HEART RATE AND BLOOD FLOW VELOCITY VARIABILITY IN THE HUMAN FETUS

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HARTSLAG- EN BLOEDSTROOMSNELHEIDSVARIABILITEIT
IN DE HUMANE FOETUS

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CONTENTS

CHAPTER 1

INTRODUCTION AND DEFINITION OF OBJECTIVES

1.1 Introduction

Much of what we know about the embryonic circulation is derived from studies of the chick embryo (Clark and Hu 1982). The similarities between the chick, rat (Nakazawa 1988) and fetal lamb (Kirkpatrick 1976) suggest that, while the details of functional change may vary, common mechanisms are expressed in these animal groups (Nakazawa 1988). Some of the mechanisms that control the cardiovascular system in the mature animal are expressed early in development (Clark 1990). The primary determinants of cardiovascular function in the embryo as in the mature animal are preload, afterload, heart rate and myocardial contractility. These factors regulate cardiac output before the development of the functioning autonomic nervous system. The Frank-Starling relationship is operative and effective in both the fetal lamb heart (Kirkpatrick 1976) and the chick embryo (Wagman 1990). After maturation of the autonomic nervous system, both the parasympathetic and sympathetic systems control cardiovascular function in the fetal lamb (Nuwayhid 1975).

We reasoned that the information gathered on cardiovascular function in animal models is also applicable to the human fetus. To explore this idea, we studied the variability of the arterial blood flow velocity waveform in early pregnancy. Modulations in haemodynamic waveform periodicity reflect the functional control of the autonomic nervous system and probably also of other mechanisms outside the central nervous system (Breborowicz 1988). We speculate that specific fetal heart variability and arterial flow velocity variability correlate directly with fetal cardiovascular health. Thus, deviation from the normal may be a sensitive and specific marker of cardiovascular dysfunction and may be highly correlated with adverse fetal outcome. Detection of cardiovascular dysfunction in early pregnancy would permit careful monitoring and potential treatment of the affected fetus.

Using combined transvaginal and transabdominal Doppler ultrasonography, it is now feasible to measure human fetal arterial and venous flow velocities during the late first and early second trimesters of pregnancy (van Splunder 1996). The late first and early second trimester of pregnancy is characterised by marked changes in fetal haemodynamics. Flow velocity waveforms recordings from the fetal descending aorta and umbilical artery have demonstrated a marked drop in pulsatility index, reflecting a reduction in fetoplacental vascular resistance (Wladimiroff 1992). This stage of pregnancy is characterised by trophoblast invasion of the spiral arteries (Brosens 1967), whereas the observed reduction in pulsatility index values is considered to be determined by microangiogenesis at placental level (Jauniaux 1992). Also, fetal heart rate changes from 175-180 bpm at 9-10 weeks to 145-150 bpm at 15 weeks of gestation with the appearance of beat-to-beat variability most likely resulting from parasympathetic nerve development (Wladimiroff and Seelen 1972). The combined use of transvaginal and transabdominal Doppler ultrasound offers us the opportunity to assess fetal heart rate variability and arterial flow velocity variability under physiological and pathophysiological circumstances during the late first and early second trimester of pregnancy.

1.2 Definition of objectives

The first objective was to identify the best method for reconstructing the blood flow velocities from the early human umbilical artery to determine the physiological changes in fetal blood flow velocity and heart rate. Furthermore, we determined the influence of maternal breathing patterns on the quality of the fetal blood flow velocity waveform. Data are presented in chapter 2.

The second objective was to obtain insight into data on normal flow velocity parameters in relation to gestational age which were obtained from the umbilical artery and the fetal descending aorta. Results of this part of the study are presented in chapter 3.

The third objective was (i) to determine whether at early gestation, changes in absolute values and variability occur for heart rate and blood flow velocity derived from umbilical artery flow velocity waveforms in women who subsequently develop pregnancy induced hypertension (PIH) and (ii) to address the role of variability in fetal heart rate and umbilical artery flow velocities in the assessment of early cardiovascular homeostasis in insulin-dependent diabetic pregnancies. Data are discussed in chapter 4.

The fourth objective was to examine whether (i) fetuses with congenital heart disease demonstrate alterations in heart rate variability and blood flow velocity

variability in the umbilical artery compared to normal controls and whether (ii) these variability measures can be used as markers for impaired homeostasis in fetal congenital heart disease (chapter 5).

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CHAPTER 2

METHODOLOGY AND SAFETY ASPECTS OF DOPPLER ULTRASOUND

2.1 Methodology of spectral analysis

Signal processing of clinical signals, like ECG, heart rate or blood pressure is widespread in biomedical research. In this chapter, we will briefly discuss the basic terminology of signal processing and review several analysis techniques that can be applied to those signals. This information will serve as a basis for the methodology employed in this thesis.

stationarity

Fundamental to many analysis techniques is the assumption of stationarity. A signal is defined stationary if its statistical parameters do not change with time. A concept of importance is that not all biological signals are stationary. Therefore, methods are used to transform the signal to be reasonably stationary. Once one can make the assumption that the signal is stationary one can begin to analyse it to test our hypothesis about the mechanisms underlying the clinical signal.

sampling and digitising

In processing biomedical signals, the analogue signal is first digitised. The signal is sampled into equally spaced samples, where the difference between two consecutive samples is the sampling period, which is the inverse of the sampling rate. Care must be exercised when digitising the analogue signal. The sampling rate must be at least twice the highest resolvable frequency or Nyquist frequency. Sampling rates below or exactly twice the Nyquist frequency violate the Nyquist Theorem (Nyquist 1928) and lead to erroneous results, which is due to a phenomenon called aliasing. Reconstruction of a sinusoid requires a minimum of two sample points per cycle. If the sample rate includes only sample point per cycle, the frequency of the sinusoid changes (Figure 1). To avoid aliasing and misinterpretation of the data, the sample rate must satisfy the Nyquist Theorem.

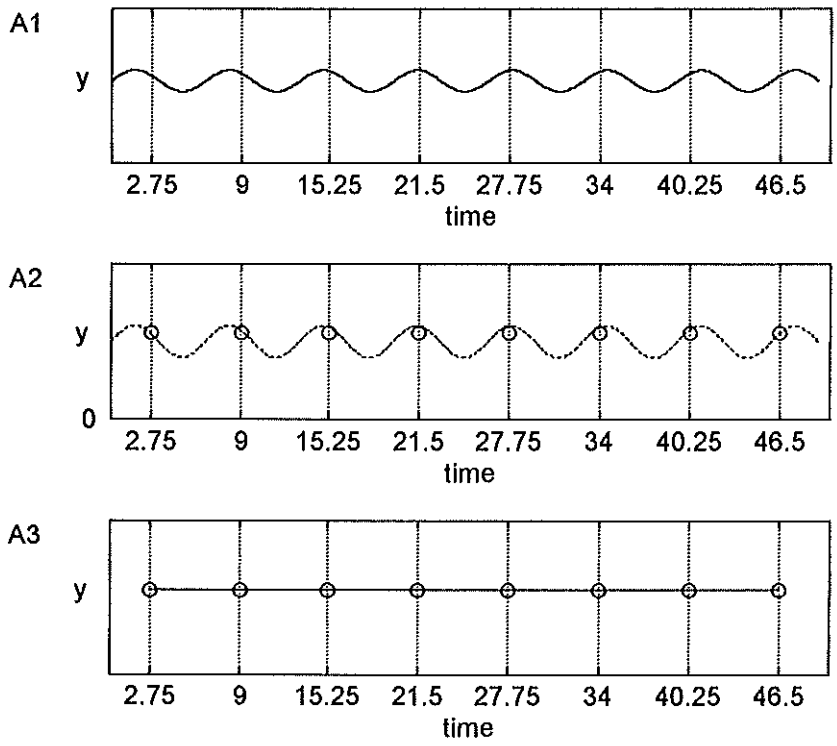

Figure 1. A1: actual changing signal during a particular time interval; A2: sampling of the signal every 6.25 seconds; A3: reconstruction of the signal. Note that the sampling rate was too low to identify the fluctuations in the actual signal.

Signals are characterised by two approaches: a time domain method and a frequency domain method. Both methods observe the same signal but represent the data in a different way, although both methods are interchangeable. The time domain method represents the signal in time, while the frequency domain method displays the signal as waveform with different frequencies.

J.B.J. Fourier (1768-1830) showed that any signal can be dissected into a sum of sine waves. Fourier transform involves the decomposition of signals into the summation of sinusoids at different frequencies. The amplitude and frequency of the individual sinusoid can be displayed in an amplitude spectrum (Figure 2). The power spectrum of the signal can be obtained by squaring the respective Fourier amplitudes.

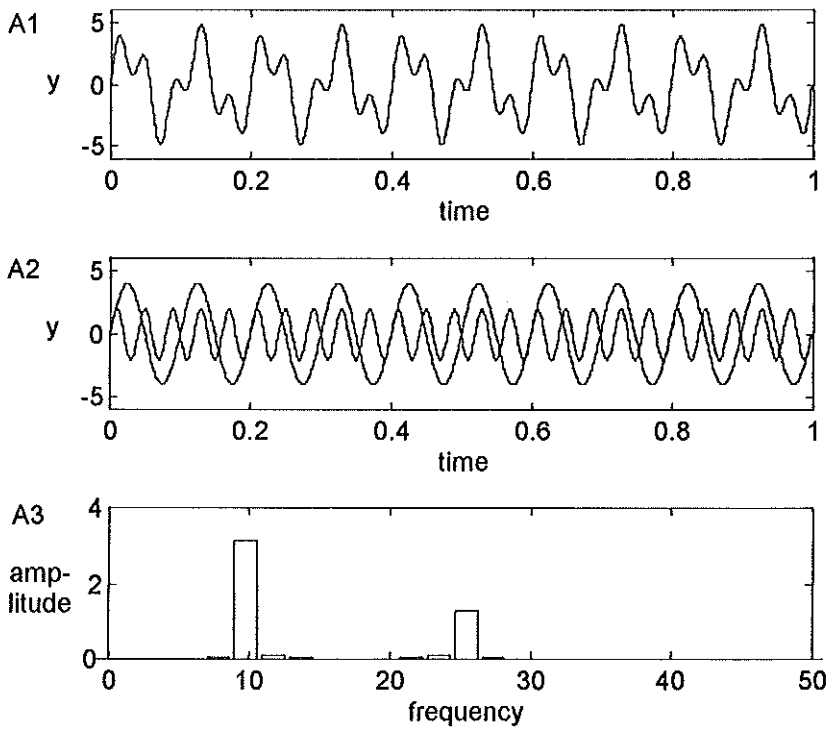

Figure 2. Graphical decomposition of a signal (A1) into its individual sinusoids (3 sin(10x); sinusoid 1 with an amplitude of 3 and a frequency of 10 and 1.5 sin(25x); sinusoid 2 with an amplitude of 1.5 and a frequency of 25 (A2) and the amplitude spectrum of the sum of individual sinusoids (A3).

For practical implementation of Fourier transform, a modified version is used in which samples are drawn so that a finite or discrete series of frequency points are chosen for analysis. This is called the discrete Fourier transform. The fast Fourier transform (FFT) is an algorithm for rapid digital implementation of the discrete Fourier transform (Challis and Kitney 1991). The principle behind fast Fourier transform method is that the transform of the sequence of N samples is decomposed into a number of transforms of shorter sequences, and leads to greater reduction in computational effort. Two basic types of decomposition are used: decimation in frequency and decimation in time. The decimation in frequency algorithm decomposes the original signal into sections of sequential samples. The decimation in time algorithm operates by dividing the original data sequence length by two by selecting alternate samples in each successive sequence (Oppenheim and Schafer 1975). Both types require that the length of the input series be an integer power of two.

leakage and windowing

Signal processing procedures are normally applied to selected section of long data recordings. The actual frequency content of the data recording is continuously disrupted, mainly at the edges of the selected sections, which will lead to a phenomenon called spectral leakage. Therefore, some of the actual frequency content "leaked out" to other parts of the amplitude spectrum (Figure 3: A1, A2). It can result in serious errors in spectral estimation where the signal being studied contains significant low-amplitude components which may be masked by leakage from high-amplitude components at other frequencies in the same signal (Challis and Kitney 1991).

The effects of spectral leakage can be controlled by a smoothing window. The process of windowing involves multiplication of the selected section by a window function, which lessens the boundary discontinuities of the selected data set (Figure 3: B1, B2). The net effect of point-to-point multiplication is to force the ends of the data segment to be nearly zero and the centre portion of data segment will thus be emphasised for the subsequent Fourier analysis. The proper choice of the window, like Hanning or Hamming can thus lead to a minimisation of spectral leakage caused by a rectangular window function.

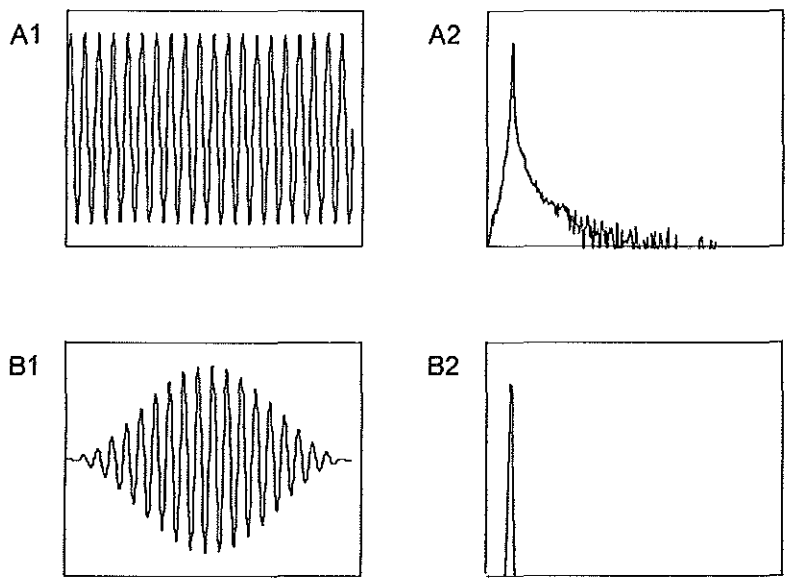

Figure 3. Sine wave and superimposed rectangular window (A1) and Hanning window (B1) and their amplitude spectra (A2 and B2). Note that windowing reduces spectral leakage but does not eliminate it.

time frequency domain methods

There are many methods available for analysing biomedical signals in the time domain. Autocorrelation is essentially the time domain comparison of a delayed version of a signal to itself, which is the inverse Fourier transform of the power spectral density representation of the signal. Autocorrelation is a widely used method in spectral analysis of speech and frequency-dependent phenomena. An advantage of the autocorrelation method is that it produces cleaner spectra and better frequency domain based methods. The autocorrelation function can be very useful for signals corrupted by noise, in which it can extract the signal from the noise. A disadvantage to autocorrelation is that it adds a second step (in the time domain) to the computation of the frequency domain representation of a signal (Kay and Marple 1981).

2.2 Physics and safety aspects of Doppler ultrasound

As two objects move toward each other, the observed frequency increases, and if they move away from each other, the observed frequency decreases. The change in observed frequency is called the *Doppler shift*, which was first described by the Austrian physicist Johann Christian Doppler in 1842. The Dutch scientist Buys Ballot applied Doppler's finding to sound one year later, showing that the frequency of a sound wave increases when the source of sound approaches the receiver and decreases when the source of sound moves away from the receiver.

The relationship between velocity of blood flow and Doppler shift is expressed by the following equation:

$$F_t - F_r = F_d = \frac{2 \cdot F_t \cdot \cos\alpha \cdot v}{c}$$

where F_d = Doppler shift (kHz), F_t = transducer transmission frequency (MHz), F_r = received frequency (MHz), α = incident angle (between ultrasonic beam and long axis of vessel), c = velocity of sound in tissue (approximately 1540 m/s), and v = velocity of blood flow (cm/s).

As mentioned above, the Doppler shift produced by a moving object is relative to the velocity of the object moving and the angle of the ultrasonic beam if the transmission frequency and velocity of sound in the tissue are known. When using Doppler ultrasonography to assess blood flow in an artery, the incident ultrasound beam is reflected not by one but by millions of red blood cells. When the ultrasound beam is reflected by moving red blood cells, it undergoes a reflective phenomenon called backscattering. Backscattering occurs because the

size of the reflective surface of the red cell is smaller than the wavelength of the ultrasonic beam. The produced echoes are scattered and reflected in all directions. The frequency shift determined by the transducer is a collection of all the Doppler shifted echoes produced by the red cells and is proportional to the velocity of the moving column of cells in the vessel. The power of the Doppler frequency shift is affected by both the red blood cells concentration (haematocrit) and the turbulence of flow within the vessels (Meyer and Jaffe 1992).

The biological effects of diagnostic ultrasound can be divided into thermal and non-thermal mechanisms. The ultrasound-induced temperature increase during ultrasonic exposure depends on the properties of both the ultrasound field parameters and the biological tissue involving ultrasound absorption, thermal conduction and blood perfusion. During prenatal life, the developing fetal brain is considered to be the most susceptible to effects of ultrasound-induced heating. Although the tissues of the central nervous system have a relatively low absorption coefficient, they are encased in the bone of the fetal skull and vertebrae, which are susceptible to significant heating by ultrasound (Barnett 1997). A diagnostic exposure that elevates embryonic and fetal in situ temperature above 41°C (4°C above normal temperature) for 5 minutes or more should be considered potentially hazardous (WFUMB 1997). The non-thermal effects such as cavitation should also be considered in assessing the safety of diagnostic ultrasound. Cavitation-related bio-effects have been reported at cell suspensions and gas/tissue interfaces, such as the lung (Barnett 1997). However, there is no evidence of cavitation effects in vivo, because thermal effects occur at lower intensities and dominate the observation (Miller 1991). The exact mechanism responsible for cavitation-related effect is yet to be identified. The World Federation for Ultrasound in Medicine and Biology (WFUMB) in its latest recommendations for thermal bio-effects states (WFUMB 1997) that "It has been demonstrated in experiments with unperfused tissue that some Doppler diagnostic equipment has the potential to produce biologically significant temperature rises, specifically at bone/soft tissue interfaces. The effects of elevated temperatures may be minimised by keeping the time for which the beam passes through any one point in tissue as short as possible. Where output power can be controlled, the lowest available power level consistent with obtaining the desired diagnostic information should be used. Although the data on humans are sparse, it is clear from animal studies that exposures resulting in temperature less than 38.5°C can be used without reservation on thermal grounds. This includes obstetric application". The WFUMB states (WFUMB 1997) in its latest recommendations for non-thermal bio-effects that "When tissue/gas interfaces or contrast agents are nor present, and there is no risk of significant temperature elevation, the use of diagnostic Doppler equipment need not be withheld because of concern for ultrasound safety. When any of the above

conditions might be present, ultrasound exposure levels and duration should be reduced to the minimum necessary to obtain the required diagnostic information".

Epidemiological studies reported possible associations between ultrasound exposure in utero and childhood cancer, left-handedness, dyslexia, delayed speech development and low birth weight. In subsequent studies, none of the associations between exposure to ultrasound in utero and childhood maldevelopment has been proven. However, for left-handedness and low birth weight after frequent Doppler ultrasound exposures no firm conclusions can be drawn (Salvesen and Eik-Nes 1995).

2.3 Waveform reconstruction methods and quality assessment of early human umbilical artery waveforms

Doppler ultrasound permits non-invasive measurements of the fetal arterial blood flow velocity as early as 8 weeks of gestation. Mechanisms that control the periodicity of the blood flow velocity profile can be studied with analysis techniques described in the previous subchapter 2.2. We speculate that computational analysis of Doppler velocity variability and fetal heart rate variability will identify women with at-risk pregnancies.

Before we can verify our hypothesis, we have to ascertain which reconstruction method is best suited to estimate blood flow velocities from the early human umbilical artery to determine the physiological changes in fetal blood flow velocity and heart rate. Secondly, we need information whether spontaneous maternal breathing influence the quality of the fetal blood flow velocity waveform. This information is essential for a correct interpretation of the variability data obtained from the umbilical artery.

2.4 Umbilical artery waveform analysis based on maximum, mean and mode velocity in early human pregnancy

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Abstract

The objective of this study was to identify the best method for reconstructing the blood flow velocities from the early human umbilical artery to determine the physiological changes in fetal blood flow velocity and heart rate. Pulsed Doppler recordings from the umbilical artery with duration of approximately seven seconds were made at 10-20 weeks of gestation. For reconstruction of the blood flow velocity from the Doppler audio signal, the maximum (envelope), mean and mode frequency reconstruction methods were used. For the assessment of variability in blood flow velocity and heart rate in the umbilical artery, the maximum flow velocity reconstruction method is preferred because it is relatively insensitive to noise, non-uniform insonation, and wall filter settings.

Introduction

Doppler ultrasound is a widely used technique for measuring flow velocities in the fetal circulation. The Doppler shifted signal from moving blood cells contains a spectrum of frequencies, of which the amplitude is related to the velocities of the blood cells. The pulsatility of the Doppler waveform can be angle-independently determined by several indices like, the pulsatility index (PI), resistance index (RI) or systolic diastolic ratio (S/D). These Doppler indices describe the haemodynamic phenomena both proximal and distal to the point of measurement, like peripheral resistance, cardiac contraction force, vessel compliance, blood viscosity and heart rate (McDonald 1964, Maulik 1982).

Flow velocities can be estimated from several Doppler shift frequency components of the Doppler power spectrum. The maximum waveform has been used to determine indices for downstream impedance. The maximum velocity is the most convenient waveform to use, but the same indices could be derived from the mean velocity waveform or mode velocity waveform. Information is lost when only the maximum velocity is considered, and this could be important when the derived waveform indices are ultimately used to make inferences about physiological function (Thompson 1986). We selected the two commonly used methods for velocity estimation (reconstruction), the maximum and mean velocity waveform, as well as the mode velocity waveform, because it is claimed that the latter is insensitive to most cases of background noise (Evans 1989a).

Pulsatility index calculations in the umbilical artery at 12-13 weeks of gestation have demonstrated that uncomplicated pregnancies are characterised by a gestational age-related increase of the end-diastolic component of the blood flow velocity waveform (Wladimiroff 1991, van Splunder 1996a), suggesting a gestational age-related decrease in the umbilical-placental downstream impedance to meet the metabolic demands of the rapidly growing fetus. The control mechanisms that mediate the haemodynamic function in the early developing fetus are probably beat-to-beat regulated (Hu and Clark 1989) and supplemented by long-term regulatory mechanisms (Kempski 1993). Fluctuations in fetal heart rate are associated with the functional state of the autonomic nervous system (Kleinhout 1977, Karin 1993, Segar 1994). Impaired control of the neural-mediated mechanisms and feedback mechanisms may lead to abnormal variability. Measurement of variability in umbilical artery flow velocity parameters and heart rate may serve as a tool for cardiac dysfunction studies in early pregnancy.

The aim of this paper was to identify any important differences in early fetal velocity and/or heart rate variability derived from the maximum flow velocity waveform, mean velocity waveform and mode velocity waveform reconstruction methods, and to investigate likely reasons for these differences.

Methods

Subjects

A total of 36 women with a normal singleton pregnancy between 10-20 weeks of gestation (median 15 weeks) consented to participate in the study. Each woman was included in the study once. The study was approved by the Hospital Ethics Committee. Maternal age ranged between 14-46 years (median 27 years). Pregnancy duration was estimated from the last menstrual period and confirmed by ultrasound measurement of the fetal crown-rump-length (10-12 weeks) or biparietal diameter (12-20 weeks). All pregnancies were uncomplicated and

resulted in a term delivery of a normal infant with a birth weight between the 10th and 90th centile corrected for maternal parity and fetal sex (Kloosterman 1970).

Doppler recordings

Ultrasound Doppler studies were performed with a Toshiba SSH 140A (Toshiba corp., Medical systems Division, Tokyo, Japan). A combined transvaginal real-time and pulsed Doppler system (carrier frequency 6 MHz and 5 MHz, respectively) was used at 10-13 weeks of gestation and a combined transabdominal real-time and pulsed Doppler system (carrier frequency 5.0 MHz and 3.75 MHz, respectively) was used at 14-20 weeks of gestation. The system operates at power outputs of < 100 mW/cm^2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specification. All Doppler studies were performed with the women in the semirecumbent position and during fetal apnoea. Doppler recordings were performed by one examiner (NTCU). The high-pass filter was set at 100 Hz and the sample volume length was 0.2-0.3 cm. Depending on the scanning depth, the pulse repetition frequency ranged between the 3-6 kHz. Flow velocity waveforms from the umbilical artery were obtained from the free floating loop. The angle of insonation was always less than 15°.

Data processing

Umbilical artery Doppler recordings were stored on sVHS videotape in PAL format using a Panasonic model AG7350 machine (Matsushita Electric Ind Co, Japan). The forward and reversed Doppler audio waveforms were digitised at 12 kHz per channel for 10-s intervals using a Pentium-based computer fitted with a 12-bit AD-card (Data Translation DT24-EZ). Flow velocity waveforms were reconstructed using analysis algorithms developed at the Central Instrumentation Department, Erasmus University Rotterdam, The Netherlands using Borland C++ for Windows® v4.5. Power spectra are calculated using a 512-point Fast Fourier Transform (FFT) with a Hanning window and an overlap of 75%. This results in a frequency resolution of 23 Hz and a time resolution of 10 ms.

From the collected spectra, the mean, mode and maximum velocity waveforms can be reconstructed, shown on a screen and subsequently analysed. The mean velocity waveform is constructed from the frequencies of the power spectra obtained by the equation:

$$\bar{f}_t = \frac{\sum_{n=0}^{n=255} P_n \cdot f_n}{\sum_{n=0}^{n=255} P_n}$$

where f_n is frequency n, P_n is the power of frequency n, and f_i denotes the intensity weighted mean frequency at time increment $t=i$. Frequencies with an intensity below a preset background noise level are set to zero. This background noise level can be set on screen by means of a slider. The mode velocity waveform is constructed from the frequencies with the highest intensity within the power spectra. The maximum velocity waveform is constructed from the maximum frequency of the power spectra. As shown by Mo et al. (1988), estimation of the maximum frequency is not as trivial as it may seem. Therefore we adopted a dual approach involving an automated estimation augmented with an interactively changeable threshold. First, the threshold is set to 1.5% of the mean intensity. Second, the user can then open an inspection window (Figure 1) that shows the power spectrum at a specific point in time, together with the threshold level presented as a horizontal line. In this window, the threshold can be changed on the screen by means of a slider. Third, the maximum curve can be recalculated, based on the new threshold. This process can be repeated to ensure the best fit for the maximum curve. A moving averaging filter of 3 points was used for smoothing and was applied for all 3 reconstructed velocity profiles.

For analysis of the blood flow in the umbilical artery, the program calculates the peak systolic velocity (PSV), end-diastolic velocity (EDV), time-averaged velocity (TAV), pulsatility index (PI) (Gosling and King 1975), and heart rate (HR), including the standard deviation of these indices. The calculations are done on a beat-to-beat basis. The interval to be analysed can be set onscreen by means of two sliders. The velocity waveform analysis was carried out on exactly the same section of the Doppler signal for the 3 velocity reconstruction methods. The duration of the umbilical artery flow velocity signal was 6 to 7 s, containing 12 to 18 heartbeats, depending on the heart rate of the fetus.

Statistical analysis

For each test period and each variable under consideration, we computed mean and standard deviation (SD) or mean and standard error of the mean (SEM), as appropriate. The Spearman correlation coefficient was used to quantify the strength of the linear relationship between two variables. Nonparametric analysis of variance (Friedman test) and the Wilcoxon signed rank test was applied to assess differences between paired variables; $p < 0.05$ was taken as the level of significance. A plot of the SD vs. the mean for each fetus showed a significant positive correlation for most variables. We, therefore, decided to examine the coefficient of variation (SD/mean), expressed as a percentage, for further analysis.

Results

Figure 1 shows a typical example of the umbilical artery velocity (cm/s) for a duration of 7 s, derived from the 3 different flow velocity waveform reconstruction methods: maximum (envelope), mean and mode velocity waveform reconstruction. The peak systolic velocity as well as the variability in velocity derived from the 3 waveform reconstruction methods are displayed in Figure 2. Mode peak systolic velocity is wrapped around the mean peak systolic

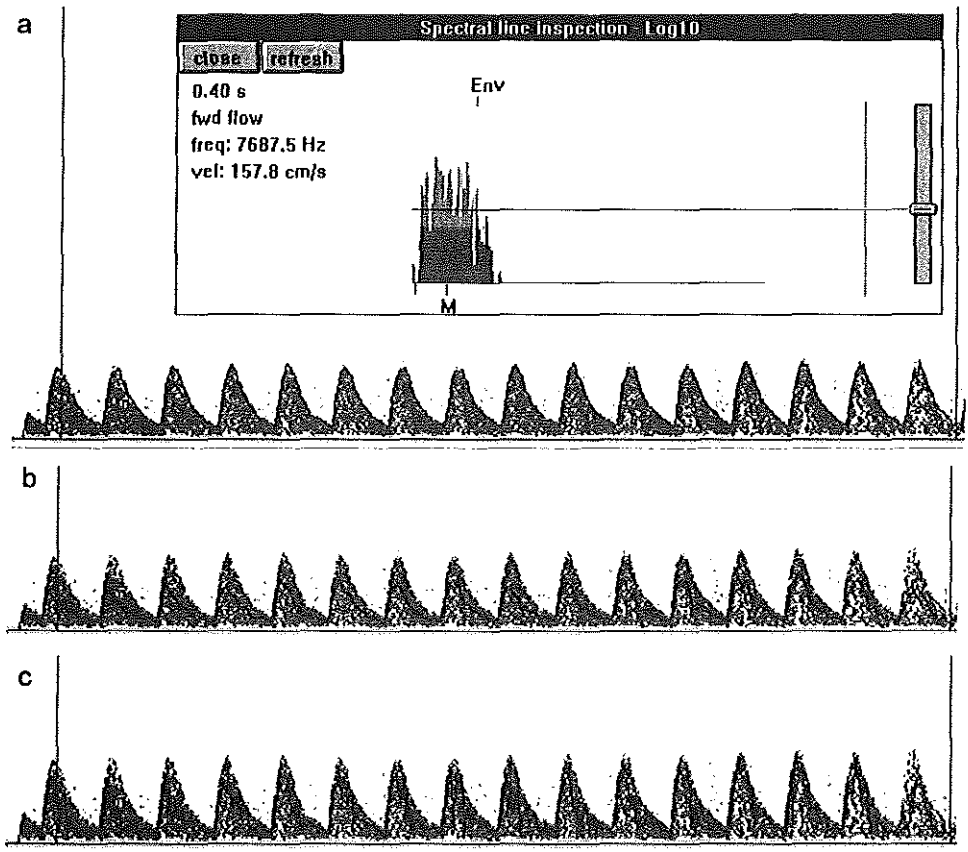

Figure 1. Example of the 3 velocity waveform reconstruction methods: a. maximum (envelope) flow velocity waveform reconstruction, b. mean flow velocity waveform reconstruction, c. mode flow velocity waveform reconstruction in a Doppler recording from the umbilical artery at 12 weeks of gestation (reconstruction length: seven seconds). The spectral inspection window demonstrated at the top of the figure displays the Doppler power spectrum: the horizontal line indicates the threshold level and the dark coloured portion of spectrum indicates the background noise.

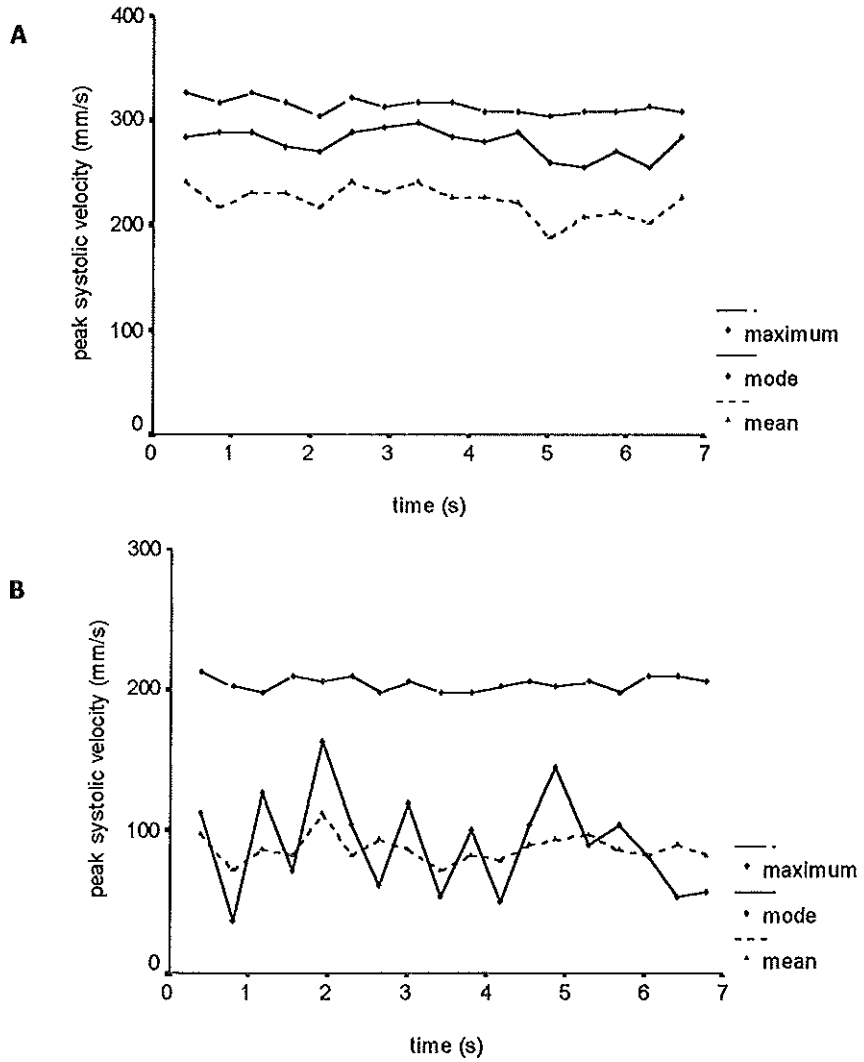

Figure 2. Example of beat-to-beat variation in umbilical artery peak systolic velocity derived from the 3 velocity waveforms reconstruction methods (maximum, mean and mode) at 12 weeks (A) and at 18 weeks (B) of gestation.

velocity at 10-13 weeks and positioned between the maximum peak systolic velocity and mean systolic peak velocity at 14-20 weeks of gestation. The mode velocity waveform clearly demonstrates a large variability in peak systolic velocity, whereas the maximum velocity waveform and, to a lesser extent, the mean velocity waveform are much more stable for the duration of the Doppler recording.

Figure 3 presents the pulsatility index (mean ± SEM) relative to gestational age for each of the flow velocity waveform reconstruction methods. The pulsatility index derived from the mode flow velocity waveform reconstruction was significantly higher ($p < 0.05$) than the pulsatility index derived from the maximum and mean flow velocity waveform reconstruction throughout the study period (10-20 weeks). No significant difference in pulsatility index could be established between the maximum and mean flow velocity waveform reconstruction, except at 10-11 weeks of gestation ($p < 0.05$).

Fetal heart rate (mean ± SEM) did not differ between the 3 flow velocity waveform reconstruction methods and ranged between 172 (± 5) beats per min at 10-13 weeks and 146 (± 9) beats per min at 18-20 weeks of gestation.

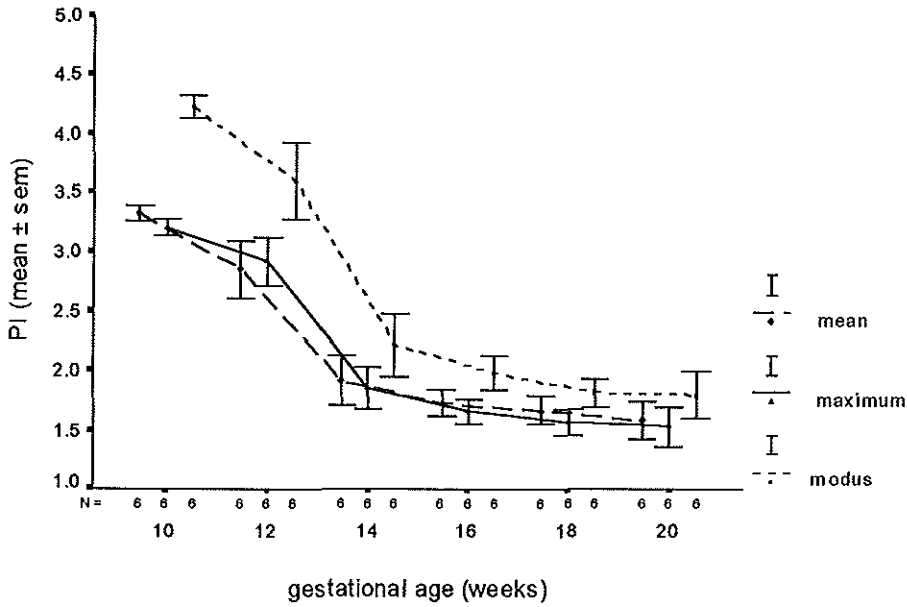

Figure 3. Pulsatility indices (PI; mean ± SEM) at 10-20 weeks of gestation derived from the 3 flow velocity reconstruction methods (maximum, mean and mode velocity waveform).

Table 1 demonstrates a significant positive correlation with gestational age for the variation in end-diastolic velocity and a significant negative correlation with gestational age for the time-averaged velocity for all 3 flow velocity waveform reconstruction methods. A significant negative correlation with gestational age also existed for the variation in peak systolic velocity, but only for the mean and mode flow velocity waveform reconstruction method. For the variation in the

pulsatility index, a significant negative correlation with gestational age could only be observed for the mode flow velocity waveform reconstruction method. No gestational age-related correlation could be established for fetal heart rate. Based on these gestational-age related observations it was decided to determine the coefficient of variation for all haemodynamic parameters for each of the flow velocity waveform reconstruction methods during the following 3 gestational age periods, 10-13, 14-17 and 18-20 weeks of gestation. Data are presented in table 2. A significant difference of the median coefficient of variation existed between the maximum and the mean flow velocity waveform reconstruction for all haemodynamic parameters except end-diastolic velocity for one or more gestational age periods. A similar difference of the median coefficient of variation could be established for both the maximum and mean velocity waveform reconstruction method as compared to the mode velocity waveform reconstruction method for all haemodynamic parameters for one or more gestational age periods.

Table 1. Spearman correlation of the coefficients of variation for all haemodynamic parameters related with gestational age for the three flow velocity waveform reconstruction methods.

	A	B	C
PSV	-0.21 (0.212)	-0.42 (0.011)	-0.67 (<0.001)
EDV	0.5 1 (0.001)	0.36 (0.032)	0.40 (0.016)
TAV	-0.5 2(0.001)	-0.65 (<0.001)	-0.69 (<0.001)
PI	0.17 (0.311)	-0.01 (0.941)	-0.58 (<0.001)

PSV: peak systolic velocity, EDV: end-diastolic velocity, TAV: time-averaged velocity, PI: pulsatility index. A = maximum flow velocity waveform reconstruction method; B = mean flow velocity waveform reconstruction method; and C = mode flow velocity waveform reconstruction method. *p*-values are given between parentheses.

Discussion

Our study demonstrates that the largest coefficient of variation for umbilical artery peak systolic velocity is found for the mode velocity waveform reconstruction method (*i.e.*, 6-fold higher at 10-13 weeks and 3-fold higher at 14-20 weeks of gestation) when compared with the maximum flow velocity waveform reconstruction method. A similar, but less pronounced increase exists for the umbilical artery time-average velocity. These findings are determined by the rather flat Doppler power spectrum, resulting in a poor determination of the exact position of the peak amplitude of the power. A flat spectrum can be expected in the presence of laminar flow, although the velocity profile is constantly changing during the cardiac cycle (McDonald 1964). Moreover, it is

likely as a result of the narrow ultrasound beam that only a small portion of the vessel cross-sectional flow field is insonated. This poor peak amplitude determination is more prominent in the early study period (10-13 weeks), probably due to the relatively low amount of reflected energy resulting in a worse signal-to-noise ratio.

Table 2. Medians and quartile ranges of the coefficient of variation (%) from the haemodynamic parameters of the umbilical artery velocity waveforms (n=36).

					A		B		C
PSV	10-13	†	‡	§	3.6	(2.7-5.4)	8.4	(7.3-11.8)	21.7	(18.4-29.3)
	14-17	†	‡	§	3.0	(2.0-3.3)	6.3	(4.8-8.1)	8.2	(6.3-18.1)
	18-20	†	‡		2.7	(2.5-4.0)	6.8	(4.7-8.3)	7.0	(4.8-10.7)
EDV	10-13				0.0	(0.0-0.0)	0.0	(0.0-15.9)	0.0	(0.0-17.2)
	14-17			§	10.1	(7.1-16.0)	11.6	(11.0-12.1)	15.8	(12.9-17.8)
	18-20		‡	§	7.9	(5.6-14.8)	11.5	(8.1-15.0)	14.8	(11.5-22.2)
TAV	10-13	†	‡	§	7.4	(6.4-9.8)	13.4	(10.3-15.1)	24.1	(19.4-29.2)
	14-17	†	‡	§	3.3	(2.8-5.1)	6.8	(4.4-9.1)	11.8	(8.3-15.7)
	18-20	†	‡	§	4.8	(3.4-5.6)	7.1	(5.4-9.0)	10.7	(6.2-14.7)
PI	10-13	†	‡	§	3.5	(2.7-5.6)	8.9	(7.4-12.2)	22.6	(18.5-29.4)
	14-17	†	‡	§	3.0	(2.0-3.3)	6.3	(4.8-8.1)	8.2	(6.3-18.1)
	18-20	†	‡		4.1	(3.4-5.1)	9.3	(7.1-10.2)	9.4	(6.4-13.1)
HR	10-13				1.6	(1.4-5.3)	1.5	(1.4-7.1)	4.9	(1.5-7.1)
	14-17		‡	§	3.6	(3.0-4.3)	7.6	(5.7-9.4)	9.1	(7.6-19.8)
	18-20	†	‡	§	1.6	(1.3-2.1)	2.2	(1.7-2.8)	6.5	(4.4-12.9)

A = maximum flow velocity waveform reconstruction method; B = mean flow velocity waveform reconstruction method; and C = mode flow velocity waveform reconstruction method. Statistical difference ($p<0.05$) between A and B (†), A and C (‡), B and C (§).

Mode flow velocity waveforms displayed a more spiky pattern when frequency components originating from the moving vessel wall were situated above the cut-off level of the wall filter. Occasionally, these low-frequency components became the mode frequency. Greene at al (1982) stated that this can be partly overcome by first locating the median frequency, which exceeds a fixed threshold. This is followed by a search for the mode frequency, which is situated in the immediate vicinity of the median frequency.

Similar to the mode velocity waveform reconstruction method, the coefficient of variation for the umbilical artery peak systolic velocity and the time-averaged velocity derived from the mean flow velocity waveform reconstruction method is significantly higher than the coefficient of variation derived from the maximum flow velocity reconstruction method. It is suggested that movements of the umbilical cord relative to the sample volume causes non-uniform insonation,

which will have a considerable effect on the mean frequency calculation (Thompson 1986, Evans 1989b).

There were differences in pulsatility index values derived from the 3 waveform reconstruction methods. At 10-11 weeks, pulsatility index values from the mean velocity waveform were significantly higher than pulsatility index values from the maximum velocity waveform. Wall filtering results in a higher mean velocity pulsatility index due to loss of data, as more of the lower diastolic velocity components fall below the cut-off level of the wall filter (Gill 1979). The observation that pulsatility index values originating from the mode velocity waveform were significantly higher than pulsatility index values derived from the maximum and mean velocity waveform may be due to the spiky pattern of the mode velocity waveform. The spiky waveform pattern generates a relatively lower time-average velocity and, therefore, elevated the pulsatility index.

However, the maximum and mean velocity waveforms are intrinsically filtered data sets because the maximum velocity reconstruction method acts as a high-pass filter within each Doppler power spectrum, and the mean velocity reconstruction method obviously acts as an averager. Additional filtering of the mode velocity waveform may improve the quality of the reconstructed signal. Filtering should be used with caution because a high filter setting may eliminate end-diastolic velocities and, as such, may preclude the identification of these velocities in the umbilical artery during the late first trimester of pregnancy.

From the results of this study, it can be concluded that the preferred method to reconstruct the Doppler signal from the human umbilical artery is the maximum flow velocity reconstruction method because it is relatively insensitive to noise, non-uniform insonation, and wall filter settings. Using this method, it is possible to establish the physiological change in flow velocity parameters and heart rate in the umbilical artery, which may serve as a tool for early detection of cardiac dysfunction.

2.5 Variability analysis of umbilical artery blood flow velocity in the week 10 to 20 human fetus

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Abstract

The fetal cardiovascular system is precisely regulated during development. We defined heart rate variability and peak systolic velocity variability in the human fetus during conditions of maternal spontaneous and stop breathing. Maternal breathing movements may affect the quality of long-term (> 14 s) fetal Doppler flow velocity waveform recordings. Doppler recordings of the umbilical artery were made during spontaneous maternal breathing and during stop maternal breathing at 10-20 weeks of gestation. Peak systolic velocity and fetal heart rate were determined for each velocity time series. The standard deviation of peak systolic velocity and heart rate was used as a measure of the variability. Peak systolic velocity variability and fetal heart rate variability were unaffected by a change in maternal breathing pattern in the normal fetus. Independent of maternal breathing, measures of umbilical artery peak systolic velocity variability and fetal heart rate variability can be used to assess fetal haemodynamics during a critical period of fetal morphogenesis and growth.

Introduction

The human fetal cardiovascular system is precisely regulated during development to match the metabolic needs of the fetus. There is a clinical need for early detection of fetal cardiovascular dysfunction particularly in view of early therapeutic intervention (Sibai 1993). For the past 15 years, pulsed Doppler ultrasound velocity waveform analysis has been a valuable tool for assessment of the fetal circulation (Groenenberg 1989). Recently, Doppler velocity information has been obtained as early as 8 weeks of gestation providing an opportunity to extending the window of physiological assessment to the late embryo (Wladimiroff 1991; Wladimiroff 1992; Van Splunder 1996b).

In the normal late second and third trimester pregnancy, changes in Doppler flow velocity waveforms define low fetal vascular resistance in the placenta, fetal trunk and brain (Griffin 1984; Schulman 1987) Doppler studies in abnormal pregnancies, like intrauterine growth retardation, display alterations in arterial and venous velocity waveforms associated with changes in fetal cardiac function (Rizzo and Arduini 1991; Hecher 1995).

The quality of long-term recordings of fetal Doppler flow velocity waveform may be influenced by maternal breathing movements. Movement of the maternal abdomen or uterus may shift the position of the Doppler sample volume and/or interrogation angle, so that different velocities will be recorded. The transabdominal approach (used after 13 weeks of gestation) may be more susceptible to maternal breathing movements than the transvaginal approach (used from 8 to 13 weeks of gestation).

Fetal heart rate variability is a marker of well being during late gestation. Heart rate variability is altered from normal in pathologic conditions, like intra-uterine growth retardation during the latter half of pregnancy (Breborowicz 1988; Ribbert 1991). However, important changes in fetal cardiovascular function may occur much earlier in development.

Non-human embryonic cardiovascular function has been investigated using pulsed-Doppler methods for the past 20 years (Keller and Clark 1993). Chicken dorsal aortic velocity waveform has particular patterns of velocity and heart rate variability (Kempski 1993). These changes may reflect haemodynamic regulation prior to autonomic innervation.

We reasoned that similar control mechanisms may be present in the early human fetus. To explore this idea, we studied human fetuses at 10-20 weeks of gestation. The objective of this study was to determine (i) if variability in peak systolic velocity and fetal heart rate are markers for cardiovascular homeostasis and (ii) if spontaneous maternal breathing affects the peak systolic velocity and fetal heart rate variability data. We found that these measures are consistent among fetuses without development of diseases.

Methods

Paired velocity time series were recorded during maternal spontaneous breathing and during maternal stop breathing from fetuses at 10-20 weeks of gestation. In ten women (10-13 weeks) the transvaginal probe was used and in ten women (14-20 weeks) the transabdominal probe was applied. All Doppler studies were performed with the women in the semirecumbent position and during fetal

apnoea. The study protocol was approved by the institutional review boards at Erasmus University and the University of Rochester. We obtained informed consent from all the mothers. The study group was recruited from patients in the normal obstetrical program at the Academic Hospital Rotterdam-Dijkzigt, The Netherlands. All pregnancies resulted in a normal healthy infant.

Pregnancy duration was determined from the last menstrual period and confirmed by ultrasound measurement of fetal crown-rump length (10-12 weeks) or fetal biparietal diameter (13-20 weeks). Recordings were made with a 5 MHz transvaginal pulsed Doppler system (Toshiba SHH-140A, Medical Systems Division, Tokyo) at 10-13 weeks of gestation and a 3.75 MHz transabdominal pulsed Doppler system at 14-20 weeks of gestation. The recordings were stored on sVHS videotape in PAL format on a Panasonic model AG7350 machine (Matsushita Electric Ind Co, Japan). The ultrasound system operated at power outputs of less than 100 mW/cm^2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specifications. Flow velocity waveforms from the umbilical artery were obtained from a straight portion of floating loop of the umbilical cord. The interrogation angle was always 20° or less. The high pass wall filter was set to 70-100 Hz. Continuous high quality Doppler audio waveforms were digitised at 12 kHz for greater than 14 s intervals using an analogue-to-digital data acquisition board (LabPC+ and BNC-2081 boards, National Instruments, Austin, TX).

In a previous study (Ursem 1998), several methods were described to reconstruct (estimate) the velocity waveform from the Doppler audio signal of the umbilical artery blood flow (Figure 1). The maximum velocity reconstruction method is preferred because it is relatively insensitive to noise, non-uniform insonation, and wall filter settings. The maximum velocity waveform was estimated from the Doppler data using computer algorithms developed in our centre using LabVIEW® software (National Instruments, Austin, TX). A detailed description of the analysis technique and the computer algorithms has been published previously (Ursem 1998).

We determined peak systolic velocity (mm/s), fetal heart rate (bpm) for each cardiac cycle. For the entire maximum velocity waveform recording, the mean and standard deviation for peak systolic velocity and heart rate were calculated to establish variability for each of these variables. The haemodynamic data during breathing and stop breathing are reported as mean and 95% confidence interval and tested for significance at $p<0.05$ by a paired t-test.

Results

Umbilical artery flow velocity waveforms were assessed by transvaginal (n=10) and transabdominal (n=10) Doppler ultrasonography. Peak systolic velocity

variability and fetal heart rate variability were not significantly different between spontaneous maternal breathing and stop breathing for both the transvaginal and

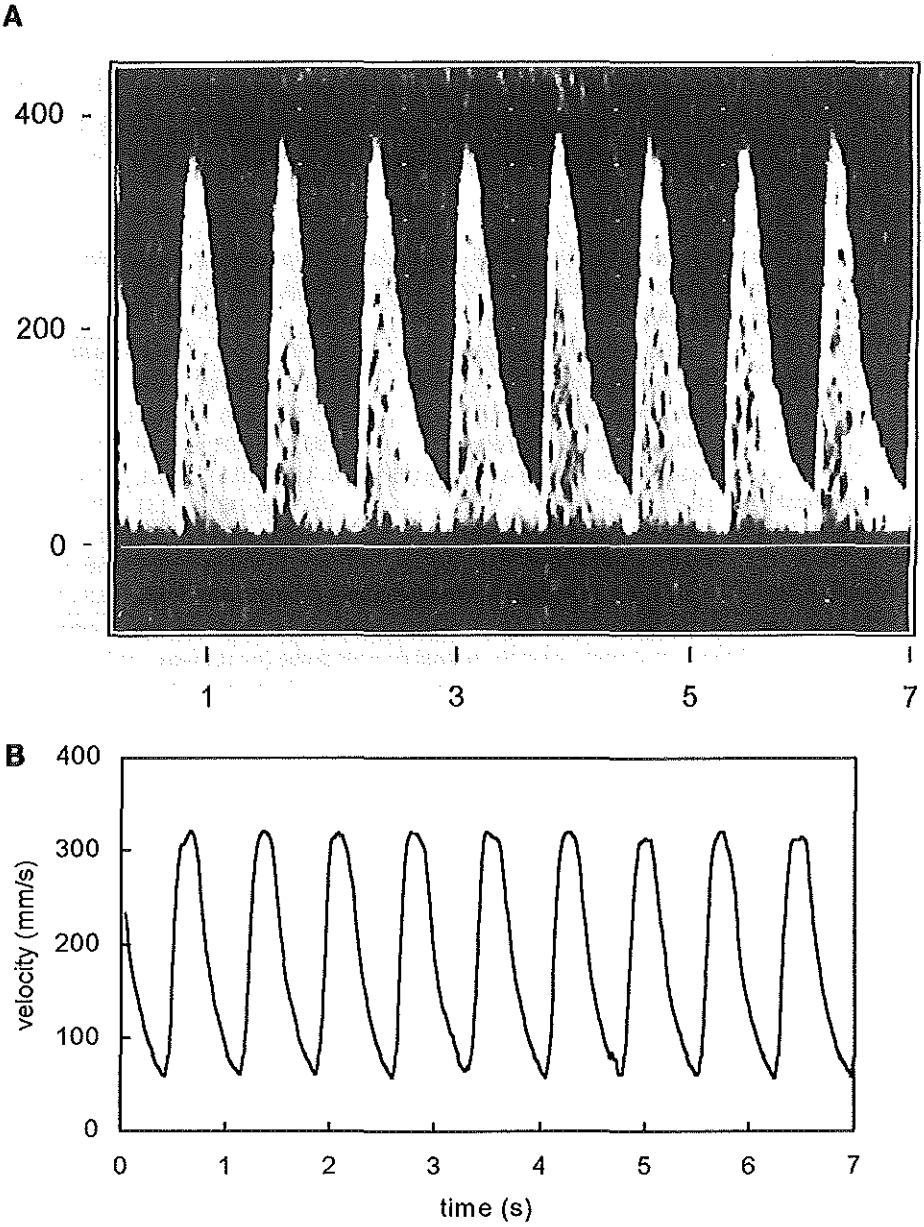

Figure 1. Doppler ultrasound recording of blood flow velocity waveforms from the free floating loop of the umbilical artery at 18 weeks of gestation (A) and schematic presentation of the estimated maximum velocity envelope of the umbilical artery at 18 weeks of gestation which is used for calculation of heart rate and velocity variability data (B).

transabdominal data sets (Table 1). Therefore, we pooled the breathing and stop breathing data sets providing the following results. The mean coefficient of variation for fetal heart rate variability was 1.1% (range: 0.7%-1.7%) at 10-13 weeks and 0.9% (range: 0.6%-1.3%) at 14-20 weeks of gestation. The mean coefficient of variation for peak systolic velocity variability was 3.8% (range: 2.2%-6.7%) at 10-13 weeks and 3.4% (range: 1.9%-6.8%) at 14-20 weeks of gestation.

Table 1. Mean (95% confidence interval) for haemodynamic parameters in the umbilical artery obtained from women while breathing or stop breathing during the Doppler fetal umbilical artery waveform recording.

Probe	Haemodynamic parameter	Breathing	Non-breathing
Transvaginal	Heart rate (bpm)	164 (157-174)	164 (156-173)
	Heart rate variability (bpm)	1.5 (1.1-2.4)	1.5 (0.9-2.1)
	Peak systolic velocity (mm/s)	199 (146-305)	193 (126-304)
	Peak systolic velocity variability (mm/s)	6.7 (3.8-10.6)	6.1 (3.0-13.2)
Transabdominal	Heart rate (bpm)	151 (146-161)	151 (145-160)
	Heart rate variability (bpm)	1.6 (1.0-3.4)	1.4 (1.1-1.8)
	Peak systolic velocity (mm/s)	297 (223-406)	307 (256-404)
	Peak systolic velocity variability (mm/s)	11.3 (6.5-16.7)	11.7 (5.8-25.9)

Doppler recordings using the transvaginal probe (n=10) were made at 10-13 weeks of gestation and Doppler recordings using the transabdominal probe (n=10) were made at 14-20 weeks of gestation. Variability data are expressed as SD. $P > 0.05$ by paired t-test.

Discussion

There is a clinical need for early detection of fetal cardiovascular dysfunction as an early point for potential intervention. A broad range of maternal fetal disease likely affects fetal haemodynamics early in the developmental process. These diseases include pregnancy induced hypertension, insulin-dependent diabetes mellitus, and collagen vascular diseases.

Analysis of Doppler flow velocity has contributed to the identification of disease states during the second half of gestation. Umbilical artery and uterine artery flow velocity waveforms are predictive of pre-eclampsia and intra-uterine growth retardation later in pregnancy (Bewley 1991; Mullick 1993). Intra-uterine growth retardation is associated with abnormal umbilical artery, ascending aortic and intracerebral artery flow velocity waveforms correlating with chronic fetal hypoxemia (Groenenberg 1989). In addition, intra uterine state may affect adult cardiovascular risk factors. Barker and colleagues have shown that in-utero events correlate with adult onset disease (Barker 1993). They found that trends of higher blood pressure among adults correlated with smaller for gestational weight and larger placental weight (Barker 1994). These and other studies emphasise the importance of fetal health on future adult onset disease.

We have pioneered the quantitative analysis of the embryonic cardiovascular system. As part of a SCOR in Pediatric Cardiovascular Disease, we studied dorsal aortic blood flow in the chick embryo during the early stages of morphogenesis (Hu and Clark 1989). We have identified velocity and heart rate variability prior to the innervation of the cardiovascular system (Kempski 1993). Early in development, velocity variability is correlated with changes in afterload of the heart (Kempski 1995). We speculate that these changes are due to circulating neuro-humoral factors acting at the level of the myocardium and peripheral vascular bed.

We have now extended this analysis of heart rate and velocity variability to the human fetus at weeks 10-20 gestation. In the current study, we found no difference in heart rate variability and umbilical artery peak systolic velocity variability between spontaneous breathing and stop breathing in the normal fetus in either the transvaginal or transabdominal groups. Thus, spontaneous maternal breathing does not affect the variability data by either shifting the interrogation angle or altering the position of the Doppler sample volume.

These human fetal observations suggest that measures of velocity and heart rate variability may serve as markers of cardiovascular homeostasis. Extension of this analytic method to cross-sectional and longitudinal analysis of normal and high risk maternal-fetal pairs is in progress. Development of sensitive and specific measure of fetal homeostasis is integral to new therapeutic strategies prior to the completion of gestation.

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CHAPTER 3

VARIABILITY ANALYSIS OF FETAL ARTERIAL WAVEFORMS IN NORMAL PREGNANCY

3.1 Introductory remarks

In order to study haemodynamics in abnormally developing fetuses, insight into the physiological development of the cardiovascular system during the late first and early second trimesters of pregnancy is necessary. We speculate that haemodynamic variability is correlated to cardiovascular function and that the variability analysis of the Doppler velocity provides us with a method to study fetal cardiovascular health. Doppler velocimetry is a non-invasive technique to study the fetal circulation as early as the first trimester of pregnancy. However, Doppler ultrasound does not allow measurements of volume flow or pressure and therefore, puts restrictions to the interpretations of the data.

The maximum flow velocity reconstruction method is the preferred method to reconstruct the Doppler signal from the human umbilical artery because it is relatively insensitive to noise, non-uniform insonation, and wall filter settings (*chapter 2.4*). Initially we used the mode reconstruction method to estimate the blood flow velocity waveform since this method relates to the most frequent velocity in a vessel (*chapter 3.2*). Later on we demonstrated that this reconstruction method is particularly sensitive to artefacts with emphasis on the 10-14 weeks gestation period and we decided that in successive studies the maximum reconstruction method should be used to estimate the velocity waveform (*chapter 3.3*).

Up till now we have focussed on the umbilical artery to study the modulations in fetal heart rate and flow velocity. We extended our study to the fetal descending aorta, since Doppler studies in the chick embryo and fetal lamb have shown that blood flow velocity waveforms in this vessel are related to fetal cardiac function (Thompson 1994; Clark and Hu 1982). Similar mechanisms should be responsible for the gestational age-related modulations in umbilical artery and descending aorta and we therefore expect no differences in variability data obtained from these two vessels (*chapter 3.4*).

In this chapter the reconstruction method, reproducibility and variability analysis of waveforms obtained from the umbilical artery and descending aorta in normal fetuses during the late first and early second trimester of pregnancy will be discussed.

3.2 An estimate of fetal autonomic state by spectral analysis of human umbilical artery blood flow velocity

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Abstract

Objective: Determination of gestational age-related fluctuations in heart rate in the umbilical artery of the early human fetus.

Methods: Doppler velocity recordings from human umbilical artery were obtained, in a cross sectional study design in 137 singleton pregnancies at 10-20 weeks of gestation. After exclusion criteria were applied, data on 117 normal pregnancies were available and subdivided into group I: 10-12 weeks (n=49); group II: 13-16 weeks (n=43); and group III: 17-20 weeks (n=25). Blood flow velocity waveforms were reconstructed from Doppler audio signals. Variability in heart rate was calculated using Fast Fourier Transforms (FFT). Individual heart rate variability power spectra were subdivided into frequency bands.

Results: Fetal heart rate variability decreases at 10-20 weeks and demonstrates a shift to lower frequencies at 17-20 weeks.

Conclusions: Fetal heart rate variability is related to gestational age and shows a shift to lower frequencies which may reflect autonomic functional development.

Introduction

The analysis of variations in cardiovascular parameters is an established non-invasive technique for investigating the autonomic control of the cardiovascular system. In man, heart rate variability estimated by spectral analysis reflects the activity of parasympathetic and sympathetic limbs of the autonomic nervous system (Akselrod 1981; Appel 1989; Karin 1993). Long and short term variations in heart rate differ with advancing gestational age and through early postnatal life coincident with maturation of the autonomic nervous system (Siimes 1990; Karin 1993). However, the analysis of heart rate variability during the development of autonomic innervation has remained limited.

In the preinnervated chick embryo, heart rate variability and blood flow velocity variability will likely indicate variations in cardiovascular output related to function, growth and morphogenesis (Kempski 1993; 1995).

We are pioneering the application of these principles in early human fetal development. Using combined transvaginal and transabdominal Doppler ultrasonography, it is now feasible to measure human fetal arterial and venous flow velocities during the late first and early second trimesters of pregnancy (van Splunder 1996a). Digital signal processing of these fetal flow velocity waveforms allowed the definition of flow velocity and heart rate variability. The objective of the present study was to determine the relationship between gestational age and fluctuations in heart rate in the umbilical artery of the normal human fetus at 10-20 weeks of gestation.

Methods

Subjects

A total of 137 women with a normal singleton pregnancy between 10-20 weeks of gestation (median 15 weeks) consented to participate in the study. Maternal age ranged from 14 to 46 years (median 28 years). Pregnancy duration was determined from the last menstrual period and confirmed by ultrasound measurement of fetal crown-rump length (10-12 weeks) or fetal biparietal diameter (13-20 weeks). Three different gestational age groups were enrolled into the study, group I: 10-12 weeks (n=49), group II: 13-16 weeks (n=43), and group III: 17-20 weeks (n=25). The study protocol was approved by the institutional review boards at the Erasmus University, Rotterdam, and both the Rochester Institute of Technology and the University of Rochester, Rochester, NY. Each woman was included in the study only once. Only pregnancies which progressed uneventfully resulting in the delivery of a normal infant with a birth weight between the 10th and 90th percentile corrected for maternal parity and fetal sex (Kloosterman 1970) were included in the data analysis.

Doppler recordings

Pulsed wave Doppler ultrasound recordings were obtained using a Toshiba SHH-140A (Toshiba Corporation, Medical Systems Division, Tokyo, Japan). A combined transvaginal real-time and pulsed Doppler system (carrier frequency 6 MHz and 5 MHz, respectively) was used at 10-13 weeks of gestation and a combined transabdominal real-time and pulsed Doppler system (carrier frequency 5 MHz and 3.75 MHz, respectively) was used at 14-20 weeks of gestation. The system operates at power outputs of less than 100 mW/cm^2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specifications. The angle of insonation was always less than 30 degrees. Sample volume length for all flow velocity waveforms ranged between 0.2 and 0.3 cm; the high-pass wall filter was set at 70-100 Hz. Doppler recordings were performed by one examiner (NTCU). All Doppler studies were carried-out with the women lying in a semirecumbant position and during fetal apnoea. Maximal flow velocity waveforms from the umbilical artery were obtained from a floating loop of the umbilical cord. Only technically high quality recordings lasting more than 16 s, and therefore containing at least 40 peak flow velocity waveforms, were analysed. This was to ensure an adequate collection of waveforms for the heart rate variability analysis. The velocity waveforms (video and Hi-Fi audio signal) were stored on sVHS videotape in PAL format using a Panasonic model AG7350 machine (Matsushita Electric Ind Co, Japan).

Data analysis

Continuous high quality Doppler audio waveforms were digitised at 44 kHz for greater than 16 s intervals using an analogue-to-digital data acquisition board (LabPC+ and BNC-2081 boards, National Instruments, Austin, TX). Flow velocity waveforms were reconstructed from the Doppler audio data using the 'DopV' collection of computer algorithms developed at the Rochester Institute of Technology using LabVIEW software (National Instruments, Austin, TX). We determined mean velocity (mm/s) and instantaneous heart rate (bpm) for each cardiac cycle. To measure instantaneous (i.e. beat-to-beat) heart rate, we used a global mean velocity threshold detection scheme. When the rising edge of the velocity signal crossed the threshold, an event marker was set (Figure 1). Instantaneous heart rate was determined from the reciprocal of the difference in successive threshold event times. Global mean heart rate was calculated by taking the average of the respective pulsatile values over the entire velocity series. Heart rate variability time series were calculated by using a piece-wise linear interpolation of the heart rate beat series. To remove the DC-drift we used a second-order polynomial fit. After quadratic de-trending, the heart rate time series were passed through a 5th order Butterworth low pass filter, with a cut-off frequency of 4 Hz. To compute the power spectral density of each variability time series, we used Fast Fourier Transforms. Power spectral density identifies the frequency-domain heart rate characteristics. For comparison, the individual heart rate variability power spectra were subdivided into 10 frequency bands of 0.15

Hz width, covering the range from zero to 1.5 Hz. Spectral power was expressed as the absolute band power values as well as the total power between 0 and 1.5 Hz. Total heart rate variability is equated to the total power (i.e. area under the curve) in the spectrum of the heart rate. The band powers were computed from the respective spectra in each pre-defined band and for the entire spectrum under consideration. The band power and total power for respective fetuses were then averaged across each gestational age group.

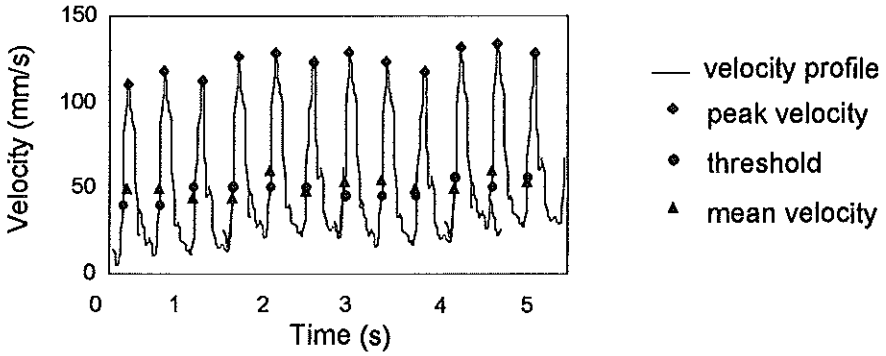

Figure 1. Schematic reconstruction of umbilical artery flow velocity waveforms with indicators for peak velocity (crosses), mean velocity (triangles) and threshold value (circles).

Statistical analysis

All data are presented as mean ± SEM (standard error of the mean) or as mean and 95% confidence interval. A logarithmic transformation of some variables was performed to obtain normal distributions. For statistical comparison of the three study groups, we selected an analysis of variance (ANOVA). If the result was significant ($p<0.05$) pairwise comparisons with a Student-Newman-Keuls correction were performed. Medians, percentiles (25th and 75th), and interquartile ranges were used to describe the characteristics of the heart rate variability frequency distribution. Statistical significance was defined by a value of $p< 0.05$. Calculations were performed with SPSS 6.1 software (SPSS Inc., Chicago, IL).

Results

Of the 137 women who consented to participate in the study, seven women were excluded independently of the study protocol because of a fetal birth weight below the 10th percentile or above the 90th percentile for gestational age and 13 women were excluded because of pregnancy pathology, such as gestational hypertension and premature labour later in gestation. Flow velocity waveforms recordings from 117 fetuses were available for further analysis.

Global mean heart rate decreased ($p<0.05$) and global mean flow velocity increased ($p<0.05$) with advancing gestational age (Table 1). An example of the heart rate time series and their power spectra from each of the age groups is depicted in Figure 2.

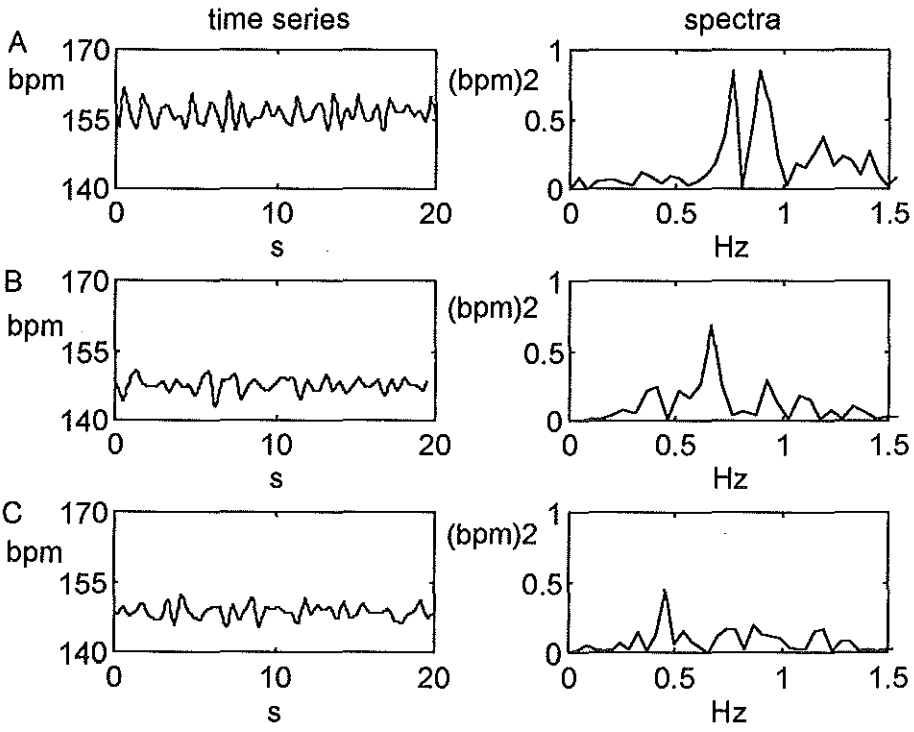

Figure 2. Examples of heart rate times series and their spectra (A-C). Group I: 10-12 weeks (A), group II: 13-16 weeks (B), and group III: 17-20 weeks (C).

Table 1. Fetal global mean heart rate and global mean flow velocity

	Group I	Group II	Group III	p
Heart rate (bpm)	169 (166, 171)#,*	151 (149, 154)#	149 (146, 152)*	<0.001
Mean velocity (mm/s)	45.9 (43.1, 48.8)#,*	81.9 (76.5, 87.6)#	94.1 (84.7, 104.6)*	<0.001

Group I: 10-12 weeks, group II: 13-16 weeks. and group III: 17-20 weeks. Data are presented as mean and 95% CI. Common symbology (#,*) indicate statistical difference between groups tested to $p < 0.05$.

Total heart rate variability decreased with gestational age from 10 to 20 weeks (Table 2). Total heart rate variability in group I was twice that in group III. Figure 3 demonstrates that the band power distribution of heart rate variability decreased in amplitude with gestational age above 0.3 Hz. Below frequency 0.3 Hz, heart rate variability was similar among the three gestational age groups

(Figure 3, Table 3). Analysis of heart rate variability distribution demonstrated that median and percentiles decreased with gestational age, indicating that later in pregnancy heart rate variability is centered around lower frequencies (Table 2).

Table 2. Descriptive statistics of heart rate variability band power distribution.

	Group I	Group II	Group III	p
Total variability $(bpm)^2$	8.84 (8.09, 10.49)#*	5.15 (5.06, 5.25)#	4.41 (3.91, 4.97)*	<0.001
Median (Hz)	0.83 (0.81, 0.87)#	0.79 (0.76, 0,82)*	0.69 (0.63, 0.76)#*	<0.001
25th percentile (Hz)	0.58 (0.56, 0.61)#	0.56 (0.53, 0.59)*	0.46 (0.40, 0.51)#*	<0.001
75th percentile (Hz)	1.09 (1.06, 1.12)#*	1.01 (0.98, 1,04)#℘	0.94 (0.89, 0.99)*℘	<0.001
Interquartile range (Hz)	0.51 (0.48, 0.53)#*	0.45 (0.42, 0.48)#	0.48 (0.44, 0.53)*	0.017

Group I: 10-12 weeks, group II: 13-16 weeks, and group III: 17-20 weeks. Values are presented as mean and 95% CI. Common symbology (# ,* ,℘) indicate statistical difference between groups tested to p<0.05.

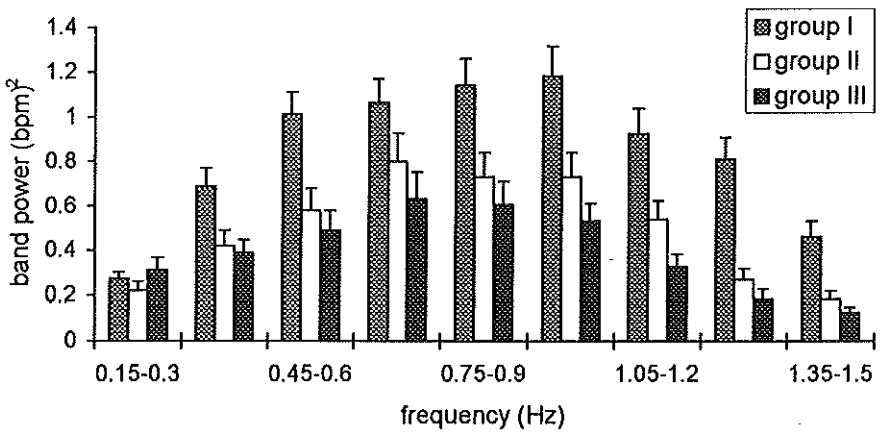

Figure 3. Heart rate variability band power spectra for gestational age groups; I: 10-12 weeks, II: 13-16 weeks, and III: 17-20 weeks. Data are presented as mean ± SEM

Discussion

During the last 15 years, there has been an increasing interest in cardiovascular beat-to-beat variations generated by the autonomic nervous system. Studies investigating heart rate variability in adults indicate the influence of three oscillating physiologic mechanisms arising from respiration, baroreceptor activity, and vasomotor activity (Akselrod 1981; Malliani 1991).

Although data from late gestation fetal heart rate variability are available, there are still many questions regarding the normal heart rate variability spectrum. Frequency analysis of heart rate in humans of all ages has revealed two prominent frequencies of variation: the high-frequency band (0.15-0.45 Hz) which is controlled by parasympathetic tone and the low-frequency band (0.03-

0.15 Hz) which is controlled by sympathetic tone (Hyndman 1971; Finley and Nugent 1983; Pomeranz 1985). Fluctuations in heart rate variability in premature and full-term neonates indicate post-term maturation of the autonomic nervous

Table 3. Summary of Analysis of Variance of the heart rate variability band power spectrum.

Spectral band	p	p<0.05 between group I and II	p<0.05 between group I and III	p<0.05 between group II and III
0.15-0.30	0.288	no	No	no
0.30-0.45	0.006	yes	Yes	no
0.45-0.60	0.001	yes	Yes	no
0.60-0.75	0.038	no	Yes	no
0.75-0.90	0.003	yes	Yes	no
0.90-1.05	<0.001	yes	Yes	no
1.05-1.20	<0.001	yes	Yes	yes
1.20-1.35	<0.001	yes	Yes	no
1.35-1.50	<0.001	yes	Yes	no

Group I: 10-12 weeks, group II: 13-16 weeks, and group III: 17-20 weeks.

system (Baldzer 1989; Chatow 1995). Long and short term variability in fetal heart rate was studied on the basis of previous findings in the instrumented chick embryo (Kempski 1993; 1995) which focus on flow velocity and heart rate variability. In our study we discarded the power spectra frequency band, 0-0.15 Hz, since the Doppler signal duration (> 16 s) was too short for reliable interpretation of frequencies below 0.15 Hz.

Animal studies done in the developing heart have demonstrated that autonomic nerves reach the heart in the late embryonic period (Gordon 1993). Histological and ultrastructural studies have shown the presence of developing nerves in the human heart at 5-6 weeks of gestation (Gardner and O'Rahilly 1976). The ontogenesis of parasympathetic cardiac innervation is considered to precede that of sympathetic innervation in chick and most mammals including man (Pappano 1977). In the early developing heart, the presence of intracardiac nerves may not reflect functional innervation (Pappano 1977; Epstein 1990). Walker (1975) showed in vitro evidence for the possibility of autonomic neuroeffector transmission in the human fetus at 16-17 weeks of gestation, yet there is no evidence of reflex activation of the autonomic nervous system by respiration, baroreceptors or vasomotor activity.

In the present study, we observed a random broad band pattern of heart rate variability in group I and group II (Figure 3), which may indicate that the autonomic control mechanisms are not yet developed. Karin *et al* (1993) demonstrated that at 23 weeks of gestation, the functionally immature fetal

autonomic nervous system generates a large variability in heart rate, resulting in a power spectrum with twice as much energy, when compared with the more mature and more stable autonomic nervous system during the last period of gestation (40 weeks). In the present study, gestational age group III (17-20 weeks) presents a further decrease in fetal heart rate variability which is centered around lower frequencies compared to group I and II (Table 2). This may reflect some degree of autonomic function during this period of fetal life.

In adults, the heart rate power spectrum reveals a clear peak in the higher frequency range, which is attributed to breathing activity. In the fetus, a widely dispersed respiration peak is observed around the 0.7 Hz centre, although not as powerful and focused as in adults (Karin 1993). The heart rate band power spectrum in our study also displayed high frequency variability. Whilst fetal breathing movements may occur as early as 11 weeks of gestation (Boddy and Dawes 1975), this high frequency component does not represent fetal breathing activity, since all our Doppler recording were performed during fetal apnoea.

It can be concluded that the data presented reflect normal fetal heart rate variability which is characterised by a decrease with advancing gestational age and a shift to lower frequencies at 17-20 weeks suggesting autonomic functional development.

3.3 Heart rate and flow velocity variability as determined from umbilical Doppler velocimetry at 10-20 weeks of gestation

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Abstract

1. The aim of this study was to define from umbilical artery flow velocity waveforms absolute peak systolic and time-averaged velocity, fetal heart rate, fetal heart rate and flow velocity variability and the relation between fetal heart rate and velocity variables in early pregnancy.
2. A total of 108 women presenting with a normal pregnancy from 10 to 20 weeks of gestation consented to participate in a cross-sectional study design. Doppler ultrasound recordings were made from the free floating loop of the umbilical cord.
3. Umbilical artery peak systolic and time-averaged velocity increased at 10-20 weeks, whereas fetal heart rate decreased at 10-15 weeks of gestation and plataued thereafter. Umbilical artery peak systolic velocity variability and fetal heart rate variability increased at 10-20 and 15-20 weeks, respectively.
4. The inverse relationship between umbilical artery flow velocity and fetal heart rate at 10-15 weeks of gestation suggests that the Frank-Starling mechanism regulates cardiovascular control as early as the late first and early second trimesters of pregnancy. A different underlying mechanism is suggested for the observed variability profiles in heart rate and umbilical artery peak systolic velocity. It is speculated that heart rate variability is mediated by maturation of the parasympathetic nervous system, whereas peak systolic velocity variability reflects the activation of a haemodynamic feedback mechanism.

Introduction

Fetal heart rate variability in the late second and third trimester of pregnancy is widely used to study the condition of the fetus in utero. Reduced heart rate variability occurs in pathophysiological states like intra-uterine growth retardation and may be related to fetal stress and diminished cardiovascular functional reserve (Breborowicz 1988). In the late first and early second trimester of human pregnancy, there is a decrease in fetal heart rate and appearance of beat-to-beat variation in fetal heart rate. These changes probably reflect maturation of vagal parasympathetic functional control (Wladimiroff and Seelen 1972; Schifferli and Caldeyro-Barcia 1973). In normal pregnancy there is a positive correlation between fetal heart rate variability and gestational age throughout the second trimester (Pillai and James 1990). Therefore, fetal heart rate variability is a marker of normal physiological and pathophysiological processes.

Combined transvaginal and transabdominal Doppler ultrasonography provides measures of human fetal arterial flow velocities during the late first and early second trimester of pregnancy (Wladimiroff 1991a; van Splunder 1996b). Moreover, using Doppler ultrasonography it has also become possible to obtain information on beat-to-beat variability in arterial blood flow velocity during this early period of pregnancy (Ursem 1998a).

Spectral analysis of blood flow velocity in the dorsal aorta of the chick embryo showed a change in velocity modulation with development, which probably reflects shifts in haemodynamic control associated with cardiovascular morphogenesis (Kempski 1995).

The aims of the present study were to determine from umbilical artery flow velocity waveforms: (i) absolute peak systolic and time-averaged velocities; (ii) fetal heart rate, fetal heart rate variability and flow velocity variability; and (iii) the interrelationship between fetal heart rate and flow velocity variables in normal pregnancies at 10-20 weeks of gestation.

Methods

Subjects

A total of 108 women with a normal singleton pregnancy between 10-20 weeks of gestation (median 15 weeks) consented to participate in the cross-sectional study. The study was approved by the Hospital Ethics Committee at the Erasmus University, Rotterdam and the University of Rochester, Rochester, NY. Forty-seven women were nulliparous. Maternal age ranged between 14-46 years (median 29 years). Pregnancy duration was estimated from the last menstrual period and confirmed by ultrasound measurement of the fetal crown-rump length (10-12 weeks) or biparietal diameter (12-20 weeks). All pregnancies were uncomplicated and resulted in the term delivery of a normal infant with a birth weight between the 10th-90th centile corrected for maternal parity and fetal sex (Kloosterman 1970).

Doppler recordings

Ultrasound Doppler studies were performed with a Toshiba SSH 140A (Toshiba Corp., Medical Systems Division, Tokyo, Japan). A combined transvaginal real-time and colour Doppler system (carrier frequency 6 MHz and 5 MHz respectively) was used at 10-13 weeks of gestation and a combined transabdominal real-time and colour Doppler system (carrier frequency 5.0 MHz and 3.75 MHz respectively) was used at 14-20 weeks of gestation. For a more detailed visualisation of fetal vessels, transvaginal ultrasonograpgy was used at 10-13 weeks, whereas due to increasing fetal size the transabdominal approach was used as from 13-14 weeks of gestation. The system operates at power outputs of < 100 mW/cm^2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specification. These output levels are clearly situated in the lower regions for acoustic output of Japanese and American diagnostic equipment (Ide 1989). The total examination time was limited to 15 minutes in each instance. Colour Doppler was used to obtain the highest velocity signal in the umbilical artery so that the angle of insonation was kept as small as

possible. The high pass filter was set at 100 Hz and the sample volume length was 0.2-0.3 cm. Depending on the scanning depth, the pulse repetition frequency ranged between the 3-6 kHz. All Doppler studies were performed with the women in the semirecumbent position and during fetal apnoea. Doppler recordings were performed by one examiner (NTCU). Flow velocity waveforms from the umbilical artery were obtained from the free-floating loop.

The change in methodology was validated by comparison of transvaginally and transabdominally collected umbilical artery waveforms in 10 normal singleton pregnancies at 12-13 weeks of gestation. This period of gestational age was selected, because at that time a transition from transvaginal to transabdominal scanning takes place.

Data processing

Umbilical artery Doppler recordings were stored on sVHS videotape in PAL format using a Panasonic model AG7350 machine (Matsushita Electric Ind Co, Japan). Umbilical artery audio waveforms were digitised at 12 kHz using an A/D data acquisition board (LabPC+ and BNC-2081 boards, National Instruments, Austin, TX, USA). In a previous study (Ursem 1998a), several methods were described to reconstruct (estimate) the velocity waveform from the Doppler audio signal of the umbilical artery blood flow. The maximum velocity reconstruction method is preferred because it is relatively insensitive to noise, non-uniform insonation, and wall filter settings.

The maximum velocity waveform was estimated from the Doppler data using computer algorithms developed in our centre using LabVIEW® software (National Instruments). The power spectra were calculated using a 512-points Fast Fourier transform with a Hanning window and an overlap of 75%. The maximum velocity envelope was estimated using a threshold level adaptive to the level of background noise (Hoskins 1991). The algorithm starts from the high-frequency end of a spectral line (power spectrum) and the highest frequency which exceeds the threshold value is called the maximum frequency. The threshold is interactively changeable so that as the background level increases, a higher threshold value can be used.

The first derivative of the velocity waveform was used to determine the instantaneous heart rate, which is the reciprocal of the time between successive peaks. For the entire maximum velocity waveform, the peak systolic velocity (PSV, mm/s), time-averaged velocity (TAV, mm/s) and fetal heart rate (FHR, bpm) per cardiac cycle were calculated. The duration of the Doppler recording of the umbilical artery ranged between 18-45 s.

Reproducibility of the umbilical artery flow velocity waveform recordings was established in a separate study of 11 singleton pregnancies at 10-20 weeks of gestation. In each of the 11 fetuses three independent measures were made at 5-min time intervals. For each of the waveform parameters (FHR, PSV and TAV) the mean coefficient of variation was determined.

Statistical analysis

For each fetus, the mean and standard deviation (SD) was calculated for fetal heart rate, peak systolic velocity and time averaged velocity. A logarithmic transformation was performed for the standard deviation to stabilise the variability with gestational age. For the expression of variability in the velocity parameters we used the coefficient of variation, because the standard deviation of peak systolic and time-averaged velocity were not independent of the mean. For all six variables, i.e. mean and standard deviation of heart rate, mean and coefficient of variation of peak systolic and time-averaged velocities, piece-wise linear regression (Neter and Wasserman 1974), also called the 'broken stick' method, was used to evaluate the relation between these variables and gestational age. If the difference between the slope before and after the breaking point was statistically significant, further analysis was performed with the resulting broken stick line. Slopes are given as result ± standard error. The p50, p10 and p90 were established using the mean and the mean±1.64 SD of the residuals. Multiple regression analysis was carried out to evaluate gestational age, fetal heart rate, parity and maternal age simultaneously regarding their predictive value. A paired *t*-test was used to establish the difference in umbilical artery flow velocity waveforms between the transvaginal and transabdominal approach. $P \leq 0.05$ was taken as the level of significance. All calculations were performed with SPSS 6.1 software (SPSS Inc, Chicago, IL, USA).

Results

The reproducibility study revealed a mean coefficient of variation of 1.1 (range: 0.2-3.5)% for fetal heart rate, of 1.9 (range: 1.1-3.3)% for peak systolic velocity, and of 2.4 (range: 1.2-3.9)% for time-averaged velocity. Comparison of transvaginal and transabdominal flow velocity waveform recordings at 12-13 weeks revealed no statistically significant difference for umbilical artery flow velocity parameters. The intra-class correlation coefficient between the two recording methods was 0.99 for fetal heart rate, 0.92 for peak systolic velocity and 0.91 for time-averaged velocity.

During the gestational age period of 10-15 weeks, mean umbilical artery peak systolic velocity increased (Figure 1) and mean fetal heart rate decreased (Figure 2). Both variables plateaued for the remainder of the study period.

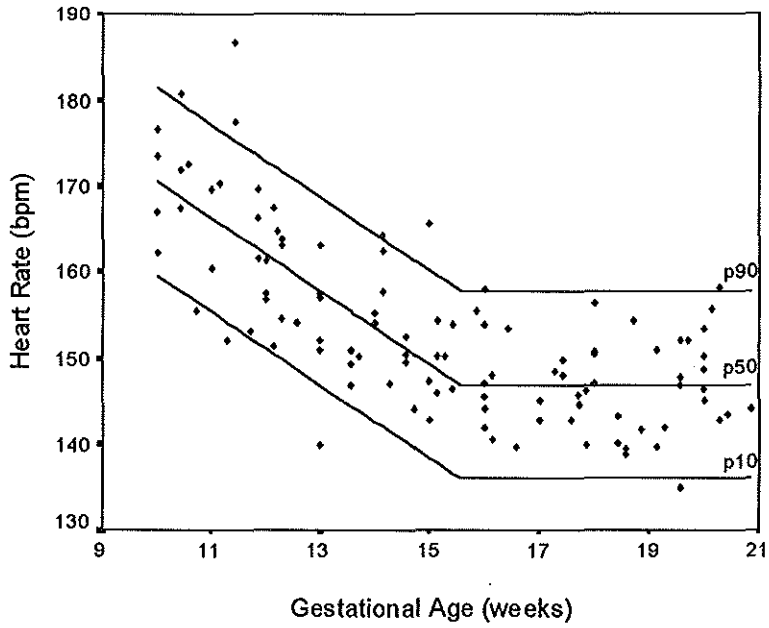

Figure 1. Individual data and centiles (p10, p50, p90) for umbilical artery peak systolic velocity relative to gestational age. The slope up to the calculated breakpoint (i.e. 15.8 ± 0.6 weeks) equals 26.6 ± 4.3 mm/s per week.

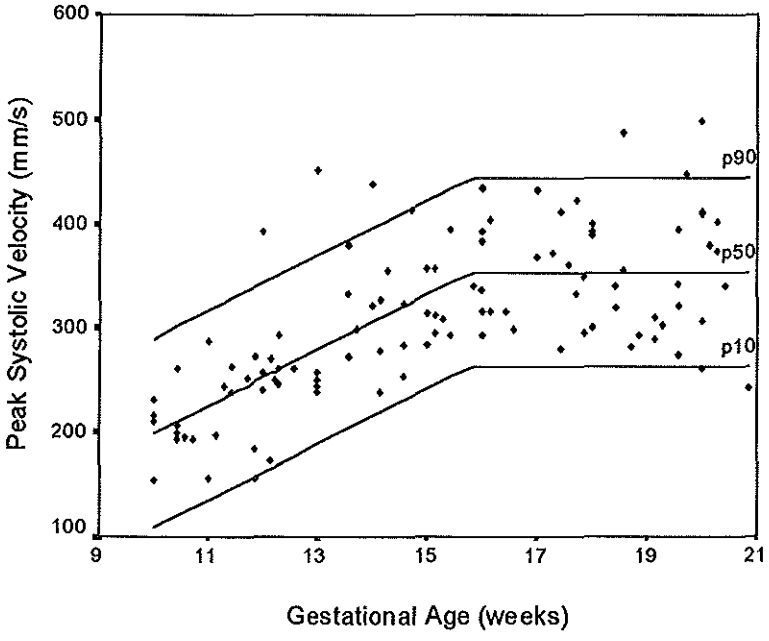

Figure 2. Individual data and centiles (p10, p50, p90) for fetal heart rate relative to gestational age. The slope up to the calculated breakpoint (i.e. 15.6 ± 0.5 weeks) equals -4.2 ± 0.5 bpm per week.

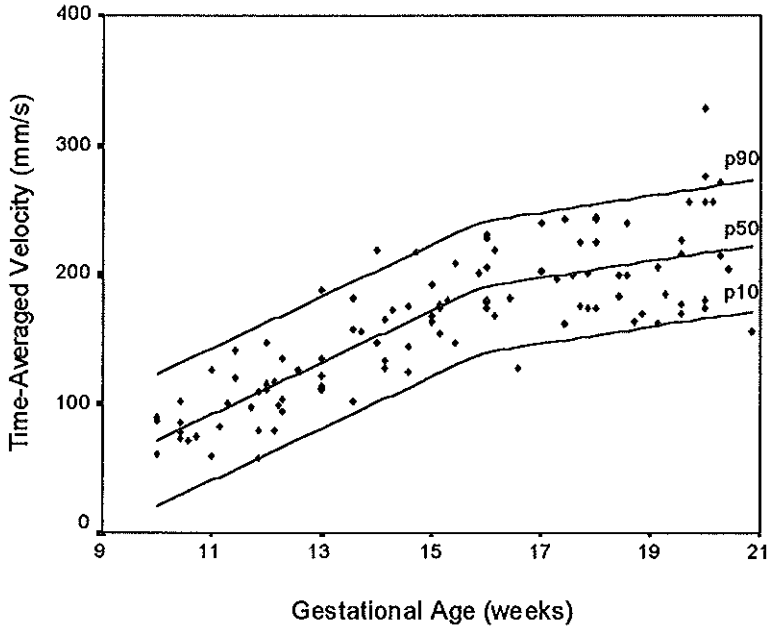

Figure 3 Individual data and centiles (p10, p50, p90) for umbilical artery time-averaged velocity relative to gestational age. The slope up to the calculated breakpoint (i.e. 15.9 ± 0.9 weeks) equals 20.1 ± 2.4 mm/s per week and thereafter 6.5 ± 3.0 mm/s per week.

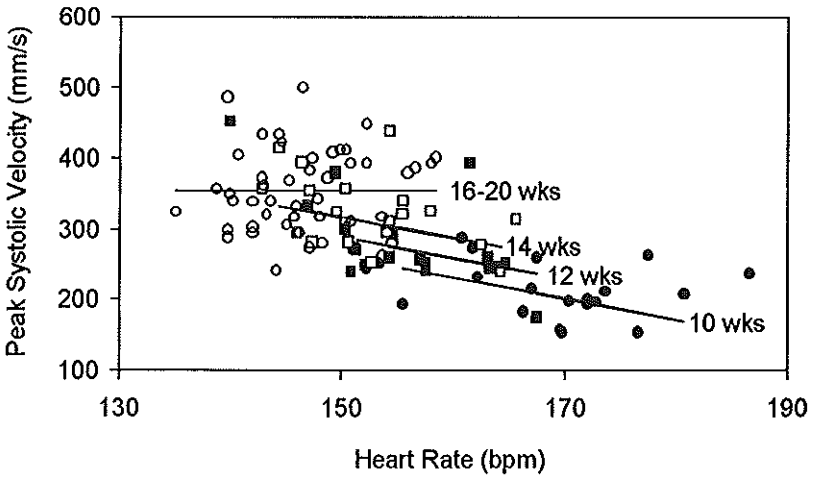

Figure 4. Umbilical artery peak systolic velocity relative to fetal heart rate. Drawn lines represent mean PSV versus FHR at different gestational ages (10, 12, 14 and 16-20 weeks of gestation). Data points indicate individual PSV values according to the different gestational age categories (● 10-11 wks, ■ 12-13 wks, □ 14-15 wks, ○ 16-20 wks).

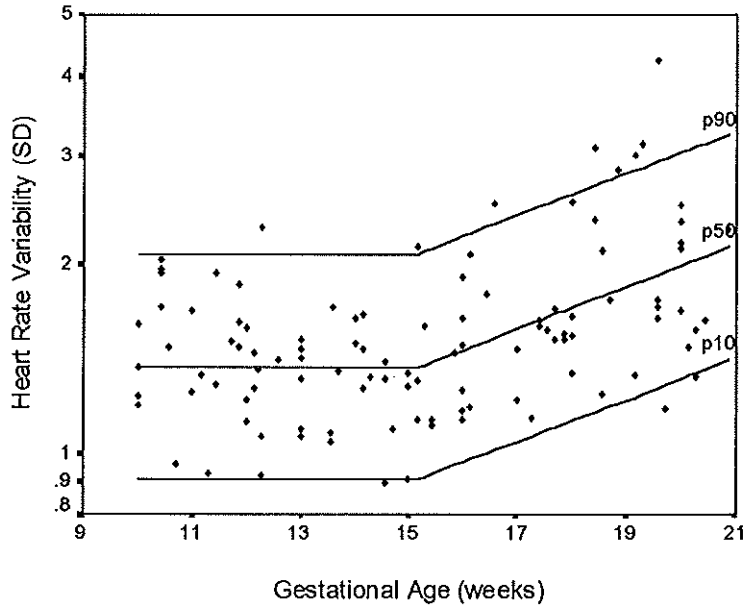

Figure 5. Individual data and centiles (p10, p50, p90) for heart rate variability relative to gestational age. The slope after the calculated breakpoint (i.e. 15.2 ± 1.0 weeks) equals 0.08 ± 0.2 bpm per weeks. Note the logarithmically transformed vertical axis.

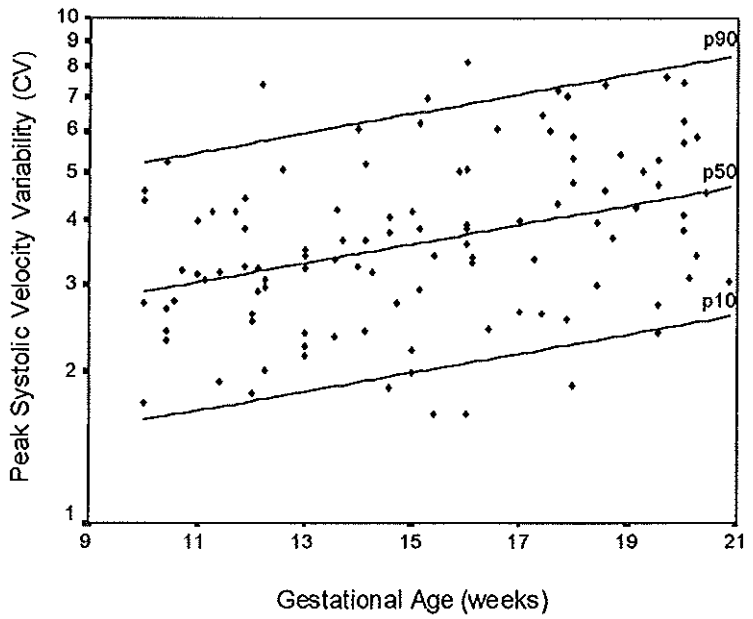

Figure 6. Individual data and centiles (p10, p50, p90) for peak systolic velocity variability relative to gestational age. (r = 0.6, p < 0.001). Note the logarithmically transformed vertical axis.

Mean umbilical artery time-averaged velocity increased between 10-20 weeks of gestation (Figure 3), but the slope of velocity differed between 10-15 and 16-20 weeks of gestation. Umbilical artery peak systolic velocity was inversely correlated with fetal heart rate at 10-15 weeks of gestation. As both peak systolic velocity and fetal heart rate were related to gestational age up to about 16 weeks, multiple regression analysis was used in this gestational age range to evaluate whether fetal heart rate and gestational age displayed an independent predictive value with regard to peak systolic velocity. Both variables demonstrated a significant relation with peak systolic velocity. This relationship becomes non-existent at 16-20 weeks of gestation. The resulting multiple regression equations are given in Table 1, while the corresponding visual display of the relationships is shown in Figure 4. A similar analysis was carried out for the time-averaged velocity, fetal heart rate and gestational age. Table 1 shows the resulting equations; the relationship between umbilical artery time-averaged velocity and fetal heart rate is different before and after 16 weeks of gestation. A statistically significant increase is demonstrated for fetal heart rate variability, expressed as SD at 15-20 weeks (Figure 5) and for umbilical artery peak systolic velocity variability, expressed as CV at 10-20 weeks of gestation (Figure 6).

The coefficient of variation for fetal heart rate variability was 0.97 (quartile range: 0.81-1.13)%, whereas for umbilical artery peak systolic velocity and time-averaged velocity variability, the median percentages were 3.63 (quartile range: 2.75-4.74)% and 3.75 (quartile range: 2.76-4.57)%, respectively.

Table 1. Multiple regression analysis.

Parameter	GA (wks)	Mathematical description	Significance	
			GA coefficient	HR coefficient
PSV (mm/s)	< 16	14.6*GA-2.9*HR+547.5	$p = 0.005$	$p = 0.001$
PSV (mm/s)	≥ 16	354.4	NS	NS
TAV (mm/s)	< 16	16.6*GA-0.8*HR+50.1	$p < 0.001$	$p = 0.050$
TAV (mm/s)	≥ 16	6.2*GA+2.2*HR-233.0	$p = 0.050$	$p = 0.025$
VarHR (bpm)	< 16	0.005*HR-0.6	NS	$p < 0.001$
VarHR (bpm)	≥ 16	0.03*GA-0.007*HR+0.7	$p = 0.012$	$p = 0.031$
VarPSV (%)	10-20	-0.004*HR+1.2	NS	$p = 0.011$
VarTAV (%)	10-20	0.56	NS	NS

GA: gestational age; HR: heart rate; PSV: umbilical artery peak systolic velocity; TAV: umbilical artery time-averaged velocity; varHR: heart rate variability (SD); varPSV: peak systolic velocity variability (CV); varTAV: time-averaged velocity variability (CV). NS = not significant.

Discussion

Doppler recordings of absolute velocities in the umbilical artery over a longer period of time (18-45 s) document variability in both fetal heart rate and flow velocity amplitude. Fetal heart rate decreased between 10-15 weeks of gestation but remained constant during the remainder of the study period. The present data agree with previous studies in which fetal heart rate decreases from approximately 175-180 bpm at 9-10 weeks to 145-150 bpm at 15 weeks of gestation and remains more or less constant during the remainder of intrauterine life (Wladimiroff and Seelen 1972; Robinson and Shaw-Dunn 1973; Martinez 1996). Colour-coded Doppler ultrasound allows recordings of peak umbilical artery velocity signals at an interrogation angle of less than 15°. A good reproducibility was established for umbilical artery peak systolic velocity, time-averaged velocity, and fetal heart rate. Both peak systolic and time-averaged umbilical artery flow velocity demonstrated a marked increase up to 15 weeks of gestation followed by plateauing or a less pronounced rise at 15-20 weeks of gestation, thus mirroring the fore-mentioned changes in fetal heart rate. Of interest is that a marked reduction in pulsatility index has been established in the descending aorta and umbilical artery at 10-15 weeks, reflecting a significant reduction in fetoplacental vascular resistance at this early stage of gestation. Placental microanglogenesis is probably the mechanism for these resistance changes (Jauniaux 1991). The change in umbilical artery flow velocity before and after 16 weeks of gestation is unrelated to the measurement technique. No difference between transvaginal and transabdominal umbilical artery flow velocity recordings could be established at 12-13 weeks of gestation.

In late pregnancy, auditory stimulation increases heart rate and decreases ventricular stroke volume without changing ventricular output (Kenny 1987). Thus, the Frank-Starling mechanism and not heart rate is the major regulator of cardiac output in the human fetus. Similar findings have been found in the fetal lamb (Kirkpatrick 1976) and chick embryo (Wagman 1990). In our study we were unable to measure blood volume flow, but the inverse relationship between umbilical artery peak systolic velocity and fetal heart rate suggests that the Frank-Starling mechanism regulates fetal cardiovascular control as early as the early second trimester of pregnancy. If the Frank-Starling mechanism is indeed operational as early as 10-15 weeks of gestation, then the pronounced rise in umbilical artery peak systolic and time-averaged velocity may not only reflect a fetal growth-determined rise in volume flow, but also an increase in cardiac stroke volume due to an increase in diastolic filling time related to the slower fetal heart rate. The less marked increase in time-averaged velocity at 16-20 weeks may be mainly growth-related since heart rate remains rather constant at that time.

No correlation existed between fetal heart rate variability and umbilical artery peak systolic velocity variability, suggesting that the gestational age related increase in fetal heart rate variability and peak systolic velocity variability are mediated by two separate control mechanisms. Maturation of the parasympathetic nervous system mediates the increase in fetal heart rate variability (Wladimiroff and Seelen 1972; Schifferli and Caldeyro-Barcia 1973). A cardiovascular feedback mechanism probably increases variability in peak systolic velocity as has been demonstrated in the chick embryo. In the chick, short-duration modulations in aortic blood flow and vascular impedance are a haemodynamic control during early development (Kempski 1993; Yoshigi 1996). Several oscillating physiologic mechanisms arising from respiration, baroreceptor activity and vasomotor activity may influence heart rate variability. Whether all these mechanisms influence heart rate variability in the early human fetus is not known. Power spectral analysis of longer lasting umbilical artery waveform recordings will be needed to investigate which mechanisms are present this early in pregnancy.

In the fetal lamb, vagal blockade by atropine increases fetal heart rate and decreases beat-to-beat variability (Silmes 1990). In the human fetus, atropine administered to the mother after 15 weeks of pregnancy results in a rise in fetal heart rate, that correlates with advancing gestation (Schifferli and Caldeyro-Barcia 1973). Parasympathetic maturation is considered responsible for the gestational age-related decrease in fetal heart rate; these changes occur in parallel with the appearance of fetal heart rate variability (Fleisher 1997). However, the present study has shown that at 10-15 weeks the fall in fetal heart rate is not associated with an increase in fetal heart rate variability. Therefore, an alternative explanation for the reduction in fetal heart rate must be considered.

There is evidence that cardiac muscle cells are immature at 9-10 weeks of gestation. During the subsequent weeks myofibrils, which form the contractility apparatus, appear in large numbers. The maturation of these myofibrils may affect the performance of the myocardium to load resulting in more efficient contractions (Robinson and Shaw-Dunn 1973). Developmental changes in the myocardial contractile system demonstrate that, in 18- and 21-day-old rabbits fetuses, the myofibrils are scarce and disorganised compared with 28-day-old rabbits (Nakanishi 1988). According to the data of Sissman (1970), 18- and 21-day-old rabbit fetuses are comparable with 10-12-week-old human fetuses and 28-day-old rabbit fetuses are comparable with 18- week-old human fetuses. Thus, improvements in myocardial contractility may be responsible for the early decrease in heart rate in the human fetus.

Fetal heart rate variability data from the present study are at variance with earlier data indicating a decrease with advancing gestational age (Ursem 1998b).

We suggest that the difference relates to the reconstruction method used to estimate the velocity waveform. Recently we found that the mode reconstruction method, (i.e. frequencies with the highest intensity within the power spectra) is particularly sensitive to noise, non-uniform insonation and wall filter settings (Ursem 1998a) with emphasis on the 10-14 weeks gestation period. Therefore, the previous method generated a larger variability in heart rate compared with the reconstruction method used in the present study.

The increase in umbilical artery flow velocity coincides with a decline in fetal heart rate at 10-15 weeks of gestation. These observations suggest that the Frank-Starling mechanism regulates cardiovascular function during this early stage of human pregnancy. The pregnancy period of 15-20 weeks is characterised by an increase in both fetal heart rate variability and umbilical artery peak systolic velocity variability. The former may be mediated by maturation of the parasympathetic nervous system, and the latter by the activation of a haemodynamic feedback mechanism.

3.4 Assessment of fetal heart rate and velocity variability by velocimetry of the descending aorta

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Abstract

Objectives Determination of gestational age-related modulations in fetal heart rate and descending aorta blood flow velocity in the early human fetus and comparison of aortic variability data with data obtained from the umbilical artery.

Methods Doppler studies of descending aorta velocity waveforms were performed at 10-20 weeks in 55 normal pregnant women. In 24 out of 55

women, Doppler recordings from both the descending aorta and umbilical artery were collected. Absolute values and variability for fetal heart rate, peak systolic and time-averaged velocity were determined from flow velocity waveforms of at least 18 s duration.

Results From 10-20 weeks of gestation, descending aorta peak systolic and time-averaged velocity increased, whereas fetal heart rate decreased. Descending aorta peak systolic variability also increased. However, time-averaged velocity variability and fetal heart rate variability remained constant during the study period. In the subset of 24 women, the fetal heart rate variability and velocity variability data from descending aorta and umbilical artery were not significantly different.

Conclusions Reproducible fetal heart rate and velocity variability data can be derived from the descending aorta and umbilical artery. The increase in heart rate variability observed in the umbilical artery was not seen in recordings obtained from the descending aorta. The rise in peak systolic velocity variability was seen in the umbilical artery as well as in the descending aorta.

Introduction

Pulsed Doppler ultrasonography serves as an essential tool for the assessment of human fetal haemodynamics (Wladimiroff 1992). The late first and early second trimester of pregnancy is characterised by marked changes in arterial flow velocity waveform patterns, reflecting a marked reduction in fetoplacental vascular resistance (Wladimiroff 1991b). Recently, we observed that variability in fetal heart rate and umbilical artery flow velocity is gestational age-related in normal pregnancies (Ursem 1998b). Studies from our centre demonstrated altered fetal heart rate variability and umbilical artery flow velocity variability under the pathophysiological conditions of pregnancy induced hypertension and pregnancies complicated by insulin dependent diabetes mellitus. Thus, fetal heart rate variability and flow velocity variability derived from the umbilical artery are likely markers for cardiovascular health in the human fetus at 10-20 weeks of gestation.

Doppler studies from the descending aorta of the chick embryo and fetal lamb showed that blood flow velocity waveforms are related to fetal cardiac function (Thompson 1994; Clark and Hu 1982). However, the simultaneous recording of dorsal aortic peripheral arterial blood velocity is technically challenging (Yoshigi 1997). In the human fetus, fetal cardiac output correlates with blood flow in the descending aorta and aortic peak systolic velocity may be related to cardiac performance (Tonge 1986; Wladimiroff and McGhie 1981). We therefore hypothesised that the variability measures observed in the umbilical circulation are also present in the fetal descending aorta.

The objective of the current study was: (i) to determine gestational age related changes in absolute values for, and beat-to-beat variability in, fetal heart rate, peak systolic velocity and time-averaged velocity derived from descending aorta flow velocity waveforms in normal pregnancies at 10-20 weeks of gestation; (ii) to compare these variables with fetal heart rate and flow velocity data derived from the umbilical artery.

Methods

Subjects

A total of 55 healthy women with a normal singleton pregnancy consented to participate in a cross-sectional study design. The study was approved by the Hospital Ethics Committee at the Erasmus University, Rotterdam and the University of Rochester, Rochester, NY. Gestational age varied between 10-20 weeks (median 14 weeks) and was estimated from the last menstrual period and confirmed by ultrasound measurement of the fetal crown-rump-length or biparietal diameter. Maternal age ranged between 16-43 years (median 28 years). All pregnancies were uncomplicated and resulted in the term delivery of normal infants with a birth weight between the 10th-90th centime corrected for maternal parity and fetal sex (Kloosterman 1970). Women were selected according to five gestational age subgroups (10-11 weeks: n=12, 12-13 weeks: n=12, 14-15 weeks: n=12, 16-17 weeks: n=10, 18-19 weeks: n=9) to guarantee a homogenous distribution. In 24 out of 55 women, both Doppler recordings from the descending aorta and umbilical artery were collected. Based on the same gestational age distribution, the numbers were as follows: 10-11 weeks: n=7; 12-13 weeks: n=6; 14-15 weeks: n=5; 16-17 weeks: n=4 and 18-19 weeks: n=2. The time interval between Doppler recording in the descending aorta and umbilical artery was always less than 15 minutes. Each woman was included in the study only once.

Doppler recordings

Ultrasound Doppler studies were performed with a Toshiba SSH 140A (Toshiba corp., Medical systems Division, Tokyo, Japan). A combined transvaginal real-time and colour Doppler system (carrier frequency 6 MHz and 5 MHz, respectively) was used at 10-13 weeks of gestation and a combined transabdominal real-time and colour Doppler system (carrier frequency 5.0 MHz and 3.75 MHz, respectively) was used at 14-20 weeks of gestation. The system operates at power outputs of < 100 mW/cm^2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specification. Doppler recordings were performed by one examiner (NTCU). Flow velocity waveforms from the lower thoracic part of the descending aorta were obtained from a sagittal cross-section through the fetal trunk, displaying a major section of the fetal spine. Flow velocity waveforms from the umbilical artery were obtained

from a straight portion of the free-floating loop of the umbilical cord. The vessel interrogation angle was < 15°. The high pass filter was set at 100 Hz and the sample volume length was 0.2-0.3 cm. All Doppler studies were performed with the women in the semirecumbent position and during fetal apnoea.

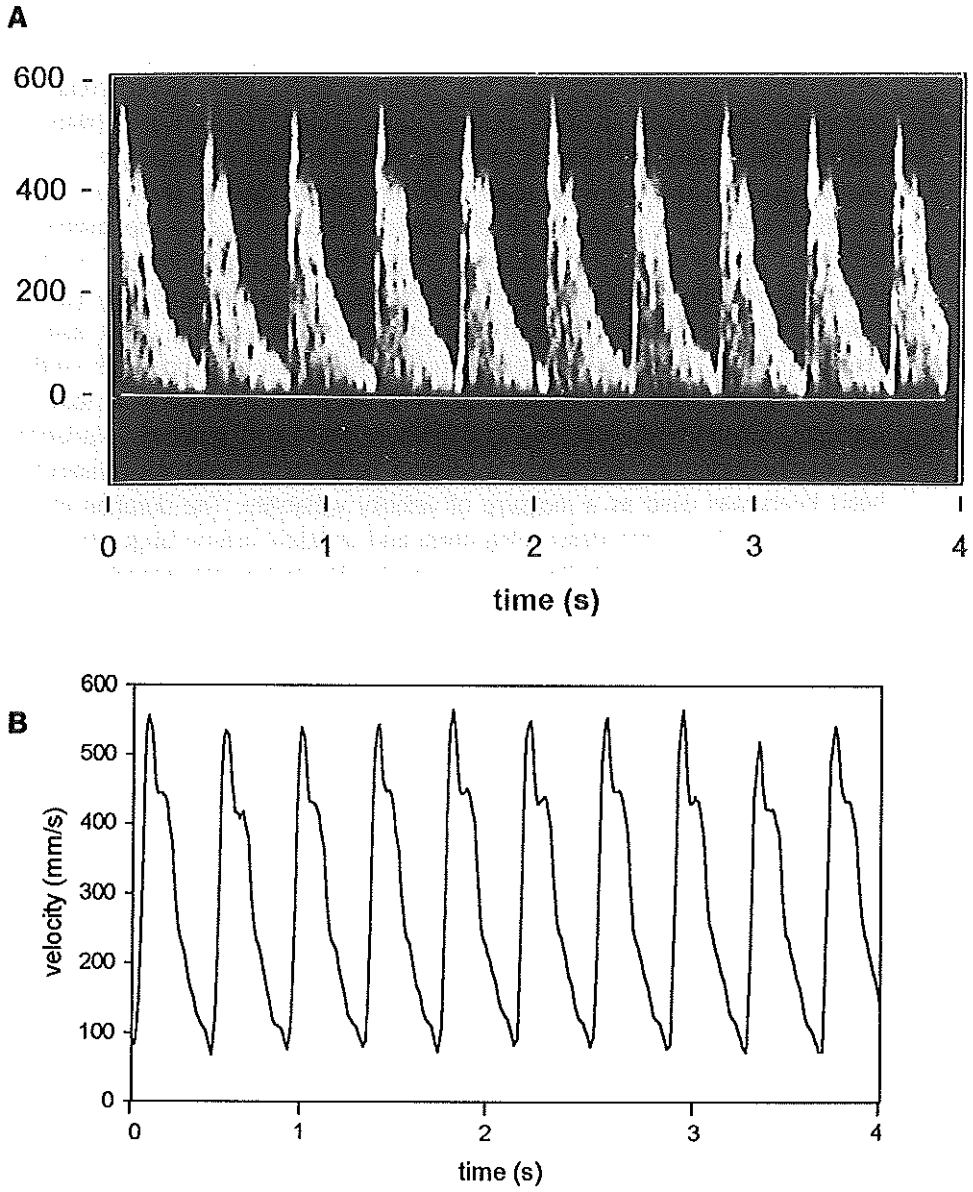

Figure 1. Doppler ultrasound recording of blood flow velocity waveforms from the thoracic part of the descending aorta at 18 weeks of gestation (A). Schematic presentation of the estimated maximum velocity envelope of the descending aorta at 18 weeks of gestation which is used for calculation of heart rate and velocity variability data (B).

Data processing

Descending aorta and umbilical artery Doppler recordings were stored on sVHS videotape in PAL format using a Panasonic model AG7350 machine (Matsushita Electric Ind Co, Japan) and digitised at 12 kHz using an AD data acquisition board (LabPC+ and BNC-2081 boards, National Instruments, Austin, TX). The maximum flow velocity waveforms from the descending aorta and umbilical artery were reconstructed from the Doppler audio data using computer algorithms developed in our centre using LabVIEW software (National Instruments, Austin, TX) (Figure 1). Here digitised audio data packets of 512 points and 75% overlap were used with Hanning windowing prior to Fourier transform spectral analysis. From the collected power spectra the maximum frequency (velocity) can be estimated. A detailed description of the analysis technique and the computer algorithms has been published previously (Ursem 1998a). For the entire maximum flow velocity waveform, the peak systolic velocity (mm/s), time-averaged velocity (mm/s) and heart rate (bpm) per cardiac cycle were calculated. The standard deviation (SD) over a period of at least 45 consecutive heart beats was used as a measure of the fetal heart rate variability and the coefficient of variation (CV) over a period of at least 45 consecutive heart beats was used as a measure of velocity variability. The duration of the Doppler recording of the descending aorta and umbilical artery ranged between 18-44 s (median 24 s), including 44-118 (median 65) high quality waveforms.

Reproducibility study

A previous reproducibility study of the umbilical artery flow velocity waveform recordings at 10-20 weeks of gestation revealed a mean CV of 1.1 (range: 0.2-3.5)% for fetal heart rate, a mean CV of 1.9 (range: 1.1-3.3)% for peak systolic velocity, and a mean CV of 2.4 (range: 1.2-3.9)% for time-averaged velocity (Ursem 1998c).

Reproducibility of the descending aorta flow velocity waveform recordings was established in a separate study of 11 singleton pregnancies at 10-20 weeks of gestation. In each of the 11 fetuses three independent measures were made at five minute time interval. For each of the waveform parameters (FHR, PSV and TAV) the mean CV was determined.

Statistical analysis

For each fetus, the mean and SD was calculated for heart rate, absolute peak systolic velocity and absolute time-averaged velocity, as well as fetal heart rate variability. For the expression of variability in the velocity parameters, we used the CV, because the SD of peak systolic and time-averaged velocity was not independent of the mean. A logarithmic transformation was performed for the absolute velocity parameters, velocity variability and heart rate variability to stabilise the variance for gestational age. For all six variables, i.e. mean and SD

of heart rate, mean and CV of peak systolic and time-averaged velocities, linear regression was used to evaluate the relationship between these variables and gestational age. The p50, p10 and p90 were established using the mean and the mean ± 1.64 SD of the residuals. $P \leq 0.05$ was taken as the limit of significance. For comparison of the velocity and heart rate parameters determined from descending aorta and umbilical artery a paired Wilcoxon's test was used. All calculations were performed with SPSS 6.1 software (SPSS Inc., Chicago, IL).

Results

The reproducibility study of the descending aorta revealed a mean CV of 0.7 (range: 0.1 - 2.3)% for FHR, of 2.2 (range: 0.3 - 4.4)% for PSV, and of 3.0 (range: 0.2 - 5.8)% for TAV.

During the gestational age period of 10-20 weeks, descending aorta peak systolic velocity (slope=0.04, r=0.44, p<0.001) and time-averaged velocity (slope=0.07, r=0.60, p<0.001) increased, whereas instantaneous fetal heart rate decreased (slope=-2.52, r=0.71, p<0.001). A statistically significant increase was demonstrated for descending aorta peak systolic at 10-20 weeks (Figure 2). Descending aorta time-averaged velocity variability (Figure 3) and fetal heart rate variability (Figure 4) remained constant during the entire study period.

Comparison of the velocity and fetal heart rate parameters between the descending aorta and umbilical artery in the subset of 24 women is displayed in Table 1. The peak systolic and time-averaged velocities were significantly higher in the descending aorta than in the umbilical artery. These differences were not gestational age dependent. The remaining parameters were not significantly different between the two vessels.

Table 1. Median and range for fetal heart rate (bpm), peak systolic velocity (mm/s), time-averaged velocity (mm/s), heart rate variability, peak velocity variability, time-averaged velocity variability determined in the both descending aorta and umbilical artery of one fetus (n=24).

	Descending Aorta		Umbilical Artery	
	median	range	median	range
Peak systolic velocity	328.8*	211.3-765.4	275.3	156.5-399.9
Time-averaged velocity	153.2*	88.1-368.6	125.9	59.3-227.5
Heart rate	154	145-187	154	146-187
Peak systolic velocity variability	3.40	1.84-6.54	3.09	1.65-7.20
Time-averaged velocity variability	4.10	1.74-6.42	3.57	1.57-8.08
Heart rate variability	1.34	0.79-4.13	1.48	1.05-2.29

Heart rate variability data expressed as standard deviation and velocity variability data expressed as coefficient of variation. * $P < 0.05$ by paired Wilcoxon's test.

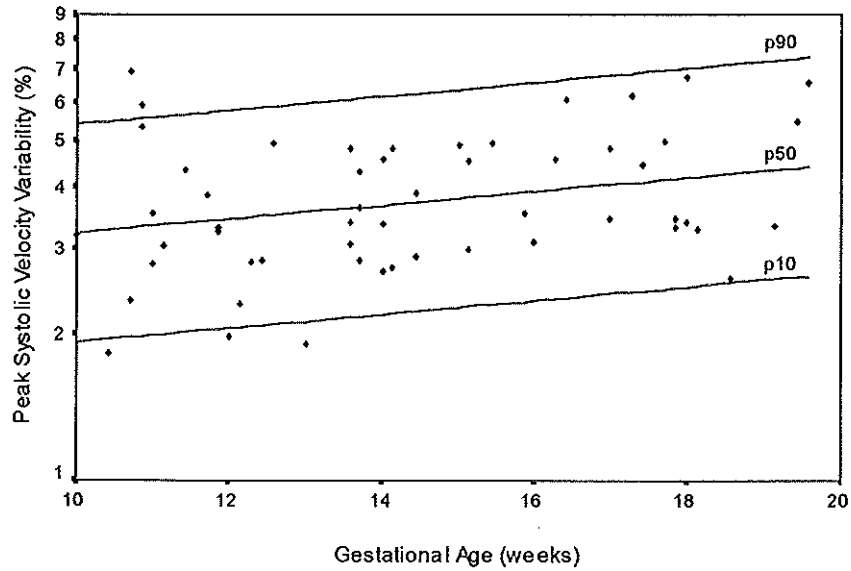

Figure 2. Individual data and centiles (p5, p50, p95) for descending aorta peak systolic velocity variability (%) expressed as CV relative to gestational age (weeks). Note the logarithmically transformed vertical axis. (slope=0.03, r=0.27, p=0.04)

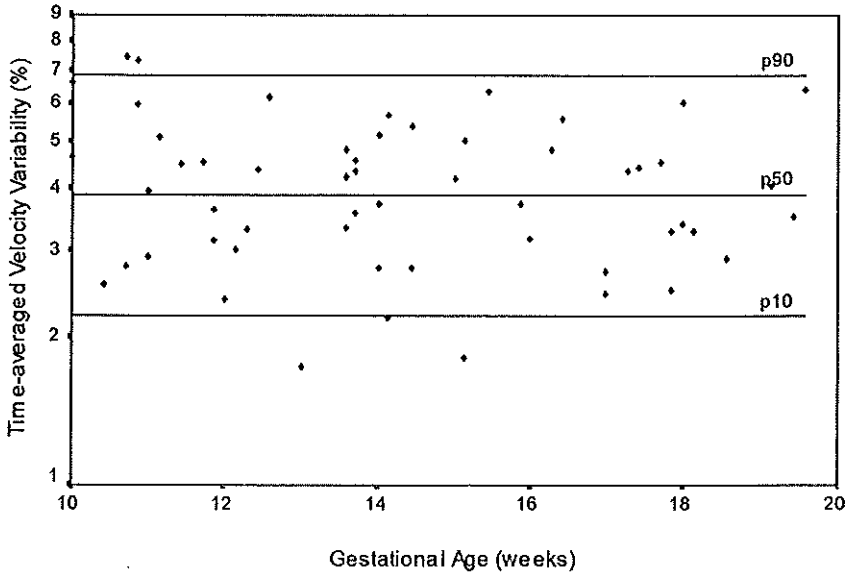

Figure 3. Individual data and centiles (p5, p50, p95) for descending aorta time-averaged velocity variability (%) expressed as CV relative to gestational age (weeks). Note the logarithmically transformed vertical axis.

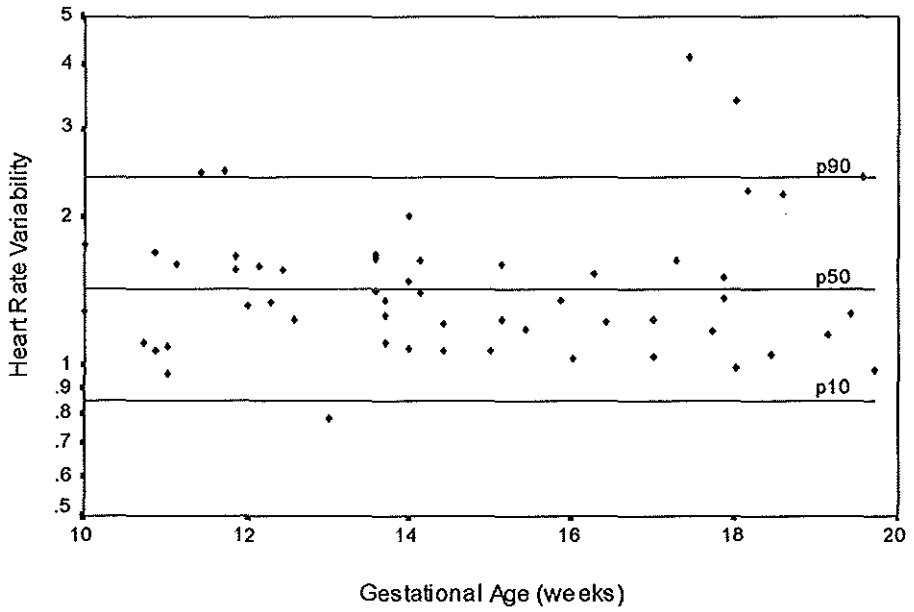

Figure 4. Individual data and centiles (p5, p50, p95) for heart rate variability (bpm) expressed as SD relative to gestational age (weeks). Note the logarithmically transformed vertical axis.

Discussion

The aim of this study was to determine the gestational age related changes in absolute values for, and beat-to-beat variability in, fetal heart rate, peak systolic velocity and time-averaged velocity derived from descending aorta flow velocity waveforms in normal pregnancies at 10-20 weeks of gestation. The aortic time-averaged velocity multiplied by cross-sectional area equals aortic blood flow. Blood flow in the descending aorta represents 70-75% of cardiac output in both human and sheep fetus in late gestation (Thompson 1994) and 90% of cardiac output in the chick embryo (Hu and Clark 1989; Yoshigi 1997). Therefore, aortic blood flow can be considered an index for cardiac output during the growth and morphogenesis of the cardiovascular system. The gestational age-related rise in descending aortic peak systolic velocity observed in the present study may be accounted for by a fetal growth determined increase in volume flow, a reduction in afterload or increased myocardial contractility. Measurements of cardiac function in 18 and 28 days-old rabbits, show marked developmental changes in the myocardial contractile system that produce a more efficient contraction pattern (Nakanishi 1988). The gestational age period studied in the rabbit at 18 to 20 days is comparable with 10-18 weeks of gestation in the human fetus (Sissman 1970). Thus, improvement in myocardial contractility may be partly

responsible for the increased fetal descending aortic flow velocities we observed. The non-invasive nature of the fetal Doppler studies does not allow differentiation between variables such as volume flow, afterload and contractility. We can, therefore, only speculate that the gestational aged related increase in descending aortic peak flow velocity may be due to one or a combination of these variables.

As expected, absolute peak systolic and time-averaged velocity in the descending aorta were higher than in the umbilical artery (Table 1), since absolute velocities decrease from central to distal level.

Doppler recordings of the descending aorta consisted of 18-44 s of data, ensuring a minimum of 44 cardiac cycles for analysis of heart rate and velocity variability. Fetal heart rate and time-averaged velocity variability determined from the descending aorta remained similar during the study period of 10-20 weeks of gestation, whereas peak systolic velocity variability significantly increased with advancing gestation. Peak systolic velocity is more susceptible to subtle maternal and/or fetal movements than time-averaged velocity. Movements such as maternal breathing may shift the position of the interrogation angle or the Doppler sample volume, so that different velocities will be recorded. However, a previous study from our centre demonstrated that spontaneous maternal breathing does not influence heart rate and peak systolic velocity variability data measured in the umbilical artery.

A recent cross-sectional Doppler flow velocity study in the umbilical artery (Ursem 1998c) demonstrated an increase in umbilical artery peak systolic velocity variability at 10-20 weeks and an increase in fetal heart rate variability at 15-20 weeks. We speculated that the increased heart rate variability is mediated by maturation of the parasympathetic nervous system, whereas the rise in peak systolic velocity variability reflects the activation of a haemodynamic feedback mechanism, similar to that demonstrated in the chick embryo (Kempski 1993).

In this study, fetal heart rate variability derived from the descending aorta remains unchanged throughout the study period. A possible explanation for the difference in heart rate variability derived from the descending aorta and umbilical artery might be the nature of the fetal activity state at the time of the Doppler survey. The recording conditions might not change in the umbilical artery despite minor fetal movements whereas assessment of flow velocity waveforms in the fetal descending aorta is only feasible when the fetus is lying completely still during the recording session. In normally developing fetuses, coupling between fetal movement and fetal heart rate has been established as early as 20 weeks of gestation (Dipietro 1996). Behaviour patterns can be observed in the human fetus, even before 20 weeks of gestation (de Vries 1986; Van Dongen and Goudie 1980). Quiescence, active movements involving all parts

of the body, isolated limb movements and strong pulsed trunk movements can be observed in fetuses between 10-12 weeks gestation, closely resembling behavioural patterns established later in pregnancy (Van Dongen and Goudie 1980). We propose that a low fetal activity state is required to obtain technically acceptable waveforms from the descending aorta, whereas waveform recordings of the umbilical artery may be technically acceptable under conditions which represents both low and high activity states, the latter is associated with fetal (minor) movements.

We conclude from this study that reproducible fetal heart rate and velocity variability data can be derived from the descending aorta. However, fetal heart rate variability data established from the descending aorta differ from the variability data obtained from the umbilical artery and therefore serial recordings during gestation should come from matched sites. In addition, different fetal activity states may be the underlying mechanism for these heart rate variability discrepancies.

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CHAPTER 4

VARIABILITY ANALYSIS OF UMBILICAL ARTERIAL WAVEFORMS IN COMPLICATED PREGNANCIES

4.1 Introductory remarks

In normal pregnancy, marked changes occur regarding absolute and beat-to-beat variability in fetal heart rate and arterial flow velocity in relation to gestational age. The mechanisms responsible for these alterations are not exactly known, but we propose that the sympathetic and parasympathetic nervous system, Frank-Starling relation and cardiovascular feedback control are involved.

We have extended our variability analysis to potentially complicated pregnancies which are at risk for poor fetal outcome. Pregnancy induced hypertension is associated with defective formation of the placenta. These morphological changes may coincide with the process of beat-to-beat regulation of fetal heart rate and flow velocities in the umbilical artery. Haemodynamic variability deviated from normal values may be indicative for fetuses at risk for pathology.

Moreover, we have studied pregnant women with diabetes mellitus type 2 who have a three-fold higher incidence for congenital heart disease than the normal population (Ferencz 1993). It has been proposed that abnormal structural and functional cardiac development occurs in diabetic pregnancies (Ferencz 1990). Fetal haemodynamic adaptations in fetuses of diabetic women may be depicted by variability analysis of the umbilical artery.

This chapter describes the possible changes in haemodynamic regulation in pregnancies complicated by pregnancy induced hypertension (*chapter 4.2*) or insulin-dependent diabetes mellitus (*chapter 4.3*) compared to normal healthy controls.

4.2 Do heart rate and velocity variability derived from umbilical artery velocity waveforms change prior to clinical pregnancy induced hypertension?

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Abstract

Objective: To investigate the hypothesis that alterations in heart rate variability, peak systolic velocity variability and time-averaged velocity variability in the human umbilical artery may predict early signs of dysfunctional fetal-placental coupling in pregnancies which later develop pregnancy induced hypertension.

Methods: Doppler flow velocity recordings from the umbilical artery were performed at 10-20 weeks of gestation in 12 nulliparous women who subsequently develop pregnancy induced hypertension. From umbilical artery velocity waveforms of at least 12 s duration we determined absolute values and beat-to-beat variability regarding fetal heart rate, peak systolic and time-averaged velocity and compared these findings with normal nulliparous pregnant women matched for gestational age.

Results: Absolute values for fetal heart rate, peak systolic and time-averaged velocity as well as beat-to-beat variability in fetal heart rate did not differ significantly between women later developing pregnancy induced hypertension and normal controls. However, variability in peak systolic velocity and time-averaged velocity were decreased in women who subsequently developed pregnancy induced hypertension.

Conclusions: Whereas fetal heart rate variability was similar, umbilical artery flow velocity variability was reduced in women developing pregnancy induced hypertension compared with controls. It is proposed that umbilical artery flow velocity variability is a marker of the mechanical properties of the resistance vessels in the placenta.

Introduction

Hypertensive disorders represent the most common complication during pregnancy, affecting approximately 7% of all pregnancies (Chesley 1978). These disorders are the most common cause of fetal growth retardation and neonatal mortality is high because of prematurity (Sibai 1983).

Studies using Doppler techniques demonstrate that a high impedance to flow in the uteroplacental circulation in the late second and third trimester of pregnancy can predict the development of pregnancy induced hypertension (PIH) (Steel 1990) and pre-eclampsia (Bower 1993). Abnormal Doppler patterns are associated with defective formation of the placenta, in particular inadequate trophoblast invasion and transformation of the spiral arteries in the placental bed (Robertson 1986). Recently, pulsed Doppler investigations of the uteroplacental circulation in the late first and early second trimester of pregnancy demonstrate abnormal blood flow indices that are correlated with subsequent development of pregnancy complications (Van den Elzen 1995; Harrington 1997).

During the last decade, spectral analysis of fetal heart rate variability has become a common non-invasive tool to investigate fetal well being. Metsälä and colleagues (Metsälä 1993) found that an increase in pulsatility index (PI) in the umbilical artery and uterine artery correlates with altered frequency-specific fetal heart rate variability during the latter weeks of gestation. A study from our own centre on normal umbilical artery flow velocity waveform at 10-20 weeks of gestation demonstrated gestational age-related changes in short-term variability for both heart rate and absolute flow velocities (Ursem 1998a).

PIH is often associated with impaired placental perfusion and reduced fetal oxygenation resulting in centralisation of the fetal circulation with redistribution of blood flow to the brain, heart and adrenals (Wladimiroff 1986; Chaoui 1996; Mari 1996). The question arises as to whether this process of haemodynamic adaptation is preceded by changes in beat-to-beat regulation of fetal heart rate and umbilical artery flow velocities. Fetal heart rate and flow velocity variability might be measures for cardiovascular function of the human fetus and deviation from the normal can be a specific marker for early cardiovascular dysfunction.

The aim of the present study is to determine whether at 10-20 weeks of gestation, changes in absolute values and beat-to-beat variability occur for heart rate, peak systolic velocity and time-averaged velocity derived from umbilical artery flow velocity waveforms in women who subsequently develop PIH. Such a marker would be valuable in identifying and guiding therapy for at risk maternal-fetal pairs.

Methods

Subjects

119 women with a singleton pregnancy were enrolled for umbilical artery flow velocity analysis in a prospective, cross-sectional study at 10-20 weeks of gestation (median 15 weeks). All women were nulliparous and did not display any pregnancy pathology at the Doppler survey. Only nulliparous women were selected to obtain a homogeneous group of patients. Successful recordings were obtained in 96 of 119 women. Twelve of 96 women developed pregnancy induced hypertension at 19-35 weeks of gestation (median 28 weeks). Pregnancy induced hypertension was defined as a blood pressure of 140/90 mmHg or higher during the second half of pregnancy in a previously normotensive woman. Three women developed pre-eclamptic toxaemia at 29, 33 and 34 weeks, respectively. Pre-eclamptic toxaemia was defined as pregnancy induced hypertension in combination with proteinuria of at least 300 mg/l. In two cases, fetal birthweight was below the 10th centile corrected for maternal parity and fetal sex (Kloosterman 1970). Baseline characteristics are demonstrated in Table 1. Twelve uneventful nulliparous pregnancies were studied within the same time frame. From our normal population, we selected the first woman with a comparable gestational age and parity as the abnormal group and they served as matched controls. These pregnancies resulted in the delivery of a healthy infant with a birthweight between 10-90th centile corrected for maternal parity and fetal sex (Kloosterman 1970).

Pregnancy duration was determined from the last menstrual period and confirmed by ultrasound measurements of fetal crown-rump length (10-12 weeks) or fetal biparietal diameter (13-20 weeks). The study protocol was approved by the institutional review boards at the Erasmus University Rotterdam, Rotterdam and the University of Rochester, Rochester, NY.

Table 1. Baseline characteristics of women developing PIH and normal controls.

	PIH (n=12)	Controls (n=12)
Gestational age at survey (weeks)	15.4 (12.5-18.8)	15.2 (12.4-18.8)
Systolic blood pressure at survey (mmHg)	125 (120-150)*	110 (105-120)
Diastolic blood pressure at survey (mmHg)	80 (70-85)	75 (70-80)
Gestational age at delivery (weeks)	39 (37-41)	39 (39-41)
Birth weight (g)	3227 (2246-3420)	3390 (3045-3550)
Placental weight (g)	560 (525-638)*	650 (586-713)
Maternal age (years)	29 (27-33)	30 (24-36)

Values are presented as medians (interquartile ranges). PIH, pregnancy induced hypertension; SD, standard deviation. * $P < 0.05$ compared to normal controls.

Doppler recordings

Pulsed wave Doppler ultrasound recordings were obtained using a Toshiba SHH-140A (Toshiba Corporation, Medical Systems Division, Tokyo, Japan). A combined transvaginal real-time and colour Doppler system (carrier frequency 6

MHz and 5 MHz, respectively) was used at 10-13 weeks of gestation and a combined transabdominal real-time and colour Doppler system (carrier frequency 5 MHz and 3.75 MHz, respectively) was used at 14-20 weeks of gestation. The system operates at power output of less than 100 mW/cm2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specifications. Using colour Doppler, maximal flow velocity waveforms from the umbilical artery were obtained from a floating loop of the umbilical cord. The angle of insonation was always less than 15°. Sample volume length for all flow velocity waveforms ranged between 0.2-0.3 cm; the high-pass wall filter was set at 70-100 Hz. Doppler recordings were performed by one examiner (NTCU). All Doppler studies were carried-out with the women in a semirecumbant position and during fetal apnoea. Only technically high quality recordings lasting at least 12 seconds were analysed to ensure a collection of at least 25 consecutive waveforms for heart rate and velocity variability analysis. The velocity waveforms (video and hi-fi audio signal) were stored on sVHS videotape in PAL format using a Panasonic model AG7350 machine (Matsushita Electric Ind Co, Japan).

Data processing

Continuous high quality Doppler audio waveforms were digitised at 12 kHz using an analogue-to-digital data acquisition board (LabPC+ and BNC-2081 boards, National Instruments, Austin, TX). The maximum flow velocity waveforms from the umbilical artery were reconstructed from the Doppler audio data using computer algorithms developed in our centre using LabVIEW software (National Instruments, Austin, TX). A detailed description of the analysis technique and the computer algorithms has been published previously (Ursem 1998b). Umbilical artery peak systolic velocity (mm/sec), time-averaged velocity (mm/s) and instantaneous heart rate (bpm) for each cardiac cycle were calculated. For the entire maximum velocity waveform, the mean and standard deviation for peak systolic velocity, time-averaged velocity and heart rate were calculated to establish beat-to-beat variability for each of these variables. The umbilical artery PI was calculated from the difference between the maximal and minimal flow velocity divided by the time-averaged velocity.

A previous reproducibility study of the umbilical artery flow velocity waveform recordings at 10-20 weeks of gestation revealed a mean coefficient of variation (CV) of 1.1% (range: 0.2%-3.5%) for fetal heart rate, a mean CV of 1.9% (range: 1.1%-3.3%) for peak systolic velocity, and a mean CV of 2.4% (range: 1.2%-3.9%) for time-averaged velocity.

Statistical analysis

Data are presented as median and interquartile range. Baseline characteristics and haemodynamic parameters were compared between women developing PIH and matched normal controls using a paired Wilcoxon's test. Statistical

significance was reached at $P < 0.05$. Calculations were performed with SPSS 6.1 software (SPSS Inc., Chicago, IL).

Results

Women developing PIH (n=12) displayed a higher systolic blood pressure at the Doppler survey and a lower placental weight compared to normal controls (Table 1). The median time interval between Doppler survey and onset of clinical PIH was 13 weeks (range 1-25 weeks). The fetal heart rate, umbilical artery peak systolic velocity, time-averaged velocity and pulsatility index did not differ significantly between the two study groups (Table 2). Beat-to-beat variability in fetal heart rate was also not significantly different among women developing PIH and normal controls, but beat-to-beat variability in umbilical artery peak systolic and time-averaged velocity was decreased among the two study groups (Table 2).

Table 2. Median and interquartile range for fetal heart rate, peak systolic velocity, time-averaged velocity, heart rate variability, peak velocity variability, time-averaged velocity variability and pulsatility index in the umbilical artery from women developing PIH and matched normal control pregnancies.

	PIH (n = 12)	Controls (n =12)
Heart rate (bpm)	152 (148-161)	151 (148-157)
Peak systolic velocity (mm/s)	320 (277-376)	311 (280-354)
Time-averaged velocity (mm/s)	186 (124-217)	173 (130-201)
Heart rate variability (bpm)	1.58 (1.19-1.80)	1.62 (1.30-1.75)
Peak systolic velocity variability (mm/s)	8.54 (5.33-11.28)*	10.90 (9.46-17.07)
Time-averaged velocity variability (mm/s)	4.92 (3.42-7.52)*	5.82 (4.35-10.28)
Pulsatility index	1.68 (1.50-2.84)	1.75 (1.32-3.07)

Variability data expressed as mean SD. PIH, pregnancy induced hypertension; bpm, beats per minute; SD, standard deviation. *$P < 0.05$ compared to normal controls.

Discussion

The present study was aimed at determining whether changes in beat-to-beat variability of fetal heart rate and umbilical artery flow velocities could detect fetal placental dysfunction early in pregnancies, which later develop pregnancy induced hypertension. Pulsatility index calculations demonstrate that uncomplicated pregnancies are characterised by a gradual gestational age-related decrease in umbilical-placental down stream impedance starting as from 12-13 weeks of gestation (Van Splunder 1996; Wladimiroff 1991). It was recently noted that heart rate variability and velocity variability in normal umbilical artery flow velocity waveforms are gestational age-related (Ursem 1998a). Increase in heart rate variability at 15-20 weeks suggests a correlation with functional maturation of the parasympathetic nervous system, whilst an increase in flow velocity variability may reflect a cardiovascular feedback control mechanism

similar to that in the chick embryo. In the chick model, short-duration modulations in aortic blood flow and vascular impedance appear to be a method for haemodynamic control during early development and prior to autonomic innervation (Kempski 1993).

Histological studies of the placental bed of pregnancies complicated by hypertension show inadequate trophoblast invasion and transformation of the spiral arteries of the placental bed in early pregnancy (Pijnenborg 1991; Robertson 1986). In cases of pregnancy induced hypertension, the physiological vascular changes are limited to some spiral arteries in the decidual segments and some remain in the same state as spiral arteries in the non-pregnant uterus (Khong 1986). Biagini and co-workers (1992) showed that in placentas of hypertensive women, there is a higher number of chorionic-villi-decidua interactions and a smaller surface area of a single interaction. Thus, the placental vascular interface between mother-fetus may be abnormal prior to the development of clinical pregnancy induced hypertension.

Based on this histological data, the search began for early haemodynamic data from the uteroplacental and fetal circulation as predictors of pregnancy induced hypertension and/or fetal growth retardation developing later in pregnancy. Uterine artery flow velocity waveforms depicting reduced end-diastolic flow velocities and early diastolic notching as early as 18-20 weeks of gestation were predictive of subsequent development of pregnancy induced hypertension (Bower 1993). Even at 12-13 weeks of gestation, Doppler detectable changes are established in the uterine artery, which were associated with subsequent development of pregnancy complications (Van den Elzen 1995; Harrington 1997). Contradictory findings were observed in the umbilical artery flow velocity waveform at this early stage of pregnancy. In one study, no changes were observed in umbilical blood flow velocity waveforms prior to the emergence of pregnancy induced hypertension (Arduini 1991), whereas in another study abnormal Doppler values in the umbilical artery were associated with premature delivery, the development of pregnancy induced hypertension and the delivery of a small-of-gestational-age baby during the late second or third trimester of pregnancy (Harrington 1997).

In the present study, there was a decrease in umbilical artery flow velocity variability, but the absolute peak systolic and time-averaged velocity were similar for women developing pregnancy induced hypertension and normal controls. Moreover, the placental weight of women developing pregnancy induced hypertension was decreased compared to normal controls. Increased arterial down stream impedance due to abnormal morphological changes of the vascular bed of the placenta may be responsible for the decrease in velocity variability.

However, the absence of a difference in umbilical artery pulsatility index values between fetuses of women developing pregnancy induced hypertension and normal controls argues against a modification in afterload (Table 2). Alternatively, changes in arterial compliance may occur without altering afterload. An *in vitro* study has shown that the concentration of lipoperoxide in the microvillus membrane of women who subsequently develop pregnancy induced hypertension is increased over normotensive pregnant women (Cester 1994; Gurtner 1983). Lipoperoxidation products are capable of inducing smooth muscle constriction (Gurtner 1983) and increase the responsiveness to angiotensin II (Hubel 1989). It is proposed that this will result in a reduced compliance of the placental vessels and therefore reduction in umbilical artery flow velocity variability in women, which later develop pregnancy, induced hypertension.

In the present study, beat-to-beat variability in fetal heart rate was similar between women developing pregnancy induced hypertension and normal controls. This suggests that parasympathetic activity, which plays an important role in the control of heart rate variability, is not altered in women who at a later stage develop pregnancy induced hypertension.

It can be concluded that beat-to-beat variability in fetal heart rate was similar for women developing pregnancy induced hypertension and normal controls. However, a significant decrease in umbilical artery flow velocity variability was observed in women developing pregnancy induced hypertension. Changes in mechanical properties of the resistance vessels in the placenta due to the release of vasoactive biochemical substances, such as lipoperoxidation products may be a mechanism for this observation.

4.3 Fetal heart rate and umbilical artery velocity variability in pregnancies complicated by insulin-dependent diabetes mellitus

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Abstract

Objectives: Variability in fetal heart rate and absolute flow velocity are possible haemodynamic markers of cardiovascular homeostasis in pregnancies complicated by diabetes mellitus.

Methods: Doppler studies of umbilical artery velocity waveforms were performed at 12-21 weeks of gestation in 16 women with well-controlled type I (insulin dependent) diabetes mellitus. From umbilical artery velocity waveforms of at least 13 seconds duration we determined absolute values and beat-to-beat variability for fetal heart rate and umbilical artery flow velocities and compared these findings with normal controls matched for gestational age.

Results: Fetuses of diabetic women displayed increased fetal heart rate variability and umbilical artery peak systolic velocity. Fetal heart rate, umbilical artery time-averaged velocity, and variability in umbilical artery flow velocity were not essentially different between the two groups.

Conclusion: Fetal heart rate variability and umbilical artery peak systolic velocity may be markers for fetal cardiovascular homeostasis in pregnancies complicated by insulin-dependent diabetes mellitus.

Introduction

The perinatal mortality and morbidity associated with diabetic pregnancies has declined, mainly due better preconceptional, antenatal and neonatal care (Miller 1981). The majority of fetal and neonatal deaths that occur in diabetic pregnancies are now due to congenital cardiovascular disease, which has a three-fold higher incidence than in the general population (Ferencz 1993).

In the human fetus, structural cardiac malformation can be diagnosed with reasonable confidence in the midtrimester of pregnancy (Copel 1986; Allan 1994). More recently, Doppler studies have been investigated as a method for identifying fetal compromise in diabetic women (Bracero 1986; Landon 1989; Salvesen 1993; Rizzo 1995). Contradictory findings were observed in the umbilical artery flow velocity waveform in the second and third trimester of pregnancy. Some studies reported similar umbilical artery S/D ratios in pregnancies complicated by diabetes mellitus and non-diabetic women (Landon 1989; Salvesen 1993), whereas in one study elevated S/D ratios in the umbilical artery of diabetic women were associated with an increased number of stillbirths and neonatal morbidity (Bracero 1986). Rizzo et al. (1995) described altered transitions of cardiac and venous blood flow patterns in fetuses of insulin-dependent diabetic mothers in the early second trimester of pregnancy. They proposed that these differences were secondary to structural alterations of the fetal myocardium reducing ventricular compliance.

In adults, heart rate variability is associated with cardiovascular pathology like myocardial dysfunction and cardiomyopathy (Baselli 1987; Anonymous 1996). In the fetus, heart rate variability is a marker of well-being during late gestation. Heart rate variability is altered in pathologic conditions, such as intra-uterine growth retardation, during the second half of pregnancy (Breborowicz 1988). We recently demonstrated that the assessment of beat-to-beat variability in fetal heart rate and in absolute umbilical artery flow velocity can be performed as early as 10 weeks of gestation (Ursem 1998c). We noted increased heart rate variability with advancing gestation suggesting a correlation with functional maturation of the parasympathetic nervous system. An increase in flow velocity variability may reflect the development of a cardiovascular feedback control mechanism.

In the present study we addressed the role of beat-to-beat variability (or variation; expressed as the standard deviation) in fetal heart rate and absolute umbilical artery flow velocities in the assessment of early cardiovascular homeostasis in insulin-dependent diabetic pregnancies.

Methods

Subjects

Sixteen women with type I (insulin dependent) diabetes mellitus and a singleton pregnancy consented to participate in a prospective, cross-sectional matched control study at 12-21 weeks of gestation (median 18 weeks). Pregnancy duration was determined from the last menstrual period and confirmed by ultra-sound measurements of fetal crown-rump length (10-12 weeks) or fetal biparietal diameter (13-20 weeks). Clinical baseline characteristics are detailed in Table 1. For all subjects, the median glycosylated haemoglobin value during the first trimester of pregnancy was 6.3% (range 6.1-7.1%) which is within the normal range for our institute. All diabetic women were treated with insulin. According to the White classification, six women belonged to group B, seven women to group C, two women to group R and one woman to group F/R (White 1978). Normal controls matched for gestational age were selected from outpatients of the department of Obstetrics and Gynaecology of the Academic Hospital Rotterdam-Dijkzigt, and all had a normal pregnancy. The study was approved by the Hospital Ethics Committee at the Erasmus University Rotterdam, Rotterdam and the University of Rochester, Rochester, NY.

Data analysis

A Toshiba SHH-140A (Toshiba Corporation, Medical Systems Division, Tokyo, Japan) unit was used for all Doppler ultrasound recordings. A combined transvaginal colour and pulsed Doppler system (carrier frequency 5 MHz) and a combined transabdominal colour and pulsed Doppler system (carrier frequency 3.75 MHz) were used. Using colour Doppler, maximal umbilical artery flow

velocity waveforms were obtained from a small straight portion of the free floating loop of the umbilical cord. The angle of insonation was always less than 15°. A previous reproducibility study of the umbilical artery flow velocity waveform recordings at 10-20 weeks of gestation revealed a mean coefficient of variation (CV) of 1.1% (range: 0.2%-3.5%) for fetal heart rate, a mean CV of 1.9% (range: 1.1%-3.3%) for peak systolic velocity, and a mean CV of 2.4% (range: 1.2%-3.9%) for time-averaged velocity. The system operates at power outputs of less than 100 mW/cm2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specifications. Sample volume length for all flow velocity waveforms ranged between 0.2-0.3 cm; the high-pass wall filter was set at 100 Hz. Doppler recordings were performed by one examiner (NTCU). The transvaginal technique was used at 12-13 weeks of gestation and the transabdominal approach at 14-21 weeks of gestation. All Doppler studies were carried-out with the women being in a semirecumbant position.

Table 1. Clinical data in pregnant women with diabetes mellitus type I (n=16) and normal pregnant controls (n=16).

	IDDM (n=16)	controls (n=16)
Maternal age (years)	32 (23-32)	32 (15-39)
Gestational age at survey (weeks)	18.0 (12-21)	18.0 (12-21)
FEW (g)	316 (88-490)	291 (92-436)
Parity (n)		
0-para	8	6
≥ 1-para	8	10
Gestational age at delivery (weeks)	38.0 (30-40)*	40.0 (37-42)
Caesarean section (n)	2	1
Assisted delivery (n)	3	2
Birth weight (g)	3750 (1440-4310)	3400 (2720-4330)
Placental weight (g)	950 (370-1050)	650 (450-750)
Fetal-placental weight ratio	0.25 (0.16-0.26)*	0.19 (0.14-0.22)
Birth weight >10th and <90th centiles (n)	8	12
Birth weight < 10th centile (n)	0	0
Birth weight >90th centile (n)	8	4

Data are presented as median (range). IDDM: insulin dependent diabetes mellitus. FEW: fetal estimated weight. *P ≤ 0.05 compared to normal controls.

Umbilical artery Doppler recordings were stored on sVHS videotape in PAL format using a Panasonic model AG7350 machine (Matsushita Electric Ind Co, Japan) and the audio signals were digitised at 12 kHz using an AD data acquisition board (LabPC+ and BNC-2081 boards, National Instruments, Austin, TX). Flow velocity waveforms were reconstructed using computer algorithms developed in our

centre using LabVIEW software (National Instruments, Austin, TX). Power spectra were calculated using a 512-point fast Fourier transform (FFT) with a Hanning window and an overlap of 75%. From the collected power spectra the maximum (envelope) velocity waveform can be estimated. The first derivative of the velocity waveform was used to determine the instantaneous heart rate which is the reciprocal of the time between successive peaks. For each cardiac cycle the fetal heart rate, peak systolic velocity and time-averaged velocity was calculated. A more detailed description of the analysis technique and the computer algorithms has been published previously (Ursem 1998b). Only technically high quality umbilical artery recordings containing at least 13 seconds during periods of fetal rest were used for analysis. This was to ensure a collection of at least 30 consecutive waveforms for the analysis of the following parameters: (1) fetal heart rate (bpm), (2) umbilical arterial peak systolic velocity (mm/s), (3) umbilical arterial time-averaged velocity (mm/s), (4) fetal heart rate variability, (5) peak systolic velocity variability and (6) time-averaged velocity variability. The standard deviation over a period of at least 30 consecutive heart beats was used as a measure for variability in both heart rate and velocity parameters. The umbilical artery pulsatility index (PI) was calculated from the difference between the maximal and minimal flow velocity divided by the time-averaged velocity. Fetal estimated weight was derived using the Hadlock formula (Hadlock 1985).

Statistical analysis

Clinical data and haemodynamic parameters were compared between diabetic women and matched normal controls using a paired Wilcoxon's test. Statistical significance was reached at $p \leq 0.05$. Calculations were performed with SPSS 6.1 software (SPSS Inc., Chicago, IL).

Results

Table 2. Median (range) for haemodynamic parameters in the umbilical artery obtained from women with diabetes mellitus type I (n=16) and normal pregnant controls (n=16).

	IDDM (n = 16)	Controls (n =16)
Heart rate (bpm)	145 (142-152)	148 (144-153)
Peak systolic velocity (mm/s)	399.4 (295.3-416.5)*	337.2 (307.5-393.4)
Time-averaged velocity (mm/s)	221.1 (174.4-273.5)	204.3 (174.8-239.7)
Heart rate variability	2.19 (1.58-2.90)*	1.57 (1.35-2.11)
Peak systolic velocity variability	15.57 (9.58-18.07)	14.20 (7.93-21.11)
Time-averaged velocity variability	8.71 (5.60-13.05)	8.97 (6.24-11.02)
End-diastolic velocity (mm/s)	56.0 (17.8-104.8)	57.8 (6.0-102.3)
Pulsatility index	1.47 (1.17-2.14)	1.50 (0.91-2.49)

Variability data are expressed as SD. *$P \leq 0.05$ compared to normal controls. IDDM: insulin dependent diabetes mellitus.

Fetuses of diabetic mothers were born earlier in gestation and displayed a higher fetal/placental ratio compared to normal controls (Table 1). Both umbilical artery peak systolic velocity and fetal heart rate variability were increased in diabetic women compared with normal controls. No difference between the two study groups was noted for fetal heart rate, umbilical artery time-averaged velocity and beat-to-beat variability in umbilical artery flow velocity (Table 2). The individual data for the haemodynamic parameters measured in the umbilical artery in fetuses of diabetic mothers and normal controls are depicted in Figure 1 (A-H).

Discussion

It has been proposed that abnormal structural and functional cardiac development occurs in diabetic pregnancies (Ferencz 1990). This is supported by the lower passive/active ventricular filling ratio in fetuses of diabetic mothers compared to non-diabetics in the early second trimester of pregnancy, which is likely the result of abnormal myocardial structure and compliance. Moreover, echocardiographic examinations of fetuses of diabetic mothers have revealed impaired myocardial contractility with concomitant reduction in stroke volume and cardiac output (Rasanen and Kirkinen 1987).

The increased umbilical artery peak systolic velocities in fetuses of diabetic women demonstrated in our study may reflect changes in systemic arteriolar placental afterload, myocardial contractility, heart rate and preload (Clark 1990). The placenta in diabetic pregnancy has morphologic changes that may result in reduced uteroplacental perfusion (Bjork and Persson 1982; Singer 1984). The absence of a difference in umbilical artery pulsatility index values between fetuses of diabetic women and normal controls argues against a modification in afterload (Table 2). On the basis of the three-element Windkessel model, increased arterial compliance may increase peak systolic velocity without altering afterload. Impaired myocardial contractility reported by others (Rasanen and Kirkinen 1987) should result in a reduction rather than a rise in arterial flow velocities which should include the umbilical artery. A third variable, i.e. fetal heart rate, was also similar between the two study groups (Table 2). This leaves us with the fourth possible factor, the preload, which is related to end-diastolic events.

It can be said that none of the separate factors that regulate cardiovascular function provide a definitive explanation for the observed increase in umbilical artery peak systolic velocity. Moreover, the fore-mentioned factors may change simultaneously in the same or opposite direction. The lack of information on blood pressure and volume flow inherent in non-invasive Doppler techniques prevents the identification of possible underlying mechanisms responsible for the

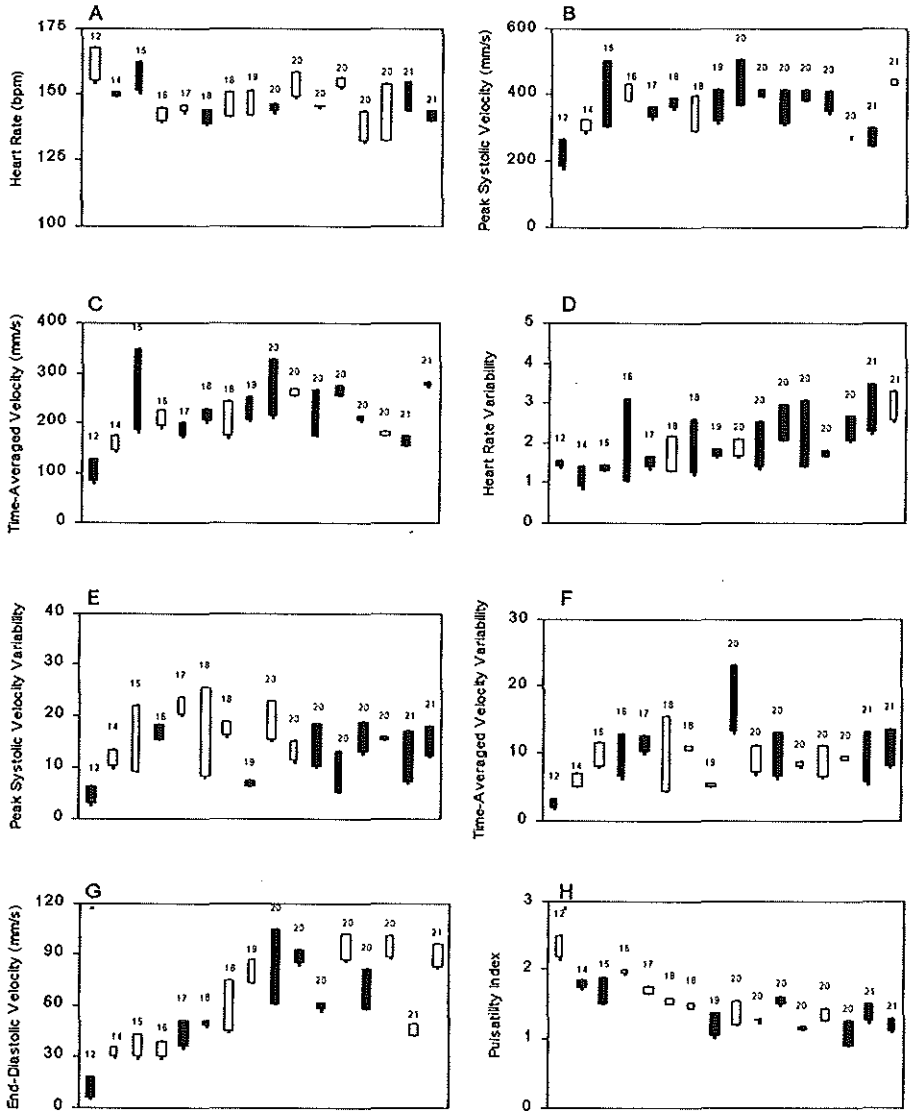

Figure 1. Individual data of haemodynamic parameters measured in the umbilical artery from women with diabetes mellitus type I (n=16) and normal pregnant controls (n=16) as function of gestational age (weeks). Solid boxes indicate that a particular value measured in diabetic women was higher compared with normal controls and open boxes indicate that a particular value measured in diabetic women was lower compared with normal controls. The data labels above the boxes indicate the gestational age. The difference between diabetic women and normal controls are depicted for heart rate (A), peak systolic velocity (B), time-averaged velocity (C), heart rate variability (D), peak systolic velocity variability (E), time-averaged velocity variability (F), end-diastolic velocity (G) and pulsatility index (H). Note that both peak systolic velocity (B) and heart rate variability (D) were significantly increased in diabetic women compared with normal controls.

altered flow velocities established in the present study. Our results are consistent with an earlier Doppler echocardiographic study showing significantly higher peak velocities at the level of the cardiac outflow tract in fetuses of diabetic mothers (Rizzo 1992). This may be explained by an increased intracardiac flow volume secondary to the relatively larger size of fetuses of diabetic mothers, since the cardiac output is a function of fetal weight (Rizzo 1992). However, the estimated fetal weight at the time of the Doppler survey was similar between the two study groups (Table 1).

Doppler studies are widely utilised to detect early fetal compromise in diabetic women. Most studies demonstrate normal Doppler indices in the uteroplacental circulation in pregnancies complicated by maternal diabetes mellitus, except in those cases complicated by pre-eclampsia or intra-uterine growth retardation (Landon 1989; Salvesen 1993). However, in one study elevated Doppler indices were found in the umbilical artery in insulin-dependent diabetes compared with controls (Bracero 1986). This highlights possible differences in the degree of metabolic control of diabetic women in these studies. We characterised diabetic women in our study to be under strict metabolic control. The increase in beat-to-beat variability in fetal heart rate was observed in insulin-dependent diabetic pregnancies at 12-21 weeks of gestation. This is at variance with a reduction in fetal heart rate variability seen in diabetic women later in pregnancy (Weiner 1996).

Morphological changes, such as increased thickness of ventricular septum and ventricular walls (Veille 1992; Macklon 1998) and cardiomegaly (Mace 1979) are a well-described features in fetuses of diabetic women. Fetuses of well controlled diabetic women show an accelerated increase of cardiac size (Rizzo 1992). The cause of the abnormal cardiac growth rate is unclear, but may relate to a different degree of sensitivity of the fetal myocardium to growth factors generated by the mother, placenta, or fetus in response to maternal diabetes. We speculate that due to the accelerated cardiac growth, the functional maturation of the cardiac parasympathetic nervous system may be altered compared with normally developing fetuses, resulting in an increase in heart rate variability.

From this study, we conclude that significant changes in umbilical artery peak systolic velocity and fetal heart rate variability occur in insulin-dependent pregnancies despite strict metabolic control. These data are likely markers of changes in fetal cardiac function in early second trimester pregnancies complicated by insulin-dependent diabetes mellitus or reflect early changes in afterload in either the fetal or placental circulation.

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CHAPTER 5

VARIABILITY ANALYSIS OF UMBILICAL ARTERIAL WAVEFORMS IN CONGENITAL HEART DEFECTS

5.1 Introductory remarks

In chapter 4, data were discussed of women who were at risk of having an infant with adverse outcome. In these cases, no malformations were observed at the time of the Doppler survey.

Studies performed in the chicken and mouse, but also in the human fetus demonstrated that structural cardiac anomalies are accompanied by marked haemodynamic changes (Shenker 1988; Broekhuizen 1996; Gui 1996). Chick embryos with induced cardiac malformations display reduced heart rate, peak systolic and mean blood flow velocities/volumes, peak acceleration and stroke volume suggesting both pacemaker and contractile dysfunction (Broekhuizen 1996). Power spectral analysis of the dorsal aortic blood velocity in this animal model showed that these embryos exhibited strong broad-spectrum heart rate variability and depressed peak and mean velocity variability when compared to control embryos (Kempski 1996). If a parallel can be drawn to the human fetus, flow velocity waveform recordings obtained by pulsed Doppler ultrasound could serve as an additional diagnostic tool in the detection of congenital heart disease.

5.2 Fetal heart rate and umbilical artery velocity variability in fetuses with congenital heart defects

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Introduction

Pulsed Doppler ultrasound velocity waveform analysis has been a tool for assessment of the fetal circulation for the past 20 years (Groenenberg 1989). Doppler velocity information may be obtained as early as 8 weeks of gestation providing an opportunity for early quantitative assessment of late embryonic and fetal cardiovascular function (Wladimiroff 1991; Splunder van 1996).

We recently established that the pregnancy period of 10-20 weeks is characterised by gestational age related changes in both fetal heart rate variability and umbilical artery peak systolic velocity variability (Ursem 1998a). In pregnancies complicated by pregnancy induced hypertension or insulin-dependent diabetes mellitus, we noted altered fetal heart rate variability and umbilical artery velocity variability compared with normal pregnancies, which may be related to impaired fetal cardiovascular homeostasis (Ursem 1998b). Heart rate variability and velocity variability can be indicative as a marker for physiological and pathophysiological haemodynamic processes.

In the chick embryo, cardiac malformations induced by retinoic acid are accompanied by marked haemodynamic changes (Broekhuizen 1995). Simultaneous dorsal aortic Doppler flow velocity/volume and pressure measurements demonstrated reduced heart rate, peak systolic and mean blood flow velocities/volumes, peak acceleration and stroke volume in chick embryos treated with retinoic acid suggesting impaired cardiac contraction force (Broekhuizen 1995). Another embryonic chicken model, the venous clip model has shown to produce specific cardiovascular malformations, like ventricular septal defects and/or dextroposed aorta (Hogers 1997). The haemodynamic parameters in this model were increased in contrast to the reduced haemodynamic parameters in the retinoic acid model (Broekhuizen 1996).

Trisomy 16 embryonic mice, presenting a model for cardiac disease, demonstrate decreased heart rate and increased cardiac outlflow peak systolic velocities (Gui 1996). Thus, in the chick and mouse embryo morphologic changes in heart development result in altered flow velocity waveform patterns. If a parallel can be drawn to the human fetus, flow velocity waveform recordings obtained by pulsed Doppler ultrasound could contribute to our understanding of altered cardiac function in the presence of congenital heart defects.

Contradictory data exist regarding the association between abnormal umbilical artery flow velocity waveforms and structural cardiac disease. In one study, umbilical artery pulsatility index values are not predictive of fetal outcome in fetuses with structural cardiac anomalies (Copel 1991), in another study absent diastolic flow in the umbilical artery indicates a poor prognosis for fetuses with congenital heart defects (Al-Gazali 1987).

The objective of the present study was to examine whether (i) fetuses with congenital heart defects demonstrate alterations in heart rate variability and blood flow velocity variability in the umbilical artery compared to normal controls and whether (ii) these variability measures can be used as markers for impaired homeostasis in fetal congenital heart defects.

Methods

Subjects

Thirteen women carrying a fetus with a congenital heart defect previously diagnosed by ultrasound consented to participate in a prospective, cross-sectional matched control study at 20-35 weeks of gestation (median 26 weeks). Maternal age ranged from 21-33 years (median 30 years). Gestational age was calculated from the last menstrual period and confirmed by ultrasound measurements of fetal crown-rump length or fetal biparietal diameter in early pregnancy. Normal controls matched for gestational age were selected from outpatients of the department of Obstetrics and Gynaecology of the Academic Hospital Rotterdam-Dijkzigt. All pregnancies progressed uneventfully and resulting in a term delivery of an infant with a birth weight between 10th and 90th percentile corrected for maternal parity and fetal sex (Kloosterman 1970). The study was approved by the Hospital Ethics Committee at the Erasmus University Rotterdam, Rotterdam and the University of Utah, Salt Lake City, Utah.

Data analysis

Doppler ultrasound recordings were performed with an Acuson-128 (Mountain View, California) unit (cases 1-5) or a Toshiba SHH-140A (140A (Toshiba Corporation, Medical Systems Division, Tokyo, Japan) unit (cases 6-14). Either a 3.75 MHz or 2.5 MHz transabdominal transducer was used, as appropriate. Using

colour Doppler, maximal flow velocity waveforms from the umbilical artery were obtained from a straight portion of floating loop of the umbilical cord. The angle of insonation was always less than 15°. Earlier in our centre (Ursem 1998c), acceptable reproducibility was established for fetal umbilical artery flow velocity waveforms, with coefficient of variation values being less than 3%.

Both systems operate at power outputs of less than 100 mW/cm2 spatial peak-temporal average in both imaging and Doppler modes by manufacturer's specifications. All Doppler studies were carried-out with the women in semi-recumbant position. Only technically high quality umbilical artery recordings lasting at least 18 seconds during periods of fetal rest were obtained for later analysis. This was to ensure a collection of at least 40 consecutive waveforms for the analysis of the following parameters: (1) fetal heart rate (bpm), (2) peak systolic velocity (mm/s), (3) time-average velocity (mm/s), (4) fetal heart rate variability (%), (5) peak systolic velocity variability (%) and (6) time-average velocity variability (%). The standard deviation of a period of at least 40 consecutive waveforms was used as a measure of variability.

The umbilical artery audio data was stored on sVHS videotape and sampled at 12 kHz. Segments of 512 data points were used for fast Fourier transform analysis. The maximum frequency was estimated from the power spectrum of each data segment which was used to reconstruct the maximum flow velocity waveform. A more detailed description of the analysis technique and the computer algorithms using LabVIEW software (National Instruments, Austin, TX) developed in our centre has been published previously (Ursem 1998a).

The umbilical artery pulsatility index (PI) was calculated from the difference between the maximal and minimal flow velocity divided by the time-averaged velocity. Fetal estimated weight was derived from the Hadlock formula (Hadlock 1985).

Statistical analysis

For the expression of variability in the hemodynamic parameters (heart rate, peak systolic velocity and time-averaged velocity) the coefficient of variation (CV) was used, because the standard deviation of heart rate and velocity were not independent of the mean. Hemodynamic parameters were compared between fetuses with congenital heart defects and matched normal controls using the paired Wilcoxon's test. Statistical significance was reached at $p \leq 0.05$. Calculations were performed with SPSS 6.1 software (SPSS Inc., Chicago, IL).

Results

Clinical information of the 13 cases of congenital heart defects (CHD) is shown in Table 1. In seven out of 13 patients the prenatal diagnosis could be confirmed by postnatal ultrasound or by obduction. In five patients obduction was refused and therefore, the prenatal diagnosis could not be confirmed. In one women (no. 6), due to maternal adipositas a prenatal diagnosis was difficult to obtain and a misdiagnosis of the fetal congenital heart anomaly was made.

Table 1. Number of fetuses with congenital heart defects, additional structural or chromosomal anomalies and outcome.

Case	Gest. age (wks)	Prenatal diagnosis	Gest. Age at delivery (wks)	Fetal/neonatal outcome
1	26	DORV	29	DORV/bicuspid aorta valve; alive
2	34	ASD/DORV/IUGR	36	DORV/SGA/VSD/t18; p.p. death*
3	21	AVSD/IUGR	22	TOP; t18; no obduction
4	35	DORV/TOF	38	ASD/TOF; surgically repaired; alive
5	29	AVSD/t21	37	AVSD/t21; alive
6	30	AVSD/VSD	37	HLH/VSD; post partum death*
7	26	AVSD/VSD	36	Expired; no obduction
8	22	ASD/DORV/VSD	23	TOP; DORV/VSD**
9	28	AVSD/IUGR/t18	28	TOP; IUGR/t18; no obduction
10	22	VSD	39	ASD/VSD; surgically repaired; alive
11	26	VSD	28	TOP; microcephaly; no obduction
12	23	AVSD	25	TOP; cor vitium; no obduction
13	20	HLH/VSD	23	TOP; ASD/HLH/VSD**

ASD: atrial septal defect; AVSD: atrioventricular septal defect; DORV: double-outlet right ventricle; HLH: hypoplastic left heart; IUGR: intra uterine growth retardation; SGA: small for gestational age; t18: trisomy 18; t21: trisomy 21; TOF: tetralogy of Fallot; TOP: termination of pregnancy; VSD: ventricular septal defect. *prenatal diagnosis confirmed by postnatal ultrasound. **prenatal diagnosis confirmed by obduction.

Six women opted for pregnancy termination due to the poor prognosis associated with the severity of the cardiac anomaly and the presence of a chromosome abnormality in two of these women (trisomy 18). Three infants died, one during labour, one two weeks and three months post partum, respectively. There were four surviving infants, two of whom after surgical repair.

In fetuses with a congenital heart defect, umbilical artery peak systolic and time-averaged velocity were significantly decreased, whereas variability in fetal heart rate and umbilical artery peak systolic and time-averaged velocity were significantly increased compared with normal controls (Table 2, Figure 1). Fetal heart rate and umbilical artery pulsatility index values were not essentially different between fetuses with congenital heart defects and normal controls (Table 2, Figure 1).

Mean fetal estimated weight at the time of the examination derived from the Hadlock formula was 508 g (range 277-2211 g) for fetuses with a congenital heart defect and 697 g (range 306-2507) for normal control fetuses. This difference was not statistically significantly different.

Table 2. Median and range for hemodynamic parameters in the umbilical artery obtained from fetuses with congenital heart defects (CHD) (n=13) and normal fetuses (n=13).

	CHD	controls	p
Heart rate (bpm)	142 (118-156)	141 (131-161)	0.422
Peak systolic velocity (mm/s)	334 (222-411)	381 (268-508)	0.050
Time-averaged velocity (mm/s)	209 (90-273)	241 (167-376)	0.046
Heart rate variability (%)	2.3 (1.1-10.3)	1.8 (0.9-5.3)	0.033
Peak systolic velocity variability (%)	4.2 (1.3-10.3)	3.1 (0.9-5.4)	0.023
Time-averaged velocity variability (%)	4.0 (1.7-12.6)	3.4 (1.3-5.7)	0.028
Pulsatility index	1.24 (1.00-4.38)	1.19 (0.76-1.52)	0.31

Variability data are expressed as coefficient of variation.

Discussion

Fetal congenital heart defects in this study were a heterogeneous group of six cases with outflow tract anomalies in combination with malalignment, five cases with inflow tract anomalies and two cases with hypoplasia of the left heart. It is well established that cardiac neural crest cells are involved in the outflow tract septation (Kirby and Waldo 1990; Poelmann 1998a) and that ablation studies of neural crest cells cause cardiac malformations like ventricular septal defects and double outlet right ventricle. (Kirby and Waldo 1990). Recent evidence suggests that the dorsal part of the neural crest cells are involved in inflow tract septation and maldevelopment of this cell population may lead to atrioventricular septal defects (Poelmann 1998b). This means that our heterogeneous group of patients is probably all neural crest-related. We included the two cases with left heart hypoplasia since these patients also displayed an outflow tract problem. We, therefore, combined the entire group of patients and investigated whether fetuses with this kind of cardiac malformation demonstrate alterations in absolute and variability values of fetal heart rate and/or blood flow velocity compared to normal healthy controls.

In our study, fetuses with congenital heart defects had decreased umbilical artery peak systolic and time-averaged flow velocity and increased variability in fetal heart rate, peak systolic and time-averaged velocity compared to normal controls. The developmental mechanism responsible for those changes in function likely relates to the autonomic nervous system.

The development of cardiac outflow tract can be separated in three stages of the looping process. The first stage is the looping of the heart tube and secondly brings the outflow tract in front of the atrioventricular canal. In the last stage the

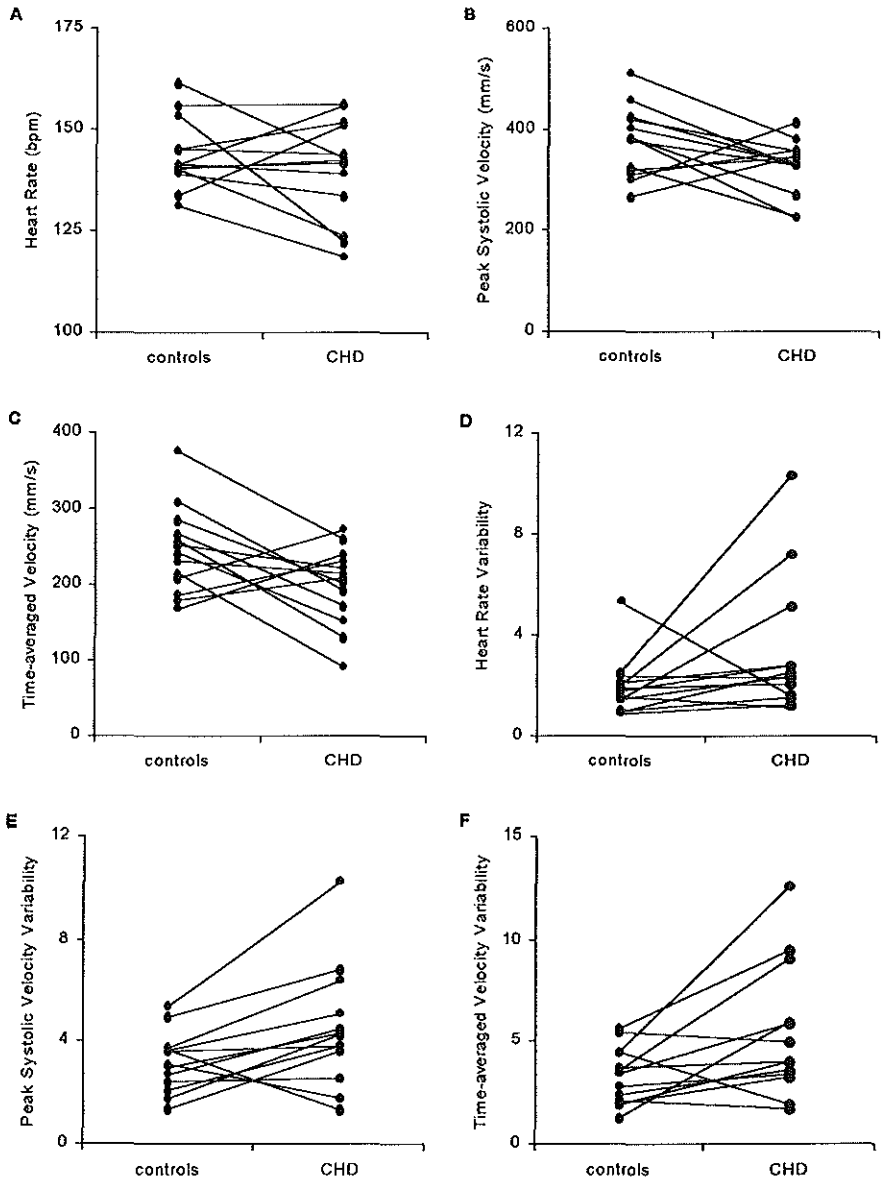

Figure 1. Individual data of haemodynamic parameters measured in the umbilical artery from fetuses with congenital heart defects (n=13) and normal fetuses (n=13). The difference between the two study groups are depicted for fetal heart rate (A), peak systolic velocity (B), time-averaged velocity (C), heart rate variability (D), peak systolic velocity variability (E) and time-averaged velocity variability (F).

aortic orifice is wedged between the tricuspid and mitral valves. Disturbances in the timing of the second stage of the looping process results in a spectrum of double outlet right ventricle to a ventricular septal defect (Gittenberger-De Groot 1997). Prerequisites for the formation of a normal atrioventricular conduction tissue axis are the presence and correct alignment of the atrial septum and their inlet and trabecular components of the ventricular mass. Absence or malalignment of any of these components will affect the disposition of the conduction tissue (Davies 1983). Additionally, infants with an atrial septal defect demonstrated electrophysiologic abnormalities caused by sinus node and atrioventricular node dysfunction (Clark and Kugler 1982). Abnormal cardiac morphology in combination with a disturbance in the atrioventricular conduction system could account for the increase in heart rate variability. In the chick embryo, administration of all-trans retinoic acid induces cardiac outflow anomalies, like double outlet right ventricle and ventricular septal defects associated with abnormal vagal innervation of the heart (Broekhuizen 1998). Disturbance of vagal nerve distribution is likely to be associated with altered cardiac function (Broekhuizen 1998). Power spectral analysis of the aortic blood flow velocity in the same intervention model showed that treated chick embryos exhibit increased heart rate variability (Kempski 1996). The possible explanations for the observed increase of heart rate variability are not applicable for the increase in velocity variability. Studies in the dorsal aorta of the normally developing chick embryo suggest a haemodynamic feedback mechanism to be responsible for the flow velocity variability in this vessel (Kempski 1993). Similar flow velocity variability patterns have been established in the umbilical artery (Ursem 1998a) and descending aorta in normal human fetal development. Whether an altered haemodynamic feedback mechanism is responsible for the increase in peak and time-averaged flow velocity variability in the umbilical artery in the presence of congenital heart disease is not clear.

The reduction in peak systolic and time-averaged velocities values in the umbilical artery in fetuses with congenital heart defects could be secondary to reduced volume flow, reduced cardiac contractility, or increased afterload. The non-invasive nature of the human Doppler studies does not allow differentiation between these variables. Although, there were three cases of mild intra-uterine growth retardation (Table 1), the estimated fetal weight at the time of the Doppler survey was not essentially different between the two study groups. Therefore, reduction in fetal weight determined volume flow could not be responsible for the observed decrease in umbilical artery peak systolic and time-averaged velocities. The same applies to downstream impedance, which reflects afterload; the umbilical artery pulsatility index values were similar between the two groups (Table 2). Support for reduced cardiac contractility as a cause of decreased umbilical artery flow velocity can be found in the chick embryo model. Treatment of stage 34 chick embryos with retinoic acid resulted in a spectrum of

a rightward shift of the aorta and a significant decrease in heart rate, aortic peak systolic and mean velocities. This was associated with a reduction in peak acceleration and cardiac stroke volume suggesting a decrease in cardiac contraction force. Impaired parasympathetic innervation through a disturbed cell migration from the neural crest and/or a direct effect of retinoic acid on the growth and differentiation of cardiac myocytes has been proposed as possible mechanisms for the reduction in cardiac contractility (Broekhuizen 1995). It is realised that comparison of the animal retinoic acid model and the human model of fetal congenital heart disease has its limitations. Nevertheless, animal intervention models allow us to pertain insight into the possible mechanisms underlying abnormal cardiac morphology and function.

In conclusion, our results show that marked cardiovascular changes occur in the fetus with congenital heart defects compared with the normal healthy fetus. We propose that variability in fetal heart rate and umbilical artery blood flow velocity are markers for impaired cardiovascular homeostasis among fetuses with structural heart defects.

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CHAPTER 6

GENERAL DISCUSSION AND CONCLUSIONS

In this thesis we compare different techniques to reconstruct fetal arterial blood flow velocity waveforms from Doppler audio signals. Variability analysis of these reconstructed signals may serve as a measure for cardiovascular health in the fetus. We studied the variability of heart rate and arterial blood flow velocity under physiological and pathologic circumstances in early pregnancy. In case-control studies we investigated whether beat-to-beat variability is a reliable method to detect cardiovascular dysfunction.

Flow velocities can be estimated from several Doppler shift frequency components of the Doppler power spectrum. The maximum velocity is the most used method, but also the mean velocity waveform or mode velocity waveform can be used to estimate the velocity waveform. We demonstrated that for heart rate variability and velocity variability studies the preferred method to reconstruct the Doppler signal from the human umbilical artery is the maximum flow velocity reconstruction method, because it is relatively insensitive to noise, non-uniform insonation, and wall filter settings. Using this method, it is possible to establish the physiological change in flow velocity parameters and heart rate in the umbilical artery, which may serve as a tool for early detection of cardiac dysfunction.

The beat-to-beat variability in normal pregnancies ranged for heart rate from 1%-4% and for umbilical artery flow velocity from 2%-8% at the late first and early second trimester of pregnancy. These physiological modulations in the fetal Doppler flow velocity waveform can be easily corrupted by non-physiologic factors like fetal or maternal movements or hand movements of the investigator. Maternal respiration is one of those factors that may influence the quality of the long-term Doppler recordings since movement of the maternal abdomen or uterus may shift the position of the Doppler sample volume and/or interrogation angle, so that different velocities will be recorded. The variability data obtained from the transvaginal approach (8-13 weeks of gestation) and the transabdominal approach (after 13 weeks of gestation) were similar between maternal spontaneous breathing and during stop breathing. Thus, spontaneous maternal breathing will not influence variability data of fetal heart rate and velocity and therefore these data can be obtained during normal maternal breathing. The above mentioned factors that influence the Doppler waveform

limit the duration of the recording to 15-25 s. A solution to overcome the problems of non-physiological modulations of the flow velocity waveform might be to apply the 3D ultrasound technique in which the sample volume is fixed to the fetal vessel (Mathews 1998, personal communication). The sample volume will be able to track the moving fetal vessel in all directions and therefore very long high quality Doppler recordings can be made. By using these Doppler recordings, we can determine both low and high frequency contents of heart rate and velocity variability. This frequency content can be obtained by power spectral analysis of arterial Doppler waveform recordings and may reveal the mechanisms that regulate homeostasis in the early human fetus.

In the pregnancy period of 10-20 weeks, reproducible fetal heart rate and velocity variability data can be derived from the umbilical artery. Fetal heart rate decreased at 10-15 weeks of gestation and platued thereafter. Peak systolic umbilical artery flow velocity demonstrated a marked increase up to 15 weeks of gestation followed by plateauing at 15-20 weeks of gestation, thus mirroring the fore-mentioned changes in fetal heart rate. The inverse relationship between umbilical artery peak systolic velocity and fetal heart rate per week suggests that the major regulator of cardiac output is the Frank-Starling mechanism and that changes in heart rate have little effect on cardiac output. For this statement the assumption has to be made that blood flow velocity relates to some extent with volume flow. The cross-sectional area of the umbilical artery is likely to be similar between fetuses of the same gestational age. Because volume flow is equal to the product of blood flow velocity and the cross-sectional area of the vessel, flow velocity could be related to volume flow. With the recent development of 3D ultrasound, we may obtain accurate measure of the vessel area and therefore be able to directly calculate volume flow. With the measurement of volume flow at normal heart rate and increased heart rate induced by auditory stimulation we may resolve the controversy regarding the role of heart rate and the Frank-Starling mechanism in regulating cardiac output during prenatal life (Kirkpatrick 1976; Gilbert 1980).

Parasympathetic maturation is considered responsible for the gestational age-related decrease in fetal heart rate in the late first trimester of pregnancy; these changes occur in parallel with the appearance of fetal heart rate variability. We demonstrated that at 10-15 weeks the fall in fetal heart rate does not coincide with an increase in fetal heart rate variability. Therefore, an alternative explanation for the reduction in fetal heart rate must be considered. The increase in the number of cardiac muscle cells and changes in myocardial properties may result in adaptation to slower contractions. This is in support of the approximately two-fold increase in ejection time between 5–14 weeks of gestation (Leiva 1994). A fundamental change in haemodynamic regulation likely occurs at 10-20 weeks of gestation, with autonomic control mechanisms

appearing subsequent to afterload modulation control. The mechanism for afterload regulation is unknown, but biochemical substances which can induce vasodilatation or vasoconstriction are likely candidates. Haemodynamic regulation in the fetus therefore may shift from biochemical to neural mediation with cardiovascular development.

Absolute and variability values of heart rate and umbilical artery flow velocity seem to discriminate between fetuses that are at risk for pathology and normal fetuses. In women who later develop pregnancy induced hypertension, umbilical artery flow velocity variability was reduced compared with normal controls. Changes in mechanical properties of the resistance vessels in the placenta due to the release of vasoactive biochemical substances, such as lipoperoxidation products may be a mechanism for the decrease in flow velocity variability

Fetuses of diabetic women displayed deviation from the normal values in umbilical artery flow velocity and heart rate variability. A mechanism behind these observations may be the accelerated cardiac growth in fetuses of diabetic women. The pathophysiology of myocardial and septal hypertrophy in fetuses from diabetic mothers is still under consideration. A different degree of sensitivity of the fetal myocardium to factors accelerating growth and/or an increase in release of growth factors might be an explanation.

Marked haemodynamic changes in both absolute blood flow values and variability data were observed in fetuses with severe cardiac malformations, similar to studies performed in animal models. The mechanisms that regulate beat-to-beat variation seem to be disturbed in fetuses with cardiac malformations. In normal fetuses the variability is clustered around specific values and not randomly distributed as in fetuses with congenital heart defects. The non-invasiveness of this study does not allow differentiation between the mechanisms responsible of these alterations, but dysfunction of myocardial contractility and impaired parasympathetic innervation are likely candidates.

Finally, variability analysis of signals such as heart rate or blood flow velocity in the frequency or time domain assume linearity of the systems that regulate these variables. Recent work in systems ranging from intact adult to isolated dog hearts suggests that the control of heart rate and blood pressure may be best described with chaotic non-linear dynamics (Chaffin 1991; Wagner 1996). Goldberger (1997) proposes that healthy physiological systems are organised in such a way that they display complex variability and unpredictability, popularly referred as to "chaos", whereas pathological states are defined by a loss of chaotic nature and may be manifest by the emergence of highly periodic and predictable dynamics (Goldberger 1997).

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SUMMARY

Chapter 1

The development of diagnostic ultrasound for measurement of blood flow velocity allows direct assessment of the fetal cardiovascular system. We hypothesise that variability in the haemodynamic waveform is directly related with fetal cardiovascular health. Deviation of these variability data is a measure of cardiovascular dysfunction that correlates with poor pregnancy outcome. The objectives of the present thesis are to document fetal heart rate and arterial flow velocity variability in a normal population at early gestation and relate these variability measures with pregnancies that are at risk for abnormal fetal developement.

Chapter 2

This chapter deals with methodological aspects of spectral analysis and the safety issues of Doppler ultrasound in the human fetus. The second part of this chapter describes the development of a computer algorithm for the analysis of arterial Doppler velocity waveform for heart rate and velocity variability. Flow velocity can be estimated from several Doppler shift frequency components (maximum, mean or mode frequency) of the Doppler power spectrum. We demonstrate that for the assessment of variability in blood flow velocity and heart rate in the umbilical artery, the maximum flow velocity reconstruction method is preferred because it is relatively insensitive to noise, non-uniform insonation, and wall filter settings. Spontaneous maternal breathing does not influence the variability data obtained from the umbilical artery in early gestation.

Chapter 3

This chapter focuses on the physiological changes in fetal haemodynamics in relation to gestational age. Umbilical artery peak systolic and time-averaged velocity increase at 10-20 weeks, whereas fetal heart rate decrease at 10-15 weeks of gestation and platued thereafter. The increase in umbilical artery flow velocity coincides with a decline in fetal heart rate at 10-15 weeks of gestation. The inverse relationship between umbilical artery flow velocity and fetal heart rate per gestational week suggests that the Frank-Starling mechanism regulates cardiovascular control this early in pregnancy. Umbilical artery peak systolic variability and fetal heart rate variability increase at 10-20 and 15-20 weeks, respectively. It is speculated that heart rate variability is mediated by maturation of the parasympathetic nervous system, whereas peak systolic velocity variability reflects the activation of a haemodynamic feedback mechanism.

Reproducible fetal heart rate and velocity variability data can be derived from the descending aorta and umbilical artery. The increase in heart rate variability observed in the umbilical artery is not seen in recordings obtained from the descending aorta. We suggest that a different fetal activity state may be the underlying mechanism for these heart rate variability discrepancies. The rise in peak systolic velocity variability is seen in the umbilical artery as well as in the descending aorta.

Chapter 4

In this chapter we extended our variability analsyis to potentially complicated pregnancies which are at risk for poor fetal outcome. Absolute values for fetal heart rate, peak systolic and time-averaged velocity as well as variability in fetal heart rate does not differ significantly between women which later developed pregnancy induced hypertension and normal controls. Umbilical artery flow velocity variability is reduced in women developing pregnancy induced hypertension compared with controls. Changes in mechanical properties of the resistance vessels in the placenta due to the release of vasoactive biochemical substances may be a mechanism for the decrease in flow velocity variability. Fetuses of diabetic women display increased fetal heart rate variability and umbilical artery peak systolic velocity. Fetal heart rate, umbilical artery time-averaged velocity and variability in umbilical artery flow velocity are similar between the two groups. We speculate that due to accelerated cardiac growth in fetuses of diabetic women, the functional maturation of the cardiac parasympathetic nervous system may be altered compared with normally developing fetuses, resulting in an increase in heart rate variability.

Chapter 5

Animal experimental work and human Doppler studies have shown marked haemodynamic changes in fetuses with cardiac malformations. In this chapter variability of fetal heart rate and umbilical artery flow velocity in fetuses with congenital heart defects are studied. In fetuses with congenital heart defects, umbilical artery peak systolic and time-averaged velocity are significantly decreased, whereas variability in fetal heart rate and umbilical artery peak systolic and time-averaged velocity increase compared with normal controls. Fetal heart rate is similar between fetuses from both subsets. In conclusion, our results show that marked cardiovascular changes occur in the fetus with congenital heart defects compared with a normal healthy fetus. We propose that raised variability in fetal heart rate and umbilical artery blood flow velocity is a marker for impaired homeostasis in fetal congenital heart defects. Disturbance of the vagal nerve distribution at cardiac level may be the underlying mechanism for these flow velocity variability findings.

SAMENVATTING

Hoofdstuk 1

De ontwikkeling van diagnostische echoscopie voor het meten van de bloedstroomsnelheid maakt het mogelijk om direct het foetale hart- en vaatstelsel te evalueren. Onze hypothese is dat variaties in het bloedstroomprofiel direct gerelateerd zijn aan de foetale cardiovasculaire functie. Afwijkingen van variabiliteit zijn mogelijk een maat voor het niet goed functioneren van het hart- en vaatstelsel hetgeen kan leiden tot een slechte afloop van de zwangerschap. De doelstellingen van dit proefschrift zijn het vroeg in de zwangerschap vastleggen van variaties in foetale hartslag en bloedstroomsnelheid in risicozwangerschappen en deze variaties vergelijken met een normale populatie zwangeren.

Hoofdstuk 2

Dit hoofdstuk behandelt de methodologische kanten van spectraalanalyse en de veiligheidsaspecten van foetale Doppler-echoscopie. Het tweede gedeelte van dit hoofdstuk beschrijft de ontwikkeling van een computerprogramma voor het bepalen van variaties in hartslag en bloedstroomsnelheid. De snelheid kan worden bepaald uit diverse componenten (maximum, gemiddelde en modale frequentie) van het Doppler-intensiteitspectrum. De beste methode voor evaluatie van variaties in hartslag en bloedstroomsnelheid in de arteria umbilicalis is de reconstructiemethode, waarbij gebruik wordt gemaakt van de maximale frequentie. De reconstructiemethode is relatief ongevoelig voor ruis, ongelijke insonatie en instellingen van de wandfilters. Vroeg in de zwangerschap heeft maternale ademhaling geen invloed op de variabiliteit van de foetale hartslag en bloedstroomsnelheid verkregen uit de Doppler-opnames van de arteria umbilicalis.

Hoofdstuk 3

Dit hoofdstuk concentreert zich op de fysiologische veranderingen in de foetale hemodynamiek in relatie tot de zwangerschapsduur. Van 10 tot 20 weken nemen de piek-systolische en gemiddelde snelheden in de arteria umbilicalis toe. De foetale hartslag neemt daarentegen af tussen de 10 en 15 weken en blijft daarna constant. Per zwangerschapsweek is snelheid in de arteria umbilicalis omgekeerd evenredig met de foetale hartslag. Dit suggereert dat het Frank-Starlingmechanisme actief is in deze periode van de zwangerschap. Er wordt gespeculeerd dat variaties in de hartslag worden beïnvloed door het parasympathische zenuwstelsel, terwijl variaties in piek-systolische snelheid de activatie van een terugkoppelingsmechanisme weergeven.

Zowel in de aorta descendens als in de arteria umbilicalis is de variabiliteit in hartslag en bloedstroomsnelheid goed reproduceerbaar. De toename in hartslagvariaties die wordt gezien in de arteria umbilicalis wordt niet waargenomen in de Doppler-opnames van de aorta descendens. Wij suggereren dat variatie in motorische activiteit een mogelijke varklaring is voor de verschillen in deze hartslagvariaties. De toename in piek-systolische snelheidsvariaties is zowel aantoonbaar in opnames van de arteria umbilicalis als in de aorta descendens.

Hoofdstuk 4

In dit hoofdstuk hebben we de variatie-analyse uitgebreid naar potentieel risicovolle zwangerschappen die een kans hebben op de slechte afloop van de zwangerschap. Bij zwangeren die later zwangerschapshypertensie ontwikkelen, zijn geen verschillen aangetoond in foetale hartslag, piek-systolische en gemiddelde snelheid en hartslagvariaties in vergelijking met gezonde zwangere vrouwen. De bloedstroomsnelheidsvariaties in de arteria umbilicalis zijn afgenomen ten opzichte van de controlegroep. Veranderingen in mechanische eigenschappen van bloedvaten in de placenta door de afgifte van vasoactieve stoffen zijn mogelijk verantwoordelijk voor de afname in snelheidsvariaties in vrouwen die later zwangerschapshypertensie ontwikkelen. De foetale hartslagvariaties en bloedstroomsnelheid zijn verhoogd bij vrouwen met diabetes mellitus in vergelijking met de controlegroep. De foetale hartslag en variaties in piek-systolische en gemiddelde snelheden van de arteria umbilicalis zijn gelijk tussen beide groepen. Wij speculeren dat de toename in foetale hartslagvariaties bij diabetische vrouwen mogelijk kan worden verklaard door een verandering van de ontwikkeling van het parasympatische zenuwstelsel. De versnelde groei van het foetale hart bij zwangeren met diabetes mellitus kan mogelijk resulteren in een verandering van het parasympatische zenuwstelsel.

Hoofdstuk 5

Doppler-studies bij dieren en in de mens hebben aangetoond dat hartafwijkingen gepaard kunnen gaan met welomschreven hemodynamische veranderingen. In dit hoofdstuk zijn de variaties in hartslag en bloedstroomsnelheid in de arteria umbilicalis bestudeerd in de foetus met een structurele hartafwijking. Bij foetale structurele hartafwijkingen zijn de piek-systolische en gemiddelde snelheden in de umbilicalis afgenomen, terwijl de variaties in hartslag en bloedstroomsnelheid verhoogd zijn in deze groep. De foetale hartslag is gelijk in beide groepen. Uit deze studie kunnen wij concluderen dat er opmerkelijke verschillen zijn in de foetale haemodynamiek tussen de groep met aangeboren hartafwijkingen en de controlegroep. Variaties in hartslag en bloedstroomsnelheid kunnen mogelijk

worden gebruikt als een maatstaf voor veranderende homeostase bij foetale aangeboren hartafwijkingen. Bij foetale structurele hartafwijkingen is de waargenomen toename in variaties van hartslag en bloedstroomsnelheid mogelijk het gevolg van abnormale vertakking van de nervus vagus op cardiaal niveau.

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