Title
In-utero ventricular pacing as a treatment for complete atrioventricular block

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In-utero Ventricular Pacing as a Treatment for Complete Atrioventricular Block: Physiologic, Functional, and Morphologic Consequences for Fetal Heart Development

by

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ABSTRACT:

Congenitally complete atrioventricular block (CCAVB) is a condition increasingly being diagnosed in the fetal period, which, when associated with an inadequate ventricular response rate, can rapidly result in hydrops fetalis and fetal demise. Epicardial pacing aimed at increasing ventricular rate and improving cardiac output in the symptomatic fetus may allow for delivery of a full term, stable neonate with preserved ventricular function. However, the effect of pacing on the structure and function of the rapidly developing fetal heart remains to be elucidated. Complete heart block (CHB) was created in fetal lambs at 80% gestation by cryoablating the atrioventricular (AV) node. Epicardial ventricular pacing was immediately instituted at 130 bpm and the fetus was returned to the uterus and allowed to continue to term. Hemodynamics and ventricular function were assessed 4-8 days after birth in seven CHB lambs and ten controls. No significant difference in right atrial ($p=0.68$), left atrial ($p=0.49$), and aortic pressures ($p=0.08$) between CHB and control lambs was found. Similarly, there was no difference between study and control lambs in functional parameters such as stroke volume ($p=0.36$), stroke work ($p=0.57$), or cardiac index ($p=0.06$) at the baseline state. Using the conductance catheter technique, the end systolic pressure-volume relationship (ESPVR) and the preload-recruitable strokework index (PRSW) were determined at different heart rates, inotropic states and pacing modalities. There was no difference in baseline contractility between the two groups as determined by the Emax of the ESPVR and the slope (m) of the PRSW index ($p=0.87$ and 0.45 respectively). The relative augmentation in contractile function with a positive inotropic agent (dobutamine) also seemed unaffected by chronic ventricular pacing (Emax: $p=0.43$; PRSW-m: $p=0.13$). However, the relative decline in contractility with negative inotropy

(esmolol) did differ between control and CHB lambs (PRSW-m: $p<0.0001$); the significance of this finding is unclear because ventricular output as measured by the cardiac index dropped significantly but similarly in CHB and control lambs with esmolol infusion. Finally, gross and histopathological post-mortem analysis failed to reveal any adverse sequelae of *in utero* ventricular pacing. To conclude, chronic fetal epicardial ventricular pacing does not appear to have negative hemodynamic, functional or histopathologic effects in the rapidly developing, immature lamb heart. While extrapolation of results in a lamb model to the human fetus is certainly limited, these findings suggest that epicardial pacing may be an appropriate therapeutic modality for CCAVB and should be further studied.

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INTRODUCTION:

Congenital complete atrioventricular block (CCAVB) may be defined as a condition in which the atria and ventricles beat independently of one another; the ventricular rate is typically slower than the atrial rate and there are no captured beats. CCAVB is the product of a failure of communication between atrial and ventricular myocardial tissues that results from a lack of continuity in the atrioventricular conduction system. The etiology of this lesion is quite heterogeneous but can be divided into those cases which occur in the setting of abnormal cardiac anatomy (e.g. congenitally corrected transposition of the great arteries) and those which occur in the anatomically normal heart. This latter catagory encompasses approximately 50 to 60% of all cases (1,2,3). Lev and colleagues have further divided CCAVB in the structurally normal heart into two pathophysiological sub-types: 1. failure of the atrial myocardium to make contact with the atrioventricular (AV) node; or 2. separation of the AV node from the ventricular contractile tissue and myocardium by fibrous tissue (4). These pathophysiologic events in the fetus with normal cardiac anatomy have been attributed to tumors, fetal endocarditis, endocardial fibroelastosis and localized fibrosis based on histologic analysis (5). In addition, CCAVB in the fetus has been associated with maternal collagen vascular diseases involving a variety of auto-antibodies particularly anti-Ro and anti-La (6,7).

Although most fetal arrhythmias involve premature contractions or irregular/regular tachyarrhthmias, CCAVB does occur in 1 in 20,000 live births (1). With recent advancements in fetal echocardiographic evaluation--in particular 2D, M-mode and Doppler ultrasound techniques--CCAVB is increasingly being identified in the fetal period as early as 16-18 weeks of gestation (8,9). Some fetuses appear to tolerate this lesion well, particularly in the presence of an adequate ventricular rate and a competent AV valve.

However, in up to 50% of the cases diagnosed, non-immune hydrops fetalis develops presumably due to inadequate cardiac output (10). Rudolph and Heymann have demonstrated that fetal ventricular outputs significantly correlate with changes in heart rate so that a reduction in fetal heart rate (e.g a slow idioventricular rate in CCAVB) results in a linear fall in ventricular output (11). Their work also suggests that atrial contraction may be quite important in diastolic ventricular filling in the fetus.

Currently, two treatment modalities exist for hydrops fetalis resulting from CCAVB. The first is pharmacologic and involves administration of sympathomimetic drugs to the mother to accelerate the fetal ventricular rate and thus enhance cardiac output. Unfortunately the disadvantages of medical management are numerous and include maternal toxicity--tremulousness, tachycardia, and potential myocardial ischemia-- and an unpredictable effect on fetal heart rate which is predominantly the result of an equally unpredictable transplacental transfer of drugs from mother to fetus. Other agents such as digoxin and furosemide have also been utilized to improve cardiac performance but have been irregular in their effects. The second form of treatment involves emergent and often preterm delivery of the hydropic fetus by Caesarian section and placement of a cardiac pacemaker to improve cardiac output and ameliorate hydrops. The disadvantages of this treatment are essentially all the complications of the delivery of a premature infant--namely, respiratory distress syndrome, necrotizing enterocolitis, intraventricular hemorrhage, thermoregulatory immaturity, etc.

A theoretical alternative to these treatments is placement of a cardiac pacemaker on the surface of the fetal heart with return of the fetus to the uterus for continued maturation and development. By providing a stable and adequate ventricular rate, fetal cardiac output should improve and hydrops

should be ameliorated. While implantation of a pacemaker in the fetus has been shown to be feasible (by this laboratory and others), this treatment option is not without complications (12). Apart from the risk of maternal exposure to laparotomy and hysterotomy and the post-operative tocolytic issues, ectopic ventricular pacing alters the electromechanical environment of the fetal heart (13-16). [Currently, atrioventricular (AV) sequential pacing is not an option in this setting because implantable pacemakers of appropriate size and adequate maximum AV pacing rate for a fetus are not available.] In a young canine model, studies have demonstrated histopathological, electrophysiologic, and hemodynamic changes in response to chronic, fixed-rate ventricular pacing (17). In addition, in the developing fetal heart, AV dysynchrony and ventricular pacing could result in morphologic changes that might influence cardiac function. It has also been suggested that ectopic ventricular depolarization can result in depressed ventricular function in an adult model (18). However, the effects of chronic *in utero* ventricular pacing and AV dysynchrony on the rapidly changing fetal heart remain to be elucidated.

This study was undertaken to determine whether fetal epicardial ventricular pacing in the setting of complete heart block (CHB) can result in the delivery of a hemodynamically stable neonate with preserved ventricular function and morphology. Ventricular function is determined by heart rate, cardiac load, and myocardial contractility. Since cardiac loading conditions and heart rate are constantly changing, measures that reflect contractility are more reliable indicators of intrinisic myocardial function than parameters such as stroke volume or cardiac output which are influenced not only by contractile state but also by load and heart rate. Measures such as stroke volume, stroke work, end diastolic volume, and cardiac output are more useful as indicators of cardiac pump function. In order to assess contractility, an index that is

independent of changes in preload, afterload and cardiac geometry should be utilized (19). Although many such "contractile indices" exist, most are limited by the degree to which they are altered by load. Systolic pre-ejection indices such as dp/dt_{max} are somewhat preload dependent, while ejection indices such as ejection fraction and velocity of circumferential fiber shortening can be both pre- and after- load dependent (19). The end systolic pressure volume relationship (ESPVR), and the preload recruitable strokework index (PRSW), however, have been shown to be relatively load independent in the range of physiologic measurement and are reliable indicators of change in contractility in both adult and neonatal models (20-25). The ESPVR and the PRSW were utilized to assess the impact of chronic *in utero* ventricular pacing on neonatal ventricular function. Pressure-volume loops were generated in order to determine these relationships in the paced, CHB neonate and these were then compared with data from age-matched controls. Measures of pump function were also utilized in this analysis along with hemodynamic and histopathological data.

METHODS:

The Model:

A pregnant ewe at 115-120 days gestation (full=145-150 days) was anaesthetized with 10mg/kg IM ketamine and 2 cc intraspinal ponticaine. She was maintained throughout the procedure on 5-20mg/kg/hr IV ketamine as necessary for adequate anaesthesia. A midline laparotomy and hysterotomy were performed to expose the right chest of the fetus. The remainder of the fetus was carefully secured within the uterus which was then returned to the maternal abdomen to maintain fetal temperature homeostasis. Following administration of 10mg/kg IM ketamine (based on estimated weight) and 20mg succinylcholine to the fetus for anaesthesia and intraoperative paralysis, a right lateral thoracotomy was performed at the 5th intercostal space. An intramuscular pocket for the pacemaker was created at this time to avoid the use of electrocautery once the pacemaker leads were affixed to the heart. The fetal heart was exposed through a pericardiotomy and the region of the coronary sinus and inferior vena cava/right atrial junction identified.

At this time, an epicardial pacing wire [Medtronics, Minneapolis, MN] was placed high in the right ventricular wall, secured with suture, and connected to an implantable pacemaker [Legend, Minix] set on VVI mode at 130bpm. Pacing was not begun until complete heart block (CHB) was achieved. Using a liquid nitrogen cryogenic system, a cryoprobe with a 2mm tip was directed at the approximate location of the atrioventricular node (at the apex of the Triangle of Koch bounded by the coronary sinus, the tendon of Todaro, and the septal leaflet of the tricuspid valve). The region was cooled to -80°C for 5 minutes during which the fetal right ventricle was paced intermittently. Several applications of the cryoprobe were performed at various locations within this region to ensure creation of CHB. Once block was reliably achieved,

the pacemaker was placed in its muscular pocket and the fetal incisions were carefully closed. The fetus was placed back in the uterus and amniotic fluid lost during hysterotomy and manipulation of the fetus was replaced with 37°C saline. Antibiotics (1 million units penicillin G potassium and 400mg gentamicin) were also instilled into the amniotic cavity to prophylax against chorioamniotic infection. The hysterotomy incision was closed and the uterus returned to the exact position in the maternal abdomen in which it was found. The abdominal incision was closed in layers and penicillin and gentamicin were administered to the ewe intramuscularly for three days. The ewe was then allowed to continue to term and deliver spontaneously

Preparation of neonatal lambs for study:

Seven chronically paced and ten control (non-instrumented siblings of paced animals) lambs were studied between 4 and 8 days of age using a study protocol approved by the Committee on Animal Research at UCSF. Each lamb was weighed prior to study and then premedicated with 5mg/kg IM ketamine and 0.1 mg/kg pancuronium bromide. The lamb was then intubated and ventilated with oxygen and air using a pressure regulated ventilator in order to maintain pO2 and pCO2 within a normal range during the study. Polyvinyl catheters were placed in an artery and vein in the hindlimb and advanced to the descending aorta and inferior vena cava respectively. The venous line was utilized for delivery of 0.9%NS at a rate of 8-12 cc/kg/hr and for administration of a ketamine drip titrated to maintain adequate general anaesthesia (5-20mg/kg/hr). Through the arterial line, blood gas measurements were taken. Such measurements were taken 5-10 minutes following the onset of mechanical ventilation in order to assess oxygenation, ventilation and acid-base status. Thereafter, arterial blood gases were measured regularly throughout the study

to ensure acid/base stability. NaHCO3 was administered to maintain base deficits <5mml/l. A surface three lead EKG was also placed to monitor atrial and ventricular rates and rhythms.

A midline sternotomy was then performed. The thymus was resected and the pericardium opened and tacked to the chest wall to provide optimum visualization of the heart and great vessels. Purse string sutures were placed in the right atrium , left atrium , pulmonary artery , ascending aorta, and left ventricular apex. Using a 5 French pressure-tip catheter [Millar Instruments, Houston,TX], pressure tracings were obtained from the right atrium, left atrium , pulmonary artery, and aorta and digitized on line to a Macintosh II computer [Apple Computers, Cupertino, CA] via data acquisition methodology to be described below. A polyvinyl catheter was then placed in the pulmonary artery for administration of inotropic agents.

The Millar pressure tip catheter was then placed through the left ventricular apical purse string suture and its position in the ventricular cavity was verified via pressure tracings on an oscilloscope. A 7 French self-sealing sheath was then placed through the aortic purse string. A 6 French 10 electrode conductance catheter (Webster Laboratories, Baldwin Park, CA) was guided through the aortic sheath such that the most proximal electrode on the catheter lay just above the aortic valve and the most distal lay in the apex of the left ventricle. The position of the conductance catheter was verified echocardiographically, via volume tracings on an X-Y oscilloscope, and via the presence or absence of paradoxical motion of volume segment signals during systole and diastole which would suggest that a particular segment was in a different chamber or in a great vessel and not in the left ventricular cavity. Finally, a 5 French atrial septostomy catheter (American Edwards Laboratories, Irvine, CA) was introduced via the right atrium to the inferior vena cava-right

atrium junction. The position of the balloon catheter was assessed via steady occlusion of the inferior vena cava during balloon inflation and assessment of volume changes on the oscilloscope.

In the chronically paced, CHB lambs, the implantable pacemaker was resected and the pacing wire mobilized. Right atrial and right ventricular pacing wires with appropriate grounds were then placed and connected to an external stimulator [Model 5328 Programmable Stimulator, Medtronic, Minneapolis, MN]. When atrioventricular sequential pacing was required as dictated by the study protocol, a sequential demand pulse generator [Model 5330, Medtronics, Minneapolis, MN] was used. The implantable pacemaker wire was then clipped and external pacing was immediately begun. CHB was verified by a failure of anterograde conduction during a brief trial of atrial pacing.

In the control (non-blocked, non-paced) lambs, right atrial and right ventricular pacing wires with grounds were placed and connected to an external stimulator for atrial or ventricular pacing as determined by the study protocol.

Study Protocol on Neonatal Lambs:

Studies were performed in the absence of cholinergic or ß-blockade due to the influence of these agents on myocardial function and heart rate (26). Instantaneous left ventricular volume and pressure were measured simultaneously using the conductance and pressure tip catheters, respectively, and thus pressure-volume loops were generated for each cardiac cycle. Immediately prior to each data acquisition period (lasting approximately 15 seconds), the lamb was disconnected from the ventilator so that all data was collected at the same point in the respiratory cycle (end-expiration). As others who have utilized this technique in neonatal subjects have observed, these brief periods of apnea were not associated with any changes in beat to beat heart rate

suggesting that chemoreceptor stimulation by hypoxia or hypercapnia does not occur (27). Pressure-volume loops were utilized to obtain various hemodynamic/functional measurements including: end systolic volume (ESV), end systolic pressure (ESP), peak pressure (PP), end diastolic pressure (EDP), end diastolic volume (EDV), stroke volume (SV), stroke work (SW), and cardiac index (CI).

In each lamb, the ESPVR was generated by changing pressure and volume conditions within the heart via inflow occlusion. Reflex changes in heart rate observed during inflow occlusion were generally <5% and therefore were not considered significant contributors to differences in contractility due to the force-frequency relationship. The inferior vena cava was occluded by inflation of the atrial septostomy balloon with1.5-2.0 cc of NaCl solution over 10 seconds. This was performed under three inotropic conditions: at baseline (no intropic agent), with infusion of 10ug/kg/min dobutamine (ß-agonist/positive inotrope), and with infusion of 150-200ug/kg/min esmolol (ß-blocker/negative inotrope) in random order. The animal was allowed to stabilize at each pharmacologic state for at least 15 minutes (as determined by heart rate, left ventricular pressure and stroke volume) and a period of 20-30 minutes was allowed between conditions for return to baseline. At each inotropic state, the lamb was paced in various modes at varying heart rates in order to examine the effect of a change in the frequency of contraction and mode of conduction on contractility in chronically paced, CHB lambs vs controls. The following protocol was employed:

State/pace mode	SA	A/AV170-210	A 210-250	V130	V170-210	V210-250
Baseline	C	C/CHB	C	CHB	C/CHB	C/CHB
Esmolol	C	C/CHB	C	CHB	C/CHB	C/CHB
Dobutamine	C	C/CHB	C	CHB	C/CHB	C/CHB

Pacing Modes:
SA=sinoatrial node/no external pacing
A=atrial pacing
AV=atrioventricular sequential pacing
V=ventricular pacing
Lamb Type:
C=interventions performed with **control** lambs
CHB=interventions performed with **CHB, chronically paced** lambs

As many of the heart rate and pacing variations as possible were performed with each lamb given the limitations of baseline heart rate. For example, during dobutamine infusion, the lamb's native heart rate often rose to 230-250 bpm making atrial pacing in the control lamb at 170-210 bpm impossible. Furthermore, the resting heart rate of our control neonates at baseline was in the realm of 160-200 bpm (normal values at this age), so lower heart rate interventions were not always possible (26). In addition, atrioventricular pacing could only be performed up to a rate of 180 bpm due to the limitations of available sequential pacemakers/pulse generators.

Data Acquisition and Analysis:

The pressure tip catheter was connected via a conditioning amplifier [Model 5900 Signal Conditioner, Gould, Cleveland, OH] to a 12 bit analog to digital (AD) converter and to an X-Y oscilloscope [Model 1604 Digital Recording Oscilloscope, Gould, Cleveland, OH]. The conductance catheter was connected to a Sigma 5 signal conditioner processor [Leycom, Oegstgeest, The Netherlands] which supplied the current to the catheter, computed instantaneous and continuous segmental conductances, and generated analog output to the

oscilloscope for generation of pressure-volume loops and to the AD converter. Pressure and volume data input to the AD converter was digitized at 200Hz. It was then acquired and recorded on the hard disk of a Macintosh II computer utilizing software written by Teitel and Klautz in the LabView programming language [National Instruments]. Data was also analyzed using this software.

The principles behind conversion of segmental conductances to absolute volumes has been discussed extensively by previous authors. (20,28-32). In short, conductance measurements, G(t), from each segment on the conductance catheter are converted to segmental volumes, V(t), via the following formula:

$$V(t)=(1/\alpha)(L^2/\sigma_b)G(t)-Vc$$

where α is a unitless slope constant, L is the interelectrode distance on the catheter, σ_b is a measure of the conductivity of the lamb's blood as determined by the Sigma 5 from a sample of the blood in a 5 ml cuvette, and Vc is a correction term reflecting "parallel" conductance or the conductance of tissues surrounding the left ventricle. Vc is determined by transiently altering blood conductivity within the left ventricle without actually changing left ventricular volume. 0.3-0.5cc of 23% saline was injected into the left ventricle via the pulmonary arterial line. The increase in G(t) coupled with an unchanging V(t) and V(c) allow one to solve for Vc (32). Volumes obtained in this manner have been shown to be accurate in the neonatal heart as reflected by a highly linear correlation with angiographic estimations of ventricular volume (33). The conductance catheter technique is superior to other modalities of beat-to-beat (instantaneous) volume measurement because it does not alter loading conditions as angiocardiography can and is not cumbersome or technically difficult in the small neonatal heart as ultrasonic crystals can be (33). Other

modes of volume measurement such as computed tomography (CT) or radionuclide imaging cannot measure beat to beat changes in ventricular volume which is necessary for determination of ESPVR and PRSW.

The ESPVR was determined by analyzing the pressure-volume loops generated as described above. In the time varying elastance model, end systole is chosen as the point of maximal elastance where elastance is defined as:

$$\frac{P(t)}{V(t)-Vd}$$

where P(t) is time varying pressure, V(t) is time varying volume and Vd is the "unstressed" volume of the ventricle (i.e. the volume at which ventricular chamber pressure is zero). Vd cannot be directly measured and therefore was determined by an iterative technique described by Baan. (28,29). The ESPVR for each run was analyzed by performing simple linear and second order polynomial regression analyses on the ESPV points from each PV loop. The slope, Emax, of this relationship was then determined. The x-intercept of the ESPVR (i.e. Vd) was also determined but since others have suggested that the ESPVR may be non-linear under extreme pressure/volume conditions, extrapolation of the ESPVR outside the range of its measurement may produce unreliable results and hence this information was not utilized in our analysis. The preload recruitable stroke work index (PRSW) was also determined from the pressure volume loops generated by this technique. The slope of this index which relates end diastolic volume to stroke work was then determined using the same points used in the calculation of the ESPVR. A representative example of pressure volume loops generated during inferior vena caval occlusion in one lamb is given in Figure 1. It is from these loops that the ESPVR and the PRSW index were determined under each condition for each lamb.

Histological Analysis:

Following euthanasia of the study animal, the heart was harvested, preserved in a 10% formalin solution, and later divided into right and left ventricular portions (septum to left) in a uniform fashion by a single operator. The two portions were weighed. Tissue sections from both right and left ventriclar free walls and atrioventricular septum at the region of the atrioventricular node were then taken and mounted using standard techniques. Sections were stained with hematoxylin-eosin for light microscopic analysis. The atrioventricular nodal sections were further stained with Masson's trichrome blue for illumination of collagen deposition.

Statistical Analysis:

All animal weight, ventricular weight and hemodynamic data {right atrial, left atrial, pulmonary arterial, and aortic pressures; end systolic volume, end systolic pressure, peak pressure, end diastolic pressure, end diastolic volume, stroke volume, stroke work, and cardiac index} are reported as mean$\pm$standard deviation (SD). Comparisons of these measurements were made by unpaired t tests and statistical significance was assumed at a p value of <0.05.

Ventricular function analysis comparing CHB to control lambs at their baseline states was carried out by unpaired t-test with results reported as mean$\pm$SD and statistical significance was assumed at a p value of <0.05. Functional analyses of contractile reserve and baseline stress levels in the two groups and the relative importance of the atrial contribution in CHB lambs were made by multiple linear regression (MLR) using dummy variables with

effects coding (with the exception of HR variable which was continuous and not effects coded). The regression models for these analyses are outlined below.

For assessment of contractile reserve and baseline stress level, the following model was employed:

$$Y=a_o+a_gG+(a_{r1}R_1+a_{r2}R_2)+a_{d1}D_1+a_{gd1}(GxD_1)$$
$$+\Sigma a_{chb_n}L_{chb_n}+\Sigma a_{con_j}L_{con_j}$$

where Y is the dependent variable of interest {Emax, PRSW-m, cardiac index (=cardiac output/animal weight}. Variable identification and effects coding is as follows: a_0 (the x intercept) is the mean value of Y over all the runs in that data set. G is the dummy variable representing group (CHB=1, Control=-1). (R_1 R_2) is the set of variables representing heart rate: heart rate<170=(-1 -1) for the two variables respectively, 170≤heart rate≤210=(1 0), and heart rate>210=(0 1). Of note, the variables (R_1 R_2) were not included in the model for cardiac index since heart rate is a component of the definition of this variable. D_1 is the variable representing the various inotropic states with coding as follows: for the assessment of contractile reserve--that is the increase in contractility with dobutamine infusion relative to the baseline/no inotrope state--runs with dobutamine are coded D_1=1 while runs with no inotropes present are coded D_1=-1; runs with esmolol present, are excluded from this comparison. For the assessment of baseline stress level--that is the relative decrease in contractility from the baseline state resulting from blockade of catacholamine ß-receptor effects-- runs with esmolol are coded D_1=1 while runs at baseline are coded D_1=-1; runs with dobutamine present are not included in this analysis. $\Sigma a_{chbn}L_{chbn}$ are the six variables representing the seven CHB lambs which incorporate inter-animal variability into the model; coding for the first lamb is (1 0 0 0 0 0), the

second (0 1 0 0 0 0) and the seventh (-1 -1 -1 -1 -1 -1). $\Sigma a_{conj}L_{conj}$ are the set of 9 variables representing the 10 control lambs; these are coded in the same fashion as described for the CHB lambs. Finally the interaction between group type and inotropic intervention is represented by the variable GxD1; this variable is assigned a value of 1 if the interaction is present, -1 if it is not.

For assessment of the significance of atrial contribution in the CHB, chronically paced lambs, the following model was used:

$$Y=a_o+a_pP+a_{hr}HR+(a_{d1}D1+a_{d2}D2)+[a_{pd1}(PXD1)+a_{pd2}(PxD2)] +a_{phr}(PxHR)+\Sigma a_{chbn}L_{chbn}$$

where Y is the dependent variable of interest (stroke volume, stroke work, end diastolic volume, cardiac index). a_o and $\Sigma a_{chbn}L_{chbn}$ are coded the same as in the model for contractile reserve above. P is the dummy variable for pacing modality where ventricular pacing=1 and atrioventricular sequential pacing=-1. HR is the continuous variable for heart rate and is therefore not effects coded. Note: this variable is not used in the model for cardiac index for the reason stated previously. (D_1 D_2) are the variables encoding inotropic state where runs during dobutamine infusion are coded (1 0), during esmolol infusion (0 1), and without any inotropes present (-1 -1). Interactive variables PxHR, PxD1, and PxD2 are assigned a value of 1 when the interaction is present and -1 when it is not.

The coefficients for each dummy variable estimate the changes in the overall mean value of the dependent variable associated with changes in that dummy variable. In an effects coded reference system, this overall mean value is not equivalent to the baseline value of the dependent variable in control lambs; rather, it represents an overall data set mean value including both

control and CHB lambs (34,35). Coefficients measure deviations from this overall mean. Coefficient values are given along with their standard error of mean (SEM) and with their statistical significance based on a t-test for single variables or on a partial F-test for sets of variables. The partial F statistic for a set of variables is determined by adding the incremental sum of squares for these variables dividing by the number of variables in the set and dividing finally by the mean square residual (36). For both t and partial F tests, statistical significance was assumed at a p value of <0.05.

RESULTS:

Survival and Growth:

Hemodynamic, functional and histological analyses were carried out in ten control and seven CHB lambs that delivered at term. Twelve fetuses in which CHB had been created did not make it to term: two had massive hematomas at the site of intraspinal injection of anaesthesia which led to a change in the mode of anaesthesia; there were no subsequent known fetal anaesthesia related losses. The mothers of five of these fetuses appeared ill and aborted both the CHB fetus and control siblings; no signs of hydrops fetalis were found in any of the CHB fetuses at autopsy suggesting surgical, infectious and/or maternal cause for loss not treatment failure. Four CHB fetuses were aborted near term or were delivered autolyzed at term while their siblings were born alive; on post-mortem examination of the non-autolyzed fetuses, there was no evidence of hydrops. No clear cause for these four losses could be elucidated but abrupt failure of the pacemaker to pace or capture or undetected infection are distinct possibilities. The mother of one fetus had to be euthanized prior to delivery due to neurological complications of spinal anaesthesia. One lamb that expired in the perinatal period was found moribund with placental membranes covering its nares; though this loss was likely the result of mechanical asphyxia, an inadequate ventricular output (due to a fixed heart rate) in the face of increasing demand during birth cannot be ruled out.

Of the CHB lambs that survived to term, mean weight at time of study was nearly identical to that of control lambs suggesting no difference in skeletal and muscular growth between these two groups in utero and in the early neonatal period (CHB=5.52$\pm$1.20 kg; Control= 5.48$\pm$1.06 kg; p=0.92)

Hemodynamics:

Right atrial, left atrial, pulmonary artery, and aortic pressures obtained prior to ventricular function studies are shown in Table 1. There was no statistically significant difference in right atrial, left atrial, and aortic pressures between control and CHB lambs. The values obtained for these measurements are within the ranges observed by other investigators (37,38). However, there was a statistically significant difference in pulmonary arterial pressures between control and CHB lambs ($p=0.0007$) despite a large standard deviation among CHB lamb values. This large standard deviation was predominately due to a single outlying value which may represent interference of a patent ductus arteriosus with pressure measurement. If this value is removed from the analysis, the statistical significance of the pulmonary pressure difference is lost ($p=0.073$). The pulmonary arterial pressures obtained for CHB lambs are within the range observed by others whereas those for control lambs were slightly low (38).

Ventricular Function:

Baseline function: CHB and control lambs were initially compared at their baseline states (i.e. no inotropes) with CHB lambs ventricularly paced at a rate of 130 bpm and control lambs in their native state following stabilization after instrumentation and catheter placement. Comparative values for several hemodynamic and functional variables including end systolic volume, end systolic pressure, peak pressure, end diastolic pressure, end diastolic volume, stroke volume, stroke work, and cardiac index are given in Table 2. There was no significant difference in these variables between control and CHB lambs. However, cardiac index was decreased by 39% in the CHB group relative to the control group without quite achieving statistical significance ($p=0.06$). In

addition, there was no significant difference in the slopes of the ESPVR (Emax: p=0.87) and the PRSW index (PRSW-m: p=0.45) between control and CHB lambs suggesting that chronic ventricular pacing in the fetus and neonate does not adversely affect baseline contractility. The results of this analysis are given in Table 3.

Contractile Reserve: To further elucidate differences in cardiac function between CHB and control lambs, contractile reserve was determined. In the analysis of contractile reserve, the augmentation of contractile function with a positive inotropic agent from the native or baseline state of the myocardium was determined by comparing Emax and PRSW-m during dobutamine infusion to the values of these indices at the baseline/no inotrope state. In the multiple linear regression models of Emax and PRSW-m, the interactive variable GxD1 represents the difference in contractile reserve between CHB and control lambs. The values for variable coefficients in the MLR analysis of contractile reserve are presented in Table 4. Correlation coefficients (R^2) and p values for the regression equation for each index are also given. The coefficient of the variable GxD1 was not significant in the regression equation for Emax or PRSW-m suggesting that there was no difference in contractile reserve between the two groups (Emax: p=0.43; PRSW-m: p=0.13). There was also no significant difference between CHB and control lambs in the relative increase in cardiac index associated with dobutamine administration (p=0.70) indicating that changes in pump function (output) paralleled changes in contractile function. It is worthy of note that there was a significant positive inotropic effect of dobutamine on PRSW-m (p=0.04) and on CI (p<0.0001) but not on Emax reflecting differences in the functional characteristics assessed by each index and shedding light on the effect of dobutamine on myocardial function in the

newborn lamb (38,39). Finally, the coefficient reflecting group differences (CHB vs control) in the regression models for PRSW-m and CI were both statistically significant suggesting that differences in contractile function and ventricular output exist between groups for the set of data points collected during dobutamine infusion and at the baseline state (PRSW-m: G coeff=-5.06, p=0.003; CI: G coeff=-0.023, p<0.0001). However, a significantly higher mean heart rate in the control group may explain the increased contractility (force-frequency relationship) and higher cardiac output (CO=HR x SV) in this group relative to CHB lambs (CHB heart rate=176 bpm; control heart rate=213 bpm, p<0.0001).

Baseline Stress Level: The values of variable coefficients and corresponding p values in the MLR analysis of baseline stress levels are presented in Table 5. As expected, esmolol infusion (ß-blockade) resulted in a significant decrease in overall mean contractile function and cardiac index for CHB and control lamb values combined (Emax: coefficient D_1=-4.45, p=0.0002; PRSW-m: coefficient D_1=-6.46, p<0.0001; cardiac index: coefficient D_1=-0.01, p=0.0001). However, while there was no group difference in the relative decrease in contractile function with esmolol from the baseline state as reflected by the coefficient of the variable GxD1 in the regression model for Emax (p=0.24), the coefficient of this variable in the regression model for PRSW-m was significant and revealed a substantially greater decrease in contractile function with esmolol in the control group than in the CHB group (GxD1 coefficient=9.36, p<0.0001). As a consequence, the mean value for PRSW-m for this set of data points was significantly lower among control lambs than CHB lambs reflecting the greater decrease in this index in the control group with esmolol infusion (p<0.0001). Despite the difference in the change in PRSW-m with ß-blockade, there was no significant difference between control and CHB lambs in the relative decrease in

cardiac index with esmolol infusion (p=0.12). Pump function, therefore, did not parallel the differential change in contractile function between groups with esmolol infusion. Of note, the mean value for cardiac index for this set of data points was lower for CHB lambs than controls as reflected by the coefficient for the variable G in the regression model for cardiac index (p=0.002). However, this is the result of a lower mean heart rate for CHB lambs than control lambs over this set of data points (CHB heart rate=172 bpm, Control heart rate=190 bpm; p=0.04).

Atrial Contribution: In order to shed greater light on differences in cardiac performance that are secondary to a lack of atrio-ventricular (AV) synchrony in the CHB lambs, the contribution of a synchronized atrial systole to ventricular output at various inotropic states was assessed. The results of this analysis are given in Table 6. CHB lambs were alternately ventricularly paced and AV sequentially paced for short periods during this portion of the study. There was no significant change in stroke volume (p=0.42), stroke work (p=0.44), end diastolic volume (p=0.96), or cardiac index (p=0.95) in CHB lambs with the loss of atrio-ventricular synchrony. This finding persisted regardless of the rate at which the lamb was paced in each mode (ventricular or AV sequential); this result is reflected in the non-significant coefficients for the interactive variable PxHR in the regression models for stroke volume (p=0.38), stroke work (p=0.46), and end diastolic volume (p=0.75). Alteration of the inotropic state of these CHB lambs with dobutamine or esmolol had significant but similar positive and negative effects (respectively) on stroke volume, stroke work, and cardiac index whether the lambs were ventricularly or AV sequentially paced; this result is reflected in the non-significant coefficients for the interactive variables PxD_1 and PxD_2 in the regression models for these measures.

In the analyses of contractile reserve, baseline stress levels and atrial contribution, interanimal variability was significant and was therefore included in the regression model for each index of contractility (Emax, PRSW-m) as well as for each load dependent measure (cardiac index, stroke volume, stroke work, end diastolic volume). The F statistic and corresponding p value for these variables are given for each MLR analysis.

Ventricular Weights:

Right and left ventricular weights for control and CHB lambs are given in Table 7. There was no statistically significant difference in right or left ventricular weights between these two groups (p=0.36 and 0.55, respectively) suggesting that muscular hypertrophy/hyperplasia did not occur to any significant extent as a consequence of chronic fetal and neonatal ventricular pacing.

Histological Analysis:

Light microscopic analysis of right and left ventricular free wall sections from CHB myocardium revealed no evidence of myofiber or myofibrillar disarray, dystrophic calcifications or any other abnormality not present in age matched controls. By Masson's trichrome stain, the atrioventricular node in the CHB lambs revealed loss of conductive fibers and extensive fibrosis corresponding to a loss of anterograde conduction. There was also a question of a small area of infarction in the region of the atrioventricular node in a few CHB specimens, but this was unclear and may have been artifact.

DISCUSSION:

The theoretical benefits of *in utero* pacing as a treatment modality for congenital complete atrioventricular block are clear. In contrast to pharmacological intervention with its maternal toxicities and unreliability and to emergent delivery with all the concomitant complications of premature infant, *in utero* pacing can reliably increase fetal heart rate and allow for continued development of the fetus to term. As Rudolph and Heymann have described, maintenance of an adequate heart rate is of particular importance in the fetus because the ability of the fetus to increase stroke volume in response to a decrease in ventricular output is limited. In their studies, as heart rate declined, there was a linear reduction in right and left ventricular outputs (11). Once ventricular outputs fall and hydrops fetalis develops, the prognosis for fetal survival becomes increasingly grim. A multicenter retrospective study of fetuses with prenatally detected CCAVB noted 0 survivors among 22 fetuses with CCAVB who developed hydrops fetalis (10).

But despite the theoretical advantages, there are many issues which must be addressed prior to implementation of such therapy. The maternal complications and tocolytic issues associated with fetal surgery are discussed extensively elsewhere and and this study did not attempt to further investigate these questions (13-16). Rather, our studies focused upon ventricular pacing and its effects on survival, hemodynamics, ventricular function and cardiac histopathology in the developing fetus and neonate. As discussed previously, CHB fetuses were ventricularly (VVI) not sequentially (V-demand or dual chambered) paced during this study because no commercially available pacemaker of appropriate size can reliably capture and pace sequentially with the high sinoatrial rates often present in the late gestation fetus. In addition the

increased output required for dual chambered pacing would decrease the longevity of the pacemaker thus increasing the risk of pacemaker failure.

Many have described potential limitations on optimal cardiac performance as a consequence of ventricular pacing in both pediatric and adult models (17, 18, 40-44). The specific etiology of these limitations are two-fold. Since the VVI mode does not sense atrial contraction, this form of pacing inherently results in atrio-ventricular (AV) dysynchrony. Ventricular contraction and atrioventricular valve closure may occur prior to the completion of ventricular filling resulting in a decreased end diastolic volume . This may result in a decrease in ventricular output (Frank-Starling) and a change in ventricular geometry due to irregular filling (17). In addition, right and left atrial pressures may rise as a consequence of incomplete emptying into the ventricular chamber. This elevation in pressure may be augmented by AV valve regurgitation and by persistent atrial contraction against a closed AV valve (45). AV valve regurgitation can occur as a consequence of a delay in valve closure relative to ventricular contraction. Normally, atrial contraction prior to the onset of systole assists in the mechanical closure of the AV valve; in the absence of such coordinated contraction, AV valve closure does not occur until ventricular contraction creates a ventriculo-atrial pressure gradient. This delay increases the chance of retrograde flow. The resulting increase in atrial pressures can lead to an elevation in atrial natiuretic peptide (ANP) levels which, through vasodilatory effects, can cause a decrease in systemic pressures (46,47). This augments the potential systemic hypotension that can result from decreased ventricular output resulting in the "pacemaker syndrome" (40). Chronic elevation in atrial pressures can also lead to atrial enlargement and thereby increase the risk of atrial arrhythmias (41). This could be problematic if AV sequential pacing is desired in post-natal life.

Ventricular pacing also alters the normal ventricular activation sequence thereby changing both the pattern of ventricular contraction and the electrophysiologic environment of the developing myocardium. Studies in a young (3-4 month old) canine model have demonstrated changes in atrial conduction and an increase in ventricular refractory periods in response to chronic ventricular pacing (17). These changes in electrophysiologic parameters may be significant if they interfere with AV synchronous pacing in later life. In addition, prominent histopathological changes have been observed in older chronically ventricularly paced hearts. The alteration in the sequence of contraction results in a change in myocardial stress vectors which can lead to myofibrillar disarray and dystrophic calcifications as part of a more diffuse myopathic process (17). It has also been shown that ectopic ventricular stimulation via abnormal depolarization of muscle fibers can impair ventricular function in adult models (18,43,48).

The question then arises: Can the experience with chronic ventricular pacing in older pediatric and adult models be extrapolated to the later gestation fetal heart? In the opinion of this author, the answer is no. The fetal heart is quite distinct in terms of hemodynamics, physiologic responses and sarcomere structure and function and is undergoing rapid change and development during this period. Most prominent among these differences are the unique fetal flow patterns and the changes in these patterns with birth. In the fetus, the right atrium fills not only the right ventricle but also the left atrium through the foramen ovale; as a result changes in atrial pressure and trans-atrial flow due to asynchronous contraction may not be as predictable as with an in-series circulation. Furthermore, the fetal myocardium is structurally immature and changing dramatically during this period (37,49-52). Sarcomeres are fewer in number per gram of cardiac tissue and myofiber disarray has been observed

normally in the fetal myocardium (53). In addition, the calcium sequestration and release function of the sarcoplasmic reticulum (SR) is incompletely developed in the fetus; it has been suggested that, however, myofilament calcium sensitivity is enhanced in the fetal heart thereby compensating somewhat for SR immaturity (50). Sympathetic innervation of the myocardium is also incomplete and the B-adrenoreceptor complex does not function at adult levels (49). These incompletely developed structures in the fetus may be more adaptable to alterations in conduction and contraction patterns than the more mature heart.

Physiologic responses in the fetus are also quite distinct from those in the adult and further complicate attempts to draw conclusions from studies of chronic ventricular pacing in older models. For example, while the Frank-Starling mechanism has been shown to be operative in the fetus, volume loading appears to be of limited utility in increasing stroke volume in the fetal heart (54-57). However, studies have suggested that the atrial contribution to ventricular filling and ventricular output in the fetal heart may be particularly important (11,58). In addition, as mentioned previously, ventricular output in the fetus is quite dependent upon heart rate due to its limited ability to increase SV in response to decreasing outputs (11,57). At birth, ventricular outputs must increase dramatically to meet increasing demand. Augmentation of myocardial contractility by circulating hormones, catacholamines and oxygen at the time of birth is a significant factor in this increase in ventricular output, but elevations in heart rate also play a major role in the matching of output to demand (38). Fixed rate VVI pacing could compromise the fetus at the time of birth by preventing an adequate increase in ventricular output. All of these issues unique to the fetus and neonate coupled with the possibility for hemodynamic, functional, and histopathological sequelae with chronic ventricular pacing as

suggested by studies in older models, necessitated a thorough investigation of the effects of chronic ventricular pacing in the fetus prior to its implementation as a treament modality.

In this study, mortality among the fetuses in which complete heart block was created exceeded the spontaneous abortion rate of approximately 30-40% observed for ewes of this species even when clearly technical/ anaesthesia related deaths were excluded. However, since no evidence of hydrops fetalis was found on post-mortem examination in any of the fetuses, it does not appear that this excess mortality was a consequence of treatment failure; surgical complications including undetected infection are more likely etiologies for these deaths. The only perinatal death appeared to be the result of mechanical asphyxiation; while an inadequate ventricular output may have contributed to this lamb's demise, the other seven chronically paced, CHB lambs that survived to term did not appear to have difficulty meeting the increased demand for cardiac output at birth despite a fixed heart rate substantially lower than that observed in normal fetuses in the perinatal period (59). Through enhanced contractility and perhaps the Starling effect, these lambs were able to increase stroke volume and consequently ventricular output during this critical time.

At the time of study at 4-8 days of life, there was no sign of cardiac dysfunction as a consequence of chronic ventricular pacing. Atrial chamber pressures were not elevated suggesting that there was adequate anterograde flow, no hemodynamically significant regurgitant flow across AV valves, and no significant atrial enlargement. Neither end diastolic volume, stroke volume, cardiac index, nor systemic (aortic) pressure was significantly different between control and chronically paced, CHB lambs further affirming the conclusion that anterograde flow was not compromised by either the chronicity or the lack of synchrony of the pacing modality employed. While pulmonary

arterial pressures appeared higher in CHB vs control lambs on initial analysis, the loss of statistical significance of this difference with removal of a markedly outlying value suggests that a patent ductus interference in pulmonary arterial pressure measurement may indeed have occurred and that no real difference in PA pressures between control and CHB lambs existed.

Furthermore, chronic ventricular pacing in the CHB lamb did not adversely affect contractile function as measured by the end-systolic pressure-volume relationship (ESPVR) and the preload recruitable stroke work (PRSW) index. Both baseline contractility and contractile reserve as measured by these indices were comparable between the two groups. Similarly, the baseline stress level, the decrease in baseline contractility with esmolol infusion, was not significantly different between CHB and control lambs as determined by the Emax of the ESPVR. However, using the PRSW index, this difference was quite significant. This apparent disparity between the two indices illustrates that, although they are both considered measures of contractile state, they are unique in terms of their load sensitivity, inotropic sensitivity and the portion of the cardiac cycle they best reflect (30). The ESPVR has been demonstrated to be less load sensitive than PRSW, but the latter appears to be more sensitive to changes in contractile state (30). Furthermore, as its name suggests, the ESPVR is a systolic (ejection) phase index, while the PRSW tends to reflect both systolic and diastolic function. Any one of these differences alone or in combination could explain the disparity in baseline stress level results. The greater decrease in baseline contractile function with esmolol infusion in control lambs as detected by the more inotropically sensitive and pan-cyclic PRSW index may be the product of differences in ß-receptor function or quantity. Normally, cardiac function in the newborn lambs is characterized by a high resting ß-adrenergic state, with little reserve for augmentation in contractility with positive

inotropic agents, but with (theoretically) profound reserve for a decrease in contractility with ß-blockade (39). While these observations were also made in our study among normal lambs, CHB lambs did not respond as dramatically to ß-blockade; diminished ß-receptor sensitivity or numbers could explain this result, though this study did not provide any evidence to support this hypothesis. The practical significance of this finding is unclear, however, for the differential reduction in contractility with ß-blockade between control and CHB lambs was not reflected in ventricular outputs; cardiac index decreased similarly in both groups with esmolol infusion. Finally, acute AV synchronous pacing in the chronically ventricularly paced, CHB lambs did not improve filling or ejection, affirming the theory that mechanisms exist to compensate for the loss of a synchronous atrial contraction and maintain output (e.g increased contractility, Starling effect) (18).

This data suggests that fetal epicardial ventricular pacing in the setting of complete heart block can result in the delivery of a hemodynamically stable lamb with preserved ventricular function and morphology. In fact, given this result and the failure of AV synchronous pacing to acutely improve filling or output in the newborn CHB lamb, sequential pacing may not necessarily be superior to ventricular pacing in the fetus and neonate. However, the leap to clinical application cannot be made on the basis of these results alone. Clearly, the extrapolation of results from a lamb model to a human fetus is difficult given possible differences in physiologic and functional responses. Furthermore, since the sequelae of congenital complete atrioventricular block would likely manifest early in gestation following cardiac development, it seems necessary to investigate the effects of a longer period of *in utero* pacing. In addition, electrophysiologic studies should be done to determine whether the changes in atrial conduction and ventricular refractory periods observed in

older models with chronic ventricular pacing occur in the fetus and neonate. In summary, this study has provided promising results and will serve as a stepping stone for further investigation into the use of epicardial pacing as a treatment modality for congenital complete atrioventricular block.

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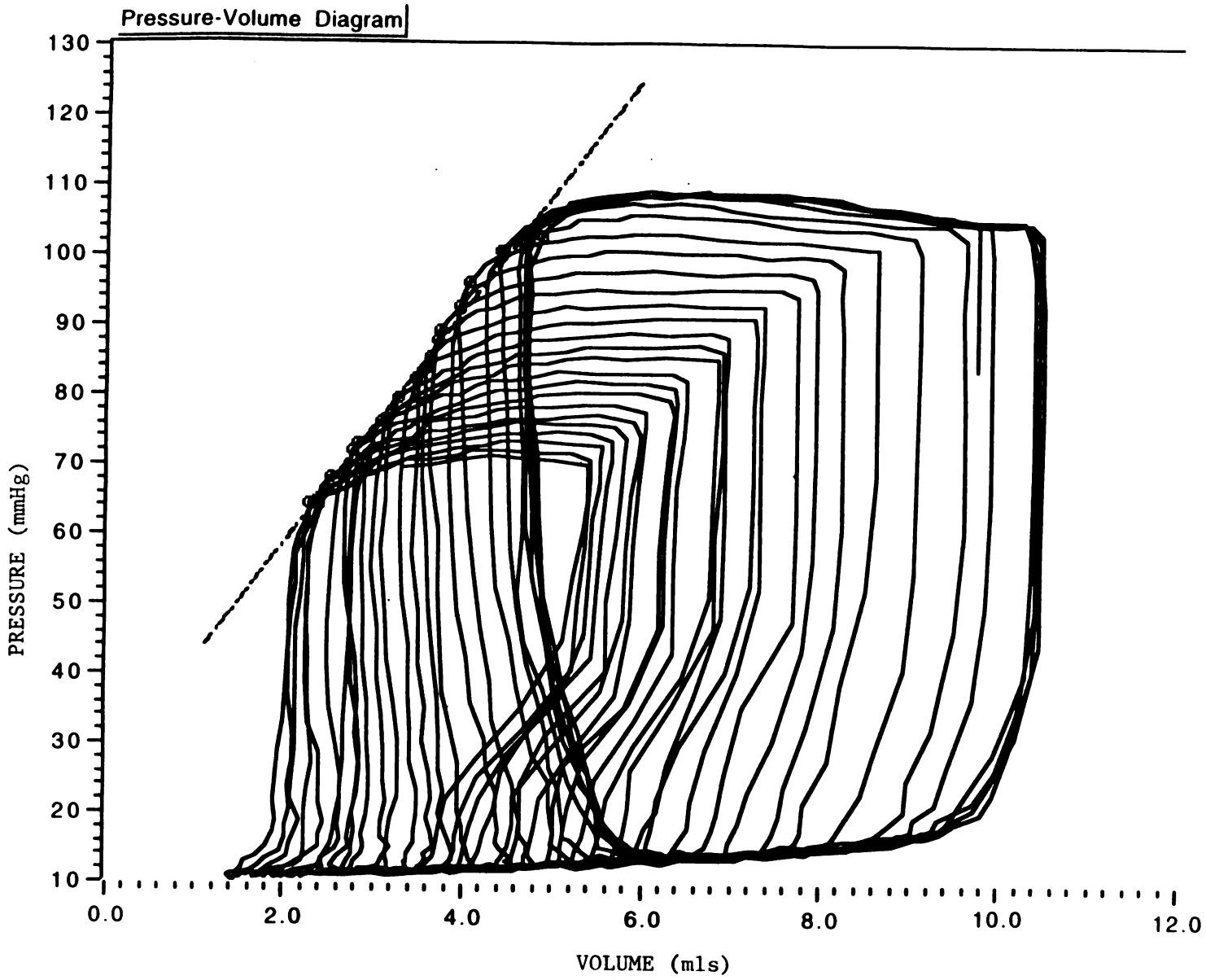

Figure 1: Representative Pressure-Volume Loops obtained during inferior vena caval occlusion in one lamb

Table 1: Atrial, Pulmonary Artery, Aortic Pressures

	Right Atrium (mmHg)	Left Atrium (mmHg)	Pulmonary Artery (mmHg)	Aorta (mmHg)
CHB (n=7)	5.42±2.0	6.14±2.91	21.0±6.99	63.5±5.97
Control (n=10)	5.9±2.69	7.20±3.71	14.83±2.79	71.5±12.23
p-value	0.68	0.49	0.0007	0.08

* values are given as mean ± standard deviation; p value significant at $p<0.05$

Table 2: Baseline Hemodynamic Parameters

	ESV (ml)	ESP (mmHg)	Peak P (mmHg)	EDP (mmHg)	EDV (ml)	SV (ml)	SW (ml-mmHg)	Cardiac Index (ml/kg)
CHB (n=7)	5.14±4.49	95.68±33.1	102.66±33.3	12.07±24.0	9.17±5.63	4.32±1.96	331.66±176.24	106.0±44.0
Control (n=10)	4.76±2.98	74.79±13.8	87.23±13.71	2.46±13.55	9.68±5.05	5.48±2.81	388.57+209.47	173.0±78.0
p value	0.83	0.09	0.20	0.31	0.85	0.36	0.57	0.06

* values given as mean ± standard deviation; p value significant at $p<0.05$.
**ESV=end systolic volume; ESP=end systolic pressure; Peak P=peak pressure; EDP=end diastolic pressure; EDV=end diastolic volume; SV= stroke volume; SW=stroke work

Table 3: Baseline Contractile Function: Emax, PRSW-m

	Emax (mmHg/ml)	PRSW-m (mmHg)
CHB (n=7)	22.95±9.72	56.31±16.40
Control (n=10)	25.05±31.35	63.19±18.74
p-value	0.87	0.45

*values given as mean ± standard deviation; p values signifiant at $p<0.05$.

Table 5: Baseline Stress Level: Results of Multiple Linear Regression Analysis for Emax, PRSW-m and Cardiac Index

Emax

Variable	a_0	G	D_1	GXD_1	R_1	R_2	Lamb Variables $\Sigma a_{chb} L_{chb}$	Lamb Variables $\Sigma a_{con} L_{con}$
a	21.34	2.76	-4.45	2.73	-3.17	5.91	F statistic for	coefficients combined =1.21
SEM	1.30	1.88	1.13	2.30	1.50	1.56		p > 0.05
p-value		0.15	0.0002	0.24	0.04	0.0003		

*105 episodes from 17 lambs $R^2=0.75$ p<0.0001

PRSW-m

Variable	a_0	G	D_1	GXD_1	R_1	R_2	Lamb Variables $\Sigma a_{chb} L_{chb}$	Lamb Variables $\Sigma a_{con} L_{con}$
a	55.13	8.40	-6.46	9.36	-4.24	8.16	F statistic for	coefficients combined =17.70
SEM	1.26	1.82	1.10	2.22	1.45	1.51		P< 0.01
p-value		<0.0001	<0.0001	<0.0001	0.004	<.0001		

*105 episodes from 17 lambs $R^2=0.76$ p<0.0001

Cardiac Index

Variable	a_0	G	D_1	GXD_1	Lamb Variables $\Sigma a_{chb} L_{chb}$	Lamb Variables $\Sigma a_{con} L_{con}$
a	0.13	-0.02	-0.01	0.01	F statistic for	coefficients combined =2.10
SEM	0.004	0.006	0.003	0.07		p < 0.05
p-value		0.002	0.0001	0.12		

*105 episodes from 17 lambs $R^2=0.84$ p<0.0001

**The coefficients for Emax are in mmHg/ml, for PRSW-m in mmHg, and for cardiac index in ml/kg. The regression equations were all statistically significant (p values given) with correlation coefficients R^2 as indicated. Variable identities are detailed in Methods: Statistical Analysis.

Table 6: Atrial Contribution: Results of Multiple Linear Regression Analysis for Stroke Volume, Stroke Work, End Diastolic Volume and Cardiac Index

Stroke Volume

Variable	a_0	P	D_1	D_2	HR	PxD_1	PxD_2	PxHR	$\Sigma a_{chb} L_{chb}$	
a	6.40	0.67	0.95	-0.712	-0.011	0.36	-0.20	-0.004	Fstatistic for	coefficients combined=29.5
SEM	0.84	0.83	0.34	0.23	0.005	0.32	0.21	0.005		p<0.01
p-value		0.42	0.007	0.003	0.03	0.26	0.34	0.38		

*70 episodes from 7 CHB lambs $R^2=0.90$ p<0.0001

Stroke Work

Variable	a_0	P	D_1	D_2	HR	PxD_1	PxD_2	PxHR	$\Sigma a_{chb} L_{chb}$	
a	479.52	50.96	71.90	-65.38	-1.00	36.52	-18.55	-0.30	Fstatistic for	coefficients combined=25.8
SEM	66.90	66.16	27.12	17.84	0.40	25.66	16.53	0.40		p<0.01
p-value		0.44	0.01	0.0006	0.02	0.16	0.27	0.46		

*70 episodes from 7 CHB lambs $R^2=0.89$ p<0.0001

End Diastolic Volume

Variable	a_0	P	D_1	D_2	HR	PxD_1	PxD_2	PxHR	$\Sigma a_{chb} L_{chb}$	
a	12.54	0.07	-0.38	0.75	-0.02	-0.09	0.10	-0.003	Fstatistic for	coefficients combined=50.2
SEM	1.34	1.32	0.54	0.36	0.0008	0.51	0.33	0.008		p<0.01
p-value		0.96	0.49	0.04	0.01	0.86	0.76	0.75		

*70 episodes from 7 CHB lambs $R^2=0.95$ p<0.0001

Cardiac Index

Variable	a_0	P	D_1	D_2	PxD_1	PxD_2	$\Sigma a_{chb} L_{chb}$	
a	0.14	-0.0005	0.04	-0.03	0.01	-0.005	F statistic for	coefficients combined=25.6
SEM	0.009	0.007	0.01	0.009	0.01	0.008		p<0.01
p-value		0.95	0.003	0.0003	0.36	0.55		

*70 episodes from 7 CHB lambs $R^2=0.87$ p<0.0001

*The coefficients for stroke volume are in mls; for stroke work, in ml-mmHg; for end diastolic volume, in mls; and for cardiac index in mls/kg. The regression equations were all statistically significant (p values given) with correlation coefficients R^2 as indicated. Variable identities are detailed in Methods: Statistical Analysis.