DOPPLER ULTRASONOGRAPHY

OF THE

HUMAN FETAL PULMONARY

CIRCULATION

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Uitgeverij Eburon
Postbus 2876
2601 CW Delft
info@eburon.nl
www.eburon.nl

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DOPPLER ULTRASONOGRAPHY OF THE HUMAN FETAL PULMONARY CIRCULATION

Doppler ultrageluidonderzoek van de humane foetale longcirculatie

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JACOBINA AVELINE MARIA LAUDIJ

GEBOREN TE SITTARD

PROMOTIECOMMISSIE

PROMOTOR:	Prof. jhr dr. J.W. Wladimiroff
OVERIGE LEDEN:	Prof. dr. D. Tibboel
	Prof. dr. J.N. van de Anker
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De hoc multi multa, omnes aliquid, nemo satis

Desiderius Erasmus

Aan Edwin en Joep

Aan mijn ouders

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CHAPTER 1

INTRODUCTION AND DEFINITION OF OBJECTIVES

1.1 Introduction

Pulmonary hypoplasia is a condition characterized by a decrease in the number of lung cells, airways and alveoli with a resulting decrease in organ size and weight.[1] The reported incidence of pulmonary hypoplasia in the general population is about 1 per 1000 live births and it is one of the most common single abnormalities found at postmortem examination.[2,3] The occurrence of pulmonary hypoplasia is mostly secondary to congenital anomalies or pregnancy complications that inhibit lung development.[4] For instance prolonged oligohydramnios due to premature rupture of membranes (PROM) or fetal renal and urinary tract malformations may lead to abnormal fetal lung development with subsequent severe respiratory distress immediately after birth and even neonatal death.[5-9]

The current trend in case of PROM favors expectant management with intravenous corticosteroids, tocolysis and/or antibiotics, if necessary. This should maximize the benefits of increasing fetal maturity and reduce the perinatal morbidity and mortality related to preterm delivery.[10] On the other hand, the risk of oligohydramnios-related pulmonary hypoplasia increases with advancing gestation. Therefore, in order to improve the clinical care, an accurate and patient-friendly prenatal test for early detecting and separating lethal from non-lethal pulmonary hypoplasia is highly desirable.

Various methods have been proposed. Most of these include fetal two-dimensional biometric indices, such as fetal thoracic circumference and ratio of thoracic and abdominal circumference or of cardiac and thoracic circumference. However, these measurements are late indicators of pulmonary hypoplasia, with a sensitivity and specificity not satisfactory for clinical management.[11,12] Data on the predictive value of the presence or absence of fetal breathing movements are contradictory.[13,14] In 1990, van Eyck et al.[15] have reported on Doppler evaluation of the fetal ductus arteriosus in the prediction of pulmonary hypoplasia. They demonstrated that blood flow velocity waveforms obtained from the fetal ductus arteriosus are modulated by fetal breathing activity during normal pregnancies. This was considered a reflection of a decreased pulmonary vascular resistance during lung expansion. In fetuses with pulmonary hypoplasia, they observed a reduced ductal peak systolic flow velocity modulation, which was hypothesized to result from impaired development of the pulmonary vascular bed.

The use of combined two-dimensional real-time and Doppler ultrasound systems has provided a non-invasive method for studying the early and late human fetal systemic circulation.[16,17] It has been demonstrated that the normally developing fetus during the second half of gestation is characterized by a low feto-placental vascular resistance with well-defined and reproducible arterial, cardiac and venous flow velocity

waveforms.[18] The addition of color Doppler has made it possible to determine flow directions at cardiac and extra-cardiac levels.[19-21]

Our knowledge of the fetal pulmonary circulation is primarily derived from invasive animal studies, particularly in lambs.[22-24] Although hemodynamic indices from these different studies may subsequently be extrapolated to the human fetus, caution should be applied since the distribution of the fetal circulation and subsequent fetal flow patterns may be different.[25]

The introduction of more sensitive color-coded Doppler systems has led to the identification of the human fetal pulmonary circulation.[26,27] Consequently, non-invasive Doppler velocimetry of the human fetal arterial and venous pulmonary circulation has become subject of our study. This information may contribute to our understanding of the normal fetal pulmonary circulation and of hemodynamic changes underlying structural pathology such as lung hypoplasia. As shown in postmortem studies, pulmonary hypoplasia is associated with underdevelopment and structural changes of the pulmonary vascular bed.[28,29] This may lead to increased pulmonary vascular resistance and decreased pulmonary arterial compliance. These alterations may effect pulmonary blood flow and consequently induce changes in pulmonary flow velocity waveforms as recorded by Doppler ultrasonography.

1.2 Definition of research objectives

The objectives of this thesis were as follows:

1. to establish the characteristics and gestational age dependency of flow velocity waveforms from proximal, middle and distal arterial and venous pulmonary branches in normal singleton human pregnancies during the second half of gestation. The results are discussed in chapter 3 and 4.

2. to assess the repeatability of flow velocity waveforms from proximal, middle and distal arterial and venous pulmonary branches. Data are presented in chapter 3 and 4.

3. to determine the relationship between flow velocity waveforms from proximal, middle and distal arterial and venous pulmonary branches. Results are discussed in chapter 3 and 4.

4. to establish the nature and gestational age dependency of the pulmonary venous flow velocity pattern into the left atrium relative to systolic and diastolic phases of the cardiac cycle. This is discussed in chapter 4.

5. to study whether, similar to the systemic circulation, behavioral state dependent changes can be demonstrated in fetal arterial and venous pulmonary blood flow velocity waveforms in normal term pregnancy. Data are presented in chapter 5.

6. to evaluate normal fetal lung volume measurements obtained by three-dimensional ultrasonography during the second half of pregnancy. Results are discussed in chapter 6.

7. to determine the predictive value of Doppler pulmonary artery flow velocity waveforms regarding the development of pulmonary hypoplasia in cases of prolonged oligohydramnios following premature rupture of membranes or due to renal pathology. This is presented in chapter 7.

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CHAPTER 2

A LITERATURE REVIEW

2.1 Introductory remarks

The study of growth and development of the lung provides insights into the pathogenesis and sequelae of congenital malformations and is important in understanding how lung growth is altered by disease and environmental factors. This chapter will give an overview of these aspects of fetal lung development and of the development of pulmonary hypoplasia in particular. Further, from the obstetrician's and pediatrician's point of view, knowledge of the clinical course and of pre- and postnatal diagnostic procedures and characteristics is mandatory to cope with the various ways pulmonary hypoplasia may present itself. This will also be discussed in detail in this chapter.

2.2 Normal fetal lung development

Human lung development is divided into five stages: embryonic, pseudoglandular, canalicular, saccular and alveolar.[1-11] One should realize that the transition between stages occurs gradually and that there is considerable overlap from one stage to the next, but also between various areas within the lung and between various gestational ages and individuals.[1-3] The anatomic and morphologic characteristics of each stage are as follows:

Embryonic stage (conception-6th weeks gestation):
The human fetal lung arises as a ventral diverticulum from the caudal end of the laryngotracheal groove of the foregut at the end of the fourth week. During the next few weeks this diverticulum grows caudally to form the primitive trachea, and then, the end of the diverticulum divides into two sacs, the lung buds. These two buds develop lobar buds that correspond to the mature lung lobes, i.e., three on the right and two on the left. The lobar buds will have further subdivided and by the end of the sixth week all bronchopulmonary segments will have been formed. The primitive lung bud is lined with endodermally derived epithelium.

Pseudoglandular stage (6th-16th weeks of gestation):
Stimulated by the presence of surrounding mesenchyme, conducting airways are formed by repeated dichotomous branching resulting in a tree of narrow, thick epithelial-lined tubules. During this phase the lung has a distinctly glandular appearance, hence the term pseudoglandular. By the 16th week of gestation the tracheobronchial tree has been formed, from the trachea up to and including the terminal bronchioles. These branches, or pre-acinar airways, may increase in size as further growth of the lungs occurs, but new branches cannot be formed after the 16th week. The mesenchyme surrounding the lung bud diverticulum differentiates to form

the early rudiments of cartilage, connective tissue, muscle, blood vessels, and lymphatics. The epithelio-mesenchymal interactions play a determining role in regulating growth and branching patterns.[8,9] Recently, it has become clear that both embryonic and pseudoglandular stages are histologically similar and should therefore be referred to as the pseudoglandular period.[11]

Canalicular stage (16th-28th week of gestation):
During this phase, the basic structure of the gas-exchanging portion of the lung is formed and vascularized. Since the cephalad segments of the lungs mature faster than the caudal parts, this stage partially overlaps the previous one. Differentiation of the airways starts with widening of the lumina and gradual thinning of the cuboidal epithelium. Proliferation of a rich vascular supply occurs and due to a relative decrease in mesenchyme the capillaries become closer to the airway epithelium. Primitive respiratory bronchioles begin to form, thus delineating the acinus. The acinus is the gas-exchanging portion of the tracheobronchial tree, composed of respiratory bronchioles, alveolar ducts, sacs and alveoli. Flattening of the acinar epithelium at approximately 22-24 weeks marks the initial differentiation of type II pneumocytes, from which type I pneumocytes will be derived later. Type II pneumocytes produce surfactant. Type I pneumocytes are responsible for gas exchange.

Saccular stage (28th-36th week of gestation):
At the beginning of the saccular stage airways terminate in large smooth-walled cylindrical structures subdivided by ridges called crests. The crests protrude into saccules, pulling a capillary network with them and creating subsaccules, which will eventually become alveoli. The end result of the saccular stage is a rapid increase in the gas-exchange surface of the lung and rapid thinning of the interstitium. During the period of 32-36 weeks, type II (surfactant producing) pneumocytes mature, completing the functional maturity of the lung.

Alveolar stage (36th week–term):
Studies by Langston *et* al [4] have shown that alveolar structures can be recognized in some fetuses at 30-32 weeks of gestation and are uniformly present at 36 weeks of gestation, challenging previous beliefs that alveoli develop after birth.[7,12-14] The alveolar stage is not completed until 8 years of age, with the greatest increase in the number of alveoli occurring during the first 2 years of life. During this stage further thinning of the blood-gas barrier, increase of surfactant production and progressive branching of the respiratory airways occurs.

Normal lung growth in the human can be summarized according to Reid by three laws[3,12-15]:

(I) the bronchial tree is developed by the 16th week of gestation;
(II) the majority of alveoli develop after birth, increasing in number until the age of eight years and in size until growth of the chest wall is finished;
(III) the pre-acinar arteries and veins parallel the development of the airways, while the intra-acinar vessels follow the development of the alveoli.

2.3 Fetal pulmonary circulation

2.3.1 *Development*

Prenatal growth and development of the pulmonary blood vessels is closely linked to that of the bronchial tree (*pre-acinar* blood vessels with the conducting airways and *intra-acinar* blood vessels with alveolar development). By the third week of gestation (embryonic period), the primitive heart anlage with its arterial and venous communications is present. The sixth branchial arches appear at about 5 weeks and develop into the main pulmonary arterial trunk with right and left branches during the next few weeks of the pseudoglandular period. By 7 weeks of gestation, the adult pattern of vessels connecting heart and lungs is established.[1,16] Just as the branching of the conducting airways is completed at by 16 weeks of gestation, all pre-acinar arteries are present by this time as well. These arteries grow further by increasing their lengths and diameters, but not in number. By contrast, the intra-acinar arteries develop relatively late in fetal life and continue to form after birth and multiply rapidly then to keep pace with alveolar multiplication.[1,2,16,17]

There are two types of pulmonary arterial branches in the lung: *conventional arteries*, which course with the conducting airway branches, and ultimately provide terminal branches to supply the acini. From these conventional arteries numerous and smaller branches arise, called *supernumerary arteries*, and pass into the adjacent respiratory tissue to supply the alveoli directly. The supernumerary arteries may serve for collateral circulation if blood flow through the conventional arteries is impeded. The pre-acinar conventional and supernumerary arteries appear simultaneously and the adult ratio of 2.5 to 3.5 supernumaries to one conventional artery is present by 12 weeks of gestation.[1,2,16-18]

Development of the pulmonary veins occurs after that of the arteries, but by 20 weeks of gestation all pre-acinar veins are present. The development of the venous system parallels that of the arteries and airways in the fetus, with the exception that the venous system of the lungs is more extensive than the arterial system: the conventional veins

correspond in number and branches to conventional arteries and conducting airways but the supernumerary veins outnumber the supernumerary arteries. Both types of veins appear together and their number maintains the same ratio (3.5-4.3 supernumerary to 1 conventional vein) to each other throughout pre- and postnatal life. The veins do not accompany the arteries and airways but lie in the intersegmental plane.[1,16,17,19]

2.3.2 *Structure*

Whereas the total adult complement of branches of blood vessels forming the pre-acinar region is completed half-way through fetal life, while the intra-acinar blood vessels develop as alveoli form, further changes of the pulmonary circulation are characterized by remodeling of the arteries, with changes in wall-thickness and in extension and distribution of muscle within their walls.[1] In the adult lung the proximal half of the pre-acinar arterial pathway down to the seventh generation has an elastic wall structure, and beyond this it is muscular. In fetal life the adult distribution of elastic structure is achieved by the 19th week of gestation.[17] The elastic arteries may play a role during fetal life as their high tensile strength may counteract the distending tendency of the blood pressure and also maintain the vessels patent.[18] The fetal pulmonary arteries are completely muscularized to the level of the terminal bronchioles, and only after birth does muscle extend to the level of alveolar ducts and beyond.[2,18] In the fetus, the arteries are more muscular than in the adult, and wall thickness being higher relative to external diameter. The wall thickness of a given sized artery in the fetus is double that in the adult.[16] This increases the resistance in the pulmonary arteries to maintain the reduced flow through the lungs seen during fetal life.[18] As gestation advances, the number of muscularized arteries rises, increasing the total amount of smooth muscle per unit area of lung tissue. In contrast to those investigators who found a rise in the amount of pulmonary arterial smooth muscle with increasing gestational age, Hislop and Reid concluded that thickness of the muscle coat of each individual artery does not change, regardless of age.[17,18,20-23]

Unlike the arteries, the intrapulmonary veins have thinner walls and muscle cells are rarely found in the wall before 28 weeks of gestation.[19]

2.3.3 *Physiology*

The in-utero pulmonary vascular circuit is a high-resistance, high-pressure, low-flow system.[24] In the fetus, normal gas exchange is placental in origin and pulmonary blood flow is low, merely supplying nutritional requirements for lung growth and some metabolic functions.[25] In the fetal lamb 3-4% of the total cardiac output supplies the lungs in early gestation, rising to about 7% near term.[25-27] In human fetuses this value is 13% at 20 weeks increasing to 25% at 30 weeks, but it remains constant during the last trimester.[28] This implies that pulmonary blood

flow increases and pulmonary vascular resistance decreases with advancing of gestation.

Fetal pulmonary blood flow is low despite the dominance of the right ventricle, which ejects about two-thirds of total cardiac output. Most of the right ventricular output is diverted away from the lungs through the widely patent ductus arteriosus to the thoracic aorta, then reaching the placenta through the umbilical circulation for oxygenation.[26,27] Since the ductus arteriosus is patent during intra-uterine life, pulmonary arterial pressure is considered to be maintained at least equal to systemic arterial pressure.[29]

Maintenance of a low-flow pulmonary vascular circuit depends on the high-resistance of the pulmonary vasculature. In the fetal lamb, lung development is characterized by a marked increase in the number of pulmonary resistance arteries.[20] Both the total vascular cross-sectional area and the total amount of vascular wall muscular tissue increase significantly during fetal development. The increased cross-sectional area of the pulmonary vascular bed produces a lower total resistance, while the greater number of small vessels with muscular walls provides more a widespread mechanism for controlling resistance.[20,24] Nevertheless, while absolute pulmonary flow increases with advancing gestation, Morin and Egan determined in fetal sheep that when corrected for wet weight of the lungs, pulmonary flow actually falls somewhat and total pulmonary resistance rises, and when corrected for dry weight of the lungs neither pulmonary blood flow nor total pulmonary resistance change with advancing gestation.[30]

2.3.4 *Regulation of fetal pulmonary vascular resistance*

During fetal life and the transition to extra-uterine air breathing, pulmonary vascular tone is regulated by a complex, interactive group of mechanisms, including mechanical influences and the release of a variety of vaso-active substances, which have been the subject of several reviews.[25,27,31-35] Much of our knowledge of fetal blood flow and its distribution is based on animal studies. Data on human fetuses and newborn are sparse because of the invasive techniques that would be necessary to obtain them.[25]

In unventilated fetal lungs, fluid filling the alveolar space compresses the small pulmonary arteries, thereby increasing pulmonary resistance. In addition, a high pulmonary vascular resistance is associated with a normally low oxygen tension in pulmonary and systemic arterial blood, whereas fetal pulmonary vascular resistance decreases by increasing oxygen tension.[30,36] This leads to the suggestion that vasoconstriction to the normal, relatively hypoxic status of the fetus is a mechanism that restricts blood flow to the expanding vascular bed in the fetal lungs, thereby protecting the fetal heart from unnecessary increases in output.[30,37]

O_2-related changes in pulmonary vascular resistance are also affected by pH. As acidosis is a pulmonary vasoconstrictor, alkalosis is a potent

pulmonary vasodilator at all stages of development.[31,38] The exact mechanism of hypoxic pulmonary vasoconstriction in the fetal pulmonary circulation is unclear. Although a change in O_2 environment could directly relax or vasoconstrict pulmonary vascular smooth muscle, it also could modify the local production and release of vaso-active substances, which then could act directly or indirectly on the pulmonary circulation.[25,33,39,40]

Arachidonic acid metabolites have been suggested to play an important role in the regulation of the pulmonary vascular tone. Leukotrienes represent one such a group of metabolites and are potent smooth muscle constrictors.[25,35,41] Prostaglandines form another group of potent vaso-active substances, although none of the prostaglandines is truly specific for the pulmonary circulation. PGI_2 seems to be the major prostaglandin with an effect on the pulmonary vascular resistance and is produced in vascular endothelial cells including in the lungs. Throughout gestation, a maturational increase in PGI_2 production parallels the decrease in pulmonary vascular resistance in the fetal third trimester.[25,33,35,38,39,41] In addition to PGI_2, vascular endothelial cells produce other vaso-active factors, clearly involved in the regulation of the vascular tone of the fetal pulmonary circulation. These include potent vasodilators, such as endothelium-derived relaxing factor, also known as nitric oxide (NO), and potent vasoconstrictors, such as endothelin type 1 (ET-1).[25,34,35,40]

Control of the perinatal pulmonary circulation probably reflects a balance between factors producing pulmonary vasoconstriction (low O_2, leukotrienes, ET-1, and other vasoconstricting substances) and those producing pulmonary vasodilatation (high O_2, PGI_2, NO, shear stress and other vasodilatating substances).[25]

2.4 Factors influencing fetal lung growth

Fetal lung development incorporates a combination of two processes: lung growth and lung maturation, which are related, but appear to be separately controlled. Normal lung growth refers to increase in cell number and seems to be influenced primarily by physical factors such as intra-uterine (i.e. amniotic fluid volume) and intra-thoracic space, lung liquid volume and pressure and breathing movements. Lung maturation refers to the distensibility or compliance of the lung and is divided into two components, structural and biochemical (surfactant). Structural maturation is regulated by physical factors, whereas biochemical maturation appears to be controlled by several endocrine organs (pituitary, adrenal, thyroid) and a host of endocrine factors including corticotrophin, cortisol, thyroid hormones and others.[2,3,5,42,43] Detailed discussion of surfactant production and regulation is beyond the scope of this thesis.

2.4.1 *Intra-uterine space*

Amniotic fluid formation and resorption is a dynamic process. Fetal urine is the primary component of amniotic fluid, but actively secreted fetal lung fluid also constitutes a significant portion, which is either swallowed or flows into the amniotic space.[44] Fetal swallowing is the principal means of amniotic fluid resorption. A normal amount of amniotic fluid seems to be important for fetal lung growth.[2,42,45] Bilateral renal agenesis, obstructive lesions of the urinary tract and early preterm rupture of membranes are the most common conditions that cause oligohydramnios.[46] Although the association between oligohydramnios and pulmonary hypoplasia is well-documented, the mechanisms of this phenomenon remain not fully elucidated. Several possibilities have been adopted such as i) decreased space for lung growth due to compression of the uterine wall upon the fetal chest and abdomen, ii) restriction of fetal breathing movements by prolonged thoracic compression and iii) increased efflux of lung liquid from the intrapulmonary space to the amniotic space, resulting in a decrease of intrapulmonary pressure.[2,42,45]

2.4.2 *Intra-thoracic space*

All abnormalities which result in a smaller than normal intra-thoracic cavity can interfere with fetal lung growth and be responsible for the development of fetal pulmonary hypoplasia both in animals and in man.[2,42,43] Among the conditions known in this respect are congenital diaphragmatic hernia, fetal hydrops, tumors of the thorax including adenomatoid malformations and skeletal anomalies deforming the thoracic cage.[15,42,45,47-50] Paralysis of the diaphragm may, apart from limiting intra-thoracic space, interfere with fetal breathing movements and this may have an additional negative effect on fetal lung growth.[42,43,51]

2.4.3 *Lung fluid*

Fetal intrapulmonary fluid is formed by active transport across pulmonary epithelium into the tracheo-bronchial lumen, where it establishes positive pressure within the developing lung. Lung-liquid volume and intra-tracheal pressure are maintained within very precise ranges by the larynx, which through unknown mechanisms regulates the efflux of lung liquid volume from the trachea to the amniotic space.[43,52] Lung liquid contributes up to one third of amniotic fluid volume.

Oligohydramnios may increase lung fluid loss with consequent decreased lung volume within the potential airways.[46] In 1977, Alcorn *et al*[53] demonstrated in fetal sheep that chronic drainage of lung liquid through a tracheal cannula led to pulmonary hypoplasia, while tracheal ligation resulted into increased lung growth, although with a negative influence on lung maturation as assessed by the enhanced ratio of type 2 to type 1 pneumocytes. Other reported that tracheal ligation resulting in increased intra-tracheal pressure, is responsible for lung growth through

cell proliferation. In these studies, however, lung maturation was normal as confirmed by measurement of the surfactant phospholipids, or by quantitative morphometrics.[52,54] Several lung hypoplasia animal experiments (tracheal ligation in combination with amniotic fluid shunting in fetal rabbits, with lung fluid drainage or bilateral nephrectomy in fetal lambs or in combination with a model of congenital diaphragmatic hernia also in fetal lambs) showed that tracheal ligation did not only reverse the pulmonary hypoplasia, but could actually accelerate lung growth even beyond normal limits.[2,46,52,55]

2.4.4 *Fetal breathing movements*

Fetal breathing movement in humans can be recorded by ultrasonography from about 10 weeks gestation. These respiratory movements produce significant changes in intra-thoracic pressure, which may influence lung development.[42,56] A fetal breathing movement is usually defined as a downward movement of the diaphragm with concomitant inward movement of the chest wall and outward movement of the abdominal wall.[57] However, different types of respiratory movements during gestation have been described.[57-59] Early in gestation fetal breathing is usually irregular and sporadic, from about 28 weeks of gestation onward it becomes more regular and episodic.[59,60] During the last 10 weeks of pregnancy, fetuses make spontaneous breathing movements approximately 30% of the time over a 24-hour period.[61] Several factors have been identified that may influence the presence and maybe also the pattern of fetal breathing movements. Among these are gestational age, time of the day, maternal food intake and maternal plasma glucose level, maternal end-tidal carbon dioxide level, fetal behavioral state, fetal distress, maternal use of nicotine, drugs or alcohol, and labor. Some of these factors may be interrelated.[58,59]

There is experimental evidence that fetal respiration is critical to lung growth and development. Wigglesworth *et al*[51] abolished fetal breathing movements in an animal model by selective destruction of the upper cervical cord, which arrested lung development. Bilateral phrenic nerve section in sheep produces pulmonary hypoplasia, but also results in diaphragmatic atrophy and raises the issue that there may be compression of the lung by abdominal contents.[5,62]. Adzick and co-workers[46] showed in an other animal study that when oligohydramnios and cervical cord transection were combined, a further significant decrease in lung growth was observed. Thus, even when fetal breathing was eliminated by cord transection, oligohydramnios caused even more hypoplasia. They concluded that although fetal breathing is clearly important for fetal lung growth, it seems unlikely that inhibition of fetal breathing is the predominant etiology of oligohydramnios-related pulmonary hypoplasia. Fetal breathing movements may stimulate lung growth by intermittently distending the lungs with fluid aspirated into the

trachea. It has been suggested that integration of fetal breathing movements and fluid secretion by the lungs is necessary for lung growth.[42,51]

2.4.5 *Normal balance of volume and pressure in the lung*

Fetal lung fluid establishes a positive pressure within the lung of the fetal lamb.[43,46] The presence of a positive pressure in the trachea relative to amniotic pressure shows that there is resistance to outflow.[43] When breathing movements are absent, the pressure in the fetal trachea is even higher, which may be important in regulating the volume of fluid within the lungs. So, there is evidence that lung fluid acts as an internal stent for the lung, distending potential airways and stimulating growth and differentiation.[46,51]

Oligohydramnios may increase lung fluid loss by compression of the lungs, thus leading to pulmonary hypoplasia, which was demonstrated by Harding and co-workers in the fetal lamb.[56] Lung fluid production was not affected by oligohydramnios according to this study. During breathing movements in the fetal lamb a considerable negative pressure is created on inspiration and the volume of fluid within the potential airways and air spaces increases when fetal breathing occurs.[42] Based on several experimental animal studies it may be concluded that positive intra-tracheal pressure, amplitude of pressure changes as well as tidal volume changes during fetal breathing movements may influence fetal lung growth.[42,43]

2.4.6 *Interference with the blood supply*

Ligation of the left pulmonary artery during the late canalicular stage of lung development in the fetal sheep creates pulmonary hypoplasia with significantly reduced lung weight and lung volume of the future gas exchange portion of the lung (future air spaces, parenchymal tissue and capillary content). These results suggest that normal pulmonary arterial flow during the canalicular and alveolar stages of fetal lung development is essential for normal lung growth.[3,63]

2.4.7 *Other factors involving fetal lung development*

Several transcription factors may play an important role in lung development, regulating cell growth and differentiation by modulating expression of target genes. Two functional domains are important in these proteins, one responsible for transcription activation and the other for DNA recognition and binding. Although the lung is the site of expression of several transcription factors, such as Hox-genes, retinoid receptors, hepatocyte nuclear factors and myc, sparse information is available on how they influence lung pattern.[64] Cardoso[64] has recently published an extensive review regarding these factors.

Important growth factors involving lung development are insulin-like growth factor I and II (IGF-I and IGF-II), platelet-derived growth factor (PDGF), fibroblast pneumocyte factor (FPF), transforming growth factor-β (TGF-β) and epidermal growth factor (EGF). These growth factors may regulate the extracellular matrix formation, which regulates cellular growth, migration, and differentiation.[65]

Pulmonary neuro-endocrine cells are amine and peptide producing cells distributed throughout the airway mucosa. Several observations indicate that these cells are involved in lung development.[66]
In case of controlling vascular growth the following growth factors have been suggested to be involved: fibroblast growth factors (FGFs), TGF-β, PDGFs, IGF-I and II and vascular endothelial growth factor (VEGF).[35]

2.5 Abnormal lung development: pulmonary hypoplasia

2.5.1 *Definition and morphologic features*

Pulmonary hypoplasia, either unilateral or bilateral, is a poorly defined condition of incomplete development of the lung, so that it fails to reach adult size.[45,50] This defective development is due to a decrease in the number of lung cells, airways and alveoli, with a resulting decrease in organ size and weight.[5,6,47,48,67-70] Based on a study of Nakamura *et al*[67] it has been postulated that impairment of pulmonary development during the pseudoglandular stage (before 16 weeks' gestation) causes reduced bronchiolar branching, cartilage development, acinar complexity and maturation, and retarded vascularization and thinning of the air-blood barrier. Insults occurring after the pseudoglandular stage (after 16 weeks' gestation) impair acinar complexity and maturation.[67] In a morphometric and biochemical study on hypoplastic lungs from human fetuses associated with oligohydramnios, Wigglesworth *et al*[47] as well as Nakamura *et al*[71,72] confirmed not only lung growth arrest but also impairment of lung maturation based on low surfactant phospholipid concentrations. Real et al[70] stated that changes in the histologic appearance were correlated with severity and were independent of the type of associated malformations.

Since growth of the pulmonary blood vessels parallels development of the airways (pre-acinar blood vessels with the conducting airways and intra-acinar blood vessels with alveolar development), it is not surprising that disturbance of the pulmonary vascular bed coincides with pulmonary hypoplasia. A decrease in total size of the pulmonary vascular bed, a decrease in the number of vessels per unit of lung tissue and increased pulmonary arterial smooth muscle have been described.[73] The latter has been expressed in an increased medial wall thickness as percentage of the external thickness.[74,75] Peripheral extension of muscle into arteries smaller

than normal have been observed[48,68,74,75] and the intrapulmonary arteries are smaller than normal but appropriate for the reduced volume of the lung.[48,68,]These features form the morphologic basis for the increased pulmonary vascular resistance.

2.5.2 *Epidemiology*

The reported incidence of pulmonary hypoplasia in the general population ranges from 9-11 per 10,000 live births and is 14 per 10,000 of all births.[76,77] This must be an underestimate of the true incidence since infants with lesser degrees of hypoplasia undoubtedly survive the neonatal period. The reported incidence of this condition in autopsies ranges from 7.8-22%[50,76-80] and more than 85% of those cases will display significant associated anomalies.[50] Perinatal mortality is high, approximately 70% in most series (55-100%).[81-86]

2.5.3 *Etiology*

Pulmonary hypoplasia may be a primary or secondary phenomenon. Bilateral pulmonary hypoplasia occurring as an isolated anomaly has been considered as extremely rare.[87,88] It has been suggested that infants with primary pulmonary hypoplasia represents a group of infants subject to decreased respiratory activity in utero, although the amount of amniotic fluid at delivery was not described in these instances.[1,87,88] Most cases of pulmonary hypoplasia are secondary to congenital anomalies or pregnancies complications that inhibit lung development. Factors important in lung growth are discussed in the previous sections. Several authors have reported on various anomalies associated with pulmonary hypoplasia.[6,10,15,45,48-50,67-70,72,79,81,82,88-94] An exhaustive list of the numerous conditions reportedly associated with pulmonary hypoplasia was compiled by Sherer *et al.*[15] Based on this list the following categories can be grouped (Table 1).

Most cases, such as renal anomalies, congenital diaphragmatic hernia and hydrops fetalis, indicate interference with lung growth during the early developmental stage mainly before 16 weeks of gestation, whereas oligohydramnios due to prolonged rupture of membranes possibly impairs pulmonary development after 16 weeks.[67] Wigglesworth *et al*[47] could detect no difference between structural appearance of the lungs after rupture of the membranes dating from before 20 weeks' gestation in the presence of normal kidneys and that seen in cases of bilateral renal agenesis.

Table 1
Etiologic categories and examples of related anomalies associated with pulmonary hypoplasia

Categories	***Anomalies***
Intra-thoracic masses	Congenital diaphragmatic hernia, congenital cystic adenomatoid malformation, bronchogenic cyst etc
Oligohydramnios based on renal or urinary tract anomalies	Bilateral renal agenesis or dysplasia, bladder outlet obstruction etc
Non-renal oligohydramnios	Prolonged preterm rupture of membranes
Skeletal malformations	Osteogenesis imperfecta, thanatophoric dwarfism, etc
Neuromuscular and central nervous system anomalies	Fetal akinesia, anencephaly, etc
Pleural effusions	Rhesus and non-rhesus hydrops, etc
Cardiac lesions	Hypoplastic right or left heart, pulmonary stenosis, etc
Abdominal wall defects	Omphalocele, gastroschisis etc
Syndromes associated with pulmonary hypoplasia	Trisomy 13, 18, 21, Robert syndrome, etc

2.5.4 *Oligohydramnios related pulmonary hypoplasia*

Renal and non-renal prolonged oligohydramnios are one of the major causes of pulmonary hypoplasia. The occurrence of bilateral renal agenesis and pulmonary hypoplasia was first recognized by Potter in 1946.[89] Later it became apparent that lethal pulmonary hypoplasia was also a consistent feature in babies with no renal function at birth because of bilateral renal dysplasia with or without cyst formation.[69,95]

The common features associated with these anomalies, are known as the Potter's syndrome: profound oligohydramnios, characteristic facial and limb deformities and pulmonary hypoplasia.[90,91,96,97] Growth deficiency, if delivered after 34 weeks, is also reported to be a common, although not a major feature in association with Potter's syndrome.[95,97] The facial abnormalities (Potter facies) include large, low-set, floppy ears, a small chin, a flattened nose, hypertelorism, epicanthic folds.[98] Limb defects such as spade-like hands, talipes equinovares and flexion contractures at the level of the elbows, knees and feet are described.[81,82,85,95]

There is no evidence that pulmonary hypoplasia is genetically or teratologically related to the various urinary tract anomalies.[72,90,99] One hypothesis for the development of Potter's syndrome is that it is the consequence of multiple early mesodermal defects[96], although the widely accepted view is that the structural abnormalities of the face, lungs and limbs are secondary to oligohydramnios.[90,91] Also other non-renal causes of prolonged oligohydramnios such as chronic amniotic fluid leakage may lead to the described non-renal features of the Potter's syndrome.[90-92] Therefore, these secondary features are also known as the oligohydramnios sequence or oligohydramnios tetrad.[91,92,100]

It has been reported that oligohydramnios due to prolonged premature rupture of membranes (PROM) may be associated with pulmonary hypoplasia[81-85,92-94,101] even in the absence of the full complex of malformations connected with the oligohydramnios tetrad.[82,84,102] PROM, mostly defined as rupture of the fetal membranes prior to the onset of labor, is a common obstetric problem which occurs in approximately 10% of all pregnancies with worldwide reports varying between 5% and 45%.[103,104] Ninety percent of the patients who present with this problem have a latent phase of less than one week.[105] The other 10%, who are largely preterm patients, present a dilemma in management.[81,104,106,107] Prolonged PROM is associated with increased chance of infection in both the mother and the fetus, perinatal death as a result of prematurity, and fetal distress during labor because of cord compression.[84,106,-108] The risks of early delivery and hyaline membrane disease must be weighed against those of expectant management like pulmonary hypoplasia, sepsis and fetal deformities. The incidence of neonatal infection following preterm membrane rupture has been reported to be between 0.5% and 25%.[104] It was demonstrated by McIntosh *et al*[85] that death from pulmonary hypoplasia is a much more serious problem following preterm prolonged membrane rupture than is infection. This was confirmed by Tibboel *et al*[93] who emphasized that in case of prolonged PROM, prenatal care should not merely focus on the occurrence and prevention of infections, but rather on the occurrence of oligohydramnios because of the risk of pulmonary hypoplasia increasing with its duration.

Even a period of oligohydramnios as short as 6 days may interfere with fetal lung development and cause pulmonary hypoplasia.[82,109] The reported incidence of pulmonary hypoplasia due to oligohydramnios based on chronic amniotic leakage varies between 8-26% of which 55-100% was lethal.[81-85] The overall mortality rate after prolonged preterm PROM in these studies ranges between 17-41% with a mortality rate due to pulmonary hypoplasia between 29-58%.

2.6 Postnatal diagnosis of pulmonary hypoplasia

2.6.1 *Clinical and radiological criteria*

In most cases, pulmonary hypoplasia is lethal. However, sub-lethal forms of lesser severity (usually proportional to the severity of the underlying etiologic condition) exist and they present a spectrum of clinical presentation, course, prognosis and eventual outcome. Clinical manifestations of pulmonary hypoplasia in neonates range from severe respiratory failure leading to neonatal death (lethal form) to respiratory insufficiency associated with pulmonary hypertension with persistent fetal circulation, pulmonary hemorrhage, bronchopulmonary dysplasia, or sub-acute lung disease, to mild or transient respiratory disease (non-lethal form).[15,81,84,94] Presently, the clinical diagnosis of pulmonary hypoplasia is difficult to make as no tissue is available for histologic examination. Although there is no extensive prospective study of neonatal findings suggestive of pulmonary hypoplasia, there are several case series describing clinical and/or radiological findings.[81,82,84,87,92,94,110-112] The clinical features of infants with pulmonary hypoplasia are characterized by an immediate onset of severe respiratory insufficiency after birth, with small lung capacity and requiring high ventilatory pressures in the absence of obstruction or atelectasis.

Other additional neonatal signs secondary to prolonged oligohydramnios are facial (Potter's facies) and skeletal deformities with a reported incidence of 21-28%.[81,82,85,86,113] Their presence correlates most strongly with the degree and duration of oligohydramnios. Thibeault *et al*[82] showed that from a group of 76 preterm infants ≤ 34 weeks gestation associated with premature rupture of membranes (PROM) for longer than 5 days, 21 infants displayed oligohydramnios and deformities at birth. Twenty of these infants showed clinical evidence of pulmonary hypoplasia. The duration of PROM was longest in infants with deformities, and the mortality in these infants was 86% compared to 52% in infants without deformities who required mechanical ventilation. In a series of McIntosh *et al*[85] 15 out of 16 infants with pulmonary hypoplasia displayed compression deformities but 9 infants with compression deformities did not show mal-development of the lungs. Rotschild *et al*[84] confirmed the presence of a strong correlation between skeletal deformities and pulmonary hypoplasia, since all cases of severe skeletal deformities in their study were associated with pulmonary hypoplasia. In their study development of skeletal deformities was associated with severe oligohydramnios and duration of PROM, but not with gestational age at PROM. Nevertheless, pulmonary hypoplasia can also occur in isolation with little or no compression.[82,84]

The radiological criteria for pulmonary hypoplasia include small lung fields with diaphragmatic domes elevated up to the seventh rib, downward sloping ribs, a bell-shaped thorax, pneumothorax or pneumomediastinum. In a study of Leonidas *et al*[110] pneumomediastinum/pneumothorax was

diagnosed in 82% of 17 infants dying from oligohydramnios related pulmonary hypoplasia and a bell-shaped chest contour in 59%. Both features were present in 53%. The bell-shaped thorax is valuable in predicting the most severe degrees of lung growth impairment and was present in all cases in which oligohydramnios was caused by bilateral renal agenesis. However, either pneumomediastinum/pneumothorax or the bell-shaped chest, when present alone, were of low diagnostic value (6.4 and 4.5%, respectively), while the chance of oligohydramnios related pulmonary hypoplasia is much higher when both X-ray manifestations were present (around 30%).

2.6.2 *Pathologic criteria*

Several pathological criteria of pulmonary hypoplasia are well established, although presently there is still uncertainty as to which method is most reliable.[79]

Lung weight or lung weight to body weight ratio:

The direct measurement of lung weight and comparison of that weight with expected values is the simplest method to determine pulmonary hypoplasia.[89] The lung weight to body weight ratio (LW/BW ratio) is the most consistently used method of diagnosing pulmonary hypoplasia.[6,15,47,78,114] The normal LW/BW weight ratio at term is 0.018-0.022.[70,78] Wigglesworth *et al*[114] proposed to use 0.012 as the lower limit of normality ≥ 28 weeks of gestation and 0.015 before 28 weeks' gestation. When either lung weight or LW/BW ratio is used to diagnose pulmonary hypoplasia, it is important to realize that lung weight is altered by conditions causing pulmonary congestion or edema. Postnatal absorption of fluid also effects lung weight. Askenazi and Perlman[78] suggested the combination of the LW/BW ratio with the radial alveolar count (see following paragraph) to address these confounding variables. They proposed the following guidelines for the diagnosis of pulmonary hypoplasia. Likely pulmonary hypoplasia: LW/BW ratio < 0.009, RAC (=radial alveolar count) not obligatory; Probable: pulmonary hypoplasia: LW/BW ratio between 0.010 and 0.012, RAC indicated for confirmation of diagnosis; Possible pulmonary hypoplasia: LW/BW ratio between 0.013 and 0.017, RAC required; Unlikely pulmonary hypoplasia: LW/BW ratio > 0.018, RAC probably not indicated.

Morphometric radial alveolar count:

The radial alveolar count (RAC) offers a simple objective assessment of alveolar development, as originally proposed by Emery and Mithal.[115] They estimated the number of alveoli cut by a line which was dropped at right angles to the epithelium from the center of terminal respiratory bronchioles to the nearest connective tissue septum. At least ten counts were made for each fetal lung, mainly from 2-3 histologic sections. The

mean value for RAC is taken as a measure of lung maturation. They found an increase of the mean RAC in relation to gestational age, which has also been confirmed by other studies.[116,117] Askenazi and Perlman[78] used a modification of the method of Emery and Mithal, meaning that they counted the alveolar septi instead of alveoli (RAC=alveolar septal count-1). In this study only two fetal age groups, less or more than 36 weeks of gestation, were evaluated and no differences between the groups were found. No detailed information on a fetal age-dependent increase in the RAC-values was obtained. The mean RAC for controls was 5.3 (SD 0.6) in this study and a RAC-value < 75% of the mean normal value as defined by each individual laboratory was recommended as diagnostic for pulmonary hypoplasia.

DNA estimation performed at autopsy:
Although the amount of lung DNA increases with gestation, it decreases in proportion to total body weight. Lung hypoplasia defined in terms of LW:BW ratio was associated with low lung DNA content for gestation, even when corrected for body weight.[114] Studies have shown that the lung DNA of infants with pulmonary hypoplasia delivered at 34-40 weeks' gestation is equivalent to the lung DNA of normal fetuses at 20-22 weeks. A cutt-off of less than 100 mg DNA per kilogram of body weight has been proposed to diagnose pulmonary hypoplasia.[114]

2.7 Prenatal prediction of pulmonary hypoplasia

An accurate prenatal test for detecting pulmonary hypoplasia is highly desirable from the obstetrician's point of view, because of the high perinatal mortality rate. Since the prediction of the non-lethal forms of pulmonary hypoplasia will not drastically change obstetric management, the reliable prenatal prediction of the lethal form might alter obstetric management. Therefore we need an accurate and patient-friendly method of separating lethal from non-lethal pulmonary hypoplasia. The following parameters/methods have been proposed.

2.7.1 *Premature rupture of membranes and persistent oligo-hydramnios*

The relationship between premature rupture of membranes (PROM) and pulmonary hypoplasia has been the subject of study of several authors.[81-84,86,94,106,118.119] Nimrod *et al*[81] studied 100 singleton pregnancies in which PROM occurred more than 1 week prior to delivery. They revealed that the greatest impact of prolonged ruptured membranes on fetal development was seen in patients in whom membrane rupture occurred prior to 26 weeks' gestation and the duration of the rupture was more than 5 weeks. Rotschild *et al*[84] reviewed 88 neonates who were born after prolonged

PROM of ≥ 7 days with the onset of the rupture before 29 weeks' gestation and in 14 of them (16%) pulmonary hypoplasia was diagnosed. They revealed that gestational age at the time of rupture of the membranes was the best predictor of pulmonary hypoplasia, with no cases of pulmonary hypoplasia when PROM occurred after 26 weeks, whereas the duration and degree of oligohydramnios showed no correlation with pulmonary hypoplasia. These authors created a relating probability of pulmonary hypoplasia to gestational age at PROM derived from estimated logistic regression. According to this curve the probability of pulmonary hypoplasia in a patient with PROM at 19 weeks is 50%. Vergani *et al*[118] calculated this estimated probability of 50% to develop pulmonary hypoplasia when the rupture of membranes occurs at 25 weeks of gestation. This difference may be due to the fact that the latter used a subgroup of patients who had amniotic fluid pockets of less then 2 cm during the latency period. In the study of Vergani[118] with a prevalence of pulmonary hypoplasia of 28% in a group of women (n=54) with PROM < 29 weeks and delivery after 25 weeks, gestational age at premature rupture of membranes and oligohydramnios were independent predictors of the occurrence of pulmonary hypoplasia. This is in agreement with the data from Kilbride *et al*[86] who studied 108 patients with PROM < 29 weeks. Of the 40 infants who died in this group, 23 had lung hypoplasia. The authors concluded that severe oligohydramnios > 14 days after premature rupture of membranes at < 25 weeks' gestation has a predicted neonatal mortality of > 90%. Of these infants 87% died of pulmonary hypoplasia. In a study of Van Dongen *et al*[83], however, neither the duration of PROM nor the gestational age at the time of rupture showed any impact on the occurrence of lung hypoplasia.

Persistent severe oligohydramnios appears to be a poor prognostic sign which is associated with a poor fetal outcome.[86,118,120-122] Whereas some authors revealed that persistent severe oligohydramnios is an independent significant predictor of lung hypoplasia[86,118], others imply that this is co-dependent of gestational age at the time of PROM, and does not predict pulmonary hypoplasia when evaluated alone.[6,84,120,121,123]

2.7.2 *Absence of fetal breathing movements*

The relationship of fetal breathing movements and pulmonary hypoplasia has been vigorously disputed. However, data on the predictive value of the presence or absence of fetal breathing movements are still contradictory. Both Blott and Greenough[124-126] showed a sensitivity and predictive value of the absence of fetal breathing for pulmonary hypoplasia up to 100%. Although in a study of Roberts and Mitchell[119] breathing movements were seen in fetuses with pulmonary hypoplasia, they found a significant reduction in this group compared to those that did not develop pulmonary hypoplasia and controls. In contrast, others have demonstrated that fetuses with oligohydramnios and pulmonary

hypoplasia spend significantly more time breathing when compared to fetuses with similar conditions and normal development.[127,128] Nevertheless, there are also reports that failed to determine any correlation between the absence of fetal breathing movements and pulmonary hypoplasia.[86,129-131] These conflicting findings are probably the result of different definitions of fetal breathing, different selection criteria resulting in different sample populations and different diagnostic criteria for pulmonary hypoplasia. The many factors which affect fetal breathing are also not always controlled.[6,132] Despite these differences most work supports the suggestion that the presence or absence of fetal breathing movements does not prevent or confirm pulmonary hypoplasia, especially in isolated examinations. Nevertheless, the persistent absence over several weeks is probably a poor prognostic sign.[6,129]

A study of Fisk *et al*[133] demonstrated that restitution of amniotic fluid volume in human pregnancies complicated by severe oligohydramnios does not acutely alter the incidence of fetal breathing movements, suggesting that impairment of fetal breathing is not the mechanism for oligohydramnios-related pulmonary hypoplasia.

2.7.3 *Biometric indices*

Since the fetal thorax normally grows at a regular rate from 16-40 weeks, a linear correlation exists between thoracic size and gestational age. Ratios between thoracic size and other biometric indices (biparietal, head circumference diameter, abdominal circumference and femur length) remain constant with a high correlation coefficient in normal pregnancies.[123,134-137] Measurements of the thoracic circumference are obtained in an axial section through the fetal thorax at the level of the four-chamber view of the heart, with the atrioventricular valves in diastole.[6] The reported measurements for thoracic circumference vary among different series, probably because of differences in the plane of section and the amount of tissue included in the measurement. For example, some series include the skin and subcutaneous tissues of the thorax in the measurements of thoracic circumference, while others measure the thoracic circumference at the outer, mid or inner perimeter of the rib cage.[137]

Several investigators have attempted to predict lethal pulmonary hypoplasia by using fetal two-dimensional biometric parameters like thoracic circumference and lung length measurements, interthoracic relationship nomograms (thoracic circumference vs biparietal diameter, head circumference, abdominal circumference or femur length), thoracic circumference to abdominal circumference ratio, thoracic area (TA) minus heart area (HA), HA/TA ratio and (TA-HA)x100/TA.[120,123,129,134,138-146] Although the first reports[123,134,138-140] on the application of fetal thoracic circumference measurements in the prediction of lung hypoplasia were quit promising, other and some more recent studies revealed that this measurement has a lower sensitivity and accuracy compared with other

methods in the prenatal prediction of lung hypoplasia.[119,141-143] Vintzileos et al[141] compared six different ultrasonographic methods for predicting lethal fetal pulmonary hypoplasia and found that the (TA-HA)x100/TA parameter had the best diagnostic accuracy. Roberts and Mitchell[129] demonstrated that lung length measurements was superior to fetal chest circumference measurements in the identification of pulmonary hypoplasia. Yoshimura et al[142] showed in 1996 that the application of lung area and the thoracic circumference/abdominal circumference ratio were the best clinical parameters for this purpose and that there were significant linear relationships between lung weight and lung area and between the lung weight/body weight ratio and the thoracic circumference/abdominal circumference ratio. Lately, Merz *et al*[143] displayed in 1999 that in comparison with other measured parameters, the lung diameter appears to be the only reliable parameter for detecting pulmonary hypoplasia.

It should be emphasized that most studies represent very heterogeneous and overall relatively small patient cohorts and use different definitions of thoracic circumference dimensions and lung hypoplasia. This makes it difficult to compare and consequently to select the best prenatal predicting test. The positive predictive prenatal value of any of these reported tests should be 100% accurate in order to alter the obstetric management as Vintzileos[146] stated in 1996. Therefore, biometry alone cannot achieve such a high degree of prediction, which is probably determined by the diversity of etiologies of lung hypoplasia.
Harstad *et al*[147] compared the ultrasonographic circumference and area measurements and their ratios of the fetal chest and heart with magnetic resonance imaging (MRI) measurements performed in a fashion similar to sonography. They concluded that MRI appears not to offer any advantages over sonographic evaluation in the diagnosis of fetal pulmonary hypoplasia.

2.7.4 *Perinasal flow*

Fetal upper respiratory tract function was studied by Fox *et al*[148] using color flow Doppler in five cases of antenatally diagnosed congenital diaphragmatic hernia and in 16 cases of uncomplicated pregnancies at gestational ages ranging from 27-38 weeks. Fetal breathing related nasal and oropharyngeal fluid flow was seen at the level of the nose in all cases with uncomplicated pregnancies. Fetal breathing movements were present in all five cases of congenital diaphragmatic hernia, so its presence or absence did not help to distinguish between those infants that died and those that survived. This was consistent with previous work from this group.[128] However, four of the fetuses who survived, demonstrated perinasal fluid flow, whereas the fetus who died of pulmonary hypoplasia did not. These preliminary observations need to be further investigated by a larger case series to point out whether this technique can be a marker for prenatal prediction of pulmonary hypoplasia.

2.7.5 *Three-dimensional lung volume measurements*

Adequate development and available volume are major contributors to total functional lung tissue.[4,149] Recent reports have demonstrated the possibility of fetal lung volume assessment using echo-planar magnetic resonance (MRI)[150], or three-dimensional ultrasonography[149,151-153] and concluded that these techniques would have useful applications in the prenatal prediction of pulmonary hypoplasia. Fetal lung volume demonstrates a close association with the gestational age[149-153] and fetal weight[152]. As expected right lung volume measured consistently greater than left lung volume.[149,153] Examinations of the fetus in-utero by MRI are limited because of relatively long acquisition times, artifacts caused by fetal movements, high costs, and limited acceptance of pregnant women, whereas three-dimensional ultrasonography offers a fast and easy means with a high patient acceptability for both surface rendering and determination of fetal organ volume.[151-156]

In the studies of Lee *et al*[151] and Laudy *et al*[152] total fetal volume was calculated by subtracting fetal heart volume from thoracic volume using the three perpendicular transverse, sagittal and frontal planes of the fetus, whereas D'Arcy *et al*[149] described a procedure based on direct contouring of both fetal lungs. Pöhls and Rempen[153] introduced another, third method. They performed volumetric evaluation of each separate lung in each of the three perpendicular planes and point at the reduced imaging conditions which may occur in the frontal and sagittal planes of the fetal lung during the second and third trimester of pregnancy. Underestimation of fetal lung volume in fetuses beyond 34 weeks of gestation occurred since complete volumes could not be obtained due to a too small swiveling angle of the probe. It was suggested that wider scanning angles and faster scanning procedures are expected to improve the volumetric measurements. The volume data of Pöhls and Rempen[153] were significantly small compared to data derived by the method of Lee and co-workers[151]. These differences may be due to inclusion of other mediastinal organs such as the thymus, vessels, trachea and esophagus within the lung volume measurements performed by Lee *et al*.[151] Measurement variation may also be expected from cardiac volume measurements since they involve the beating fetal heart with varying volumes depending on the phase of the cardiac cycle at the time of the measurement.

General limiting factors of three-dimensional ultrasound result from non-favorable scanning conditions, such as oligohydramnios, severe obesity and the absence of tissue borderlines on the one hand and moving artifacts like body movements, breathing motion and heart action, on the other hand.[153,154]

The applicability of three-dimensional ultrasonography in the prediction of lung hypoplasia still needs to be determined.

2.7.6 *Doppler velocimetry*

Doppler ultrasound is a technique for measuring the velocity of red blood cells or other moving fluid that reflects echoes from the area of isonation of the ultrasonic bream. The difference (f_D) between the frequency of the ultrasound beam (f_0) and the received echo is determined by the Doppler equation: $f_D = (2f_0 \cos\alpha)/c.$, where α is the solid three-dimensional angle between the ultrasonic beam and the direction of flow and c is the speed of ultrasound in the medium.[157,158] Flow velocities can be estimated from several Doppler shift frequency components of the Doppler power spectrum. The maximum waveform is the most convenient waveform to use and has been used to determine indices for downstream impedance.[159] These Doppler indices as the pulsatility index (PI=(PSV-EDV)/TAV)[160], resistance index (RI=(PSV-EDV)/PSV)[161] or systolic/diastolic ratio (S/D)[162], are angle-independent and can determine the pulsatility of the Doppler waveform; *(PSV, peak systolic velocity; EDV, end-diastolic velocity; TAV, maximum time-averaged velocity)*. These ratios 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.[158]

Combined two-dimensional real-time and Doppler ultrasonography offers a unique non-invasive technology for investigation of the circulatory system. Since its introduction, a host of information has appeared on the early and late human fetal and placental blood flow under normal[163-168] and abnormal circumstances.[169-172] It has been demonstrated that the normally developing fetus during the second half of gestation is characterized by a low feto-placental vascular resistance with well-defined and reproducible arterial, cardiac and venous flow velocity waveforms.[165,173] The addition of color Doppler has made it possible to determine flow directions at cardiac and extra-cardiac levels.[174-176]

In 1990, Van Eyck *et al*[177] reported on Doppler evaluation of the fetal ductus arteriosus in the prediction of pulmonary hypoplasia. Ductal blood flow in the fetal lamb is modulated by lung expansion[178], as a result of opening of the pulmonary vascular bed with subsequent reduced shunting of right ventricular output through the ductus arteriosus. In the study of Van Eyck[177], breathing related modulation of blood flow was calculated by subtracting the peak systolic velocity during expiration from the peak systolic velocity during inspiration in the ductus arteriosus. Normal ductal blood flow velocity modulation values were associated with normal neonatal lung performance reflecting a decreased pulmonary vascular resistance during lung expansion. Reduced ductal blood flow velocity modulation values were associated with pulmonary hypoplasia which was hypothesized to result from impaired development of the pulmonary vascular bed.

Reed *et al*[179] used two-dimensional and pulsed Doppler ultrasound techniques to examine the pulmonary artery and aorta. They revealed

that the human fetal pulmonary artery is usually larger than the aortic outflow region (ratio 1.2:1), while maximal Doppler flow velocities are usually higher in the aortic outflow region (<31 weeks ratio 1.1:1, ≥31 weeks ratio 1.2:1). Maximal and mean pulmonary artery and mean aortic outflow Doppler flow velocities did not change with advancing gestation, whereas maximal aortic outflow Doppler velocity increased with gestation. Overall, volume flow calculated across the pulmonary valve was greater than volume flow across the aortic valve (ratio 1.3:1). This is consistent with earlier work[180] demonstrating a 1.3:1 ratio of transvalvular volume across the tricuspid compared to the mitral valve and confirms the evidence of right-heart dominance in the human fetus. Blood flow through the lungs was investigated in 38 normal fetuses by St John Sutton *et al*[181] and was assessed indirectly as the difference between flow in the pulmonary artery and ductal flow. This flow increased exponentially with gestational age by almost four-fold between 18 and 37 weeks of gestation.

The introduction of more sensitive color-coded Doppler systems has lead to identification of the human fetal pulmonary circulation.[24,182-184] Consequently, Doppler velocimetry has recently been used to study both the arterial[24,185-197] and venous[24,198-202] pulmonary circulation in normal human fetuses. Most Doppler studies of the human fetal pulmonary circulation have been performed in the proximal arterial pulmonary branches and demonstrated a unique Doppler waveform pattern.[185-194] Agreement exists regarding the nature of the systolic component of the proximal arterial pulmonary waveform, which is characterized by a rapid initial flow acceleration phase followed by an equally rapid deceleration phase. However, opinions differ towards the end-diastolic component, ranging from forward flow being absent[24], present[187-189,193] or a combination of present and absent flow[186]. Further, discrepancies appear to exist regarding reported peak systolic velocities in the proximal arterial pulmonary branches, which vary from a significant increase[24,186,188,192,193] to gestational age independency[185,187]. Finally, in the same vessel the pulsatility index as well as the resistance index[190,191] has been described as remaining constant[24,187,190,191,193,194] or demonstrating a gestational age-related decrease[186,188,191,192]. Probably sample site and equipment-related differences (high-pass filter setting) play a role in the occurrence of these discrepancies. This emphasizes the need for standardizing recording techniques. The physical basis for the shape of the human fetal pulmonary arterial flow velocity waveform remains speculative, and is probably determined by several factors such as vascular pressure, resistance, impedance, vessel compliance and ventricular contractility.[24] This profile partly resembles the waveforms recorded in pulmonary arteries in fetal lambs by chronically implanted cuff-type electromagnetic flow transducers.[36] These profiles show forward flow with a sharp peak early in systole with almost no flow during mid-systole, and reverse flow during late

systole and early diastole; throughout the remainder of diastole there is no significant forward or backward flow. This velocity pattern has been explained on the basis of a high (relative to systemic) pulmonary vascular resistance and the presence of a patent ductus arteriosus.[27,36]

Several studies have reported on Doppler velocimetry of fetal peripheral arterial pulmonary branches in uncomplicated pregnancies.[186,191,195-197] Also here, methodological differences such as defining the Doppler sample site for distal pulmonary arteries have made a comparison difficult. Rasanen *et al*[186] examined fetal distal branch pulmonary arterial vascular impedance and analyzed the relationships between proximal (after the bifurcation of the main pulmonary artery) and distal (beyond the first bifurcation of the branch pulmonary artery) pulmonary arterial blood velocity waveforms. Achiron *et al*[196] defined peripheral pulmonary artery blood flow as flow located 10 to 15 mm beyond the first bifurcation of the pulmonary artery branch. Data in the study by Mitchell *et al*[191] and Laudy *et al*[197] were obtained in the middle portion of the fetal lung and by Rizzo *et al*[195] and Laudy *et al*[197] in the most distal area of the fetal lung. Nevertheless, Rasanen[186] observed a decrease in pulsatility index until 31 weeks of gestation followed by a constant pattern thereafter, whilst Mitchell[191] and Rizzo[195] established a significant reduction in pulsatility index (or resistance index) throughout the second half of gestational age. Achiron *et al*[196] showed that the PI did not change with gestation, although there was a statistically significant increase when comparing the period of 14-17 weeks and the remainder of pregnancy. In the study of Laudy *et al*[197], the pulsatility index from the middle arterial pulmonary branches showed a significant decrease from 20 to 29 weeks followed by a significant increase as from 32 weeks to term and from the distal arterial pulmonary branches a significant decrease from 20-26 weeks and a rise as from 31 weeks until term. None of all these Doppler flow velocity studies could detect significant differences between right and left arterial pulmonary branches.

Increased pulmonary vascular resistance and reduced pulmonary arterial compliance may be the result of underdevelopment and structural changes (decreased total size of the pulmonary vascular bed, reduced vessel count per unit of lung tissue and increased pulmonary vascular muscularization) of the pulmonary vascular bed as seen in post-mortem findings of pulmonary hypoplasia.[73,74] These alterations could effect pulmonary blood flow and consequently induce changes in pulmonary flow velocity waveforms as recorded by Doppler ultrasonography. From this point of view, it has been hypothesized that fetal arterial pulmonary Doppler velocimetry may play a role in the prediction of pulmonary hypoplasia.

So far, only a few papers including two abstracts have dealt with this particular issue.[191-194,196,203,204] Laudy and co-workers[203] observed a significant reduction in pulmonary artery systolic and diastolic velocities in a case of fetal pulmonary hypoplasia due to bilateral obstructive uropathy.

Further, there were marked changes in the shape of the pulmonary arterial waveform, characterized by a second needle-shaped peak occurring during mid-systole, even higher than the first systolic peak. The presence of reduced volume flow and/or raised downstream impedance may have been responsible for these Doppler velocity changes.[203] Mitchell and colleagues[191] studied a group of 10 fetuses with multicystic dysplastic kidney disease and pulmonary hypoplasia. This group did not have significantly different Doppler waveforms in the main pulmonary artery and in the proximal pulmonary arteries compared to the control group. However, Doppler recordings from the peripheral pulmonary arteries demonstrated a high-resistance pattern quite different from that of normal fetuses. Achiron *et al*[196] demonstrated that PI measurements from the right peripheral pulmonary artery in 4 fetuses with proven lung hypoplasia were within the 95% confidence limits of those for normal fetuses (n=96). Based on these preliminary data, they suggest that the PI of the lung circulation is a poor indicator of lung hypoplasia. Two abstracts appeared on the value of arterial pulmonary Doppler flow cases of lung hypoplasia associated with diaphragmatic hernia.[193,194] The first one[193] reported reversed diastolic flow in the right pulmonary artery from three fetuses with diaphragmatic hernia, who all died after birth from lung hypoplasia. In the second one[194] five fetuses were examined with congenital diaphragmatic hernia, although the exact sampling size was not given. A normal or reduced pulsatility index was shown in two of these fetuses who died from hypoplasia, whereas increased PI was found in the other three fetuses who had surgery and a good outcome. More recently, two other reports questioned the usefulness of Doppler velocimetry of the proximal arterial pulmonary circulation in the prediction of pulmonary hypoplasia.[192,204] Yoshimura *et al*[192] investigated five fetuses at risk for pulmonary hypoplasia (2 hydrops fetalis, 2 thanatophoric dwarfism and 1 bilateral agenesis). They revealed that pulsatility index values were high and the peak systolic flow was significantly lower than in the healthy fetuses. In the group of Chaoui *et al*[204] nine cases were examined with autopsy proven lung hypoplasia after termination of pregnancy (19-23 weeks, 4 bilateral renal malformations, 2 diaphragmatic hernia, 3 miscellaneous malformations including heart defects). Normal values were found in the end-systolic reversal flow in all cases, decreased peak systolic velocity in 3 of 9 cases and increased pulsatility index in 6 of 9 cases. The latter six cases were all fetuses with renal malformations and diaphragmatic hernia and it was suggested that increased PI is the best parameter to detect flow abnormalities in this condition.

It should be pointed out that all these reports present data from very small heterogeneous patient series and do not represent cases of premature rupture of membranes, which is considered one of the most common causes of pulmonary hypoplasia.[191-194,196,203,204] Nevertheless, they suggest that Doppler velocity waveform changes - although different in each of the

papers - may occur in well-established cases of pulmonary hypoplasia. However, whether this implies that Doppler assessment of the fetal arterial pulmonary circulation allows accurate differentiation between lethal and non-lethal pulmonary hypoplasia, needs to be substantiated in prospective larger series. Only then a more realistic view of the clinical significance of Doppler velocimetry in the detection of lethal lung hypoplasia can be obtained.[205]

2.8 References

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CHAPTER 3

DOPPLER ASSESSMENT OF THE ARTERIAL PULMONARY CIRCULATION

3.1 Introductory remarks

Until recently, the arterial pulmonary circulation had provoked only limited interest. This has changed through the introduction of more sensitive color coded Doppler techniques. Arterial pulmonary vasculature can now be visualized as from 20 weeks gestation with identification of the proximal arterial branches as early as 14 weeks of gestation.

To answer the question whether Doppler velocimetry of the arterial pulmonary circulation contributes to the prenatal detection of fetal lung hypoplasia, information is needed on methodology, reproducibility and normal data. This is presented in the next two research papers.

3.2 Doppler velocimetry in branch pulmonary arteries of normal human fetuses during the second half of gestation

J.A.M. Laudy[1], M.A.J. de Ridder[2], J.W. Wladimiroff[1]
Department of Obstetrics and Gynaecology[1], University Hospital Rotterdam, Institute of Epidemiology and Biostatistics[2], Erasmus University Medical School, Rotterdam, The Netherlands

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ABSTRACT
The objective of the present study was to determine the characteristics of Doppler flow velocity waveforms in branch pulmonary arteries in relation to gestational age. A total of 111 singleton normal pregnancies were studied during the second half of pregnancy using a combined color-coded Doppler and two-dimensional real-time ultrasound system. Pulsed Doppler measurements of the most proximal branch of the right or left pulmonary artery were attempted during fetal apnea from a transverse cross-section of the fetal chest at the level of the cardiac four-chamber view after visualization with color Doppler. The success rate in obtaining the pulmonary arterial waveform was 85%. The waveform displayed a rapid systolic velocity acceleration, followed by an initially rapid but then more gradual velocity deceleration which was interrupted in most cases by a short reversed flow interval at the beginning of the diastolic phase of the cardiac cycle. The diastolic phase was characterized by forward flow. Peak systolic, end-diastolic and time-averaged velocity, pulsatility index and systolic integral remained constant during gestation. Changes in vessel diameter or compliance may play a role in this. A gestational age-dependent rise was established for peak diastolic velocity, diastolic integral and early peak diastolic reverse flow, whereas a gestational age-determined

decline was found for peak systolic/peak diastolic ratio. Fetal heart rate demonstrated a statistically significant increase relative to gestational age. However, the observed relation between the flow velocity waveform parameters, pulsatility index calculations and gestational age was independent of fetal heart rate. It is speculated that peak diastolic velocity, diastolic integral and peak systolic/peak diastolic ratio rather than the pulsatility index are useful in detecting gestational age related changes in human fetal pulmonary vascular resistance.

INTRODUCTION

Studies on fetal lambs have demonstrated that pulmonary flow increases and pulmonary vascular resistance decreases with advancing gestational age.[1] However, when corrected for wet weight of the lungs, pulmonary blood flow does not change and total pulmonary resistance even increases with advancing gestation.[2]

The use of combined two-dimensional real-time and color coded Doppler systems has provided a host of information on the early and late human fetal systemic circulation.[3,4] It has been demonstrated that the normally developing fetus during the second half of gestation is characterized by a low feto-placental vascular resistance with well defined and reproducible arterial, cardiac and venous flow velocity waveforms.[5]

Lately, data have become available on Doppler velocimetry in the human fetal pulmonary circulation, which is mainly due to the introduction of more sensitive color coded Doppler systems.[6-8] In the present cross-sectional study we addressed the characteristics of branch pulmonary artery flow velocity waveforms in the human fetal lung during the second half of pregnancy. This information may contribute to our understanding of the normal fetal pulmonary circulation and of hemodynamic changes underlying structural pathology such as lung hypoplasia.[9]

PATIENT AND METHODS

Study design

During a period of 24 months, a total of 111 non-smoking women with a normal singleton pregnancy consented to participate in a cross-sectional study design. All women were recruited from the routine antenatal clinic of the Department of Obstetrics and Gynecology of the University Hospital Rotterdam-Dijkzigt. The study protocol was approved by the Hospital Ethics Committee. Gestational age at entering the study ranged between 20 and 40 wk (median 30 wk) and maternal age varied between 18 and 46 y (median: 28 y). Pregnancy duration was determined from the last reliable menstrual period and confirmed or adjusted by ultrasonic measurement of the fetal crown-rump length (8-12 wk) or fetal biparietal diameter (12-20 wk). None of these pregnancies was associated with congenital anomalies including lung pathology. All pregnancies were uneventful with normal fetal biometry and normal amniotic fluid volumes. As expected, 80% of the fetal

birth weights was situated between the 10th and 90th centile according to the Kloosterman Tables[10], adjusted for maternal parity and fetal sex. Women were selected according to 10 gestational age subgroups (20-22, ..., 38-40 wk), to guarantee a homogeneous distribution. Each woman was included in the study only once.

Recording technique

Ultrasound Doppler studies were performed using a Toshiba SSH 140 with combined transabdominal real-time and color Doppler imaging facilities (Toshiba Corp., Medical Systems Division, Tokyo, Japan). The Doppler carrier frequency was 5 MHz. The spatial peak temporal average power output was less than 100 mW/cm^2 in both imaging and Doppler modes according to manufacturers' specifications.

After obtaining a clear sonographic transverse cross-section of the fetal chest at the level of the cardiac four-chamber view, color Doppler was switched on to visualize the pulmonary circulation. Depending on fetal position, the pulsed Doppler sample volume (0.1-0.3 cm) was placed over the most proximal branch of the right or left pulmonary artery. The angle between the Doppler cursor and the assumed blood flow direction was always kept below 20 degrees. The high-pass filter was set at 70 Hz. Recordings were performed with the woman in the semi-recumbent position and during fetal apnea, since the latter may modulate the flow velocity waveform.[11]

All flow velocity waveforms were recorded on hard copies. Waveform analysis was performed by one examiner (J.A.M.L.) using a microcomputer (Olivetti M24; Olivetti BV, Leiden, The Netherlands) linked to a graphics tablet. Branch pulmonary artery flow velocity waveforms (Figure 1) are characterized by a rapid systolic velocity acceleration, followed by an initially rapid but later more gradual velocity deceleration which in most instances is interrupted by a short reverse flow component at the beginning of the diastolic phase of the cardiac cycle.[6,9] Although slight variations in the waveform morphology were observed, these were not essentially different from the waveform demonstrated in Figure 1. In all women forward flow could be observed during the remainder of the diastolic phase of the branch pulmonary artery velocity waveform.[9]

Three consecutive flow velocity waveforms with the highest velocity and of similar appearance were used to calculate the different parameters in each vessel. For each woman the mean value of the following waveform parameters was determined (Figure 1):

(i) peak systolic velocity (cm/s), peak diastolic velocity (cm/s), end-diastolic velocity (cm/s) and time-averaged velocity (TAV, cm/s); (ii) peak systolic/peak diastolic ratio (S/D ratio); (iii) early peak diastolic reverse flow velocity (RV, cm/s); (iv) pulsatility index (PI= (PSV-EDV)/TAV), according to Gosling and King[12]; (v) systolic and diastolic velocity integral (cm).

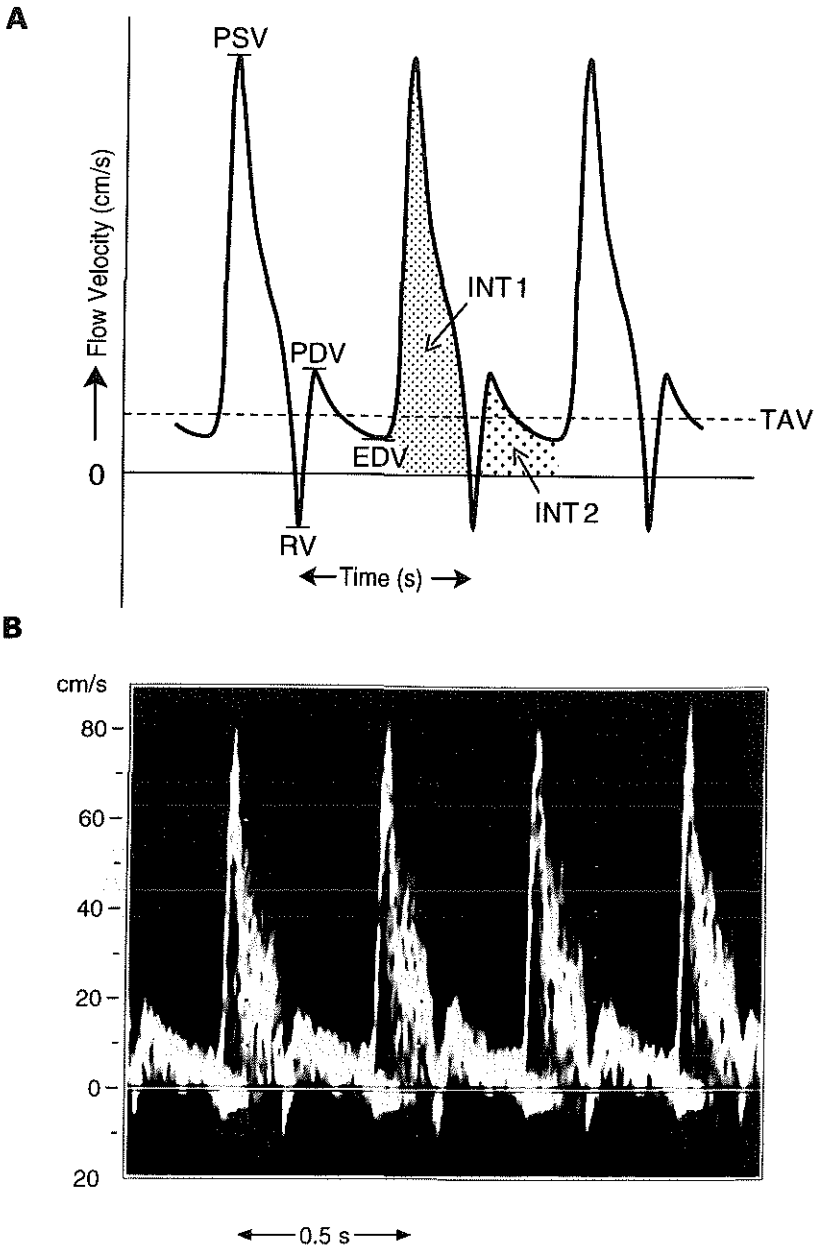

Figure 1
(A) Schematic presentation of three Doppler flow velocity waveforms from the branch pulmonary artery. PSV, peak systolic velocity; RV, early peak diastolic reverse flow velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; INT 1, systolic integral; INT 2, diastolic integral; TAV, time-averaged velocity. (B) Doppler ultrasound recording of four consecutive left branch pulmonary artery velocity waveforms at a gestation of 34 wk.

Statistical analysis

For the construction of reference centiles (P5, P50 and P95) the method described by Royston[13] was used and, if necessary, the standard deviation was modeled as a function of gestational age as described by Altman[14].
We tested for significant regression. If it was significant, the 50th centile (P50) was established by modeling the outcome variable, *y*, by a straight line regression model with gestational age as predictor. Further, it was tested whether adding the quadratic term was significant or whether the distribution of *y* depending on gestational age was skewed. If the regression was not significant, the 50th centile was established by using the mean of the outcome variable *y*. Next, it was checked if the residuals depended on gestational age. If so, straight line regression was used to model the absolute value of the residuals, and from this model a regression equation for the standard deviation, depending on gestational age, was derived. Using this standard deviation, the 5th (P5) and 95th (P95) centile were constructed. Also was tested, whether the relation between the outcome variable *y* and gestational age was influenced by fetal heart rate (FHR) by adding FHR in the regression model.

Left and right branch pulmonary artery flow velocity waveforms were compared using the Student's *t* test. The level of statistical significance was set at $p<0.05$.

RESULTS

Technically acceptable flow velocity waveforms were collected in 94 out of 111 women, resulting in a success rate of 85%. Recording failures were determined by fetal breathing movements, unfavorable fetal position or maternal obesity. There was no relationship between the recording failures and the left or right lung or gestational age, although recording failures seem to occur slightly more often after 35 wk of gestation due to fetal breathing movements. The median gestational age at delivery was 40 wk (36-42 wk) and the median birth weight was 3190 g (1930-4760 g).

Regression slopes defining the relation between branch pulmonary artery velocity parameters and gestational age (5th, 50th and 95th centiles) are presented in Table 1. A statistically significant increase with advancing gestational age was found for peak diastolic velocity ($p<0.0001$; Figure 2) and diastolic integral ($p<0.05$; Figure 3), whereas a statistically significant gestational age-related decrease was demonstrated for the S/D ratio ($p<0.0001$; Figure 4). RV was noted in 91 (97%) out of 94 women. Maximum RV revealed a statistically significant increase with advancing gestational age ($p<0.0001$; Figure 5). Pregnancy duration in the three women with absent RV ranged between 20 and 23 weeks. No statistically significant gestational age-related change existed for the TAV, peak systolic and end-diastolic velocity, PI (Figure 6) and systolic integral.

Table 1
Regression equations for the proximal arterial pulmonary branch Doppler measurements and their ratios with gestational age

Velocity parameters	*Equations of velocity parameters relative to GA*	*r*	*Significance*
TAV (cm/s)	P50: y = 17.38 (mean) P95/P5: y = 17.38 ± 1.645x3.58	0.002	NS
PSV (cm/s)	P50: y = 61.76 (mean) P95/P5: y = 61.76 ± 1.645x11.48	0.09	NS
EDV (cm/s)	P50: y = 6.64 (mean) P95/P5: y = 6.64 ± 1.645x1.63	0.03	NS
PI	P50: y = 3.22 (mean) P95/P5: y = 3.22 ± 1.645x(0.023+0.012xGA)x1.2533	0.16	NS
Systolic integral (cm)	P50: y = 5.49 (mean) P95/P5: y = 5.49 ± 1.645x1.21	0.01	NS
PDV (cm/s)	P50: y = 0.31xGA + 4.75 P95/P5: y = 0.31xGA + 4.75 ± 1.645x3.11	0.48	$p<0.0001$
Diastolic integral (cm)	P50: y = 0.02xGA + 1.41 P95/P5: y = 0.02xGA + 1.41 ± 1.645x0.52	0.21	$p<0.05$
S/D ratio	P50: y = -0.08xGA + 6.98 P95/P5: y = -0.08xGA + 6.98 ± 1.645x0.91	-0.43	$p<0.0001$
RV (cm/s) (>23 wk GA)	P50: y = -0.59xGA + 7.77 P95/P5: y = -0.59xGA + 7.77 ±1.645x(-1.37 + 0.13xGA)x1.2533	-0.62	$p<0.0001$

If regression was significant, P50: y = slope x GA + C, P95/P5: y = slope x GA + C ± 1.645xSEM. If regression was not significant, P50 = mean, P95/P5: mean ± 1.645xSD. GA = gestational age, C = constant.

In our study group FHR demonstrated a statistically significant increase ($p<0.04$) in relation to advancing gestational age. However, the observed relation between the flow velocity waveform parameters, PI calculations and gestational age was independent of FHR.

In nearly all cases left and right branch pulmonary artery flow velocity waveforms were not obtained from the same patient. When separating left (n=37) and right (n=57) branch pulmonary flow velocity waveforms, no statistically significant mean differences were observed for any of the flow velocity parameters studied.

DISCUSSION

The present study describes characteristics of the human fetal branch pulmonary artery flow velocity waveform during the second half of normal pregnancy. The total complement of pulmonary arterial and venous branch vessels, which is formed in the pre-acinar region of the lung is attained by 20 wk of gestation.[15]

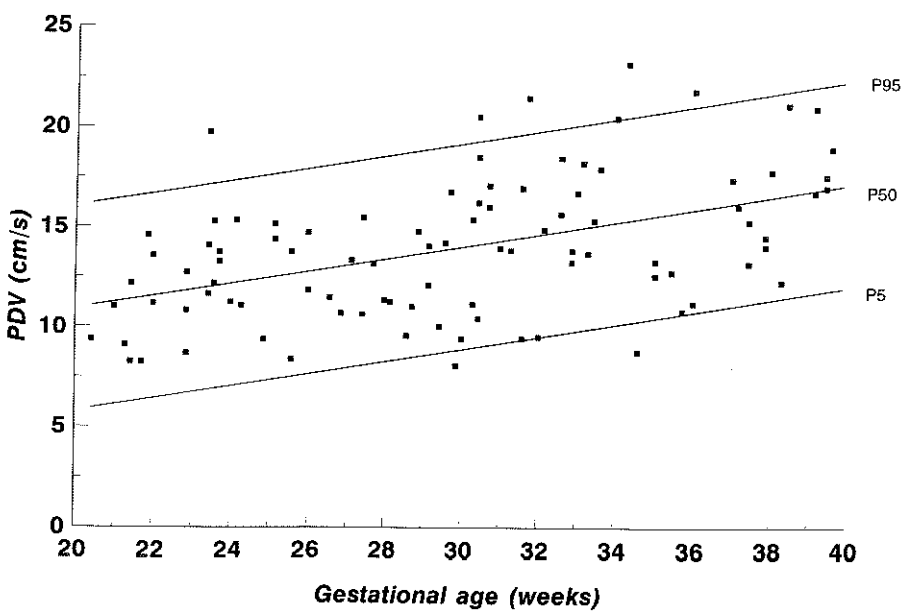

Figure 2
Individual values and reference ranges (mean (P50), 5th (P5) and 95th (P95) centiles) for the peak diastolic velocity (PDV) of the branch pulmonary artery relative to gestational age (wk).

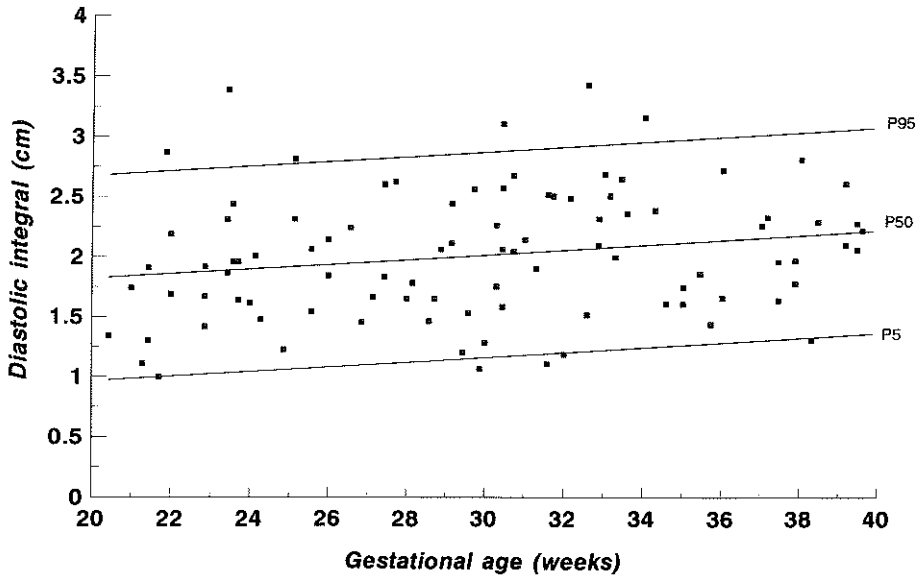

Figure 3
Individual values and reference ranges (mean (P50), 5th (P5) and 95th (P95) centiles) for the diastolic integral of the branch pulmonary artery relative to gestational age (wk).

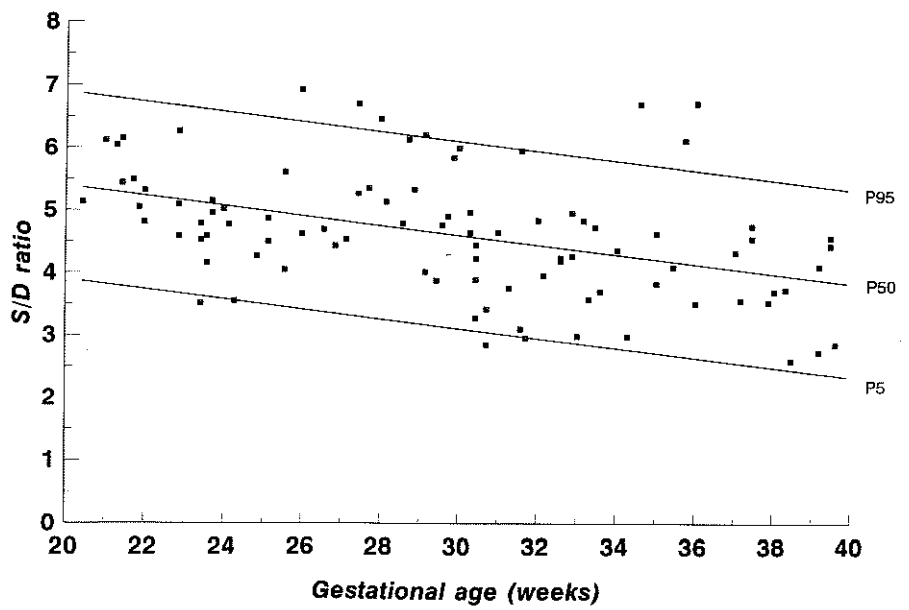

Figure 4
Individual values and reference ranges (mean (P50), 5th (P5) and 95th (P95) centiles) for the S/D ratio of the branch pulmonary artery relative to gestational age (wk).

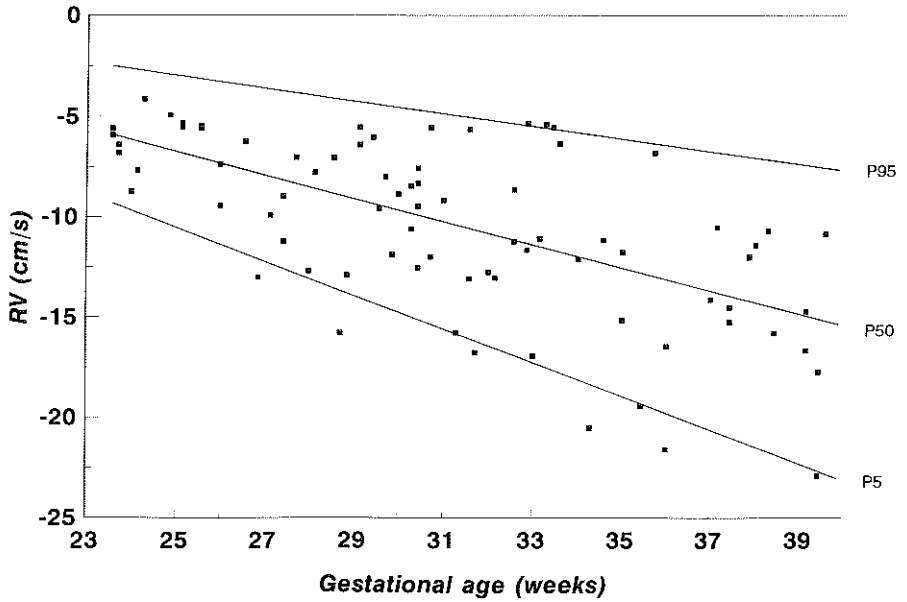

Figure 5
Individual values and reference ranges (mean (P50), 5th (P5) and 95th (P95) centiles) for the RV of the branch pulmonary artery relative to gestational age (wk).

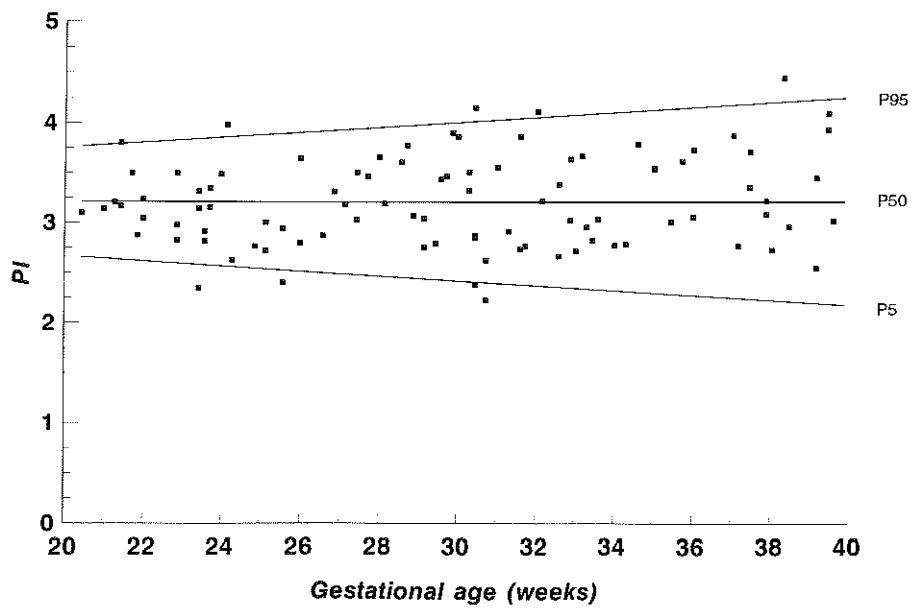

Figure 6
Individual values and reference ranges (mean (P50), 5th (P5) and 95th (P95) centiles) for the PI of the branch pulmonary artery relative to gestational age (wk).

The human fetal pulmonary artery velocity waveform obtained from the most proximal branch of the left or right pulmonary artery displays a unique Doppler waveform pattern characterized by a rapid initial flow acceleration phase and an equally rapid deceleration phase, producing a needle-shaped systolic peak. This is followed by a more gradual decline in flow velocity which in nearly every instance is interrupted by a short reverse flow pattern at the beginning of diastole.[6] Forward flow was observed throughout the remainder of diastole.[9] The latter is at variance with pulmonary artery flow velocity waveform recordings in the fetal lamb, which are characterized by absent end-diastolic flow.[16] This may be due to the invasive recording techniques applied in fetal lamb studies or anatomical differences between lamb and human. However, also in the human fetus different descriptions of end-diastolic flow in the branch pulmonary artery have been given. Although we observed forward flow during end-diastole, absent end-diastolic flow was reported by Emerson and Cartier[6], and present or absent forward end-diastolic flow was established by Rasanen *et al*[8]. One explanation for these somewhat different findings could be the setting of the high-pass filter, which was lower in our recording equipment (70 Hz) than in the study by Rasanen[8] (100 Hz), but not reported in the study by Emerson and Cartier[6].

The physical basis for the shape of the fetal branch pulmonary artery flow velocity waveform remains speculative and is probably determined by several factors, such as vascular pressure, resistance, impedance and ventricular contractility.[6] Most of our knowledge of the fetal pulmonary circulation is derived from fetal lambs.[1,16] Blood flow through the lungs is low during fetal life, which has been explained on the basis of a high pulmonary vascular resistance. Flow velocity profiles have been recorded in pulmonary arteries of fetal lambs by chronically implanted cuff-type electromagnetic flow transducers.[16] These profiles show forward flow with a sharp peak early in systole with almost no flow during mid-systole, and with reverse flow during late systole and early diastole; throughout the remainder of diastole there is no significant forward or backward flow. This velocity pattern, which partly resembles the profile found in our study, has been explained on the basis of a high (relative to systemic) pulmonary vascular resistance and the presence of a patent ductus arteriosus.[16,17] During early systole, blood is ejected at high velocity through the pulmonary arterial circulation. As velocity falls, blood passes preferentially through the ductus arteriosus into the descending aorta. Because pulmonary vascular resistance is high, reverse flow is recorded as blood flow from the branch pulmonary arteries through the ductus arteriosus in association with elastic recoil of these vessels.[17,18] A late systolic to early diastolic reversal of flow, coming from the ductus arteriosus and increasing with gestational age, has previously been demonstrated in the human fetal aortic isthmus.[19] This would suggest that an open ductus arteriosus will also

permit pressure and flow changes in the systemic circulation to affect the pulmonary velocity profile.

The present study revealed no significant change in TAV, peak systolic and end-diastolic velocity with advancing gestational age. Similar findings were done by Stanley *et al*[20] for peak systolic velocity in the right pulmonary artery of normally developing human fetuses. Changes in vessel diameter and/or compliance may play a role in velocity parameters, such as TAV, peak systolic and end-diastolic velocity remaining constant in the present study. The presence of elastic tissue in the proximal half of the pre-acinar arteries may also be responsible for this observation because of the high tensile strength which counteracts the distending tendency of blood pressure and maintains the patency of the pulmonary arterial vessels.[21] Because we did not measure pulmonary vessel area, no information is available on volume flow. In the fetal lamb, the absolute total rise of pulmonary blood flow[1,22], has been attributed to the expansion of the total cross-sectional pulmonary vascular bed with advancing gestational age.[23]

The regulation of fetal pulmonary flow depends on the high resistance of the pulmonary vasculature. A low total cross-sectional pulmonary vascular area appears to be the major factor determining the high vascular resistance.[6,24] During fetal life, the wall thickness of the muscular and partially muscular pulmonary arteries is considerable, and the lumen is small.[21] In the human fetus pulmonary artery, wall thickness is twice that in the adult, which results in a relatively high pulmonary vascular resistance to maintain reduced lung blood flow during fetal life.[21] Fetal lamb studies revealed a decrease of pulmonary vascular resistance with advancing gestational age.[17,18,22] Levin *et al*[23] demonstrated in the fetal lamb that increased pulmonary blood flow and decreased vascular resistance with advancing gestational age are due to a rise in the total number of vessels and vasomotor activity is related to an increase in total amount of smooth muscle while the muscle thickness in individual vessels remains constant.

Of interest is, however, that when corrected for wet weight of the lungs, pulmonary artery flow remains constant and total pulmonary vascular resistance rises.[2] This suggests that the rising pulmonary artery flow does not keep pace with the increase in pulmonary vascularity.[6]

In the present study, the PI, which at fetal trunk and placental level has been used as a measure of downstream impedance, remained constant. This is in contrast to Rasanen *et al*[8], who attributed a drop in branch pulmonary artery PI up to 34-35 weeks of gestation to a reduction in pulmonary vascular resistance. However, recently the same group[25] reported that weight-indexed pulmonary vascular resistance appears to increase after 30 weeks gestation, which is consistent with fetal lamb data. As in our study, Emerson and Cartier[6] failed to identify a fall in downstream impedance in the high resistance pulmonary circuit. They proposed that this was due to insensitivity of the PI to small changes in resistance, rather than the constancy of the resistance in the fetal pulmonary circulation. We were

able to establish significant gestational age-related changes in the diastolic component of the branch pulmonary artery waveform. Both the peak diastolic velocity and diastolic integral displayed a significant increase with advancing gestational age, whereas S/D ratio showed a significant decrease, suggesting reduced pulmonary vascular resistance.

An approximately 2.5 fold increase was established for RV, which probably represents the preferential escape of blood from the branch pulmonary arteries through the ductus arteriosus into the descending aorta.[17,18] The increase in RV, therefore, may be the result of diminishing arterial downstream impedance at lower body and placental level, but also at the level of the upper body and brain[19]. Comparison with other data[8] was possible for the peak systolic velocity and PI; a similar scatter was observed.

It can be concluded that technically acceptable flow velocity waveforms can be obtained from human fetal branch pulmonary arteries during the second half of gestation applying combined color-coded and pulsed Doppler techniques. Changes in vessel diameter and/or compliance may play a role in the constancy of TAV, peak systolic and end-diastolic velocity in branch pulmonary arteries. We speculate that the measurement of peak diastolic velocity, diastolic integral and S/D ratio rather than the PI is useful in detecting gestational age-related changes in human fetal pulmonary vascular resistance. The clinical implications of these findings will be investigated in relation to pathological conditions such as lung hypoplasia.

3.3 Human fetal pulmonary artery velocimetry: repeatability and normal values with emphasis on middle and distal pulmonary vessels

J.A.M. Laudy[1], M.A.J. de Ridder[2], J.W. Wladimiroff[1]
Department of Obstetrics and Gynaecology[1], University Hospital Rotterdam, Institute of Epidemiology and Biostatistics[2], Erasmus University Medical School, Rotterdam, The Netherlands

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ABSTRACT

Objectives: To establish the nature and gestational age dependency of flow velocity waveforms from fetal middle and distal arterial pulmonary branches in the second half of normal pregnancy and to determine repeatability and inter-relationship of flow velocity waveform recordings from proximal, middle and distal arterial pulmonary branches.

Design: cross-sectional study.

Subjects/Methods: A total of 111 singleton normal pregnancies between 20 and 40 weeks of gestation were studied using a color coded Doppler ultrasound system. Pulmonary waveforms were obtained at the level of the fetal cardiac four-chamber view. Repeatability was tested from two recordings at 15 minute time-intervals in 25 separate normal pregnancies.

Results: Acceptable repeatability of flow velocity waveforms from fetal arterial pulmonary branches was established with coefficients of variation below 15%. The nature of middle arterial pulmonary flow velocity waveforms was similar to that of proximal waveforms and showed a gestational age related change for diastolic velocity parameters, peak systolic/peak diastolic ratio and pulsatility index. The distal arterial pulmonary branch displayed a monophasic forward flow velocity profile throughout the cardiac cycle. All velocity parameters of the distal branch remained unchanged with advancing gestation, with the exception of the pulsatility index. Significant inter-pulmonary changes were found for all pulmonary arterial waveform parameters.

Conclusions: Alteration in pulmonary vascular resistance may play a role in gestational age related changes, whereas changes in vessel branching / diameter and in the distance between the heart and more distal arterial pulmonary vessels may cause inter-pulmonary differences.

INTRODUCTION

Pulmonary hypoplasia is associated with underdevelopment and structural changes of the pulmonary vascular bed.[26,27] This may affect pulmonary blood flow and consequently induce changes in pulmonary flow velocity waveforms as recorded by Doppler ultrasonography.[9]

Color-coded Doppler studies of the human fetal arterial pulmonary circulation have so far focused primarily on the proximal arterial branches.[6,8,28,29] Information on more distal pulmonary flow velocity waveforms may further our understanding of pulmonary hemodynamics in the human fetus. The objectives of the present study were threefold:

(i) to determine the repeatability of flow velocity waveform recordings from proximal, middle and distal arterial pulmonary branches;

(ii) to establish the characteristics and gestational age dependency of middle and distal arterial pulmonary branches;

(iii) to determine the relationship between proximal, middle and distal arterial pulmonary branches.

PATIENT AND METHODS

Study design and recording techniques

One hundred and eleven non-smoking women with a normal singleton pregnancy at 20-40 weeks of gestation (median 30 weeks) were recruited into this cross-sectional study. This cohort of women has provided data which has been previously published elsewhere.[29] All women gave informed consent and the study protocol was approved by the Hospital Ethics Committee.

Ultrasound Doppler studies were performed using a Toshiba SSH 140 with combined transabdominal real-time, color and pulsed Doppler imaging facilities (Toshiba Corp., Medical Systems Division, Tokyo, Japan). The carrier frequency was 5 MHz (real-time) or 3.75 MHz (Doppler). The spatial peak temporal average power output was less than 100 mW/cm^2 in both imaging and Doppler modes according to the manufacturer's specifications. After obtaining a clear cross-section of the fetal chest at the level of the cardiac four chamber view, the color Doppler was switched on to visualize the pulmonary circulation. Depending on fetal position, the right or left lung was examined. Firstly, the pulsed Doppler sample volume (0.1-0.3 cm) was placed over the most proximal branch of the pulmonary artery.[29] The sample volume was then moved to a point in the middle of the fetal lung, equidistant from the outer border of the fetal heart and the inner thoracic wall. Arterial pulmonary blood flow velocity waveforms were collected at this point and then from the most distal branch of the pulmonary artery as close as possible to the fetal inner thoracic wall (Figure 1, page 198) The angle between the Doppler cursor and the assumed blood flow direction was always kept below 20°. The high pass filter was set at 100 Hz. When absent early diastolic reverse flow or absent end-diastolic flow were present, it was reduced to 70 Hz.

Recordings were performed with the woman in the semi-recumbent position and during fetal apnea, since the latter may modulate the flow velocity waveform.[11] All flow velocity waveforms were recorded on hard copy. Waveform analysis was performed by one examiner (J.A.M.L.) using a microcomputer (Commodore 386 CX) linked to a graphics tablet. At least three consecutive flow velocity waveforms with the highest velocity and of similar appearance were used to calculate the different parameters in each vessel.

In each fetus the following maximum waveform parameters from proximal, middle and distal branches of the pulmonary artery were determined from the same lung i) time-averaged maximum velocity (TAV, cm/s), peak systolic velocity (cm/s), peak diastolic velocity (cm/s), end-diastolic velocity (cm/s) and early peak diastolic reverse flow velocity (RV, cm/s); ii) peak systolic/peak diastolic ratio (S/D ratio); iii) pulsatility index (PI= (peak systolic velocity - end-diastolic velocity)/TAV), according to Gosling and King[12]; iv) systolic and diastolic velocity integral (cm). Fetal heart rate (FHR, beats/min) was calculated from the time interval (ms) between the onset of the systolic velocity component of two successive arterial pulmonary flow velocity waveforms.

Repeatability

To investigate the repeatability of arterial pulmonary branch flow velocity waveform recordings, Doppler waveforms from proximal, middle and distal arterial pulmonary branches were collected in 25 separate women with an uncomplicated singleton pregnancy. Pregnancy duration ranged between 22 and 26 weeks in 15 cases and between 32 and 36 weeks in 10 cases. In each of the 25 fetuses, two recordings were made from each of the three Doppler sampling locations previously described at a time interval of approximately 15 min. Each recording resulted in at least three waveforms per hard copy. These hard copies did not reveal the identity or gestational age of the patient, or the date or time of recording. They were coded by number, shuffled into random order and analyzed. Both Doppler recording and waveform analysis was performed by the same investigator (J.A.M.L.).

For assessment of repeatability, the mean and standard deviation (SD) of differences, and repeatability coefficient of the two repeated tests within patients were calculated.[30] In estimating the SD of the differences between the two repeated tests, it was assumed that the true mean difference equals zero. The repeatability coefficient has been defined as 1.96 times the standard deviation of differences between repeated measurements, so that plus/minus the repeatability coefficient gives the 2.5 and 97.5% probability limits, the so-called limits of agreement for the difference between two repeated measurements.[30] Further, the coefficient of variation (CV) was calculated from the two repeated tests, defined as the SD of the error in a single test and expressed as a percentage of the population mean of the test variable considered. Finally the relationship

between the variability in repeated tests, defined as the absolute value of the difference between the tests and 1) gestational age and 2) FHR was tested.

Statistical analysis

Fifth (P5), 50th (P50) and 95-th (P95) reference centiles were constructed[13] and, where necessary, the SD was modeled as a function of gestational age.[14] Linear regression was used to establish how the outcome variable related to gestational age and thus to define the 50th centile. Where there was no significant relationship the mean of the outcome variable y was used. In other cases a straight line or quadratic equation was used. Where the distribution of y dependent on gestational age was skewed, a logarithmic transformation was applied and the resulting transformed variable was remodeled. The association between the absolute residuals and gestational age was evaluated. Linear regression was used, where possible, to model the absolute value of the residuals and a regression equation for the SD calculated. Using this residual SD, the 5th and 95th centiles were constructed. Where a quadratic relationship was the best descriptor, the age range with significant increase or decrease of the outcome variable was calculated using the first derivative. We also tested whether the relationship between the outcome variable y and gestational age was influenced by FHR, by adding FHR to the regression model.

A paired t-test was used to establish the difference in mean values of all velocity parameters between proximal and middle, proximal and distal, and middle and distal arterial pulmonary branches. We also tested whether these mean differences were related to gestational age. The level of statistical significance was set at $p<0.05$.

RESULTS

Technically acceptable middle and distal pulmonary artery flow velocity waveforms with well-defined systolic and diastolic components were obtained in 92 (83%) and 85 (77%) of 111 women respectively. Recording failures were determined by fetal (breathing) movements, unfavorable fetal position or maternal obesity. There was no relationship between the recording failures and the left or right lung or gestational age. The median gestational age at delivery was 40 week (range 36-42 week) and the median birth weight was 3190 g (range 1930-4760 g).

Repeatability

The repeatability data are presented in Table 1. The mean differences of the two repeated tests were statistically significant for the end-diastolic velocity from the proximal arterial branch waveform and for PI from the distal arterial branch waveform. This may have happened by chance, since the overall probability of two or more significant results among 22 tests at a 5% level each, is 0.30 under the null hypothesis that all true mean differences

are equal to zero. Hence, this null hypothesis cannot be rejected. The differences of the two repeated measurements were not related to the mean of both tests. There was no significant difference in the relationship between the two repeated tests and gestational age or FHR. Therefore, data from all 25 patients could be combined for analysis.

Table 1

Repeatability of Doppler recordings from fetal proximal, middle and distal **arterial** pulmonary branches in 25 pregnancies

Doppler velocity parameters	***MD***	***SD of differences***	***Repeatability Coefficient***	***CV***
Proximal branch				
TAV (cm/s)	0.28	1.55	± 3.03	7 %
PSV (cm/s)	0.63	2.35	± 4.61	3 %
PDV (cm/s)	0.67	2.05	± 4.01	11 %
EDV (cm/s)	0.56*	1.28	± 2.05	13 %
RV (cm/s)	-0.40	1.37	± 2.68	13%
S/D ratio	-0.25	0.78	± 1.53	10 %
PI	-0.04	0.30	± 0.59	6 %
Systolic integral (cm)	-0.07	0.45	± 0.88	6 %
Diastolic integral (cm)	0.04	0.33	± 0.65	14 %
Middle branch				
TAV (cm/s)	0.33	1.55	± 3.04	12 %
PSV (cm/s)	0.86	2.67	± 5.23	7 %
PDV (cm/s)	-0.04	1.30	± 2.56	11 %
EDV (cm/s)	0.22	1.00	± 1.97	15 %
RV (cm/s) (n=9)	-0.26	0.58	± 1.14	11 %
S/D ratio	0.13	0.40	± 0.78	9 %
PI	0.03	0.37	± 0.73	11 %
Systolic integral (cm)	0.03	0.37	± 0.73	12 %
Diastolic integral (cm)	-0.02	0.26	± 0.52	14 %
Distal branch				
TAV (cm/s)	-0.02	0.93	± 1.83	12 %
PSV (cm/s)	-0.32	1.36	± 2.67	11 %
EDV (cm/s)	0.13	0.50	± 0.98	11 %
PI	-0.09*	0.14	± 0.28	9 %

*TAV, time-averaged maximum velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; RV, early peak diastolic reverse flow; S/D, peak systolic/peak diastolic; PI, pulsatility index. MD, mean difference of repeated tests; * MD of repeated tests is significant ($p<0.05$). SD, standard deviation of differences of repeated tests; CV, coefficient of variation.*

For the proximal arterial pulmonary branch waveform parameters and their ratios, the coefficient of variation (CV) varied between 3 and 14% (lowest: peak systolic velocity, highest: diastolic integral), for the middle arterial pulmonary branch the CV ranged between 7 and 15% (lowest: peak systolic velocity, highest: end-diastolic velocity) and for the distal arterial pulmonary branch the CV varied between 9 and 12%.

Waveform characteristics

As with the proximal arterial pulmonary waveform[29], the waveform from the middle arterial pulmonary branches is characterized by a needle-shaped systolic peak, although with lower maximum peak systolic velocities (Figure 2a and 2b). This is followed by a more gradual decline in flow velocity, which in 45% of cases is interrupted by a short reverse flow interval at the beginning of diastole. Forward flow could be observed throughout the remainder of diastole. The distal arterial pulmonary branch demonstrated blood flow velocity waveforms that are characterized by a monophasic profile with low forward flow velocities throughout the cardiac cycle (Figure 2c).

Arterial pulmonary branch velocity parameters relative to gestational age

Middle arterial pulmonary branches (Table 2, Figures 3 and 4)

For the middle arterial pulmonary branches, a statistically significant increase with advancing gestational age was found for peak diastolic velocity. End-diastolic velocity and the diastolic integral however, showed a significant increase only until 31 weeks of gestation. The S/D ratio depicted a significant reduction up to 31 weeks of gestation. No significant change in these three parameters was identified between 31 weeks and term. The PI demonstrated a significant decrease between 20 and 29 weeks of gestation, but a significant increase from 32 weeks to term. No statistically significant gestational age-related change was demonstrated for TAV, peak systolic velocity and systolic integral. RV was noted in 41 of 92 women (45%). In this subset RV revealed a statistically significant increase with advancing gestational age. In five of 92 cases (5%) the early diastolic interruption was characterized by zero flow and in 39 of 92 cases (42%) by forward flow. Presence of early diastolic reverse flow could not be identified in the remaining seven cases (8%), due to interference in the waveforms from the adjacent pulmonary vein.

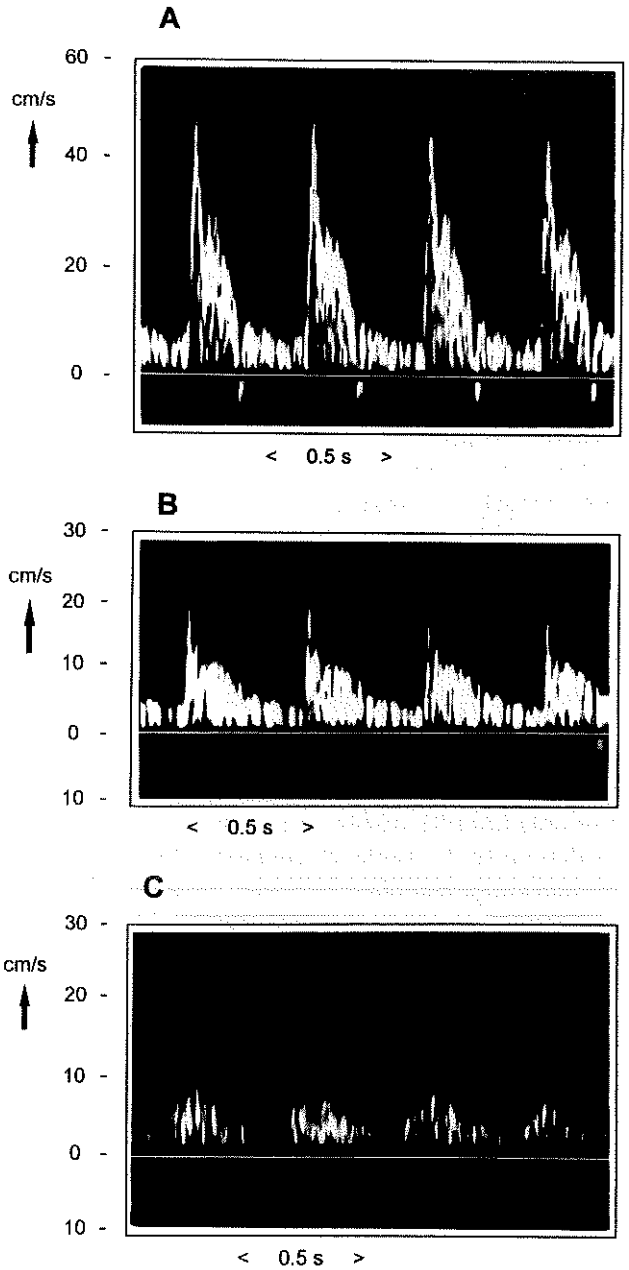

Figure 2
Doppler recording of blood flow velocity waveforms from the (A) proximal, (B) middle and (C) distal branch of the pulmonary artery from the same fetal lung at 25 weeks of gestation.

Table 2
Regression equations for various arterial pulmonary branch Doppler measurements and their ratios with gestational age

Doppler velocity parameters	*Transformation*	*Constant*	*A x GA*	*B x GA²*	*SD Constant / CxGA*		*R*	*p*
Proximal branch[29]								
Middle branch								
TAV (cm/s)*	-	9.15			1.62		0.15	NS
PSV (cm/s)*	-	27.75			5.82		0.11	NS
PDV (cm/s)	log (PDV)	1.65	0.016		-0.012	0.0058	0.39	0.0001
EDV (cm/s)	-	-2.62	0.46	-0.0069	-0.31	0.034	0.31	0.01
RV (cm/s)	-	-1.95	-0.069		0.89		0.37	0.02
S/D ratio	log(S/D+0.25)	3.15	-0.12	0.0018	0.21		0.36	0.002
PI	-	8.09	-0.38	0.0062	0.54		0.34	0.005
Diastolic integral (cm)	-	-1.78	0.20	-0.0030	-0.028	0.0093	0.40	0.0005
Systolic integral (cm)*	-	2.53			0.49		0.11	NS
Distal branch								
TAV (cm/s)*	-	5.59			1.036		0.09	NS
PSV (cm/s)*	-	8.40			1.77		0.15	NS
EDV (cm/s)*	-	3.32			-0.0027	0.016	0.18	NS
PI	-	2.25	-0.095	0.0016	0.15		0.31	0.01

*TAV, time-averaged maximum velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; RV, early peak diastolic reverse flow; S/D, peak systolic/peak diastolic; PI, pulsatility index. A, coefficient for linear components; GA, gestational age; B, coefficient for quadratic component; C, coefficient for linear component if the absolute residuals depended on GA; SD, residual standard deviation (as a linear function of GA, if necessary); R, multiple correlation coefficient; *, when the regression was NS, the mean and SD are presented.*

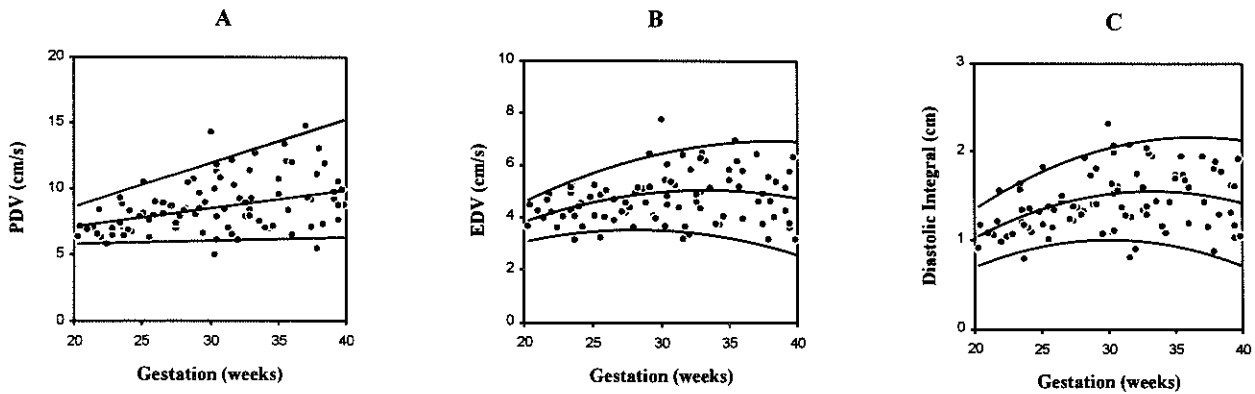

Figure 3
*Individual values and reference ranges (mean, 5th and 95th centiles) for (A) peak diastolic velocity (PDV, cm/s), (B) end-diastolic velocity (EDV, cm/s) and (C) diastolic integral (cm) of the **middle arterial** pulmonary branch relative to gestational age*

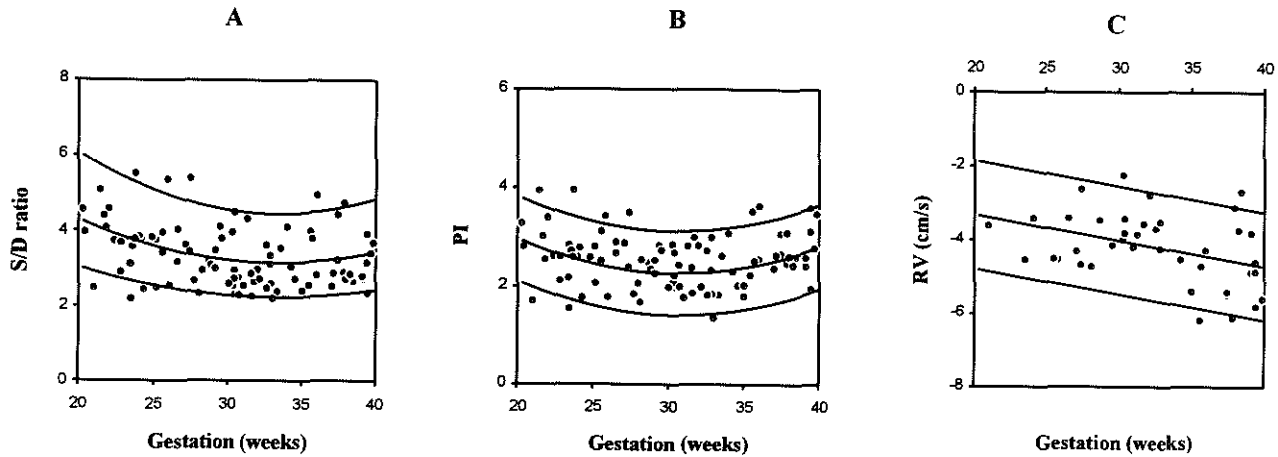

Figure 4
*Individual values and reference ranges (mean, 5th and 95th centiles) for (A) S/D ratio (B) PI and (C) early peak diastolic reverse flow (RV, cm/s) of the **middle arterial** pulmonary branch relative to gestational age.*

Distal arterial pulmonary branches (Table 2, Figure 5)
The distal arterial pulmonary flow velocity waveform demonstrated a monophasic forward flow profile. Therefore, waveform parameters such as peak diastolic velocity, S/D ratio, RV, systolic and diastolic integral could not be calculated. The PI showed a significant decrease between 20 and 26 weeks, but a rise from 31 weeks to term. No statistically significant change was observed in peak systolic velocity, TAV and end-diastolic velocity.

The FHR demonstrated a statistically significant increase ($p<0.04$) with advancing gestational age. However, the observed relationship between the arterial flow velocity waveform parameters and PI values of both middle and distal pulmonary branches and gestational age was independent of FHR.

Inter-pulmonary (proximal, middle, distal) differences in arterial blood flow velocity parameters
All mean differences in Doppler flow velocity parameters between proximal and middle, proximal and distal and middle and distal arterial pulmonary branches were statistically significant (Table 3). In all arterial pulmonary recordings, the mean differences in Doppler flow velocity parameters were unrelated to gestational age, with the exception of the mean differences in peak diastolic velocity and RV between proximal and middle arterial pulmonary branches (Table 3). These showed a statistically significant increase with advancing gestational age.

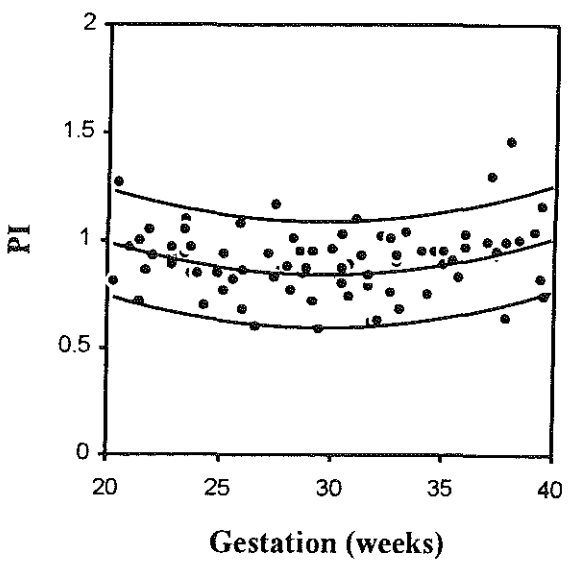

Figure 5
Individual values and reference ranges (mean, 5th and 95th centiles) for the PI of the distal arterial pulmonary branch relative to gestational age.

Table 3
Differences in Doppler flow velocity parameters between proximal, middle and distal **arterial** pulmonary branches

	No. of pairs	***MD****	***SD***
TAV (cm/s)			
Proximal-distal	81	11.77	3.66
Proximal-middle	82	8.33	3.65
Middle-distal	80	3.60	1.77
PSV (cm/s)			
Proximal-distal	81	52.92	11.08
Proximal-middle	82	34.55	11.17
Middle-distal	80	19.27	6.20
PDV (cm/s)			
Proximal-middle	82	5.48**	3.24
EDV (cm/s)			
Proximal-distal	81	3.33	1.71
Proximal-middle	82	2.00	1.72
Middle-distal	80	1.44	0.94
RV (cm/s) Proximal-middle	36	-7.66§	4.67
S/D ratio Proximal-middle	82	1.28	1.03
PI			
Proximal-distal	81	2.29	0.45
Proximal-middle	82	0.70	0.63
Middle-distal	80	1.60	0.58
Systolic integral (cm/s)			
Proximal-middle	82	2.96	1.14
Diastolic integral (cm/s)			
Proximal-middle	82	0.63	0.51

*TAV, time-averaged maximum velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; RV, early peak diastolic reverse flow; S/D, peak systolic/peak diastolic; PI, pulsatility index; MD, mean differences; *, All MD were statistically significant ($p<0.0001$); **, positive significant correlation with gestational age ($p<0.05$); §, negative significant correlation with gestational age ($p=0.0001$).*

DISCUSSION

The present study focuses on the characteristics and repeatability of flow velocity waveforms in different areas of the human fetal arterial pulmonary vasculature and their relationship to gestational age during the second half of pregnancy. It should be emphasized that non-invasive Doppler studies of the human fetal circulation allow assessment only of flow velocities. The absence of volume flow and pressure measurements restrict the interpretation of our data. The increasing failure to obtain satisfactory recordings rate when moving from proximal to distal arterial lung vessels may reflect the gradual reduction in vessel size.

The coefficient of variation for all velocity parameters, S/D ratio and PI were below 15% for proximal, middle and distal arterial pulmonary branches. Overall, the parameters of the proximal branch showed the lowest coefficients of variation. However, to evaluate repeatability in terms of clinical practice, measurement variability, expressed as ± the repeatability coefficient, should be considered; 95% of the differences between two repeated tests in the same arbitrary patient should lie within this interval. This variability may be due to a combination of within-patient physiologic variation and intra-observer variation.

The middle arterial pulmonary Doppler flow velocity waveform pattern is similar to that of the proximal branch[2], but displays a much lower peak systolic velocity and a less frequent reverse flow component (45% versus 97% in the proximal branch).[29] The wall filter setting of 70 Hz (1.44 cm/s) may have hidden minimal retrograde flow in some cases, but we did not wish to use the lowest high pass filter (50 Hz) since this might induce too much interference from the vessel wall. Other workers have demonstrated that pulmonary branch arteries, observed further downstream in the lungs, produce waveforms similar to those of more proximal arteries, but with a progressive fall in peak systolic velocity and a reduction in time of the systolic component as the pulsed Doppler interrogation proceeds more distally.[6] It is thought that the subsequent drop in flow velocity following ejection of blood through the pulmonary arterial circulation during early systole is associated with preferential blood flow through the ductus arteriosus into the descending aorta.[18] As pulmonary vascular resistance is high, this preferential flow will be recorded as early diastolic retrograde flow.[18] Unlike proximal and middle arterial pulmonary waveforms, distal arterial pulmonary waveforms are characterized by a monophasic flow profile with low forward flow throughout the cardiac cycle. This may be explained by the increase in vessel branching and distance between the heart and distal arterial pulmonary vessels, resulting in nearly complete dampening of the arterial pulsatile waveform.

Based on animal studies, the proportion of pulmonary blood flow within the combined cardiac output is estimated to be less than 10%.[18] However, this may be considerably higher in the human fetus, with an increase from 13% to 25% at 20-30 weeks of gestation and a constant

pattern thereafter.[25] Despite the increase in total pulmonary flow during the second half of gestation, the present study revealed no significant gestational age related change in TAV and peak systolic velocity at middle or distal arterial pulmonary branch level. Similar results were established for the proximal arterial branch.[29] It appears that the increase in vessel diameter, rather than a rise in flow velocities, determines the increase in pulmonary artery volume flow during the second half of gestation.[25]

The gestational age independence of end-diastolic velocity in both distal and proximal[29] arterial pulmonary branches, contrasting with a significant rise, until 31 weeks, in middle arterial pulmonary branches, is difficult to explain. Changes in morphology, in particular arterial muscular wall structure, vessel compliance and/or downstream stream impedance may be responsible for this finding.

As with the proximal arterial pulmonary branches[29], peak diastolic velocity, early peak diastolic reverse flow (RV), diastolic integral and S/D ratio in the middle arterial pulmonary branches demonstrated significant change with gestation. Whereas peak diastolic velocity and RV changed linearly in the second half of pregnancy, diastolic integral and S/D ratio changed only during the second and early third trimesters of pregnancy. During the same study period, both the middle arterial and distal arterial PI were characterized by a significant decrease followed by a significant increase during the last 8-10 weeks of gestation. The latter increase supports the hypothesis of Rasanen and colleagues[25] that pulmonary vascular resistance may increase during this time frame. With the exception of the RV, all these observations may reflect changes in pulmonary vascular resistance and/or compliance. The PI in the proximal arterial branches did not change significantly with gestation.[29] To our knowledge, there is no information available to explain the difference in PI between the proximal and more distal parts of the pulmonary artery with gestational age. Referring to animal studies, differences in wall structure and/or sensitivity to oxygen changes or other factors such as prostaglandins, may play a role in these findings.[2,23,31] As with the proximal arterial branches[29], the increase in early diastolic reverse flow in the middle arterial branches - expressing the preferential escape of blood from the branch pulmonary arteries through the ductus arteriosus - may be the result of a gestational age-related reduction in arterial downstream impedance in the systemic circulation.

Only two other studies[8,32] have reported Doppler velocimetry findings in human fetal peripheral arterial pulmonary branches. Differences in defining the Doppler sample site for distal pulmonary arteries have made comparison with our data difficult. Rasanen and colleagues[8] investigated fetal distal branch pulmonary arterial vascular impedance and analyzed the relationships between proximal (after the bifurcation of the main pulmonary artery) and distal (after the first bifurcation of the branch pulmonary artery) pulmonary arterial blood velocity waveforms. It should be noted that their

definition of "distal", is more analogous to our definition of "middle" than to our definition of "distal". As with middle and distal arterial pulmonary waveforms obtained in this study, Rasanen and colleagues observed a linear decrease in distal pulmonary artery PI until 31 weeks of gestation.[8] By contrast Rizzo and colleagues[32] using power Doppler imaging in the most distal area of the fetal lung, reported a decrease in PI throughout the second half of gestation.

When the inter-relationship between proximal, middle and distal arterial pulmonary flow velocity waveforms was considered, all velocity parameters, including the PI, changed significantly as pulsed Doppler interrogation proceeded more distally. The mean peak systolic velocity dropped by a factor of 2 from proximal to middle and by a factor of 7 to 8 from proximal to distal sample sites while the mean TAV dropped by a factor of 2 and 3, respectively. Mean PI decreased by a factor of 1.3 from proximal to middle and by a factor of 3.5 from proximal to distal sampling sites. Changes in vessel diameter and/or vessel compliance, increase in vessel branching and distance between the heart and distal arterial pulmonary vessels may be responsible for these observations. Of interest is the remarkable fall in PI with low absolute PI values (mean 0.91) in the distal arterial pulmonary branches. This is similar to that seen in the umbilical artery and suggests a low pulmonary vascular resistance.

In conclusion, it is possible to obtain Doppler velocity waveforms in more distal parts of the human fetal pulmonary artery during the second half of pregnancy. Acceptable repeatability of flow velocity waveforms, with coefficients of variation below 15%, has been demonstrated. The Doppler flow velocity waveform pattern does not change significantly from the proximal to the mid-pulmonary sections, but turns into a monophasic profile in the distal part of the lung. At mid-arterial level a gestational age-related change was found for peak diastolic velocity, diastolic integral, S/D ratio and PI, suggesting changes in pulmonary vascular resistance. At distal arterial level pulmonary Doppler velocity waveform parameters remain unchanged with advancing gestational age, with the exception of the PI, which showed low absolute values compared with more proximal arterial branches. There was a significant decrease in peak and time-averaged velocities and in PI as Doppler interrogation moved from the proximal to more distal parts of the fetal lung. This may be due to increase in vessel branching and distance between the heart and distal arterial pulmonary vessels. The characteristics of the arterial pulmonary circulation and the reference charts of the various Doppler velocity parameters demonstrated in this study will improve our knowledge of the normal hemodynamics of the human fetal pulmonary circulation. This information may be useful in the detection of gestation-related changes of pulmonary vascular resistance in pregnancies at risk of impaired lung development such as pulmonary hypoplasia.

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CHAPTER 4

DOPPLER ASSESSMENT OF THE VENOUS PULMONARY CIRCULATION

4.1 Introductory remarks

Next to the arterial circulation, information on venous flow in the fetal lungs is needed to obtain a full picture of the fetal pulmonary circulation. Venous flow velocity patterns can now be visualized across the entire lung area. Moreover, venous inflow into left heart can be studied relative to the different systolic and diastolic time-intervals of the cardiac cycle. This was established by relating venous pulmonary inflow velocities to simultaneous recordings of transmitral and aorta flow velocity waveforms.

In this chapter, methodology, reproducibility and normal Doppler data from proximal, middle and distal venous lung vessels are presented with special attention for venous inflow into the left atrium.

4.2 Normal fetal pulmonary venous blood flow velocity

J.A.M. Laudy[1], T.W.A. Huisman[2], M.A.J. de Ridder[3], J.W. Wladimiroff[1]
Department of Obstetrics and Gynaecology[1], University Hospital Rotterdam, Department of Obstetrics and Gynaecology[2], Medical Center, Alkmaar, Institute of Epidemiology and Biostatistics[3], Erasmus University Medical School, Rotterdam, The Netherlands

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ABSTRACT

Our objectives were to describe the normal pulmonary venous blood flow velocity waveform and to establish reference ranges for the second half of pregnancy in healthy human fetuses. A total of 123 women with uncomplicated pregnancies between 20 and 40 weeks was examined, using a combined color-coded Doppler and two-dimensional real-time ultrasound system. Pulsed Doppler flow velocity waveforms of pulmonary venous drainage into the left atrium were obtained from a transverse cross-section of the fetal chest at the level of the cardiac four-chamber view. All waveforms were obtained during fetal apnea.

The success rate in obtaining the pulmonary venous waveform was 81%. The waveform displayed a biphasic forward flow profile with a systolic and diastolic component. Peak systolic, peak diastolic and time-averaged velocities demonstrated a gestational age-related rise, whereas peak systolic/peak diastolic ratio shows a gestational age-related reduction.

The nature of the fetal pulmonary venous flow velocity waveform pattern suggests positive pressure towards the left atrium throughout the cardiac cycle. We speculate that an increase in volume flow and in pulmonary venous pressure gradient play a role in the gestational age-related changes in pulmonary venous flow velocities.

INTRODUCTION

Doppler ultrasound provides a non-invasive method for visualizing the fetal circulation. The addition of color Doppler have made it possible to determine flow directions at cardiac and extra-cardiac levels.[1-3] Several investigations have been carried out to examine fetal vessels; however, sparse information is available on pulmonary venous flow into the left atrium. This information may improve our understanding of total venous return to the fetal heart in the normally developing pregnancy and in the presence of structural and functional pathology, such as congenital heart disease and lung hypoplasia. Development of the pulmonary veins occurs in the canalicular phase (16-24 weeks of gestation) and all pre-acinar veins will be present by 20 weeks of gestation.[4]

The purpose of this study was to determine the nature of the fetal pulmonary venous blood flow velocity waveform proximal to the entrance into the left atrium and to evaluate its change during second half of pregnancy.

PATIENTS AND METHODS

Study subjects

A total of 123 women with a normal singleton pregnancy, recruited from our routine antenatal clinic, consented to participate in the study. The study protocol was approved by the Hospital Ethics Committee. Gestational age, varying between 20 and 40 weeks, was calculated from a reliable menstrual history or from an early ultrasonic measurement of fetal crown-rump length or biparietal diameter. Women were selected according to ten gestational age subgroups (ten 2-week divisions between 20 and 40 weeks), to guarantee a homogeneous distribution. None of these pregnancies were associated with fetal congenital heart disease or lung pathology. All pregnancies were uncomplicated. As expected, 10% of the newborn had a birth weight below the 10th percentile. Each woman was included in this cross-sectional study once.

Recording technique

We used two systems, the Toshiba SSH 140A (Toshiba Corp., Medical Systems Division, Tokyo, Japan, Doppler carrier frequency, 5 MHz) and the Hitachi EUB 515 (Hitachi Medical Corp., Tokyo, Japan, Doppler carrier frequency 3.5 MHz), both with two-dimensional real-time and color Doppler facilities. It had been established earlier that there was no difference in flow velocity measurements between these two systems.[5] In both systems the spatial peak temporal average power output was less than 100 mW/cm^2 in both imaging and Doppler modes according to manufacturers' specifications. Recordings were performed with the women in the semi-recumbent position. After a clear sonographic transverse cross-section of the fetal chest was obtained at the level of the four-chamber view, color Doppler was switched on to visualize one of the pulmonary veins. Depending on the fetal position, the sample volume was placed over the best visualized vein of the right or

left lung, just proximal to the entrance into the left atrium (Figure 1, page 198). Doppler sample volume length was 0.1-0.3 cm. The angle between the Doppler cursor and the assumed direction of blood flow was always kept below 20°. Maximum flow velocity waveform envelopes were collected during fetal apnea, since fetal breathing movements modulate venous Doppler waveforms.[6,7]

Data analysis

During each recording, at least three consecutive technically acceptable flow velocity waveforms were documented on hard copies. A microcomputer (Olivetti M24; Olivetti BV, Leiden, The Netherlands) linked to a graphics tablet was used for analysis of the Doppler recordings.

For each woman the mean value of the following waveform parameters was determined (Figure 2): peak systolic and peak diastolic velocity (cm/s), time-averaged velocity (TAV, cm/s), end-diastolic velocity (cm/s), peak systolic /peak diastolic ratio (S/D ratio).

Statistical analysis

For the construction of reference centiles (mean (P50), 5th centile (P5) and 95th centile (P95)) the method described by Royston[8] was used and, if necessary, the standard deviation was modeled as a function of gestational age as described by Altman[9]. This resulted in the following procedure.

The 50th centile (P50) was established by modeling the outcome variable, *y*, by a straight line regression model with gestational age as predictor. It was tested whether adding the quadratic term was significant. If the distribution of *y* depending on gestational age was skewed, a logarithmic transformation was applied. It was then checked whether the residuals depended on gestational age. If so, straight line regression was used to model the absolute value of the residuals and from this model a regression equation for the standard deviation, depending on gestational age, was derived. Using this standard deviation the 5th (P5) and 95th (P95) centiles were constructed.

RESULTS

The median gestational age at delivery was 39.85 weeks (35.14 - 42.28) and the median birth weight was 3182 g (1920 - 4200). Technically acceptable flow velocity waveforms were collected in 100 out of 123 women, resulting in a success rate of 81%. Failures were determined by fetal movements, unfavorable fetal position or maternal obesity. There was no relationship with gestational age.

The waveform (Figure 2) consists of a systolic and early diastolic forward flow component and a late diastolic forward component coincident with atrial contraction. The relationship between the various components of the pulmonary venous flow velocity waveform and gestational age is presented in Table 1.

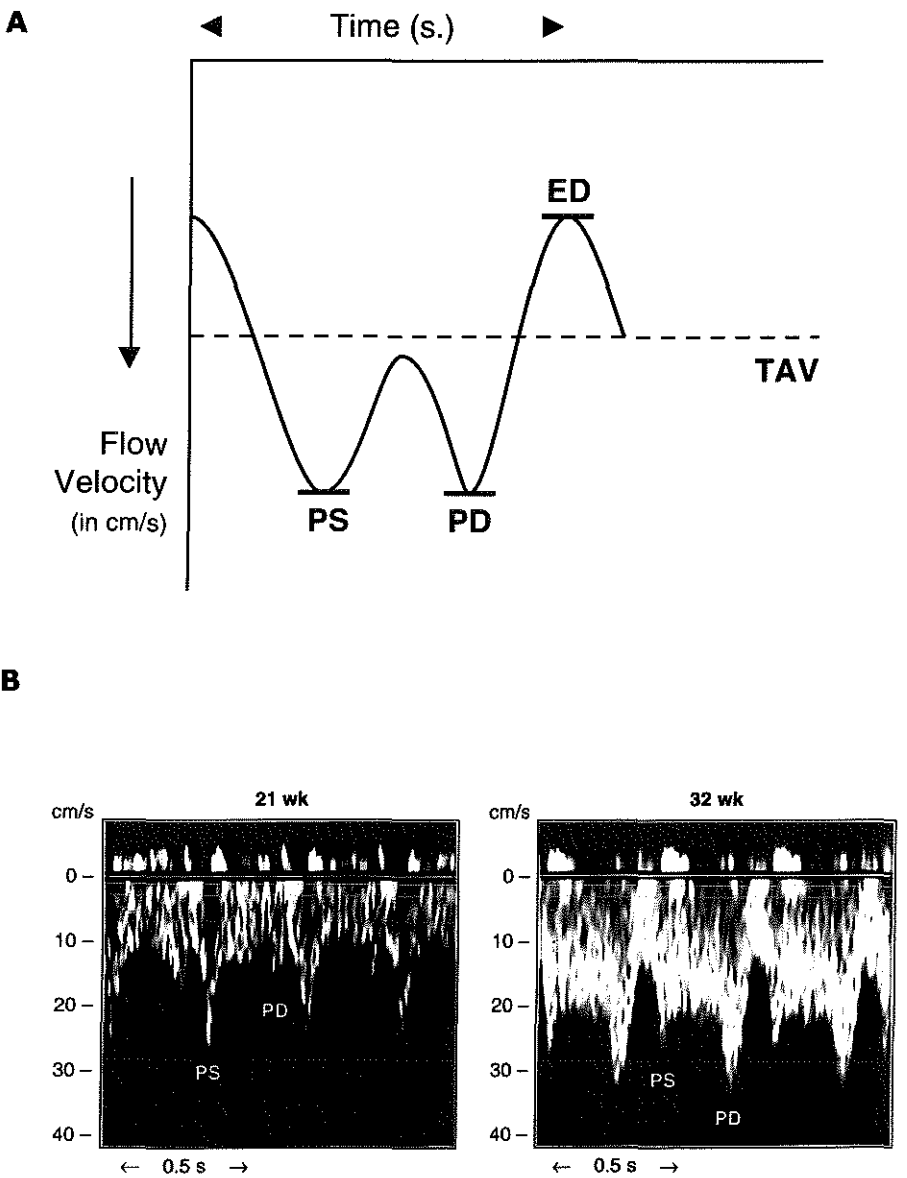

Figure 2:
(A) Pulmonary venous flow velocity waveform, consisting of biphasic forward flow components. PS, peak systole; PD, peak diastole; ED, end-diastole; TAV, time-averaged velocity. (B) Examples of the pulmonary venous flow velocity waveform at 21 weeks and 32 weeks of gestation. Note the higher peak diastolic velocity at 32 weeks in relation to the peak systolic velocity.

A statistically significant increase with advancing gestational age was found for all velocity components (Figures 3-6), apart from the S/D ratio (Figure 7) which demonstrated a statistically significant decrease with advancing gestational age. The S/D ratio was > 1 in 77%, equal to 1 in 6% and < 1 in 17%. This was independent of gestational age.

Table 1
Data from regression equations for the Doppler velocity profile measurements and their ratios relative to gestational age

Velocity parameters	***Trans-formation***	***Constant***	***Slope***	***p-value***	***SD***	
					Constant	***Slope***
PSV (cm/s)	-	9.391	0.554	<0.0001	6.584	-
PDV (cm/s)	-	2.261	0.695	<0.0001	-0.019	0.175
EDV (cm/s	log	1.067	0.032	0.0001	0.435	-
TAV (cm/s)	log	1.937	0.031	<0.0001	0.234	-
S/D ratio	log	0.416	-0.0098	0.0008	0.400	-0.0083

PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; TAV, time-averaged velocity; S/D ratio, peak systolic/peak diastolic ratio; SD, standard deviation

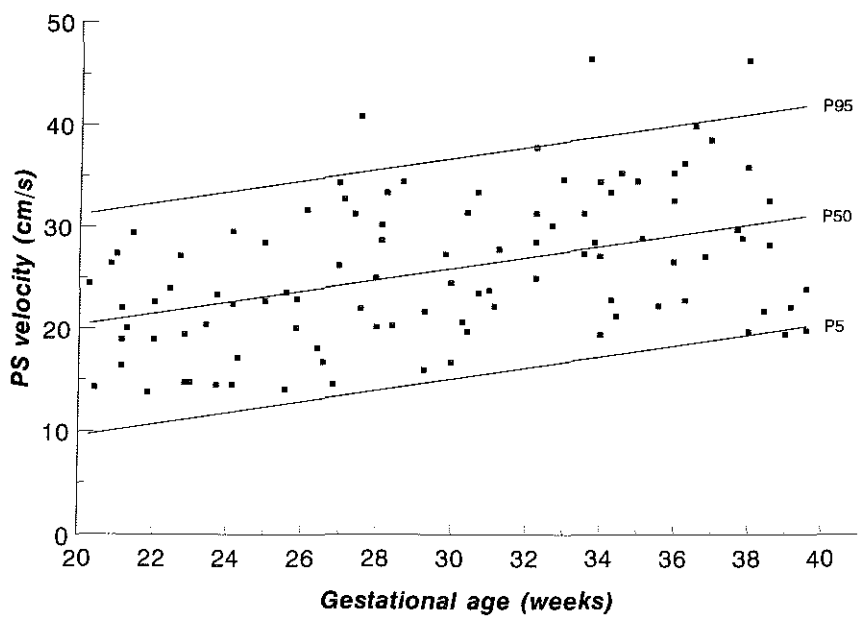

Figure 3
Individual values and reference ranges (mean, 5th and 95th centiles) for the peak systolic (PS) velocity of the pulmonary venous inflow into the left atrium relative to gestational age.

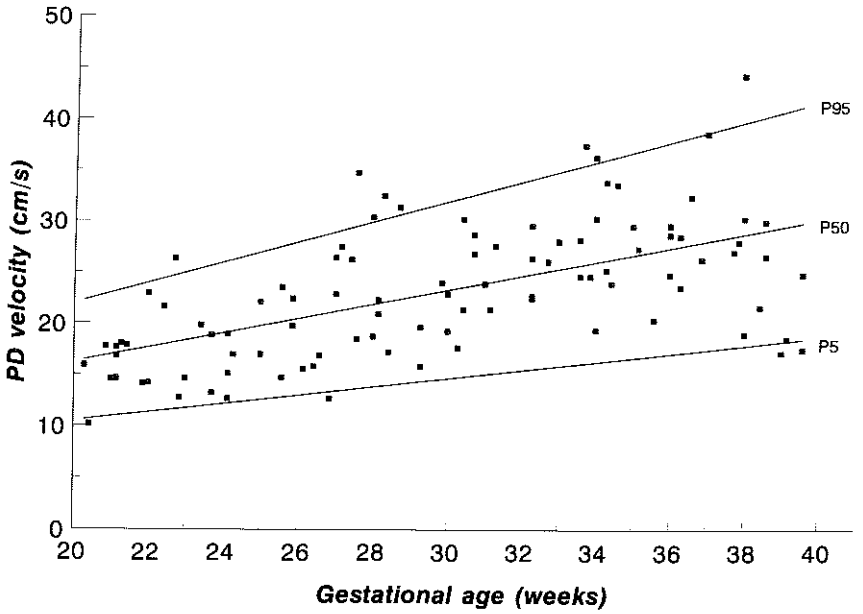

Figure 4
Individual values and reference ranges (mean, 5th and 95th centiles) for the peak diastolic (PD) velocity of the pulmonary venous inflow into the left atrium relative to gestational age.

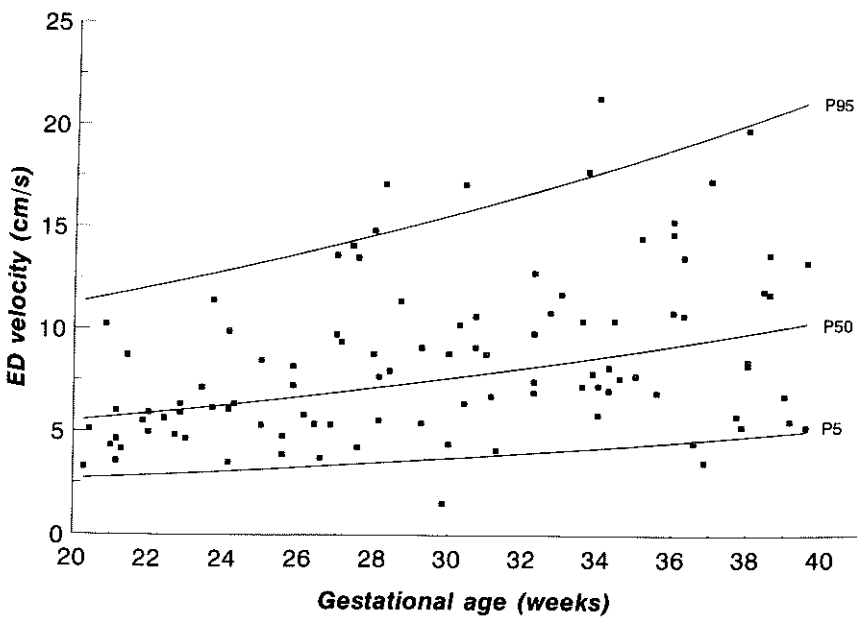

Figure 5
Individual values and reference ranges (mean, 5th and 95th centiles) for the end-diastolic (ED) velocity of the pulmonary venous inflow into the left atrium relative to gestational age.

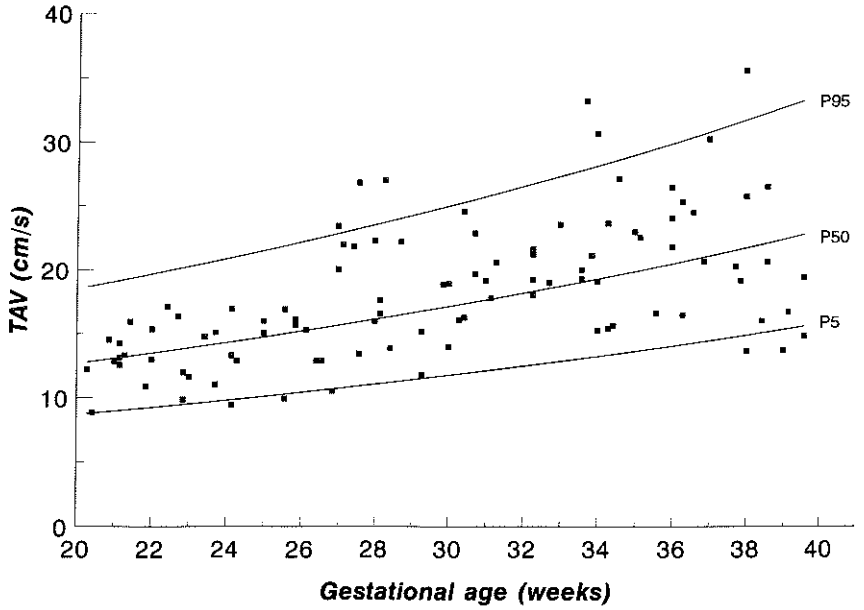

Figure 6
Individual values and reference ranges (mean, 5th and 95th centiles) for the time-averaged velocity (TAV) of the pulmonary venous inflow into the left atrium relative to gestational age.

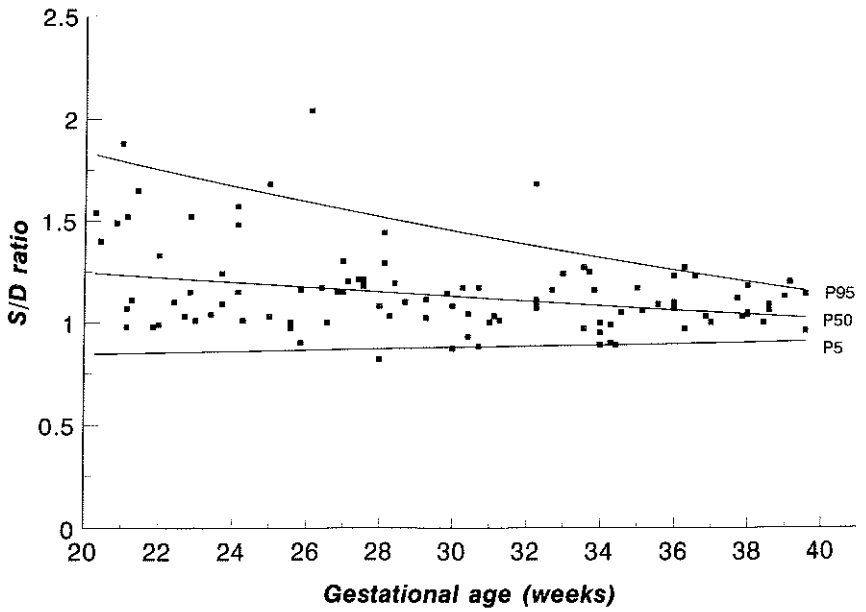

Figure 7
Individual values and reference ranges (mean, 5th and 95th centiles) for the peak systolic/ peak diastolic (S/D) ratio of the pulmonary venous inflow into the left atrium relative to gestational age.

DISCUSSION

Several studies have reported on the development and morphology of the lungs in general and on the pulmonary venous system in particular, both in the sheep and human fetus.[4,10-12] Whereas fetal pulmonary venous blood flow has been identified using color Doppler[13,14] or pulsed wave Doppler[15], normal data on flow velocities have only been described postnatally.[16-18] The present study describes the characteristics of the human fetal pulmonary venous blood flow velocity waveform relative to gestational age during the second half of normal pregnancy.

The pulmonary venous blood flow velocity waveform shows a pulsatile forward flow velocity pattern, equal to that established postnatally in sheep and humans.[16,17] Ramjagopalan and collegues[16] demonstrated in adults that the pulmonary venous velocity waveform mirrors the atrial pressure wave, resulting in an almost exact correspondence between the pulmonary venous velocity waveform and the inverse of the left atrial pressure waveform. If a similar relationship is assumed in the fetus, then the systolic component of the pulmonary venous flow velocity waveform coincides with a reduction in atrial pressure, whereas the diastolic component coincides with rapid emptying of the left atrium during ventricular relaxation. This would mean that pulmonary venous flow is determined by suction of blood from the pulmonary veins into the left atrium and left ventricle.[17] In contrast to the adult with virtually absent pulmonary venous flow during late diastole[17], forward flow is present during this stage of the cardiac cycle in the fetus, signifying blood flow from the pulmonary venous vessels into the left atrium during atrial contraction.

A 1.5 to 2.5-fold increase was established for the systolic and diastolic component of the pulmonary flow velocity waveform during second half of pregnancy. Several factors may play a role in the gestational age related rise in flow velocity such as an increase in volume flow and a rise in pressure gradient between the pulmonary venous system and the left atrium.[19] The 1.25 fold decline in S/D ratio is determined by the more pronounced rise in peak diastolic velocity relative to peak systolic velocity. Based on the role of left atrial pressure changes in the initiation of pulmonary venous flow, this would suggest a further reduction in atrial pressure after the rapid filling wave across the mitral valve during diastole with advancing gestational age.

Venous inflow at atrial level is also determined by the ductus venosus[3,20,21] and inferior vena cava[3,5]. The ductus venosus flow velocity waveform closely resembles the pulmonary venous flow velocity waveform. Absolute values are, however, 2-3 fold higher in the ductus venosus compared with the pulmonary vein, which may be due to the higher pressure gradient between ductus venosus and atrium[22] or due to a difference in vessel diameter.

It can be concluded that technically acceptable flow velocity waveforms can be obtained from human fetal pulmonary veins. The waveform consists of biphasic forward flow components, similar to those in the adult, suggesting that pulmonary venous flow is influenced by dynamic changes in left atrial pressure as result of atrial and ventricular contraction and relaxation. A gestational age-dependent rise in flow velocities was established for all waveform velocity parameters, except for the S/D ratios which demonstrate a gestational age-determined decline. We speculate that increase in volume flow and in pulmonary venous pressure gradient plays a role in this. The clinical implications of these findings will be investigated in relation to pathological conditions such as lung hypoplasia.

4.3 Doppler velocimetry of normal human fetal venous intrapulmonary branches

J.A.M. Laudy[1], N.T.C. Ursem[1], P.G.H. Mulder[2], J.W. Wladimiroff[1]

Department of Obstetrics and Gynaecology[1], University Hospital Rotterdam; Institute of Epidemiology and Biostatistics[2], Erasmus University Medical School Rotterdam, The Netherlands

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ABSTRACT

***Objectives*:** To describe the nature of flow velocity waveforms from fetal middle and distal venous pulmonary branches in the second half of normal pregnancy in relation to gestation and to test repeatability and inter-relationships of flow velocity waveform recordings from proximal, middle and distal venous pulmonary branches.

***Design*:** Cross-sectional study.

***Subject/Methods*:** A total of 111 singleton normal pregnancies between 20 and 40 weeks was studied using a color-coded Doppler ultrasound system. Pulmonary waveforms were obtained at the level of the fetal cardiac four-chamber view. Repeatability was tested from two recordings at 15-minute time intervals in 25 separate normal pregnancies.

***Results*:** The nature of middle and distal venous pulmonary flow velocity waveforms was comparable with that of proximal waveforms. Acceptable repeatability of pulmonary venous flow velocity wave forms with coefficients of variation below 15% was established for nearly all velocity parameters and their ratios. A gestational age-dependent change was found for all flow velocity waveform parameters including pulsatility index for veins at both middle and distal venous levels. Significant inter-pulmonary changes are observed for nearly all pulmonary venous waveform parameters.

***Conclusions*:** It is speculated that increase in volume flow and venous pulmonary pressure gradient plays a role in the gestational age-dependent changes, whereas changes in vessel diameter and distance between the heart and more distal venous pulmonary vessels are responsible for the inter-pulmonary changes.

INTRODUCTION

Our knowledge of the fetal pulmonary circulation is primarily derived from invasive animal studies, particularly in lambs.[23-25] The venous pulmonary blood flow in particular has mainly been studied postnatally both in humans and animals.[16,17,26] Although hemodynamic indices from all these different studies may subsequently be extrapolated to the human fetus, caution should be applied, since the distribution of the fetal circulation and subsequent fetal flow patterns may be different.[27]

The introduction of color-coded Doppler systems has led to identification of the human fetal venous pulmonary circulation.[13,14,28] Consequently, non-invasive Doppler studies of the human fetal venous pulmonary circulation with emphasis on the pulmonary venous drainage into the left atrium have been reported.[29-31] However, to our knowledge, information on more distal human fetal venous pulmonary hemodynamics is not available. Doppler velocimetry of proximal, middle and distal parts of the venous pulmonary circulation may improve our knowledge of the total human fetal pulmonary vascular system under normal and abnormal circumstances, such as in pulmonary hypoplasia. We therefore investigated, during the second half of normal singleton pregnancy:

(i) the repeatability of flow velocity waveforms from proximal, middle and distal venous pulmonary branches;
(ii) the nature and gestational age-dependency of middle and distal venous pulmonary flow velocity waveforms (data on the nature and gestational age-dependency of proximal venous pulmonary flow velocity waveforms have been reported earlier[29]);
(iii) the relationships between flow velocity waveforms from proximal, middle and distal venous pulmonary branches.

PATIENTS AND METHODS

Study subjects

A total of 111 non-smoking women with a normal singleton pregnancy consented to participate in this cross-sectional study. Women were recruited from the routine antenatal clinic at the University Hospital Rotterdam. The study protocol was approved by the Hospital Ethics Committee. Gestational age at entering the study ranged between 20 and 40 weeks (median 30 weeks) and maternal age varied between 18 and 46 years (median 28 years). Pregnancy duration was determined from the last reliable menstrual period and confirmed or adjusted by ultrasonic measurement of the fetal crown-rump length (8-12 weeks) or fetal biparietal diameter (12-20 weeks). None of these pregnancies was associated with congenital anomalies, including lung pathology. All pregnancies were uneventful, and had normal fetal biometry and normal amniotic fluid volumes. As expected, 80% of the fetal birth weights was situated between the 10th and 90th percentiles according to the Kloosterman Tables[32], adjusted for maternal parity and fetal sex. Women

were selected according to ten gestational age subgroups (20-22, ..., 38-40 weeks), to guarantee a homogeneous distribution. Each woman was included in the study only once.

Recording technique

Ultrasound Doppler studies were performed using a Toshiba SSH 140 machine with combined transabdominal real-time, color and pulsed Doppler imaging facilities (Toshiba Corp., Medical Systems Division, Tokyo, Japan). The carrier frequency was 5 MHz (real-time) and 3.75 MHz (Doppler). The spatial peak temporal average power output was less than 100 mW/cm^2 in both imaging and Doppler modes, according to manufacturers' specifications.

After obtaining a clear sonographic transverse cross-section of the fetal chest at the level of the cardiac four-chamber view, color Doppler was used to visualize the pulmonary circulation. Depending on fetal position, the right or left lung was examined. Firstly, the pulsed Doppler sample volume (0.1-0.3 cm) was placed over the most prominent vein, just proximal to the entrance into the left atrium.[29] The sample volume was then moved to a point in the middle of the fetal lung, at equal distance from the outer border of the fetal heart and the inner thoracic wall. Venous pulmonary blood flow velocity waveforms were collected at this point and from the most distal branch of the pulmonary vein as closely as possible to the fetal inner thoracic wall.

The angle between the Doppler cursor and the assumed blood flow direction was always kept below 20°. The high-pass filter was set at 70-100 Hz. Recordings were performed with the woman in the semi-recumbent position and during fetal apnea, since the latter may modulate the flow velocity waveform.[33] All flow velocity waveforms were recorded on hard copies. Waveform analysis was performed by one examiner (J.A.M.L.) using a microcomputer (Commodore 386 CX) linked to a graphics tablet. At least three consecutive flow velocity waveforms with the highest velocity and of similar appearance were used to calculate the different parameters in each vessel. In each woman the following waveform parameters from the proximal, middle and distal branch of the pulmonary vein were determined: time-averaged maximum velocity (TAV; cm/s), peak systolic velocity (cm/s), peak diastolic velocity (cm/s), end-diastolic velocity (cm/s), peak systolic/peak diastolic ratio (S/D ratio) and pulsatility index for veins (PIV= (peak systolic velocity - end-diastolic velocity)/TAV) according to Gosling and King[34].

Fetal heart rate (FHR; beats/min) was calculated from the time interval (ms) between the onset of the systolic velocity component of two successive venous pulmonary flow velocity waveforms.

Statistical analysis

Reference centiles (P5, P50 and P95) were constructed[8] and, if necessary, the standard deviation (SD) was modeled as a function of gestational age.[9] To find the 50th centile (P50), linear regression was used to establish how the outcome variable was related to gestational age. If there was no significant relationship, the mean of the outcome variable *y* was used. Otherwise, a straight line or quadratic equation was used. If the distribution of *y* depending on gestational age was skewed, a logarithmic transformation was applied and this transformed variable was modeled again. Further, it was checked whether the absolute residuals depended on gestational age. If so, straight line regression was used to model the absolute value of the residuals and, from this model, a regression equation for the SD, depending on gestational age, was derived. Using this residual SD, the 5th (P5) and 95th (P95) centile were constructed. In case of a quadratic relationship, the age range with significant increase or decrease of the outcome variable was found, using the first derivative. We also tested whether the relationship between the outcome variable *y* and gestational age was influenced by FHR, by adding FHR into the regression model.

The paired *t*-test was applied to establish the difference in mean values of all velocity parameters between proximal and middle, proximal and distal, and middle and distal venous pulmonary branches. We also tested whether these mean differences were related to gestational age. The level of statistical significance was set at $p<0.05$.

Repeatability

To examine repeatability of venous pulmonary branch flow velocity waveform recordings, Doppler waveforms from proximal, middle and distal venous pulmonary branches were collected in 25 separate women with an uncomplicated singleton pregnancy. Pregnancy duration ranged was between 22 and 26 weeks in 15 cases and between 32 and 36 weeks in ten cases. In each of the 25 fetuses, two recordings were made from each of the Doppler sampling locations at a time interval of approximately 15 minutes. Each recording resulted in at least three waveforms per hard copy. These hard copies did not reveal the identity or gestational age of the patient, nor the date or time of recording. They were coded with number, shuffled in random order and analyzed. Both Doppler recording and waveform analysis was performed by the same investigator (J.A.M.L.).

For assessment of repeatability, mean differences and SD of differences of the two repeated tests within patients and the repeatability coefficient were calculated.[35] In estimating the SD of the difference between the two repeated tests, it was assumed that the true mean difference equals zero. The repeatability coefficient has been defined as 1.96 times the SD of differences between repeated measurements, so that

plus/minus the repeatability coefficient gives the 2.5 and 97.5% probability limits, the so-called limits of agreement for the difference between repeated measurements.[35] Further the coefficient of variation was calculated from the two repeated tests, defined as the SD of the error in a single test and expressed as a percentage of the population mean of the test variable considered. Last, it was tested if the variability in repeated tests, defined as the absolute value of the difference between the tests, was related to gestational age and FHR.

RESULTS

Technically acceptable flow velocity waveforms from the middle and distal pulmonary veins were collected in 96 (86%) and in 81 (73%), respectively, out of 111 women. Recording failures were determined by fetal (breathing) movements, unfavorable fetal position or maternal obesity. There was no relationship between the recording failures and the left or right lung or gestational age, although recording failures seem to occur slightly more often after 35 weeks of gestation due to fetal breathing movements. The median gestational age at delivery was 40 weeks (range 36-42 weeks) and the median birth weight was 3190 g (range 1930-4760 g).

Waveform characteristics

Proximal, middle and distal venous pulmonary flow velocity waveforms are demonstrated in Figure 1. The nature of the middle and distal venous pulmonary branch blood flow velocity waveform exhibited a profile comparable with that of the proximal venous pulmonary branch[29], indicating a biphasic pattern consisting of a systolic and early diastolic forward flow component and forward flow during late diastole. However, in 3% of middle venous pulmonary flow velocity waveforms and in 16 % of distal venous pulmonary flow velocity waveforms, no end-diastolic forward could be observed. Since the lowest wall filter setting was 70 Hz (1.44 cm/s), flow velocities lower than 1.44 cm/s were assumed to be zero.

Repeatability

Outcomes of repeatability are demonstrated in Table 1. Mean differences of the two repeated tests were not statistically significant. The differences of the two repeated measurements were not related to the mean of both tests. All absolute differences between the two repeated tests were not significantly related to fetal heart rate. Since the homogeneity of variances between the two gestational age groups was not significantly different at the 1% level using the *F*-test, all data of the 25 patients could be combined; a significance level of 1% was used because of multiple testing.

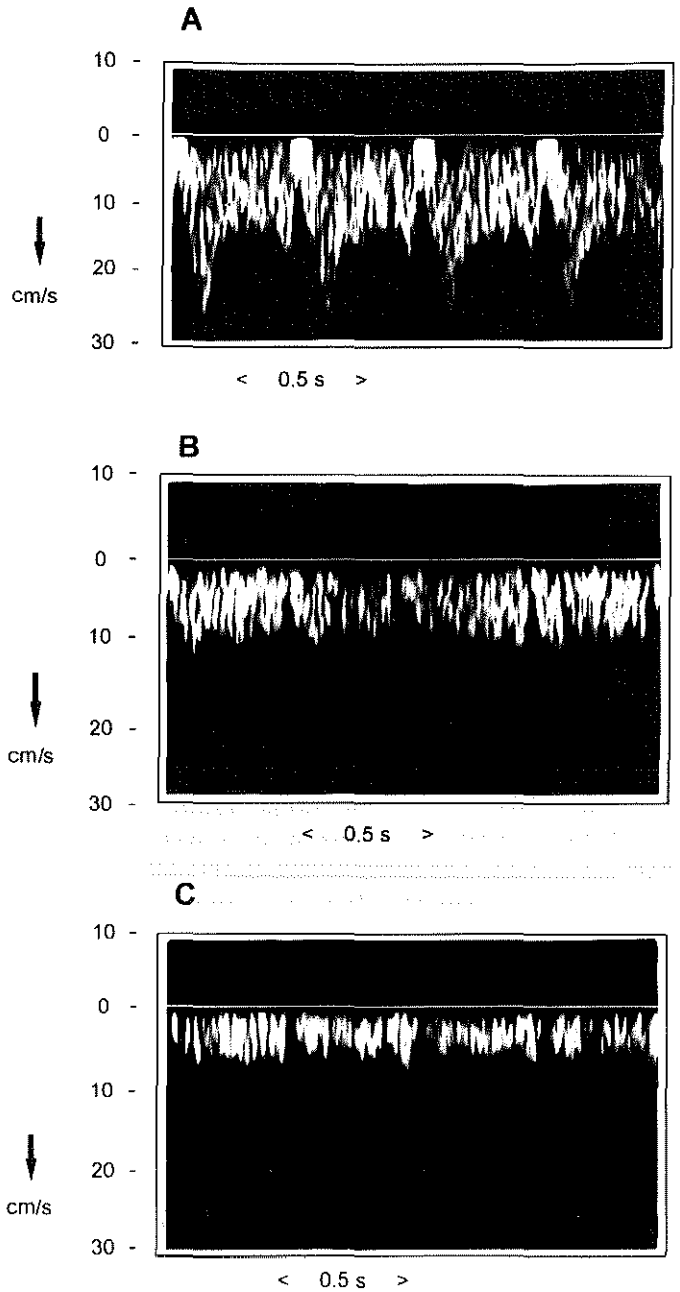

Figure1
Doppler ultrasound recording of blood flow velocity waveforms from the (A) proximal, (B) middle and (C) distal branch of the pulmonary vein at 25 weeks of gestation.

Table 1
Outcomes of repeatability of Doppler recordings from the fetal proximal, middle and distal **venous** pulmonary branches in 25 pregnancies

Doppler velocity parameters	*MD*	*SD of differences*	*Repeatability coefficient*	*CV*
Proximal branch				
TAV (cm/s)	0.22	2.00	± 3.92	9%
PSV (cm/s)	0.04	2.55	± 4.99	9%
PDV (cm/s)	-0.16	1.66	± 3.26	6%
EDV (cm/s)	-0.10	1.19	± 2.34	13%
S/D ratio	-0.01	0.10	± 0.20	7%
PIV	-0.03	0.17	± 0.34	12%
Middle branch				
TAV (cm/s)	0.31	1.58	± 3.09	11%
PSV (cm/s)	0.26	1.53	± 2.99	8%
PDV (cm/s)	-0.01	2.12	± 4.16	11%
EDV (cm/s)	-0.20	1.12	± 2.19	23%
S/D ratio	0.02	0.10	± 0.20	6%
PIV	0.10	0.14	± 0.28	10%
Distal branch				
TAV (cm/s)	-0.21	0.96	± 1.90	12%
PSV (cm/s)	-0.29	0.98	± 1.93	9%
PDV (cm/s)	-0.15	0.97	± 1.90	9%
EDV (cm/s)	-0.32	0.85	± 1.67	24%
S/D ratio	-0.02	0.10	± 0.20	5%
PIV	0.01	0.17	± 0.34	15%

MD, mean difference of repeated tests; SD, standard deviation of differences of repeated tests; Plus/minus the repeatability coefficient, express the limits of agreement for differences between repeated measurements; CV, coefficient of variation. TAV, time-averaged maximum velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; S/D, peak systolic/peak diastolic; PIV, pulsatility index for veins.

For the proximal venous pulmonary branch waveform parameters, the coefficient of variation (CV) varied from 6 to 13% (lowest, peak diastolic velocity; highest, end-diastolic velocity). For the middle venous pulmonary branch waveform parameters, the CV ranged between 6 and 23% (lowest, S/D ratio; highest, end-diastolic velocity) and for the distal venous pulmonary branch waveform, between 5 and 24% (lowest, S/D ratio; highest, end-diastolic velocity).

Venous pulmonary branch velocity parameters relative to gestational age

The regression equations for Doppler blood flow velocity parameters of middle and distal venous pulmonary branches relative to gestational age are described in Table 2. Statistically significant gestational age-related changes in middle and distal venous pulmonary flow velocity parameters are depicted in Figure 2-5.

For the *middle venous pulmonary branches*, a statistically significant increase with advancing gestational age was found for all velocity components and S/D ratio, apart from the PIV, which demonstrated a statistically significant reduction with advancing gestation (Figures 2 and 3). A significant increase existed for peak systolic velocity (Figure 2a) and TAV (Figure 3a) until 31-32 weeks of gestation and for end-diastolic velocity (Figure 2c) until 33 weeks of gestation. The peak systolic velocity and end-diastolic velocities did not change significantly thereafter until term, whereas the TAV showed a significant reduction from 35 weeks to term. The peak diastolic velocity (Figure 2b) displayed a significant rise from 20 to 31 weeks and a significant decline as from 33 weeks until term. The S/D ratio (Figure 3b) increased significantly as from 29 weeks until term.

In the *distal venous pulmonary branches*, peak systolic velocity (Figure 4a), peak diastolic velocity (Figure 4b), end-diastolic velocity (Figure 4c) and TAV (Figure 5a) demonstrated a statistically significant increase with advancing gestational age. Both TAV and peak diastolic velocity increased significantly until 31 weeks, followed by no significant change until term. The PIV (Figure 5b) displayed a significant decrease, whereas the S/D ratio remained unchanged throughout the second half of pregnancy.

The observed relationship between the venous flow velocity waveform parameters, PIV calculations of both middle and distal pulmonary branches and gestational age was independent of FHR.

Table 2

Regression equations for various venous pulmonary branch Doppler measurements and their ratios with gestational age

Doppler velocity parameters	*Transformation*	*Constant*	*A x GA*	*B x GA²*	*SD*		*R*	*p*
					Constant	*CxGA*		
Proximal branch[29]								
Middle branch								
TAV (cm/s)	-	-17.54	1.82	-0.028	1.96	-	0.50	<0.0001
PSV (cm/s)	-	-14.10	1.76	-0.026	2.44	-	0.46	<0.0001
PDV (cm/s)	-	-23.68	2.52	-0.040	2.62	-	0.47	<0.0001
EDV (cm/s)	-	-14.86	1.15	-0.016	0.34	0.04	0.54	<0.0001
S/D ratio	log (S/D)	0.43	-0.037	$6.9x10^{-4}$	0.09	-	0.34	0.003
PIV	log (PI)	0.25	-0.014	-	0.23	-	0.32	0.001
Distal branch								
TAV (cm/s)	-	-4.74	0.63	-0.0095	1.14	-	0.35	0.005
PSV (cm/s)	-	4.03	0.096	-	1.35	-	0.35	0.001
PDV (cm/s)	-	-6.03	0.83	-0.013	1.48	-	0.33	0.01
EDV (cm/s)	log (EDV)	0.53	0.016	-	0.28	-	0.30	0.01
S/D ratio*	log (S/D)	-0.02	-	-	0.09	-	0.16	NS
PIV	log (PI)	0.24	-0.015	-	0.32	-	0.24	0.03

*GA, gestational age; A, coefficient for linear components; B, coefficient for quadratic component; C, coefficient for linear component if the absolute residuals depended on GA; SD, residual standard deviation (as a linear function of GA if necessary); R, multiple correlation coefficient; p, significance of regression; *, when the regression was not significant, the mean and SD are presented.*

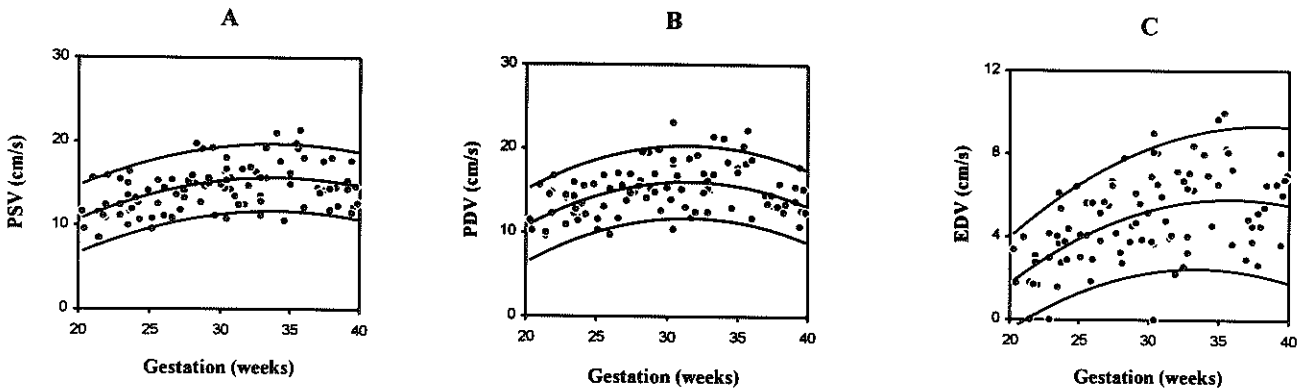

Figure 2

*Individual values and reference ranges (mean, 5th and 95th centiles) for (A) peak systolic velocity (PSV), (B) peak diastolic velocity (PDV) and (C) end-diastolic velocity (EDV) of the **middle venous** pulmonary branch relative to gestational age.*

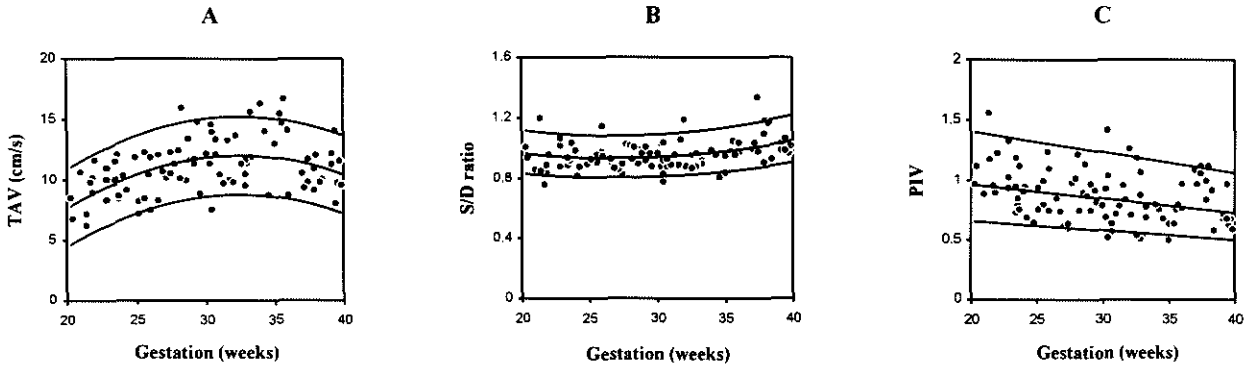

Figure 3
*Individual values and reference ranges (mean, 5th and 95th centiles) for (A) time averaged maximum velocity (TAV), (B) S/D ratio and (C) pulsatility index (PIV) of the **middle venous** pulmonary branch relative to gestational age.*

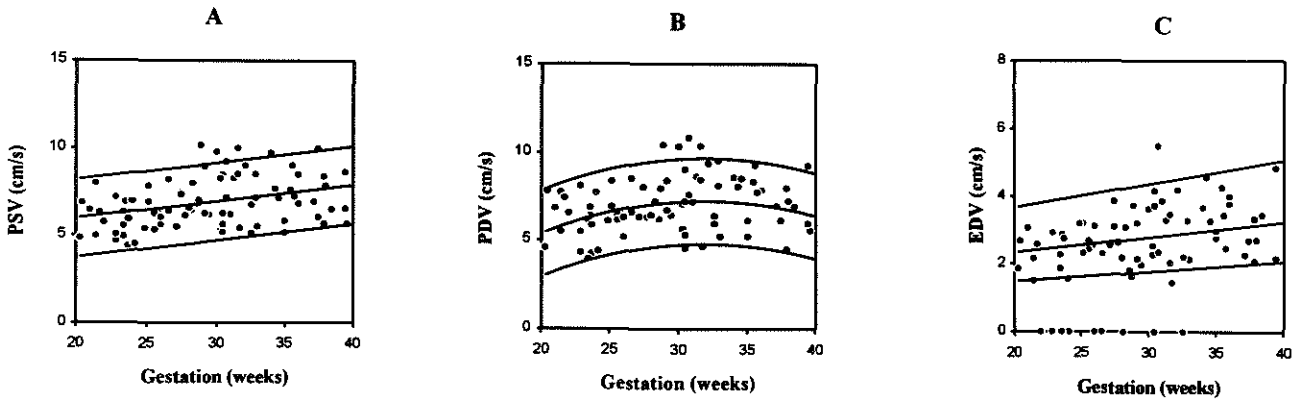

Figure 4
*Individual values and reference ranges (mean, 5th and 95th centiles) for (A) peak systolic velocity (PSV), (B) peak diastolic velocity (PDV) and (C) end-diastolic velocity (EDV) of the **distal venous** pulmonary branch relative to gestational age.*

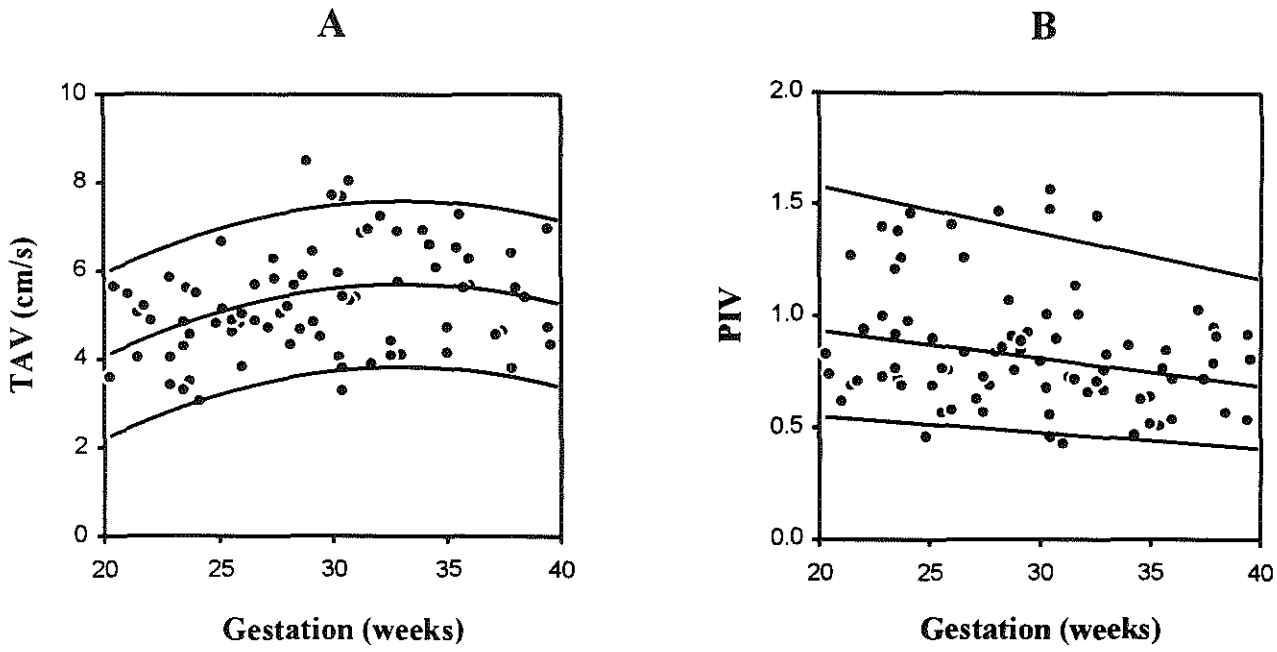

Figure 5
*Individual values and reference ranges (mean, 5th and 95th centiles) for (A) time-averaged maximum velocity (TAV) and (B) pulsatility index (PIV) of the **distal venous** pulmonary branch relative to gestational age.*

Inter-pulmonary (proximal, middle, distal) differences in venous blood flow velocity parameters

All mean differences in the Doppler flow velocity parameters between proximal and middle, between proximal and distal and between middle and distal venous pulmonary branches were statistically significant apart from the mean difference in PIV between middle and distal venous pulmonary flow velocity wave forms, which was not significant (Table 3).

No significant gestational age-related change existed for mean differences in (Table 3):

(i) peak systolic velocity, peak diastolic velocity, PIV and S/D ratio between middle and distal venous pulmonary branches;

(ii) end-diastolic velocity and PIV between proximal and middle venous branches;

(iii) PIV between proximal and distal venous branches.

Mean differences in S/D ratio between proximal and middle and between proximal and distal branches of the pulmonary vein revealed a significant decrease, whereas all other remaining mean differences displayed a significant rise with advancing gestational age (Table 3).

DISCUSSION

The present study describes characteristics and repeatability of Doppler flow velocity waveforms obtained from different locations of the human fetal pulmonary venous circulation during the second half of normal pregnancy. The cross-sectional study design adopted in the present study puts some limitation to the interpretation of the collected data. Venous pulmonary blood flow velocity waveforms obtained from one of the pulmonary veins just proximal to the entrance of the left atrium of the human fetus display a biphasic forward flow profile with a systolic and diastolic component reflecting dynamic changes in left atrial pressure as a result of atrial and ventricular contraction and relaxation.[17,28,29] These flow velocity waveform characteristics continue to be present in both middle and distal venous pulmonary branches although with lower peak velocities. Only in a few cases, no end-diastolic flow could be detected in the middle and distal venous pulmonary branches during atrial contraction. A late diastolic reverse flow component at pulmonary venous orifice level has been reported in 38%[30] and 18% of cases[31]. This was never seen in the present study, whether at proximal, middle or distal venous level. Both the sampling site, i.e. immediately proximal to the entrance into the left atrium, and FHR may play a role, since both measurements at the venous orifice level and at lower heart rates as seen in infants[26] were associated with well-defined late diastolic reverse flow. Alternatively, the wall filter setting of 70 Hz (1.44 cm/s) may have hidden minimal end-diastolic (reverse) flow in some instances. To our knowledge no other reports are available on Doppler velocimetry from more peripheral venous pulmonary branches.

Table 3
Differences in Doppler flow velocity parameters from proximal, middle and distal **venous** pulmonary branches in the second half of gestation

	NR of pairs	***MD****	***SD***	***Relation MD and GA***
TAV (cm/s)				
Proximal-distal	72	12.86	4.70	<0.0001
Proximal-middle	84	6.54	4.46	<0.0001
Middle-distal	80	5.87	2.18	0.03
PSV (cm/s)				
Proximal-distal	72	19.22	6.48	<0.0001
Proximal-middle	84	11.08	6.14	0.0002
Middle-distal	80	7.58	2.58	NS
PDV (cm/s)				
Proximal-distal	72	16.55	6.55	<0.0001
Proximal-middle	84	7.82	5.59	<0.0001
Middle-distal	80	8.14	2.94	NS
EDV (cm/s)				
Proximal-distal	72	6.99	3.67	0.0003
Proximal-middle	84	4.38	3.27	NS
Middle-distal	80	2.37	1.78	0.005
S/D ratio				
Proximal-distal	72	0.15	0.19	0.007§
Proximal-middle	84	0.18	0.18	0.01§
Middle-distal	80	-0.03	0.11	NS
PIV				
Proximal-distal	72	0.09	0.30	NS
Proximal-middle	84	0.07	0.24	NS
Middle-distal	80	0.03**	0.23	NS

*TAV, time-averaged maximum velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; S/D, peak systolic/peak diastolic; PIV, pulsatility index for veins; MD, mean differences; NR, number; MD, mean difference; SD, standard deviation; GA, gestational age; *, All MD were statistically significant (p<0.05), except the MD in PIV (**) between mid and distal venous branches. §, negative correlation with gestational age.*

The coefficients of variation for all velocity parameters, S/D ratios and PIV was below 15% for proximal, middle and distal branches, apart from the end-diastolic velocity of middle and distal venous pulmonary flow velocity waveforms (23% and 24%, respectively). Overall, the parameters from the proximal branch flow velocity waveform showed the lowest coefficients of variation. However, to judge repeatability for clinical practice, measurement variability expressed as plus/minus the repeatability coefficient should be taken into account; 95% of the differences between two repeated tests in the same arbitrary patient, will lie within this interval. This variability may be due to within-patient as well as intra-observer variation. There is only one other report[31] that has studied repeatability, although only for waveforms obtained from the proximal venous pulmonary branches and not from more distal branches. Unfortunately, it is not possible to compare our repeatability data with the outcomes from that particular study, since it did not report from which velocity parameters the recounted mean differences were calculated.[31]

In a previous study[29], a gestational age-dependent rise in flow velocities (TAV, peak systolic, peak diastolic and end-diastolic velocities) was established from the proximal venous pulmonary branches. It was speculated that an increase in volume flow and pressure gradient between the pulmonary venous system and the left atrium may play a role in this.[29] Rasanen and collegues[36] demonstrated in the human fetus an increase from 13% to 25% in the proportion of pulmonary flow when related to combined cardiac output at 20-30 weeks of gestation. This proportion remained unchanged during the remainder of pregnancy. This is consistent with the present observation that, for the middle and distal venous pulmonary branches, a gestational age-dependent rise in flow velocities was obtained for all waveform velocity parameters, mainly evident in the second trimester and first part of the third trimester.

The pulsatile pattern of flow velocity waveforms in the pulmonary venous system is the result of changes in pressure difference between the pulmonary venous system and the left atrium.[3] Based on the linear decrease in PIV for both the middle and distal venous pulmonary branches with gestation, an increase in pulmonary venous pressure gradient is suggested. This may be the result of a decline in proportion of volume flow through the foramen ovale[36] and/or a decrease in cardiac afterload, due to a reduction in placental resistance.[3,23] The decrease in cardiac afterload causes a decrease in end-diastolic ventricular pressure and therefore an increase in blood flow velocity during atrial contraction. This is demonstrated by the significant increase in end-diastolic velocity in the proximal[29], middle and distal venous pulmonary branches. Unfortunately, we cannot compare our outcomes on middle and/or distal human fetal venous pulmonary hemodynamics with other studies, since these, to our knowledge, are not available.

When the inter-relationship between proximal, middle and distal venous pulmonary flow velocity waveforms, all velocity parameters demonstrated a significant change from proximal to distal level. Mean peak systolic velocity and peak diastolic velocity drop 1.8- and 1.6-fold from proximal to middle and 3.7- and 3.4-fold from proximal to distal, whereas mean end-diastolic drops 1.9-fold and 3.8-fold, respectively. Changes in vessel diameter, increase in vessel branching and increase in distance between the heart and distal venous pulmonary vessels may account for these observations.[11] Although the PIV remained unchanged as pulsed Doppler interrogation proceeds from middle to distal, it changed significantly from proximal to middle and from proximal to distal. The mean differences of PIV between the three locations were not related to gestational age. This suggests that the pulsatility of the venous pulmonary system is also influenced by the distance between the heart and the site of Doppler interrogation and/or vessel diameter.

It can be concluded that color-coded Doppler systems allow reliable Doppler velocity waveform recordings to be obtained in the more distal parts of the human fetal pulmonary venous system during the second half of pregnancy. Pulmonary venous flow velocity waveforms are not essentially different at proximal, middle or distal levels. Acceptable repeatability of flow velocity waveforms with coefficients of variation below 15% was established for nearly all velocity parameters and their ratios. A gestational age-dependent change was found for all flow velocity waveform parameters including PIV at both middle and distal level. It is speculated that increases in volume flow and venous pulmonary pressure gradient play a role in these findings. Nearly all waveform parameters alter significantly as pulsed Doppler interrogation proceeds from proximal to more distal venous lung regions. This may be due to changes in vessel diameter and distance between the heart and more distal venous pulmonary vessels.

4.4 Fetal pulmonary venous flow into the left atrium relative to diastolic and systolic cardiac time intervals

C. Brezinka[1], J.A.M. Laudy[1], N.T.C. Ursem[1], W.C.J. Hop[2], J.W. Wladimiroff[1]

Department of Obstetrics and Gynaecology[1], University Hospital Rotterdam; Institute of Epidemiology and Biostatistics[2], Erasmus University Medical School Rotterdam, The Netherlands

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ABSTRACT

Objective: To establish the nature and gestational age dependency of the pulmonary venous flow velocity pattern into the left atrium relative to systolic and diastolic phases of the cardiac cycle.

Design: This was a cross-sectional study of Doppler measurements of fetal pulmonary venous inflow velocities, which were correlated with simultaneous recordings of transmitral and aortic flow velocity waveforms based on an equal cardiac cycle length (± 5%).

Results: Successful recordings were obtained in 28 out of 60 (47%) normal singleton pregnancies at 20-36 weeks of gestation. Reproducibility of waveform analysis of the various phases of the cardiac cycle was satisfactory with within-patient variance ranging between 1.7% and 6.5%. A statistically significant increase ($p< 0.05$) in pulmonary venous time-averaged velocity and velocity integral with advancing gestational age was established. A statistically significant increase ($p< 0.05$) of pulmonary flow velocity integral was also found when related to each of the systolic and diastolic segments of the cardiac cycle with the exception of isovolemic relaxation time. The duration of each of the diastolic and systolic segments of the cardiac cycle, as well as the pulmonary venous velocity integral expressed as percentage of the cardiac cycle, remained constant with advancing gestational age.

Conclusions: The second half of pregnancy is characterized by pulmonary venous inflow into the left atrium throughout the cardiac cycle. Pulmonary venous inflow into the left atrium occurs predominantly during the filling and ejection phase of the cardiac cycle. Absolute cardiac diastolic and systolic time intervals as well as the percentage distribution of pulmonary venous flow velocity integral between these cardiac time intervals remain unchanged with advancing gestational age.

INTRODUCTION

Recently, a number of reports have appeared on pulmonary venous flow velocity patterns during fetal life.[28-31,37] These patterns, which show great similarity with flow velocity waveforms originating from the ductus venosus, are characterized by a systolic and early diastolic forward flow component followed by a late diastolic forward flow[29-31], absent or reverse flow[30,31] component coincident with atrial contraction.

Simultaneous recordings of transmitral and aortic flow velocities have provided detailed information on diastolic and systolic time intervals in the developing heart.[38] A study of pulmonary venous inflow into the left atrium corresponding with these different cardiac time intervals may further deepen our understanding of normal cardiac function and may serve as a reference for fetal developmental pathology. The objective of the study was to establish the nature and gestational age dependency of the pulmonary venous flow velocity pattern into the left atrium relative to systolic and diastolic phases of the cardiac cycle.

PATIENTS AND METHODS

Study subjects

A total of 60 healthy, non-smoking women with a normal singleton pregnancy recruited from our routine antenatal clinic consented to participate in the study. Gestational age varied between 20 and 36 weeks (mean 27 weeks) and was calculated from a reliable menstrual history or from early ultrasonic measurement of crown-rump length or biparietal diameter. Maternal age ranged between 16 and 36 years (median 24 years); parity varied between 1 and 5 (median 2). Women were selected to obtain a homogeneous distribution over the gestational age period studied. Each woman participated in the study only once. All pregnancies were uncomplicated. All women gave birth to a healthy infant with a birth weight between the 10th and 90th centile of weight for gestation.[32]

Recording technique

Ultrasound Doppler studies were performed on a Toshiba SSH 140A (Toshiba Corp. Medical Systems Division, Tokyo, Japan) with a combined transabdominal real-time and pulsed Doppler system (carrier frequency 3.75 MHz in Doppler mode and 5 MHz in B-mode). Spatial peak temporal average power output was less than 100mW/cm2 in both imaging and Doppler modes, according to manufacturers' specifications. Recordings were performed with the woman in a semi-recumbent position. After a clear sonographic transverse cross-section of the fetal chest was obtained at the level of the five-chamber view, the sample volume was set at 3-4 mm to cover the area immediately distal to the mitral and the aortic valves. Pulsed wave Doppler was switched on and transmitral wave flow patterns were recorded simultaneously with ventricular ejection patterns into the ascending aorta. Only transmitral waveforms consisting of a clear E- and A-

wave and a distinctive aortic flow pattern were accepted. The smallest detectable time interval of the Doppler equipment used was 1 ms. The angle of insonation was kept below 30 degrees. Subsequently, color Doppler was used to visualize one of the pulmonary veins. Depending on the fetal position, the sample volume was placed over the best visualized vein of the right or left lung, just proximal to the entrance into the left atrium. Maximum flow velocity envelopes were collected during fetal apnea, since fetal breathing movements modulate venous Doppler waveforms.[6] For the sake of depicting single waveforms with maximum accuracy, the fastest possible scrolling speed option of 12.5 cm/s was selected. All Doppler recordings were performed by one examiner (C.B.). Pulmonary venous flow velocity recordings and simultaneous recordings of transmitral and ascending aortic flow velocity waveforms were selected on the basis of equal cardiac cycle length (±5%). Comparison of pulmonary venous inflow velocity and simultaneously registered transmitral and transaortic flow velocities was feasible through synchronization of the transmitral peak A-wave velocity and the pulmonary venous end-diastolic trough, both of which coincide with atrial contraction (Figure 1).[37,38] The mean value was calculated from measurements during two consecutive cardiac cycles.

Waveform analysis

Technically acceptable flow velocity waveforms were documented on hard copies during each of the recordings. The largest possible format of hardcopies was chosen (12.5 x 10 cm graph size). Off-line waveform analysis was performed on a microcomputer (Commodore 386 CX) linked to a graphics tablet as described previously.[39]

First, the time-averaged velocity (cm/s) and flow velocity integral (cm) for the entire pulmonary venous waveform were determined. This was followed by the calculation of filling time (ms) and ejection time (ms) on the simultaneous recording of transmitral and ascending aortic flow velocities. Filling time is defined as the time interval between the beginning and the end of the transmitral flow curve, ejection time is defined as the time interval between the beginning and the end of the aortic flow curve. Isovolemic relaxation time (ms) represents the time interval between the end of the aortic flow velocity waveform and the onset of the transmitral flow velocity waveform, whereas isovolemic contraction time (ms) lasts from the end of the transmitral flow velocity waveform to the onset of the aortic flow velocity waveform.

This was followed by dividing up the pulmonary venous waveform into four segments corresponding to the systolic (ejection time and isovolemic contraction time) and diastolic (filling time and isovolemic relaxation time) phases of the cardiac cycle. Flow velocity integral measurements of these four segments were subsequently performed on the pulmonary vein tracing.

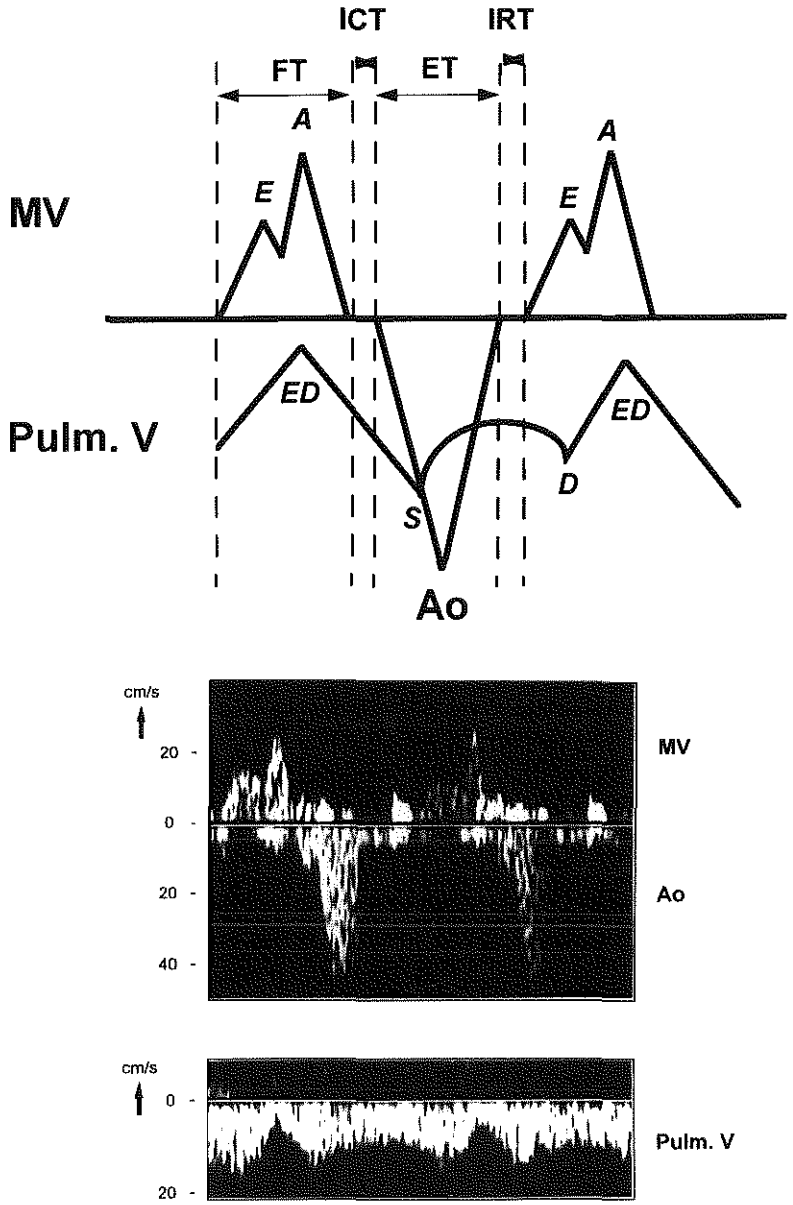

Figure 1
Top: Schematic presentation of pulmonary venous flow velocity waveforms relative to simultaneous recordings of mitral and aortic flow velocity waveforms. MV, transmitral flow velocity waveform with E- and A-waves; Ao, aortic flow velocity waveform; PV, pulmonary venous flow velocity waveform with S (peak systolic; cm/s), D (peak diastolic; cm/s) and ED (end-diastolic; cm/s) velocities. FT, filling time (s); ICT, isovolemic contraction time (s); ET, ejection time (s); IRT, isovolemic relaxation time (s). Bottem: Example of Doppler recordings of pulmonary venous flow velocity waveforms relative to simultaneous recordings of mitral and aortic flow velocity waveforms.

Reproducibility study

In order to assess reproducibility of the measurements of pulmonary vein flow corresponding to the different phases of the cardiac cycle, a reproducibility study was performed. A total of 15 women, with acceptable Doppler recordings were selected: five at 21-25 weeks, five at 26-30 weeks and five at 31-35 weeks of gestation. Five copies were made of each hard copy, resulting in a total of 75, which were numbered, shuffled into random order and analyzed on the graphics tablet in the manner described.

Statistical analysis

All data obtained during the measurements were analyzed using the SPSS for Windows package (Torrance, CA, USA). The relationships between the various parameters and gestational age were analyzed by least squares linear regression. Pearson's correlation coefficients (r) are given.

To assess the reproducibility, analysis of variance was used in which the total variation was divided into the variation between fetuses and the variation between hard copy analyses within fetuses. Data are represented as mean $\pm$ SD. Statistical significance was set at $p < 0.05$.

RESULTS

Technically acceptable flow velocity waveforms were collected in 28 out of 60 women, resulting in a success rate of 47%. Recording failures were caused by fetal movements, fetal breathing, maternal obesity and unfavorable fetal position. In 12 patients (38% of all failures) recordings were omitted from further analysis since, there was a cardiac cycle difference of more than 5% between the measurement of aortic/mitral waveform and pulmonary vein waveforms.

Data on reproducibility are shown in Table 1. Within patient variance as percentage of total variance was always situated below 7%.

Table 1

Reproducibility of the pulmonary venous flow velocity integral measurement corresponding to each of the diastolic and systolic phases of the cardiac cycle.

	Within-patient variance ***(cm)***	***Between-patient variance*** ***(cm)***	***Within-patient variance as percentage of total variance*** ***(%)***
Filling time	0.0132	0.6552	1.7
Isovolemic contraction time	0.004	0.0749	5.4
Ejection time	0.0079	0.6816	1.1
Isovolemic relaxation time	0.0027	0.0386	6.5

Cardiac cycle length remained constant at 428 ± 21.1 ms throughout the observation period. A statistically significant increase ($p< 0.05$) in pulmonary venous time-averaged velocity (Figure 2) and velocity integral (Figure 3) with advancing gestational age was established. A statistically significant increase ($p< 0.05$) of pulmonary flow velocity integral was also found when related to each of the systolic and diastolic segments of the cardiac cycle with the exception of isovolemic relaxation time (Figures 4-7).

The duration of each of the diastolic and systolic segments of the cardiac cycle (Table 2) as well as the pulmonary venous velocity integral expressed as percentage of the cardiac cycle (Table 3) remained constant with advancing gestational age.

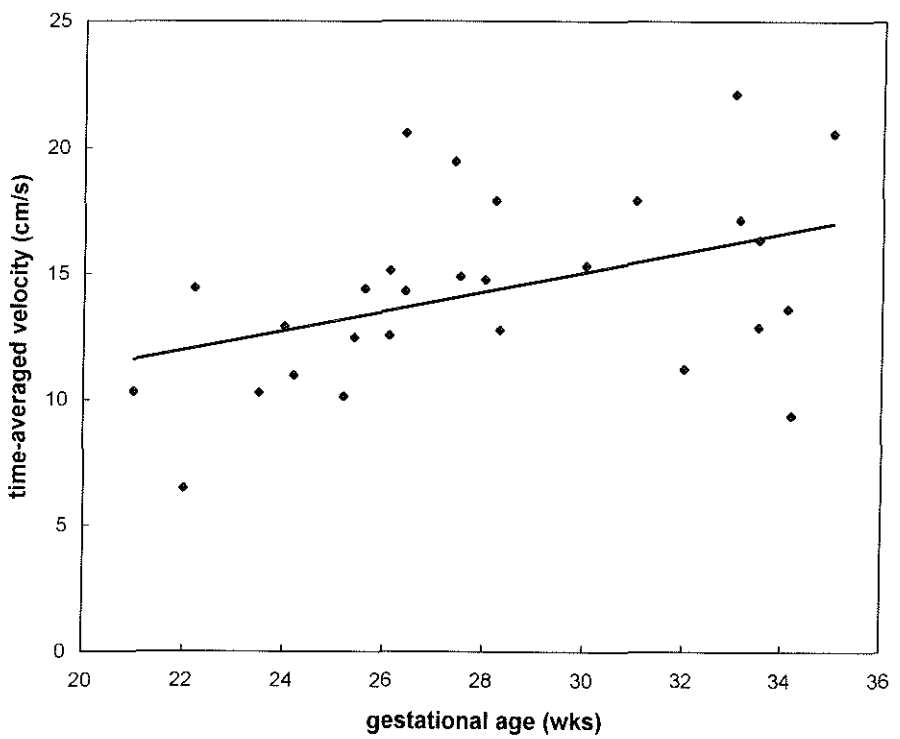

Figure 2
Fetal pulmonary venous time-averaged velocity relative to gestational age. Regression line, with slope = 0.37cm/s per week; r=0.41, p=0.03

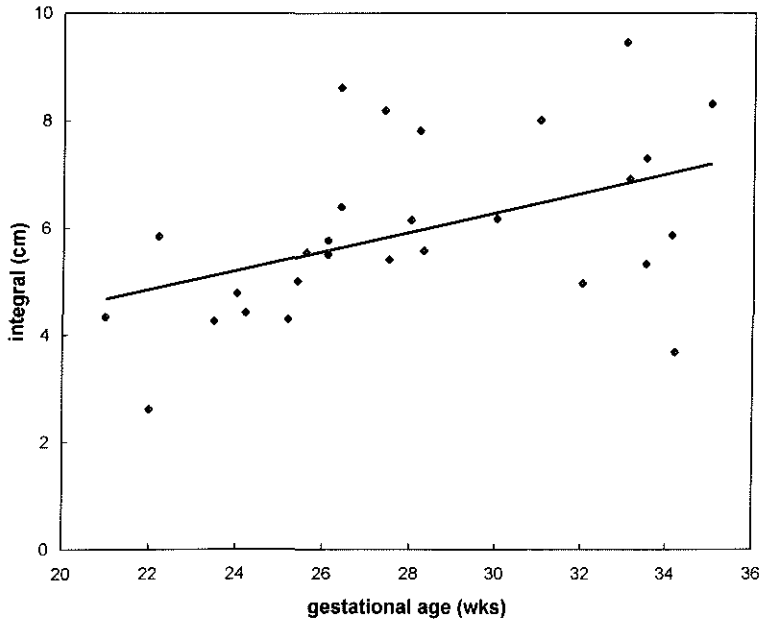

Figure 3
Fetal pulmonary venous flow velocity integral relative to gestational age. Regression line, with slope = 0.16 cm/week; r=0.40; p=0.03

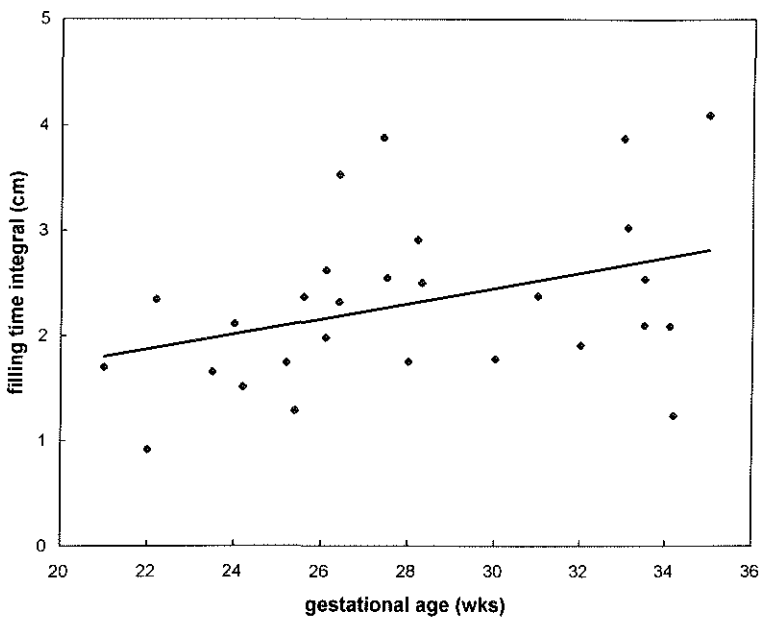

Figure 4
Fetal pulmonary venous flow velocity integral during filling time relative to gestational age. Regression line, with slope = 0.07 cm/week; r=0.38; p=0.047

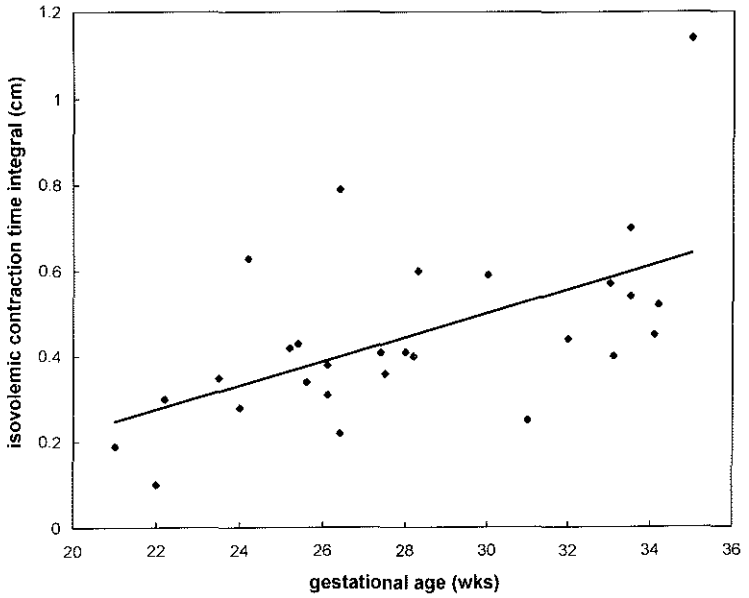

Figure 5
Fetal pulmonary venous flow velocity integral during isovolemic contraction time relative to gestational age. Regression line, with slope = 0.02 cm/week; r=0.50; p=0.007

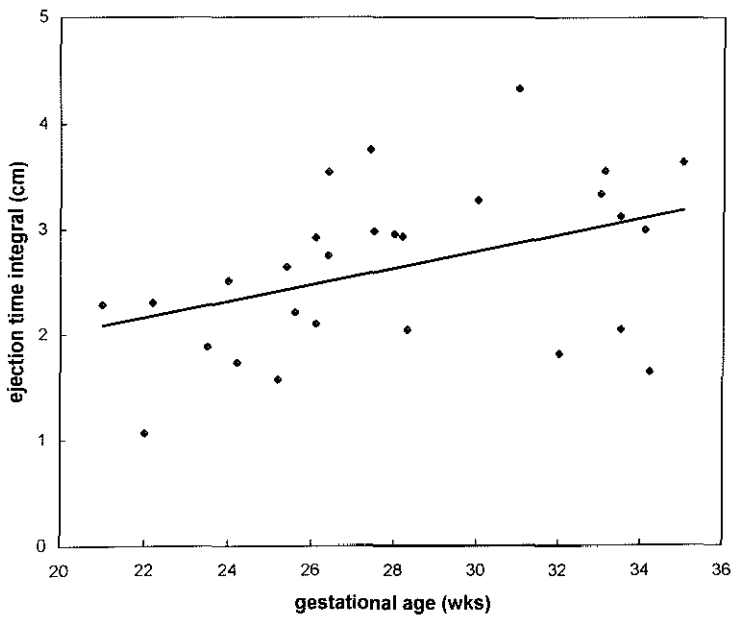

Figure 6
Fetal pulmonary venous flow velocity integral during ejection time relative to gestational age. Regression line, slope = 0.08 cm/week; r=0.41, p=0.03

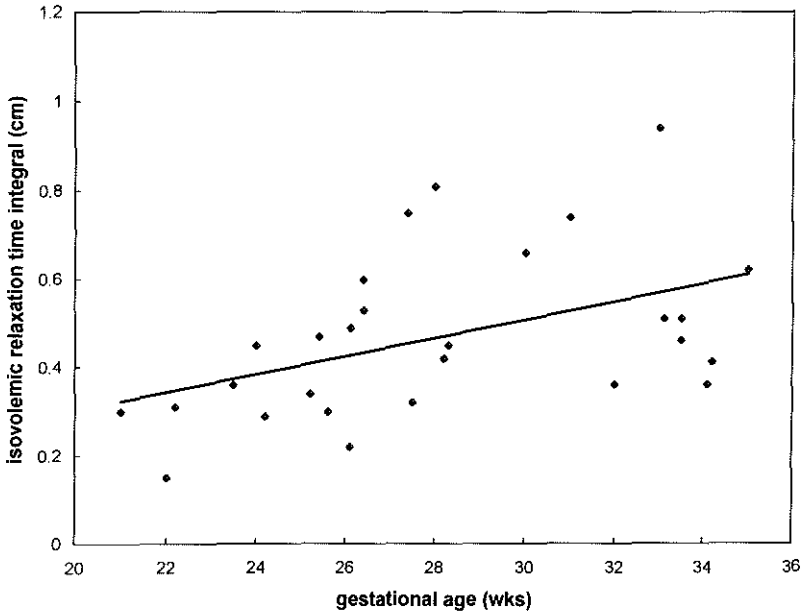

Figure 7
Fetal pulmonary venous flow velocity integral during isovolemic relaxation time relative to gestational age. Regression line, with slope = 0.02 cm/week, r=0.35, NS

Table 2
Mean ±SD for each of the diastolic and systolic phases of the cardiac cycle (ms)

	mean	***SD***
Filling time (ms)	184.5	23.9
Ejection time (ms)	179.8	16.6
Isovolemic contraction time (ms)	26.1	8.1
Isovolemic relaxation time (ms)	37.6	9.3
Heart cycle length (ms)	428.0	21.1

Table 3
Mean ±SD and percentage of pulmonary venous integral (cm) corresponding to each of the diastolic and systolic phases of the cardiac cycle.

	mean	***SD***	***%***
Filling time (cm)	2.32	0.80	39
Ejection time (cm)	2.65	0.78	45
Isovolemic contraction time (cm)	0.45	0.20	7.5
Isovolemic relaxation time (cm)	0.47	0.18	8.5

DISCUSSION

The present study presents first data on pulmonary venous inflow into the left atrium relative to the different systolic and diastolic time components of the fetal cardiac cycle. The fetal pulmonary venous waveform consists of a systolic and early diastolic forward flow component followed by a late diastolic forward flow component coincident with atrial contraction.[29] There is pulmonary venous inflow into the left atrium throughout the cardiac cycle. The trough caused by atrial contraction is followed by an increase in velocity towards atrial diastole. As atrial capacitance decreases, flow velocity stabilizes but increases again as the mitral valve opens. The lowest velocity of the fetal pulmonary venous flow velocity waveform pattern is reached at the peak of the mitral A-wave when there is rapid emptying of the left atrium into the left ventricle. In the adult, there is virtually no measurable flow from the pulmonary vein into the left atrium at this point of late diastole[17], whereas in the fetus forward[29-31], absent or even reverse flow[30,31] flow may be observed during atrial contraction. In the adult the pulmonary venous inflow pattern mirrors the left atrial pressure waveform.[16] If a similar relationship is assumed in the fetus, then the systolic component of the pulmonary venous flow velocity waveform would coincide with a reduction in atrial pressure.

The increase in both pulmonary venous time average flow velocity and flow velocity integral with advancing gestational age may reflect a rise in volume flow as a result of an increase in pulmonary vasculature in the growing fetus.[19,25] Van Splunder and Wladimiroff[38] have demonstrated that it is possible to determine diastolic and systolic time relationships from simultaneous left ventricular inflow and outflow tract flow velocity waveforms. Our data demonstrate that a gestational age-related increase in pulmonary venous flow velocity integral was also observed for each of the diastolic and systolic cardiac time intervals, except for the isovolemic relaxation time. These data further support the view that the previously mentioned increase in flow velocity is volume flow determined. Alternatively, the increase in pulmonary venous flow velocity could be explained by a rise in pulmonary venous pressure gradient with advancing gestational age as a result of increasing relaxation in both the ventricles for the systolic increase and the atria for the diastolic increase.[40,41]

Our reproducibility study of the pulmonary venous flow velocity integral relative to the different diastolic and systolic cardiac time intervals demonstrates that between-patient variance is considerably larger than within-patient variance. The latter, expressed as percentage of total variance, is excellent for filling time (1.7%) and ejection time (1.1%), and acceptable for isovolemic contraction time (5.4%) and isovolemic relaxation time (6.5%). For both the isovolemic contraction and relaxation time, the time intervals are relatively short, corresponding to 2-6 mm paper length on the hard copy x axis. The increase in total variance can be explained by lack of precision in the manual measurements of very short tracings for the

latter two components of the cardiac cycle. This may also explain the lack of change in isovolemic relaxation time with advancing gestational age.

When looking at the absolute values for diastolic and systolic cardiac time intervals, no significant change could be established with advancing gestational age. This suggests that intrinsic myocardial properties do not alter during the normal second half of pregnancy. Also, pulmonary venous flow velocity integral expressed as percentage of the cardiac cycle does not change when related to the different cardiac time intervals. Approximately 84% of pulmonary venous inflow into the left atrium occurs during the filling and ejection phase of the cardiac cycle.

It can be concluded that the second half of pregnancy is characterized by pulmonary venous inflow into the left atrium throughout the cardiac cycle. Pulmonary venous inflow into the left atrium occurs predominantly during the filling and ejection phase of the cardiac cycle. Absolute cardiac diastolic and systolic time intervals as well as the percentage distribution of pulmonary venous flow velocity integral between these cardiac time intervals remain unchanged with advancing gestational age.

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CHAPTER 5

FETAL BEHAVIOR STATES

5.1 Introductory remarks

It has been demonstrated that fetal blood flow is modulated by intrinsic fetal variables such as breathing movements and behavior states. Doppler velocimetry has shown that behavior state dependent changes occur at all three fetal shunts, e.g. the ductus venosus, foramen ovale and ductus arteriosus during the last few weeks of normal pregnancy. Information on possible changes in arterial and venous pulmonary flow velocity waveforms would provide us with a more complete picture of the total fetal circulation during the different behavior states. It could be anticipated that the clear shift of blood from the right to the left heart which was established during active sleep, is associated with changes in pulmonary blood flow.This chapter deals with Doppler velocimetry in the fetal lung relative to quiet sleep (behavior state 1F) and active sleep (behavior state 2F) in the normally developing term fetus.

5.2 Behavior-state-dependent changes in human fetal pulmonary blood flow velocity waveforms

N.S. Macklon[1], J.A.M. Laudy[1], P.G.H. Mulder[2], J.W. Wladimiroff[1]
Department of Obstetrics and Gynaecology[1], University Hospital Rotterdam, Institute of Epidemiology and Biostatistics[2], Erasmus University Medical School, Rotterdam, The Netherlands

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ABSTRACT

Objective: To establish the influence of fetal behavior states on venous and arterial pulmonary flow velocity waveforms in the normally developing term fetus.

Methods: The relation between venous and arterial pulmonary blood flow velocity waveforms and fetal behavior states was investigated in 18 normal term fetuses. Recordings of the venous pulmonary blood flow velocity waveforms were obtained just proximal to the entrance in the left atrium, and of the arterial pulmonary blood flow velocity waveforms were taken from the most proximal branch of the pulmonary artery in the same lung using color Doppler imaging. Time-averaged, peak systolic, peak diastolic and end-diastolic flow velocity, peak systolic/peak diastolic ratio, pulsatility index and fetal heart rate were calculated from both venous and arterial Doppler recordings obtained during behavior state 1F (quiet sleep) and 2F (active sleep). Fetal behavior states were determined from combined recordings of fetal eye and body movements.

Results: Recordings of sufficient quality for analysis were obtained from ten fetuses. Venous pulmonary blood flow velocity waveforms demonstrated a statistically significant increase in time-averaged, peak diastolic and end-diastolic velocity during fetal behavior state 2F. No behavior-state-related changes were observed for the arterial pulmonary blood flow velocity waveform.

Conclusions: The data suggest an increased pressure gradient between the pulmonary venous system and the left atrium during behavior state 2F. Flow velocity waveforms from the proximal arterial pulmonary branch are independent of behavioral state.

INTRODUCTION

A classification of behavior states in the full-term newborn was introduced by Prechtl and Beintema[1] on criteria such as opened or closed eyes, regular or irregular respiration, and present or absent body movements. With the advent of diagnostic ultrasound, the emergence of fetal behavior was demonstrated in late pregnancy. Eventually, well-defined behavior states could be identified with fetal behavior state 1F (quiet sleep) and 2F (active sleep) being most prevalent.[2]

Doppler ultrasound studies suggested that fetal behavior states are associated with specific hemodynamic adaptations. An increase in blood flow to the left heart may help to meet raised energy demands at fetal trunk and cerebral level during the active sleep state.[3,4] This is supported by the observed increase in time-averaged velocities in the ductus venosus[5] and at the level of the foramen ovale[6], indicating a rise in venous inflow into the left heart during this behavior state.

The fetal pulmonary circulation has been the subject of recent study, and reference ranges have been described for both the fetal venous pulmonary blood flow velocity waveform[7,8] and the fetal arterial pulmonary branch blood flow velocity waveform[9,10]. The clinical value of fetal pulmonary circulation assessment remains to be fully evaluated, but recent data have suggested that the study of pulmonary flow velocity waveforms may aid in the detection of lung hypoplasia.[11] Knowledge of behavior-state dependency of fetal pulmonary hemodynamics may further our understanding of circulatory adaptation to behavior-state changes and improve the clinical interpretation of fetal pulmonary blood flow velocity waveforms. In the present study, we addressed whether, as in the systemic circulation, behavior-state-dependent changes can be demonstrated in fetal arterial and venous pulmonary blood flow velocity waveforms in normal term fetuses.

MATERIAL AND METHODS

Study design

The study protocol was approved by the Hospital Ethics Committee. Over a period of 6 month, 18 women at 37 weeks' gestation or more with a normal singleton pregnancy were recruited to the study from the antenatal clinic. Gestational age at entering the study was determined from the last reliable menstrual period and confirmed or adjusted by sonographic measurement of fetal crown-rump length or biparietal diameter (8-12 weeks). Maternal age varied between 18 and 38 years (median 28 years). All participants were nonsmokers and, except for iron tablets, were receiving no medications. Birth weight was between the 10th and 90th percentiles, corrected for maternal parity and fetal sex.[12] There were no structural anomalies.

Recording technique

Subjects were examined in the semisupine position. Blood flow velocity waveforms were obtained from the pulmonary circulation using a combined curved linear-array and pulsed Doppler system (Toshiba SSH-140,Toshiba Corp., Medical Systems Division, Tokyo, Japan). The carrier frequency was 5 MHz (real-time) and 3.75 MHz (Doppler). The high-pass filter was set at 70-100 Hz. The spatial peak temporal average power output was less than 100 mW/cm^2 in imaging and Doppler modes, according to manufacturer's specifications.

Recordings of venous and arterial pulmonary blood flow velocity waveforms were obtained at the level of the cardiac four-chamber view. First, the real-time transducer was angled to image the pulmonary venous drainage into the left atrium.[7] Depending on fetal position, the sample volume was placed over the best-visualized vein of the left or right lung just proximal to the entrance in the left atrium. When the pulmonary vessels were difficult to image, color Doppler imaging was used to aid identification. After obtaining technically acceptable pulmonary venous recordings, we placed the sample volume over the most proximal branch of the pulmonary artery in the same lung using color Doppler imaging.[10] For both venous and arterial recordings, the Doppler sample length was 0.1-0.3 cm and the angle of interrogation was maintained below 20°.

As described previously, the venous waveform[7] consists of a systolic and early diastolic forward component of flow and a late diastolic forward component of flow coincident with atrial contraction (Figure 1A). The arterial waveform[10] consists principally of a rapid systolic acceleration phase, followed by an initially equally rapid deceleration and a second acceleration to forward flow in diastole (Figure 1B).

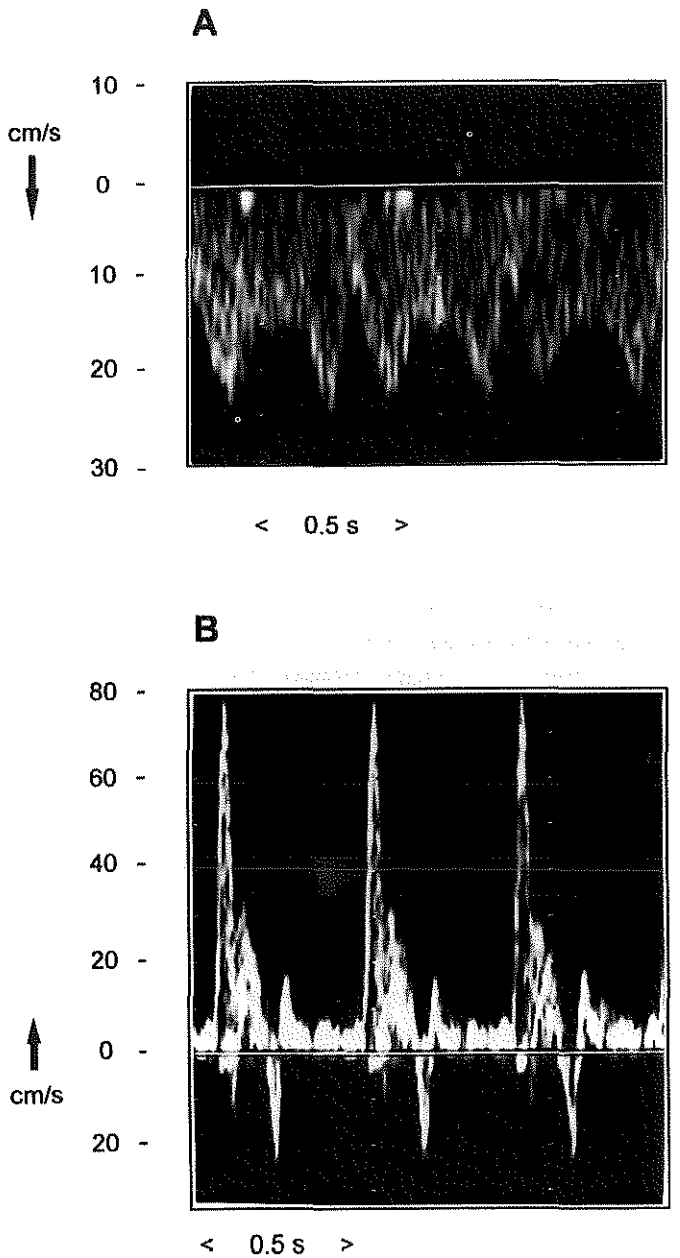

Figure 1
Doppler ultrasound recordings of blood flow velocity waveforms from the pulmonary vein just proximal to the entrance into the left atrium (A) and from the most proximal branch of the pulmonary artery (B) in the term fetus.

All recordings were performed by one examiner (N.S.M.) during absence of fetal breathing movements. Recordings were continued until three consecutive, technically satisfactory venous and arterial pulmonary waveforms during a particular behavior state were obtained. Hard copies were made of these venous and arterial waveforms during behavior states 1F (quiet sleep) and 2F (active sleep). A microcomputer (Olivetti M24; Olivetti BV, Leiden, The Netherlands) linked to a graphic tablet was used to analyze the Doppler recordings. Both venous and arterial were subject to analysis of the following indices: time-averaged maximum velocity (TAV, cm/s), peak systolic velocity (cm/s), peak diastolic velocity (cm/s), peak systolic/peak diastolic ratio (S/D ratio), and end-diastolic flow velocity (cm/s). The pulsatility index (PI) of both venous and arterial waveforms was calculated by dividing the difference between peak systolic velocity and end-diastolic flow velocity during atrial contraction by time-averaged maximum velocity, according to Gosling and King.[13] Fetal heart rate (FHR) was established from the time interval between peak systolic velocities from two consecutive cardiac cycles.

Repeatability

In a different study, the repeatability of venous and arterial pulmonary blood flow velocity waveforms was determined in 25 women with a normal, uncomplicated singleton pregnancies. Venous and arterial recordings were obtained at approximately 15-minute intervals. Repeatability was expressed by the coefficient of variation and the reliability index from two repeated tests. The coefficient of variation was defined as the standard deviation (SD) of the error in a single test and was expressed as a percentage of the population mean of the test variable considered. The reliability index was defined as the intraclass correlation coefficient between the two repeated tests, which equals the between subjects variance as a fraction of the total variance. For the proximal venous pulmonary waveform indices, the reliability index varied between 0.75 and 0.97, with the lowest reliability index for PI and the highest for peak diastolic velocity. The coefficient of variation ranged from 6% to 13% (lowest: peak diastolic velocity; highest: end-diastolic velocity). For the proximal arterial pulmonary branch waveform, the reliability index varied between 0.57 and 0.98 (lowest: end-diastolic velocity; highest: peak systolic velocity). The coefficient of variation ranged between 3% and 13% (lowest: peak systolic velocity, highest: end-diastolic velocity) (unpublished data).

Fetal behavior states

To establish fetal behavior states 1F and 2F, we recorded the following variables.[2] First, fetal eye movements were observed by ultrasonic visualization of the fetal eye lens in a transverse scanning plane through the orbits using the two-dimensional real-time scanner. Immediately after establishing the presence or absence of eye movements, we scanned the

fetal trunk in a sagittal plane to confirm body movements. Fetal behavior state 1F was characterized by the combination of complete absence of fetal eye movements and quiescence of fetal body movements, with occasional startles. Fetal behavior state 2F was defined as the continuous presence of fetal eye movements and frequent periods of gross fetal body movements. Flow velocity waveforms were accepted for analysis when a clear fetal behavior state had been identified and when this state had been present for at least 3 minutes. Fetal heart rate, which constitutes another variable for establishing the fetal behavior state, was not recorded because of interference between the two-dimensional real-time transducer and Doppler ultrasound cardiotocograph.

Statistical analysis

The paired Student's *t* test was used to test the null hypothesis that there was no difference in the individual waveform components between behavior states 1F and 2F. For each of the Doppler waveform indices, statistical significance was considered to be present at $p<0.05$. Data are presented as mean ± SD.

RESULTS

Data from the behavior state study are presented in Tables 1 and 2 (next page). Doppler signals of adequate quality could not be obtained in eight women, leaving ten women for further analysis. Failure was due to either fetal position or fetal breathing movements both in behavior states 1F and 2F. Venous pulmonary blood flow velocity waveforms demonstrated a statistically significant increase in TAV, peak diastolic and end-diastolic velocity during fetal behavior state 2F. No behavior-state-related changes were observed for the arterial pulmonary branch blood flow velocity waveform.

DISCUSSION

In normal third-trimester pregnancies, fetal neurologic development, expressed by the emergence of fetal behavior and eventually resulting in well-defined behavior states, is clearly associated with specific hemodynamic adaptations in the fetal systemic circulation, as demonstrated by Doppler flow velocimetry.

In the present study, we examined the behavior-state dependency of the normal fetal pulmonary circulation at term. An increase of approximately 40% was established for pulmonary venous TAV during fetal behavior state 2F. This increase may arise from a reduction in venous vessel diameter, an increase in pressure gradient between the pulmonary venous system and the left atrium, or an increase in volume flow through the pulmonary vein. Accurate determination of volume flow by ultrasound may be prone to error, mainly caused by inaccuracies in the determination of the vessel diameter.

Table 1
Comparison of venous pulmonary Doppler blood flow velocity indices measured during behavior states 1F and 2F.

Doppler waveform index (n=10)	***1F***	***2F***	***Mean difference (1F-2F)***	***95% CI of difference***
TAV (cm/s)	14.4 ±4.2	20.2 ±5.1	-5.8 ±6.9 [a]	-11.1, -0.5
PSV (cm/s)	22.2 ±6.4	27.2 ±4.5	-5.1 ±8.2	-11.4, 1.2
PDV (cm/s)	19.4 ±4.8	26.5 ±6.1	-7.1 ±8.3 [b]	-13.5, -0.7
S/D ratio	1.1 ±0.2	1.1 ±0.1	0.1 ±0.2	-0.1, 0.3
EDV (cm/s)	5.7 ±3.0	10.0 ±3.9	-4.3 ±5.6 [c]	-8.6, -0.1
PI	1.2 ±0.4	0.9 ±0.3	0.3 ±0.5	-0.1, 0.6
FHR (beats/min)	133 ±9.6	136 ±8.4	-3.7 ±8.0	-9.8, 2.5

CI, confidence interval; TAV, time-averaged velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; S/D ratio, peak systolic/peak diastolic ratio; EDV, end-diastolic velocity; PI, pulsatility index; FHR, fetal heart rate. Data are presented as mean ± standard deviation. a) p = 0.035; b) p = 0.034; c) p = 0.048.

Table 2
Comparison of arterial pulmonary Doppler blood flow velocity indices measured during behavior states 1F and 2F

Doppler waveform index (n=10)	***1F***	***2F***	***Mean difference* (1F-2F)***	***95% CI of difference***
TAV (cm/s)	16.8 ±6.8	16.4 ±4.6	0.5 ± 4.2	-2.6, 3.5
PSV (cm/s)	67.0 ±21.8	63.4 ±14.6	3.7 ±11.3	-4.5, 11.8
PDV (cm/s)	17.1 ±6.1	16.4 ±4.7	0.7 ± 3.4	-1.7, 3.1
S/D ratio	4.1 ±0.9	4.0 ±0.7	0.1 ± 0.6	-0.3, 0.6
EDV (cm/s)	6.0 ±3.2	5.9 ±2.3	0.2 ± 2.8	-1.9, 2.0
PI	3.9 ±1.1	3.6 ±0.7	0.3 ± 0.9	-0.4, 0.9
FHR (beats/min)	137 ±5.7	137 ±7.5	0.4 ± 7.0	-4.6, 5.4

*CI, confidence interval; TAV, time-averaged velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; S/D ratio, peak systolic/peak diastolic ratio; EDV, end-diastolic velocity; PI, pulsatility index; FHR, fetal heart rate. Data are presented as mean ± standard deviation. *: none of the mean differences were statistically significant.*

Further, our data indicate behavior-state independency in the arterial pulmonary system. This renders an increase in volume flow unlikely. The comparable increases in pulmonary venous peak systolic velocity (23%) and peak diastolic velocity (37%) also suggests a raised pressure gradient from the pulmonary vein into the left atrium during this behavior state. This would be consistent with earlier reports on a behavior state 2F showing a rise in TAV indicative of raised volume flow at venous inflow level (ductus venosus),[5] foramen ovale level[6], and mitral valve level[14]. Moreover, the approximately 75% rise in pulmonary venous end-diastolic velocity suggests that this increase in pressure gradient may arise from reduced downstream impedance to venous drainage into the left side of the heart during behavioral state 2F.

The respiratory function of the pulmonary circulation is redundant in utero. Increased fetal activity does not, therefore, require increased arterial blood supply to the lungs. Reduced downstream impedance has been previously established at the fetal trunk[3,15] and cerebral level[4] during behavior state 2F. The present flow velocity data from the proximal arterial pulmonary branch therefore suggest that the arterial pulmonary vascular bed does not contribute to the general redistribution of blood flow to the left side of the heart. Increased activity after birth produces increased demands for pulmonary oxygenation. Although volume flow through the pulmonary circulation was not measured, the present data suggest that before birth, the arterial pulmonary blood supply remains constant during behavior state 2F and is, therefore, protected from redistribution of blood flow, favoring the muscular-skeletal and cerebral systems during this behavior state in the normally developing term fetus.

The raised end-diastolic velocity in behavior state 2F may reflect an increased pressure gradient between the pulmonary venous system and the left atrium resulting from reduced downstream impedance to venous drainage. The behavior state should be taken into account in future studies on pulmonary venous blood flow velocity waveforms in normal term pregnancies. Blood flow velocity waveforms from the proximal arterial pulmonary branch are independent of the behavior state.

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CHAPTER 6

MEASUREMENT OF FETAL LUNG VOLUME

6.1 Introductory remarks

Lung hypoplasia is represented by reduced lung size. An alternative way of establishing lung hypoplasia could be through measurements of lung volume. Three-dimensional sonographic lung volume measurements allow successful determination of lung volume by measuring lung area in three perpendicular planes. Also here, technique and normal data have to be described before establishing the validity of 3-dimensional lung volume measurements in the prenatal detection of fetal lung hypoplasia.

6.2 Three-dimensional ultrasonography of normal fetal lung volume: a preliminary study

J.A.M. Laudy[1], M.M.M. Janssen[1], P.C. Struyk[1], Th. Stijnen[2], J.W. Wladimiroff[1]

Department of Obstetrics and Gynaecology[1], University Hospital Rotterdam, Institute of Epidemiology and Biostatistics[2], Erasmus University Medical School, Rotterdam, The Netherlands

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ABSTRACT

The objective of this preliminary study was to evaluate three-dimensional ultrasonographic lung volume measurement in the normally developing fetus in the second half of pregnancy.

Total fetal lung volume was determined by subtraction of fetal heart volume from thoracic volume, using the perpendicular transverse, sagittal and frontal planes of the fetus. Technically acceptable lung volume measurements were obtained in 29 out of 34 women with an uncomplicated pregnancy. A statistically significant increase in normal fetal lung volume was established with advancing gestational age and with increasing fetal estimated weight, demonstrating an approximately seven-fold rise in fetal lung volume during the second half of pregnancy.

Three dimensional ultrasonography can be applied for estimation of fetal lung volume. Whether this technique is useful in the prenatal prediction of pulmonary hypoplasia remains to be determined.

INTRODUCTION

Pulmonary hypoplasia is defined as a reduction in the number of lung cells, airways and alveoli, with a resulting decrease in organ size and weight.[1] The occurrence of this condition has been documented in both renal and non-renal causes of prolonged oligohydramnios.[2] Absence of fetal breathing movements[3] and reduced thoracic circumference[4], lung length[5] and ratio of thoracic circumference to abdominal circumference[6], have been proposed to predict pulmonary hypoplasia. However, the sensitivity and specificity of these parameters do not allow reliable clinical decision making.[7]

More recent reports have indicated the value of fetal lung volume measurement using echo-planar magnetic resonance imaging[8] or three-dimensional ultrasonography[9] in predicting lung hypoplasia. Particularly, the latter technique is easy to perform and has a high patient acceptability.[9]

The purpose of this preliminary study was to evaluate normal fetal lung volume measurements obtained by three-dimensional ultrasonography in our clinic during the second half of pregnancy.

MATERIAL AND METHODS

Study design

During a period of 1 month a total of 34 women with a normal singleton pregnancy consented to participate in a cross-sectional study design. All pregnancies were uneventful and resulted in the delivery of a healthy infant with a birth weight appropriate for gestational age. Women were recruited from the routine antenatal clinic at the University Hospital Rotterdam-Dijkzigt. The study protocol was approved by the Hospital Ethics Committee. Gestational age at entering the study ranged between 19 and 39 weeks (median 29 weeks) and maternal age varied between 21 and 40 years (median: 30 years). Pregnancy duration was determined from the last reliable menstrual period and confirmed by ultrasonic measurement of the fetal crown-rump length (8-12 weeks) or fetal biparietal diameter (12-20 weeks). None of these pregnancies was associated with congenital anomalies including lung pathology. Fetal biometry and amniotic fluid were always normal. Each woman was included in the study only once.

Recording technique

The ultrasound equipment used for this study was a standard Kretz Combison 530 system (Kretztechnik AG, Zipf, Austria), with a 5.0 MHz VSW 3-5 annular array transducer for three-dimensional volume scanning. The internal mechanism of this transducer facilitated the recording of a truncated pyramidal volume. Depth, as well as longitudinal and transverse dimensions of this volume are adjustable with an initial area of 6.6 cm x 3.3 cm, up to a maximum area of 28 cm x 22 cm at a depth of 20 cm. This provides a maximum scanned volume of 5.9 l. The maximum resolution in the longitudinal direction is 512 ultrasound lines for each of the maximum of 500 slices in the transverse (sweep) direction. For the

present study, the opening angle of each plane was 70° and the angle of volume sampling was limited to a maximum of 60°. The depth of the volume was restricted to 12-16 cm, with a resolution of 210 slices per volume. Scanning of each volume took an average of about 8 s. To avoid variation, recordings were performed by one examiner (J.A.M.L.) with the patient in the semi-recumbent position. All ultrasound data were stored by the system and presented on the screen in three perpendicular planes immediately after the scanning. Each plane could be rotated, translated and magnified in any arbitrary direction for optimal positioning. The system provided storage of the complete volume data set on a removable hard disk (IOMega Jaz Drive) for off-line analysis and enhanced evaluation.

For lung volume sampling, we used transverse, sagittal and frontal planes of the fetus. The total fetal lung volume was calculated by subtraction of fetal heart volume from the thoracic volume.[9] Fetal thoracic volume measurement (Figure 1) was performed by area tracing of a series of carefully positioned transverse cross-sections of the thorax excluding vertebrae and ribs. The clavicles and the middle part of diaphragm were used as upper and lower limits of the fetal thorax. These two landmarks could always be visualized in one of the used planes. The system automatically kept track of the distances between the sections and calculated total volume measurement after finishing each area measurement. Fetal heart volume (Figure 1) was derived from area tracing using an ellipsoid geometric model after obtaining a transverse cross-section of the fetal chest at the level of the cardiac four-chamber view. All measurements were performed by one examiner (M.M.M.J.) to eliminate variations in tracing technique.

Statistical analysis

Fetal estimated weight (FEW) was derived from the Hadlock formula[10]. The relationships between: (a) human fetal heart volume and gestational age (GA); (b) thoracic volume and GA; (c) lung volume and GA; (d) heart volume and FEW; (e) thoracic volume and FEW; and (f) lung volume and FEW, were assessed by linear regression analysis. All fetal lung volume data were plotted against GA and against FEW. To describe variability around the estimated regression curves for these two relations, centile curves (P5 and P95) were computed by adding and subtracting 1.645 times the residual standard deviation, and the 95% confidence intervals for the predicted means were given. The level of statistical significance was set at $p<0.05$.

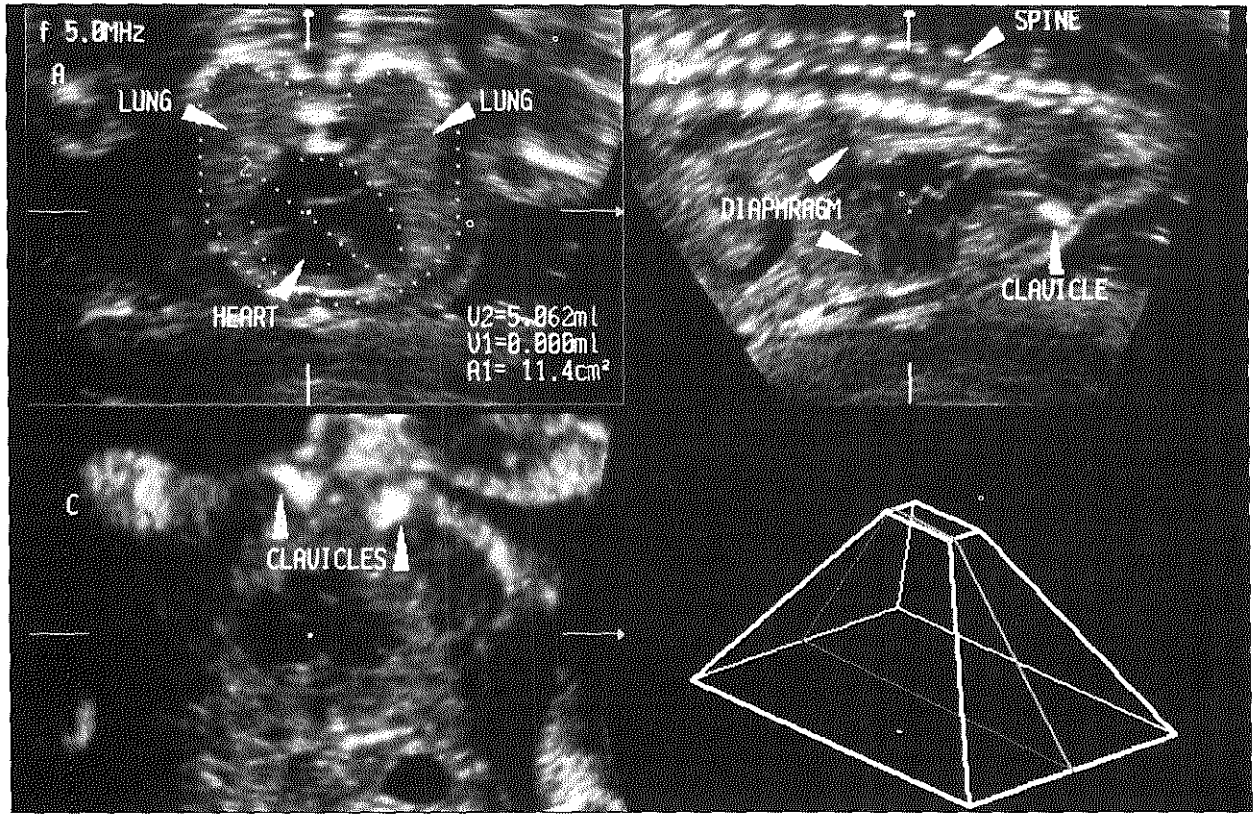

Figure 1 *Image of the fetal thorax in transverse (A), sagittal (B) and frontal (C) perpendicular planes. The fetal thoracic volume measurement was performed by area tracing in transverse cross-sections of the thorax excluding vertebrae and ribs (A). The clavicles and diaphragm were used as upper and lower limits of the fetal thorax (B,C). Fetal heart volume was derived from area tracing (A) using a ellipsoid geometric model in a transverse cross-section of the fetal chest at the level of the cardiac four-chamber view.*

RESULTS

Technically acceptable lung volume measurements were obtained in 29 out of 34 women with an uncomplicated pregnancy, resulting in a success rate of 85%. Recording failures were determined by fetal (breathing) movements, unfavorable fetal position or maternal obesity.

The regression equations for fetal heart, thoracic and lung volume relative to gestational age and fetal estimated weight are presented in Table 1. A statistically significant increase in normal fetal heart, thoracic and lung volumes was established with advancing gestational age ($p<0.0001$) and with increasing fetal estimated weight ($p<0.0001$). The relationship between lung volume and gestational age is plotted in Figure 2 and the relation between lung volume and fetal estimated weight is presented in Figure 3. Mean fetal lung volume data (P50) ranged between 13 ml at 20 weeks' gestation and 96 ml at 38 weeks' gestation.

Table 1

Regression equations for the fetal heart, thoracic and lung volume (ml) relative to gestational age (GA, weeks) and fetal estimated weight (FEW, g). SD, residual standard deviation; R^2, proportion explained variability; p, significance of regression.

	Regression equation	***SD***	***R^2***	***p***
Heart volume	y= -43.43+2.15xGA	4.42	0.85	<0.0001
Thoracic volume	y= -128.28+ 6.70xGA	7.79	0.94	<0.0001
Lung volume	y= -84.83 + 4.54xGA	6.46	0.92	<0.0001
Heart volume	y= 0.22 + 0.013xFEW	3.94	0.88	<0.0001
Thoracic volume	y= -5.97 + 0.06xFEW-($7.02x10^{-6}$)xFEW^2	6.75	0.96	<0.0001
Lung volume	y= -5.13 + 0.049xFEW-($6.51x10^{-6}$)xFEW^2	6.14	0.94	<0.0001

DISCUSSION

Since its introduction, three-dimensional ultrasonic imaging has been adopted for both surface rendering images and determination of fetal organ volume.[11-13] Three-dimensional ultrasonography was recently promoted by Lee and co-workers[9] to be a fast and easy means of volume estimation of the fetal lung. They calculated fetal lung volume indirectly by subtracting fetal heart volume from fetal thoracic volume. In the present study we followed the same procedure, although heart volume measurement was different. Lee and colleagues[9] determined fetal heart volume by area tracing of the fetal heart in a series of fetal thoracic cross-sections, which included different stages of heart contraction. The resulting volume represented a mean value between maximum and minimum heart volume. We derived fetal heart volume from area tracing of the outer border of the heart using an ellipsoid geometric model after obtaining a transverse cross-section of the fetal chest at the level of the cardiac four-chamber view.

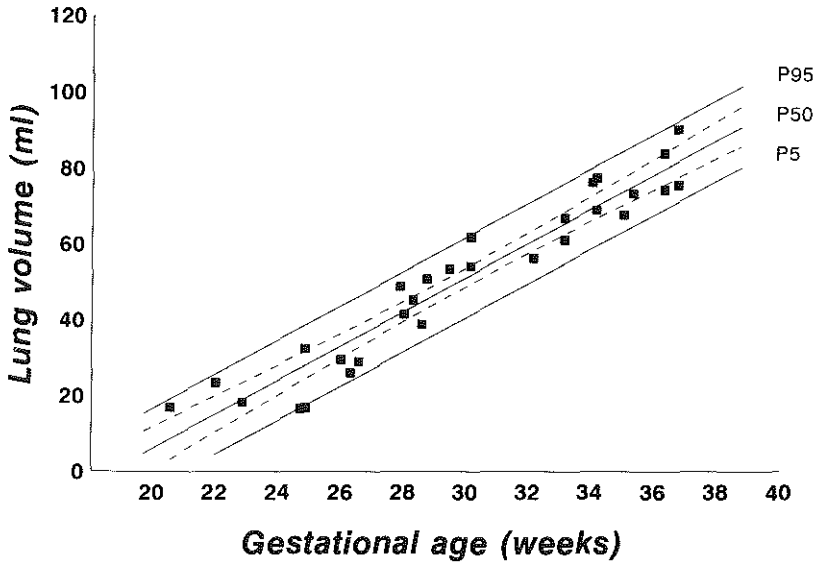

Figure 2
Individual normal values and centile curves (P5, P50 and P95) for lung volume relative to gestational age with 95% confidence intervals for the P50 (dashed curves)

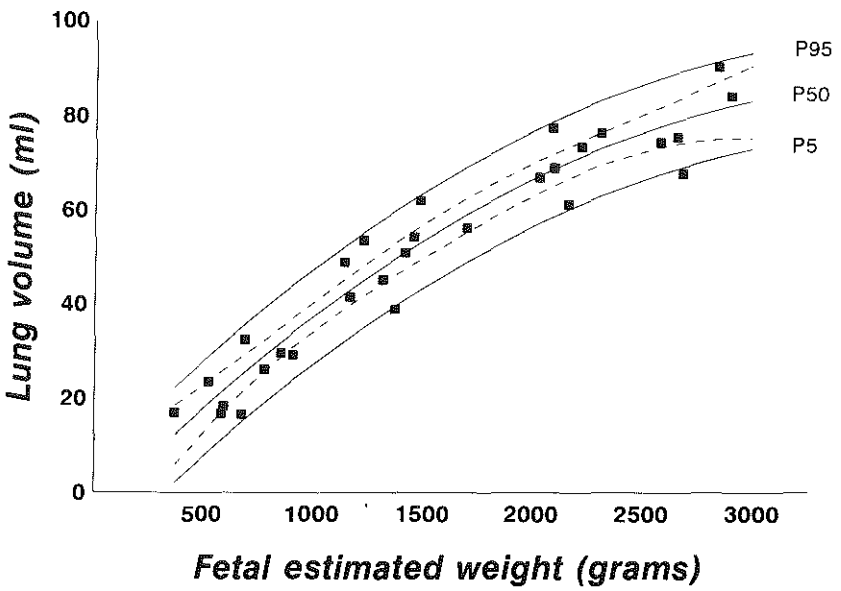

Figure 3
Individual normal values and centile curves (P5, P50 and P95) for lung volume relative to fetal estimated weight with 95% confidence intervals for the P50 (dashed curves).

The results of our preliminary study confirm that it is technically possible to obtain fetal lung volume measurements by subtraction of fetal heart volume from thoracic volume. However, using this method, we should realize that not only both lungs but also blood vessels and the thymus gland are included in the thoracic volume measurement. Based on three-dimensional ultrasonography, Chang and associates[14] recently suggested a slight adjustment to the calculation of fetal heart volume, when this calculation was obtained from the ellipsoid geometric model.

The relationship between fetal lung volume measurements and gestational age showed a trend similar to that observed by Lee and co-workers.[9] A close correlation between fetal lung volume and gestational age and fetal estimated weight was demonstrated by us with an approximately seven-fold increase in fetal lung volume during the second half of pregnancy.

Since three-dimensional ultrasonography can be applied for estimation of fetal lung volume, the question arises as to whether this technique is useful in the prenatal prediction of pulmonary hypoplasia. Although we speculate that three-dimensional fetal lung measurement may identify the fetus at-risk for developing lung hypoplasia, further three-dimensional volume studies are needed to substantiate whether this method is accurate in predicting pulmonary hypoplasia. In this respect, new digital imaging techniques producing better resolution, may allow accurate direct assessment of fetal lung volume.

It can be concluded that three-dimensional ultrasonography allows indirect determination of fetal lung volume. There is an increase in fetal lung volume related to both gestational age and estimated fetal weight. Its significance in the prenatal detection of fetal lung hypoplasia still remains to be elucidated.

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CHAPTER 7

DOPPLER ASSESSMENT OF THE FETAL PULMONARY CIRCULATION ASSOCIATED WITH PULMONARY HYPOPLASIA

7.1 Introductory remarks

Both the high success rate in obtaining technically acceptable flow velocity waveform recordings and the acceptable repeatability of the waveforms created a firm basis for further Doppler waveform studies in pregnancies at risk of pulmonary hypoplasia. There are two questions which need to be addressed: (i) Can hemodynamic changes be expected in the presence of lung hypoplasia? The answer should be positive. Several postmortem studies have demonstrated increased pulmonary vascular muscularization in hypoplastic lungs. This may lead to raised pulmonary vascular resistance and reduced pulmonary arterial compliance. (ii) Will hemodynamic changes be reflected in arterial pulmonary Doppler flow velocity waveform recordings in the presence of pulmonary hypoplasia? The morphological changes in the pulmonary vascular bed associated with lung hypoplasia should have a bearing on pulmonary blood flow and may, therefore, result in changes in arterial pulmonary flow velocity waveforms. Both questions are dealt with in the subchapters 7.2 and 7.3.

7.2 Doppler Ultrasound Imaging: a new technique to detect lung hypoplasia before birth? A case report.

J.A.M. Laudy[1], J.L.J.Gaillard[2], J.N. v.d. Anker[3], D. Tibboel[4], J.W. Wladimiroff[1]
Department of Obstetrics and Gynaecology[1], Department of Pathology[2], Department of Neonatology[3], Department of Pediatric Surgery[3], University Hospital Rotterdam, The Netherlands

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ABSTRACT
Combined color coded Doppler and pulsed Doppler ultrasonography allows visualization of the fetal pulmonary circulation and study of pulmonary blood flow velocity waveforms. Systolic and diastolic changes were observed in fetal pulmonary artery flow velocity waveforms in a case of fetal pulmonary hypoplasia at 34 weeks of gestation. This observation supports our hypothesis that Doppler velocimetry can detect lung hypoplasia based on postmortem examination of decreased total size of the pulmonary vascular bed, decreased number of pulmonary vessels per unit lung tissue and increased pulmonary vascular muscularization.

INTRODUCTION

Combined color-coded Doppler and pulsed Doppler ultrasonography provides the opportunity to identify and record pulmonary arterial and venous waveforms in both normally developing human fetuses and in fetuses with structural or functional pathology, such as congenital heart disease and lung hypoplasia.

Only a few reports are available on Doppler velocimetry in the pulmonary circulation of the normally developing human fetus[1-4] and fetal lamb[5]; there is no information on pulmonary hemodynamics in the presence of lung pathology.

Several postmortem studies have shown increased pulmonary vascular muscularization in hypoplastic lungs.[6,7] This may lead to increased pulmonary vascular resistance and decreased pulmonary arterial compliance. These hemodynamic alterations of the pulmonary vascular bed should have a bearing on pulmonary blood flow and thus may result in changes in pulmonary flow velocity waveforms as recorded by Doppler ultrasonography.

The present case report demonstrates for the first time pulmonary artery flow velocity waveforms originating from a fetus with lung hypoplasia.

CASE REPORT

A 32-year old woman at 34 weeks of gestation was referred to our Prenatal Diagnosis Center for a suspected fetal renal anomaly. Detailed ultrasound examination revealed anhydramnios, bilateral enlarged kidneys with hydronephrosis, bilateral dilated ureters and a distended bladder with bladder wall hypertrophy. Cordocentesis revealed a normal fetal karyotype (46,XY). A diagnosis of bilateral obstructive uropathy was made. An additional finding was a thoracic circumference below the 5th centile[8] relative to an abdominal circumference between the 25th and 50th centile[9], which was suspicious of lung hypoplasia. After informed consent the fetal pulmonary circulation was examined by means of combined color coded Doppler and two-dimensional real-time ultrasound (carrier frequency 5 MHz, high pass filter 100 Hz; Toshiba SSH 140A, Toshiba Corp., Medical Systems Division, Tokyo, Japan). After a transverse cross-section of the fetal chest was obtained at the level of the four-chamber view, color Doppler was switched on to visualize the pulmonary circulation. Owing to the fetal position, the left lung was in the most accessible position for pulsed Doppler flow velocity examination.

The sample volume (0.1-0.3 cm) was placed over the left primary branch of the pulmonary artery (Figure 1, page 199) and maximum flow velocity waveform envelopes were recorded. No fetal breathing movements were observed. Figure 2 shows blood flow velocity waveforms obtained in this case of suspected lung hypoplasia.

At 34 weeks and 5 days of gestation, the patient went into spontaneous labor and subsequently delivered from a male infant weighing 2765 g. Apgar scores were 9 and 8 at 1 and 5 min., respectively; arterial umbilical cord pH was 7.24 with a base excess of -3 mmol/l. Physical examination revealed a typical Potter facies. Nasotracheal intubation was carried out because of life threatening respiratory distress due to suspected pulmonary hypoplasia and worsened as a result of bilateral pneumothorax and complicating, therapy-resistent, persistent pulmonary hypertension. Owing to the inability to obtain normal oxygenation during maximal ventilatory support (peak inspiratory pressure 65 cm H_2O; respiratory rates of 75 breaths/ min), treatment was stopped because of the severity of the lung problems together with the obstructive uropathy. The infant died 4.5 hours following birth.

Postmortem examination confirmed the presence of lung hypoplasia. In lung hypoplasia one would expect a reduced lung/body-weight ratio.[10,11] In our case, the ratio was only borderline (0.0127), due to the presence of the hyaline membrane disease with focal oedema. The latter condition is associated with an increased fetal lung weight.[12] The radial alveolar (septal) count (RAC) was 3.2, which is reduced. Normal values of RAC are: mean 5.3, SD 0.6, range 4.1-6.3.[11] Macroscopically, atrophic microcystic kidneys with histologically typical Potter type II dysplasia in combination with obstructive uropathy, Potter type IV, was found.

Figure 2 demonstrates fetal pulmonary artery flow velocity waveforms from a normally developing fetus of 34 weeks of gestation. Similar waveforms were seen in six other fetuses at this gestational age. These waveforms are characterized by a rapid systolic velocity acceleration[1,13], followed by an initially rapid but then more gradual velocity deceleration that is interrupted by a short reversed flow interval at the beginning of the diastolic phase of the cardiac cycle. The diastolic phase is characterized by forward flow.

Table 1 presents peak systolic, time-averaged (TAV) and end-diastolic velocities as well pulsatility index (PI) and heart rate for both normal and the lung hypoplasia pulmonary artery flow velocity waveforms. An average of three consecutive heart beats was used for each value. The PI was calculated from the difference between the peak systolic and end-diastolic flow velocity divided by the TAV. The significance of the difference between the pathological case and mean values of the control group ($n=7$) was determined by the Student t test. p-Values of < 0.05 were considered statistically significant.

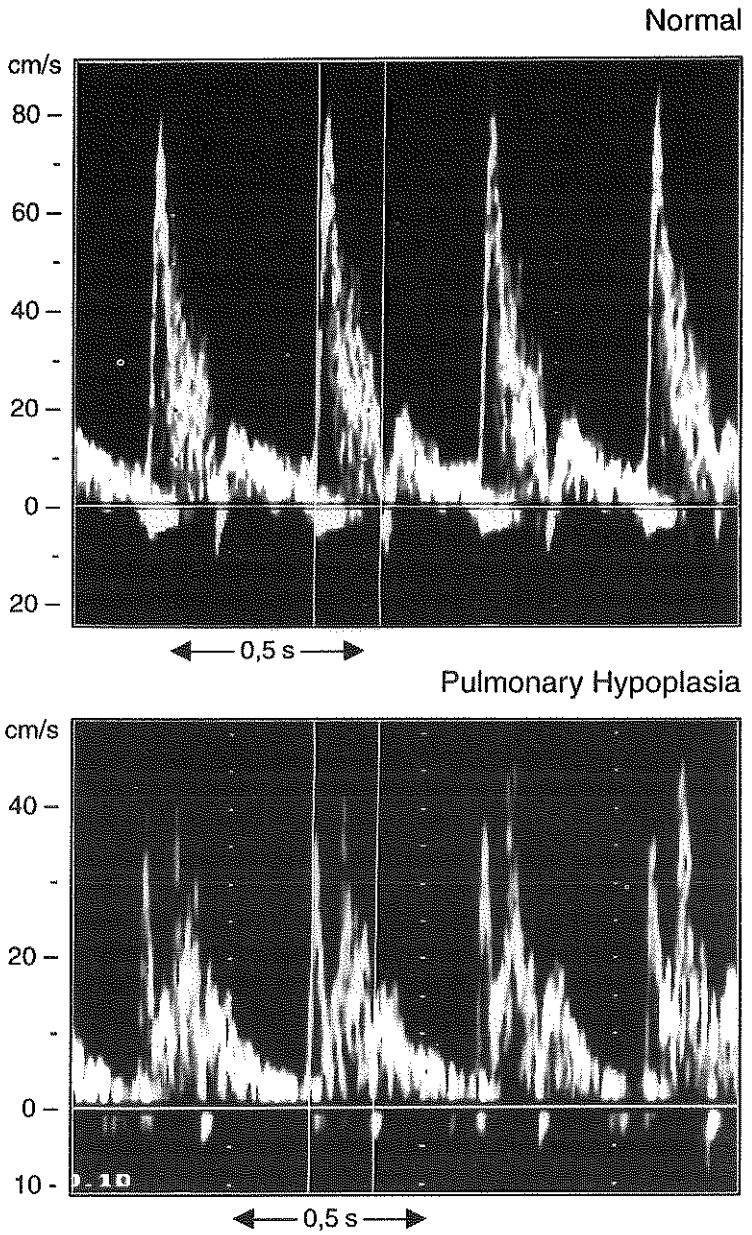

Figure 2
Doppler flow velocity waveforms from the left branch of the fetal pulmonary artery in a normally developing fetus (top) and a fetus with lung hypoplasia (bottem) at 34 weeks gestation. Note the difference in velocity scale (cm/s) used in both figures.

Table 1
Mean fetal pulmonary artery flow velocities and fetal heart rate in the presence of normal fetal lung development and individual values for the one case of fetal lung hypoplasia at 34 weeks of gestation. Mean values and standard deviation are given.

Blood Flow velocity waveform parameters	***Normal lung development (n = 7)***	***Lung hypoplasia (n = 1)***
Peak systolic velocity (cm/s)	78.6 (8.7)	38.5*
Time averaged velocity (cm/s)	23.7 (3.3)	16.0
End-diastolic velocity (cm/s)	8.7 (1.2)	4.9*
Pulsatility index	3.0 (0.3)	2.1*
Fetal heart rate (bpm)	141.0 (11.5)	147.0

*, *p-value < 0.05.*

Blood flow velocity waveforms obtained in our case of suspected lung hypoplasia differed from the normal waveforms in three ways:
(i) there was a mid-systolic peak, which is even higher than the first systolic peak;
(ii) peak systolic and end-diastolic velocities were statistically significantly lower than under normal circumstances (peak systolic velocity mean difference (cm/s) 40.06, SD 8.68, $p = 0.008$; end-diastolic mean difference 3.78, SD 1.26, $p = 0.03$);
(iii) the PI was statistically significantly lower than in the control group (PI mean difference 0.87, SD 0.25, $p = 0.03$).
The TAV was not significantly different from the normal cases.

DISCUSSION

Bilateral obstructive uropathy but also premature rupture of the membranes may cause long-term oligohydramnios with consequently fetal pulmonary underdevelopment and neonatal death.[14-18] Several factors play a role in the process of growth and maturation of the fetal lung, such as the available intrathoracic and intrauterine space, and also the balance between pulmonary volume and pressure.[19,20] Sonographic measurements of the fetal thoracic circumference and lung length are useful[21] but these are late parameters for detection of lung hypoplasia, as was shown for the case presented in this paper. Information about the predictive value of fetal breathing activity is still contradictory.[22,23]

Expansion of the total pulmonary vascular bed with advancing gestational age induces an increase in absolute pulmonary flow and a decrease in pulmonary vascular resistance.[1,24,25] However, particularly during the canalicular phase of lung development (18-26 weeks of gestation), a prolonged and pronounced oligohydramnios may cause a delay or even an arrest in pulmonary vascular development, resulting in a decreased lung volume and a raised pulmonary vascular resistance.[6,7,17,26]

It can be assumed that changes in the pulmonary vascular bed associated with developing lung hypoplasia may be detected by Doppler velocimetry. Our case report supports this suggestion. A marked reduction in left pulmonary artery systolic and diastolic velocities was established in the presence of lung hypoplasia. Based on postmortem findings[6,7,26] of decreased total size of pulmonary vascular bed, reduced vessel count per unit lung tissue and increased pulmonary vascular muscularization in lung hypoplasia, reduced volume flow and/or raised downstream impedance may be responsible for these Doppler velocity changes. However, changes in vessel diameter and compliance cannot be ruled out. The presence of raised downstream impedance is further supported by marked changes in the shape of the pulmonary artery waveform characterized by a second needle shaped peak occurring during mid-systole, even higher than the first systolic peak. This second peak may be responsible for the fact that the TAV was not statistically significantly different compared with the control group. Since both peak systolic and end-diastolic velocities were reduced with an unchanged TAV, PI cannot be considered to be a true reflection of downstream impedance. Also, Emerson and Cartier[1] mentioned this failure of the PI to identify resistance changes in the normal pulmonary arteries. They suggested that other Doppler data may identify the expected gestational age-related change in resistance in the human fetal pulmonary circulation.

We have to consider possible errors in our measurements, such as an increased chance of measuring the velocity in a more peripheral section of the pulmonary artery than in the normal fetus, because of the smaller dimensions of the hypoplastic lung. However, we were reasonably confident of the position of the sample volume, as described earlier.

It can be concluded that our data suggest a change in nature and absolute velocities of the fetal pulmonary artery waveform in lung hypoplasia. This needs to be substantiated in further Doppler studies of the fetal pulmonary circulation.

7.3 Prenatal prediction of fetal pulmonary hypoplasia: clinical, biometric and Doppler velocity correlates.

J.A.M. Laudy[1], D. Tibboel[2], S.G.F. Robben[3], R.R. de Krijger[4], M.A.J. de Ridder[5], J.W. Wladimiroff[1]
Department of Obstetrics and Gynaecology[1], Department of Pediatric Surgery[2], Department of Radiology[3], Department of Pathology[4], University Hospital Rotterdam, Institute of Epidemiology and Biostatistics[5], Erasmus University Medical School, Rotterdam, The Netherlands

Submitted

ABSTRACT

Objectives: To determine the value of Doppler flow velocity waveforms from the fetal arterial pulmonary branches relative to fetal biometric indices and clinical correlates in the prenatal prediction of lethal lung hypoplasia in prolonged oligohydramnios.

Study design: 42 singleton pregnancies with oligohydramnios associated with premature rupture of membranes (PROM, n=31) or bilateral renal pathology (n=11) were examined using a combined color coded Doppler and 2D real-time ultrasound system. Thoracic (TC), cardiac (CC) and abdominal circumference (AC) and the largest vertical amniotic fluid pocket were measured. Pulsed Doppler measurements of the arterial pulmonary branches were made at the level of the cardiac four-chamber view after visualization with color Doppler. Diagnosis of lung hypoplasia was based on pathological, clinical and radiological criteria.

Results: The incidence of lethal lung hypoplasia was 43%. In the PROM-subset, combination of onset of PROM ≤ 20 weeks; duration of oligohydramnios ≥ 8 weeks and degree of oligohydramnios ≤ 1 cm, presented the highest clinical prediction rate for lethal lung hypoplasia. For both the total group and the PROM-subset, the highest prediction rate for lethal lung hypoplasia from biometric and Doppler parameters was presented by TC/AC ratio, peak systolic velocity in the proximal branch and time-averaged and end-diastolic velocity in the middle branch of the pulmonary artery. In the PROM subset, combination of all three clinical, biometric and Doppler parameters revealed the most favorable combination to predict lethal lung hypoplasia, (PPV 100%, accuracy 93%, and sensitivity 71%).

Conclusion: Doppler velocimetry may detect changes in blood velocity waveforms from the arterial branches of the fetal pulmonary circulation in the presence of lung hypoplasia, but fails to be the ultimate test for the prenatal prediction of lethal lung hypoplasia. The best prediction can be achieved by combining clinical, biometric and Doppler parameters.

INTRODUCTION

Pulmonary hypoplasia is the result of a primary or secondary developmental delay in pulmonary development, which is classically characterized by an abnormal low value of the lung/body weight ratio and radial alveolar count.[10,11,27] The occurrence of pulmonary hypoplasia is mostly secondary to congenital anomalies, notably renal tract malformations, diaphragmatic hernia, cardiac disorders, skeletal anomalies, and neuromuscular diseases or to pregnancy complications such as premature rupture of membranes, that inhibit lung development.[27] Most commonly, prolonged oligohydramnios due to premature rupture of membranes (PROM) or renal and urinary tract malformations may lead to abnormal fetal lung development with subsequent severe respiratory distress immediately after birth and even neonatal death.[16,27,28] Therefore, in order to improve the clinical care, an accurate and patient-friendly prenatal test for early detection and separation of lethal from non-lethal pulmonary hypoplasia is highly desirable.

Various methods have been proposed in this respect. Most of these include fetal two-dimensional biometric indices, such as fetal thoracic circumference and ratio of thoracic and abdominal circumference or ratio of cardiac and thoracic circumference.[29,30] However, these measurements are late indicators of pulmonary hypoplasia, with a sensitivity and specificity not satisfactory for clinical management. Data on the predictive value of the presence or absence of fetal breathing movements are contradictory.[22,23] Furthermore, the applicability of three-dimensional ultrasonography, computerized tomography and/or magnetic resonance imaging for accurate measurement of fetal lung volume in the prediction of lung hypoplasia still needs to be determined. The introduction of more sensitive color coded Doppler systems has led to the visualization of the human fetal pulmonary circulation. Information on normal fetal pulmonary circulation has been obtained in our center.[31,32]

It has been shown in postmortem studies, that pulmonary hypoplasia is associated with underdevelopment and structural changes of the pulmonary vascular bed. A decrease in total size of the pulmonary vascular bed, a decrease in the number of vessels per unit of lung tissue and an increase of pulmonary arterial smooth muscle thickness have been described.[6,16,33] We hypothesized that these changes may lead to increased pulmonary vascular resistance and decreased pulmonary arterial compliance. These alterations may effect pulmonary blood flow and consequently may induce changes in both systolic and diastolic components of the pulmonary artery flow velocity waveform as suggested before.[34]

On the basis of this hypothesis, we addressed the question whether:

(i) Doppler flow velocity waveforms from the fetal arterial pulmonary branches in the presence of prolonged oligohydramnios are predictive of lethal lung hypoplasia;

(ii) and if so, whether the predictive value of these Doppler recordings is superior to that of fetal biometric indices.

PATIENTS AND METHODS

Patients and study design

After informed consent was obtained, a total of 42 women with a singleton pregnancy and prolonged oligohydramnios participated in the study according to a cross-section design. Oligohydramnios was either the result of premature rupture of membranes (PROM) before 30 weeks of gestation and lasting more than one week (n=31) or due to the presence of bilateral renal pathology (n=11). The latter subset consisted of bilateral renal agenesis (n=5), bilateral cystic/dysplastic kidneys (n=4) and obstructive uropathy (n=2), suspected prenatally by ultrasonography and all confirmed postnatally by post-mortem examination. The degree of oligohydramnios was defined as severe, moderate or mild if the largest vertical amniotic fluid pocket was measured below 1 cm, between 1 and 2 cm and between 2 and 3 cm, respectively.[28]

The study protocol was approved by the Hospital Ethics Committee. Pregnancy duration varied between 20 and 36 weeks (mean 28 weeks, SD 4.3) and was determined from the last menstrual period and confirmed or adjusted by ultrasonic measurement of the fetal crown-rump length (8-12 wk) or fetal biparietal diameter (12-20 wk). No other pregnancy pathology was established.

The mean onset of PROM was 23 weeks (SD 3.9) and the mean duration of PROM was 8 weeks (SD 4.4). Within the PROM subset, 11 cases (35%) had a vertical amniotic fluid pocket of ≤1 cm, 13 cases (42%) of >1 cm and ≤ 2 cm and 7 cases (23%) of > 2 cm and ≤ 3 cm. All cases with renal pathology displayed amniotic fluid pockets less than 1 cm. Table 1 presents the demographics of both patient subsets. The mean time interval between the sonographic biometric and Doppler measurements and delivery was 6 days (PROM subset) and 4 days (renal subset), respectively.

Recording technique

The real-time ultrasound and Doppler studies were performed using a Toshiba SSH 140 with combined transabdominal real-time, color and pulsed Doppler imaging facilities (Toshiba Corp., Medical Systems Division, Tokyo, Japan). The carrier frequency was 5 MHz (real-time) and 3.75 MHz (Doppler). The spatial peak temporal average power output was

Table 1
Demographics of the subset of PROM and the subset of bilateral renal pathology

	PROM *n=31*	***Renal pathology*** *n=11*
Maternal age (y), mean (SD)	28.8 (4.6)	29.8 (3.6)
Gravida, median [range]	3 [1-6]	2 [1-6]
Para, median [range]	1 [0-4]	1 [0-4]
Interval US-birth (wk), median [range]	0.86 [0.14-6.71]	0.57 [0.14-7.00]
Gestation at birth (wk), mean (SD)	30.48 (2.95)	27.79 (7.13)
Birth weight (g), median [range]	1305 [780-2750]	730 [303-2765]
Mode of delivery		
Spontaneous	25 (81%)	4 (36%)
Cesarean section	6 (19%)	0
Pregnancy termination	0	7 (64%)
Gender male / female	18 / 13	9 / 2

US= ultrasonographic examination.

less than 100 mW/cm² in both imaging and Doppler modes according to manufacturers' specifications. All recordings were performed with the woman in the semi-recumbent position and during fetal apnea.

First, sonographic measurements of thoracic, cardiac and abdominal circumference and the largest vertical amniotic fluid pocket were obtained. The thoracic and cardiac circumference were determined from a cross-section of the fetal chest at right angles to the fetal spine at the level of the atrioventricular valves displaying the cardiac four-chamber view. The cardiac circumference was defined from the outer border of the fetal heart in ventricular diastole. The thoracic circumference was obtained from the outer border of the bony fetal chest. The abdominal circumference was obtained from a transverse plane of the fetus at the level of the stomach and the portal vein. This was followed by the pulsed Doppler measurements of the arterial pulmonary branches from a transverse cross-section of the fetal chest at the level of the cardiac four-chamber view after visualization with color Doppler. Depending on fetal position, the right or left lung was examined. Doppler waveforms (sample volume 0.1-0.3 cm) were first obtained from the most proximal branch of the pulmonary artery, then in the middle lung region at equal distance from the outer border of the heart and the inner thoracic wall and subsequently in the distal lung region as closely as possible to the fetal inner thoracic wall (Figure 1, page 199).[31,32]

The interrogation angle was always kept below 20°. The high pass filter was set at 100 Hz and if no end-diastolic or early diastolic reverse flow velocities were present, the filter was set at 70 Hz. All flow velocity waveforms were recorded on hard copies. Each patient was studied by one and the same examiner (J.A.M.L.) who performed also the waveform analysis. This analysis always took place before delivery using a microcomputer (Commodore 386 CX) linked to a graphics tablet. At least three consecutive flow velocity waveforms with the highest velocity and of similar appearance were used to calculate the different parameters in each vessel. Clinicians were blinded to the Doppler results.

From the proximal, middle and distal arterial pulmonary branches, the following Doppler flow velocity parameters were determined from the same lung: (i) peak systolic velocity (cm/s), peak diastolic velocity (cm/s), end-diastolic velocity (cm/s) and time-averaged velocity (TAV, cm/s); (ii) the pulsatility index (PI= (peak systolic velocity - end-diastolic velocity)/TAV), according to Gosling and King[35].

Postnatal diagnosis of lung hypoplasia

All newborn in the subset of PROM were delivered as inborn in the Sophia Children's Hospital either following spontaneous birth or planned delivery. An experienced neonatologist was always present at the time of birth for immediate resuscitation if needed according to a standardized clinical treatment protocol. The subset of newborn associated with bilateral renal anomalies was partly delivered in the Sophia Children's Hospital (n=4) and partly in a regional hospital (n=7), either spontaneously or after early pregnancy termination.

After birth, lung hypoplasia was diagnosed according to clinical and radiological criteria and eventually on pathological data. The pathological diagnosis was based on lung/body weight ratios and radial alveolar counts (RAC) according to the criteria established by Askenazi and Perlman[11]. If the RAC could not be determined, which was particularly the case in early pregnancy (20-22 weeks), pulmonary hypoplasia was defined as wet lung/body weight ratio of ≤ 0.015 prior to 28 weeks' gestation, or ≤ 0.012 at 28 weeks or later, including the developmental stage.[10] The clinical criteria were defined as an immediate onset of severe respiratory insufficiency after birth, small lung capacity, and requirement of high ventilatory pressures in the absence of obstruction or atelectasis.[28,36] The following features were used as radiological criteria: small lung fields with diaphragmatic domes elevated up to the seventh rib, downward sloping ribs, a bell-shaped chest, and/or pneumothorax/ pneumomediastinum.[37,38] The radiologist (S.G.F.R.) who scored the X-rays of the neonatal chest, was blinded to the sonographic measurements and neonatal outcome.

Lethal lung hypoplasia was defined as death from lung hypoplasia based on the described pathological and/or combined clinical and radiological criteria. Fetuses with autopsy proven lung hypoplasia after

early pregnancy termination were also included in this subset. Neonates with non-lethal lung hypoplasia developed respiratory insufficiency compatible with clinical and radiological signs of pulmonary hypoplasia, but survived. Absent lung hypoplasia was represented by (i) neonates which did not show any clinical and/or radiological sign of pulmonary hypoplasia and (ii) fetuses in which no pulmonary hypoplasia could be diagnosed on post-mortem examination after early pregnancy termination.

From a clinical standpoint, an accurate prenatal prediction of a lethal form of pulmonary hypoplasia is important to both obstetric management and parental counseling. It was decided, therefore, to separate out lethal lung hypoplasia from non-lethal and absent lung hypoplasia in the present study.

Data analysis

Clinical parameters (PROM subset only)

The onset of PROM and the duration and degree of oligohydramnios served as clinical parameters for the prediction of lethal lung hypoplasia. Differences in clinical parameters between lethal lung hypoplasia and combined non-lethal and absent lung hypoplasia were tested (independent sample t-test). For the onset of PROM we examined the levels of $\leq$ 26 weeks, $\leq$ 24 and $\leq$ 20 weeks of gestation; for the duration of oligohydramnios the levels of $\geq$ 4 weeks, $\geq$ 6 and $\geq$ 8 weeks, and for the degree of oligohydramnios a largest vertical amniotic fluid pocket of $\leq$ 2 cm and $\leq$ 1 cm. Further, sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and overall accuracy for lethal lung hypoplasia were calculated at the different cut-off levels for each of these clinical parameters separately and in combination. Accuracy was defined as the sum of true "positives" and true "negatives" divided by the total population studied. Exact 95% confidence intervals were computed with the package StatXact 4 for the combined clinical parameters. Data were considered abnormal when situated below a particular cut-off level for onset of PROM or degree of oligohydramnios and above a particular cut-off level for duration of oligohydramnios.

Biometric and Doppler parameters

Data on thoracic circumference (TC) and ratios of cardiac/thoracic (CC/TC) circumference and thoracic/abdominal (TC/AC) circumference, as well as pulmonary Doppler flow velocities, were plotted in nomograms constructed from an earlier patient cohort of 111 uncomplicated singleton pregnancies.[31,32] All biometric and Doppler blood flow velocimetry parameters were subsequently converted into standard deviation scores (SD-scores) in order to be independent of gestational age. This score was obtained by taking the difference between the observed value and the predicted value according to gestational age and dividing the result by the

standard deviation of the normal control values. It was tested whether the SD-scores of both the lethal lung hypoplasia subset and combined non-lethal and absent lung hypoplasia subset were statistically different from the SD-scores of the mean of the normal controls (= 0) using the one-sample t-test. For the latter subset, it was also tested whether this result was influenced by the addition of the three cases of non-lethal lung hypoplasia to the subset of absent lung hypoplasia. The level of statistical significance was set at $p<0.05$.

Further, sensitivity, specificity, predictive values, and overall accuracy of the separate and combined biometric and Doppler parameters were calculated for the total study group to evaluate the efficacy of prenatal prediction of lethal pulmonary hypoplasia. A similar procedure was carried out within the PROM subset, including combination with the three clinical parameters, i.e. the onset of PROM and the duration and degree of oligohydramnios. Exact 95% confidence intervals (CI) were computed with the package StatXact 4 for all tested combinations. Measurements below the 5th centile were considered abnormal for TC, TC/AC, and the velocity parameters TAV, PSV, PDV, and EDV, and measurements above the 95th centile were considered abnormal for CC/TC and PI.

RESULTS

Pregnancy outcome (Figure 2)

The incidence of lethal lung hypoplasia in the total study group was 43% (18/42), of which 9 were in the PROM subset (9/31=29%) and 9 were in the renal subset (9/11=82%). Non-lethal lung hypoplasia was established in 3 cases associated with PROM, whereas absent lung hypoplasia existed in the remaining 21 cases of which 19 were related to PROM.

In the renal subset, the mortality rate was 100% (11/11); two cases (bilateral renal agenesis; bilateral cystic kidneys) displayed normal lung/body weight ratio's after termination of pregnancy at 21 weeks of gestation. Of the remaining 9 cases displaying lethal lung hypoplasia, 4 died within 5 hours after normal delivery and 5 died after early pregnancy termination.

In the PROM subset, the overall mortality rate was 39% (12/31). Of the 9 fetuses with lethal lung hypoplasia, 78% (n=7) died within 24 hours after birth of which 70% (5/7) died in the first hour. Despite vigorous artificial ventilation with 100% inspiratory oxygen and frequencies ranging from 30 to 100 per minute and peak inspiratory pressures reaching 40 cm H_2O in isolated cases, adequate oxygenation was never reached in severely affected neonates. In absent lung hypoplasia (n=19), 3 neonates died from sepsis (n=2) or severe asphyxia (n=1). The remaining 16 neonates survived, although 81% (13/16) of them displayed mild to severe respiratory complications: e.g. respiratory distress syndrome (RDS) (n=8), transient tachypnea of the newborn (TTN) (n=5),

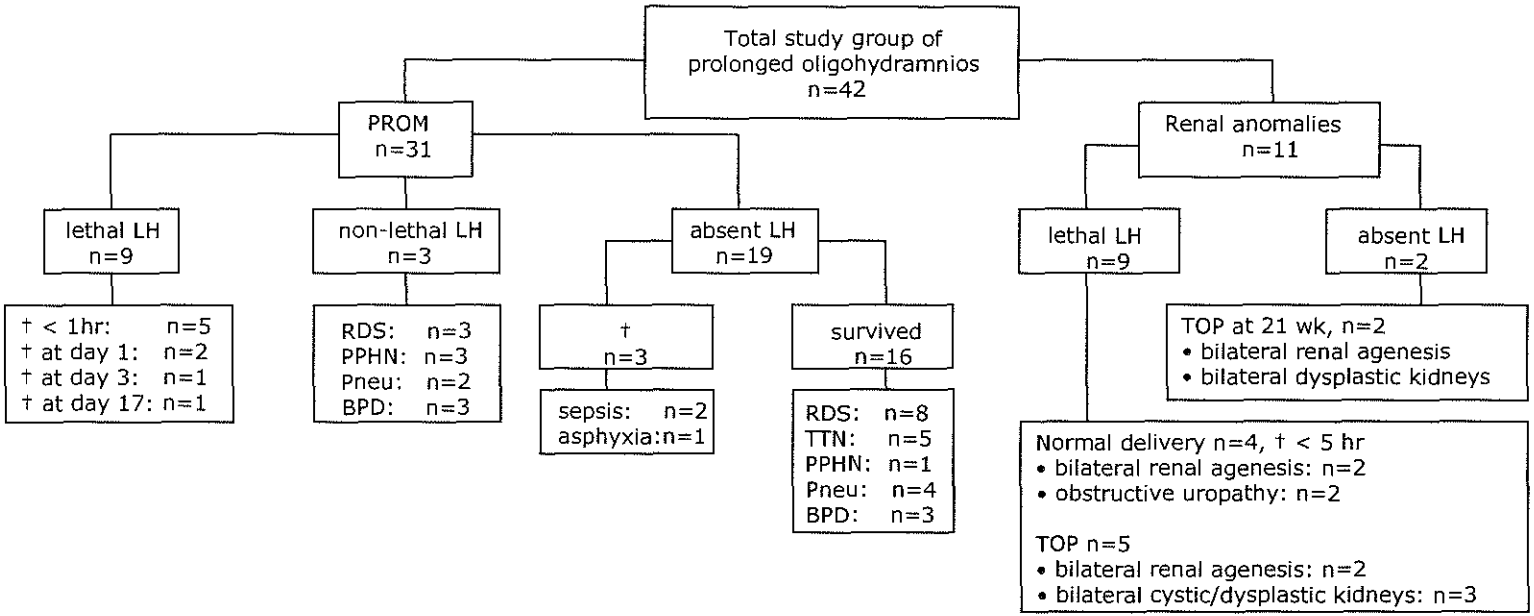

Figure 2
Flow chart of the pregnancy outcome from the total study group. PROM, premature rupture of membranes. LH, lung hypoplasia; RDS, respiratory distress syndrome; TTN, transient tachypnea of the newborn; PPHN, persistent pulmonary hypertension of the newborn; Pneu, pneumothorax; BPD, bronchopulmonary dysplasia; TOP, termination of pregnancy.

pneumothorax (n=4), persistent pulmonary hypertension of the newborn (PPHN) (n=1).[39] In 3 patients bronchopulmonary dysplasia (BPD) was diagnosed at a later stage after birth.[40] The intubation period in the 19 neonates with absent lung hypoplasia ranged from 0 to 40 days (median 1 day, mean 4.4 days (SD 9.3)), whereas the stay in the neonatal intensive care unit varied from 0 to 170 days (median 10 days, mean 22.2 days ((SD 39.7)). All 3 neonates with non-lethal lung hypoplasia in this PROM subset developed RDS, complicated by PPHN and later on BPD, two of them underwent successful treatment of a pneumothorax in the newborn period.

Clinical parameters (PROM subset only)

The median time interval between the onset of PROM and sonographic examination was 7 weeks (range 1 week – 20 weeks). The mean onset of PROM was significantly earlier (19.7 weeks (SD 2.1) vs 23.9 weeks (SD 3.9), p=0.005), the mean duration of oligohydramnios was significantly longer (10.0 weeks (SD 1.2) vs 6.8 weeks (SD 4.9), p=0.008) and the mean degree of oligohydramnios was significantly more pronounced (0.9 cm (SD 0.5) vs 1.7 cm (SD 0.8), p=0.01) in the subset of lethal lung hypoplasia compared with the subset of combined non-lethal and absent lung hypoplasia. When considering each of the clinical parameters separately, the highest prediction rate for lethal lung hypoplasia was presented by the following abnormal conditions:

(i) onset of PROM ≤ 20 weeks;
(ii) duration of oligohydramnios ≥ 8 weeks;
(iii) degree of oligohydramnios of ≤ 1 cm.

Moreover, an even higher prediction rate was reached when combining all these three abnormal conditions (PPV 86% (CI: 42-100%), accuracy 87% (CI: 70-96%)). (Table 2)

Biometric parameters

TC and CC/TC ratio could be calculated in 98% of all patients and in 100% of the PROM subset. For the TC/AC ratio, the percentages were 95% and 97%, respectively. Figure 3 demonstrates the TC, CC/TC and TC/AC ratios for the total study group plotted in the normal reference charts. The SD-scores of all three biometric parameters were statistically significantly different from the SD-scores of the mean of normal controls for both the subset of lethal lung hypoplasia and the subset of combined non-lethal and absent lung hypoplasia. For the TC and the CC/TC, this was independent of the addition of the three cases of non-lethal lung hypoplasia to the subset of absent lung hypoplasia. TC/AC ratio displayed the best positive predictive value and overall accuracy in the prenatal prediction of lethal lung hypoplasia. (Tables 3 and 4)

Table 2
Efficacy of the clinical parameters in the prenatal prediction of lethal lung hypoplasia in the subset of PROM (n=31)

Clinical parameters	*Cut-off levels*	*Sens %*	*Spec %*	*PPV %*	*NPV %*	*Accuracy %*
Onset of PROM	≤ 20 wk[a]	78	77	58	89	77
	≤ 24 wk	100	45	43	100	61
	≤ 26 wk	100	32	38	100	52
Duration of Oligohydramnios	≥ 8 wk[b]	89	59	47	93	68
	≥ 6 wk	100	50	45	100	65
	≥ 4 wk	100	41	41	100	58
Degree of oligohydramnios	≤ 1 cm[c]	78	82	64	90	81
	≤ 2 cm	100	32	38	100	52
Combination of the parameters with the most accurate cut-off level (a+b+c)		67	95	86	88	87

Sens, sensitivity; Spec, specificity; PPV and NPV, positive and negative predictive value.

Doppler velocity parameters

Technically acceptable proximal, middle and distal arterial pulmonary branch flow velocity waveforms were obtained in 88%, 81% and 55% for the total study group and in 94%, 84% and 68% for the PROM subset. The success rate in obtaining technically acceptable distal arterial velocity waveform recordings was significantly lower (p=0.03) in lethal lung hypoplasia (6/18=33%) compared with combined non-lethal and absent lung hypoplasia (17/24= 71%). Therefore, distal arterial velocity waveform recordings were omitted from further analysis.

For the total study group, the SD-scores of TAV, peak systolic, peak diastolic and end-diastolic velocities from both the proximal and middle arterial pulmonary velocity waveforms were statistically significantly different from the SD-scores of the mean of normal controls for both lethal lung hypoplasia, and combined non-lethal and absent lung hypoplasia. This was independent of the addition of the three cases of non-lethal lung hypoplasia to the subset of absent lung hypoplasia. For the PI, this only applied to the proximal arterial velocity waveforms.

The highest positive predictive values and overall accuracy in the prenatal prediction of lethal lung hypoplasia were achieved for the peak systolic velocity of the proximal arterial pulmonary branch flow velocity waveforms and for the TAV and end-diastolic velocity of the middle arterial flow velocity waveforms. (Tables 3 and 4) Individual data for these

three flow velocity parameters relative to the normal reference charts, are demonstrated in Figure 4. Individual PI data, representing the (nearly) lowest positive predictive values and overall accuracy (Tables 3 and 4), are given in Figure 5.

Table 3

Efficacy of the biometric and Doppler parameters in the prenatal prediction of lethal lung hypoplasia in the total study group (n=42)

Incidence LLH 43%	***Success rate***	***Sens %***	***Spec %***	***PPV %***	***NPV %***	***Accuracy %***
Biometric parameters						
TC	98% (41/42)	94	38	52	90	61
CC_TC	98% (41/42)	76	50	52	75	61
TC_AC [a]	95% (40/42)	69	71	61	77	70
Doppler parameters						
Proximal pulm. branch	88% (37/42)					
TAV		69	76	69	76	73
PSV [b]		63	90	83	76	78
PDV		44	90	78	68	70
EDV		50	86	73	69	70
PI		56	76	64	70	68
Middle pulm. branch	81% (34/42)					
TAV		71	80	71	80	76
PSV		43	80	60	67	65
PDV		43	70	50	64	59
EDV		57	90	80	75	76
PI		29	75	44	60	56
Combination						
Biometry + Doppler	86% (36/42)	40	100	100	70	75

LLH, lethal lung hypoplasia; Sens, sensitivity; Spec, specificity; PPV and NPV, positive and negative predictive value; TC, thoracic circumference; CC/TC, ratio of cardiac and thoracic circumference; TC/AC, ratio of thoracic and abdominal circumference; Pulm., pulmonary; TAV, time-averaged velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; PI, pulsatility index. a and b: variables used for the biometric and Doppler combination.

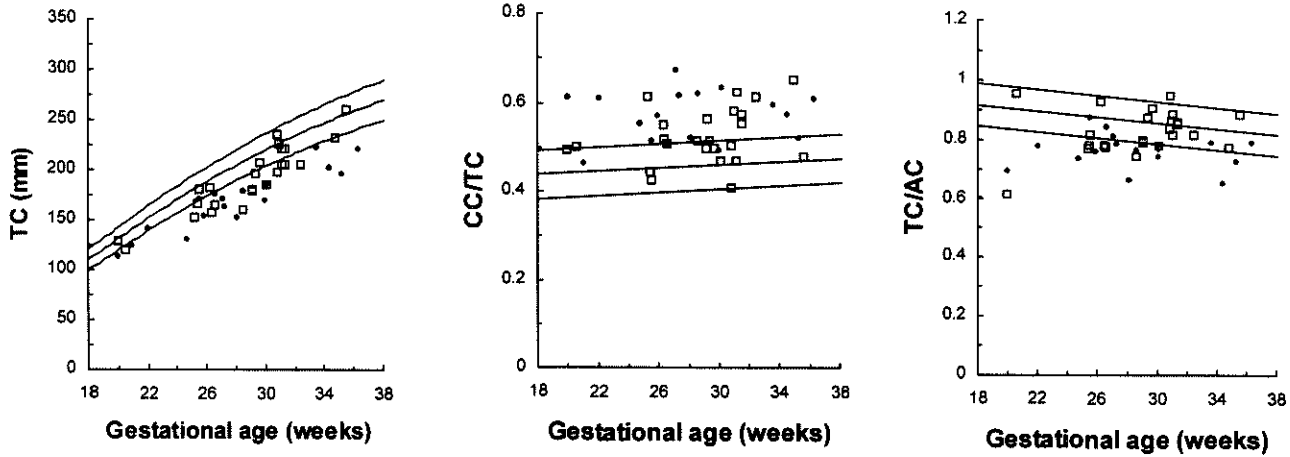

Figure 3

Individual values from the total study group compared to reference ranges (mean, 5th and 95th centiles) for (A) thoracic circumference (TC, mm), (B) ratio of cardiac and thoracic circumference (CC/TC) and (C) ratio of thoracic and abdominal circumference (TC/AC) relative to gestational age. The solid circles represent fetuses with lethal lung hypoplasia and the open blocks represent fetuses with non-lethal and absent lung hypoplasia.

Table 4
Efficacy of the biometric and Doppler parameters including the combination with the clinical parameters in the prenatal prediction of lethal lung hypoplasia in the subset of PROM (n=31)

Incidence LLH 29%	*Success rate*	*Sens %*	*Spec %*	*PPV %*	*NPV %*	*Accuracy %*
Biometric parameters						
TC	100%	100	36	39	100	55
CC_TC	100%	78	50	39	85	58
TC_AC [a]	97% (30/31)	63	73	45	84	70
Doppler parameters						
Proximal pulm. branch						
TAV	94% (29/31)	63	76	50	84	72
PSV [b]		63	90	71	86	83
PDV		25	90	50	76	72
EDV		38	86	50	71	72
PI		38	76	38	76	66
Middle pulm. branch	84% (26/31)					
TAV		71	84	63	89	81
PSV		43	84	50	80	73
PDV		14	68	14	68	54
EDV		57	95	80	86	85
PI		29	79	33	75	65
Combinations						
Clinical (Table 2)	100%	67	95	86	88	87
Clinical+Biometry	97% (30/31)	38	100	100	81	83
Clinical+Doppler	94% (29/31)	50	100	100	84	86
Biometry+Doppler	90% (28/31)	29	100	100	81	82
Clinical+Biometry+ Doppler	94% (29/31)	71	100	100	92	93

LLH, lethal lung hypoplasia; PROM, premature rupture of membranes; Sens, sensitivity; Spec, specificity; PPV and NPV, positive and negative predictive value; TC, thoracic circumference; CC/TC, ratio of cardiac and thoracic circumference; TC/AC, ratio of thoracic and abdominal circumference; Pulm., pulmonary; TAV, time-averaged velocity; PSV, peak systolic velocity; PDV, peak diastolic velocity; EDV, end-diastolic velocity; PI, pulsatility index. a and b: variables used for the biometric and Doppler combination. Clinical: combination of the 3 clinical parameters with the most accurate cut-off points in the prediction of lethal lung hypoplasia, i.e. onset of PROM ≤ 20 weeks, duration of oligohydramnios ≥ 8 weeks, degree of oligohydramnios of ≤ 1 cm.

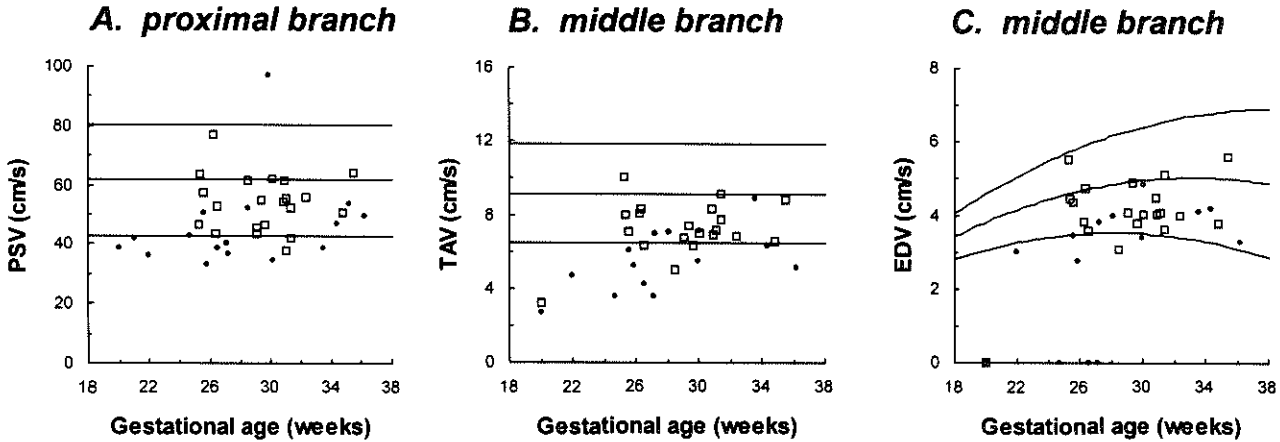

Figure 4

Individual values from the total study group compared to reference ranges (mean, 5th and 95th centiles) for (A) peak systolic velocity (PSV, cm/s) of the proximal arterial pulmonary branch, (B) time-averaged velocity (TAV, cm/s) and (C) end-diastolic velocity (EDV, cm/s) of the middle arterial pulmonary branch relative to gestational age. The solid circles represent fetuses with lethal lung hypoplasia and the open blocks represent fetuses with non-lethal and absent lung hypoplasia.

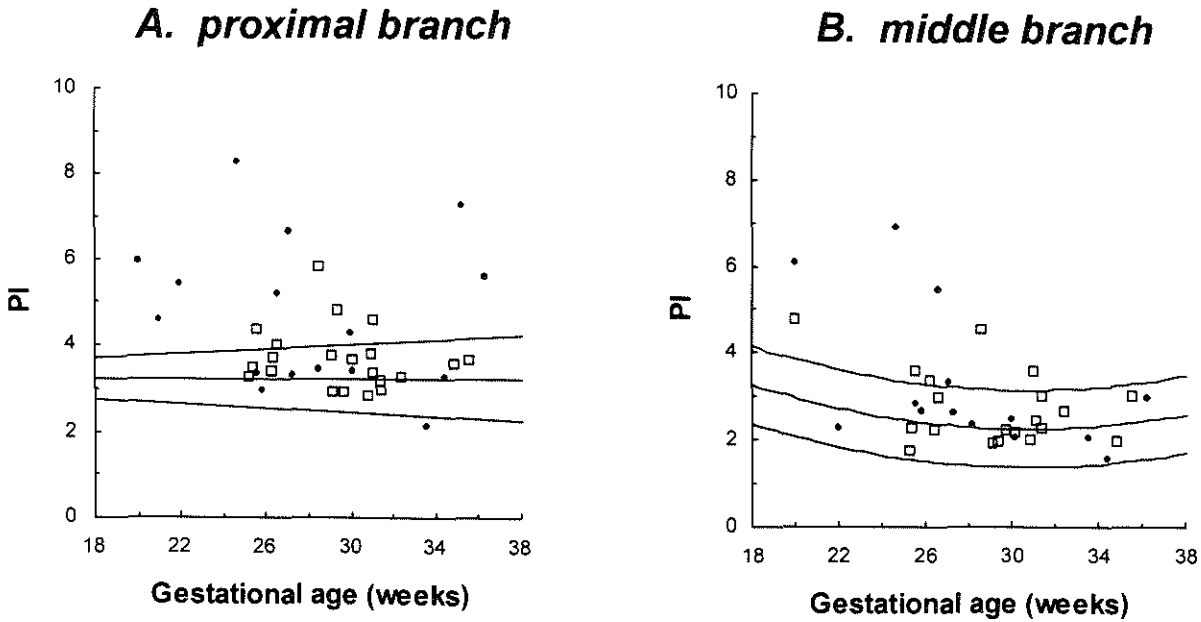

Figure 5
Individual values from the total study group compared to reference ranges (mean, 5th and 95th centiles) for (A) pulsatility index (PI) of the proximal arterial pulmonary branch and (B) pulsatility index (PI) of the middle arterial pulmonary branch relative to gestational age. The solid circles represent fetuses with lethal lung hypoplasia and the open blocks represent fetuses with non-lethal and absent lung hypoplasia.

Combination of clinical, biometric and Doppler parameters

In the total study group, TC/AC and proximal arterial pulmonary peak systolic velocity (PSV) were combined to examine whether this combination could increase the efficacy of prenatal prediction of lethal lung hypoplasia when compared with the prenatal predictive value by each of the parameters separately. The success rate of obtaining this combination of parameters was 86% (36/42). Lethal lung hypoplasia existed in 3 of the 6 unsuccessful cases, leaving 15 out of 18 cases of lethal lung hypoplasia for analysis of the combined data. Lethal lung hypoplasia was predicted, when both TC/AC and PSV were situated below the 5th centile (< P5) of the normal reference charts. This biometric and Doppler combination resulted in a positive predictive value of 100% (CI: 54-100%) and an overall accuracy of 75% (CI: 58-88%), with a drop in sensitivity to 40% (6/15, CI: 16-68%). (Table 3) Thus, lethal lung hypoplasia was detected in 6 out of 18 cases.

In the subset of PROM, it was established whether the predictive value of lethal lung hypoplasia based on the combined clinical parameters, could be further improved by the following combinations (Table 4):

(i) combined clinical parameters and TC/AC;
(ii) combined clinical parameters and proximal PSV;
(iii) combined TC/AC and proximal PSV;
(iv) combined clinical parameters, TC/AC and proximal PSV.

In the first three combinations (i-iii), lethal lung hypoplasia was considered present when both variables were abnormal. The success rate in obtaining the first combination was 97% (30/31), with one unsuccessful case of lethal lung hypoplasia, resulting in 8 out of 9 cases of lethal lung hypoplasia for further analysis. This combination showed a positive predictive value of 100% (CI: 29-100%), an overall accuracy of 83% (CI: 65-94%) and a sensitivity of only 38% (3/8, CI: 9-76%). The success rate of the second combination was 94% (29/31). Lethal lung hypoplasia existed in 1 of the 2 unsuccessful cases, leaving 8 out of 9 cases of lethal lung hypoplasia for analysis of the combined data. The positive predictive value of this combination also reached the level of 100% (CI: 40-100%) with an accuracy of 86% (CI: 68-96%) and a sensitivity of 50% (4/8, CI: 16-84%). The third combination was successful in 90% (28/31), with 2 cases of lethal lung hypoplasia out of the 3 unsuccessful cases, resulting in 7 out of 9 cases of lethal lung hypoplasia for analysis. The positive predictive values improved to 100% (CI: 16-100%) with an accuracy of 82% (CI: 6-94%) and a sensitivity of 29% (2/7,CI: 4-71%). With these three combined tests, lethal lung hypoplasia could be detected in 3, 4 and 2 out of 9 cases, respectively. The success rate in obtaining the last combination (iv), was 94% (29/31). Lethal lung hypoplasia existed in both unsuccessful cases, leaving 7 out of 9 cases of lethal lung hypoplasia for analysis of the combined data. Lethal lung hypoplasia was predicted when at least two of the three variables were abnormal. Positive predictive

value, overall accuracy and sensitivity, demonstrated an increase up to 100% (CI: 48-100%), 93% (CI: 77-99%) and 71% (5/7, CI: 29-96%), respectively (Table 4). Lethal lung hypoplasia was detected in 5 out of 9 cases.

DISCUSSION

The present study describes the role of clinical, biometric and Doppler parameters, separate or in combination, in the prenatal prediction of lethal lung hypoplasia in pregnancies associated with prolonged oligohydramnios of renal or non-renal (PROM) origin.

A normal amount of amniotic fluid is important for optimal fetal lung growth. Although the association between oligohydramnios and pulmonary hypoplasia is well-documented, the underlying mechanisms for this phenomenon have not been fully elucidated. Several explanations have been put forward such as i) decreased space for lung growth due to pressure of the uterine wall upon the fetal chest and abdomen, ii) restriction of fetal breathing movements by prolonged thoracic compression and iii) increased efflux of lung liquid from the intrapulmonary space to the amniotic space, resulting in a decrease of intrapulmonary pressure.[19,41]

In case of pulmonary hypoplasia associated with oligohydramnios, the severity of this abnormal condition depends on the degree and duration of oligohydramnios and the stage of lung development.[28,42] The present study confirms the existence of a significant relation between onset of PROM, duration and degree of oligohydramnios and the development of lethal lung hypoplasia. The positive predictive value of a prenatal test for detecting lethal lung hypoplasia, should be 100% in order to allow adjustment of obstetric management. Onset of PROM, duration and degree of oligohydramnios, as separate variables, displayed the highest prenatal prediction of lethal lung hypoplasia at the cut-off levels of $\leq$ 20 weeks, $\geq$ 8 weeks and $\leq$ 1 cm, respectively. However, neither the corresponding positive predictive values of 58%, 47% and 64% for each of the clinical parameters separately, nor a positive predictive value of 86% and an accuracy of 87% for the combined clinical parameters, is acceptable for clinical application.

It has been attempted to predict lethal pulmonary hypoplasia from fetal thoracic circumference and sagittal lung length or transversal lung diameter measurements, as well as from cardiac/thoracic and thoracic/abdominal circumference ratios.[29,30,43] Most biometric studies represent heterogeneous and relatively small patient cohorts and use different definitions of thoracic circumference and lung hypoplasia. Moreover, none of these biometric tests were reliable enough to be applied in clinical management. Also in the present study, the three biometric parameters TC, CC/TC and TC/AC, failed to provide an acceptable prenatal prediction of lethal lung hypoplasia, both in the total

study group and the subset of PROM. In the latter, the clinical parameters reached an even better prediction rate than the biometric parameters.

Remarkable is the finding that not only the mean SD-scores of the biometric parameters from the lethal lung hypoplasia subset, but also from the combined non-lethal and absent lung hypoplasia subset, were significantly different from the SD-score of the mean of the normal controls. For the TC and the CC/TC, this was also the case for absent lung hypoplasia alone. We propose that mechanical compression associated with marked oligohydramnios and/or possible interference with fetal breathing movements may play a role in this phenomenon, independent of the occurrence of lethal lung hypoplasia.

Since prenatal fetal biometry has failed to provide an accurate prediction of pulmonary hypoplasia, other methods had to be explored. A prolonged and pronounced oligohydramnios, particularly during the canalicular phase of lung development (from 18 to 26-28 weeks of gestation), may cause a delay or even arrest in pulmonary vascular development, resulting in reduced lung volume and raised pulmonary vascular resistance.[16,33] Consequently, the present study further focussed on the possible contribution of Doppler velocimetry of the arterial pulmonary circulation for the improvement of prenatal prediction of lung hypoplasia. In the total study group, the success rate of obtaining technically acceptable proximal and middle arterial pulmonary branch flow velocity waveforms was 88% and 81%, respectively, which is similar to that reported earlier in uncomplicated pregnancies.[31,32] Regarding the subset of lethal lung hypoplasia alone, the percentage of technically acceptable distal waveforms was significantly less (33%) compared with the subset of combined non-lethal and absent lung hypoplasia (71%). This suggests that failure to record distal arterial flow velocity waveforms may be indicative of lethal lung hypoplasia. So far, comparison with other studies is not possible, due to differences in definition of Doppler sample site for distal pulmonary arteries.[44,45]

Similar to the fetal biometric parameters, nearly all mean SD-scores of the proximal and middle arterial pulmonary flow velocity waveforms from the subset of combined non-lethal and absent lung hypoplasia, were significantly different from the SDS of the mean of normal controls, independently of the addition of the three cases of non-lethal lung hypoplasia to the subset of absent lung hypoplasia. In the normally developing fetus, fluid leaves the pulmonary vasculature, passes through the interstitium of the lungs entering the potential air spaces and flows out of the trachea. It has been postulated that oligohydramnios increases efflux of lung liquid from the intrapulmonary space into the amniotic space, resulting in a decrease of intrapulmonary pressure.[19,41] We propose that changes in lung liquid dynamics, even without dramatic influences on lung growth resulting in lung hypoplasia, may affect pulmonary blood flow and consequently pulmonary blood flow velocity waveforms.

The most reliable Doppler velocity parameters in the detection of lethal lung hypoplasia were peak systolic velocity in the proximal arterial pulmonary velocity waveform and TAV and end-diastolic velocity in the middle arterial pulmonary velocity waveform with positive predictive values ranging between 71% and 83% and an accuracy varying between 76% and 78% in the total study group. The majority of the Doppler velocity parameters from both proximal and middle arterial pulmonary branches showed even higher positive predictive values and overall accuracy than the biometric parameters. Nevertheless, also Doppler velocimetry of the arterial pulmonary circulation as a single test, failed to be reliable enough for clinical application. In the subset of PROM, the three combined clinical parameters, displayed an even higher positive predictive value and accuracy than Doppler velocimetry.

The lowest positive predictive value for both the proximal and middle arterial pulmonary branches (64% and 44%, respectively) and overall accuracy (68% and 56%, respectively) was presented by the PI in the total study group. The PI is known as a reliable Doppler parameter for the detection of changes in feto-placental resistance. Contradictory views have been reported regarding the clinical significance of the PI in the prediction of lethal lung hypoplasia.[44-47] These reports present data from very small heterogeneous patient series and do not include cases of premature rupture of membranes, which is considered one of the most common causes of pulmonary hypoplasia.

Since we demonstrated that also Doppler velocimetry of the arterial pulmonary circulation fails to be the single and ultimate prenatal test in the prediction of lethal lung hypoplasia, we addressed the question whether combining clinical, biometric and pulmonary Doppler parameters could improve the predictive value of lethal lung hypoplasia. In the total study group, first the most accurate biometric parameter, i.e. TC/AC, and the most accurate Doppler parameter, i.e. the proximal arterial pulmonary PSV, were combined. The positive predictive value improved to 100%. However, this result could be achieved in only 6 out of 18 cases with lethal lung hypoplasia, which would limit its clinical applicability. When a similar exercise was carried out in the subset of PROM, all three combinations of: (i) combined clinical parameters and TC/AC, (ii) combined clinical parameters and proximal PSV, and (iii) TC/AC and proximal PSV reached a positive predictive value of 100%. Also here, a correct prediction could only be made in 2 to 4 out of 9 cases with lethal lung hypoplasia. Combination of all three clinical, biometric and Doppler parameters not only demonstrated a positive predictive value of 100%, but also a considerable improvement in overall accuracy (93%) and sensitivity (71%). Here, a correct prenatal diagnosis of lethal lung hypoplasia was made in 5 cases out of 9 cases with lethal lung hypoplasia, constituting the most favorable combination in the subset of PROM.

It can be concluded that Doppler velocimetry may detect changes in blood velocity waveforms from the proximal and middle arterial branches of the fetal pulmonary circulation in the presence of pulmonary hypoplasia. The best prediction was achieved in the subset of PROM, when a combination of clinical, biometric and Doppler parameters was applied. The clinical significance of this combined test, however, appears to be limited due to the restrictions in obtaining the necessary components of this test and the low sensitivity of the combination.

7.4 References

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CHAPTER 8

GENERAL CONCLUSIONS AND DISCUSSION

The introduction of high resolution color-coded Doppler facilities has opened the possibility of studying the lung circulation during fetal life. It was argued that well-defined changes in arterial pulmonary vascular morphology established on postmortem examination in lethal lung hypoplasia, justifies an exploration of the functional aspects of the fetal arterial pulmonary circulation during the second half of gestation. It was realized that assessment of fetal arterial lung flow is limited to a descriptive analysis of Doppler flow velocity waveforms. As yet, no technique is available which provides information on volume flow and intra-arterial pressure in the human fetal lung circulation. Moreover, our understanding of the exact meaning of fetal arterial and venous pulmonary waveforms is hampered by the scarcity of similar studies under animal experimental conditions. Nevertheless, the above-mentioned morphological changes in the lung vascularization associated with lung hypoplasia, provide a challenge in establishing the nature of fetal pulmonary hemodynamics under physiological conditions and in determining the prenatal predictive value of the arterial pulmonary waveforms in the development of lethal lung hypoplasia.

In this thesis, Doppler assessment of the fetal pulmonary circulation was first performed in the normally developing fetus applying combined color-coded and pulsed Doppler techniques. Pulsed Doppler measurements of the right or left pulmonary arterial and venous branches were attempted during fetal apnea from a transverse cross-section of the fetal chest at the level of the four-chamber view after visualization with color Doppler.

Firstly, it became possible to obtain arterial and venous flow velocity waveforms across the left and right fetal lung, including the most distal vascular branches close to the inner thoracic wall in at least three-quarter of all recordings. Recording failures were determined by fetal (breathing) movements, unfavorable fetal position or maternal obesity.

Secondly, acceptable repeatability of flow velocity waveforms from both fetal arterial and venous pulmonary branches was established with coefficients of variation below 15% for nearly all flow velocity waveforms parameters and their ratios, with proximal arterial and venous pulmonary branches displaying the lowest coefficients of variation.

Thirdly, we were able to define the exact pattern of both arterial and venous waveforms.

Both proximal and middle *arterial* pulmonary branches were characterized by a rapid systolic velocity acceleration, followed by an equally rapid deceleration producing a needle-shaped systolic peak, although with lower maximum peak systolic velocities in the middle arterial branch. Of interest was the short reverse flow interval at the

beginning of diastole which was noted in nearly all proximal and just under half of the middle arterial branches. The distal arterial pulmonary branch is characterized by a monophasic flow velocity profile with low forward flow velocities throughout the cardiac cycle. Based on knowledge mainly derived from fetal lamb studies of the pulmonary arterial circulation, high downstream impedance and the presence of a patent ductus arteriosus may be responsible for the nature of this particular profile.[1,2] Nevertheless, the physical basis for the shape of the human fetal arterial pulmonary branch flow velocity waveforms remains speculative and is probably determined by several factors, such as vascular pressure, resistance, impedance, and ventricular contractility.[3]

Normal proximal, middle and distal pulmonary *venous* blood flow velocity waveforms exhibit a biphasic pattern at all the three locations, consisting of a systolic and early diastolic forward component and forward flow during late diastole coincident with atrial contraction. However, in a small percentage of middle venous pulmonary flow velocity waveforms and particularly distal venous pulmonary flow velocity waveforms, no end-diastolic forward could be observed. These venous velocity profile characteristics suggest positive pressure towards the left atrium throughout the whole cardiac cycle. A similar pattern has been established in animals and adult humans, in which it seems to represent the inverse of the left atrial pressure waveform.[15,16] This implies that pulmonary venous blood flow is influenced by dynamic changes in left atrial pressure as a result of atrial and ventricular contraction and relaxation.

A fourth and important step in our Doppler examination of the fetal lung circulation was to relate waveforms to gestational age.

On the *arterial* side of the fetal lung circulation, we found that time-averaged velocity (TAV) and peak systolic velocity did not change significantly with advancing gestational age. It appears that the increase in vessel diameter and/or compliance rather than a rise in flow velocities determines the increase in pulmonary artery volume flow during the second half of gestation.[4-6] The gestational age independency of end-diastolic velocity in both proximal and distal arterial pulmonary branches, as opposed to a significant rise until 31 weeks in middle arterial pulmonary branches remains difficult to explain. Changes in morphology, in particular arterial muscular wall structure, vessel compliance and/or downstream impedance may be responsible for this finding.[5] A gestational age-related change was found for peak diastolic velocity, early peak diastolic reverse flow, diastolic integral, and peak systolic/peak diastolic ratio, which are all characteristic parameters for the proximal and middle arterial branch, suggesting changes in pulmonary resistance. The pulsatility index (PI) displayed no significant change in the proximal branch velocity waveform in relation to gestational age, whereas both middle and distal arterial PI depicted a significant decrease during the second and early third trimester of

pregnancy followed by a significant increase during the latter part of the third trimester. The significance of the PI as a measure of downstream impedance in the fetal lung circulation is questionable, which is supported by contradictory data from other studies.[7-9] The pulsatility/resistance index has been reported as remaining constant or demonstrating a gestational-related decrease. Further, differences appear to exist regarding peak systolic velocities in the proximal branch of the pulmonary artery, varying from a significant increase[3,7,10,11] to no change[12,13]. Other contradictory data have been reported on the end-diastolic component, ranging from forward flow being absent[3], present[10,13,14], or a combination of present and absent[7]. Doppler sample site and equipment-related differences (high-pass filter settings) are likely to be responsible for these discrepancies and emphasize the need for a standardized methodological approach to the recording procedure in pulmonary flow velocity studies. Based on our Doppler results in normal pregnancies, we speculate that measurement of diastolic velocity parameters rather than the Pulsatility Index may be useful in detecting gestational age-related changes in human fetal pulmonary vascular resistance.

On the *venous* side of the fetal lung circulation, a gestational age dependent change was found for all flow velocity waveform parameters including the PI for veins at proximal, middle and distal level. It is speculated that increase in volume flow and venous pulmonary pressure gradient play a role in these findings. Investigation of the pulmonary venous flow relative to the different systolic and diastolic time-intervals of the cardiac cycle, revealed that pulmonary venous inflow into the left atrium occurs predominantly during the filling and ejection phase of the cardiac cycle. Absolute cardiac diastolic and systolic time intervals as well as the percentage distribution of the pulmonary venous flow velocity integral between these cardiac time-intervals remain unchanged with advancing gestational age.

We also investigated in both the arterial and venous lung circulation, the inter-pulmonary relation between flow velocity waveforms at proximal, middle and distal level. A significant decrease in peak and time-averaged velocities and in PI was observed as Doppler interrogation moves from proximal to distal parts of the fetal lung. This may be mainly due to increase in vessel branching and/or diameter and distance between the heart and distal arterial pulmonary vessels.[5,17]

It has been firmly established in earlier studies from our center that fetal blood flow is modulated by intrinsic fetal variables such as breathing movements and behavior states. Behavior states dependent changes have been observed in Doppler flow velocity parameters obtained from the three fetal shunts, i.e. ductus venosus, foramen ovale and ductus arteriosus in the normally developing term fetus.[18-20]

It has been proposed that the increase in blood flow from the right to the left heart during the active sleep state (=behavior state 2F), could have some reflections on pulmonary hemodynamics. However, we were able to show that blood flow velocity waveforms from the proximal arterial pulmonary branch are behavior state independent during the last few weeks of normal pregnancies. This suggests that the arterial pulmonary vascular bed does not contribute to the general redistribution of blood flow to the left side of the heart. On the other hand, venous pulmonary blood flow velocity waveforms demonstrated a marked increase for both TAV and end-diastolic velocity during fetal behavior state 2F. This may reflect an increased pressure gradient between the pulmonary venous system and the left atrium resulting from reduced downstream impedance to venous drainage in behavior state 2F. Based on these findings, it may be concluded that behavior state should be taken into account in future studies on pulmonary venous blood flow velocity waveforms in normal term pregnancies.

Defective development of the fetal lung may result in a decrease in organ size and organ weight. A normal fetal lung volume is one of the major contributors of normal fetal lung development. In order to explore new non-invasive diagnostic methods for the prenatal prediction of lethal lung hypoplasia, recent reports have indicated the possible value of fetal lung volume measurements using echo-planar magnetic resonance[21], or three-dimensional ultrasonography[22-24]. Especially, the latter technique has a high patient acceptability.

Three-dimensional ultrasonographic measurements of fetal lung volume demonstrate a close association with gestational age and fetal weight. Nevertheless, it should be realized that these measurements are indirect determinations of lung volume, which are calculated by subtracting fetal heart volume from fetal thoracic volume. The significance of three-dimensional ultrasonography in the prediction of lung hypoplasia still needs to be determined. However, prolonged severe oligohydramnios, as one of the most common causes of lung hypoplasia, may put some restrictions on the applicability of this technique. New digital imaging techniques producing better resolution may allow more accurate assessment of fetal lung volume in fetuses at risk of the developing of lung hypoplasia.

Pulmonary hypoplasia is associated with a high perinatal mortality rate. An accurate prenatal test for detecting pulmonary hypoplasia is highly desirable. Various methods have been proposed during the last two decades. Most of these include fetal two-dimensional thoracic and lung measurements and their ratios with other fetal biometric parameters.[25-27] However, not a single one possesses enough predictive value regarding the development of lethal fetal lung hypoplasia allowing reliable clinical decision making. Further, data on the presence of fetal breathing movements in the prediction of lung hypoplasia remain contradictory.[28,29]

To be used in obstetric management, a particular test should allow differentiation between lethal and non-lethal lung hypoplasia and display a positive predictive value of 100%.

Based on our work on normal arterial pulmonary Doppler velocimetry, we addressed the validity of Doppler velocimetry of the fetal arterial pulmonary circulation in the prediction of lethal pulmonary hypoplasia. Increased pulmonary vascular resistance and reduced pulmonary arterial compliance may be the result of underdevelopment and structural changes of the pulmonary vascular bed as seen in postmortem findings of pulmonary hypoplasia.[30-32] These alterations, which constitute a decreased total size of the pulmonary vascular bed, a reduced vessel count per unit of lung tissue and increased pulmonary vascular muscularization, could effect pulmonary blood flow and consequently induce changes in pulmonary flow velocity waveforms as recorded by Doppler ultrasonography.

We observed a significant reduction of both systolic and diastolic velocities in the proximal arterial pulmonary branches in a case of fetal pulmonary hypoplasia due to bilateral obstructive uropathy. Further, the shape of the pulmonary arterial waveform had altered, depicting a second needle-shaped peak during mid-systole, which was even higher than the first systolic peak. The presence of reduced volume flow and/or raised downstream impedance may have been responsible for these Doppler velocity changes. So far, Doppler arterial pulmonary waveform data associated with pulmonary hypoplasia have only been reported from small heterogeneous patient series. Contradictory views exist regarding the clinical significance of the PI in the prediction of lethal lung hypoplasia.[8-11] A larger well-defined patient cohort was needed to establish the role of Doppler velocimetry in the prenatal prediction of lethal lung hypoplasia.

This leads to our final study, which focussed on the role of Doppler velocimetry in relation to clinical and biometric parameters in the prenatal prediction of lethal lung hypoplasia associated with prolonged marked oligohydramnios, due to premature rupture of membranes (PROM) or bilateral renal pathology. Despite marked oligohydramnios, recording of technically acceptable proximal and middle arterial pulmonary branch flow velocity waveforms was as successful as in normal pregnancies. However, in the presence of lethal lung hypoplasia, the percentage of technically acceptable distal waveforms was significantly reduced. It was hypothesized that unsuccessful distal arterial pulmonary flow velocity waveforms recordings might be suspicious of lethal lung hypoplasia.

When looking at the different components of the arterial pulmonary waveforms, the most reliable parameters in the detection of lethal lung hypoplasia were peak systolic velocity in the proximal arterial pulmonary velocity waveform, and TAV and end-diastolic velocity in the middle arterial pulmonary velocity waveform with positive predictive values of more than 70% and an accuracy of more than 75%. Our previous

speculations that measurement of diastolic velocity parameters may be important in detecting gestational age-related changes in human fetal pulmonary vascular resistance, could therefore not be supported. velocity. A possible explanation for this contradiction is that the raised pulmonary vascular resistance based on vascular changes as seen in fetal lung hypoplasia is more pronounced than the gestational age-related changes in pulmonary vascular resistance as witnessed in the normally developing fetus. The latter is a result of both a decrease in vessel compliance and volume flow. In the total study group, the lowest positive predictive value for both the proximal and middle arterial pulmonary branches and overall accuracy was presented by the PI, which is in agreement with our previous observations that the PI is not useful in the detection of changes in human fetal pulmonary vascular resistance.

Doppler velocimetry of the arterial pulmonary circulation displayed a better positive predictive value and overall accuracy than fetal chest and its cardiac and abdominal ratios. But, in the subset of PROM, early onset of prolonged and marked oligohydramnios was more predictive for lethal lung hypoplasia than Doppler velocimetry. Doppler velocimetry of the arterial pulmonary circulation as a single test, therefore, fails to be reliable enough for clinical application.

Next, we questioned whether combining clinical, biometric and pulmonary Doppler parameters could improve the predictive value of lethal lung hypoplasia. In both the total study group and the subset of PROM, a positive predictive value of 100% was achieved for the biometric and Doppler combination, but the sensitivity was low (≤ 50%). In the subset of PROM alone, a similar result was established for the combinations of (i) combined clinical and biometric parameters and (ii) combined clinical and Doppler parameters. This again would limit its applicability in clinical management. Finally, in the PROM subset, the combination of all three clinical, biometric and Doppler parameters not only revealed a positive predictive value of 100%, but also a considerable improvement in overall accuracy (93%) and sensitivity (71%). A correct prenatal diagnosis of lethal lung hypoplasia could be reached in 55% of all cases with lethal lung hypoplasia, constituting the most favorable combination in the subset of PROM. Nevertheless, also here the clinical significance appears to be limited due to the restrictions in obtaining the necessary Doppler and biometric components of the combinations as well as the relatively low sensitivity.

Whether these findings may be reliable enough to improve both obstetric management and parental counseling, needs to be substantiated in a prospective study design. Further improvement in equipment technology may lead to a higher success rate in obtaining the necessary combination of Doppler and biometric data. It seems clear though that currently Doppler velocimetry alone is not accurate enough in predicting the lethal

form of lung hypoplasia. Whether addition of more recent techniques such as digital three-dimensional ultrasonography or fast MRI may contribute to a further improvement in the prenatal prediction of lethal lung hypoplasia, may become clear in the not too distant future.

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SUMMARY

CHAPTER 1

Prolonged oligohydramnios due to premature rupture of membranes (PROM) or renal and urinary tract malformations may lead to abnormal fetal lung development with subsequent severe respiratory distress immediately after birth and even neonatal death. Therefore, an accurate and patient-friendly prenatal test for early detection and separation of lethal from non-lethal pulmonary hypoplasia is highly desirable. Up till now, there is no test with a sensitivity and specificity satisfactory for clinical management.

The introduction of more sensitive color-coded Doppler systems has led to the visualization of the human fetal pulmonary circulation. It has been hypothesized that Doppler velocimetry may detect lung hypoplasia based on postmortem examination of decreased total size of the pulmonary vascular bed, decreased number of pulmonary vessels per unit lung tissue and increased pulmonary vascular muscularization. Consequently, non-invasive Doppler velocimetry of the human fetal arterial and venous intrapulmonary circulation in normally developing fetuses and in fetuses at risk for lung hypoplasia due to prolonged oligohydramnios has become the main subject of our studies in this thesis.

CHAPTER 2

A literature survey is presented on normal and abnormal lung development with emphasis on pulmonary hypoplasia. Normal anatomical and physiological aspects of fetal lung development including the fetal pulmonary circulation are described. Factors which may influence fetal lung growth are discussed. Further, the pathogenesis of pulmonary hypoplasia is highlighted with description of it's clinical, radiological and pathologic features. Finally, an extended overview is presented on the different methods having been put forward to play a role in the prenatal prediction of pulmonary hypoplasia.

CHAPTER 3

Doppler measurements of the arterial intrapulmonary branches in the normally developing human fetus are performed from a transverse cross-section of the fetal chest at the level of the cardiac four-chamber view applying combined color-coded and pulsed Doppler techniques. The proximal and middle arterial pulmonary waveform profile display a needle-shaped systolic peak, followed by a more gradual decline in flow velocity, which may be interrupted by a short reverse flow interval at the beginning of diastole. Forward flow can be observed throughout the remainder of diastole. A high downstream impedance may be responsible for this shape. The distal arterial pulmonary branch blood flow velocity waveform shows a monophasic profile with low forward flow velocities throughout the cardiac cycle. Acceptable repeatability of all these flow velocity waveforms, with coefficients of variation below 15%, is demonstrated.

No significant change in time-averaged velocity (TAV) and peak systolic velocity with advancing gestational age is found for proximal, middle and distal pulmonary branches. Changes in vessel diameter and/or compliance may play a role in this. At both proximal and mid-arterial level a gestational age-related change is found for peak diastolic velocity, early peak diastolic reverse flow, diastolic integral and peak systolic/peak diastolic ratio suggesting changes in pulmonary vascular resistance. At distal arterial level, pulmonary Doppler velocity waveform parameters remain unchanged with advancing gestational age. Whereas the pulsatility index (PI) from the proximal pulmonary artery does not change significantly during gestation, both middle and distal arterial PI reveal a significant decrease followed by a significant increase during the last 8-10 weeks of gestation in the same study period.

A significant decrease in peak and time-averaged velocities and in PI is established as Doppler interrogation moves from the proximal to more distal parts of the fetal lung. This may be due to increase in vessel branching/diameter and in distance between the heart and distal arterial pulmonary vessels.

CHAPTER 4

In the human fetus, technically acceptable flow velocity waveforms can be obtained from the most prominent branch of the pulmonary vein immediately proximal to the entrance into the left atrium and from the pulmonary veins in the middle and distal lung region after visualization with color Doppler at the level of the cardiac four-chamber view. On all these three locations, venous pulmonary waveforms demonstrate a biphasic pattern, consisting of a systolic and early diastolic forward flow component and a late diastolic forward component coincident with atrial contraction. This is similar to that in the adult, suggesting that venous pulmonary flow is influenced by dynamic changes in left atrial pressure as a result of atrial and ventricular contraction and relaxation. Acceptable repeatability of flow velocity waveforms with coefficients of variation below 15% is established for nearly all velocity parameters and their ratios.

A gestational age dependent change is found for all venous flow velocity waveform parameters including the PI for veins at proximal, middle and distal level. It is speculated that increase in volume flow and in venous pulmonary pressure gradient plays a role in these findings.

Nearly all waveform parameters alter significantly as pulsed Doppler interrogation proceeds from proximal to more distal venous lung regions. This may be due to changes in vessel branching/diameter and distance between the heart and more distal venous pulmonary vessels.

Further, nature and gestational age dependency of the pulmonary venous flow velocity pattern into the left atrium relative to systolic and diastolic phases of the cardiac cycle are described. Venous pulmonary inflow into the left atrium occurs predominantly during the filling and ejection

phase of the cardiac cycle. Absolute cardiac diastolic and systolic time intervals as well as the percentage distribution of venous pulmonary flow velocity integral between these cardiac time intervals remain unchanged with advancing gestational age.

CHAPTER 5

This chapter deals with the influence of fetal behavior states on venous and arterial pulmonary blood flow velocity waveforms in the normally developing term fetus. Both proximal venous and arterial Doppler blood flow velocity waveforms are obtained in the same lung using color Doppler imaging during behavior state 1F (quiet sleep) and 2F (active sleep). Fetal behavior states has been determined from combined recordings of fetal eye and body movements. Venous pulmonary blood flow velocity waveforms demonstrate a statistically significant increase in TAV, peak diastolic velocity and end-diastolic velocity during fetal behavior state 2F, suggesting an increased pressure gradient between the pulmonary venous system and left atrium during behavior state 2F. Behavior state independency exists for blood flow velocity waveforms from the proximal arterial pulmonary branch.

CHAPTER 6

This chapter focuses on 3-dimensional ultrasonographic lung volume measurement in the normally developing fetus in the second half of pregnancy. Total fetal lung volume is determined by subtraction of fetal heart volume from thoracic volume, using the perpendicular transverse, sagittal and frontal planes of the fetus. A statistically significant increase in normal fetal lung volume is established with advancing gestational age and with increasing fetal estimated weight, demonstrating an approximately 7-fold rise in fetal lung volume during the second half of pregnancy.

CHAPTER 7

Combined color-coded Doppler and pulsed Doppler ultrasonography provides not only the opportunity to identify and study the intrapulmonary circulation in the normally developing human fetus, but also in the fetus at risk for pulmonary hypoplasia. Sub-chapter 7.2 presents the first report of arterial pulmonary flow velocity waveforms originating from a fetus with lung hypoplasia. In this case, a marked reduction in systolic and diastolic velocities of the proximal arterial pulmonary waveform is observed, suggesting that Doppler velocimetry can play a role in the prenatal prediction of lung hypoplasia.

Consequently, the value of arterial intrapulmonary Doppler velocimetry relative to fetal biometric indices and clinical correlates is evaluated (sub-chapter 7.3) in the prenatal prediction of lethal lung hypoplasia due to prolonged oligohydramnios associated with premature rupture of membranes (PROM, n=31) or with bilateral renal pathology

(n=11). In the total study group, thoracic, cardiac and abdominal circumference and the largest vertical amniotic fluid pocket are measured, followed by Doppler assessment of the arterial pulmonary branches similarly performed as described in previous chapters. Diagnosis of lung hypoplasia is based on pathological, clinical and radiological criteria. Lethal lung hypoplasia could be determined in 43% of the total group.

In the PROM-subset, combination of onset of PROM ≤ 20 weeks; duration of oligohydramnios ≥ 8 weeks and degree of oligohydramnios ≤ 1 cm, presents the highest clinical prediction rate for lethal lung hypoplasia. For both the total group and the PROM-subset, the highest prediction rate for lethal lung hypoplasia is presented by thoracic/ abdominal circumference ratio, peak systolic velocity in the proximal branch, and TAV and end-diastolic velocity in the middle branch of the pulmonary artery. In the PROM subset, combination of all three clinical, biometric and Doppler parameters reveals the most favorable combination to predict lethal lung hypoplasia, (positive predictive value 100%, accuracy 93%, and sensitivity 71%).

It is concluded that although Doppler velocimetry may detect changes in arterial intrapulmonary waveforms in lung hypoplasia, it fails as an accurate prenatal predictor of lethal lung hypoplasia. The best prediction can be achieved by combining clinical, biometric and Doppler parameters.

SAMENVATTING

HOOFDSTUK 1

Een langdurig tekort aan vruchtwater (oligohydramnion) door vroegtijdig gebroken vliezen (PROM) of door een afwijking aan foetale nieren c.q. urinewegen kan bij de menselijke foetus leiden tot een onderontwikkeling van de longen (longhypoplasie) en dientengevolge ernstige ademhalingsproblemen na de geboorte of zelfs neonataal overlijden. Om die reden is een zekere en patiëntvriendelijke prenatale test voor de vroege herkenning van letale en niet-letale longhypoplasie zeer gewenst. Vandaag de dag is er nog geen test beschikbaar met een zodanige sensitiviteit en specificiteit die noodzakelijk is voor toepassing in de klinische praktijk.

Met de introductie van meer gevoeligere kleuren echoscopische Doppler technieken werd het mogelijk om de humane foetale longcirculatie op non-invasieve wijze te visualiseren. Weerstandsveranderingen door onderontwikkeling en structurele veranderingen in het longvaatbed zoals optredend bij longhypoplasie, zullen zich mogelijk uiten in meetbare veranderingen in long bloedstroomsnelheidpatronen en dus te detecteren zijn door middel van Doppler ultrageluidonderzoek. Deze hypothese vormt de basis van het Doppler ultrageluidonderzoek naar de arteriële en veneuze longcirculatie in de zich normaal ontwikkelende menselijke foetus en in de foetus met een verhoogd risico op de ontwikkeling van longhypoplasie door langdurig oligohydramnion.

HOOFDSTUK 2

Een literatuuroverzicht wordt gegeven over de normale en abnormale longontwikkeling met speciale aandacht voor de ontwikkeling van longhypoplasie. Normale anatomische en fysiologisch aspecten van foetale longontwikkeling inclusief de foetale longcirculatie worden beschreven. Factoren die mogelijk van invloed zijn op de foetale longgroei passeren de revue. De pathogenese van longhypoplasie wordt verder besproken met uiteenzetting van de klinische, radiologische en pathologische kenmerken van dit ziektebeeld. Tot slot wordt een uitgebreid overzicht gegeven van de verschillende methoden die zijn beschreven in het belang van de prenatale predictie van longhypoplasie.

HOOFDSTUK 3

Doppler ultrageluidonderzoek van de arteriële longcirculatie in de zich normaal ontwikkelende menselijke foetus kan worden uitgevoerd vanuit een echoscopische dwarsdoorsnede van de foetale borstkast, op het niveau van het 4-kamer beeld van het hart, na visualisatie met de kleuren Dopplertechniek. Het bloedstroomsnelheidsprofiel van de proximale en midden pulmonale takken wordt gekenmerkt door een naaldvormige systolische piek, gevolgd door een geleidelijke afname in bloedstroomsnelheid, waarbij een kort 'reverse flow' interval kan worden gezien aan het begin van diastole. De diastolische fase toont vervolgens voorwaartse flow. Een hoge stroomafwaartse impedantie lijkt verantwoordelijk voor dit

karakteristieke bloedstroomsnelheidsprofiel. Het distale arteriële long bloedstroomsnelheidsprofiel laat een monofasisch patroon zien met lage voorwaartse snelheden gedurende de gehele hartcyclus. De reproduceerbaarheid betreffende het verkrijgen van deze bloedstroomsnelheidsprofielen met een variatie coëfficiënt < 15%.

De gemiddelde snelheid en de piek systolische snelheid tonen geen significante verandering met het vorderen van de zwangerschapsduur in zowel de proximale, de midden als de distale arteriële pulmonale takken. Veranderingen in bloedvatdiameter en/of compliantie spelen hierbij mogelijk een rol. Zowel proximaal als midden in het arteriële longvaatbed wordt een significante verandering met een toenemende zwangerschapsduur gevonden voor de piek diastolische en vroege piek diastolische reverse flow snelheid, de diastolische integraal en de piek systolische/piek diastolische ratio, mogelijk ten gevolge van veranderingen in longvaatweerstand. De bloedstroomsnelheidsparameters in het distale deel blijven constant in de loop van de zwangerschap. De pulsatiliteits index in de proximale arterie pulmonalis toont geen verandering in relatie tot de zwangerschapsduur, terwijl tijdens de dezelfde onderzoeksperiode, deze index in de midden en distale takken een significante daling laat zien gevolgd door een significante toename gedurende de laatste 8 à 10 weken van de zwangerschap.

Bij onderzoek naar de relatie tussen de intra-pulmonaal gemeten bloedstroomsnelheden wordt van proximaal naar meer distaal in het longvaatbed een significante afname gevonden in zowel piek snelheden, gemiddelde snelheden als in pulsatiliteits index. Dit wordt verklaard door de toename in het aantal bloedvatvertakkingen/diameter en in afstand tussen het hart en de meer distale arteriële longvaten.

HOOFDSTUK 4

In dit hoofdstuk staat de veneuze longcirculatie van het ongeboren kind centraal. Het is mogelijk om in de tweede helft van de zwangerschap ook in dit deel van de circulatie technisch acceptabele bloedstroomsnelheden te verkrijgen met behulp van de kleuren Dopplertechniek op het niveau van het 4-kamer beeld van het foetale hart. Het Doppler bloedstroomsnelheidsprofiel van zowel het meest proximale deel van de vena pulmonalis, vlak voor de inmonding in het linker atrium, als ook van het midden en distale deel van het veneuze longvaatbed, toont een bifasisch patroon. Dit patroon bestaat uit een systolische en een vroege diastolische voorwaartse flow component en een laat diastolische voorwaartse flow component tijdens atriale contractie. Een vergelijkbaar patroon werd ook postnataal gevonden bij zowel mens als dier, waarbij dit bloedstroomsnelheidsprofiel de inverse blijkt te zijn van het drukprofiel van het linker atrium. Dit suggereert dat de veneuze pulmonale bloedstroom wordt beïnvloed door dynamische drukveranderingen in het linker atrium, die het gevolg zijn van atriale en ventriculaire contractie en relaxatie. De

reproduceerbaarheid van deze bloedstroomsnelheidsprofielen is, evenals in het arteriële deel, acceptabel met variatie coëfficiënten < 15% voor vrijwel alle snelheidsparameters.

Alle bloedstroomsnelheidsparameters, inclusief de veneuze pulsatiliteits index, laten zowel op proximaal, midden als distaal longniveau, een significante verandering met het vorderen van de zwangerschapsduur zien. Een stijging in volume flow en in veneuze pulmonale drukgradiënt spelen hierbij mogelijk een rol.

Evenals in de arteriële longcirculatie is er bij onderzoek naar de relatie tussen de intra-pulmonaal gemeten bloedstroomsnelheden een significante afname te zien van proximaal naar distaal in het veneuze longvaatbed voor vrijwel alle bloedstroomsnelheidsparameters. Dit wordt verklaard door veranderingen in bloedvatvertakkingen/diameter c.q. afstand tussen het hart en de distale veneuze longvaten.

Vervolgens wordt het zwangerschapsduur afhankelijke bloedstroomsnelheidsprofiel van de veneuze inflow in het linker atrium beschreven in relatie tot de systolische en diastolische fasen van de hartcyclus. De veneuze pulmonale bloedinstroom in het linker atrium vindt met name plaats tijdens de vullings- en ejectiefase van de hartcyclus. Absolute diastolische en systolische tijdsintervallen evenals de percentuele spreiding van de veneuze pulmonale bloedstroomsnelheidsintegraal over deze cardiale tijdsintervallen zijn onafhankelijk van de zwangerschapsduur.

HOOFDSTUK 5

In dit hoofdstuk wordt de invloed van foetale gedragsfasen op de veneuze en arteriële long bloedstroomsnelheidsprofielen beschreven in de zich normaal ontwikkelende à terme foetus. Zowel de veneuze als de arteriële proximale Doppler bloedstroomsnelheidsprofielen werden verkregen in dezelfde long na visualisatie met kleuren Doppler in zowel gedragsfase 1F (rustige slaap) als gedragsfase 2F (actieve slaap). De foetale gedragsfasen werden bepaald op basis van aanwezigheid van foetale oogbewegingen en lichaamsbewegingen. Het veneuze pulmonale bloedstroomsnelheidsprofiel toont een significante toename in gemiddelde, piek diastolische en eind-diastolische snelheid tijdens gedragsfase 2F. Dit impliceert een toename in drukgradiënt tussen het veneuze longvaatbed en het linker atrium tijdens gedragsfase 2F. Daarentegen zijn de arteriële bloedstroomsnelheidsprofielen onafhankelijk van de foetale gedragsfasen.

HOOFDSTUK 6

Dit hoofdstuk richt zich op het 3-dimensionaal echoscopisch meten van het longvolume in de zich normaal ontwikkelende foetus gedurende de tweede helft van de zwangerschap. Het foetale hartvolume en thoraxvolume worden bepaald gebruikmakend van drie loodrecht op elkaar staande vlakken, te weten het transversale, het sagittale en het frontale

vlak van de foetus. Het totale foetale longvolume wordt vervolgens berekend door dit hartvolume van het thoraxvolume af te trekken. Er wordt een significante toename (circa 7 maal) gevonden in normaal foetaal longvolume in relatie tot het vorderen van de zwangerschapsduur en in relatie tot een toename in geschat foetaal gewicht.

HOOFDSTUK 7

Doppler ultrageluidonderzoek biedt niet alleen de mogelijkheid om de longcirculatie te bestuderen in de zich normaal ontwikkelende menselijke foetus, maar ook in de foetus met een verhoogd risico op de ontwikkeling van longhypoplasie. Paragraaf 7.2 beschrijft het eerste case-report over Doppler bloedstroomsnelheidsprofielen verkregen uit de arteriële longcirculatie van een foetus met longhypoplasie. In deze casus wordt een afname waargenomen in systolische en diastolische snelheden gemeten in de proximale arterie pulmonalis. Dit impliceert dat Doppler ultrageluidonderzoek van betekenis kan zijn in de prenatale diagnostiek van longhypoplasie.

Om die reden is de waarde van Doppler ultrageluidonderzoek van de foetale arteriële longcirculatie onderzocht ten opzichte van foetale biometrie en klinische parameters in de prenatale voorspelling van letale longhypoplasie door langdurig oligohydramnion (paragraaf 7.3). De totale studiegroep voor dit onderzoek bestond uit 42 zwangerschappen met een langdurig oligohydramnion op basis van PROM (n=31) danwel ten gevolge van foetale bilaterale nierpathologie (n=11). De foetale thorax-, hart- en buikomtrek werden echoscopisch gemeten en tevens het diepste verticale vruchtwaterpocket. Vervolgens werden Doppler bloedstroomsnelheidsprofielen van het arteriële longvaatbed verzameld zoals beschreven in voorgaande hoofdstukken. De diagnose longhypoplasie werd na de geboorte gesteld op basis van pathologische, klinische en radiologische criteria. Letale longhypoplasie werd vastgesteld in 43% van de totale studiegroep

In de PROM sub-groep, levert combinatie van het moment van PROM $\leq$ 20 weken, de duur van het oligohydramnion $\geq$ 8 weken en de mate van oligohydramnion $\leq$ 1 cm, de beste klinische voorspelling op ten aanzien van de aanwezigheid van letale longhypoplasie. Zowel voor de totale groep als voor de PROM sub-groep, wordt de beste voorspelling voor letale longhypoplasie verkregen door de thorax/buikomtrek ratio, de piek systolische snelheid proximaal en de gemiddelde en eind-diastolische snelheid midden in het arteriële longvaatbed gemeten. In de PROM subgroep, biedt combinatie van klinische, biometrische en Doppler parameters de meest gunstige prenatale voorspelling van letale longhypoplasie (positief voorspellende waarde 100%, 'accuracy' 93%, sensitiviteit 71%)

Concluderend wordt gesteld dat Doppler ultrageluidsonderzoek veranderingen in bloedstroomsnelheden kan detecteren in geval van

longhypoplasie, doch op zichzelf geen accurate prenatale predictie geeft van letale longhypoplasie. De beste voorspelling hiervoor wordt bereikt door combinatie van klinische, biometrische en Doppler parameters.

COLOR PICTURES

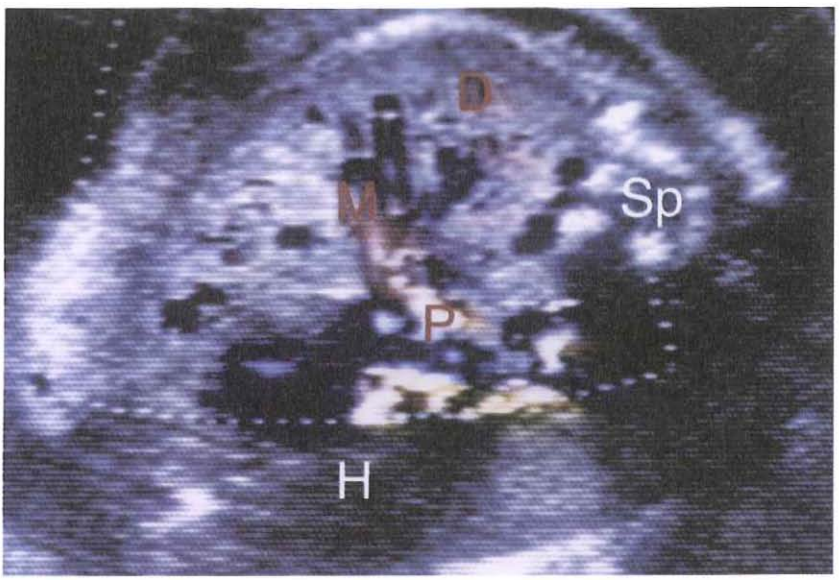

Figure 1 (Chapter 3.3, page 67)
Cross-section of the fetal chest at the level of the cardiac four chamber view with color Doppler switched on to visualize the pulmonary circulation. H, fetal heart; Sp, fetal spine; P, M, D (in red): proximal, middle and distal Doppler sample sites in the arterial pulmonary branch.

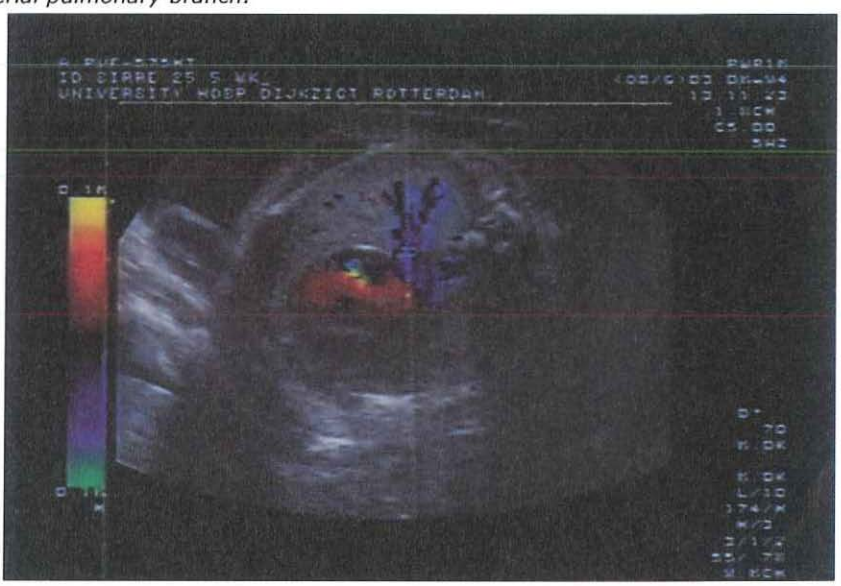

Figure 1 (Chapter 4.2, page 86)
Cross-section of the fetal chest at the level of the four chamber view. After visualizing the pulmonary vein with color Doppler, the sample volume was placed just proximal to the entrance into the left atrium.

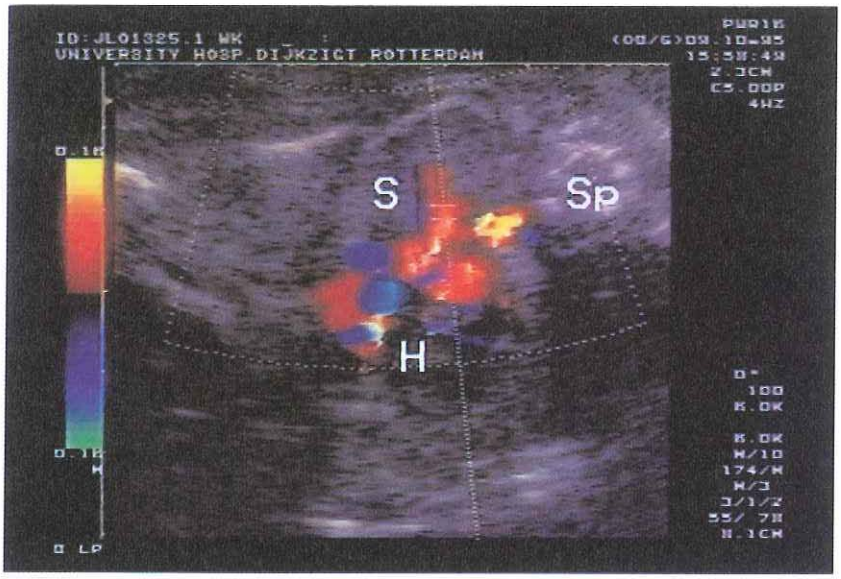

Figure 1 (Chapter 7.2, page 147)
Cross-section of the fetal chest at the level of the four-chamber view. The sample volume (S) was placed over the primary left branch of the pulmonary artery after visualization by color Doppler. Sp, spine; H, heart.

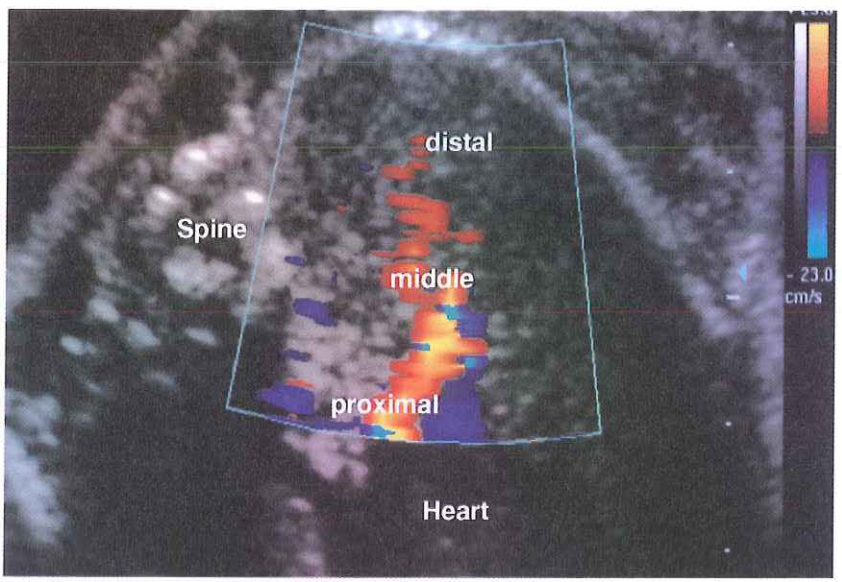

Figure 1 (Chapter 7.3, page 155)
Cross-section of the fetal chest at the level of the cardiac four chamber view with color Doppler switched on to visualize the pulmonary circulation. Proximal, Middle, Distal: Doppler sample sites in the arterial pulmonary branch.

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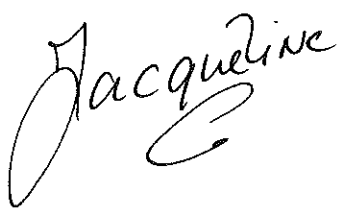

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CURRICULUM VITAE

1965	Geboren te Sittard
1978 -1984:	Gymnasium ß Serviam R.K. Scholengemeenschap, Sittard
1984 -1991:	Studie Geneeskunde Rijksuniversiteit Limburg, Maastricht
juni 1991 - dec 1991:	AGNIO Gynaecologie/Verloskunde Maasland Ziekenhuis, Sittard
dec 1991 - dec 1992:	IVF-arts Academisch Ziekenhuis Rotterdam
dec 1992 - dec 1993:	AGNIO Gynaecologie/Verloskunde Academisch Ziekenhuis Rotterdam
dec 1993 - nov 1999:	AGIKO Gynaecologie/Verloskunde Opleidingscluster Rotterdam:
• dec 1993 - dec 1995:	Start promotie-onderzoek Erasmus Universiteit Rotterdam Promotor prof. dr. J.W. Wladimiroff
• dec 1995 - dec 1996:	Start klinische opleiding Reinier de Graaf Gasthuis, Delft Opleider dr. J.C. Kuijpers
• dec 1996 - mrt 1998:	Vervolg promotie-onderzoek Academisch Ziekenhuis Rotterdam
• mrt 1998 - nov 1999:	Vervolg klinische opleiding Academisch Ziekenhuis Rotterdam Opleider prof. dr. Th.J.M. Helmerhorst
nov 1999 - heden:	Arts Prenatale Diagnostiek Academisch Ziekenhuis Rotterdam na voortijdig stoppen AGIKO-opleiding
aug 2000:	Start Master of Science opleiding Clinical Epidemiology Erasmus Universiteit Rotterdam