Fetal Ventricular Diastolic Filling Characteristics in a Primate Model: The Role of Fetal Heart Rate and Pulmonary Vascular Impedance

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Abstract

Objectives: We hypothesized that fetal ventricular diastolic filling characteristics are related to fetal heart rate (FHR) and pulmonary vascular impedance. **Methods:** Rhesus monkeys underwent Doppler ultrasonography at near-term gestation. Tricuspid (TV) and mitral valve (MV) blood velocity waveforms were used to calculate maximum velocity (V-max) and velocity time integral (VTI) E/A ratios (early filling/ventricular filling during atrial contraction) at baseline and during maternal hyperoxygenation. Right pulmonary artery (RPA) pulsatility indices (PIs) and FHR were measured. **Results:** Hyperoxygenation significantly decreased FHR and RPA PI. The TV V-max and the MV VTI E/A ratios increased significantly and correlated with a decrease in FHR but not with a decrease in RPA PI. The TV and MV A-wave V-max decreased during hyperoxygenation and their decrease correlated significantly with a drop in FHR. **Conclusions:** Fetal ventricular diastolic filling characteristics are related to FHR but not to pulmonary vascular impedance.

Keywords

Echocardiography, Doppler, fetal circulation, physiology, pregnancy

Introduction

Ventricular filling characteristics are commonly used as an indirect measure of diastolic cardiac function in adults.¹ The fetal circulation contains unique physiologic features and the factors affecting ventricular inflow patterns are poorly understood. In the first 9 weeks of pregnancy, the filling of the ventricle depends entirely on the contraction of the atrium (A-wave), which is seen as a monophasic inflow pattern. Toward the end of the first trimester the ventricular inflow pattern becomes biphasic, with the addition of the E-wave, which represents the early filling of the ventricle during the active relaxation phase of diastole.² The relative contributions of the E-wave (early ventricular filling) and A-wave (ventricular filling during atrial contraction) to diastolic filling change with advancing gestation, resulting in an increase in the E/A ratio. After birth, the continued increase in the mitral valve (MV) E/A ratio has been attributed to the increase in the E-wave filling of the left ventricle.³ Although a similar increase is initially seen in the tricuspid valve (TV) E/A ratio, it has been shown to immediately plateau after birth.⁴

In the current study, we used a near-term experimental primate model in which the fetal heart rate (FHR) and pulmonary circulation were modified by increasing fetal oxygenation, to

test the hypothesis that fetal right and left ventricular diastolic filling characteristics are related to FHR and pulmonary vascular impedance.

Methods

Subjects

A total of 23 rhesus macaque monkeys (*Macaca mulatta*) with singleton gestations underwent fetal cardiac Doppler ultrasonography at 150 to 157 gestational days (term gestation is 165 days). Subjects were housed at the Oregon National Primate Research Center. The animal care program is compliant with federal and local regulations regarding care and use of research animals and is accredited by AAALAC (Association

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Table 1. Changes in Cardiac Indices With Hyperoxygenation

Variable	Baseline Mean (SD)	Hyperoxygenation Mean (SD)	P Value
RPA PI	30.70 (21.67)	6.11 (3.74)	.0001
DA PI	2.37 (0.33)	2.72 (0.37)	.001
RVCO (mL/min)	144 (33)	131 (30)	.008
LVCO (mL/min)	90 (25)	82 (24)	.052
FHR (bpm)	165 (20)	149 (16)	.0001

Abbreviations: RPA, right pulmonary artery; PI, pulsatility index; DA, ductus arteriosus; RVCO, right ventricular cardiac output; LVCO, left ventricular cardiac output; FHR, fetal heart rate.

for Assessment and Accreditation of Laboratory Animal Care) International. The Oregon Health and Science University Animal Care and Use Committee approved all experiments reported in this article.

Each subject was anesthetized for ultrasound examination. Anesthesia was induced and maintained with a 10 mg/kg intramuscular injection of Ketamine HCl (Ketaved, Bioniche Teoranta, Inverin, Co Galway, Ireland). Once anesthetized, each subject was positioned in dorsal recumbency and intubated with an endotracheal tube. Measurements were obtained while subjects breathed room air, and then repeated while subjects breathed 100% oxygen delivered via a non-rebreathing anesthesia circuit to decrease pulmonary vascular resistance and to increase pulmonary venous return.⁵ Examinations were completed within 40 minutes. After ultrasound examination, subjects recovered from anesthesia and were returned to their home cages.

Ultrasonographic Measurements

Image-directed pulsed and color Doppler equipment (GE Voluson 730 Expert, Kretztechnik, Zipf, Austria) with a 5 to 9 MHz sector probe was used for ultrasonographic data collection. The lowest high-pass filter level was used (100 Hz) and an angle of less than or equal to 15 degrees between the vascular flow and Doppler beam was deemed acceptable.⁶ After baseline ultrasonographic measurements were collected, fetal oxygenation was increased through maternal hyperoxygenation with 100% oxygen. After a period of 5 minutes, the primates were continued on the 100% oxygen, while ultrasonographic data identical to baseline measurements were obtained.

Ventricular diastolic filling was evaluated using Doppler tracings of blood velocity waveforms through the TV (Tricuspid valve) and the MV. Tricuspid valve and MV E- and A-wave maximum velocities (V-max; m/s) and velocity time integrals (VTIs; cm) were obtained for the calculation of the corresponding E/A ratios.⁷

Blood velocity waveforms for the ductus arteriosus (DA) and right pulmonary artery (RPA) were obtained to calculate their pulsatility index (PI = [peak systolic velocity – end diastolic velocity]/time-averaged maximum velocity over the cardiac cycle) values. The RPA blood velocity waveforms were collected in the proximal part of the RPA immediately after the bifurcation of the main pulmonary artery. Fetal heart rate (FHR) was measured from the RPA blood velocity waveform.

The left ventricular isovolumic relaxation time (IVRT), filling time (FT), isovolumic contraction time (IVCT), ejection time (ET), and total cardiac cycle were obtained.⁷ Diastolic function was evaluated by calculating the left ventricular IVRT %, which is the proportion of the total cardiac cycle comprised by the IVRT or the time needed for ventricular pressure to drop from a systemic to an atrial level.⁸ Systolic function was evaluated by calculating the IVCT %, where the IVCT represents the time needed for ventricular pressure to rise from an atrial level to a systemic level. Global ventricular function was evaluated by the index of myocardial performance (IMP = [IVRT + IVCT]/ET), which describes the combined systolic and diastolic function of the heart.⁹

To calculate fetal cardiac outputs, pulmonary valve (PV) and aortic valve (AoV) diameters were measured from Doppler images of these valves in systole using the leading edge-to-leading edge method. Mean values of 3 separate measurement were used to calculate the cross-sectional area (CSA = [valve diameter/2]² × π) of each valve. Cross-sectional area calculations were based on the assumption that the cross sections of the valves were circular. From the blood flow velocity waveforms across the PV and AoV, VTIs were obtained by planimetry of the area underneath the Doppler spectrum. Volumetric blood flows across the PV (right ventricular cardiac output [RVCO], mL/min) and AoV (left ventricular cardiac output [LVCO], mL/min) were calculated using the formula (CSA × VTI × FHR).⁶ All ultrasonographic measurements were performed by a single investigator (J.R.).

Statistical Analysis

SPSS (version 17) was used for statistical analysis. Comparisons between baseline and hyperoxygenation Doppler measurements were made with paired *t* tests. Pearson correlation coefficients were calculated for the change in various cardiac parameters from baseline to hyperoxygenation. Statistical significance was established at a *P* < .05. All values are given as mean (standard deviation).

Results

Maternal hyperoxygenation resulted in a significant reduction in the RPA PI and FHR and a significant increase in DA PI. Right ventricular cardiac output significantly decreased with hyperoxygenation, whereas LVCO remained unchanged (Table 1).

Table 2. Changes in Ventricular Filling Characteristics and Ventricular Functional Parameters With Hyperoxygenation

Variable	Baseline Mean (SD)	Hyperoxygenation Mean (SD)	P Value
TV V-max E (m/s)	0.41 (0.07)	0.40 (0.07)	.350
TV V-max A (m/s)	0.51 (0.09)	0.44 (0.08)	.0001
TV V-max E/A	0.82 (0.17)	0.92 (0.20)	.030
TV VTI E (cm)	1.96 (0.88)	2.26 (0.92)	.110
TV VTI A (cm)	2.83 (0.72)	2.39 (0.58)	.009
TV VTI E/A	0.79 (0.49)	1.04 (0.51)	.048
TV VTI Total (cm)	4.78 (0.67)	4.65 (0.94)	.451
MV V-max E (m/s)	0.36 (0.06)	0.33 (0.05)	.066
MV V-max A (m/s)	0.49 (0.1)	0.43 (0.07)	.004
MV V-max E/A	0.75 (0.16)	0.80 (0.15)	.330
MV VTI E (cm)	1.82 (0.80)	2.09 (0.75)	.056
MV VTI A (cm)	2.77 (0.685)	2.45 (0.51)	.050
MV VTI E/A	0.70 (0.33)	0.89 (0.36)	.017
MV VTI Total (cm)	4.57 (1.04)	4.57 (0.84)	1.000
IVRT %	10.2 (2.1)	11.2 (1.9)	.131
IVCT %	6.7 (1.9)	6.5 (2.0)	.607
IMP ([IVRT + IVCT]/ET)	0.40 (0.1)	0.40 (0.1)	.729

Abbreviations: TV, tricuspid valve; V-max, maximum velocity; E, early ventricular filling; A, atrial contraction ventricular filling; VTI, velocity time integral; MV, mitral valve; IVRT %, isovolumic relaxation time %; IVCT %, isovolumic contraction time %; IMP, index of myocardial performance; ET, ejection time.

The TV V-max and VTI E/A ratios significantly increased with hyperoxygenation. The TV E-wave V-max remained unchanged, whereas there was a significant decrease in the A-wave V-max. The MV VTI E/A ratio increased, but the MV V-max E/A ratio remained unchanged, which was because of the relative decreases in both the E and A-wave maximum velocities. Left ventricular diastolic, systolic, and global functions were not affected by hyperoxygenation as demonstrated by the unchanged IVRT %, IVCT %, and IMP (Table 2).

The change in FHR with hyperoxygenation was negatively correlated with the change in TV V-max E/A ratio, where a larger decrease in FHR was associated with a larger increase in the TV E/A ratio (Figure 1). There was no correlation between the change in FHR and the change in the TV E-wave V-max (Figure 2A); however, there was a positive correlation between the change in FHR and the change in the TV A-wave V-max (Figure 2B). The decrease in FHR with hyperoxygenation was correlated with the increase in the MV VTI E/A ratio and the decrease in the MV A-wave Vmax (Table 3).

The change in RPA PI with hyperoxygenation was not correlated with the changes seen in either TV and MV V-max E/A ratios or VTI E/A ratios, nor the change in TV and MV E-wave V-max or A-wave V-max (Figure 3 and Table 3). No correlation was found between the change in FHR and the change in RPA PI ($r = .231$, $P = .302$).

Discussion

In the current study, we showed that maternal hyperoxygenation led to a decrease in FHR and pulmonary vascular impedance. Right ventricular filling characteristics changed with hyperoxygenation, including an increase in the TV V-max and VTI E/A ratios. We found that the increase seen in the TV V-max E/A ratio was the result of a decrease in the A-wave

V-max, whereas the E-wave V-max remained unchanged. Although the increase in the TV V-max E/A ratio significantly correlated with the decrease in the FHR, there was no such association between the TV V-max or VTI E/A ratios and pulmonary vascular impedance. During hyperoxygenation, the MV VTI E/A ratio increased and this change was correlated with a decrease in FHR. However, both MV V-max and VTI E/A ratios were not related to the decrease in pulmonary vascular impedance and the associated relative increase in pulmonary venous blood return contribution to left ventricular preload.

Unlike previous studies, which have investigated changes in ventricular inflow blood velocity waveforms with advancing gestation, this study investigated the immediate changes in ventricular filling characteristics following a decrease in FHR and a reduction in pulmonary vascular impedance induced by maternal hyperoxygenation. It has been shown in human pregnancies that maternal hyperoxygenation after 32 weeks gestation significantly decreases pulmonary vascular impedance and increases pulmonary volume blood flow, thus modifying right ventricular afterload. In addition, maternal hyperoxygenation changes left ventricular preloading by increasing pulmonary venous volume blood flow return and decreasing volume blood flow across the foramen ovale into the left atrium.⁵ In the current study, the TV V-max and VTI E/A ratios increased significantly, indicating an increased contribution of early ventricular filling to the right ventricular output. Unlike previous observational studies, we were able to demonstrate that the immediate increase in the TV V-max E/A ratio seen with hyperoxygenation was the result of a decrease in the A-wave V-max, rather than the traditionally accepted increase in E-wave V-max. In line with a prior study of fetal sheep that demonstrated that hypoxia can augment atrial contractility,¹⁰ our study suggests that an increase in fetal oxygenation can have an opposite effect on atrial contractility.

Figure 1. Correlation between the change in the tricuspid valve (TV) maximum velocity (V-max) E/A ratio and the change in fetal heart rate (change = baseline value – hyperoxygenation value). $r = -.664$, $P = .001$.

Although some researchers indicate that the longitudinal increases in the E/A ratio during gestation are due to structural changes in the heart that lead to improved ventricular compliance and relaxation,^{4,11-13} we showed that the immediate changes in the E/A ratio seen with hyperoxygenation are independent of ventricular diastolic function. If ventricular relaxation and compliance were improved, we would anticipate seeing a decrease in IVRT % and an increase in the E-wave V-max. However, both of these parameters remained unchanged during the experiment. These findings are consistent with the longitudinal study of 49 fetuses from 14 weeks to term gestation, which showed that despite a decrease in FHR, diastolic function remained unchanged.⁸

We found that the increase in the TV V-max and MV TVI E/A ratios correlated with a decrease in FHR, which is agreement with prior studies.¹⁴ A longitudinal study of 79 fetuses demonstrated that FHR gradually decreases throughout gestation and that these changes coincided with serial increases in the E/A ratio.⁴ Although the effect of FHR on ventricular diastolic filling characteristics has been recognized, the exact mechanism of how it alters these characteristics is less understood. With hyperoxygenation, we were able to show that the decrease in FHR was correlated with an increase in the TV V-max and MV TVI E/A ratios. The decrease in the A-wave V-max, which was responsible for the observed increase in the TV V-max E/A ratio in this study, was also correlated to the change in FHR. This relationship was also demonstrated in newborns, where the heart rate was significantly associated with A-wave V-max but not with E-wave V-max.³

In this study, the reduction in pulmonary vascular impedance seen with hyperoxygenation was not correlated with the changes in ventricular filling characteristics. Initially, we hypothesized that pulmonary vascular impedance was related to right ventricular filling characteristics, given that a decrease

Figure 2. A, Correlation between the change in the tricuspid valve (TV) E-wave maximum velocity (V-max) and the change in fetal heart rate (change = baseline value – hyperoxygenation value). $r = -.219$, $P = .328$. B, Correlation between the change in the tricuspid valve (TV) A-wave maximum velocity (V-max) and the change in fetal heart rate from baseline to hyperoxygenation (change = baseline value – hyperoxygenation value). $r = .664$, $P = .001$.

in pulmonary vascular impedance could modify right ventricular afterloading and alter ventricular function. This altered ventricular loading could decrease the early diastolic filling pressure in the right ventricle, resulting in an increase in the TV E-wave V-max. Instead we found that diastolic, systolic, and global ventricular function remained unchanged with hyperoxygenation, as demonstrated by an unchanged IVRT %, IVCT %, and IMP. Overall, this finding supports previous work in children undergoing valvuloplasty for PV stenosis, which found that ventricular afterload was not a direct cause of right ventricular filling abnormalities.¹⁵

Table 3. Correlation Between Changes in Cardiac Indices With Hyperoxygenation

Variable Changes (baseline–hyperoxygenation)		Correlation Coefficient (r)	P Value
TV V-max E (m/s)	RPA PI	.258	.234
	RVCO (mL/min)	.072	0.750
	FHR (bpm)	−.219	.328
TV V-max A (m/s)	RPA PI	.311	.148
	RVCO (mL/min)	.183	.414
	FHR (bpm)	.662	.001
TV V-max E/A	RPA PI	.045	.837
	RVCO (mL/min)	−.021	.925
	FHR (bpm)	−.664	.001
TV VTI E/A	RPA PI	.164	.454
	RVCO (mL/min)	.173	.440
	FHR (bpm)	−.345	.116
MV V-max E (m/s)	RPA PI	.202	.356
	LVCO (mL/min)	.177	.430
	FHR (bpm)	−.003	.988
MV V-max A (m/s)	RPA PI	.180	.411
	LVCO (mL/min)	.224	.317
	FHR (bpm)	.470	.027
MV V-max E/A	RPA PI	.050	.822
	LVCO (mL/min)	−.135	.550
	FHR (bpm)	−.418	.053
MV VTI E/A	RPA PI	−.173	.442
	LVCO (mL/min)	−.231	.314
	FHR (bpm)	−.475	.030
IVRT %	RPA PI	.025	.941
	LVCO (mL/min)	−.030	.930
	FHR (bpm)	.293	.381
IVCT %	MV V-max E/A	.016	.964
	MV VTI E/A	−.025	.942
	RPA PI	−.007	.984
IMP	LVCO (mL/min)	−.384	.244
	FHR (bpm)	.254	.450
	MV V-max E/A	.199	.558
	MV VTI E/A	−.009	.978
	RPA PI	−.030	.930
	LVCO (mL/min)	−.441	.174
	FHR (bpm)	−.013	.969

Abbreviations: TV, tricuspid valve; V-max, maximum velocity; E, early ventricular filling; A, atrial contraction ventricular filling; VTI, velocity time integral; MV, mitral valve; RPA PI, right pulmonary artery pulsatility index; RVCO, right ventricular cardiac output; LVCO, left ventricular cardiac output; FHR, fetal heart rate; IVRT %, isovolumic relaxation time %; IVCT %, isovolumic contraction time %; IMP, index of myocardial performance; ET, ejection time.

At near-term gestation, a rise in fetal oxygen partial pressure leads to an increase in pulmonary venous volume blood flow return to the left atrium and a concomitant decrease in volume blood flow across the foramen ovale. We hypothesized that this could affect left ventricular filling characteristics. Although we were able to demonstrate an increase in the MV VTI E/A ratio with maternal hyperoxygenation, we found no significant difference in the MV V-max E/A ratio. The observed changes in either MV E/A ratio were not correlated with the changes seen in pulmonary vascular impedance. Similar to what was seen with the TV, we found that the decrease in FHR was significantly correlated with the decrease in MV A-wave V-max. Altogether, our results demonstrate that left ventricular diastolic filling characteristics are not affected by the shift in contribution for left ventricular preload originating from the foramen ovale to the pulmonary circulation.

Fetal RVCO decreased significantly with maternal hyperoxygenation. Previously, it has been shown in human pregnancies, that maternal hyperoxygenation with 60% oxygen does not affect fetal cardiac output or heart rate.⁵ One explanation for these different findings could be that in the current study we used 100% oxygen, which could further improve fetal oxygenation compared with 60% oxygen. In support of our current study, it has been shown that hyperoxemia is associated with a decrease in human FHR in term gestations.¹⁶

Our current experimental study contains limitations. We did not directly measure fetal arterial blood gas values during the experiment to demonstrate the exact magnitude of increase in fetal oxygenation obtained through maternal hyperoxygenation. However, maternal administration of 55% oxygen has been shown through fetal umbilical cord blood sampling to significantly increase human fetal oxygen partial pressure.¹⁷ In

Figure 3. Correlation between the change in the tricuspid valve (TV) maximum velocity (V-max) E/A ratio and the change in right pulmonary artery pulsatility index (RPA PI) (change = baseline value – hyperoxygenation value). $r = .045$, $P = .837$.

addition, using 60% oxygen with a protocol similar to our current study, significant changes in the distribution of the fetal cardiac output, as well as pulmonary circulation have been demonstrated.⁷ Experimental studies on fetal sheep have also shown that manipulation of maternal inhaled oxygen content significantly affects fetal oxygenation.¹⁸ Concerning the accuracy of ultrasonography to measure volume blood flow, validation studies in fetal sheep have shown that Doppler volume flow estimations correlate well with invasive measurements.¹⁹ In addition, the intraobserver variabilities of Doppler ultrasonographic measurements of fetal sheep volume flow have been shown to be comparable with those in human fetal studies during the second half of gestation.^{5,18}

In summary, in our experimental primate model maternal hyperoxygenation significantly reduced pulmonary vascular impedance and FHR. The TV V-max and VTI E/A ratios and the MV VTI E/A ratio increased during maternal hyperoxygenation. The increase in the TV V-max E/A ratio and the MV VTI E/A ratio correlated with a decrease in FHR but not with the reduction in pulmonary vascular impedance. The changes in loading conditions for both right and left ventricles induced by a decrease in pulmonary vascular impedance did not affect either right or left ventricular diastolic filling characteristics.

Declaration of Conflicting Interests

The author(s) declared no conflicts of interest with respect to the authorship and/or publication of this article.

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