



A pressure overload model to track the molecular biology of heart failure[☆]

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Abstract

Objectives: Pressure overload plays an important role in left ventricular remodelling and the development of heart failure. The underlying molecular mechanisms behind these processes are poorly understood at the myocyte level. To investigate this, we developed an ovine model of pressure overload-induced heart failure, in which serial left ventricular biopsies were obtained. **Methods:** Adult male sheep were chronically banded with a novel variable aortic constriction device. This was progressively inflated via a subcutaneous port to increase left ventricular afterload. The animals were monitored clinically and echocardiographically. Serial left ventricular endomyocardial biopsies were obtained via the right external carotid artery under fluoroscopic guidance. They were used to measure mRNA expression of the genetic regulators of apoptosis by reverse transcription polymerase chain reaction. In a subset of the animals, once left ventricular failure had been established, the constriction device was deflated to produce unloading of the left ventricle. **Results:** Ten of the 17 sheep banded developed left ventricular failure. Over the first 3–4 weeks, left ventricular mass index increased acutely (88 ± 18 vs. 44 ± 10 g/m², $P < 0.01$) followed by gradual left ventricular dilatation (diastolic left ventricular internal diameter 4.1 ± 0.7 vs. 3.2 ± 0.3 cm, $P < 0.01$). Ventricular function remained stable until 7–8 weeks postoperatively, when there was significant deterioration (fractional shortening 17 ± 8 vs. $40 \pm 8\%$, $P < 0.01$) associated with clinical heart failure. Expression of the pro-apoptotic genes (bax and Fas) increased significantly following inflation of the constriction device and persisted through the transition to left ventricular failure. Following deflation of the constriction device, myocardial contractility gradually improved over a 3 week period (fractional shortening 32 ± 1 vs. $17 \pm 8\%$). **Conclusions:** Progressively increasing the afterload on the left ventricle produces a clinical and echocardiographical picture of chronic heart failure. Obtaining myocardial tissue during this transition will allow the molecular correlates of pressure overload-induced heart failure and potential myocardial recovery to be investigated.

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1. Introduction

In response to pressure overload, myocardial hypertrophy is a compensatory mechanism, which attempts to normalize myocardial wall stress [1]. If pressure overload persists, the left ventricle dilates with impairment of contractile state and subsequent dysfunction [2]. The myocyte molecular mechanisms involved in the transition from compensated hypertrophy to myocardial dysfunction

and heart failure are poorly understood. Human studies are limited by access to myocardial tissue, usually restricted to end-stage heart failure patients at transplantation.

A number of animal models have attempted to reproduce the changes induced by pressure overload. Some employ small animals that are killed at different stages of hypertrophy and heart failure but do not obtain tissue from one animal throughout the transition [3]. Other models use juvenile animals with a fixed aortic constriction that causes ‘progressive’ pressure overload as the animal grows [4]. However, the juvenile response to pressure overload differs from that of adult [5]. There have been some models of variable aortic occlusion previously described using hydraulic constrictors, inflatable cuffs or angioplasty balloons [6–9]. Some of these models have only induced

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hypertrophy [8], whereas other models have encountered problems of acute congestive failure and aortic rupture whilst attempting to induce chronic heart failure [9]. This report describes a large animal model of heart failure induced by staged pressure overload, during which serial left ventricular biopsies are obtained. This strategy will allow investigation of the molecular mechanisms that accompany the transition from hypertrophy to heart failure. To demonstrate this strategy, the biopsies were used to investigate the expression of the genetic regulators of cardiomyocyte apoptosis by using reverse transcription polymerase chain reaction (RT-PCR) analysis.

2. Materials and methods

2.1. Model of variable aortic constriction

Seventeen adult male Naemy sheep weighing 34–42 kg were used in the study. Following induction of general anaesthesia, a left antero-lateral thoracotomy was performed through the third intercostal space. After retracting the left lung, the pericardium was opened to expose the main pulmonary artery, which overlies the ascending aorta in this position. The pulmonary artery was retracted and a circumferential window created around the ascending aorta by dissecting periaortic fat and surrounding fascia. Care was taken to avoid damage to the aortic vaso vasorum network. A variable aortic constriction device was then passed around the ascending aorta, just proximal to

the origin of the innominate artery, and sutured in place with 3/0 prolene. The device consisted of a Gortex[®] cuff enclosing the balloon of a Foley catheter (Fig. 1). Inside the cuff/balloon device, another piece of Gortex[®] was positioned to protect the aorta from balloon-induced pressure necrosis. The proximal port of the device was brought out subcutaneously, caudal to the wound, for subsequent inflation and deflation of the balloon. Post-operatively, the sheep were monitored until cardio-respiratory stability had been established. All procedures were carried out in compliance with the 'Guide for the Care and Use of Laboratory Animals' published by the National Institute of Health and with full approval of the local Animal Ethics Committee.

2.2. Echocardiographic monitoring

A Vingmed 720 CPM echocardiography machine was used with a 3.25 and 5 MHz transducer and a continuous wave Doppler probe. The sheep were placed in the left lateral decubitus position, without sedation or anaesthesia. Standard two-dimensional long and short axis trans-thoracic views from the right sternal edge were obtained to measure left ventricular cavity and wall dimensions in systole and diastole. These included left ventricular internal diameter in diastole and systole (LVIDd and LVIDs), posterior wall thickness (PWTd and PWTs) and interventricular septum thickness (IVSd and IVSs). The measurements were taken in M-mode at the level of the mitral valve papillary muscles. This allowed the left ventricular mass index (LVMI) and

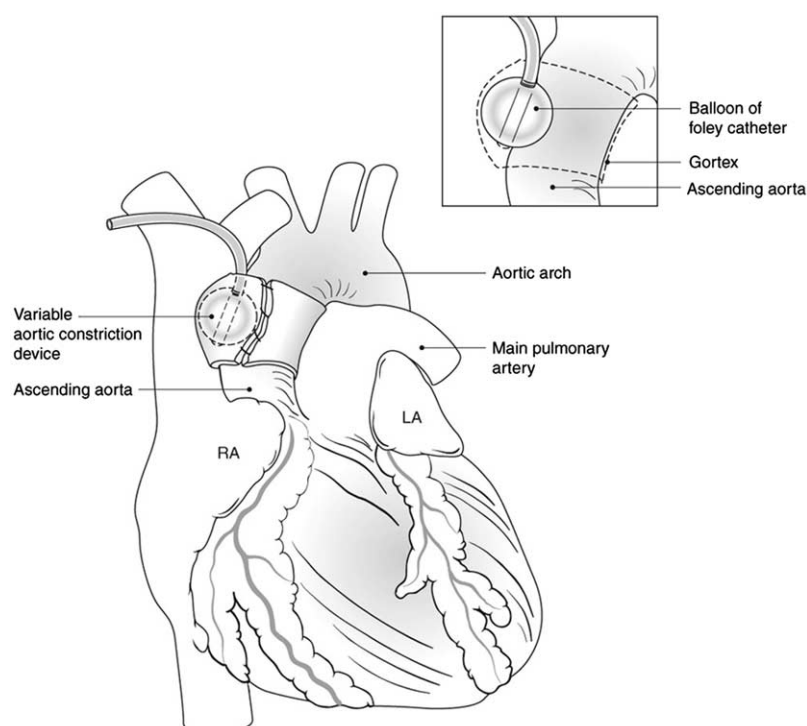


Fig. 1. Variable aortic constriction device.

fractional shortening (FS), as a measure of ventricular contractility, to be calculated [10]. The peak aortic Doppler velocity was obtained from the suprasternal position.

$$\text{LVM (g)} = 1.04[(\text{LVIDd} + \text{PWTd} + \text{IVSd})^3 - \text{LVIDd}^3] - 13.6$$

$$\text{BSA (m}^2\text{)} = 0.112 (\text{weight in kg})^{2/3}$$

$$\text{LVMI (g/m}^2\text{)} = \text{LVM/BSA}$$

$$\text{FS} = \frac{\text{LVIDd} - \text{LVIDs}}{\text{LVIDd}} \times 100$$

where LVM is the left ventricular mass and BSA is the body surface area.

2.3. Study protocol

The balloon of the variable constriction device was inflated during the operation to achieve an aortic Doppler velocity of approximately 2 m/s. The sheep were echoed daily for the first few postoperative days and then twice weekly. Four weeks and 6 weeks postoperatively, the balloon was inflated further to produce an aortic Doppler velocity of about 3 and 4.5 m/s, respectively. Left ventricular endomyocardial tissue was obtained at the initial operation, at regular intervals during the study and when the animals developed clinical and echocardiographical signs of left ventricular failure. The biopsies were carried out using a 7F endoluminal biptome (Cordis, USA) passed via the right external carotid artery under fluoroscopic guidance. These biopsies were stored in 10% neutral buffered formalin for histological studies or snap frozen in liquid nitrogen and then stored at -80°C for molecular analyses. Serum and plasma were also obtained with the biopsies for subsequent investigations. In a subset of animals with left ventricular failure, the aortic constriction device was deflated and the sheep monitored clinically and echocardiographically during the recovery phase.

2.4. RT-PCR

RNA was extracted from the frozen biopsies by homogenizing with 1 ml TRI reagent (Molecular Research Centre). After addition of 200 μl chloroform and centrifuging at $12,000 \times g$, the RNA was precipitated, washed with isopropanol and 75% ethanol and finally resuspended in 25 μl RNase free water. Total cDNA was obtained from the RNA by reverse transcription using a RT kit (Promega, USA). A polymerase chain reaction was then set up using 25 μl Taq master mix (Taq polymerase, MgCl_2 , dNTPs; Qiagen, USA), 1 μl sense primer, 1 μl antisense primer, and 1 μl cDNA in a total reaction volume of 50 μl . After denaturation at 96°C for 5 min, PCR cycles were performed in a PTC-200 thermocycler (MJ Research): 1 min at 94°C , 1 min at primer-specific annealing temperature (Table 1) and 1 min at 72°C . After extension of the cDNA amplification at 72°C for 7 min, the PCR products were electrophoretically separated using a 2% agarose gel containing 0.5 mg/ml ethidium bromide. The DNA gels were visualized under UV light and intensities measured using a densitometer (Bio-Rad, Melville, NY). Expression of the genes was normalized to GAPDH, used as an internal control.

2.5. Statistical analysis

All results are expressed as mean \pm standard deviation. A paired Student's *t*-test was used to compare values within the groups. Statistical significance was accepted at the level of $P < 0.05$.

3. Results

Two of the 17 sheep died of acute heart failure within 24 h of the operation. Death was caused by over-inflation of the balloon during the surgical learning curve. Two further animals died of acute heart failure following subsequent balloon inflation. Late aortic rupture caused sudden death in two of the sheep. Dysrhythmia following endomyocardial

Table 1
RT-PCR primers

Gene	Strand	Sequence	Product length (bp)	Annealing temperature ($^\circ\text{C}$)	Cycles
Bax	Sense	CAG GAT GCA TCC ACC AAG AAG C	164	59.2	36
	Antisense	TTG AAG TTG CCG TCG GAA AAC ATT			
Bcl-2	Sense	GAC TTC TCC CGC CGC TAC CG	341	65	36
	Antisense	GAC AGC CAG GAG AAA TCA AAC			
Fas	Sense	TCC GGG ATC TGG GTT CAC T	293	57.5	40
	Antisense	TTG TCA GAA TGA TGG CTC TTG TCT			
GAPDH	Sense	CGG GAA GCT GTG GCG TGA TG	398	55–65	25
	Antisense	TCC ACC ACC CTG TTG CTG TAG C			

Table 2
Echocardiographic changes induced by progressively increasing afterload on the left ventricle

	Weeks								
	0	1	2	3	4	5	6	7	8
IVSd (cm)	0.73 ± 0.06	0.88 ± 0.08	0.91 ± 0.06	0.89 ± 0.06	0.88 ± 0.08	0.80 ± 0.10	0.76 ± 0.07	0.70 ± 0.07	0.65 ± 0.06
LVIDd (cm)	3.15 ± 0.29	3.38 ± 0.28	3.40 ± 0.39	3.81 ± 0.47	3.93 ± 0.36	4.05 ± 0.52	4.12 ± 0.66	4.26 ± 0.58	4.27 ± 0.55
PWTd (cm)	0.72 ± 0.07	0.87 ± 0.08	0.89 ± 0.06	0.84 ± 0.09	0.81 ± 0.12	0.77 ± 0.12	0.75 ± 0.11	0.71 ± 0.06	0.59 ± 0.09
IVSs (cm)	1.16 ± 0.15	1.29 ± 0.11	1.34 ± 0.15	1.33 ± 0.16	1.33 ± 0.20	1.22 ± 0.09	1.15 ± 0.19	1.09 ± 0.17	0.98 ± 0.13
LVIDs (cm)	1.89 ± 0.29	1.89 ± 0.23	1.86 ± 0.40	2.13 ± 0.48	2.09 ± 0.41	2.59 ± 0.66	2.81 ± 0.69	3.20 ± 0.69	3.23 ± 0.65
PWTs (cm)	1.24 ± 0.19	1.41 ± 0.18	1.31 ± 0.18	1.32 ± 0.20	1.29 ± 0.10	1.18 ± 0.31	1.13 ± 0.24	0.95 ± 0.19	0.77 ± 0.13
FS (%)	40.0 ± 7.62	44.6 ± 5.58	44.5 ± 9.72	45.2 ± 5.87	42.4 ± 10.7	36.3 ± 9.79	31.2 ± 9.15	22.2 ± 11.0	17.0 ± 8.12
LVMI (g/m ²)	44.4 ± 9.61	70.2 ± 14.1	72.2 ± 14.7	87.5 ± 18.5	90.8 ± 15.6	86.0 ± 15.5	86.7 ± 24.4	84.5 ± 17.8	53.4 ± 11.3
Weight (kg)	37.0 ± 2.04	35.8 ± 2.01	35.2 ± 1.85	34.5 ± 2.33	34.1 ± 3.24	32.6 ± 2.10	31.5 ± 1.67	31.1 ± 1.48	30.8 ± 1.87

All results are given as mean ± standard deviation.

biopsy caused the death of one of the sheep. Ten sheep (59%) survived the duration of the study.

Forty-five satisfactory endomyocardial biopsies were obtained. Ventricular arrhythmias encountered were usually self-limiting or treated successfully by lignocaine. Other morbid events included carotid haematoma after endomyocardial biopsy in two sheep and thoracic wound infections in two. In one case, there was failure of the aortic constriction device through balloon rupture. This animal was subsequently excluded from the study group.

Table 2 shows the echocardiographic changes induced by progressively increasing the afterload on the left ventricle. The sheep developed hypertrophy of the left ventricle over the first 3–4 weeks, shown by a marked increase in LVMI (88 ± 18 vs. 44 ± 10 g/m², $P < 0.01$). Following progressive inflation of the aortic constriction device, the left ventricle gradually dilated (LVIDd 4.1 ± 0.7 vs. 3.2 ± 0.3 cm, $P < 0.01$). Ventricular function remained stable until 7–8 weeks post banding, when there was a significant deterioration (FS 17 ± 8 vs. 40 ± 8%, $P < 0.01$). At this stage, the LVMI had fallen off and the left ventricle remained significantly dilated. Associated with the deterioration in myocardial contractility, the animals displayed reduced mobility, anorexia and were tachypnoeic. A mean peak aortic Doppler velocity of 4.66 m/s (which equates to an aortic-left ventricular pressure gradient of 89.6 mmHg (using modified Bernoulli equation $p = 4v^2$, where p is the left ventricular-aortic pressure gradient and v is the peak aortic Doppler velocity)) was required to produce left ventricular failure. Following deflation of the constriction device in three of the animals, the FS remained poor over the first few days, following which it gradually improved over a 3 week period (FS 32 ± 1 vs. 17 ± 8%, Table 3). Associated with the recovery in myocardial contractility, there was an increase in mobility and feeding and a reduction in respiratory rate.

Biopsies taken at these echocardiographically distinct stages (LV hypertrophy, LV dilatation and LV failure), during the transition to heart failure, were then compared to biopsies taken prior to the operative procedure (Fig. 2).

After the development of LV hypertrophy, there was a significant increase in expression of both pro-apoptotic genes, bax and Fas. This increase persisted as the left ventricular phenotype changed from hypertrophy through dilatation to failure. However, no significant change in mRNA expression of the anti-apoptotic gene bcl-2 was observed by RT-PCR during the transition to heart failure.

4. Discussion

The need for further animal models of heart failure was highlighted a few years ago in a special report by the National Heart, Lung and Blood Institute [11]. Currently, the most popular model, rapid ventricular pacing, is simple to produce but represents a condition that is not frequently found in human heart failure. Also the physiological changes do not persist beyond removal of the pacing stimulus and ventricular dilatation occurs without an initial hypertrophic response [12]. Other methods include volume overload and coronary ligation. In these models, the degree of damage to the heart and severity of heart failure are unpredictable [13].

In this study, we have developed a reproducible model of chronic heart failure that results in marked systolic

Table 3
Echocardiographic changes during unloading of the left ventricle

	Weeks			
	0	1	2	3
IVSd (cm)	0.65 ± 0.06	0.84 ± 0.13	0.80 ± 0.12	0.80 ± 0.15
LVIDd (cm)	4.27 ± 0.55	3.95 ± 0.89	3.86 ± 0.74	3.79 ± 0.82
PWTd (cm)	0.59 ± 0.09	0.71 ± 0.11	0.69 ± 0.06	0.71 ± 0.08
IVSs (cm)	0.98 ± 0.13	1.17 ± 0.12	1.15 ± 0.08	1.16 ± 0.07
LVIDs (cm)	3.23 ± 0.65	2.80 ± 0.64	2.67 ± 0.48	2.57 ± 0.52
PWTs (cm)	0.77 ± 0.13	1.07 ± 0.18	0.90 ± 0.01	0.91 ± 0.04
FS (%)	17.0 ± 8.12	29.0 ± 4.58	31.0 ± 2.65	32.0 ± 1.00
LVMI (g/m ²)	53.4 ± 11.3	79.0 ± 28.0	75.4 ± 24.7	75.2 ± 26.3
Weight (kg)	30.8 ± 1.87	32.1 ± 0.76	33.1 ± 0.30	34.0 ± 0.40

All results are given as mean ± standard deviation.

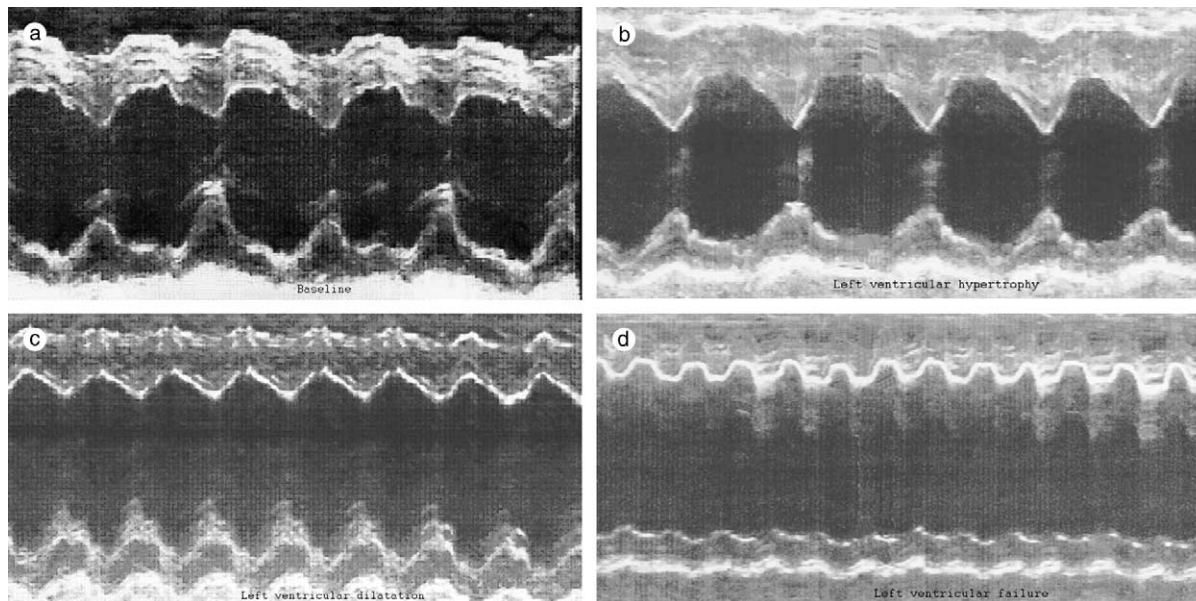


Fig. 2. mRNA expression of genetic regulators of apoptosis in the transition to heart failure.

dysfunction. The use of a variable constrictor allowed the pressure overload to be gradually increased without the need for re-operation. It also allowed the pressure overload to be removed if the animal suffered acute heart failure due to over-inflation of the balloon. This model of chronic heart failure allows multiple endomyocardial biopsies to be obtained during the transition to heart failure. The use of RT-PCR to correlate changes in expression of the genetic regulators of apoptosis with left ventricular phenotype demonstrates how this model can be used to investigate the underlying cellular and molecular mechanisms behind the development and progression of left ventricular failure and the remodelling process. Increased expression of pro-apoptotic factors, bax and Fas, associated with no significant change in expression of anti-apoptotic bcl-2 indicates that upregulation of the apoptotic cascade is associated with the transition to heart failure. Further studies using this model will allow investigation of whether apoptosis or other molecular processes play a causal role in the development of this myocardial dysfunction.

This is the first large animal model of pressure overload that has staged the progression of heart failure echocardiographically (Fig. 3). Echocardiography is an essential component of this model as it provided an ideal non-invasive method to monitor the progression of remodelling and a way to measure the pressure overload applied to the left ventricle using Doppler measurements. This ensured that the sheep were subjected to similar amounts of pressure overload and provided consistent results in the study group. The M-mode measurements of the left ventricle showed a significant increase in the LVMI during the transition from baseline to left ventricular hypertrophy. In post-mortem studies, Devereux et al. showed that LVMI was the most accurate measure of hypertrophy, when using the corrected Penn-cube

formula [10]. Progression through left ventricular dilatation and left ventricular failure stages correlated with a significant increase in LVID and significant decrease in FS, respectively. The measurements were made in conscious sheep to avoid the effects of sedation or general anaesthesia.

The risk of aortic rupture during the study was similar to previous animal models of pressure overload-induced heart

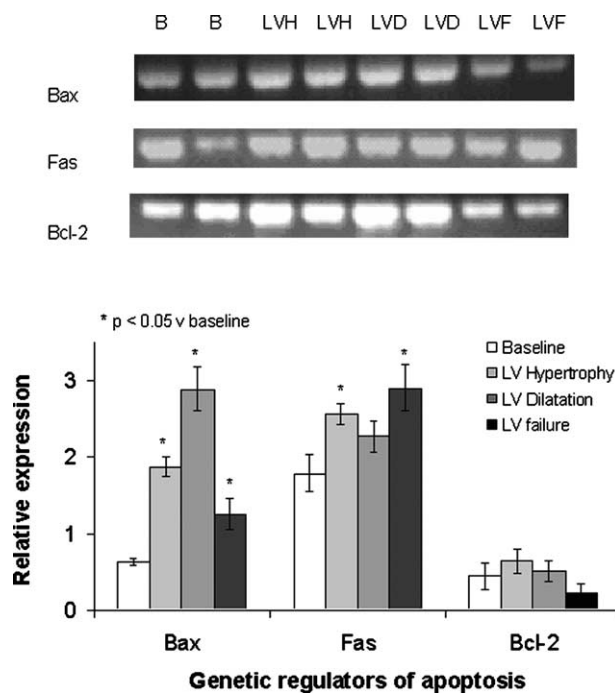


Fig. 3. M-mode echocardiography showing changes in left ventricular dimensions induced by progressive pressure overload. The transition from baseline (a) to left ventricular hypertrophy (b) is illustrated by thickening of the ventricular walls, to left ventricular dilatation (c) by enlargement of the ventricular cavity, and to left ventricular failure (d) by markedly reduced excursion of the ventricular walls in systole.

failure [9]. A number of factors are thought to be involved [14]. Firstly, external compression by the inflated balloon together with systemic blood pressure can cause pressure necrosis. Secondly, creation of the aortic-pulmonary window for the passage of the balloon constriction device may damage the supporting vaso-vascular network, leaving the aortic wall open to ischaemic injury. Thirdly, the presence of the balloon-gortex prosthesis may induce a chronic inflammatory reaction. In our two animals that died of aortic rupture, a peri-prosthetic abscess was noted at post-mortem. It is likely that the infective process will have compounded the above factors in weakening the aortic wall and leaving it susceptible to subsequent rupture. We were able to lower the incidence of aortic rupture in comparison with previous studies [6], by using a broad Gortex® band to evenly distribute the constriction pressure of the balloon device over a larger area. An inner band of Gortex® between the balloon and aortic wall may have protected the aorta from pressure necrosis and further reduced the propensity to rupture.

In three sheep in which heart failure had been induced, the balloon was deflated to unload the left ventricle. This ensured that the state of chronic heart failure induced was stable in the short term, followed by gradual recovery in left ventricular contractility. Endomyocardial biopsies taken during the unloading phase enable the molecular changes of myocardial recovery to be followed.

4.1. Study limitations

Although the model was designed to simulate the changes associated with pure pressure overload, the placement of the constriction device in a supra-coronary position leaves the coronary arteries exposed to elevated systolic pressures and hence may affect coronary perfusion. Another potential criticism of this model is whether acute or chronic heart failure has been induced. Although the variable constriction device allowed the gradual imposition of increased afterload, it is recognized that this occurred in a much shorter time period compared to that experienced in human pressure overload-induced heart failure. Furthermore, the mortality rate, whilst being relatively high, is comparable to other studies that have tried to achieve a reproducible animal model of heart failure and not just hypertrophy. To investigate echocardiographic and molecular changes following unloading, a larger number of animals would be required to achieve statistical significance.

In conclusion, we have developed a reproducible large animal model of heart failure induced by progressive pressure overload. By obtaining serial left ventricular biopsies, we can investigate the molecular changes that accompany the transition to heart failure and the onset of recovery. The model also provides the opportunity for potential therapeutic agents that attenuate the progression of heart failure to be tested.

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