudden increased of aortic flow through a less extendible excision site (Markel et al., 1986).

Furthermore, other authors studying flow-mediated dilation (FMD) argued a possible intrinsic dysfunction of the upper vascular bed, not only as a result of the postcoarctectomy stiffer segment (Guenthard J and Wyler F, 1995).

EIH can be found even in endovascular stenting treated patients (De Caro et al., 2010), even if it is reported in a limited number of patients (Bazan, 2010). EIH is associated to increased intima-media thickness, a clinical condition

predictive of atherosclerosis (Vriend et al., 2005). Recently, a prospective study revealed the predictive value of EIH in the development of chronic hypertension: maximal systolic value during exercise was positively correlated to the incidence of persistent hypertension at follow-up. (Luijendijk et al., 2011).

Chronic hypertension is diagnosed several years after intervention: in a retrospective study evaluating 244 patients (mean age at intervention 20yrs) 30 years after intervention only 30% of subjects remained normotensive (Presbitero et al., 1987).

Even if less frequently, hypertension occurs in early treated patients (O'Sullivan et al., 2002): in a cohort of children undergoing surgery at a mean age of 0.2 years, at 10 year follow-up the incidence of hypertensive patients was 20-30%. Cohen et al. (1989) reported that hypertension was present in 7% of individuals who had surgery under one year of age, whereas in those operated later (older than 14 years) the incidence rose up to 33%.

The physiopatologic mechanisms behind hypertension in effectively decoarctated patient are still poorly known.

Different studies have underlined the potential role of various factors involved in hypertension after COA repair. These factors include mechanical, functional, and structural abnormalities in the systemic arterial bed, particularly of the precoarctation region and malfunction of blood pressure (BP) regulatory systems some of which appear to exist within the fetal or neonatal period before repair. In particular, impaired response to vasoactive agents, stiffness and geometry of the proximal aorta, baroreceptor and renin - angiotensin system dysfunction have been investigated.

2.1 Alteration in vascular physiology in decoarctated patients

Investigators proposed two theories to explain vascular alterations found in effectively decoarctated patients: that the isthmic narrowing represents only a

feature of a diffuse anomaly of the vascular bed or that coarctation itself can determine irreversible alterations to the upper vascular bed prior the intervention (Celermajer and Greaves, 2002).

Several works support the latter theory, underlining histological differences between specimens of the proximal aorta and those obtained from segments distal to the lesion.

Ultrastructural evaluation of pathologic aortic specimens obtained after surgery showed significantly more collagen and less smooth muscle cells in the aorta above rather than below the coarctation. These differences were associated to altered response to *in vitro* stimulation with vasoactive agents (potassium, noradrenaline and prostaglandin F2 alpha). Postcoarctational aortic ring specimens showed a significantly greater contractility than precoarctational rings (Sehested et al., 1982). Specimens were obtained from patients aged 6 to 35 years at surgery, but these ultrastructural changes were identical compared to two aortic fragments obtained from autopsy of two affected individuals died under 2 years of age. Reduced nitric oxide bioavailability is present in the upper vascular bed of experimentally coarctated animal models (Barton et al., 2001).

Non invasive vascular clinical studies were performed looking for NO-dependent vasodilation (*flow-mediated dilation* o FMD) and endothelium independent vasodilation (*Glyceril trinitrate-mediated dilation*, GTND). De Divitiis e coll. (2003), studying a cohort of 72 patients (60% treated before one year of age) showed that FMD and GTND were reduced several years after intervention in comparison with normal control subjects. The authors hypothesized that this anomaly in the upper vascular bed physiology could be related to abnormal smooth muscle cells relaxation or to ultrastructural changes such as reduced elastin and collagen deposition.

The same Authors (2001) described that brachial FMD and GTND were reduced in 64 patients (20% hypertensive) in contrast to normal values found in lower limbs, disclosing the presence of an increased rigidity of conduit arteries proximal to coarctation site. FMD and GTND are reduced even in patients treated early in life; these parameters are independent of age at intervention or presence of residual gradient (Trojnarska et al., 2011a).

2.2 Altered aortic arch stiffness and morphology

MRI evaluation of the post coarctectomy aortic arch shape in 105 patients (medium age at intervention 22 months) showed a correlation between hypertension and "gothic-shaped" aortic arch (defined by the evidence of an acute angle between ascending and descending aorta) (Ou et al., 2004).

Before intervention, the altered aortic elastic properties were confirmed by Xu et al., (1997) who assessed aortic stiffness by intravascular echocardiography. They observed that in the ascending aorta, patients with aortic coarctation had higher stiffness index and lower aortic distensibility and compliance than the descending aortic segments. This is the opposite of what is usually found in healthy subjects, in whom the distal aorta is stiffer than the proximal aorta (Mohiaddin et al., 1989). Successful balloon angioplasty increaased distensibility and compliance whereas the stiffness index remained unchanged. The authors explained their findings through the lack of dependence of stiffness index on blood pressure changes. Therefore, aortic stiffness was still increased despite the removal of aortic obstruction and blood pressure reduction (Xu et al., 1997) and this finding was not related to the age at intervention.

Transesophageal echographic evaluation of 26 normotensive adult patients treated early in life revealed impaired elastic proprieties of the aortic arch (Doppler tissue imaging wall velocities during systole (S(w)), peak systolic strain (εAo) (Vitarelli et al., 2008).

One work focused on the role of a mild residual narrowing in the development of hypertension (decoarctation site pressure gradient < 20 mmHg) and argued that the ratio between the diameter at the site of repair and in diaphragmatic aorta is a strong and independent predictor of average daytime systolic pressure at 24-h monitoring and intima-media thickness in the carotid artery (Vriend et al., 2005).

Several papers report Pulse Wave Velocity (PWV) higher in effectively treated patients than in control subjects (de Divitiis et al., 2003; Trojnarska et al., 2011b; Mizia-Stec et al., 2011). Interestingly, PWV seems to be influenced

by different surgical procedures: in a small cohort of early treated patients PWV was higher in individuals undergoing subclavian flap rather than those who had end to end anastomosis. This abnormality was revealed in the upper vascular bed, while PWV was normal when measured in the lower arteries (Kenny et al., 2010).

With applanation tonometry it is possible to measure noninvasively central pulse pressure waveform. Augmentation index seems to be increased in patients with CoA (Moutafi et al., 2011; Trojnarska et al., 2010).

Recently Senzaki and coll. (2008) showed that stiffness is not a vascular exclusive feature, but it is also an intrinsic propriety of the left ventricle in affected patients.

Furthermore, an MRI study evaluating postcoartectomy aortic flow identified a high rate of vortices in the branches of the supra-aortic arches, suggesting a basis for future studies on the development of hypertension, intima-media thickening, and central aortic flow wave possibly related to hypertension (Frydrychowicz et al., 2011). As there is a correlation between altered vessel wall function and coarctation, the Authors hypothesize that the underlying changes in blood flow patterns may cause, trigger, or promote such changes by shear-stress mediated mechanotransduction.

2.3 Baroreceptor dysfunction

Frydrychowicz in his mentioned study (2011) also supposed that baroreceptors located in the carotid sinus may be exposed to abnormal vortical flow and thus could be stimulated or irritated.

Several works argue that baroreflex dysfunction (reduced baroreflex sensitivity) can be related to hypertension pathogenesis (Davrath et al., 2003). Before treatment, an increase in collagen and a decrease in smooth muscle in the precoarctation aorta increase stiffness and decrease distensibility that are responsible for elevation of systolic blood pressure at rest, disproportionate increase in systolic pressure during isotonic exercise, and resetting of the carotid

baroreceptors to operate at higher pressures (Perloff, 2010).

Autonomic dysfunction has been shown in animal models of aortic coarctation, even if surgical correction completely reversed this dysfunction (Igler et al., 1981).

Histology showed increased collagen concentration and decreased smooth muscular cells in proximal segments of surgically excised aortas. This ultrastructural anomaly can interfere with baroreceptorial function, because stretch receptors can be less stimulated in a stiffer aorta (Sehested et al., 1982).

Beekman and colleagues (1983) studied baroreflex sensitivity in 6 decoarctated hypertensive patients studying the relationship between mean direct arterial pressure changes and R-R interval range. They discovered that in hypertensive patients with corrected coarctation, arterial baroreflex is reset to a higher pressure and has a diminished sensitivity to blood pressure changes. This dysfunction can be found also in effectively treated patients: sBRV (spontaneous baroreflex sensitivity) seems to be abnormal in normotensive patients (Kenny et al., 2001).

Interestingly, Polson and colleagues (2006) studied baroreflex function in newborns with CoA before intervention: in 10 affected neonates sBRV and heart rate variability were significantly reduced compared to healthy controls. Blood pressure variability was increased. These data indicate that affected newborns already have autonomic dysfunction that can be implied in the pathophysiology of long term hypertension.

2.4 Renin-angiotensin system impairment

The role of the renin-angiotensin system in the development of post-coarctectomy hypertension is poorly understood.

Plasma renin activity (PRA) was significantly higher in a group of hypertensive patients at peak exercise (Ross et al., 1992). This data were not supported by Simsolo et al. (1988) who showed that patients with repaired coarctation have an exercise-induced rise in PRA comparable to the rise seen in

healthy controls or essential hypertension. Hauser and colleagues reported elevation of plasma renin activity in patients with history of paradoxical hypertension at the time of surgery (2000).

In the studies by James and Kaplan (1973) and Freed et al. (1979) patients with repaired coarctation showed a significant increase in the pressure gradient across the isthmus at exercise, which was probably due to the residual post-operative isthmus restriction, which created a significant gradient when cardiac output triples. The authors hypothesized that the pressure gradient between upper and lower body stimulates the activity of the renin angiotensin system that could itself induce a blood pressure rise.

Some authors hypothesized that the reduction of renal blood flow is the cause of stimulation of renin angiotensin system (the so called Goldblatt-type phenomenon), but in some patients with coarctation of the aorta, the presence of an extensive collateral circulation can reduce the mean pressure gradient to almost zero and ensure an adequate perfusion pressure to the kidney; in these cases PRA is not stimulated in comparison to normal subjects (O'Rourke et al., 1971). It is now well known that the renin-angiotensin activity is not only systemic, but is present in most tissues and organs; the autocrine and paracrine activity seem to be even more relevant than the systemic activity in both body homeostasis and pathologic states. It is reasonable to hypothesize that an increase in the renin-angiotensin system contributes to the arterial hypertension in patients with coarctation. However, its role in the development of high blood pressure at rest and during exercise years after a successful surgical repair is still unclear (de Divitiis et al., 2005).

3. The sheep as an animal model in cardiovascular research

The choice of the most appropriate experimental model to address a defined scientific hypothesis, and the way in which results can be extrapolated from experiments using different animal models to answer biomedical research questions, are complex and not straightforward: there are no rules regarding the choice of the proper animal nor are there rules for the extrapolation of the results from the model to another animal species or man (Festing, 2000).

Significant differences exist with regard to several cardiac characteristics when rodents are compared with humans. For this reason, large animal models like dog, sheep and pig have a well established role in cardiac research (Gandolfi et al., 2011). These are currently used as animal models for various cardiovascular surgical procedures.

Dogs, pigs and sheep have been used for cardiovascular studies. The arterial morphology of the pig is the most similar to humans. They exhibit a tendency toward hypercoagulability, and their fibrinolytic system is not as active as the canine. When compared with dogs, however, they are not as easy to handle, are less tolerant to anesthesia, and their arteries are smaller, making it difficult to use large introducers and prostheses. The sheep is considered to be a suitable model for cardiovascular surgery because of its ease of handling, size, and vascular anatomy which bears close resemblance to the human (Narayanaswamy et al., 2000; Geens et al., 2009). Furthermore, the sheep coagulation system is closer to the human than either dogs or pigs. The sheep is an easy animal to manage in the laboratory and to use for testing of vascular devices.

Sheep can harbor a rickettsial infection that is transmissible to humans (Q fever) (Narayanaswamy et al., 2000). Other limitations of working with sheep, being ruminants, derive from their gastrointestinal anatomy and thoracic contours that are substantially different from those of monogastric species. This makes some imaging approach difficult, specifically ultrasonic imaging that may

require an invasive approach as opposed to transthoracic imaging applicable to other models (Gandolfi et al., 2011).

Recently, scientific research has used this animal model to induce myocardial infarction secondary to platelet aggregates inoculation (Kwiatkowski et al., 2010) or via circumflex arteries ligation (Chaput et al., 2009). Ovine models have been involved in models of non-ischemic cardiomyopathy (Psaltis et al., 2008), pulmonary hypertension (Sato, 2008) and systemic hypertension via renal artery clamp (Lau et al., 2010).

The sheep has been used for coronary surgery studies because of its adequate anatomical resemblance with human anatomy and physiology, adequate size, and resemblance of biological response to mechanical injury and to other interventions on vessel walls (Shofti et al., 2004). In particular, its dimensions permit the use of devices such as CP stents intended for human used. In 1986, Balko et al. reported the first endoluminal exclusion of an experimentally created abdominal aortic aneurysm using endovascular grafts in sheep.

The similarities between human and ovine coagulation and fibrinolytic systems make these animals good candidates for the evaluation of in-stent restenosis occurring after percutaneous coronary interventions. The similar coronary anatomy favors its use: the responses to stent-induced injury in ovine coronary arteries parallel those of porcine coronary arteries. An added benefit to the ovine model, compared to the swine model, is the availability of appropriately sized bifurcations of the coronary vasculature, which may make this model more appropriate for the evaluation of bifurcation devices (Perkins, 2010).

When postinfarction congestive heart failure has to be studied, the sheep is a suitable model because of the lack of collateral blood flow to the infarcted area, maintenance of normal blood flow to uninfarcted areas, and the moderate size of the infarcted myocardium allow to faithfully reproduce the three major clinical causes of this pathology (Mukherjee et al., 2003).

Sheep has been used for other endovascular studies, such as mitral valve replacement using percutaneous transvenous valve-in-Ring (Shuto et al., 2011),

stent graft placement in aortic aneurysm treatment (Macierewicz et al., 2011), percutaneous implantation of an aortic valve prosthesis (Laborde et al., 2005; Webb et al., 2004) and in neointimal proliferation after nitinol stent placement (Meyer et al., 2003).

4. Aim

Coarctation of the aorta (CoA) is one of the most frequent congenital cardiovascular anomalies, as it accounts for 5-7% of all congenital heart conditions with an incidence of 1:12,000. It is the fourth most common condition requiring cardiac catheterization or surgery during the first year of life. CoA can be present alone or in association with other anomalies and can be symptomatic in the neonate or in pediatric age. Coarctation treatment in most patients does not determine complete remission of symptoms, because of a high rate of restenosis and residual hypertension, occurring in up to 50% of cases (especially if patients are treated later in life).

Residual hypertension leads to ventricular hypertrophy and major cardiovascular events such as aneurysm rupture, heart failure, atherosclerosis, myocardial infarction, stroke and sudden death. Life expectancy is reduced in this group of patients. Recently, endovascular balloon expandable stent has been introduced for native CoA and recoarctation treatment to prevent risk of restenosis.

To date, the long term effects of stent positioning in a growing aorta, its effects on the risk of developing hypertension and ventricular hypertrophy are poorly understood. Consistent literature indicates animal models as dogs, pigs and sheep to be suitable for stent application studies. Relatively short life expectancy of sheep (approximately 12 years) and its fast growth to adult size (reached at 15 months of age) makes this animal model suitable to reach significant results in relatively short period of time.

Aim of this study is, therefore, to determine feasibility of stent implantation in the aortic isthmus of an ovine model and to study pressor, hemodynamic and hormonal changes induced in the growing animal. By extrapolating the obtained data from the model to human it will be possible to state if aortic stent is not only acutely feasible to relief the anatomic obstacle, as already described in literature, but also safe and free of complications in the mid and long term.

5. Materials and methods

5.1 Study design

After selection in the breeding farm, the animals were randomly assigned to the STENT or SHAM groups and entered the study protocol reported in fig. 10.

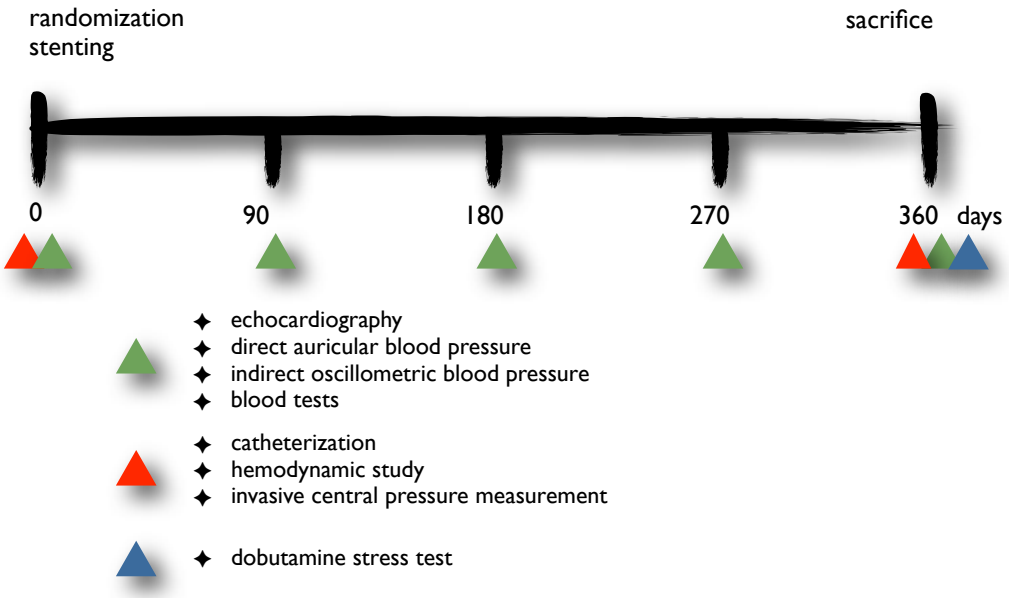

Fig. 10: study protocol.

The study protocol can be divided in 3 phases. In phase 1 the animal is introduced in the study and catheterization performed. In phase 2 the animal is kept in kennels with other individuals for 12 months and undergoes periodic check-ups. In phase 3 the animal is sacrificed after a second hemodynamic study and a dobutamine stress test.

New animals were introduced in the study gradually in order to maintain a constant number of subjects in the kennels: every sacrificed sheep is replaced by a new one, as kennels contained a maximum of 5 animals.

5.2 Animals and housing

The Faculty of Veterinary Medicine of Padua University has the approval for use of sheep as Laboratory Animals (art. n°12 D.Lgs.116 27.01.1992) and the study project was communicated to the Italian Ministry of Health according to the Italian law for use of laboratory animals (art. N°7 D.Lgs 116 27.01.1992) under project registration number 90/08 C21.

Initially, two *alpagota ovis* sheep, one male and one female, were introduced in a pilot study. This race was then abandoned because the animal dimensions were inappropriate for use of pediatric endovascular equipment. Therefore, the animals subsequently enrolled in the study were 12 female *bergamasca ovis aries.* The *bergamasca* sheep is bigger, permits a suitable catheterization and correct stent positioning.

Only female animals were chosen because females are easier to handle, and their less pronounced musculature permits easier venous catheterization with lower risk of post-procedural hemorrhage. All animals were 3 to 5 months old and weighed from 21 to 33 kg.

Few days before intervention the animals were introduced in the kennel: upon arrival at the Veterinary Faculty animal facility all the lambs underwent a complete physical examination in order to assess their health status and prophylactic antiparasitic medication was administered orally: 7.5 mg/kg netobimin (Hapadex® 5% Schering-Plough, Milan, Italy). After 20 days a feces flotation was performed and in case of a positive response, a course of anthelminthic treatment was repeated.

Health status was assessed by normal clinical examination, complete blood count (CBC) and a biochemical panel (including total proteins, albumin, globulins, urea, glucose, total bilirubin, creatinine, AST, γ-GT, LDH, CK, ALT, ALP, calcium, phosphorus, magnesium, sodium, potassium, chloride).

Before beginning the study protocol, the animals were housed in two multiple pens for at least 2 weeks to acclimatize with the new housing environment. The experimental period lasted about 1 year and during this period animals were fed a commercial pellet (CP, NDF, NEg) (Compli Sheep, Tecnozoo, Piombino Dese, Italy) and ad libitum hay. Fresh water was always

available. Lamb health status was assessed daily by a technician and veterinary consultation was requested in case of suspect medical conditions.

The animals were fasted for 24 hours before any procedure in order to reduce the repletion status and the gas content of the rumen that could be a source of artifacts during echocardiographic examination.

5.3 Anesthesia protocols

Table 1 reports the drugs used to induce anesthesia and analgesia during all the procedures. During periodic sedation for echocardiograms and check-ups different protocols were chosen after verifying that they did not alter the hemodynamic and pressure values.

Table 1: different anesthetic protocol used for all procedures

	Catheterization	Periodic sedation	Sacrifice
premedication	midazolam 0,3 mg/kg methadone 0,2mg/kg	• midazolam 0,3mg/kg or • midazolam 0,3mg/kg + butorphanol or • midazolam 0,3mg/kg + pentazocine	midazolam 0,3 mg/kg methadone 0,2mg/kg
induction	propofol 4-6 mg/kg (dose effect)	propofol 4-6 mg/kg (dose effect)	propofol 4-6 mg/kg (dose effect)
mantainance	isoflurane (dose-effect)	isoflurane (dose-effect)	isoflurane (dose-effect)
other drugs	Hepaine (Clarisco) 150 IU/kg cefazoline 25 mg/kg Amoxicillin + clavulanic acid EOD 7 days after intervention meloxicam 5-7 days post surgery		Hepaine (Clarisco) 150 IU/ kg embutramide + mebrenzonio + iodure tetracaine (euthanasia)

5.4 Hemodynamic study and stenting

STENT animals were prepared for intervention, and venous and arterious accesses were obtained through auricular vascular catheterization. After premedication, the coat was clipped in the cervical region, and in the anterior and posterior limbs. Induction was then performed and after intubation the animal was brought to the surgical table and positioned in right lateral recumbency.

After surgical scrub of the neck, vascular access was obtained using the modified Seldinger method: the left common carotid artery was surgically isolated and punctured with a sharp hollow 12F needle (trocar). A round-tipped guidewire was then advanced through the lumen of the trocar, the trocar withdrawn and the hole in the skin around the wire enlarged with a scalpel. A “sheath” or a blunt cannula with the dilator could now be passed over the guide wire into the vessel. Dilator and guide wire were removed while the sheath remained in the vessel. Fig. 11 shows the different steps of the catheterization.

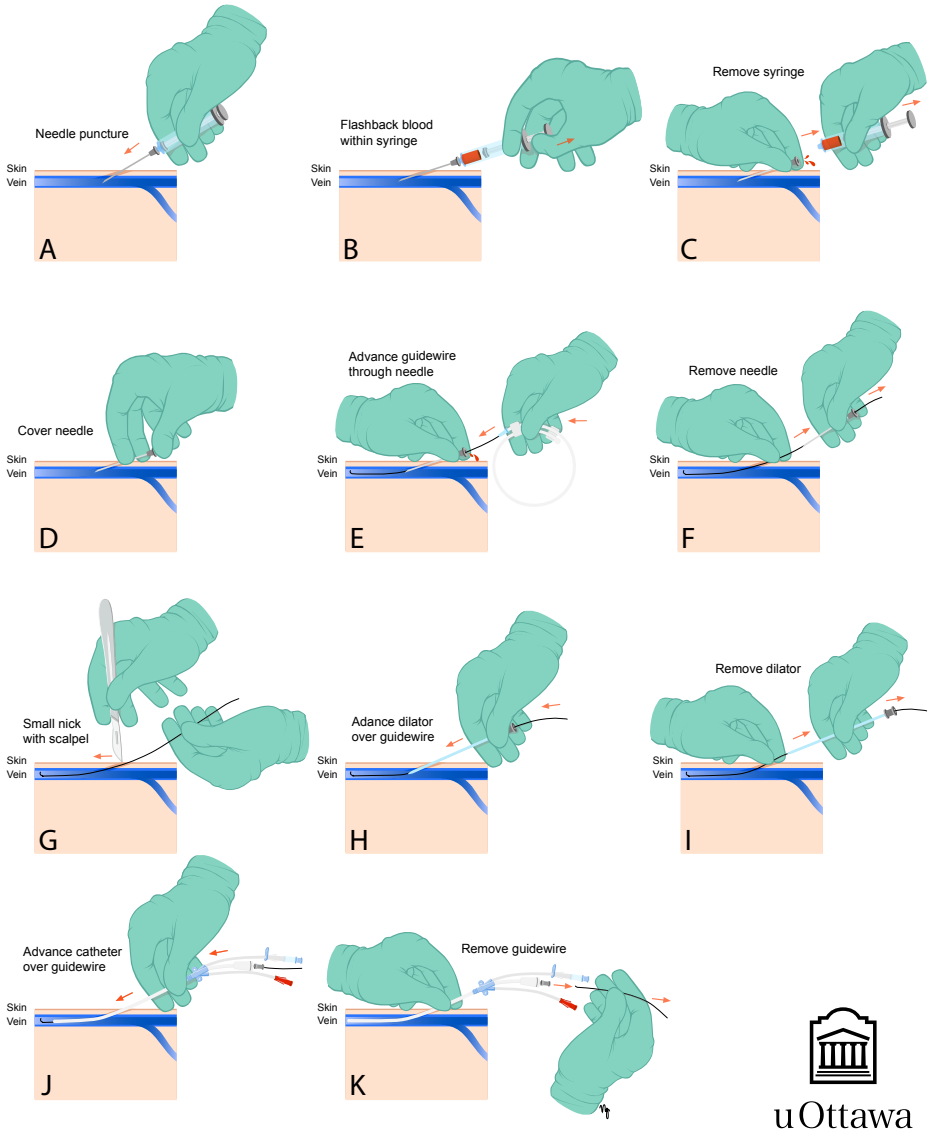

Fig. 11: schematic representation of vascular catheterization unsing the Seldinger technique.

The catheter was introduced using the sheath: two different catheters were used, a 5Fr "Gensini" (Balt Extrusion Montmorency, France) or a "right Judkins" angiographic catheter (Balt Extrusion Montmorency, France).

Under fluoroscopic guidance the tip of the catheter was placed in the left ventricle and contrast media was injected to perform morphologic evaluation. Contrast-enhanced fluoroscopy permits a correct evaluation of aortic diameter and optimal stent diameter choice. (fig. 12)

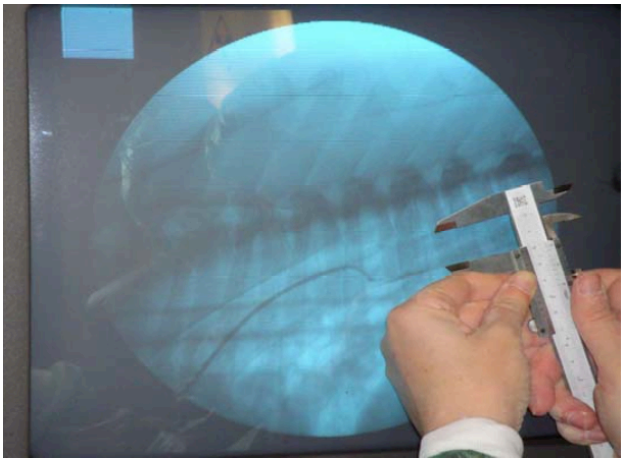

Fig. 12: fluoroscopic evaluation of aortic diameter after contrast injection.

For stent preparation, a 39 cm long CP stent™ (NuMED Cornwal, ON, Canada) was manually crimped on a 23x40 mm valvuloplastic balloon catheter (Balt Extrusion Montmorency, Francia) as shown in fig. 13. The CP Stent™ is composed of 0.013" platinum / iridium wire that is arranged in a “zig” pattern, laser welded at each joint and over brazed with 24K gold. It allows expansion from 12.0 mm to 24.0 mm.

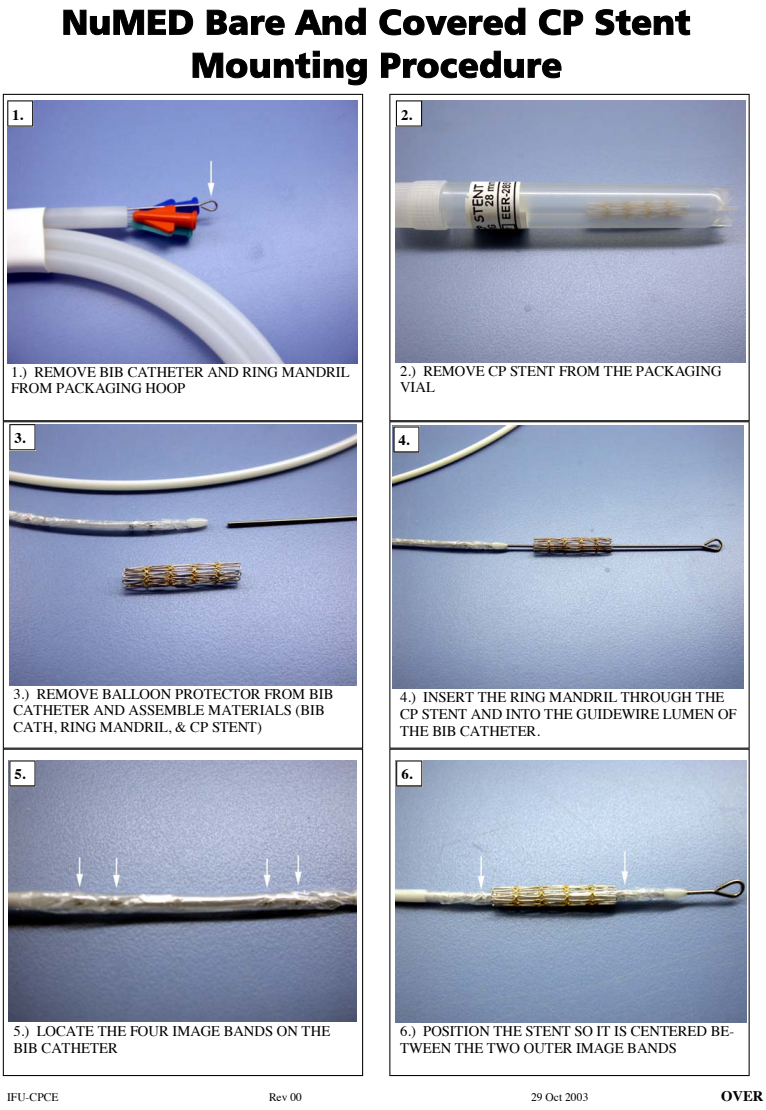

Fig. 13: manual CP stent mounting procedure.

After pressure measurements a 0,035 inch Cordis EMERALD Guidewire™ (Cordis Johnson & Johnson, Miami USA) was introduced and the previously mounted balloon expandable stent (fig. 13) was pulled forward to the isthmic region. Once correct positioning is reached, manual inflation of the balloon permits correct stent placement. After placement a contrast media was injected to evaluate correct stent positioning and absence of major vascular injuries.

Central pressure was measured again as described above. After this procedure the vascular access was sutured and the animal was awakened.

SHAM animals underwent the same procedure described above, including catheterization, ventricular and aortic contrast evaluation and direct central pressure measurements, but no stent placement is performed.

5.5 Direct and indirect blood pressure measurement.

Blood pressure was measured invasively and non invasively in both STENT and SHAM animals.

To obtain invasive blood pressure measurement, a catheter in the auricular artery on the abaural surface of the ear was connected to a monitor (Dathex-Ohmeda, GE Healthcare): catheterization of the auricular artery is easily achieved and is done in awake sheep, so it is possible to obtain direct measurement of peripheral arterial pressure in animals before any sedation. Care was taken in handling the animal to reduce stress conditions. All animals were allowed to remain quietly in the measurement room for 5-10 minutes before attempting BP measurement in order to minimize white coat effect that could alter pressure values. The same peripheral blood pressure was monitored during sedation and anesthesia. Values were registered every 5 minutes during the overall procedure and mean values were used for statistical analysis.

During the echocardiographic exam, once the sedated animal was placed in lateral recumbency, inflatable cuff was used to obtain non-invasive oscillometric limb pulse pressure. Cuffs (Critikon®, GE Healthcare) were placed alternatively to the anterior and posterior limbs, and care was taken to choose the cuff of the correct diameter in relation to the limb dimension and to read values while the inflated cuff was placed at the same height of the heart. Mean values of all measurements were used for statistical analysis. This indirect pressure measure is crucial in identifying pressure differentials between anterior and posterior limbs: anterior limb pressure reflects blood pressure of the circulatory bed upon the stent, so that a pressure differential can possibly relate to a “stenotic effect” of the device.

Direct central pressure was measured during catheterization by connecting the catheter to a Dathex-Ohmeda (GE Healthcare) pressure monitor. Before any measurement, care was taken to appropriately calibrate values by setting as 0 the transducer allowed to measure atmospheric pressure.

Initially, after connecting the transducer to the catheter, the tip of the catheter is positioned inside the ventricular chamber and correct positioning assessed under fluoroscopic guidance, to evaluate pressure values and wave morphology. Pulling back the catheter through the aortic valve, pressure was then measured in the ascending aorta before the isthmic region and in the descending aorta distal to isthmus: the ascending aortic pressure waveform was recorded at the level of one vertebral body thickness above the aortic valve. The descending aortic pressure waveform was recorded at the level of the diaphragm.

Pressure waves displayed in the monitor are recorded with simultaneous ECG tracing (fig. 14).

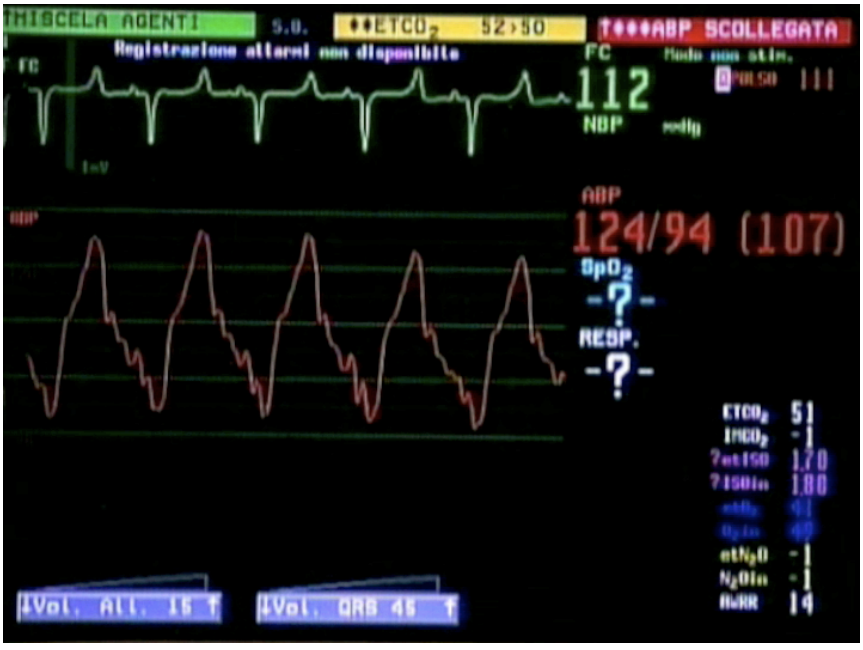

Fig. 14: simultaneous ECG and ascending aorta central pressure measurement.

In STENT animals, ventricular and aortic pressure measurement was repeated right after the device positioning.

The waveform displayed in the monitor was recorded on a digital tape using a digital camera. In order to calculate augmentation index (AIx), the inflection point was detected in every wave cycle. The inflection point is defined as the point on pressure wave at which the curvature changes (Fig. 15).

AIx is calculated using the formula:

$$AIx = \frac{\text{Augmentation}}{\text{Pulse Pressure}} \times 100$$

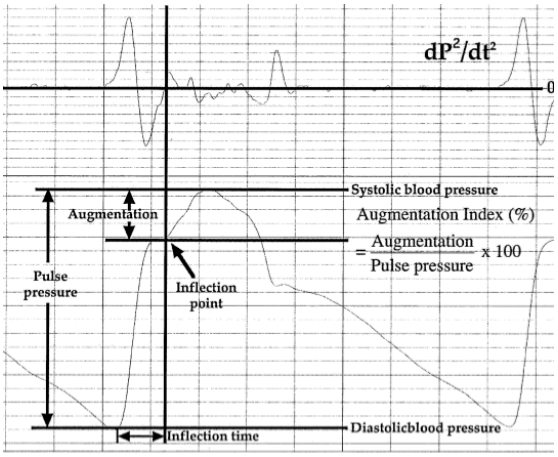

Fig. 15: schematization of a central pressure wave.

AIx was calculated from height in pixels (Fig. 16). AIx was the mean of at list 20 waveforms measures whenever inflection point was detectable.

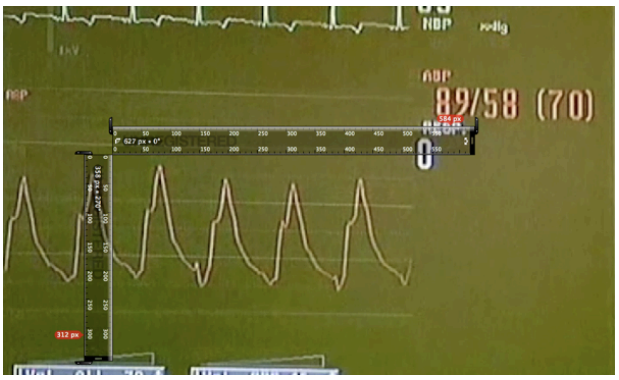

Fig. 16: measurement of the augmentation index.

5.6 Dobutamine stress test

After completion of the hemodynamic study, baseline invasive pressure was measured by positioning the catheter in the ventricular chamber, in the ascending aorta before the stent and in the descending aorta right after the stent.

Dobutamine (David Bull, Victoria, Australia), a synthetic sympathomimetic with potent alpha1-adrenoceptor-mediated inotropic actions as well as minor β2- and alpha1-adrenoceptor effects, was infused continuously intravenously in incremental steps of 2.5, 5 and 10 μg/kg/min using a roller pump (model MS 4-Reglo, Ismatec, Zurich, Switzerland). When steady-state conditions had been attained, i.e. 5–10 min after each dobutamine dose change, central pressure and heart rate measurements were repeated, pressure curves recorded and dobutamine infusion increased to the next dose.

After completion of the measurements at the highest dose, euthanasia was induced by administering intravenously a solution of embutramide, mebenzonium iodide and tetracaine hydrochloride (Tanax®, Intervet, Milano).

5.7 Echocardiography

Transthoracic parasternal echocardiography was performed in each animal before intervention. The examination was then repeated quarterly for 12 months until sacrifice by two independent different expert operators.

After sedation, thoracic left and right parasternal areas were clipped from 2nd to 7th intercostal space. The animal was positioned in right lateral recumbency on an echocardiographic table. Ultrasonographic gel was applied to the cutaneous surface. The echocardiographic exam was performed using a LOGIQ P5 echocardiograph (GE Healthcare) using a 6-10 MHz micro convex probe or 2-3 Mhz phased-array probe.

The heart was initially imaged through a 4 chambers right parasternal long axis view. The aortic annulus was then measured by rotating the probe counter clockwise until reaching a standard 5 chamber long axis parasternal view. The short axis parasternal view at the level of chordae tendinae was used to obtain the left ventricular M-mode images. M-mode images were used to obtain

measures of systolic and diastolic interventricular septum thickness (IVSd, IVSs), left ventricular inner diameters (LVIDd, LVIDs) and left ventricular free wall thickness (PWd, PWs). To perform M-mode measurements American Society of Echocardiography "leading edge to leading edge" method was followed (Sahn DJ et al., 1978). After optimizing the 2 dimension short axis view to visualize mitral leaflets, M-mode images were obtained to measure the minimum distance of the mitral anterior leaflet to the interventricular septum (E point septal separation, EPSS). Tilting the probe dorsally the bidimensional image of aortic root is achieved. From this image the aortic root / left atrium ratio is measured as described by Rishniw and Erb (2000). From this view, pulmonary blood flow is measured by PW doppler.

Through the left parasternal views bidimensional images were optimized to obtain transmitral flow spettral doppler study (E wave and A wave velocities, E/A ratio, E wave deceleration time), and aortic flow study (aortic wave integral, VTI). Ejection time (ET), pre-ejection period (PEP) and isovolumic relaxation times (IVRT) were measured. Color Doppler was used to exclude turbulent flow or valvular defects through cardiac valves.

The following indices were also calculated:

FS =fractional shortening

$$FS\ (\%) = [(LVIDD + LVIDS)/LVIDD]/100$$

h/r = sphericity index

$$h/r = [(IVSd + PWd)/2]/(LVIDd/2)$$

Each parameter was measured as an average of at least 3 measures. Every parameter was normalized to weight using the formulas already reported in the literature (Berman A, 2003):

• $0{,}09 \times W^{0,67}$ for animals weighing from 24 to 38kg

• 0,14 x $W^{0,57}$ for animals weighing more than 41 kg

The reliability of the measures was assessed by intra- and interoperator variability tests. Fig. 17 shows examples of the obtained echocardiographic images.

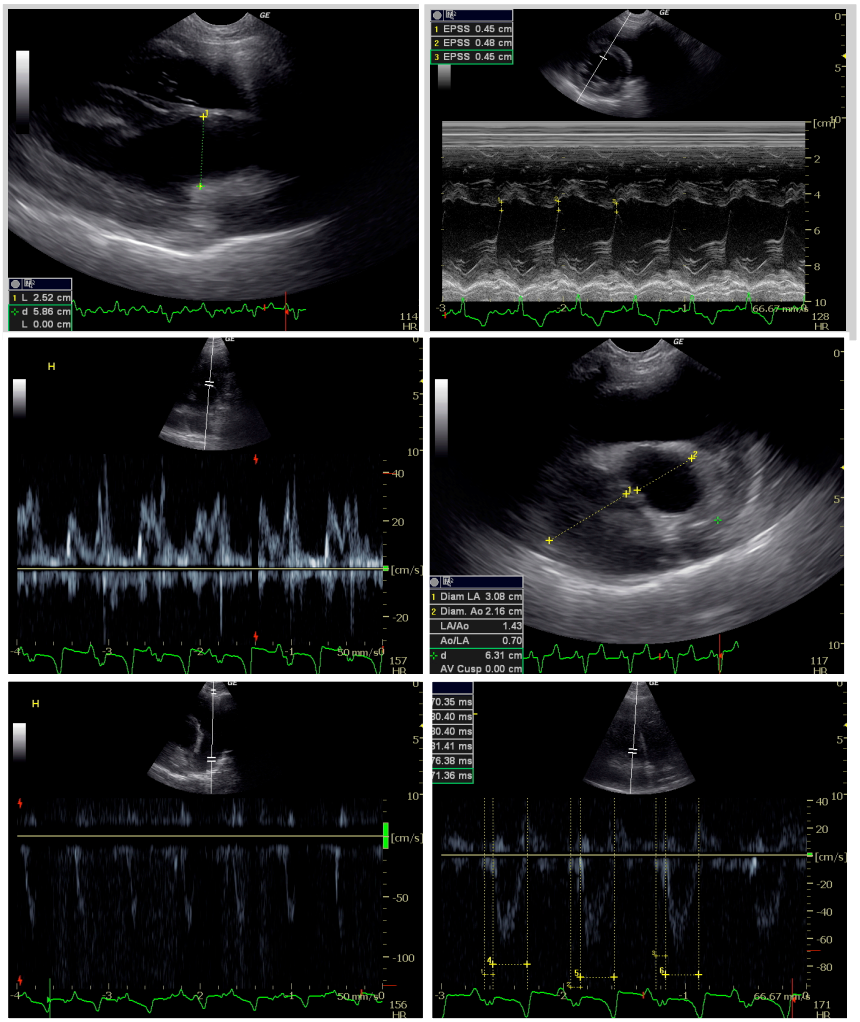

Fig. 17: images obtained from the echocardiographic exhamination: (A) Right parasternal long axis view at the level of the aortic root, (B) EPSS M-mode measurement, (C) Transmitral pulsed doppler flow, (D) Left atrium to Aortic diameter ratio measurement, (E) pulmonar and (F) aortic flow.

5.8 Bood tests

Periodic blood samples were obtained and stored for determination according to standard biochemical procedures of:

• complete blood count with differentials

• total proteins, albumin, globulin

• BUN, creatinine

• Total bilirubin, AST, ALT, GGT, LDH, CK, ALP

• Ca, P, Mg, Na, K, Cl

• glucose

5.9 Pathological examination

After sacrifice, necropsy was performed by a trained veterinary pathologist to exclude concomitant pathologic conditions that could alter results.

After a complete gross inspection, the heart is isolated from the bronchial tree and pericardium and is weighed. Myocardial, aortic and left kidney fragments are isolated. From isolated organs, specimens are obtained and rapidly collected in an mRNA later solution and stored in liquid nitrogen at -80°C to preserve genetic material. Similar fragments are collected in buffered formalin for histological preparations.

The following specimens were obtained:

- 3 samples of ventricular septal myocardium
- 3 samples of ventricular free wall myocardium
- 3 samples of left ventricle outflow tract myocardium
- 3 samples of renal tissue
- ascending aortic wall
- descending aortic wall

Aortic segment with intraluminar stent is recovered and stored in buffered formalin for histologic study to investigate local intimal reactions to the device. The stented vessels were dissected and cut lengthwise, photographed, and tissue submitted for microscopic examination.

5.10 Gene expression

Specimens from the descending and ascending aorta were isolated and

preserved in RNAlater® solution (Qiagen, Hilden, Germany), which is an aqueous, nontoxic tissue storage reagent that rapidly permeates tissues to stabilize and protect cellular RNA. After removal of RNAlater, the tissue sample was immersed in liquid nitrogen and conserved at -80 °C until use.

To perform RNA extraction, pieces of aortic tissue from sheep were minced using a mortar in order to obtain fractions smaller than 30 mg, which were then homogenized using a MagNA Lyser Instrument (Roche) which allows the complete tissue destruction. Then, total RNA extraction was performed using the RNeasy Fibrous Tissue kit (Qiagen). RNA quantification and purity were determined by spectrophotometric measurement (260/280 nm). RNA integrity was checked with a 2100 BioAnalyzer Agilent using RNA Nano LabChips.

Quantitative Real Time RT-PCR (Q-PCR): first-strand cDNA was synthesized with 1 µg of RNA extracted using iScript cDNA synthesis kit (Bio-Rad, Hercules, CA) according to the manufacturer's instructions. Quantitative real-time polymerase chain reaction assay was performed in a Thermal Cycler (CFX96, Bio-Rad, Hercules, CA). In brief, cDNA was amplified in a real-time PCR reaction containing 400 nmol of each primer and 5X SYBR Green SuperMix (Bio-Rad, Hercules, CA). All the reactions were performed in 96-well plates, in triplicate. A negative control containing all reagents but no cDNA template was included in all runs. Real-time PCR was performed following the thermal protocol: 95°C for 3 min to denature, 45 cycles of 95°C for 30s for denaturing and 60°C for 1 min for annealing and extension. Primers were designed from sequences derived from the GenBank database using Primer 3 (provided by the Whitehead Institute, Cambridge, Massachusetts, USA) and Operon's Oligo software (Operon Technologies Inc., Alameda, California, USA) and were purchased from Eurofins MWG (Ebersberg, Germany). Primer sequences were the following:

Caspase-3 (Ref Seq. AF068837):
AATGTTGGAATTAATGAGCGAC (forward)

AGGTGCTGTAGAATATGCATA (reverse)

MMP-9 (Ref Seq. FJ185130):
CCGACGACATGCTCTGGTGC (forward)
TCGTAGTTAGCAGTGGTGGC (reverse)

β-actin (Ref. Seq.NM_001009784):
CTGGCACCCAGCACGATGAA (forward)
CCTGCGCTCCAACAAGTCTT (reverse)

β-actin was used as housekeeping gene. Data analyses were performed with the iQTM Optical System Software (Bio-Rad, Hercules, CA). The comparative cycle threshold method (ΔΔCt), which compares the difference in cycle threshold values between groups, was used to obtain the relative fold change in gene expression.

5.11 Statistical analysis

The statistical analysis was carried out using the SPSS software package (version 15.1; SPSS Inc. Chicago, Illinois, USA) and MedCalc (MedCalc version 9.3.7.0; MedCalc Software, Mariakerke, Belgium). Parametric and non parametric tests were used to compare means. Relations between variables were assessed using Pearson correlation coefficient and Spearman's ρ correlation for continuous variables and $\chi 2$ or Fisher's exact test for categorical variables. The significance level was set to alpha=0.05. The data are expressed as mean ± SD.

6. Results

6.1 Survival and health status

The study started in July 2008 with the first stenting and ended in March 2011 after the sacrifice of the last two sheep.

Fig. 18 shows the timeline of all the animals progressively introduced in the study.

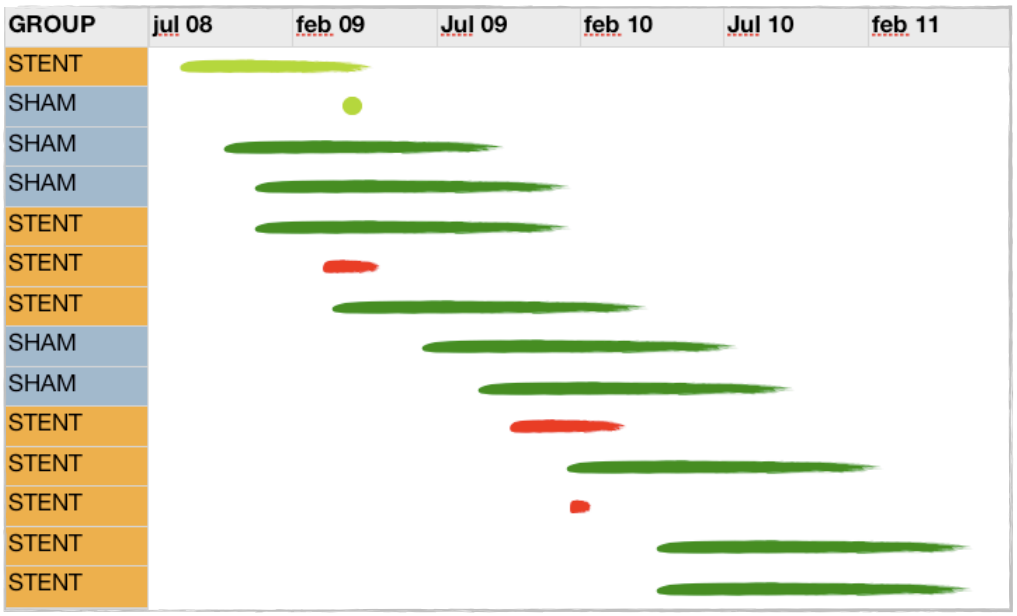

Fig. 18: time planning of the study with all animals introduced. Green lines represent animals that completed the study. Red lines represent animals accidentally died before the end of the study. Light green lines: *Alpagota sheep,* dark green lines: *Bergamasca sheep.*

All lambs resulted in good health at initial physical examination and CBC and biochemistry parameters resulted within the reference ranges.

The first two animals introduced were *alpagota sheep,* one male and one female. The male sheep died for acute hemorrhage at vascular access after catheterization. These two animals were not included in the statistical analysis because of different race. The other animals were female *bergamasca.* One animal was excluded after initial echocardiography because of excessive weight and catheterization was not performed.

One subject died during catheterization for iatrogenic aortic dissection secondary to excessive balloon diameter.

Another one died a few days after intervention for heart failure of unknown origin. One animal was found dead 4 months after intervention. Necropsy revealed severe gastroenteritis as cause of death. One subject developed aortic insufficiency after catheterization. Nine animals reached the end of the study.

6.2 Body weight and growth rate

Body weight (BW) increased from 29 Kg (27–36 Kg) at the beginning of the study to 60 kg (range 53–65 Kg) at the end. Average daily gain (ADG) progressively increased until the age class of 301-400 days and then decreased indicating that the growing period was complete by the end of the experiment (Fig. 19). Mean growth in both STENT and SHAM groups is depicted in fig. 20 which indicates that stent positioning does not affect growth.

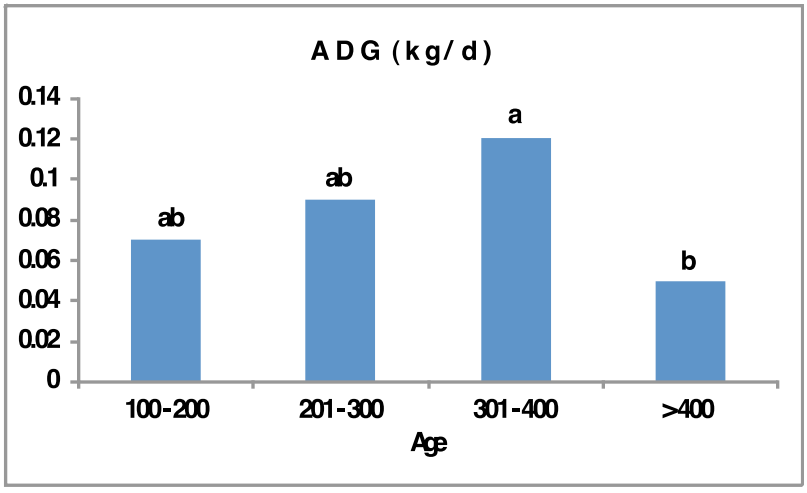

Fig.19: average daily gain of all animals (ADG) by class of age (days).

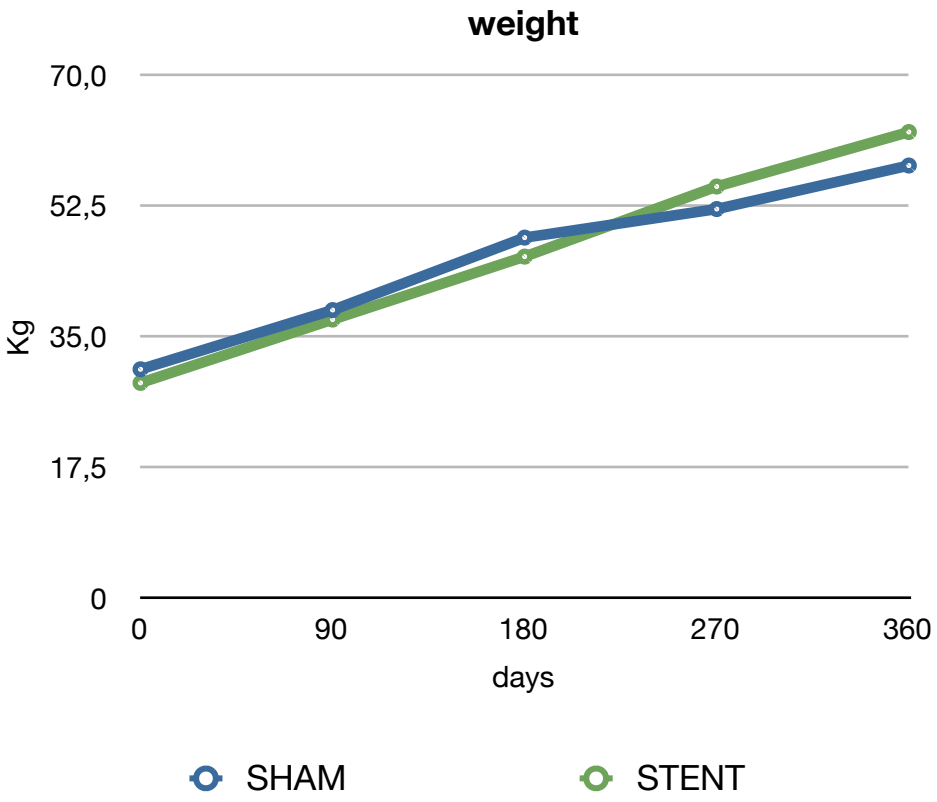

Fig. 20: mean weight of STENT and SHAM animals during periodic checks

6.3 Stenting and follow-up

The initial approach with femoral artery catheterization was abandoned because percutaneous puncture of the femoral artery was possible, but postinterventional vascular closure was too difficult. A safer carotid artery approach was then chosen and it did not determine any post excision hemorrhagic complication.

Ventricular catheterization was safe for all animals. The only major complication was a hemodynamically significant aortic insufficiency in a sheep most likely due to the passage of the catheter through the valve. The animal survived, was followed up for 12 months and remained clinically stable. Echocardiographic exams revealed a progressive enlargement of sisto-diastolic left ventricular diameters consistent with volume overload.

Stented balloon dilation determined aortic dissection in one case. Incorrect

choice of balloon cross diameter was the cause of an over stretch of the local aortic wall leading to dissection. Euthanasia was then induced. This traumatic event was documented by contrast enhanced fluoroscopy (Fig. 21).

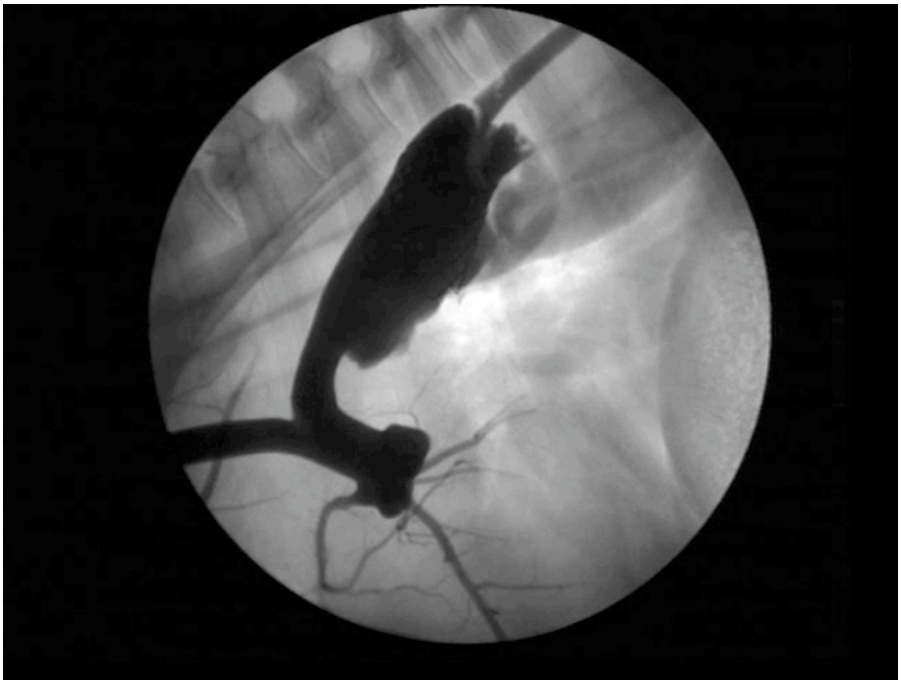

Fig. 21: contrast-enhanced aortography revealing descending aortic wall damage secondary to eccessive balloon stretching.

Stenting was successfully performed without complications in 8 subjects. None in SHAM group encountered complications during the procedure.

Stenting did not change ventricular and aortic pressure (Fig. 22 and 23).

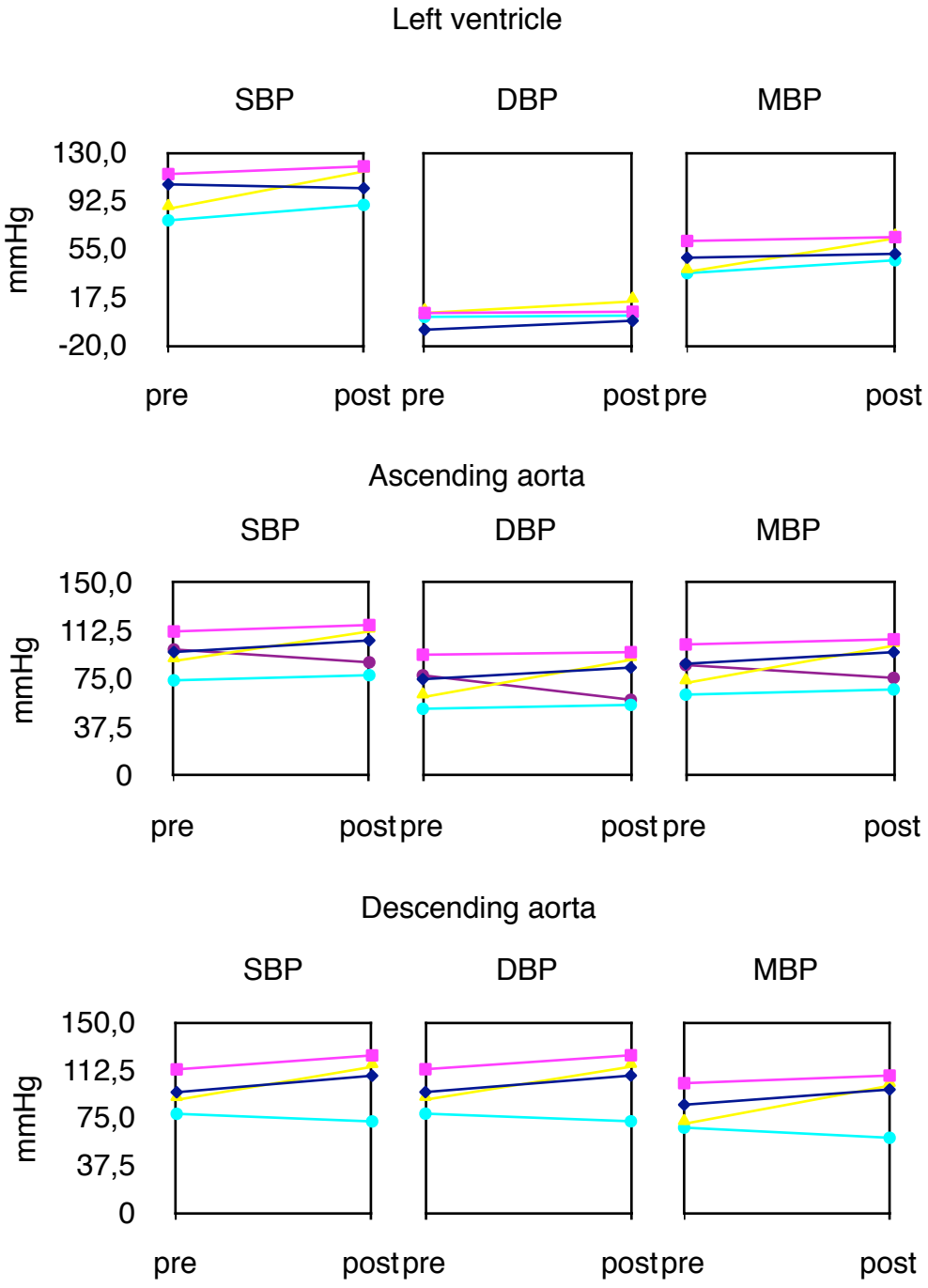

Fig. 22: central systolic (SBP), diastolic (DBP) and mean (MBP) pressure of all STENT animals measured during cahteterization before (pre) and after (post) stenting.

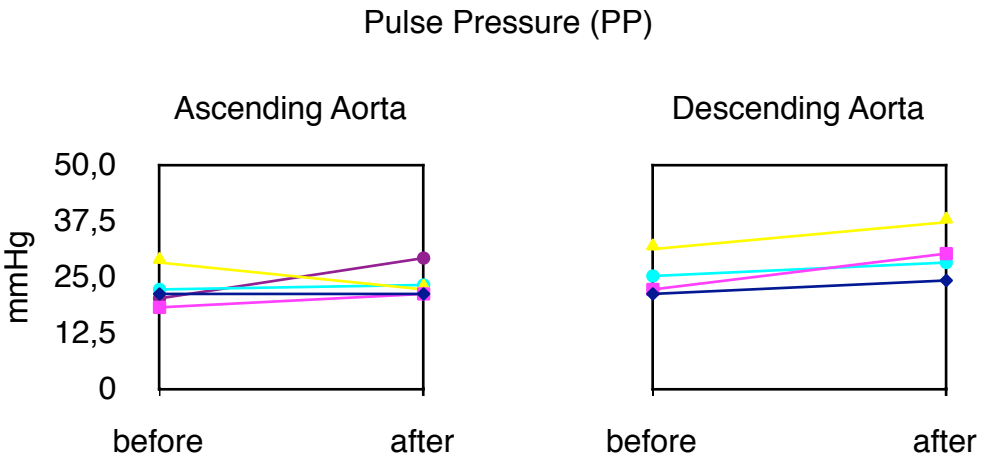

Fig. 23: Pulse pressure measured in all animals before and after stenting in ascending and descending aorta.

However in a few experiments the contour of aortic pressure wave showed modification of the inflection point (Fig. 24).

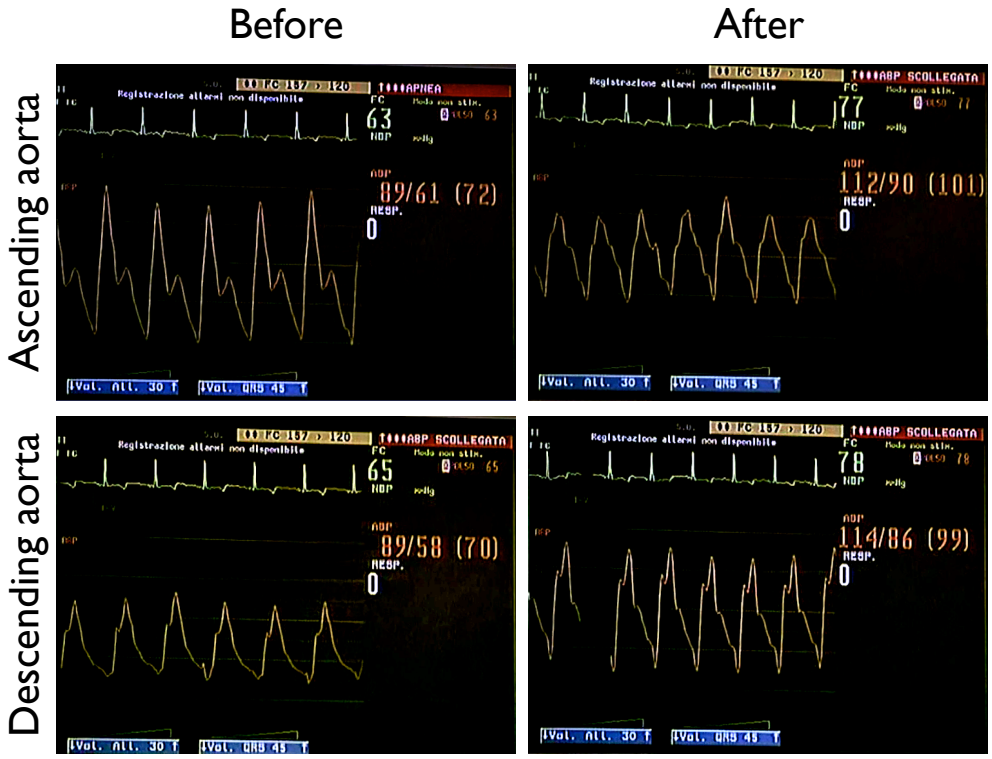

Fig. 24: Central pressure waveforms before (left column) and after (right column) stenting in ascending (first row) and descending (second row) aorta in an animal.

6.4 Blood pressure at baseline and during follow-up

The sedation protocol did not affect blood pressure: mean auricular blood

pressure was 112 + 12/78 + 9 mmHg in non sedated animals and 111 + 13/82 + 14 mmHg during sedation (NS/NS, n= 34).

Awake auricular systolic and diastolic blood pressure by time and treatment are reported in table 2. Blood pressure did not change during follow-up and it was not significantly different in sham and stent animals.

Table 2: direct auricular blood pressure (mmHg ± SD) measured before sedation in SHAM and STENT groups . SBP systolic blood pressure; DBP diastolic blood pressure; PP pulse pressure. T time in days after first catheterization

GROUP		T0	T90	T180	T270	T360
SHAM	SBP	107	112±10	119±8	111±12	108±14
	DBP	74	76±4	85±7	80±7	75±9
	PP	33	35±8	34±10	31±8	34±6
STENT	SBP	121±8	102±11	119±8	115±15	112±8
	DBP	96	73±14	76±10	82±9	82±6
	PP	31	29±11	43±6	33±11	30±5

The corresponding values after sedation are reported in table 3, confirming no difference by time and treatment.

Table 3: direct auricular blood pressure (mmHg ± SD) measured during sedation in SHAM and STENT groups . SBP systolic blood pressure; DBP diastolic blood pressure; PP pulse pressure

GROUP		T0	T90	T180	T270	T360
SHAM	SBP	103±11	102±4	107±9	107±12	108±7
	DBP	80±11	77±7	80±11	82±14	65±12
	PP	23±0,7	24±5	24±4	24±3	42±18
STENT	SBP	115±9	112±18	116±9	121±25	111±8
	DBP	85±7	86±13	91±17	89±12	76±12
	PP	30±2	26±8	25±12	33±13	35±16

Diastolic blood pressure was significantly lower in the posterior limbs

than in the anterior both in the SHAM and the STENT animals with no difference between groups (Table 4).

Table 4: mean systolic (SBP) diastolic (DBP) and pulse pressure (PP) values obtained during periodic check ups using indirect oscillometric method at anterior and posterior limbs (mmHg ± SD).

		Anterior	Posterior	p
SHAM	SBP	128±14 (14)	123±12 (14)	n.s.
	DBP	81±15 (14)	72±12 (14)	0,0028
	PP	48±7 (14)	51±4 (14)	n.s.
STENT	SBP	125±19 (19)	121±13 (19)	n.s.
	DBP	84±15 (19)	74±13 (19)	0,020
	PP	40 ±13 (19)	47±11 (19)	0,051

Twelve months after stent implantation, pulse pressure in the ascending aorta decreased from the values immediately after stent implantation while blood pressure in the descending aorta was unchanged (table 5).

Table 5: Mean systolic (SBP), diastolic (DBP) and pulse pressure (PP) in STENT animals measured immediately before (Acute) and 12 month after (Chronic) stenting in ascending (Asc Ao) and descending (Desc Ao) aorta (mmHg ± SD). *p=0.05.

		Acute	Chronic
Asc Ao	SBP	97±17	107±12
	DBP	73±20	90±7
	PP	24±4*	17±5*
Desc Ao	SBP	96±24	108±15
	DBP	68±25	88±7
	PP	28±3	20±9

6.5 Effects of stenting and growth on echocardiographic parameters.

Good quality echocardiographic right parasternal views were obtained.

In left cranial and caudal projections difficulties were encountered in obtaining a good alignment with mitral valve inflow to measure doppler transmitral flow.

Stent Effect

The M-mode echocardiographic parameters (IVSd, LVIDd, LVIDs, LVPWd) and M-mode derived ventricular volumetric measurements (EDV and ESV) are reported in fig. 25.

STENT group at any time did not differ form SHAM. Indexing data to the body surface area did not reveal any difference as well.

Doppler derived measurements, such as E-vave, A-wave IVRT PEP/ET ratio were not statistically different.

All echocardiographic parameters did not differ, indicating that stent positioning does not change echocardiographic parameters (Fig. 26 and 27).

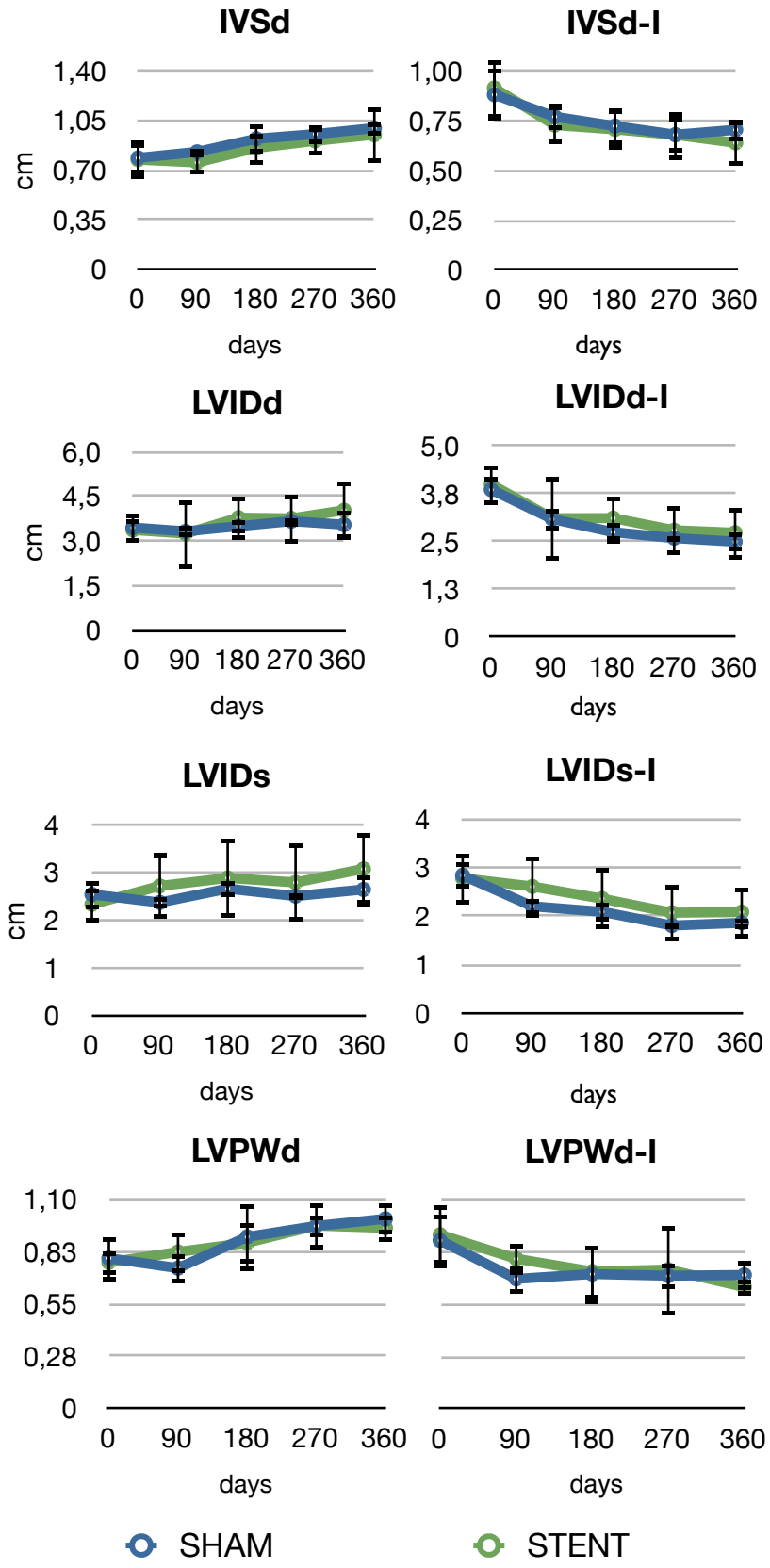

Fig. 25: mean M-mode parameters in SHAM and STENT groups during periodic echocardiographic examinations. In the right column data are displayed indexed to the body surface area.

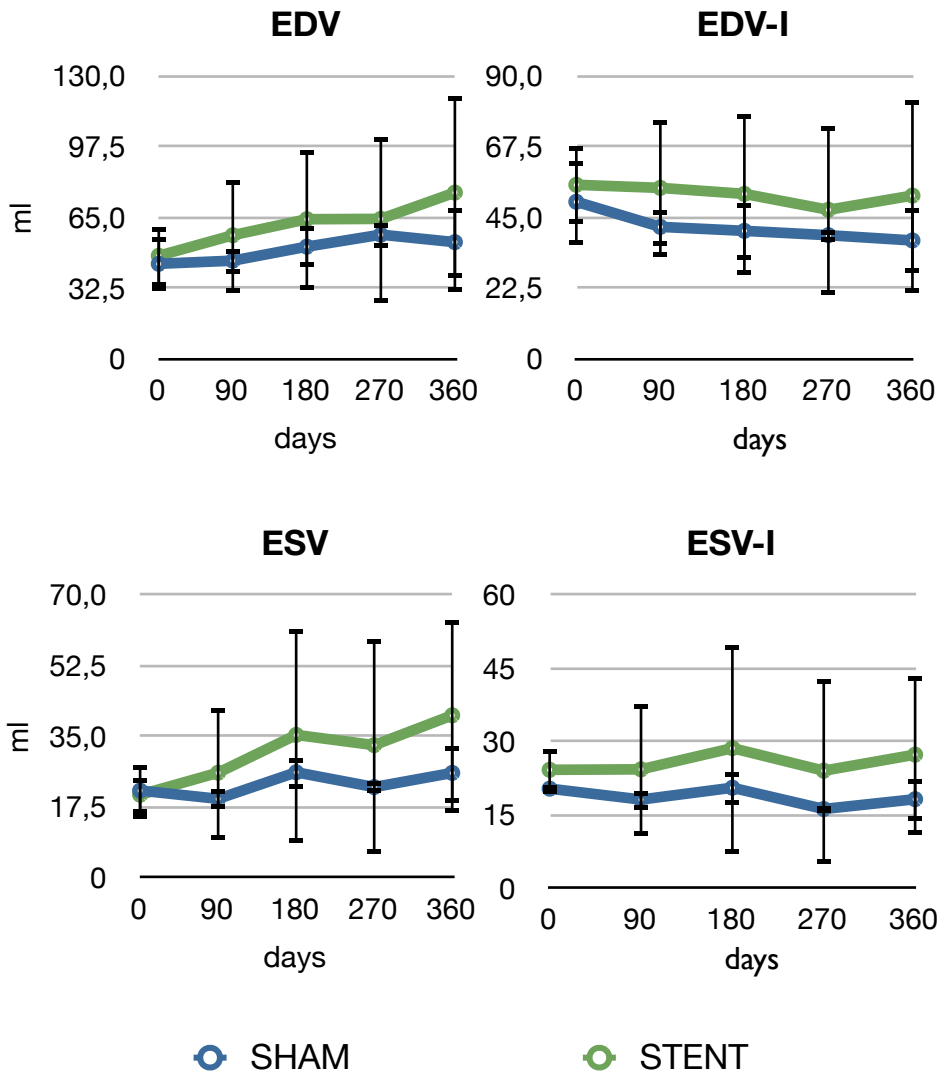

Fig. 26: mean M-mode derived end-diastolic volume (EDV) and end-systolic volume in SHAM and STENT groups during periodic echocardiographic examinations. Right colums shows values indexed on the body surface area (EDV-I and ESV-I).

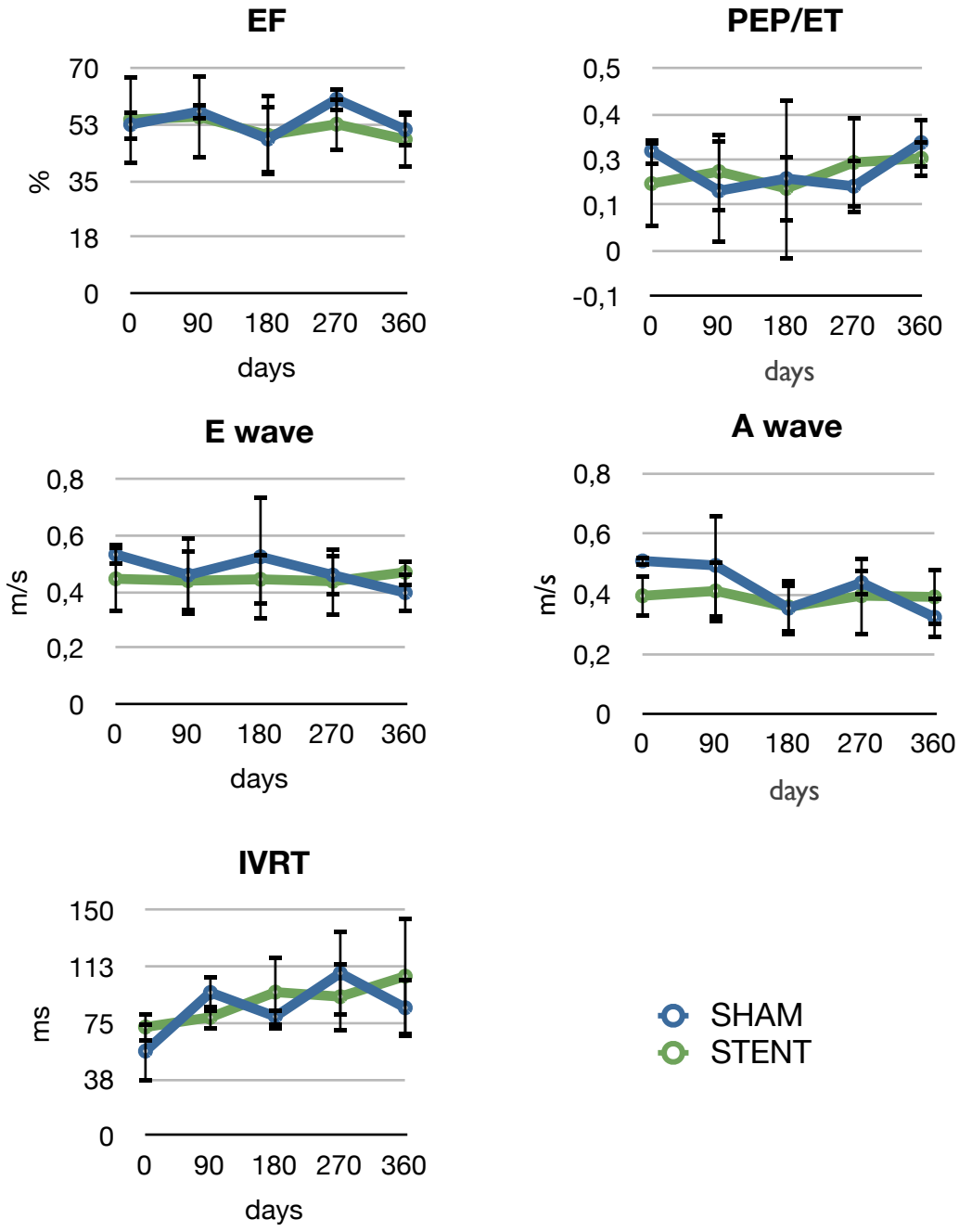

Fig. 27: M-mode derived ejection fraction (EF), pre-ejection period to ejection time ratio (PEP/ET), transmitral E and A wave doppler velocities (E-wave, A-wave) and isovolumic relaxation time (IVRT) in SHAM and STENT groups. Mean and SD.

Growth effect

To study the effect of growth on echocardiogrphic parameters the animal were pooled and divided in quintiles of age.

Table 6: Least squares means and S.E. (pooled standard error) of age, body weight (BW), body surface area (BSA), average daily gain (ADG), hearth rate (HR) and echocardiographic parameters according to class of age.

Age class(d)	<=100	101 - 200	201 - 300	301 - 400	> 400	S.E.	P
n. records	5	6	10	7	12		
Age (d)	88^{e}	162^{d}	257^{c}	362^{b}	462^{a}	12	<0.001
BW (kg)	29^{c}	35^{c}	42^{b}	55^{a}	60^{a}	2	<0.001
BSA (m2)	0.86^{c}	0.96^{c}	1.11^{b}	1.32^{a}	1.39^{a}	0.03	<0.001
HR (bpm)	121	88	94	89	89	6	<0.01.
LVIDs (cm)	2.46	2.47	2.56	2.57	2.66	0.10	n.s.
LVIDd (cm)	3.30	3.37	3.54	3.46	3.59	0.08	n.s
IVSs (cm)	1.05^{b}	1.03^{b}	1.06^{b}	1.19^{ab}	1.24^{a}	0.06	<0.001
IVSd (cm)	0.81^{b}	0.81^{b}	0.81^{b}	0.93^{ab}	0.97^{a}	0.04	<0.01
LVPWs (cm)	1.16^{c}	1.25^{bc}	1.28^{bc}	1.43^{ab}	1.47^{a}	0.06	<0.001
LVPWd (cm)	0.71^{b}	0.79^{b}	0.82^{b}	0.96^{a}	0.95^{a}	0.04	<0.001
FS (%)	24.75	26.84	26.93	25.76	25.93	2.08	n.s.
EPSS (cm)	0.25^{b}	0.27^{b}	0.34^{ab}	0.40^{a}	0.40^{a}	0.04	<0.01
AR (cm)	2.04^{c}	2.16^{bc}	2.34^{ab}	2.30^{ab}	2.45^{a}	0.07	<0.01
LA (cm)	2.39^{b}	2.36^{b}	2.79^{a}	2.78^{a}	2.87^{a}	0.08	<0.001
PEP (ms)	55.10	60.91	47.93	70.62	73.98	6.66	n.s.
ET (ms)	258	331	319	297	337	22	n.s.
PEP/ET	0.19	0.20	0.16	0.23	0.23	0.02	n.s.
MV E (m/s)	0.48	0.45	0.44	0.38	0.41	0.03	n.s.
MV A (m/s)	0.44	0.43	0.41	0.35	0.35	0.03	n.s.
MV E/A	1.16	1.02	1.11	1.21	1.15	0.09	n.s.
AO Vmax (m/s)	0.81	0.96	0.83	0.69	0.70	0.05	n.s.
PA Vmax (m/s)	0.67	0.75	0.80	0.76	0.79	0.04	n.s.

Different letters along row mean different values for P as reported
Acronyms: LVIDs: left ventricular inner dimension at end systole; LVIDd: left ventricular inner dimension at end diastole; IVSs: thickness of interventricular septum at end systole ;IVSd: thickness of interventricular septum at end diastole; LVPWs: left ventricular posterior wall at end systole; LVPWd: left ventricular posterior wall at end diastole; AR: aortic roor diameter; LA: left atrium diameter
* HR was included in models as covariate

The normality test revealed a normal distribution for all the variables.

Least squares means and pooled standard errors (S.E.) of age, BW, BSA, ADG, HR, 2-D, M-mode and Doppler parameters in different age classes are reported in Table 6. Statistical analysis revealed that age class was a significant factor for almost all the 2-D and M-mode echocardiographic parameters except for LVIDd, LVIDs and FS. A significant increment in M-mode parameters according to age was generally found until 4th age class (mean age 360 days) when the definitive dimensions are reached. At variance, EPSS and 2-D measurements (LA and AR) increase their dimension until the third age class (mean age 256 d).

No significant age effect was revealed for Doppler derived parameters. Correlation coefficients show strong relation among either Age, BW and BSA ($r>0.94$, $P<0.001$) (Table 7).

Table 7: Correlation coefficients (r) for Age, BW, BSA and HR.

Variables	Age	BW	BSA	HR
Age	-	0.94***	0.94***	-0.36*
BW		-	0.99***	-0.42**
BSA			-	-0.43**

Only significant r values are reported.*** P<0.001; **0.001<P<0.01; * 0.01<P<0.05

For this reason these variables can be chosen interchangeably to study the correlation with the echocardiographic parameters. As BW is the simplest parameter to obtain, it was chosen for regression analysis instead of BSA. Table 8 evidences a significant correlation between BW and all the variables except for LVIDd and LVIDs, which are weakly and negatively correlated with HR.

Regression equations calculated for the echocardiographic parameters significantly correlated to BW are shown in table 9 and illustrated in fig. 28 which show the individual values, the estimated line equation and the 95% predicted and 95% ellipsis confidence.

Table 8: correlation coefficients (r) for the echographic parameters versus BW and HR.

Variables	BW	HR
LVIDs	n.s.	-0.44**
LVIDd	n.s.	-0.51***
IVSs	0.48**	n.s.
IVSd	0.46**	n.s
LVPWs	0.55***	n.s
LVPWd	0.61***	n.s
FS	-0.06	n.s
EPSS	0.40*	-0,42**
LA	0.54***	n.s.
AR	0.56***	n.s.
PEP	0.36*	0.27
ET	0.18	0,55***
PEP/ET	0.20	n.s
MV E	-0.31	n.s
MV A	-0.36*	0.39*
MV E/A	0.14	n.s
AO Vmax	-0.33*	n.s
PA Vmax	0.21	n.s

Table 9: regression model for echocardiographic parameters and BW.

	Intercept	Slope	P	S.E.	R^2
IVSs	0.83	0.0065	0.0019	0.15	0.23
IVSd	0.65	0.0050	0.0030	0.12	0.21
LVPWs	2.14	0.0456	0.0002	0.16	0.31
LVPWd	0.56	0.0065	<0.0001	0.10	0.38
EPSS	0.15	0.0042	0.0092	0.10	0.18
PEP	30.76	0.6719	0.0209	19.00	0.15
LA	2.09	0.0130	0.0004	0.25	0.29
AR	1.80	0.0106	0.0002	0.19	0.32
MV A	0.52	-0.0029	0.0439	0.09	0.11
AO Vmax	1.03	-0.0055	0.0030	0.12	0.23

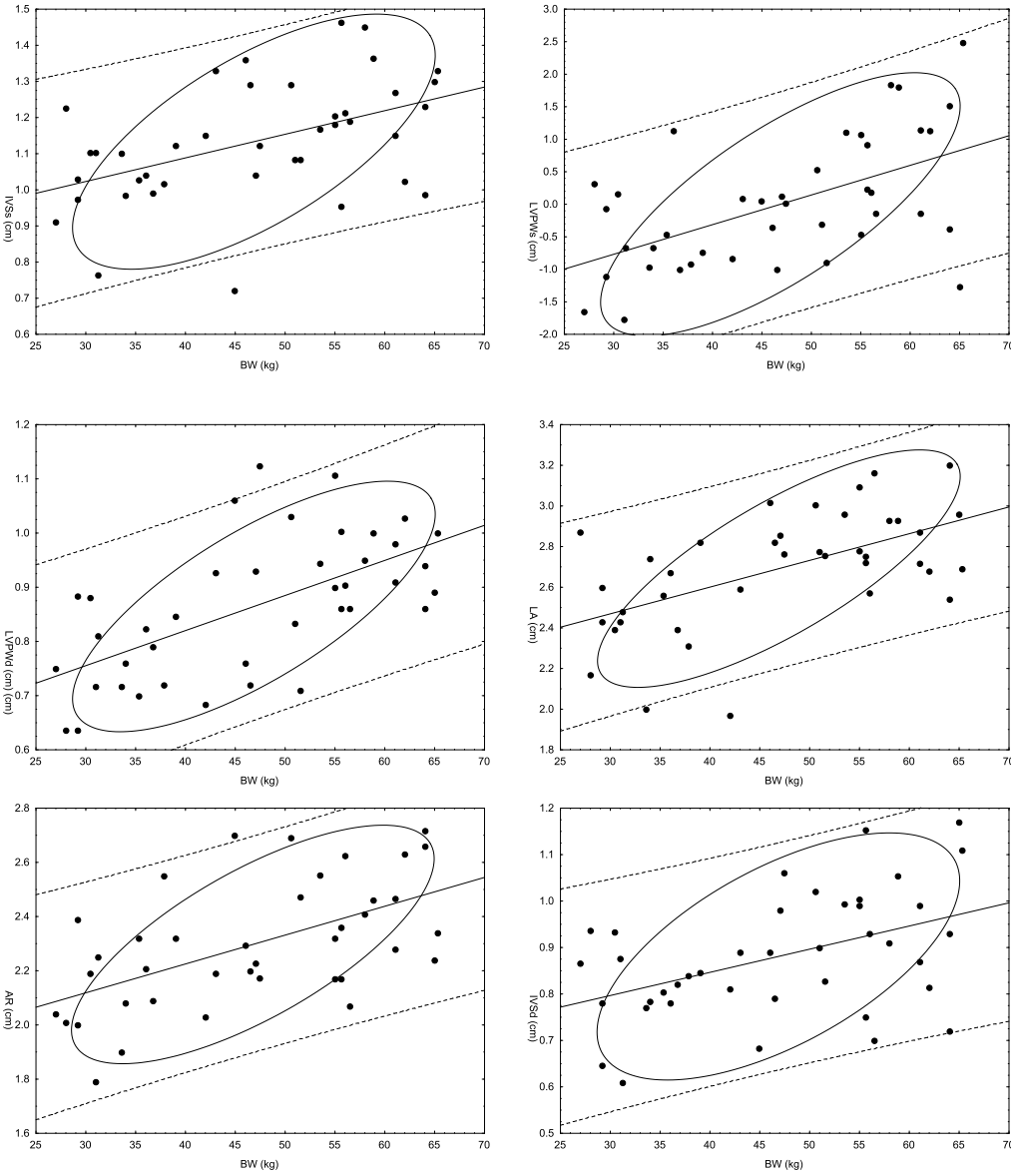

Fig. 28: individual values, the estimated line equation and the 95% predicted and 95% ellipsis confidence.

6.6 Dobutamine Test

Dobutamine dose dependently increased heart rate and systolic blood pressure without any difference between SHAM and STENT (Fig. 29 and 30).

Fig. 29: mean heart rate values in STENT and SHAM groups at different dobutamine doses.

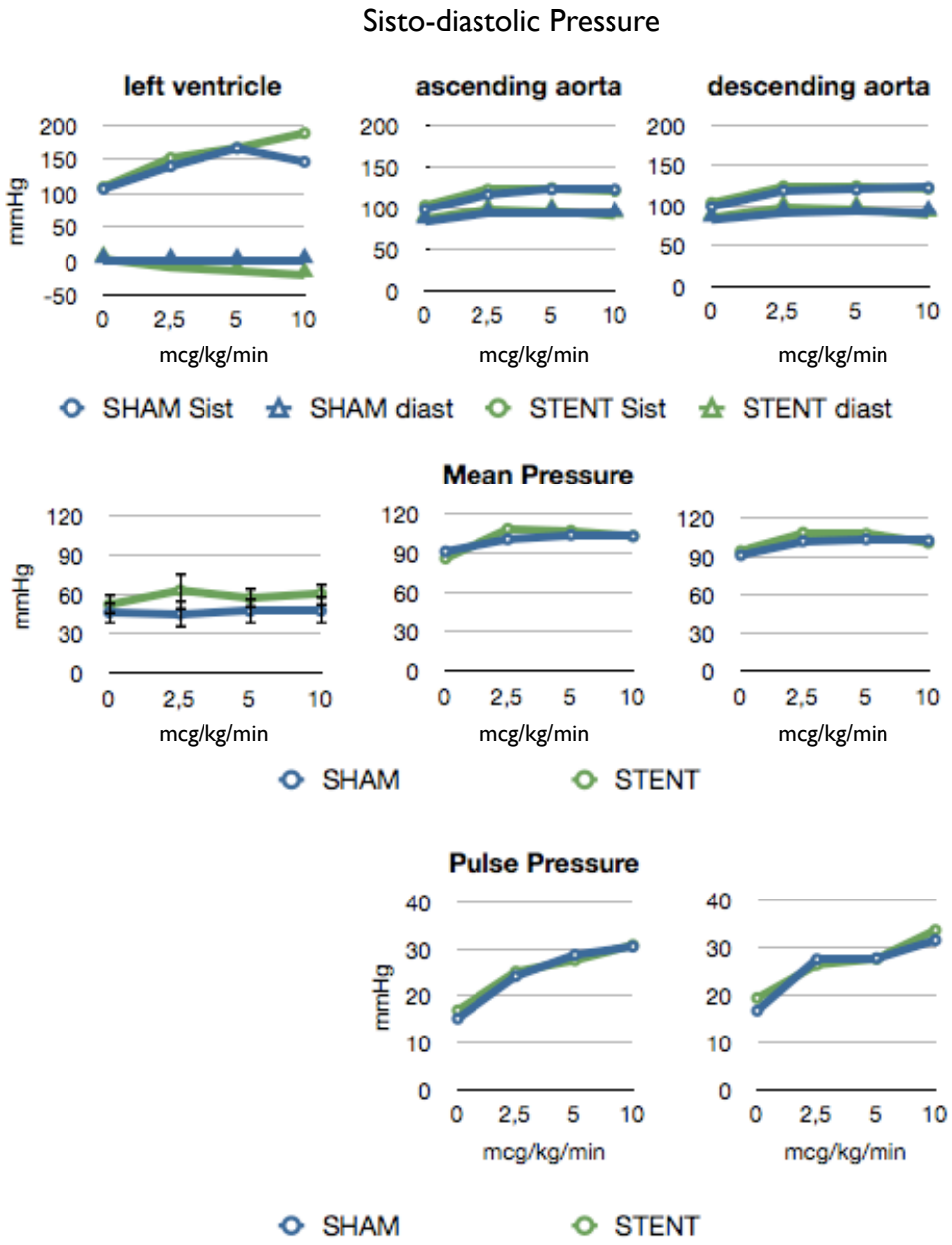

Fig. 30: mean pressure values in left ventricle (first column), ascending (second column) and descending aorta (third column) in STENT and SHAM groups at different dobutamine doses.

6.7 Blood tests

Fig. 31 shows CBC in STENT and SHAM. Fig. 32 shows biochemical parameters investigated. None of parameters was consistently different among groups during time.

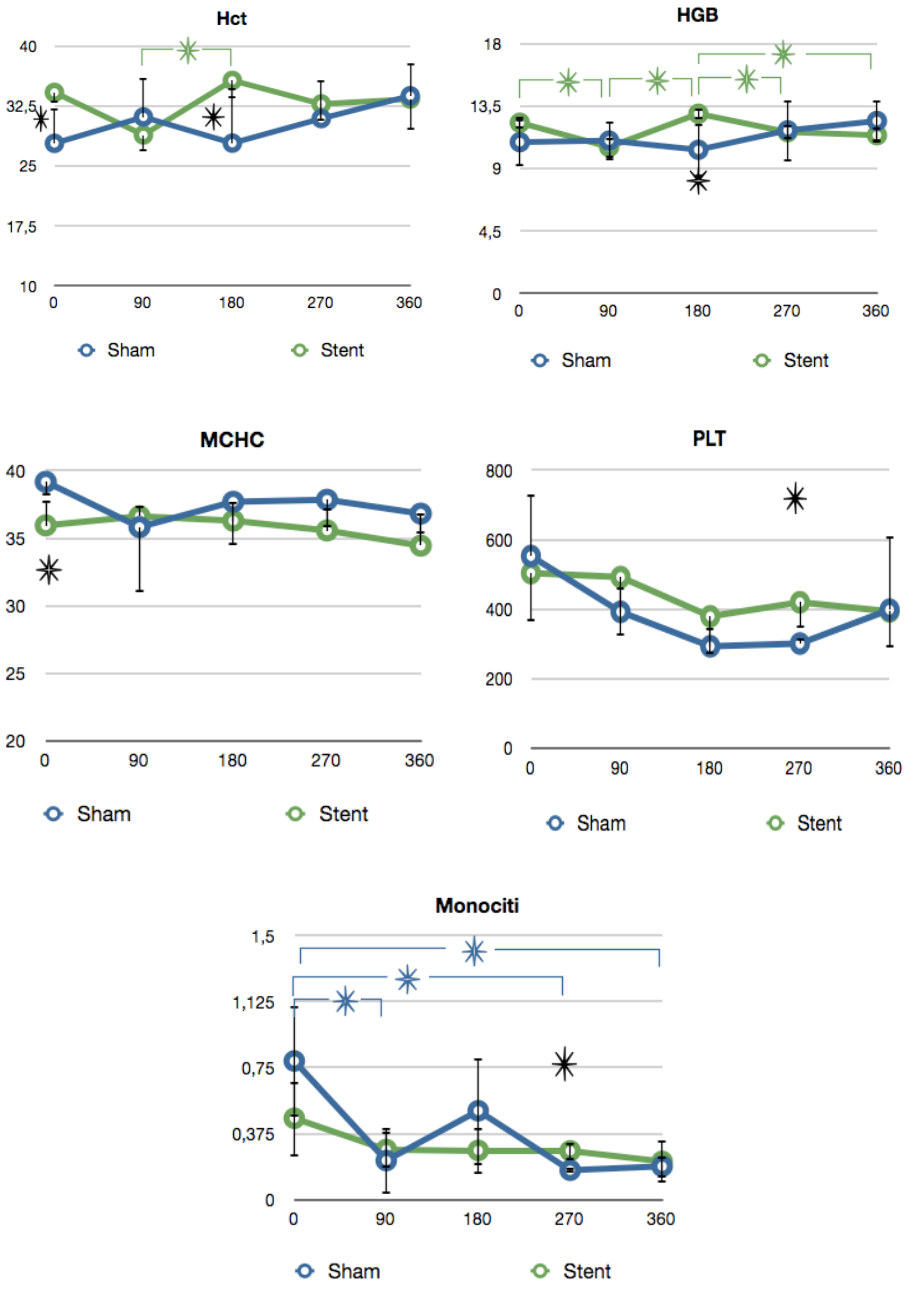

Fig. 31: mean parameters obtained from emogram analysis at different dobutamine doses. Hct: Hematocrit; HGB: Hemoglobin; MCHC: mean corpuscular hemoglobin concentration PLT: platelet.

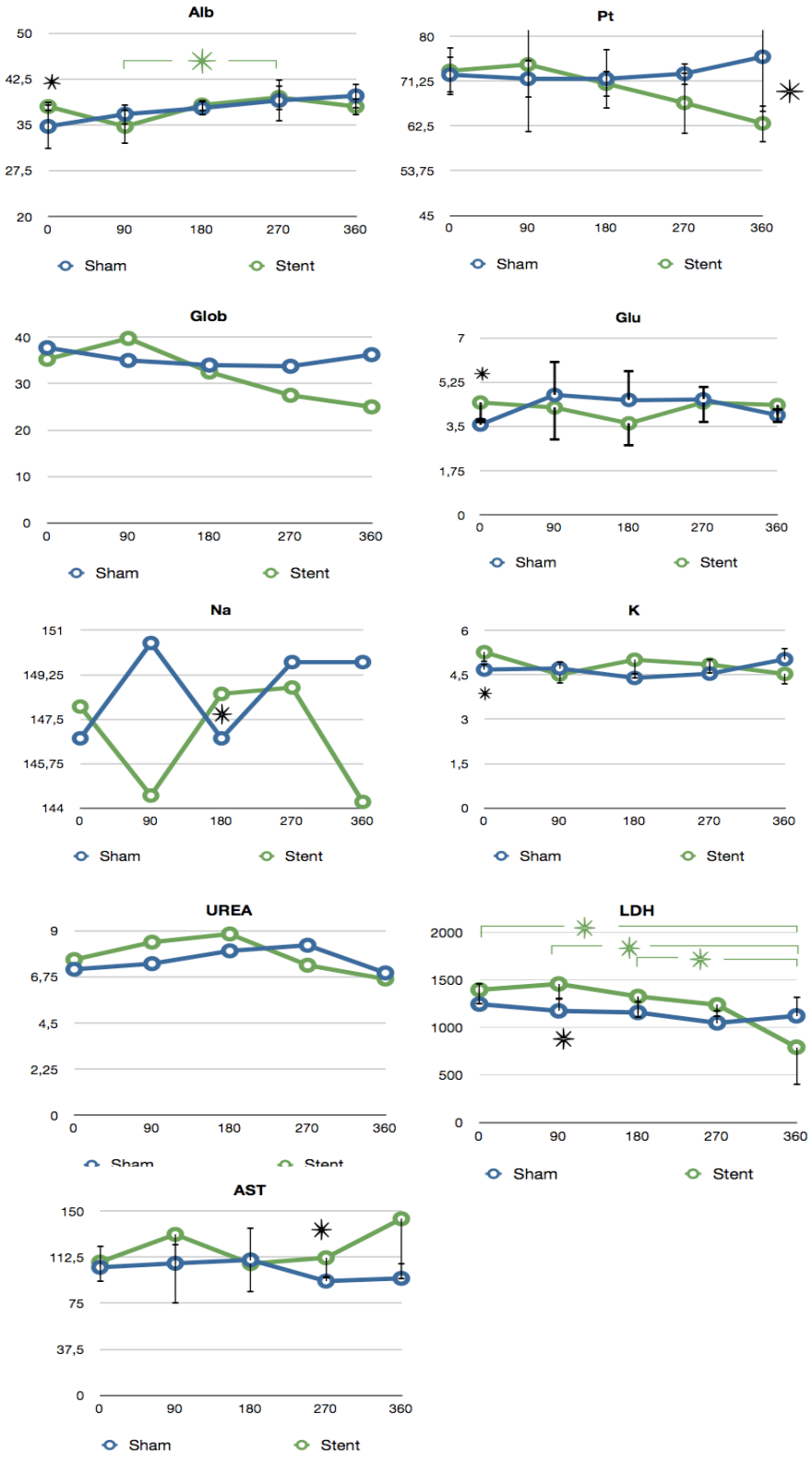

Fig. 32: biochemical parameters. Alb: albumin; Pt: total protein; Glob: globulin; Glu: glucose; Na: Sodium concentration; K potassium concentration; LDH: lactate dehydrogenase; AST: aspartate aminotransferase.

6.8 Pathological examination

Local stent effect:

Fig. 33 shows formalin-fixed aortic stented segment after section of the wall.

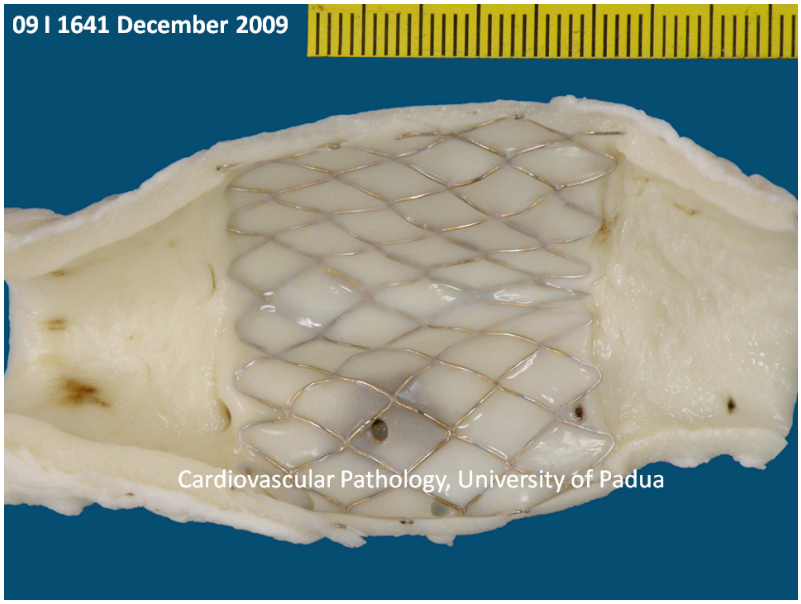

Fig. 33: stent-aortic wall relationship in formalin fixed aortic specimens.

Histological examination of stented segments of the aorta revealed mild neointimal proliferation partially covering stent mesh, never determining stenosis of the lumen. Stent mesh are not always uniformly distended and neointima covering is incomplete in some areas.

Aortic tunica media seems structurally disarranged, but this finding is present also in SHAM specimens, reflecting more a species-related finding rather than an abnormal stent-related feature.

Despite neointima proliferation, aortic wall thickness at stent level is reduced from segments above and below the device (fig. 34). This feature most likely reflects mechanical stretch locally induced by the stent.

No histological differences were found between aortic segments above

and below the stented area.

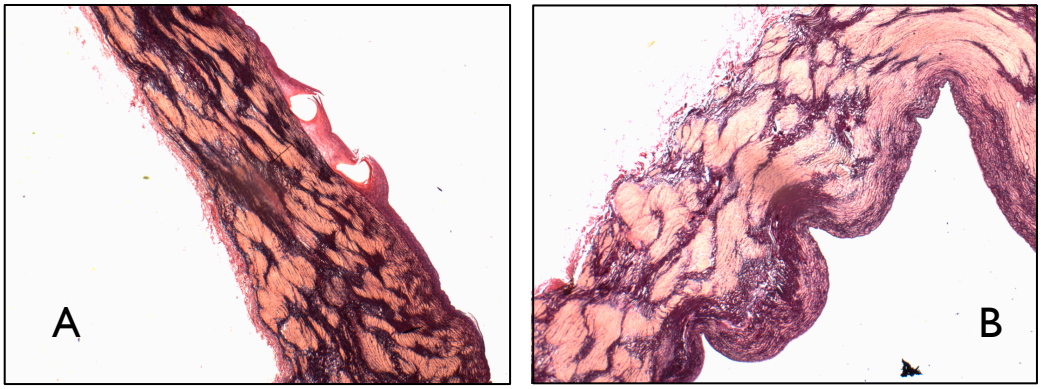

Fig. 34: hystologic sections of aortic wall. Stented aortic segment (A) with mild neointimal prolferation around the meshes. Ascending aorta above the stent (B). Wall thickness at stent level is reduced from segments above and below the device.

6.9 Gene expression of MMP9 and CASP3 in aorta.

CASP3 and MMP9 expression in ascending and descending aorta were not significantly different (table 10). On the contrary, MMP9 expression was greater in the ascending and lower in the descending aorta in stent animals than in sham (p=0.06).

Table 10: CASP3 and MMP9 in ascending (Asc Ao) and descending (Desc Ao) aorta.

	CASP3		MMP9	
	Asc Ao	Desc Ao	Asc Ao	Desc Ao
SHAM	0,96 ± 0.10	0,95 ± 0.04	0.94 ± 0.25	1.26 ± 0.31
STENT	0.97 ± 0.10	1.02 ± 0.11	1.67 ± 0.68	0.96 ± 0.09

7. Discussion

To date, this is the first long-term study to investigate the consequences of stenting of the aorta in an animal model.

Balloon dilation and stenting is now universally considered the treatment of choice for recurrent postoperative coarctation in both children and adults. The basic hemodynamic consequences of implantation, however, require further characterization.

Whether or not this technique represents the best choice in treatment of native coarctation in children or adults is still controversial: at many institutions, the transcatheter approach has become the elective treatment, but unfortunately follow-up has been limited, making it difficult to draw any meaningful conclusions as to which treatment option is superior. Furthermore designing an observational study is difficult, because several confounding factors can alter medium and long term results: CoA can be alone or associated to other hemodynamically disturbing anomalies such as bicuspid aorta, interventricular defect or patent ductus arteriosus, age at the intervention and residual isthmic pressure gradient are associated to worse outcome and hypertension development.

No efforts were made by investigators in studying whether aortic coarctation morphology and associated anomalies have direct influence on outcome (and long term hypertension development).

Recently, the first large multi-institutional observational study comparing surgery to stent treatment of aortic coarctation was published (Forbes et al., 2011), following a cohort of 350 treated patients. Mid term follow up (18 to 60 months after intervention) revealed a higher incidence of hypertensives in the 77 STENT patients (incidence: 18% high SBP, 31% under antihypertensive medications) respect to the SURGERY group (4% high SBP, 13% under antihypertensive medications), but age at intervention and age at follow up were significantly higher in the STENT group. Furthermore, subgroup analysis restricting patients' age to 6 to 12 years was conducted and stenting resulted the

procedure associated with better short term outcome.

Increased reflected pressure wave found in children after surgical CoA treatment has been involved in determination of ventricular hypertrophy and hypertension (Moutafi et al., 2011; Trojnarska et al., 2010). Since CoA cannot be defined as a simple segmental narrowing of aortic lumen, but is rather an "aortic syndrome", it is difficult to clearly understand the hemodynamic effect determined by the device: one can state that presence of a stent in aorta can itself create an area of regional increased rigidity that can alter aortic arch physiology.

The animal model helps in better understanding the long-term effects of stenting the aorta, without the confounding factor of the pathological aortic arch in which it is applied in clinical conditions. Compliance mismatch between the relatively noncompliant stent and the native vessel may have hemodynamic and mechanical consequences locally and beyond in the peripheral vascular tree.

Our study showed aortic stenting as feasible and safe in sheep. After first difficulties in defining the safest procedural protocol, stented animals survived without any problem. Sheep dimensions permit the use of the same devices used in children.

Stent positioning did not determine signs of ventricular hypertrophy or systemic hypertension, suggesting that hypertensive complication in treated patients could be related to the vascular anomaly rather than to the stent itself. Furthermore, no differences were found between groups in central hemodynamic parameters. Stenting did not alter cardiac systo-diastolic function assessed by echocardiography.

Myocardial and aortic histologic examination did not reveal any early sign of myocardial or vascular damage, and this is also reflected to a ultrastructural level, where no early signs of apoptosis nor extracellular matrix remodeling were found, according to normal CASP 3 and MMP 9 tissue expression even if differences of MMP9 expression of borderline significance were seen in the ascending aorta as a sign of increased collagen turn over.

Proteins of the matrix metalloproteinase (MMP) family are involved in the breakdown of extracellular matrix, The enzyme encoded by this gene degrades

collagen type IV and V. Interestingly, several candidate gene analysis revealed MMP9 gene overexpressed in abdominal aortic aneurysm (Thompson et al., 2008). Recently, another study found other metalloproteinase genes, MMP 14 and 19, associated with thoracic aortic aneurysms (Jackson et al., 2011). Furthermore a study found that the ACE-inhibitor perindopril downregulates MMP9 reducing degeneration in experimental abdominal aortic aneurysms in rat model, suggesting a role of RAAS in the development of abdominal aortic aneurysm (Alsac et al., 2011). To date, MMP's expression in patients with CoA, in treated patients or in patients with poststenotic aneurysm has not been studied yet.

This work supports the use of stenting as a safe procedure to treat native aortic coarctation, and it demonstrates that it does not contribute to determine chronic hypertension: the aortic segmental stiffness induced by the stent is not responsible of any pressor short and long term modification.

Hemodynamic evaluation indicates that pulsatile flow through the stent is preserved, and no differences are noted in the circulatory system below the device.

As previously mentioned, in clinical conditions decoarctated patients can be normotensive at rest, but can develop *exercise-induced hypertension*. Dobutamine test was performed in order to simulate exercise and investigate hemodynamic and pressor consequences to it. Incremental doses of dobutamine induced a progressive rise of heart rate and blood pressure in ascending and descending aorta, but pressure values in stented did not differ from those documented in SHAM animals: the sudden increase of aortic flow through the stent does not determine inappropriate hypertension found in operated children.

The less invasiveness in relation to surgical treatment makes this procedure preferable. It is sufficient to mention scholiosis as a frequent late complication of the posterolateral thoracotomy necessary as surgical access to aortic arch (Roclawski et al., 2009).

Limitations of the study

Several considerations have to be made considering the animal model. First of all animals were observed for 12 months, and reached at maximum 17

month of age. Obvious economic and practical aspects make a longer follow-up difficult, but as the average daily gain reduces at 300-400 days of age, we can speculate that adult weight is reached before the end of the study.

Other differences has to be made considering the animal model. Despite similar heart and aortic dimensions, a herbivore has cardiocirculatory system inevitably different from humans. Quadruped posture implies a different heart position and aortic arch morphology.

8. Conclusions

Aortic stenting is a safe procedure. Stent is well tolerated and it does not alter vascular physiology in the animal model. Late hypertension after stenting in CoA is unlikely caused by the device but most likely induced by the aortopathy associated to CoA.

9. List of participants to the study

Semplicini A, Ceolotto G, Stella F, (Department of Medicine), Milanesi O, Maschietto N, Valerio E (Department of Pediatrics “Salus Pueri”) Poser H, Iacopetti I, Gerardi G, De Benedictis G, Pilla T, Bernardini D (Department of Veterinary Clinical Sciences), Aresu L (Department of Public Health, Comparative Pathology and Veterinary Hygiene), Basso C (Department of Medical Diagnostic Sciences and Special Therapies)

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