# THE EARLY HUMAN FETAL DUCTUS ARTERIOSUS

Cover: The first known illustration of the ductus arteriosus is found in "De formatu fetu" Tab VI, Fig XV, of **Hieronymus Fabricius ab Aquapendente (1537 - 1619)**
Original description on the back cover. *E - ductus arteriosus, D - ascending aorta , F -pulmonary trunk , G - left pulmonary artery, C - left lung, A - descending aorta*
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# THE EARLY HUMAN FETAL DUCTUS ARTERIOSUS

## a morphological and hemodynamic study

**De foetale ductus arteriosus in de vroege zwangerschap**
**een morfologisch en haemodynamisch onderzoek**

*proefschrift*
*Ter verkrijging van de graad van doctor*
*aan de Erasmus Universiteit Rotterdam*
*op gezag van de Rector Magnificus*
*Prof.Dr.P.W.C.Akkermans M.Lit.*
*en volgens besluit van het college van dekanen*
*De openbare verdediging zal plaatsvinden op*
*woensdag 22 Juni 1994 om 15.45 uur*

door
Christoph Andreas Brezinka
geboren te Salzburg(Oostenrijk)

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*La natura delle cose sta nel loro nascimento*
*Giambattista Vico (1668 - 1744)*

# Chapter 1

## Introductory remarks and definition of objectives

The uniqueness of the anatomical structures of the fetal circulation was first recognized by Galen (130-200 A.D.) who in two passages (Galen, op.cit.) of his immense opus gives the description of a vessel that could only be the Ductus Arteriosus (Harris, 1973). The first description was later attributed to Leonardo Botallio (ca.1530-1600). This misattribution found its way into the Basel Nomenclature (BNA) of 1895 and despite several revisions the ductus arteriosus is still described as "ductus arteriosus Botallii" or "ductus Botallii" in the clinical literature of the 1980s (Deeg et al. 1987). In the French literature the foramen ovale was attributed to the same Botallio, being called "canal de Botal" (Tuchmann-Duplessis and Haegel, 1979). These misattributions support the case against giving structures, whether anatomical or geographical, the names of their reputed discoverers.

The functional understanding of the fetal circulation was only to develop after Harvey's discovery of the circulation (French, 1978) and of Malpighi's and Walaeus' discovery of the existence of capillaries (Schouten, 1974; Reubi, 1985). The first visualization of the Ductus Arteriosus in vivo, using an X-ray technique, took place in 1939 (Barclay et al. 1939).

The ductus arteriosus has been studied at biochemical, ultrastructural and histological level as well as at haemodynamic level in animal experiments (Cassels, 1973). Lately, the introduction of pulsed and coloured Doppler ultrasound has opened the possibility of carrying out non-invasive studies in the human fetus. Attention has been particularly focused on late second and third trimester pregnancies resulting in data on ductal flow velocities associated with normal and complicated pregnancies, maternal indomethacin administration and various cardiac anomalies (Saenger et al. 1992). Transvaginal

ultrasound allows more detailed structural information on fetal development (Wladimiroff et al. 1991a). Both improvement in transvaginal real-time imaging as well as the introduction of transvaginal pulsed Doppler and colour Doppler facilities has provided in-depth information on flow-velocity waveform patterns both at cardiac and extra-cardiac level in the late first trimester human fetus. These transvaginal techniques as well as improved transabdominal real-time imaging has enabled us to study flow velocity waveform patterns in the ductus arteriosus as a function of right ventricular performance as early as the late first and early second trimester of pregnancy.

Morphological studies in human fetal ductus arteriosus during this early stage of pregnancy will allow the study of possible changes in the positional relationship between the ductus arteriosus and the descending aorta. Morphologic development may affect haemodynamic performance and vice versa. It was, therefore, that a combined morphologic and pulsed Doppler study in early human pregnancy was performed to try and determine the presence and the nature of such an interrelationship.

Later in pregnancy ductus arteriosus flow velocities like flow velocities in many other fetal vessels appear to be modulated by intrinsic fetal variables such as fetal breathing movements and fetal behavioural states. As from 35-36 weeks well defined quiet (1F) and active (2F) sleep states can be recognized (van der Mooren et al. 1989).

At 27-28 weeks of gestation there are no well-defined behavioural states. Instead the developing fetus displays rest-activity states as demonstrated in the fetal heart rate pattern (Visser et al. 1987). Rest-activity dependent flow velocity changes have been established in the descending aorta at this state of pregnancy (van Eyck et al. 1988). It is assumed that similar changes may exist in the ductus arteriosus.

Based on recent morphological and haemodynamic work in the human fetus and the further improvement in Doppler flow recording techniques the following questions were addressed:

1) is it technically feasible to record flow velocities as early as the first trimester of pregnancy ? If so , what is the reproducibility of these recordings ?

2) What is the normal flow velocity waveform pattern in the ductus arteriosus in the late first and early second trimester of pregnancy ?

3) How do ductal flow velocity waveform patterns, reflecting right ventricular performance, relate to pulmonary artery flow velocity waveform patterns ?

4) What is the anatomical relationship between the ductus arteriosus and the descending aorta in the first and second trimester fetus and what are the haemodynamic consequences ?

5) Do rest-activity state-related changes exist in fetal ductal flow velocity waveforms during the early 3rd trimester of pregnancy ? If so, what is the nature of these changes ?

The study is part of a larger study on flow velocity waveforms in early pregnancy which was reviewed by the local ethics review board. All patients included in this thesis consented to participate in the flow studies.

# Chapter 2

# The ductus arteriosus, a literature review

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## 2.1 Introduction

It has long been known that the fetal circulation is essentially different from that in the infant and adult (Moore, 1988). In the fetus, well-oxygenated blood passes from the placenta through the umbilical vein into the ductus venosus, thus bypassing the liver and its vessels. It merges with poorly oxygenated blood from the inferior vena cava at subdiaphragmatic level, the venous vestibulum (Huisman et al. 1992) and then proceeds through the foramen ovale into the left atrium and left ventricle and ultimately to the head and upper extremities. Only a small fraction of blood that has followed this pathway reaches the fetal abdomen and lower extremities through the aortic isthmus.

The second pathway unique to the fetal circulation is that via the right ventricle, pulmonary trunk and ductus arteriosus.

In the fetus very little blood reaches the lungs. Pulmonary vascular resistance and pressure is higher than the systemic vascular resistance and pressure and thus blood is mainly shunted through the ductus arteriosus into the descending aorta (Friedman et al. 1983; Clyman et al. 1989a).

The return to the placenta begins in the internal iliac artery through the symmetric umbilical arteries that meet the umbilical vein at the umbilicus and, curling around the straight vein in characteristic fashion, run through the umbilical cord towards the placenta.

In the newborn, the vessels of the fetal circulation loose their function, obliterate and eventually change into strands of

connective tissue. The ductus venosus can be used as an infusion route shortly after birth. There is a similar closing pattern in all vessels of fetal circulation: contraction leads to potentially reversible functional closure which is followed by anatomical sealing and transformation into ligamentous strands. After anatomical sealing of the vessel lumen the abdominal part of the umbilical vein becomes the ligamentum teres hepatis, the ductus venosus becomes the ligamentum venosum, the umbilical arteries are transformed into the medial umbilical ligaments with just their proximal part still serving as vessels to supply blood to the bladder as the arteriae vesicales superiores. The ductus arteriosus is being transformed into the ligamentum arteriosum. A similar development is seen in the foramen ovale, which is, however, not a vessel: post partum it forms the septum interventriculare with only the limbus fossae ovalis still marking the former shape of the foramen ovale.

## 2.2. Embryology and structure of the ductus arteriosus; concepts of ductal maturation

Intrauterine development of the ductus arteriosus must be seen in the light of its function, i.e. to remain patent before birth and to close rapidly after birth. During embryogenesis the ductus arteriosus originates from the left 6th branchial arch artery (Congdon, 1922).This pulmonary arch is the only remaining vessel from a plexus of splanchnic vessels which connect the pulmonary plexus with the dorsal aortae at a very early stage (DeRuiter et al. 1993). The ductus arteriosus is different from the adjacent great arteries in that it is a muscular artery, whereas the pulmonary arteries and the aorta are elastic arteries. Virchow is credited with being the first to note the histological difference between the ductus arteriosus and the other great arteries and to point out the clinical significance for postpartum closure of this observation (Gräper, 1921). Histological distinction between the ductal vessel wall and that of the adjacent arteries is possible from 8-9 weeks of gestation. By that time the ductal vessel wall contains more

muscle cells and less elastic tissue than the other great arteries (Odé, 1951). It has been proposed, that this different development of the ductal vessel wall is made possible by a sling of the recurrent laryngeal nerve surrounding the ductus arteriosus compartment of the left sixth aortic arch during embryogenesis (Leonard et al. 1983). Whether mere mechanical support or the action of neurotransmitters from the laryngeal nerve is responsible for the unique development of the ductus, is still subject to debate. In this context it is interesting to note that the dipnoean lungfish (*protopterus a.*) which needs a patent ductus throughout its life, has powerful ductus innervation by the vagal nerve (Fishman et al. 1985). The ductal wall differs from the vessel wall of other muscular arteries in that it is a relatively loose structure with mucoid substance in the media that eventually concentrates in "mucoid lakes", most probably as a result of media contraction. The borderline between the ductus arteriosus and the pulmonary trunk is slightly distal to the origin of the pulmonary arteries. Muscular ductal tissue spreads into the aortic wall, occupying up to one-third of the aortal circumference at its point of insertion (Elzenga and Gittenberger de Groot, 1983; Russell et al. 1991).

The intrauterine development of the ductus can be seen as a preparation for postpartum closure. This closure takes place in two steps: physiological closure is effected by the muscle cells in the media of the vessel wall that are arranged longitudinally and spirally to make effective contraction possible (Hayek, 1936; Strengers, 1988). Physiological closure is supported by a decrease in the ductal lumen through intimal proliferation and intimal cushion formations, which is characteristic of the mature ductus(Gittenberger de Groot, 1978).

The next step, anatomical closure, leading to the formation of the ligamentum arteriosum, takes place during the first weeks of neonatal life. Whereas physiological closure can still be reversed, anatomical closure is definite.

Sub-endothelial edema formation is the first step in the intima cushion development in the ductus and seems to be specifically related to hyaluronic acid accumulation (DeReeder et al. 1988; Slomp et al. 1992). Increased chondroitin sulfate and dermatan sulfate may

impair assembly of newly synthesized elastin in the media of the ductus associated with the development of intimal thickening (Hinek et al. 1991). A role of the elastin receptor in the process of intimal thickening has also been proposed (DeReeder et al. 1990).

The mechanisms of ductal closure have been studied in several animal species: In the dog, for example, normal ductal closure takes place from the pulmonary artery to the aortic insertion (Gittenberger de Groot et al. 1985). Histological features of the normal and the persistent ductus in the dog resemble those in the human, suggesting similar pathogenesis and making the dog a valid model. The normal ductus in the dog is characterized by the development of intimal thickening in late gestation, a process that does not occur in canines from strains with genetically persistent ductus (DeReeder et al. 1989).

A stepwise process of maturation during which endothelial cushions and mucoid lakes develop in the vessel wall has been proposed (Gittenberger de Groot, 1977). Whereas some authors see an immediate relationship between gestational age and histological maturation of the ductus (Tada et al. 1985b), others have cautioned against a strict correlation between gestational age, birth weight and histological maturation of the ductus arteriosus (Gittenberger de Groot et al. 1980).

### 2.3 Role of prostaglandines in maintaining ductal patency

During fetal life the maturation process leading to post partum ductal closure must proceed without affecting ductal patency.

Arachidonic acid is liberated from lipid storage depots by phospholipase A2 (Ramwell et al. 1980). Cyclo-oxygenase then converts arachidonic acid to the intermediate prostaglandine $G_2$ ($PGG_2$). $PGG_2$ is converted into a number of prostaglandines

(Prostacyclin, $PGE_2$, $PGF_{2\alpha}$, $PGD_2$) and into thromboxane. This process is catalyzed by several enzymatic steps. Indomethacin and to a lesser extent aspirin and other non-steroidal anti-inflammatory drugs (NSAIDs) block the action of cyclooxygenase. These drugs, however, do not affect the lipoxygenase pathway that converts arachidonic acid into leukotrines and the hydroxy-eicosatetraenoic acids (HETE) (Barst and Gersony, 1989).

Prostaglandines are responsible for maintaining ductal patency. However, prostaglandines do not dilate the ductus beyond its resting dimension. Constriction is part of an active process.(Friedman et al. 1983) All prostaglandins except $PGF2_{\alpha}$ have a relaxing effect on the ductus. $PGE_2$ has the most significant relaxing effect at the lowest concentration when compared with the other prostaglandines. Although only a minor product of prostaglandine production, the tissue's marked sensitivity to $PGE_2$ make it the most significant prostaglandin in regulation of vessel patency (Clyman et al. 1978b). The ductus supplies its own intrinsic $PGE_2$, being equipped with an enzyme system for the synthesis of $PGE_2$ (Coceani et al. 1986; Clyman, 1987). Immature ductal tissue produces more prostaglandines than mature tissue (Clyman et al. 1978a).

Intrinsic $PGE_2$ and $PGE_2$ produced in the lung contribute to the effect of maintaining ductal tone. In isolated ductal rings of near term lambs reactive $O_2$ metabolites (Hypoxantine and Xanthinoxidase) relax the ductus arteriosus. However, the vasoactive effects of reactive metabolites in the ductus arteriosus appear to be mediated exclusively through the generation of $PGE_2$ (Clyman et al. 1989b)

At birth, oxygen exerts its effect on the plasma membrane of the ductal muscle cells at which level a membrane-bound cytochrome p-450 mechanism probably functions as the signal transducer for $O_2$ in the formation of a constrictor agent (Coceani et al. 1984; Olley and Coceani, 1987; Coceani et al. 1989b). Together with endothelin the p-450 mechanism effects the contractile response of the vessel to oxygen (Coceani et al. 1989a). Oxygen triggers closure of the DA at birth by causing a conformational change in a specific cytochrome p-450 which in turn provides the signal for the synthesis of

the constrictor entdothelin 1 (Coceani and Kelsey, 1991; Coceani et al. 1992) .

The ductus arteriosus response to oxygen is dependent on gestational age (Noel and Cassin, 1976).

Although $PGI_2$ is at least 10 times more abundant in fetal circulation than $PGE_2$, it does not relax the ductus arteriosus but is a potent pulmonary vasodilator (Coceani et al. 1980; Sideris et al. 1985). There is, however, some evidence that $PGI_2$ may have a significant role in intimal cushion formation (Slomp et al. 1992). An increase in $PGI_2$ may produce a concurrent inhibition of smooth muscle cell growth (DeReeder et al. 1989)

## 2.4 Doppler flow velocity waveforms in the ductus arteriosus

Doppler echocardiographic studies in the human fetal ductus suggest an increase in stroke volume and volume blood flow associated with falling afterload as pregnancy progresses (van der Mooren et al. 1991b). Breathing-related changes occur in peak systolic velocity but not in acceleration time and peak diastolic velocity. Breathing-related modulation in peak systolic velocities is reduced or even absent in pulmonary hypoplasia (van Eyck et al. 1990c). This suggests breathing related changes in pulmonary blood flow. Recent developments in colour-coded Doppler techniques have opened the possibility of arterial and venous blood flow velocity waveforms as far as in the periphery of the pulmonary circulation. Changes in ductal flow velocity waveforms have also been observed relative to quiet and active sleep states in normal late third trimester pregnancies. These sleep states are based on the absence or presence of fetal eye movements, so-called type A and type B heart rate patterns and incidence of gross body movements (Nijhuis, 1992). Whereas during active sleep there is a reduction in peak systolic and time-average velocities in the ductus arteriosus, a rise in these velocities was observed at the level of the foramen ovale suggesting increased blood flow to the left heart with the purpose to maintain optimal oxygen

supply to the fetal cerebrum and trunk during this high-energy behavioural state (van der Mooren et al. 1989).

It has recently been demonstrated that the use of the prostaglandine-synthetase inhibitor indomethacin may lead to fetal ductal constriction as determined by a significant rise in ductal peak systolic and peak diastolic velocities (Huhta et al. 1987b; Moise et al. 1988). This constrictive effect of indomethacin may be observed for a significant period of time after administration. Ductus arteriosus constriction in utero was defined as a systolic blood flow velocity above 140 cm/s and a diastolic velocity of above 35 cm/s. To differentiate increased ductal peak velocity caused by increased volume flow from increased velocity caused by indomethacin-induced ductal constriction, use of the pulsatility index (PI) has been proposed (Tulzer et al. 1991). A pulsatility index below 1.9 is indicative of ductal constriction. When indomethacin constricts the ductus arteriosus, blood flow velocity through the vessel increases. With increasing constriction, holosystolic tricuspid regurgitation appears with velocities of up to 200 cm/s (Moise et al. 1988). A new classification, proposed recently, uses a combination of ductus arteriosus peak systolic velocity (PSV), end diastolic velocity (EDV) and the extent of tricuspid regurgitation to assess the severity of ductal constriction under maternal indomethacin medication (Mari et al. 1993). The pulsatility index of the fetal umbilical artery, however, is not affected by maternal indomethacin administration (Moise et al. 1990).

In growth retarded fetuses due to impaired placental perfusion a marked reduction in peak systolic flow velocities has been established in all cardiac outflow tract vessels, i.e. pulmonary trunk, ascending aorta and ductus arteriosus (Groenenberg et al. 1989). Several explanations have been put forward for these decreases in outflow tract peak systolic velocities. A decrease in volume flow, a change in contractile function of the cardiac ventricles, an increase in valve or vessel size or rise in afterload have been suggested as an explanation for the forementioned peak systolic velocity changes. The non-invasive nature of Doppler ultrasound does not allow differentiation between these explanations.

## 2.5 Effects of indomethacin and steroids on the fetal ductus arteriosus

In the healthy term neonate a rapid process of closure of the ductus arteriosus sets in after birth. Blood flow that up until now has been solely right-left becomes bi-directional and subsequently changes into left-right flow until ductal closure (Shiraishi and Yanagisawa, 1991). On the second day of life shunting through the ductus takes place in only 18% of normal neonates with none on the third day (Mahoney et al. 1985; Evans and Archer, 1990). Timing of ductal closure is not affected by the mode of delivery (Mirro and Gray, 1986). When left-to-right shunting through the patent ductus arteriosus contributes to respiratory distress in the newborn infant, this is defined as symptomatic persistant ductus arteriosus (PDA or SPDA) (Cotton, 1987).

Spontaneous intrauterine closure of the ductus arteriosus was first described in 1847 (Chevers, 1847). It is a rare occurrence with only a small number of cases reported since the first description (Becker et al. 1977; Kohler, 1978). An association with malformations that exert pressure on the fetal thorax has been observed (Tada et al. 1985a).

Prenatal constriction and closure of the ductus arteriosus may be the result of prostaglandine-synthetase antagonists like indomethacin and steroids like betamethason. Both play an important role in modern obstetric care. Closure of the patent ductus arteriosus in the neonate using indomethacin was first performed in the 1970s (Heymann et al. 1976; FDA, 1985; Knight, 1992). Until then, surgical ligation of the patent arteriosus - which had been first performed in 1938 and had marked the beginning of modern heart surgery - was the only therapy available (Crafoord, 1980).

In the neonate there is an enormous variation in indomethacin plasma levels when the drug is applied to treat persistent ductus arterious. This might be caused by different metabolic pathways. In one study, 14% of premature newborns deacylated

indomethacin whereas 58% demethylated indomethacin. With demethylation there is a shorter half life, a smaller area under the plasma concentration curve and increased plasma clearance (Friedman et al. 1991). Efforts have been made to create a pharmacodynamic concentration-response curve for the serum concentration of the newborn (Gal et al. 1991). Several methods to monitor indomethacin serum plasma levels have been developed (Traeger et al. 1973; Ou and Frawley, 1984; Bhat et al. 1980; Thalji et al. 1980; Brash et al. 1981). Much experience with indomethacin use in neonatology had already accumulated when the tocolytic properties of indomethacin were first demonstrated in a clinical trial (Zuckerman et al. 1974).

Whereas beta-mimetics like ritodrine, salbutamol and fenoterol are still the most widely used tocolytic drugs, their significant side effects and their lack of proven effect in randomized studies have brought them increasing discredit (King et al. 1988; The Canadian Preterm Labor Investigators Group, 1992). Tocolytics like alcohol (Zlatnick and Fuchs, 1972) no longer have a place because of their maternal and fetal side effects. Magnesium sulphate, which initially received much enthusiasm (Elliott, 1983), is only effective in arresting premature contractions in women who are not actually in preterm labour. Moreover, it has significant side effects (King et al. 1988; Holcomb et al. 1991; Cox et al. 1990).

This makes indomethacin, until now a second-choice drug when other tocolytics have failed to stop labour, a valid first choice for acute tocolysis. The benefits of preventing premature birth must be weighed against the risks incurred by constricting the fetal ductus arteriosus and reducing amniotic fluid: constriction and subsequent closure of the fetal ductus arteriosus leads to tricuspid regurgitation, followed by hydrops and ultimately congestive heart failure. In the fetal sheep ductal ligation leads to a 200% increase in pulmonary blood flow and a 22% increase in pulmonary artery pressure (Morin and Egan, 1989). If the fetus survives ductal closure it will be at substantial risk of developing chronic pulmonary hypertension post partum (Levin et al. 1979; Morin, 1989; Abman and Accurso, 1989; Abman et al. 1989; Manchester et al. 1976). Cases of fetal death caused by

congestive heart failure as a consequence of maternal indomethacin administration have been reported (Truter et al. 1986). Twin pregnancies are more likely to be in need of indomethacin, both for tocolysis and for the relief of polyhydramnios. A significant part of the cases of fetal hydrops and death caused by maternal indomethacin administration happened to be twin pregnancies (Mogilner et al. 1982; Chaoui et al. 1989; Hallak et al. 1991; Demandt et al. 1990). Usually only one of the twins is affected, which demonstrates the differences in maturation of the ductus arteriosus and subsequent variation in reaction to indomethacin (Gittenberger de Groot et al. 1980; Clyman et al. 1985; Eronen, 1993). In twin pregnancies, discordance in ductal reaction to maternal indomethacin administration may even after birth result in one twin suffering from symptomatic persistent ductus arteriosus whereas in the other twin the ductus undergoes normal closure (Atad et al. 1987).

Several reports on the tocolytic use of indomethacin which appeared prior to the widespread introduction of fetal echocardiography provided a generally optimistic picture. When used together with ritodrine there was no efect on the ductus (Katz et al. 1983). No ductal damage was observed in 164 pregnancies following indomethacin tocolysis (Dudley and Hardie, 1985). Forty-six infants examined postnatally after having been exposed to indomethacin during maternal tocolysis showed no signs of premature ductal closure (Niebyl and Witter, 1986). Furthermore, a French study reported "negligible" cardiovascular effects in the infants of 304 women who had undergone indomethacin tocolysis (Marpeau et al. 1988).

With advancing gestational age the fetal ductus arteriosus is more likely to constrict during maternal indomethacin therapy: the majority of fetuses will begin to show constriction of the ductus arteriosus at 27 - 30 weeks of gestation (Vandenveyver et al. 1993). Moise observed an increase in fetal ductal constriction when indomethacin was administered after 32 weeks of gestation both in singleton and in twin fetuses and advised to restrict indomethacin therapy to gestational ages below 32 weeks (Moise, 1993).

The effect of maternal indomethacin administration may

range from mild to marked reduction in amniotic fluid volume (Nordstroem and Westgren, 1992). Oligohydramnios has been reported as a result of maternal indomethacin medication (Hendricks et al. 1990). There may be a massive decline in hourly fetal urinary output during indomethacin tocolysis (Kirshon et al. 1988). This makes regular ultrasound monitoring of amniotic fluid volume necessary for the duration of therapy. This effect of indomethacin may also be used therapeutically in cases of severe polyhydramnios. Doppler monitoring of the fetal ductus arteriosus has been advised when embarking on indomethacin treatment (Kirshon et al. 1990; Mohen et al. 1992). It is of interest to note, however, that during maternal indomethacin administration there is no change in fetal renovascular parameters even when the ductus arteriosus is constricted (Mari et al. 1990). Although indomethacin-induced narrowing of the ductus arteriosus is considered reversible, there is one report documenting how prolonged therapy of more than two weeks' duration led to irreversible ductal constriction (Mohen et al. 1992). A recent retrospective study of very small preterm infants born between 24 - 30 weeks of gestation after failed indomethacin tocolysis showed a significantly increased risk of necrotizing enterocolitis, patent ductus arteriosus, intracranial hemorrhage, and renal dysfunction in these infants in comparison with a control group whose mothers had not received indomethacin for the treatment of premature labor (Norton et al. 1993).

Antenatal steroid therapy has been used for more than twenty years to enhance fetal lung maturation (Liggins and Howie, 1972; Garite et al. 1992). It is associated with a doubling of survival rates of infants in the 500-799 g birth weight range at 24-26 weeks of gestational age (Doyle et al. 1992). A transient constriction of the fetal ductus arteriosus has been observed after betamethason administration (Wasserstrum et al. 1989). Similar observations have been made in fetal rats (Momma et al. 1981; Pulkkinen et al. 1986). In rats, ductal constriction was substantially increased by combined administration of indomethacin and betamethasone (Momma and Takao, 1989). Betamethasone appears to prepare the fetal ductus arteriosus for rapid postpartum closure. Premature infants who had been exposed to

betamethason before birth showed a much lower incidence of persistent ductus arteriosus than untreated infants of the same gestational age (Waffarn et al. 1983; Tsai and Brown, 1987; Papageorgiou et al. 1989; Morales et al. 1989).

A further problem of indomethacin administration is the potentially undetected presence of a ductus dependent fetal cardiac malformation (Menahem, 1991). Routine fetal echocardiography before the commencement of indomethacin tocolysis has been advocated (Saenger et al. 1992). Infants with various forms of aortic stenosis, juxtaductal aortic coarctation, hypoplastic left heart and pulmonary atresia depend on postpartum ductal patency in order to survive the neonatal period. In severe cases the entire systemic blood flow must pass from the right heart through the ductus arteriosus. Intrauterine constriction of the ductus arteriosus by indomethacin would further jeopardize the chances of survival. The majority of ductus-dependent malformations can be excluded by a normal four-chamber view (Reed et al. 1988b): For instance a right ventricle disproportionally larger than the left ventricle should point at the possibility of coarctation of the aorta (Benacerraf et al. 1989). In this case, maternal indomethacin administration should be abandoned.

Nevertheless, the rarity in a given population of ductus-dependent cardiac malformations compared with the high incidence of premature labour necessating tocolysis (Meis et al. 1987; Keirse et al. 1989; Weiner et al. 1988) would make a policy of withholding indomethacin tocolysis from all women in whom the fetal heart was not previously examined on ultrasound, highly questionable. Essential is a thorough ultrasound examination within 48 hours of commencing indomethacin, registering the amount of amniotic fluid, the pulsatility index in the ductus arteriosus and the cardiac 4-chamber view. In case of any abnormal observation, discontinuation of indomethacin therapy should be considered.

Not all maternal drugs acting on the fetal ductus lead to its constriction: Prostaglandine $E_2$, mostly in the form of gel or vaginal suppositories has found widespread application for the induction of labour (Graves et al. 1985; Calder et al. 1977). It was observed that

postnatal ductal closure may take longer in infants born after induction of labor with $PGE_2$ (Sung et al. 1990).

## 2.6 Conclusion

The intrauterine development of the ductus arteriosus is a continuous preparation for rapid postpartum closure. Gradual changes in the vascular wall structure have a narrowing effect on the lumen of the vessel. This leads to ductal velocities being higher than anywhere else in the fetal vascular system. Tocolytic agents such as indomethacin and to a lesser extent betamethasone, may constrict the fetal ductus arteriosus. Constriction may be transient and reversible or, in case of prolonged drug administration, eventually lead to congestive heart failure. The introduction of Doppler in fetal ultrasound has opened the possibility of studying ductal flow velocity waveforms in normal and pathologic conditions. Drug related effects on ductal flow velocity waveforms have been established with emphasis on maternal indomethacin administration. Knowledge of normal ductal anatomy and normal Doppler waveform patterns is necessary to correctly assess the eventual effect maternal drug intake can have.

# Chapter 3

## Reproducibility of fetal ductus arteriosus flow velocity waveforms in early pregnancy

### 3.1 Introduction

Flow velocity waveform recording at cardiac level allows angle-dependent measurement of absolute velocities. This applies to both the trans-vaginal and the trans-abdominal approach. The course of the ductus arteriosus allows interrogation angles of less than 10 degrees. This should permit reliable information on blood flow velocities in this particular vessel.

Recent studies have demonstrated an acceptable reproducibility for the ascending aorta and pulmonary artery as early as 11 - 12 weeks (Huisman et al. 1993). In chapter 3.1 the reproducibility of Doppler flow velocity waveforms obtained from the fetal ductus arteriosus between 11 and 25 weeks of gestation will be established.

## 3.2 Doppler flow velocity waveforms in the fetal ductus arteriosus in the first half of pregnancy; a reproducibility study

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### Abstract

Reproducibility of flow velocity waveform recording was studied in the fetal ductus arteriosus in 52 normal pregnancies between 11 and 25 weeks of gestation. The flow velocity parameters studied were the peak systolic velocity, mean velocity, end-diastolic velocity, flow velocity integral and acceleration time. In each patient two consecutive measurements were performed with a time delay of 15 minutes. An

acceptable reproducibility was achieved for all flow velocity parameters, except for the acceleration time and end-diastolic velocity.

## Introduction

Since the introduction of fetal ductal flow velocity waveform recording by Huhta et al (Huhta et al. 1987b) several reports have appeared on ductal flow velocities relative to normal late pregnancy (van der Mooren et al. 1991a), indomethachin administration (Huhta et al. 1987b; Kirshon et al. 1990; Tulzer et al. 1992), fetal behavioural states (van der Mooren et al. 1989) and fetal breathing movements (van Eyck et al. 1990b).

Reproducibility studies of Doppler measurements in the fetal ductus arteriosus during late pregnanacy have demonstrated satisfactory results for peak-systolic and mean velocity, but a poor reproducibility for the acceleration time (Groenenberg et al. 1991).

Recently, combined transvaginal and transabdominal Doppler ultrasound has provided flow velocity data from the fetal ductus arteriosus as early as 11 weeks of gestation (Brezinka et al. 1992). Reproducibility studies during late first and second trimester pregnancies are needed to test the potential clinical significance of ductal flow velocity recordings during this early stage of gestation.

In the present study the question was addressed whether reproducibility of ductal flow velocity waveforms during early pregnancy is comparable with that established in late pregnancy.

## Material and methods

A total of 52 women consented to participate in the study. Gestational age varied between 11 and 25 weeks (mean 17 weeks). Pregnancy duration was derived from the last menstrual period and by ultrasonic measurements of the fetal crown-rump length or biparietal diameter.

A combined curved linear array and pulsed wave Doppler

system (Hitachi 450) with vaginal and abdominal probes was used. The carrier frequency of the vaginal probe was 6.5 MHz and of the abdominal probe 3.5 MHz. The cut-off level of the high-pass filter was set at 100 Hz. Vaginal and abdominal probes operate at power outputs of less than 100 mW/cm$^2$ spatial peak/temporal average in both imaging and Doppler modes by manufacterer's specifications. Up until 13 weeks of gestation the vaginal approach was used whereas afterwards waveforms were collected using the abdominal probe with the woman in the semi-recumbent position. First a two-dimensional image of the conventional short-axis view was obtained displaying the right ventricular outflow tract including the pulmonary artery and ductus arteriosus. The interrogation anlge between the Doppler beam and ductus was kept below 10 degrees. All blood velocity waveforms were obtained during fetal apnoea.

In each woman Doppler recordings of the Ductus arteriosus were performed twice at times t1 and t2. The time delay between the two measurements was approximately 15 minutes. Hardcopies printed on a Sony A4 printing device were made of each recording. These hardcopies were encoded so as not to reveal the identity or gestational age of the patient nor date and time of the recording. After all hardcopies had been collected and encoded they were shuffled in random order and analyzed. Four consecutive waveforms of similar appearance were analyzed for t1 and t2 respectively. Both Doppler recordings and waveform analysis were performed by the same investigator (C.B.). Waveform analysis was carried out by using a conventional microcomputer linked to a graphics tablet as described previously (Groenenberg et al. 1991). The analyzing programme uses 400 datapoints to describe the four waveforms on one hardcopy.

The following parameters were determined: peak systolic velocity, mean velocity, end diastolic velocity, flow velocity integral, acceleration time and period time. Peak systolic velocities were measured from the zero line to the highest point of the Doppler velocity tracing. Mean velocity was calculated by dividing the sum of velocities over one period time by the number of data points. End diastolic velocities were obtained by measuring from the zero line to the highest point at the end of the diastole. Flow velocity integral was determined by multiplying mean velocity with period time. Acceleration time was defined as the time interval between the onset of the waveform and peak systolic velocity.

## Statistical analysis

For the periods 11-17 weeks and 18-25 weeks of gestation, the intra-individual and inter-individual standard deviations were computed for each parameter using analysis of variance after checking the assumption of equal variances across patients. Reproducibility was expressed as the intra-individual coefficient of variation (i.e. intra-individual standard deviation divided by overall mean).

To investigate the possible relationship of reproducibility with time over the entire observation period, the relative difference of the t1 and t2 measurements were plotted against gestational age (i.e.(t1-t2)/(t1+t2)/2) against age). To test for a trend in reproducibility with gestational age the absolute value of this relative difference was correlated with age using Spearman's correlation coefficient.

## Results

In nine women, of which seven below 16 weeks, no acceptable flow velocity recordings from the fetal ductus arteriosus could be obtained at t1 or t2. This was due to maternal obesity and/or gross fetal movements, leaving 43 women for further analysis.

Ductal end-diastolic velocity was absent until 13 weeks, appeared in some women at 14 weeks and was present in all women as from 17 weeks of gestation. Results of the comparison between reproducibility of flow velocity parameters before 18 weeks (n=23) and after 18 weeks (n=20) are provided in table 1.

There is no significant gestational age related change in the percentage difference between t1 and t2 for all flow velocity parameters studied except for the flow velocity integral (r Spearman = -0.31, P = 0.04).

| | group 1 < 18 wks (n = 23) | | | | group 2 ≥ 18 wks (n = 20) | | | |
|---|---|---|---|---|---|---|---|---|
| | a | b | c | d | a | b | c | d |
| PV | 44 | 4.0 | 10.1 | 9.1 % | 70.8 | 6.9 | 10 | 9.8 % |
| MV | 18.4 | 2.2 | 4.9 | 11.9 % | 29 | 2.7 | 5 | 9.3 % |
| EV | 3 | 0.6 | 3.7 | 18.8 % | 8.8 | 1.3 | 2 | 14.7 % |
| FI | 7 | 0.9 | 2 | 12.5 % | 11.6 | 1.2 | 2 | 10.0 % |
| AT | 51.7 | 11.5 | 14 | 22.2 % | 64.9 | 8.5 | 13.5 | 13.1 % |
| PT | 377 | 10.6 | 16.3 | 2.8% | 401 | 15.1 | 9.4 | 3.7 % |

**Table 1**
Mean value (a), intra-individual (b) and between-individual standard deviation (c) as well as intra-individual coefficient of variation (d) for peak-systolic velocity PV (cm/s), mean velocity MV (cm/s), end-diastolic velocity EV (cm/s), flow velocity integral FI (cm), acceleration time AT (ms) and period time PT (ms) before and after 18 weeks of gestation.

## Discussion

The present results show that reproducibility of flow velocity waveform recording expressed as the intra-individual coefficient of variation does not change with advancing gestational age during the period of 11-25 weeks of pregnancy. The gestational age related change observed for the flow velocity integral appears to be coincidental. Whereas peak systolic flow velocities display a significant rise with advancing gestational age (Brezinka et al. 1992), variations between measurements in the same subject remained at < 10% before and after 18 weeks. A similar pattern was observed for mean velocity and flow velocity integral. If end-diastolic velocities were present between 13 and 16 weeks, they were present both during t1 and t2. Similarly, when end-

diastolic velocities were absent during t1, they remained absent during t2. Reproducibility of acceleration time was poor as has been reported previously for late pregnancies (Groenenberg et al. 1991).

Although in the present study, the sample volume was always placed in the distal part of the ductus there may have been minor variations in sample volume position between patients. Several reports have appeared on the effect of sample volume position regarding the acceleration time (Panidis et al. 1986; Shaffer et al. 1990). There were no significant differences in period time between the two consecutive recording periods. The large between-tests variation which was established for some parameters is therefore not affected by variations in heart rate.

All measurements were carried out after the completion of embryonic structural development. The output levels for the transvaginal Doppler transducer are clearly situated in the lower region for output of Japanese and American ultrasonic diagnostic equipment (Ide, 1989).

It can be concluded that in the ductus arteriosus, acceptable reproducibility in the determination of peak systolic velocity, mean velocity and flow velocity integral can be achieved, indicating that these parameters can be used for assessment of cardiac function as early as the late first and second trimester of pregnancy. The reproducibility in determining acceleration time and end diastolic velocity during this stage of pregnancy is poor.

# Chapter 4

## Normal flow velocity waveforms in the fetal right ventricular outflow tract during late first and second trimester pregnancies

### 4.1 Introduction

In this chapter attention is focused on Doppler flow velocity waveform patterns in the ductus arteriosus and pulmonary trunk as early as 11 weeks of gestation. The data here presented will add to our understanding of early fetal haemodynamics. This will be of particular interest since recent combined transvaginal and transabdominal Doppler studies have shown marked changes in waveform patterns in cardiac, extra-cardiac arterial (umbilical artery, descending aorta) and extra-cardiac venous (ductus venosus, inferior vena cava) vessels.

In chapter 4.2 data will be presented on normal ductal flow velocities during the first half of pregnancy, whereas in chapter 4.3. the relationship between flow velocity waveform patterns between the pulmonary trunk and ductus arteriosus in early gestation will be highlighted.

# 4.2 Normal Doppler flow velocity waveforms in the fetal ductus arteriosus in the first half of pregnancy

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## Abstract

Ductus arteriosus flow velocity patterns were studied in 298 women between 9 and 25 weeks of gestation using transvaginal or transabdominal Doppler ultrasound. Technically acceptable recordings were first obtained at 11 weeks resulting in 231 women for further analysis. Ductal waveforms show a significant change in shape from early pregnancy to mid-pregnancy, in that end-diastolic velocities are absent until 13 weeks, are present in 50% at 15 weeks and are present in each instance as from 17 weeks. Regression analysis demonstrated a statistically significant increase with advancing gestational age for all flow velocity parameters except for the pulsatility index which remained stable during the entire study period.

## Introduction

With the advent of high-resolution real-time ultrasound and in particular colour Doppler imaging, a number of reports has appeared on fetal ductal flow velocity waveforms during late second and third trimester pregnancies (Huhta et al. 1987b; van der Mooren et al. 1991a) . It has been demonstrated that under physiological conditions fetal ductal flow velocities are behavioural state dependent (van Eyck et al. 1990c; van der Mooren et al. 1989) . Ductal constriction will occur as a result of maternal indomethacin administration in premature labour (Tulzer et al. 1991; Moise et al. 1988; Eronen et al. 1991).

The introduction of trans-vaginal Doppler ultrasound has

opened the possibility of studying the fetal circulation as early as 10 - 11 weeks of pregnancy (Dolkart and Reimers, 1991; Wladimiroff et al. 1991b) . Between 10 and 16 weeks cardiac and extra-cardiac flow velocity waveforms suggest a change from a high to a low feto-placental vascular resistance associated with increased ventricular compliance (Wladimiroff et al. 1991a). These changes may also be reflected in ductal flow velocities. Information on these velocities would therefore be of interest.

The objective of the present study was to establish the fetal ductal flow velocity waveform pattern in normal late first and second trimester pregnancies.

## Material and Methods

A total of 298 women with a clinically uneventful singleton pregnancy consented to participate in the study. Pregnancy duration, which was confirmed by ultrasonic measurements of the crown-rump length and/or biparietal diameter, varied between 9 and 25 weeks of gestation (mean 15 weeks).

Fetal ductal flow velocity waveform recording was carried out using either a Hitachi 450 (Tokyo, Japan) or a Toshiba 270 (Tokyo, Japan), both equipped with a combined sector scanner and pulsed wave Doppler system with vaginal and abdominal probes. For the Hitachi, the carrier frequency of the vaginal probe was 6.5 MHz and of the abdominal probe 3.5 MHz. For the Toshiba, the carrier frequency of the vaginal probe was 5 MHz and of the abdominal probe 3.75 MHz. In a previous study intermachine variation was calculated between these two ultrasound systems. The Toshiba 270 depicts slightly lower values than the Hitachi, but these differences never exceed 10% of the standard deviation per parameter (Huisman et al. 1991). Vaginal and abdominal probes operate at power outputs of less than 100 mW/cm2 spatial peak/temporal average in both imaging and Doppler modes by manufacturer's specifications. Until 13 weeks of gestation a transvaginal

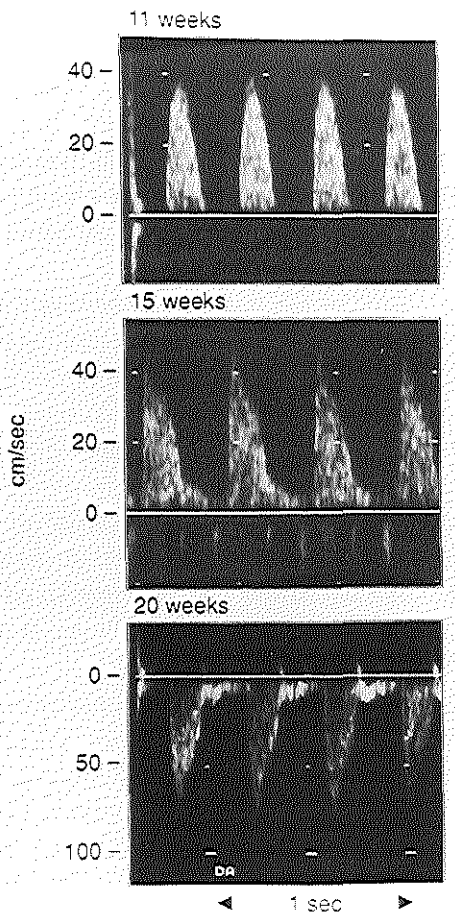


**Figure 1** Ductus arteriosus flow velocity waveforms change in appearance from (a) week 11 through (b) week 15 to (c) week 20. End-diastolic velocities appear between 14 and 17 weeks of gestation.

approach was adopted whereas afterwards waveforms were collected using trans-abdominal ultrasound. In order to limit the exposure of the fetus to transvaginally applied energy levels (Miller, 1991), a maximum recording time of 15 minutes was introduced.

Two dimensional imaging was used to ensure the correct

position of the Doppler interrogation beam both before and after each Doppler tracing was obtained. Maximum flow velocity waveforms from the ductus arteriosus were collected from the conventional short axis view. The Doppler beam was positioned closely to the junction of the ductus arteriosus and descending aorta (Kirshon et al. 1990). Doppler tracings were accepted when the angle between the Doppler beam and the assumed direction of flow was 20 degrees or less.

All blood flow velocity waveforms were obtained during fetal apnoea since later in pregnancy ductal waveforms are modulated by breathing movements (van Eyck et al. 1990b).

All blood velocity waveforms were stored on videotape. From hardcopies printed on a Sony A4 printing device the analysis was performed on a computerized off-line system described previously (Groenenberg et al. 1991) . Four consecutive waveforms of similar appearance were analysed in each case using a conventional microcomputer linked to a graphics tablet. Resolution of the analysing programme was 0.325 mm for the x-axis and 0.5 mm for the y-axis of one hardcopy. Flow velocity waveform analysis consisted of tracing the outer border of the densest part of the Doppler spectrum envelope of each waveform with a cursor and defining the onset, the maximum and the end point of each waveform.

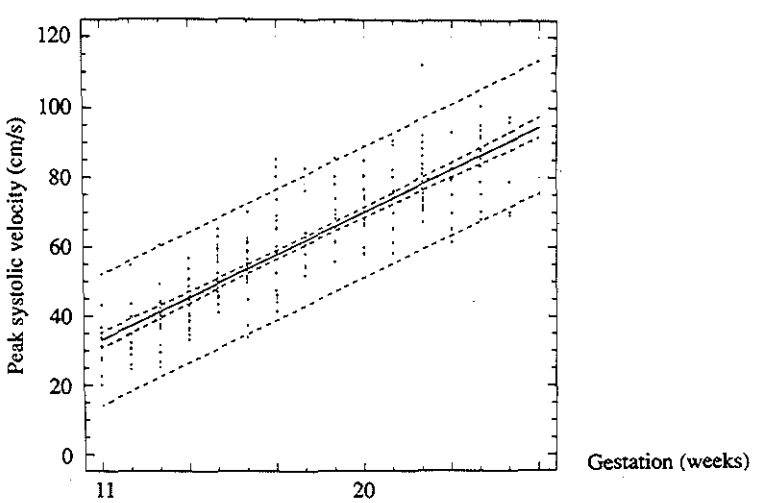


**Figure 2** Ductus arteriosus peak velocities between 11 and 25 weeks of gestation with two-sided 95%-prediction band

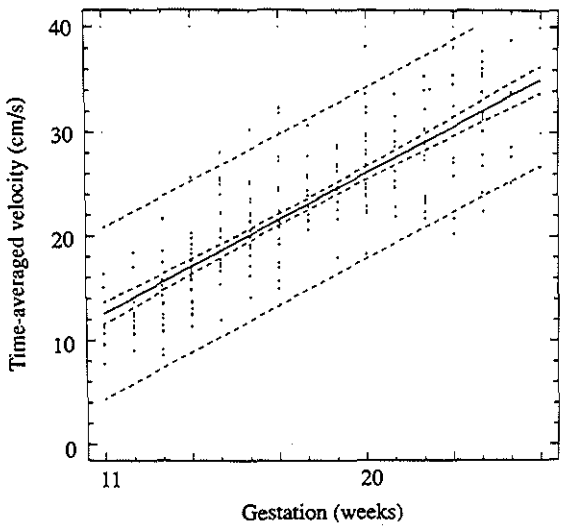


**Fig 3:** ductus arteriosus mean (time-averaged) velocity between 11 and 25 weeks of gestation with two-sided 95%-prediction band

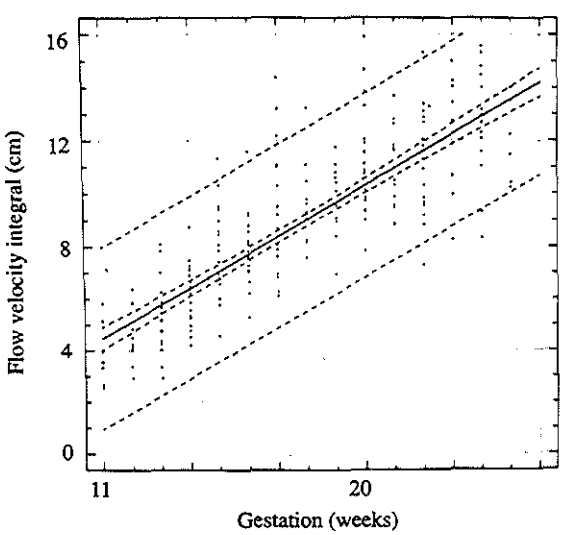


**Figure 4** ductus arteriosus flow velocity integral between 11 and 25 weeks of gestation with two-sided 95%-prediction band

The following parameters were determined: peak systolic velocity (PSV; cm/sec), acceleration time (ACT; ms), time-average velocity (TAV; cm/sec), end-diastolic velocity (EDV; cm/sec), flow velocity integral (FVI; cm), pulsatility index (PI=PSV-EDV/ TAV) and period time(ms).

Peak systolic velocities (PSV) were measured from the zero line to the highest point of the Doppler velocity tracing. Acceleration time (ACT) was defined as the time interval between the onset of ejection and peak systolic velocity. Time-average velocity (TAV) was calculated by dividing the sum of velocities over one period time by the number of data points. End-diastolic velocities (EDV), were obtained by measuring from the zero line to the lowest point at the end of the diastole. The flow velocity integral (FVI) was determined by multiplying time average velocity with period time.

Collected data were entered into a DBASE IV (Ashton Tate, Torrance, CA USA) database and exported to the STATGRAPHICS (Rockville, Md, USA) statistical programme.

The relationship between each waveform parameter and gestational age was first analyzed by means of a simple linear regression analysis. For each parameter the best fitting line

$y = ax + b$ (with y = the flow velocity parameter, x = gestational age, a = the intercept of the regression line and b = slope of the regression line) was determined, and a two-sided 95% prediction band was calculated. As this provided a skewed distribution at gestational ages beyond 20 weeks for end diastolic velocity and period time, a quadratic equation was chosen for these two parameters and a two-sided 95% prediction band calculated.

## Results

No ductal flow velocity recordings were obtained at 9 - 10 weeks of gestation.The percentage of technically acceptable recordings rose from 50% at 11 - 13 weeks to 90% at 16 weeks, resulting in a cross - sectional study population of 231 women between 11 and 25 weeks of

gestation for further analysis.

Ductal waveforms show a significant change in shape from early pregnancy to mid-pregnancy (Fig.1), in that end-diastolic velocities are absent until 13 weeks, are present in 50 % at 15 weeks and are present in each instance as from 17 weeks.

| Parameter | Intercept (SE) | Slope (SE) | p-value |
|---|---|---|---|
| PV | - 12.09 (2.86) | 4.10 (0.16) | < 0.0001 |
| MV | - 3.93 (1.39) | 1.24 (0.07) | < 0.0001 |
| PI | 2.76 (0.12) | - 0.014 (0.0068) | < 0.12 |
| AT | 26.42 (5.03) | 1.39 (0.28) | < 0.0001 |
| FI | - 2.69 (0.53) | 0.65 (0.03) | 0.0001 |

**Table 1** Regression analysis, linear model y = a + bx where a = intercept, b= slope and p-value of analysis of variance (SE: standard error). PV peak systolic velocity (cm/s), MV mean velocity (time-average velocity) cm/s, PI pulsatility index, AT acceleration time (ms), FI flow velocity integral (cm)

| parameter | intercept | linear term | quadratic term |
|---|---|---|---|
| EDV | -29.28 (3.58) | 3.36 (0.41) | 0.078 (0.01) |
| PT | 112.51 (32.22) | 28.09 (3.71) | -0.68 (0.10) |

**Table 2** Regression analysis, quadratic model for end-diastolic velocity (cm/s) and period time (ms). The p-value is <0.001 for both parameters (SE: standard error)

The values for a and b for each flow velocity parameter as well as the p-values are listed in Table 1. A statistically significant linear increase with advancing gestational age was observed for peak systolic and time-average velocity as well as flow-velocity integral ($p < 0.001$; Fig. 2-4) and acceleration time ($p < 0.001$). Using the quadratic relationship, a statistically significant linear increase was established for end-diastolic velocity (Fig. 5) and period time ($p > 0.001$)(Table 2). No significant change with advancing gestational age existed for PI (Fig. 6).

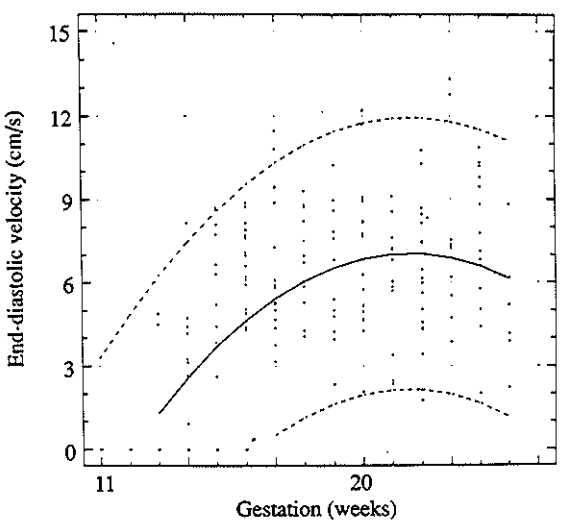


**Figure 5** ductus arteriosus end-diastolic velocity between 11 and 25 weeks of gestation with two-sided 95%-prediction band

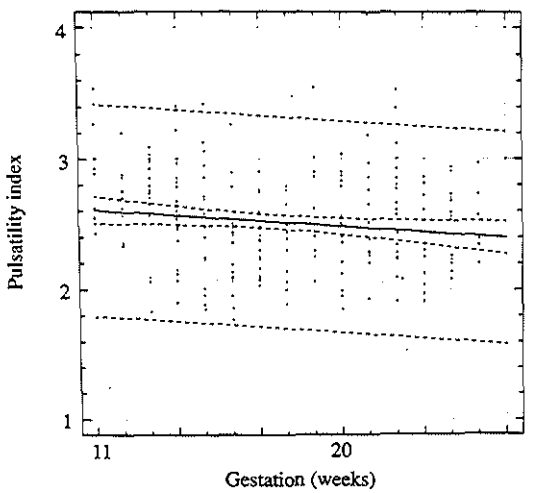


**Figure 6** Pulsatility index of ductus arteriosus flow between 11 and 25 weeks of gestation with two-sided 95%-prediction band

## Discussion

To our knowledge, this is a first report on human fetal ductal flow velocity waveform recordings as early as the late first trimester of pregnancy. Earliest recordings were collected at 11 weeks, since only then it gradually became possible to visualize the important landmarks such as the pulmonary artery and the aortic arch. Recording failure was highest (50%) before 14 weeks, which is partly due to the small size of the relevant structures and partly due to the extreme mobility of the fetus this early in gestation. End- diastolic flow velocities first appear at 13-14 weeks which coincides with the appearance of end-diastolic velocities in the descending aorta and umbilical artery and could therefore be the result of reduced umbilical-placental vascular resistance.

The ductus arteriosus acts as a right-to left shunt in the fetal heart, preventing blood from passing through both cardiac chambers. An increase with advancing gestational age was found for all flow velocity parameters concerned. The gestational age-related rise in ductal peak systolic and time-average velocities is in agreement with previous observations on ductal flow velocities between 18 weeks and term(van der Mooren et al. 1991a) and may be determined by both an increase in ventricular stroke volume and a decrease in right ventricular aferload. Right ventricular afterload is largely determined by placental vascular resistance which has been shown to decrease with advancing gestational age (Trudinger, 1987).

The flow velocity integral, calculated from time-average velocity and period time, could be used to determine volume flow if information on the ductal diameter would be available. This is not possible with present techniques. However, assuming that the ductus is maximally dilated (Friedman et al. 1983) the age-related rise in flow velocity integral suggests a steady increase in right ventricular stroke volume and output during the observation period.

The PI varies between 2 and 3 and does not significantly change with gestational age. A PI below 1.9 is seen as reflecting ductal constriction as a result of maternal indomethacin administration (Tulzer et al. 1992). The pulsatility index has been widely used as an indicator of placental and fetal peripheral vascular resistance (Giles et al. 1985). A gradual decline in umbilical PI has been demonstrated as from 12-13 weeks gestation suggesting a decrease in placental vascular resistance. The reduction in ductal PI will only be observed as a result of vessel constriction during maternal indomethacin administration (Moise et al. 1990). Therefore a change in ductal PI will represent a pathophysological phenomenon which is completely different from that seen in the umbilical artery because ductal waveforms do not directly reflect peripheral vascular resistance.

As acceleration time is a fraction of period time, the increase in acceleration time over the period observed is not unusual. However, a large scatter of data on acceleration time was observed. This is in agreement with an earlier report from our own group (Groenenberg et al. 1991) in which it was demonstrated that acceleration time determined from fetal ductal flow velocity waveforms during the second and third

trimester of pregnancy displayed a relatively poor reproducibility compared with the other waveform parameters.

### Conclusion

It can be concluded that ductal flow velocities may be recorded as early as 11 weeks of gestation. End diastolic flow velocities first appear at 13 weeks of gestation, probably as a result of a decreasing afterload. Both increase in right ventricular stroke volume and decrease in afterload may be responsible for the rise in ductal peak systolic and time-average velocities.

## 4.3 Relationship between fetal pulmonary trunk and ductus arteriosus flow velocity waveforms in early normal pregnancy

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### Abstract

To establish the relationship between flow velocity waveform patterns from the fetal pulmonary trunk and ductus arteriosus in normal late first and second trimester pregnancies, Doppler ultrasound examinations in these vessels were performed in 133 healthy women between 9 and 25 weeks of gestation. Differentiation between pulmonary and ductal blood flow was possible as from 12 weeks onwards resulting in a study population of 78 women. A linear gestational age dependent increase in peak systolic velocity was found for both the pulmonary trunk

and the ductus arteriosus. Ductal peak systolic velocity rose significantly faster with gestational age than pulmonary peak systolic velocity. This may be determined by differences in morphology and effective lumen between these two vessels.

## Introduction

Cardiac and extra-cardiac flow velocity waveform studies between 10 and 16 weeks of gestation suggest a change from a high to a low fetal-placental vascular resistance associated with increased ventricular compliance in the normally developing human fetus (Wladimiroff et al. 1991a). Outflow tract studies have demonstrated flow velocities in the ascending aorta to be higher than in the pulmonary artery (Sutton et al. 1991; Dolkart and Reimers, 1991). This suggests a different afterload to both ventricles (Wladimiroff et al. 1991b).

Recently, combined transvaginal and transabdominal flow velocity waveform studies in the ductus arteriosus revealed a significant increase in flow velocity with advancing gestational age and the appearance of end-diastolic flow velocities in this vessel at 15-16 weeks of gestation (Brezinka et al. 1992). In prenatal life, right ventricular output will mainly be shunted through the pulmonary artery and ductus arteriosus to the descending aorta. Marked differences in peak velocities between pulmonary artery and ductus arteriosus have been established in late pregnancy with peak velocities as high as 1.5-2 m/sec in the ductus (Huhta et al. 1987a; van der Mooren et al. 1991a).

The objective of the present study was to establish the relationship between flow velocity waveform patterns from the fetal pulmonary trunk and ductus arteriosus in normal late first and second trimester pregnancies.

## Material and Methods

A total of 133 women with a clinically uneventful singleton pregnancy consented to participate in the study. Pregnancy duration which was confirmed by ultrasonic measurements of the crown-rump

length and/or biparietal diameter, varied between 9 and 25 weeks of gestation (mean 15 weeks).

Flow velocity waveform recording of the pulmonary trunk and the ductus arteriosus was carried out using a Hitachi 450 (Tokyo, Japan) combined sector scanner and pulsed wave Doppler system with a transvaginal and transabdominal probe with carrier frequencies of 6.5 MHz and 3.5 MHz, respectively. The cut-off level of the high pass filter was set at 100 Hz. Both probes operate at power outputs of less than 100 mW/cm$^2$ spatial peak/temporal average in both imaging and Doppler modes by manufacturer's specifications. All recordings were made by the same operator (C.B.).

Transvaginal Doppler recordings were attempted between 9 and 11 weeks and transabdominal recordings were made as from 12 weeks onwards. In order to limit the exposure of the fetus during the scanning procedure (Miller, 1991), the cumulative recording time for both vessels was limited to 20 minutes.

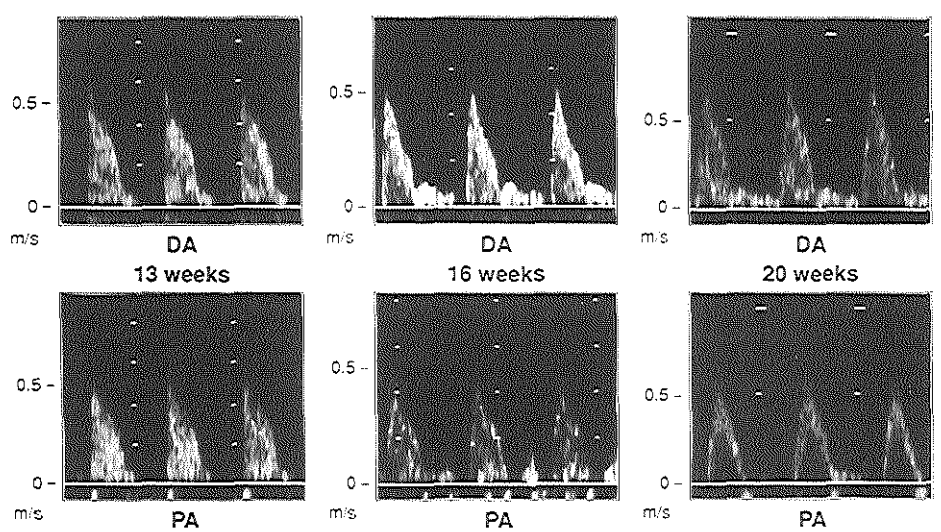



**Figure 1:** Flow velocity waveforms from the ductus arteriosus (DA) and the pulmonary trunk (PA) at 13, 16 and 20 weeks of gestation

Two dimensional imaging was used to ensure the correct position of the Doppler interrogation beam both before and after each Doppler tracing was obtained. Maximum flow velocity waveforms from pulmonary trunk and the ductus arteriosus were recorded from the conventional short axis view (Reed et al. 1988a). Pulmonary artery waveforms were obtained immediately distal to the pulmonary valve. Ductal waveforms were collected close to the junction of the ductus arteriosus and descending aorta (Kirshon et al. 1990). Doppler tracings were only accepted when the angle between the Doppler beam and the assumed direction of flow was 20 degrees or less. Sample size was 2-3 mm. Since both arterial and venous flow velocity waveforms are modulated by fetal breathing movements, all blood flow velocity waveforms were obtained during fetal apnoea (van Eyck et al. 1990b).

All flow velocity waveforms were stored on videotape. From hardcopies printed on a Sony A4 printing device the analysis was performed on a computerized off-line system described previously (Groenenberg et al. 1991) . Four consecutive waveforms of similar appearance were analysed in each case using a conventional microcomputer linked to a graphics tablet. Resolution of the analysing programme was 0.325 mm for the x-axis and 0.5 mm for the y-axis of one hardcopy. Flow velocity waveform analysis consisted of tracing the outer border of the densest part of the Doppler spectrum envelope of each waveform with a cursor and defining the onset, the maximum and the end point of each waveform.

The peak systolic velocity (PSV; cm/sec), was determined in both the pulmonary trunc and the ductus arteriosus. Peak systolic velocity (PSV) was measured from the zero line to the highest point of the Doppler velocity tracing. To study the relationship between the pulmonary trunk and ductus arteriosus flow velocity waveform, the ratio for peak systolic velocity from both vessels was calculated. Collected data were entered into a database and exported to the STATGRAPHICS (STSC, Rockville, Md, USA) statistical programme.

The relationship between each waveform parameter and gestational age was analyzed by means of regression analysis.

## Results

Ductal flow velocity recordings were first obtained at 11 weeks and pulmonary trunk flow velocity recordings first at 12 weeks of gestation (Fig 1). Simultaneous registration of pulmonary and ductal flow velocity waveforms was therefore feasible as from 12 weeks onwards. The percentage of technically acceptable recordings from both vessels rose from 30% at 12 - 14 weeks to 60% at 16 weeks, resulting in a cross-sectional study population of 78 normal singleton pregnancies between 12 and 25 weeks of gestation for further analysis.

Regression analysis confirmed that the peak systolic data could be adequately described by a linear relationship. For each parameter the best fitting line $y = ax + b$ ($y$ = the flow velocity parameter, $x$ = gestational age) was determined, and a two-sided 95% prediction band was calculated. The same procedure was applied to the ratios of the flow velocity parameters of the two vessels.

In both the pulmonary trunk and the ductus arteriosus a statistically significant linear increase with advancing gestational age was observed for peak systolic velocity (Fig.2a and 2b) ($p < 0.001$) and the ratio of ductal to pulmonary peak systolic velocity (Fig 2c) ( $p < 0.001$) Data for the slope (se) and intercept (se) for each of the variables are demonstrated in Table 1.

| | Slope (se) | Intercept (se) | p-value |
|---|---|---|---|
| **PT peak** | 2.03 (0.24) | 8.57 (4.39) | < 0.0001 |
| **DA peak** | 4.12 (0.31) | -10.94 (5.65) | < 0.0001 |
| **ratio DA/PT** | 0.029 (0.006) | 0.87 (0.12) | < 0.0001 |

**Table 1**
Regression analysis, linear model y = a+bx with a = slope b= intercept (se = standard error) of analysis of variance: Pulmonary trunk (PT) and ductus arteriosus (DA) peak velocities and the ratio of the two peak velocities are given.

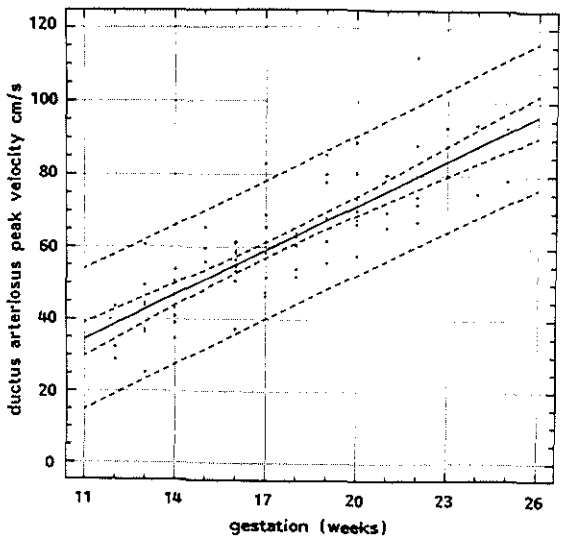
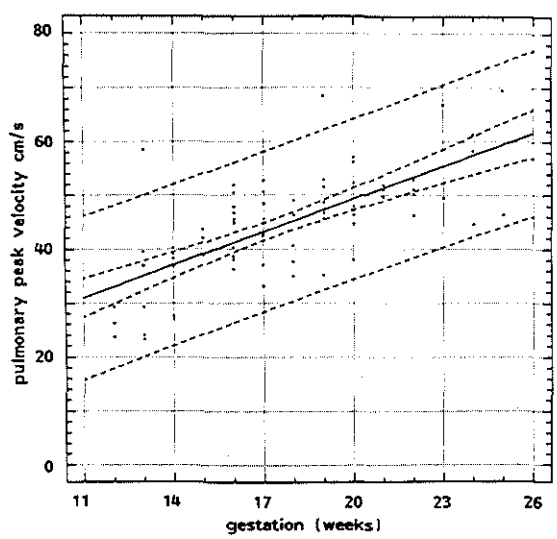

ductus arteriosus peak velocity cm/s
120
100
80
60
40
20
0
11
14
17
20
23
26
gestation (weeks)

**Fig 2 a)**


pulmonary peak velocity cm/s
80
60
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20
0
11
14
17
20
23
26
gestation (weeks)

**Fig 2 b)**

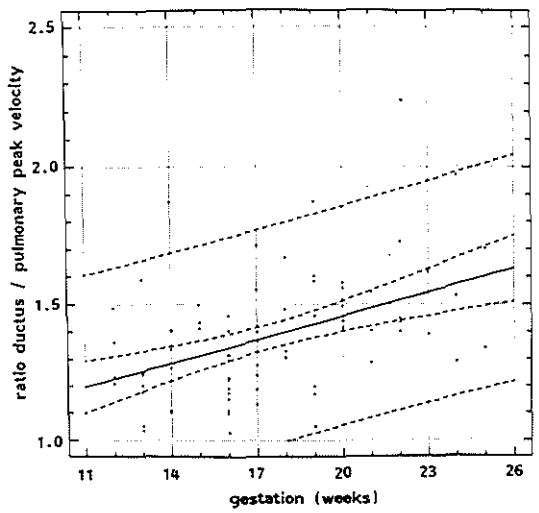


**Fig 2 c)**

**Figure 2 a - c :** Peak systolic velocities (m/sec) from the ductus arteriosus (a) and pulmonary trunk (b) and ratios of ductus arteriosus to pulmonary trunk peak systolic velocities (c) relative to gestational age (weeks)

## Discussion

It is known from studies in the second half of pregnancy that blood flow velocities in the fetal ductus are higher than in any other cardiac or extra-cardiac vessel (Huhta et al. 1987b). Both preductal and postductal blood flow velocities are significantly lower. The postductal slowing of the blood flow velocities in the descending aorta can be explained by the wider vessel diameter of this vessel.

Flow velocity in the ductus arteriosus is determined by the degree of pressure difference between pulmonary trunk and aorta and vessel size of the ductus. The reduction of 133 to 78 normal pregnancies being included in the waveform analysis was solely determined by the failure to obtain technically acceptable waveforms in late first and early second trimester pregnancies. The gestational age related increase in peak systolic velocity in both the pulmonary artery and ductus arteriosus is in

agreement with observations in late second and third trimester pregnancies (van der Mooren et al. 1991b; van der Mooren et al. 1991a). Data from the present study show that already at 12 weeks of gestation peak systolic velocity in the pulmonary trunk appears to be somewhat lower than in the ductus arteriosus. This difference in peak systolic velocity becomes even more apparent as pregnancy progresses. At the same time there appears to be a drop in fetal and umbilical placental vascular resistance (Wladimiroff et al. 1991a) with appearance of intervillous flow at 13-14 weeks of gestation (Juniaux et al. 1992).

The relationship between pulmonary trunk and ductus arteriosus diameter is difficult to ascertain in-vivo and in-vitro. In post mortem studies the ductus arteriosus has shown to be extremely susceptible to mechanical manipulation, to fixatives like formaldehyde and even to short term storing of the fetus before fixation. All these changes lead to the presence of a deformed and reduced ductal lumen (Hörnblad and Larsson, 1967; Meurs van Woezik and Klein, 1974). The development at histological level in the ductus arteriosus vessel wall can be seen as a preparation for postnatal closure throughout the fetal period. Whereas the vessel wall of the pulmonary trunk contains elastic tissue that is insensitive to oxygen, the vessel wall of the ductus is composed of longitudinal muscle cells that permit rapid constriction after birth (Elzenga, 1986).

Alvarez (Alvarez et al. 1990) developed a geometric model of the pulmonary trunk representing a truncated cone and the ductus arteriosus representing two truncated cones joined at their smaller bases. This implies a wider lumen at the level of the pulmonary trunk, which is supported by echocardiographic data (Cartier et al. 1987). This would explain the lower flow velocities in the pulmonary trunk than in the ductus arteriosus. An alternative explanation for our findings could be the relative elasticity of the vessel wall of the pulmonary trunk when compared with the muscular vessel wall of the ductus arteriosus. If the elasticity of the pulmonary trunk is to exert an effect on blood velocity this would result in a widening of this vessel during systole. However, this was not observed in a study that compared echocardiographic images with post-mortem sections of this particular vessel (Angelini et al. 1988). It can be assumed that the muscular vessel wall of the ductus arteriosus is well suited for the purpose of sustaining shear powers due to velocities

higher than elsewhere in the fetal vascular system.

It has been suggested that of the two pulmonary arteries that branch off the pulmonary trunk, the right artery is often larger and the left artery occasionally larger than the ductus arteriosus (Alvarez et al. 1991; Angelini et al. 1988). We believe this will not affect blood flow velocities in the ductus since pulmonary vascular resistance and pressure is higher than that in the systemic circulation (Clyman et al. 1989a). This will prevent significant blood flow to the lungs.

It can be concluded that present Doppler ultrasound equipment allows flow velocity waveform recordings in the pulmonary trunk and ductus arteriosus as early as 12 weeks of gestation. Peak systolic velocities in both vessels display a significant rise with advancing gestation, with ductal velocities rising signficantly faster than pulmonary trunk velocities.

# Chapter 5
# The ductus arteriosus and aorta: a combined morphological and sonographical study

## 5.1. Introduction

Morphological studies of the fetal ductus arteriosus date back to the 16th century. For a long period scant information has been available with respect to the basic processes responsible for normal and abnormal cardiac development. Lately exciting new data have emerged on the role of the neural crest, in particular regarding the development of the cardiac outflow tract. In animal experimental study designs, interference with neural crest cell migration has resulted in outflow tract anomalies such as truncus arteriosus and double outlet right ventricle (Kirby, 1990; Kirby, 1987).

Combined anatomical and Doppler studies of the developing heart would allow information on the interaction between morphology and hemodynamics under normal and abnormal circumstances. Such studies have been carried out successfully in the chick embryo model. Normal relationship between morphology and hemodynamics have been reported in detail by Clark and co-workers in Rochester, N.Y. (Clark et al. 1986; Hu and Clark, 1989). Recently, using micro-Doppler and micro-pressure systems, marked hemodynamic changes have been established in the dorsal aorta of chick embryos which were pre-treated with retinoic acid, resulting in double-outlet right ventricle (Broekhuizen et al. 1992; Broekhuizen et al. 1993).

Hemodynamic studies of early cardiovascular development are now also possible in the human fetus. The introduction of Colour Velocity Imaging (CVI) has opened new possibilites to make detailed *in vivo* observations of fetal haemodynamics (Huisman et al. 1994). Combined investigation of cardiovascular morphology and hemodynamics would greatly improve our knowledge of morphological relationships at cardiac outflow tract level, in this case between ductus arteriosus and the aorta. In chapter 5.1 the anatomical and sonographic correlation of the fetal ductus arteriosus in first and second trimester pregnancies will be discussed.

# 5.2 Anatomical and sonographic correlation of the fetal ductus arteriosus in first and second trimester pregnancy

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## Abstract

Ultrasonic visualization of the ductus arteriosus in first and second trimester pregnancies was compared with postmortem preparations. Twenty human fetal postmortem specimen from 8 to 19 weeks menstrual age were examined, eleven with microscopic reconstruction, nine with macroscopic dissection. The angle between ductus arteriosus and aortic isthmus (upstream) and ductus arteriosus and descending aorta (downstream) was determined. In 52 normally developing fetuses between 14 and 27 weeks the angle between the ductus arteriosus and the thoracic spine as visualized in real-time ultrasound was determined. In a further 19 normally developing fetuses between 14 and 25 weeks ductal blood flow was visualized by Colour Velocity Imaging (CVI).

In anatomical preparations the upstream angle was always less than 90°, the downstream angle was always 80° or more. These angles were unrelated to menstrual age. In both real-time and CVI ultrasound the angle between ductus arteriosus and thoracic spine remained at approximately 90°. CVI showed highest blood flow velocities at the point of ductal insertion into the aorta.

When performing Doppler ultrasound examinations in the fetal ductus arteriosus, no menstrual age dependent angle adjustment appears to be necessary.

## Introduction

It has long been established that the intrauterine development of the ductus arteriosus, its embryology and histology, are so unique that it sets this vessel apart from all other cardiac and peripheral fetal vessels (DeRuiter et al. 1989; Clyman et al. 1989b; Coceani and Kelsey, 1991). Doppler ultrasound studies in the fetal ductus arteriosus have shown that flow velocities in this vessel change in fetal growth retardation (Groenenberg et al. 1989) and during maternal indomethacin administration (Moise et al. 1988)

The ductus arteriosus derives from the left 6th branchial arch artery, also termed the pulmonary arch artery (Congdon, 1922). The ductus arteriosus is a muscular vessel unlike the other branchial arch derivates which have predominantly elastic walls. The smooth muscle cells in the ductus arteriosus are highly sensitive to prostaglandines and their antagonists (Olley and Coceani, 1987). Another unique feature of the ductus arteriosus is the development of intimal cushions protruding into the lumen. Intrauterine morphological development of the ductus arteriosus prepares for effective closure after birth (Gittenberger de Groot et al. 1980).

In recent years it has become possible to observe the fetal ductus with ultrasound and to measure blood flow velocities in this vessel using pulsed wave and continuous wave Doppler (Huhta et al. 1987b). We recently applied transvaginal ultrasound to observe ductus arteriosus flow velocities as early as 11 weeks menstrual age (Brezinka et al. 1992). With these advances in ultrasound technology attempts have been made to correlate ultrasound with the developing vascular anatomy of the normal and the malformed fetus (Allan et al. 1984; Angelini et al. 1988). There is scant and conflicting information as to the changing anatomical relationship between the ductus arteriosus and aorta with advancing gestational age (Odé, 1951; Mancini, 1951; Roeder, 1902). This would have implications for the technique of Doppler flow velocity waveform recording in the ductus arteriosus of the developing fetus. Further in vitro studies would be necessary to clarify the exact anatomic relationship between these two vessels and to relate findings to the in vivo situation as studied by ultrasound.

The following questions were addressed

(i) what is the angle between the ductus arteriosus and aortic isthmus (upstream) and descending aorta (downstream) in vitro; do these angles display an age-dependent change ?

(ii) How do these in vitro observations relate to the in vivo situation as studied by real-time ultrasound and Colour Velocity Imaging?

Attention was focussed on the late first and second trimester of pregnancy because whilst ductal behaviour in the perinatal period is well documented (Gittenberger de Groot et al. 1980), a systematic study of ductal and aortic relation pertinent to the present clinical diagnostic tools has not been performed in these stages of pregnancy.

## Material and Methods

Several methods were used to obtain a complete picture of the spatial relationship between the ductus arteriosus and the aorta in the first and second trimester of pregnancy. Postmortem investigation was performed on three-dimensional reconstructions of serially sectioned fetuses and on macroscopic preparations of fetuses preserved *in toto*. In vivo observations were obtained from real time ultrasound images of the right cardiac outflow tract from the pulmonary valve to the descending aorta (Reed et al. 1988a) and from colour velocity imaging (CVI) visualizations of the same structures (Pesque, 1990). CVI makes it possible to differentiate velocities within a given vessel (Huisman and Wladimiroff, 1992).

*a) 3-D reconstructions of serially sectioned fetuses (Figure 1a and Figure 1b)*

Eleven fetuses between 9 - 19 weeks menstrual age, from the collection of the Leiden Department of Anatomy and Embryology, were studied microscopically. These embryos were serially sectioned at 10μm, mounted on glass slides and alternately stained with haematoxylin-eosine, resorcine-fuchsine, a modified van Gieson stain, Azan and Methyl-green pyronine. The fetuses were also examined for abnormalities. One showed stigmata of fixation artefacts and was not included in the present study. To prevent a skewed reconstruction, care was taken to use the

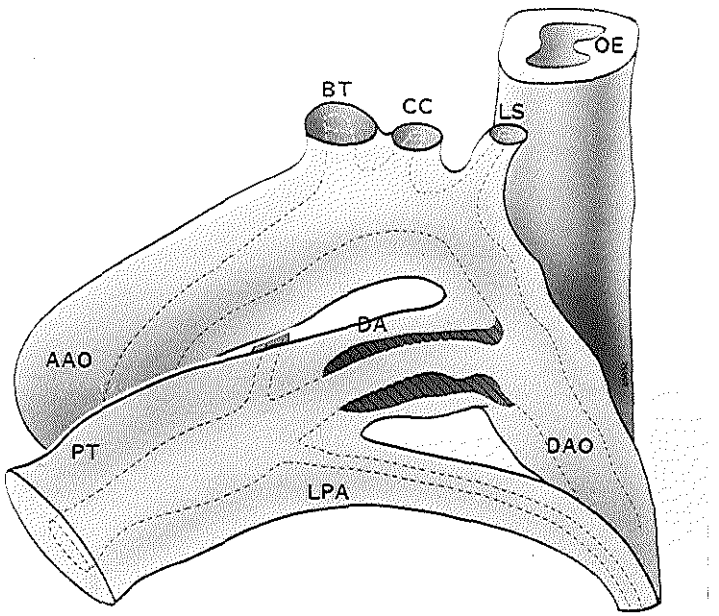



**Fig.1 a)** Graphic reconstruction of a 45 mm CRL (10 weeks) fetus with the Ductus Arteriosus (DA) leading horizontally towards the aorta. Ductal tissue is shaded dark. (AAo ascending aorta, BT brachiocephalic trunk, CC common carotid artery, LS left subclavian artery, PT pulmonary trunk, LPa left pulmonary artery, DAo descending aorta, Oe oesophagus)

oesophagus as a reference structure since it is generally accepted as the second best parallel in the absence of the fetal spine. Outer and inner contours of each 10th section of the aortic arch, the pulmonary trunk, the pulmonary arteries, the ductus arteriosus and the upper part of the descending aorta were drawn after enlargement using a Zeiss microscope with a drawing attachment. When it could be discerned, ductal tissue was highlighted. The outside wall and the lumen of the aortic arch and the ductus arteriosus were reconstructed three-dimensionally in an antero-lateral view using the method described by Tinkelenberg (Tinkelenberg, 1979).

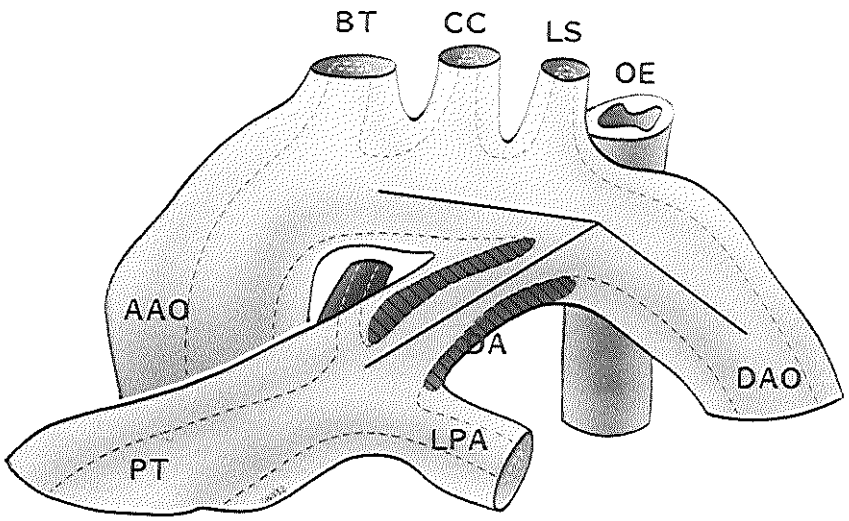



**Fig 1 b)** Graphic reconstruction of a 12 cm CRL (16 weeks) fetus with a straight line drawn through the ductus arteriosus (DA). It forms angle a, the "upstream angle" with the line drawn through the aortic arch and angle b, the "downstream angle", with the line pointing towards the descending aorta (DAo).

It was attempted to make angle measurements by drawing a line through the middle of the ductus arteriosus, which at the point of insertion into the aorta would cross with **a)** a line going upstream through the aortic isthmus and **b)** a line going downstream into the descending aorta. The centre of the vascular lumen of all vessels was used to place these lines, using the method described by Mancini(1951). Thus two angles, one upstream (angle a) and one downstream (angle b) were obtained (Figure 1b). The upstream and downstream angles would not necessarily add up to 180 degrees, as in most cases flow direction changes markedly after the insertion of the ductus arteriosus.

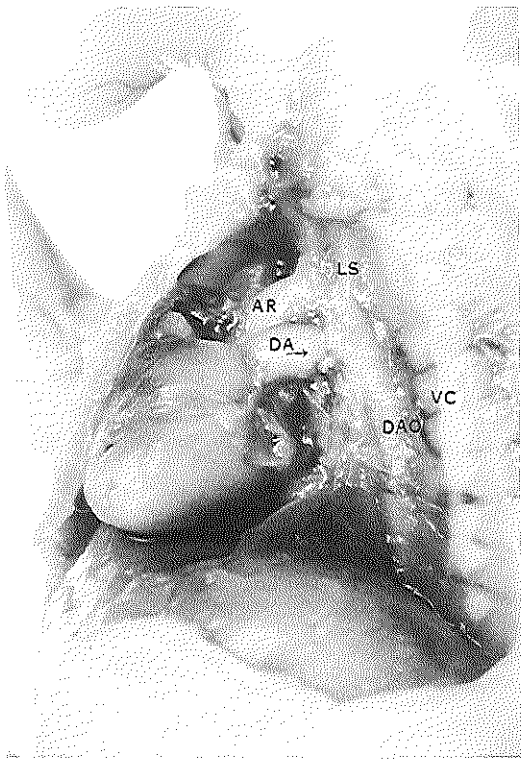



**Fig 2 a)** a fixated 90 mm CRL (13 weeks) fetus with the left rib cage and left lung and thymus removed to enable an antero-lateral view of the ductus arteriosus (DA) entering the Aorta (DAo) shortly below the left subclavian (LS) artery has branched off the aortic arch (Ar). The descending aorta runs parallel to the vertebral column (VC).

*b) macroscopic preparation of fixated fetuses (Figure 2a and Figure 2b)*

Another nine fetuses between 12 - 19 weeks menstrual age from the Leiden collection were studied macroscopically. The specimen had been fixed in 4% phosphate buffered formalin or in Bouin's solution. The thorax was opened, the rib cage of the left side removed, the mediastinum incised and the left lung and the thymus removed to obtain an unobstructed view of the right cardiac outflow tract. The fetuses were subsequently mounted on glass plates in a standardized position

permitting the same antero-lateral view on the vessels of interest as had been used for the reconstructions under a) for macro-photography. As in the serially sectioned fetuses, both the upstream and the downstream angle of the ductus arteriosus were determined by drawing lines on the vessels on the photographs. Furthermore, the angle between the ductus arteriosus and the thoracic part of the vertebral column was determined.

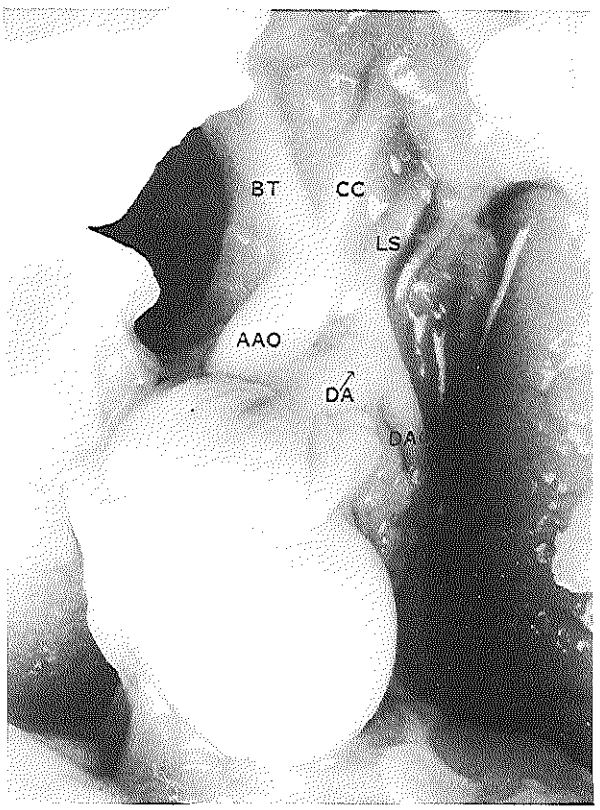
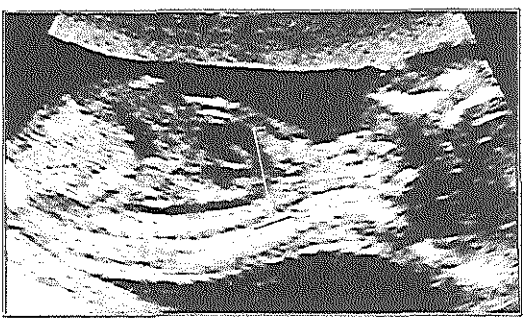
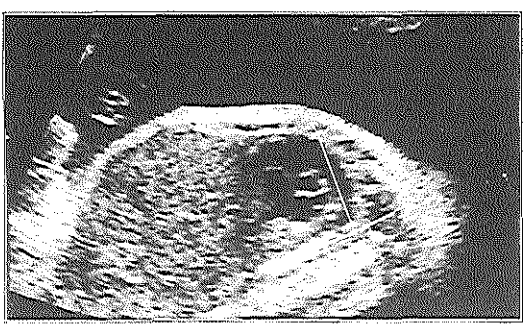



**Fig 2b**) an anterior view of a fixated 80 mm (12 weeks) fetus with the left pulmonary artery and left bronchus severed to enable the view onto the ductus arteriosus (DA). The ascending aorta (AAo) forms the aortic arch giving off the brachiocephalic trunk (BT), the common carotid artery (CC) and the left subclavian artery (LS). This is the view that should be attempted when performing Doppler measurements of the ductus arteriosus.



17 wk



25 wk

**Fig 3** View of the right cardiac outflow tract in a healthy fetus at 17 (Fig 3a) and 25 weeks of gestation (Fig 3b). Lines are drawn through the ductus arteriosus where it follows an approximately longitudinal course. A second line is drawn throug the thoracic part of the vertebral column. In fetal apnea an angle between 85° and 115° (mean 93°) was observed.

*c) ultrasound real time images of the ductus arteriosus (Fig 3a and Fig 3b)*

In 52 normally developing fetuses from singleton pregnancies between 14 and 27 weeks menstrual age referred to the Division of Prenatal Diagnosis of the Department of Obstetrics and Gynecology of Rotterdam Erasmus University Hospital for ultrasound screening, an image of the full length of the right cardiac outflow tract from the right ventricle through the pulmonary trunk and the ductus arteriosus into the descending aorta was obtained using a Toshiba 270 real time scanner

with a 3.75 MHz abdominal probe. Because the aortic arch is in a more oblique and dorsal plane compared with the sagittal plane of the right cardiac outflow tract, it was not possible to simultaneously visualize the two structures on ultrasound and thus measure upstream angles in vivo. Images were obtained during fetal apnoea since fetal breathing movements lead to upward and downward displacement of the heart and the ductus arteriosus. Once an image was obtained it was electronically enlarged and printed on A4-size videoprint-paper. A line was drawn through that part of the ductus arteriosus where it appears to follow an approximately longitudinal course. A second line was drawn through the upper part of the thoracic vertebral collumn opposite the cardiac outflow tract. Measuring downstream (caudally) the angle between these two lines was determined.

*d) Colour flow velocity imaging (Fig 4)*

Nineteen normally developing fetuses between 14 and 25 weeks menstrual age recruited from the same group as those described under c) were examined with a Philips P 700 real time and colour velocity imaging (CVI) scanner with a 3.5 MHz transducer. The high axial resolution of CVI makes it possible to differentiate between velocities within a particular vessel using a highly developed form of B-mode ultrasound. Instead of depicting waveform patterns, maximum velocities within a give area are depicted with a colour code on what otherwise is a B-mode image. Once a satisfactory image of the short-axis view as described above was obtained, the colour option was activated. From 14 weeks onwards, it was possible to differentiate between the ductus arteriosus and the aortic arch. We then proceeded to use the CVI colour code to locate the point of highest blood flow velocity inside the ductus arteriosus. Colour prints were made on a SONY Mavigraph colour printer. For the purpose of angle determination a line was drawn through the ductus arteriosus and the thoracic part of the the fetal vertebral column.

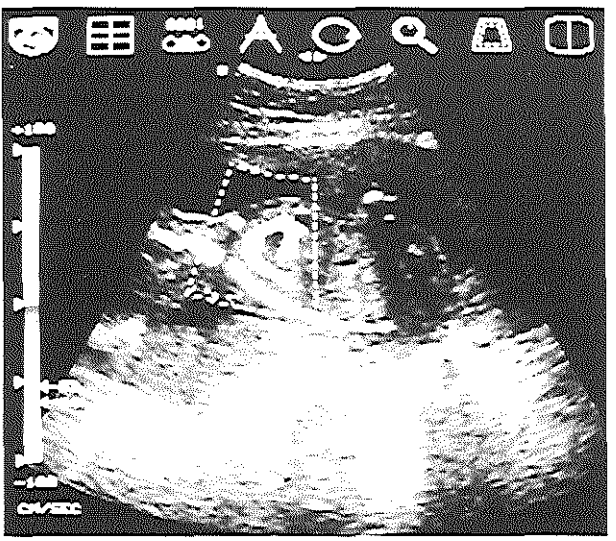


**Fig 4.** Black and white reproduction of a CVI-image of the right cardiac outflow tract of a healthy fetus of 20 weeks of gestation. Blood flow is depicted in white. The curved nature of the pulmonary trunk - ductus arteriosus part of the right cardiac outflow tract is clearly visible.

## Results

*a) microscopic reconstructions*

The upstream angle varied between 30° and 80° (mean 60.90° SD 13.93°), the downstream angle ranged between 80° and 120° (mean 106.5°, SD 14.15°). There was no statistically significant relationship with menstrual age: R-value for the upstream angle = +0.07, for downstream angle = +0.15.

*b) macroscopic study of fetuses*

The upstream angle varied between 45° and 80° (mean 61.87° SD 10.66°), the downstream angle ranged between 90° and 115° (mean 99.37°, SD 7.28°). There was no statistically significant relationship with menstrual age, the R-value for the upstream angle being -0.56 and for the downstream angle 0.40. The mean angle between the ductus arteriosus and the thoracic spine was 88.33 degrees (SD 4.08°).

*c) ultrasound images*

The angle between the ductus arteriosus and the thoracic spine ranged between 85° and 115°( mean 93.26°, SD 6.09°). There was no statistically significant relationship with menstrual age as shown by an R-value of +0.07.

*d) CVI registrations*

Blood velocities increased as blood passed from the pulmonary trunk into the ductus arteriosus. Blood in the ductus always displayed a different colour-code, signifying a higher velocity, than that in the pulmonary trunk. Highest overall velocities inside the ductal lumen were shown to be near the inferior surface of the ductus arteriosus at the point of insertion into the aorta, i.e. blood on the "inside track" of the slightly curved vessel displayed the highest flow velocities. Measurements of the angle between the ductus and the thoracic spine, resulted in data ranging from 85° to 100° (mean 90.26°; SD 3.52°); no statistically significant relationship with menstrual age was observed, the R-value being -0.30.

## Discussion

Proper angling is essential for sonographic ductal velocity measurements. The smaller the interrogation angle between Doppler ultrasound beam and ductal flow direction, the more accurate will be the absolute velocities measured inside this vessel.

In the anatomical part of the study, the upstream angle between the ductus arteriosus and the aortic isthmus was always less than 90° and the downstream angle between the ductus arteriosus and the descending aorta always 80° or more. There was, however, a wide distribution of data. The angle between the ductus arteriosus and thoracic spine was more consistent, at approximately 90°. In both the micro-preparations and macro-preparations none of these angles changed significantly with menstrual age.,

Interest in the spatial relationship between the fetal ductus arteriosus and surrounding vessels, in particular the aorta, has been expressed in several reports with emphasis on both the upstream angle

(Roeder, 1902; Mancini, 1951) and downstream angle (Faber, 1912; Odé, 1951). A specific angle between the ductus and aorta was thought to be instrumental in ductal closure. Strassmann(1894), believed that due to the acute angle with which the ductus inserted in the aorta, a thin wedge of tissue at the upper part of the insertion would be pushed into the ductal orifice after birth under the pressure of increased blood flow through the aortic arch and thus effect ductal closure. Based on in situ measurements of mature newborns Roeder (1902) stated that the (upstream) angle between the ductus arteriosus and the aortic arch was 33°. He stressed the importance of the acute angle in the prevention of turbulent flow at the point of aortic insertion of the ductus. In a series of postmortem studies on premature and full term infants upstream angles ranged between 25° and 37.5° (Mancini, 1951). This is considerably smaller than that observed in the present study, which may be due to the difference in menstrual age between both studies. More in agreement with the present study, downstream measurements after birth have been reported with angles of approximately 90° and a pointing downwards of the ductal orifice into the aortic lumen (Faber, 1912). Odé (1951), performing downstream measurements, was the first to measure angles in very early gestation (fetal crown-rump length 10 mm) and ending with mature newborns. Early in gestation an angle of 85° was established which increased to 140° at 24 weeks and decreased to 100° at term.

In the present study we have tried to correlate postmorten findings in preserved fetuses with in-vivo ultrasound images. There is no way of establishing the position of the heart inside the thorax at the time the preserved fetuses expired. During fetal breathing movements the heart moves upwards and downwards with the diaphragm and as a result the direction of the ductus arteriosus changes. Agonal changes like contractions of the diaphragm but also fixation procedures (Hörnblad and Larsson, 1967; Meurs van Woezik and Klein, 1974) may lead to the heart, the ductus arteriosus and the aortic arch being preserved in a displaced position. A further problem arises from the fact that angles are a linear geometrical concept whereas the ductus arteriosus together with the pulmonary trunk represent a slightly arched structure similar to the aortic arch with which it eventually converges. Moreover, the arch described by the aortic arch is not necessarily completed at the point of ductal insertion: when the ductus arteriosus inserts higher into the aortic

arch, the immediate downstream part of the descending aorta will be a continuation of the arch over a few millimeters and only becomes the straight vessel that runs parallel to the oesophagus (Fig 1 b). It might be argued that angle determination in arched structures should not be attempted at all because of these pitfalls. However, describing the spatial relationship between two eliptically arched tubes that fuse into each other necessitates extensive mathematical calculations that can not be applied in clinical practice. Angle determination still has a place and correct angle assessment is of paramount importance in Doppler ultrasound.

One of the aims of the present study was to determine whether sonographers measuring fetal ductal flow velocities would have to take into account menstrual age dependent changes in the spatial relationship between the ductus arteriosus and the aorta.

Our macro- and microanatomical data as well as our in vivo results from real-time and CVI ultrasound studies show that during the late first and second and early third trimester of pregnancy menstrual age dependent angle adjustments appear not to be necessary when performing ductal flow velocity measurements.

# Chapter 6

## Fetal ductus arteriosus and rest-activity states

### 6.1.Introduction

A host of reports has appeared on the influence of fetal variables such as breathing movements, heart rate and behavioural states on fetal hemodynamics (Marsal et al. 1984; van Eyck, 1987; van Eyck et al. 1991; van Eyck et al. 1990a). Fetal ductal flow velocity waveforms are markedly changed by breathing movements (van der Mooren et al. 1991c), behavioural states (van der Mooren et al. 1989) and cardiac arrhythmias (van der Mooren et al. 1992).

Quantitative and qualitative ultrasound studies of fetal breathing and body movements throughout pregnancy have deepened our insight in the process of maturation of the central nervous system of the developing fetus (de Vries, 1987; van Vliet, 1985; Roodenburg et al. 1991). At the same time studies on fetal heart rate relative to well-defined movement and behavioural patterns have appeared (Nijhuis et al. 1982; Nijhuis, 1992; van Woerden, 1989).

Rest-activity state related low and high fetal heart rate variation emerges at approximately 27 weeks (Visser et al. 1981). A previous study from our own centre has revealed significant changes in the flow velocity waveform of the descending aorta at 27-29 weeks (van Eyck et al. 1988). This does not seem to be the case in flow velocity waveform patterns obtained from the ductus arteriosus at this stage of pregnancy as will be discussed in chapter 6.1

# 6.1 There are no rest-activity dependent changes in fetal ductus arteriosus flow velocity patterns at 27-29 weeks of gestation

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## Abstract

Blood flow velocity waveforms recorded in the ductus arteriosus were related to fetal heart rate pattern (FHRP) in 13 normal pregnancies at 27-29 weeks of gestation. Recording time was always 65 minutes or more. In three women no low fetal heart rate (FHRP-A) variability was present, in the remaining 10 women high fetal heart rate (FHRP-B) variability was established in 80% of the recording time. There was no statistically significant difference between FHRP-A and FHRP-B pattern for all ductal flow velocity parameters, indicating rest-activity state independency in late second and early third trimester pregnancy.

## Introduction

In the normal term fetus well-defined periods of coincidence between fetal heart rate, eye movements and body movements or so-called behavioural state patterns have been established (Nijhuis et al. 1982). A clear relationship has been demonstrated between these fetal behavioural states and flow velocities at cardiac and extra-cardiac level in the normal term fetus (van Eyck et al. 1985; van Eyck et al. 1987; van Eyck et al. 1988; van der Mooren et al. 1989). During fetal behavioural

state 2F (active sleep) flow velocity increase at foramen ovale level is associated with a flow velocity reduction in the ductus arteriosus, suggesting a redistribution of left and right ventricular output in favour of the left side of the heart (van Eyck et al. 1990a).

During the late second and early third trimester of pregnancy the above-mentioned state variables act independently of each other. Only fetal heart rate displays recognizable episodes of low and high heart rate variability (FHRP-A and FHRP-B patterns).

Flow velocity waveform recordings from the fetal descending aorta at this state of pregnancy have demonstrated that the FHRP-B pattern is associated with a significant reduction in pulsatility index in this vessel, suggesting a reduced peripheral vascular resistance at fetal trunk level (van Eyck et al. 1987).

In the present study the question was addressed as to whether this FHR-pattern-related flow velocity waveform change was also present in the fetal ductus arteriosus at 27-29 weeks of gestation.

## Subjects and Methods

A total of 13 healthy women with normal singleton pregnancies at 27-29 weeks of gestation consented to participate in the study. Gestational age had been calculated from a reliable menstrual history and early ultrasound measurement of fetal crown-rump length or biparietal diameter. All subjects were non-smokers, no medication was prescribed. All pregnancies were uneventful, resulting in the delivery of a healthy infant at term with a birth weight between the 10th and the 90th centile for gestational age, corrected for maternal parity and fetal sex (Kloosterman, 1970). All recordings were made by the same investigator (CB) two hours after breakfast or lunch with the subject in the semirecumbent position.

For the Doppler recordings a Hitachi 450 combined linear array and pulsed Doppler system was used. The carrier frequency of the abdominal probe was 3.5 MHz, operating at less than 100 mW/cm$^2$ spatial peak/temporal average in both imaging and Doppler mode by

manufacturer's specifications. The ductus arteriosus was visualized on a short axis view which included the right ventricle and pulmonary trunk. The sample volume (0.3 - 0.4 cm) was placed in the ductus near the ductal-aortic junction. The interrogation angle was always kept below 10 degrees. Blood flow velocity waveforms were recorded during fetal apnoea. The fetal heart rate (FHR) was obtained from a Doppler ultrasound cardiotocograph (Hewlett-Packard 8040 A, carrier frequency 1 MHz).

In each subject 7 - 10 recordings of 20 seconds of simultaneous Doppler and FHR were obtained. Each recording consisted on average of 40 consecutive cardiac cycles. From each flow velocity recording at least 10 optimal flow velocity waveforms were selected. Total examination time was at least 65 minutes. Doppler recordings from the ductus arteriosus were analyzed with an off-line computer system linked to a graphics tablet described previously (Groenenberg et al. 1991). Analysis consisted of measurement of the peak systolic (cm/sec), mean and end-diastolic velocity (cm/sec), flow velocity integral (cm) and pulsatility index (PI)(Gosling and King, 1975).

FHR was classified as pattern A (FHRP-A) and pattern B (FHRP-B). The first pattern is characterized by a stable heart rate with an oscillation band of < 10 bpm (low variability). The second pattern is characterized by an oscillation band of > 10 bpm (high variability). Fetal eye and body movements were not included, since they do not depict clear rest-activity cycles. Doppler flow velocity waveform parameters were related to FHRP-A and FHRP-B patterns using the paired Student's t-test.

## Results

In three women no FHRP-A pattern could be observed. These cases were excluded from the study, leaving 10 subjects for further analysis. Episodes of low FHR variations (FHRP-A) were observed in 20% and episodes of high FHR variations (FHRP-B) were observed in 80% of the total recording time. Table 1 gives the mean values ($\pm$ SD) for each of the Doppler flow velocity waveform parameters relative to the FHR pattern. There was no statistically significant difference between

FHRP-A and FHRP-B for any of the ductal waveform parameters. The same applied to baseline fetal heart rate.

| | **FHRP-A** $x \pm SD$ | **FHRP-B** $x \pm SD$ |
|---|---|---|
| peak systolic velocity | $94.2 \pm 8.6$ | $94.1 \pm 12.4$ |
| mean velocity (cm/s) | $34.3 \pm 5.4$ | $35.8 \pm 6.8$ |
| end-diastolic velocity | $9.6 \pm 2.6$ | $9.7 \pm 2.6$ |
| velocity integral (cm) | $14.2 \pm 2.6$ | $14.4 \pm 2.8$ |
| pulsatility index | $2.51 \pm 0.36$ | $2.37 \pm 0.26$ |
| FHR (bpm) | $146 \pm 7.5$ | $140 \pm 9.5$ |

Table 1 Fetal ductus arteriosus flow velocity waveform paramters relative to low variability fetal heart rate (FHRP-A) and high variability fetal heart rate (FHRP-B) pattern.

## Discussion

The data presented here are in agreement with the existence of fetal rest-activity cycles (Sterman and Hoppenbrouwers, 1971) and their relationship with low and high heart rate variation as early as 27-29 weeks of gestation (Dawes et al. 1982; Visser et al. 1981). The recording time of at least 65 minutes was not enough to display FHRP-A in three cases. In the remaining 10 cases, there was a clear predominance of FHRP-B over FHRP-A pattern with the former pattern taking up 80% of the total recording time which is similar to that observed by others (Arduini et al. 1986).

Both the absolute velocity parameters for the ductus arteriosus flow and ductal PI were not essentially different between FHRP-A and FHRP-B. This is in contrast to behavioural state dependent

changes in peak systolic velocity in the fetal ductus arteriosus observed in the healthy fetus at term (van der Mooren et al. 1989). It has been suggested on the basis of rest-activity state related PI changes in the fetal descending aorta at 27-28 weeks that the human fetus is under increased baroreceptor sensitivity during this stage of pregnancy (van Eyck et al. 1988). This is not supported by the present data.

The physiological significance of rest-activity state independency of flow velocity waveforms in the ductus arteriosus at 27-29 weeks of gestation is not clear. Whereas at term the behavioural state dependent changes in fetal flow velocities may serve to meet changing oxygen demands at tissue level, this may not be the case during changes from low to high FHR variability and vice versa in the late second and early third trimester of pregnancy. Since in the normal term fetus, behavioural state dependent changes have been observed in flow velocity waveforms at venous inflow, cardiac and arterial level, a similar more complete picture will be necessary in the late second and early third trimester of pregnancy to understand the relationship between fetal haemodynamics and rest-activity states.

# Chapter 7

## General Conclusions

The fetal ductus arteriosus has intrigued anatomists for centuries. A full understanding still has to be achieved of the complex biochemical pathways involved in the agonist - antagonist network effecting ductal patency before and ductal closure after birth.

The introduction of pulsed Doppler and colour Doppler ultrasound equipment has opened new possibilities for in-vivo research of the fetal ductus arteriosus. Constriction of the fetal ductus arteriosus has been observed during maternal indomethacin administration. Assumptions based on anatomical studies performed on fixated embryos may not be valid to explain spatial relationships in the cardiac outflow tract of the living fetus.

The accuracy of Doppler measurements depends on correct angle assessment. In early pregnancy, simultaneous visualization of the ductus arteriosus and Doppler recording in the same vessel is not yet possible with present ultrasound resolution. Instead, estimations of flow directions are based on detailed anatomical knowledge of the cardiac outflow tract. Combined 2-dimensional real-time and colour velocity imaging (CVI) has demonstrated that for practical reasons ductal flow directions can be assumed to be at approximately right angles to the thoracic spine.

Previously published data obtained by transabdominal ultrasound have shown the unique rise in systolic ductal velocities during the second half of pregnancy. Using transvaginal Doppler ultrasound it became possible to study flow velocities in the ductus arteriosus as early as the late first trimester of pregnancy. Reproducibility of ductal waveforms collected before 18 weeks of gestation appeared not to be different from the reproducibility of waveforms collected after 18 weeks. This demonstrated that fetal mobility and ultrasound image resolution are the only limiting factors

when attempting to record flow velocities in the ductus arteriosus in late first and early second trimester pregnancies.

In the present study ductal flow velocity waveforms were collected as early as 11 weeks of gestation. At this stage there is only systolic and no end-diastolic flow as has been demonstrated for all extracardiac arterial vessels except the intracerebral circulation. End-diastolic flow appears between 14 and 16 weeks of gestation. Systolic flow velocities rise significantly as pregnancy progresses, rendering flow velocities in the ductus arteriosus to be the fastest in the fetal circulation. From our preliminary study on CVI imaging it appears that velocity grading with the CVI colour code to locate the point of highest blood flow velocity will permit more detailed information on flow and wall shear stress patterns in the pulmonary trunk and ductus arteriosus in early gestation.

From a hemodynamic point of view, blood flow velocities in the right cardiac outflow tract should be highest in the pulmonary trunk and then subsequently decrease through the ductus arteriosus into the aorta. This, however, is not the case. From the pulmonary trunk into the ductus arteriosus there is a significant acceleration of blood flow velocities with blood in the ductus arteriosus streaming faster than in the pulmonary trunk at all times of gestation. This is followed by a decrease of blood flow velocities in the descending aorta. High velocities in the ductus arteriosus can be explained by the lumen being smaller and the vessel wall being thicker and more muscular than that of the upstream and downstream vessels.

Many reports have appeared on the impact of fetal variables on fetal hemodynamics. Whereas fetal breathing movements affect cardiac and extra-cardiac arterial and venous flow velocity waveforms throughout pregnancy, this is not so for changes in fetal heart rate variability. In the term fetus there is behavioural state dependency of the fetal circulation. This is less clear at 27-29 weeks when rest-activity related changes in fetal heart rate appear. No rest-activity

related changes in ductal flow velocities could be established. This is at variance with earlier observations in the fetal descending aorta in which rest-activity state dependent flow velocity waveform changes have been documented. A more complete picture including cardiac venous inflow and arterial outflow measurements relative to rest-activity states is needed to clarify these contradictive findings.

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# Samenvatting

## hoofdstuk 1

De foetale ductus arteriosus werd voor het eerst beschreven door Galen in de 2e eeuw A.D. en niet door Leonardo Botallio in de 16e eeuw. Pas naar de ontdekking van de circulatie door Harvey en de ontdekking van de capillairen door Waleus, werd de unieke functie van de ductus arteriosus goed begrepen. De foetale en neonatale ductus arteriosus zijn uitgebrijd bestudeerd. Morfologische onderzoekingen naar de histologie en ultrastructuur gecombineerd met biochemische en histochemische onderzoeken hebben angetoond dat ductuswefsel nogal verschillend is van het wefsel van andere grote cardiale vaten. Zo sluit de ductus zich spoedig na de geboorte. De introductie van real-time, gepulsed en kleuren-Doppler ultrageluid alsmede de recente ontwikkeling van de zogenaamde colour velocity imaging (CVI) biedt de mogelijkheid om niet-invasieve in-vivo onderzoekingen in de ductus arteriosus van de menselijke foetus uit te voeren. In dit hoofdstuk worden de doelstellingen van het in dit proefschrift beschreven onderzoek omschreven.

## Hoofdstuk 2

Een literatuuroverzicht wordt gepresenteerd van de morfologische, histologische, ultrastructurele, biochemische en klinische oonderzoekingen met betrekking tot de prenatale ontwikkeling van de ductus arteriosus. De ductus arteriosus verschilt van naburige grote arterien da het een musculair vat betreft terwijl de truncus pulmonalis en de aorta elastische arterien zijn. De intrauterine ontwikkeling van de ductus arteriosus kan worden beschouwd als een voorbereiding op de sluiting van dat vat na de geboorte hetgeen wordt bereikt door een stapsgewijs rijpingsproces dat gekennmerkt is door ontwikkeling van de intimaverdikkingen en "mucoid meren" in de vatwand. Prostaglandines zijn verantwoordelijk voor doorgankelijkheid van de

ductus, het meest belangrijke prostaglandine is prostaglandine $E_2$ dat door de ductus zelf (intrinsiek $PGE_2$) en door de long wordt gevormd. De toepassing van de prostaglandine-synthetase remmer indomethacine als weenremmer kan leiden tot constrictie van de ductus en vervolgens tricuspidaal regurgitatie en foetale hydrops. Het samenknijpend effect van maternaal toegediende indomethacine op de foetale ductus arteriosus kan reeds vanaf de 27e zwangerschapsweek worden waargenomen. Een ander effect van maternale indomethacine-toedining is de matige tot soms uitgesproken afname van het vruchtwatervolume. Betamethason-toediening ter bespoediging van de fetale longrijping kan eveneens met een vorbijgaande contractie van de ductus arteriosus gepaard gaan. Tijdens maternale indomethacine-toediening is routinematige monitoring van de bloedoorstroming in de ductus arteriosus met Doppler-ultrageluid noodzakelijk om eventuele eerste teken van ductus-constrictie vast te kunnen stellen.

## Hoofdstuk 3

Reproduceerbaarheid van de bloedstroomsnelheidsprofielen verkregen in de foetale ductus arteriosus werden in 52 zwangeren met normale zwangerschap tussen de 11e and de 25e zwangerschapsweek bestudeerd. Piek-systolische stroomsnelheid, gemiddelde stroomsnelheid en einddiastolische stroomsnelheid alsmede de integraal van het stroomsnelheidsprofiel werden vergeleken in opeenvolgende registraties die met intervallen van vijftien minuten werden gemaakt. En acceptabele reproduceerbaarheid werd voor de verschillende stroomsnelheidsvariabelen verkregen. Wanneer de reproduceerbaarheid voor de 18e zwangerschapsweek werd vergeleken met die na de 18e zwangerschapsweek, werd geen verschill waargenomen.

## Hoofdstuk 4

Bloedstroomsnelheidsprofielen in de ductus arteriosus werden onderzocht in 295 zwangeren tussen de 9e en de 25e zwangerschapsweek, waarbij gebruik gemaakt werd van transvaginaal

of transabdominaal ultrageluid. Technisch acceptabele registraties werden verkregen vanaf de 11e zwangerschapsweek hetgeen betekent dat 231 zwangere voor verdere analyse beschikbaar waren. Terwijl in het laatste deel van de eerste zwangerschapstrimester bloedstroomsnelheidsprofielen in de ductus arteriosus gekenmerkd waren door afwezigheid van einddiastolische bloedstroomsnelheid, werden deze snelheden voor het eerst gezien naar 14-15 weken graviditeit in sommige ongeborenen en in alle gevallen vanaf de 17e zwangerschapsweek. Piek-systolische stroomsnelheden in de foetale ductus arteriosus toonen een statistisch significante toename met het vorderen van de zwangerschapsduur, terwijl de pulsatiliteitsindex onveranderd is gedurende de gehele onderzoeksperiode. In een aanvullend onderzoek werden de bloedstroomsnelheidsprofielen van de ductus arteriosus vergeleken met die verkregen in de foetale truncus pulmonalis, afkomstig van 133 zwangeren tussen de 9e en de 25e zwangerschapsweek. Hoewel het verkrijgen van technisch acceptabele registraties vanuit beide vaten niet eenvoudig was, waren uitendelijk 78 zwangeren beschikbaar vor verder analyse. Er was sprake van significante toename in de piek-systolische bloedstroomsnelheid met het vorderen van de zwangerschap in beide vaten waarbij snelheden in de foetale ductus arteriosus meer toenamen dan bloedstroomsnelheden in de truncus pulmonalis. Dit betekend dat de stroomsnelheid toeneemt wanneer het bloed vanuit de truncus pulmonalis in de ductus arteriosus doorstroomt om vervolgens weer in snelheid af te neemen wanneer de aorta descendens wordt bereikt.

## Hoofdstuk 5

In dit hoofdstuk wordt een combinatie van anatomische en ultrageluidstechnieken toegepast om inzicht te krijgen in de mate waarin de uitstroombaan van de rechter ventrikel van de geprepareerde foetus kan worden vergeleken met de uitstroombaan in de levende foetus in utero zoals vastgestelt met behulp van real-time ultrageluid en zogenaamde colour velocity imaging (CVI). Twintig menselijke foetale preparaten van de Leidse collectie werden bestudeerd tussen de 8e en

19e zwangerschapsweek met behulp van microscopische reconstructie en macroscopische dissectie. De hoek tussen de ductus arteriosus en de isthmus van de aorta (stroomopwaarts) en de ductus arteriosus en de aorta descendens (stroomeerwaarts) werd bepaald. De opwaartse hoek bedroeg altijd minder dan 90 graden terwijl de neerwaartse hoek altijd 80 graden of meer bedroeg zonder dat er overigens sprake was van een samenhang met de zwangerschapsduur. Dit gold voor zowel de microscopische als de macroscopische preparaten. In 52 zich normaal ontwikkelende zwangerschappen tussen 14 en 27 weken werd de hoek tussen de foetale ductus arteriosus en de thoracale wervelkolom met behulp van real-time ultrageluid vastgesteld. In een andere groep van 19 zich normaal ontwikkelende zwangerschappen tussen de 14e en de 25e zwangerschapsweek werd de bloeddoorstroming in de ductus arteriosus met behulp van zoegenaamde "colour velocity imaging" (CVI) zichtbaar gemaakt. Beide ultrageluidstechniken wezen op een hoek tussen de ductus arteriosus en de thoracale wervelkolom van ongeveer 90 graden. Met behulp van CVI werden de hoogste bloedstroomsnelheden waargenomen daar waar de ductus arteriosus in de aorta insereert. Bij ultrageluidsonderzoek van de foetale ductus arteriosus met hulp van Doppler-ultrageluid kan van een hoek van ongeveer 90 graden tussen de ductus arteriosus en de thoracale wervelkolom worden uitgegaan.

## Hoofdstuk 6

Eerdere onderzoekingen hebben angetoond dat in de a terme zwangerschap rust-activiteit-afhankelijke veranderingen in bloedstroomsnelheidsprofielen afkomstig uit de ductus arteriosus optraden. Tevens zijn rust-activiteit-afhankelijke veranderingen in de aorta descendens reeds waargenomen in de zwangerschapsperiode van 27 - 29 week. In dit hoofdstuk werd nagegaan of de veranderingen die zijn waargenomen in de aorta descendens gedurende de tweede helft van het tweede en begin van het derde zwangerschapstrimester tevens in de ductus arteriosus konden worden vastgesteld. Met behulp van Doppler ultrageluid werden bloedstroomsnelheidsprofielen

geregistreerd in de foetale ductus arteriosus afkomstig van 13 normale zwangerschappen in de zwangerschapsperiode van 27 tot 29 weken. Simultane CTG-registraties werden gemaakt om na te gaan of lage hartfrequentievariabiliteit (FHRP-A) en hoge hartfrequentievariabiliteit (FHRP-B) gepaard gaan met veranderingen in bloedstroomsnelheden in de ductus arteriosus. Er werd geen statistisch significant verschill tussen FHRP-A en FHRP-B patronen gezien voor alle bloestroomsnelheidsparameters in de ductus arteriosus. Dit geeft aan dat bloedstroomsnelheidsprofielen afkomstig uit de ductus arteriosus niet gerelateerd zijn aan rust-activiteit stadia in het late tweede en vroege derde trimester van de zwangerschap.

# Summary

## Chapter 1

The fetal ductus arteriosus was first described by Galen in the 2nd century AD and not by Leonardo Botallio in the 16th century. The uniqueness of its function was only fully appreciated after Harvey's discovery of circulation and Walaeus' discovery of capillaries. The fetal and neonatal ductus arteriosus has been studied extensively: morpholgical studies on the histological and ultrastructural level combined with biochemical and histochemical studies have demonstrated that ductal tissue is uniquely different from the tissue of the other great cardiac vessels enabling it to close and obliterate rapidly after birth. The introduction of real-time, pulsed and colour Doppler ultrasound as well as the most recent addition of colour velocity imaging (CVI) has opened the possibility of carrying out non-invasive in-vivo studies in the ductus arteriosus of the human fetus. In this chapter the objectives of the study are defined.

## Chapter 2

A literature review is presented on morphological, histological, ultrastructural, biochemical and clinical studies concerning the prenatal development of the ductus arteriosus. The ductus arteriosus is different from the adjacent great arteries in that it is a muscular artery, whereas the pulmonary arteries and the aorta are elastic arteries. Its intrauterine development can be seen as a preparation for postpartum closure which is achieved by a stepwise process of maturation during which intimal cushions and mucoid lakes develop in the vessel wall.

Prostaglandines are responsible for maintaining ductal patency, the most important prostaglandin being $PGE_2$ which is

supplied by the ductus itself (intrinsic $PGE_2$) and by the fetal lung.

The use of the prostagalandin-synthetase-inhibitor indomethacin for tocolysis may lead to ductal constriction and cause tricuspid regurgitation and fetal hydrops. The constrictive effect of maternal indomethacin ingestion on the fetal ductus arteriosus begins as early as 27 weeks of gestation.

Another effect of maternal indomethacin administration is mild to marked reduction of amniotic fluid volume.

Betamethason administration to enhance fetal lung maturation may lead to a transient constriction of the fetal ductus arteriosus.

Under maternal indomethacin therapy, routine monitoring of ductus arteriosus blood flow with Doppler ultrasound to detect signs of ductal constriction is necessary to ensure the safety of the fetus.

## Chapter 3

Reproducibility of flow velocity waveform recording in the fetal ductus arteriosus was studied in 52 patients with normal pregnancies between 11 and 25 weeks of gestation. Peak systolic velocity, mean velocity, end diastolic velocity and flow velocity integral were compared in recordings taken at 15 minute time intervals. An acceptable reproduciblity for the different flow velocity variables was established. When comparing reproducibility in those subjects before 18 weeks of gestation with those at 18 weeks or older, there was no statistically significant difference.

## Chapter 4

Ductus arteriosus flow velocity patterns were studied in 295 women between 9 and 25 weeks of gestation using transvaginal and transabdominal ultrasound. Technically acceptable recordings were obtained as from 11 weeks of gestation , leaving 231 women for further analysis. Whereas ductal waveforms display no diastolic flow in

the late first trimester of pregnancy, end-diastolic velocities begin to appear at 14/15 weeks in some fetuses and are present in all fetuses at 17 weeks of gestation. Peak systolic velocities of the fetal ductus arteriosus show a statistically significant rise with advancing gestational age while the pulsatility index remains stable during the entire observation period.

In a further study blood flow patterns in the fetal ductus arteriosus were compared with those from the pulmonary trunk in 133 women between 9 and 25 weeks of gestation using Doppler ultrasound. As technically acceptable recordings were difficult to achieve in both vessels, only 78 subjects remained for further analysis. There was a significant rise in peak systolic velocities with advancing gestational age in both vessels with velocities in the fetal ductus arteriosus rising faster than those in the pulmonary trunk. Thus blood velocities increase as blood passes from the pulmonary trunk into the ductus arteriosus and then decrease again as it progresses down the descending aorta.

## Chapter 5

In this chapter a combination of anatomical and sonographic techniques is used to elucidate to what extent the right cardiac outflow tract of the preserved fetus can be compared with that observed in the living fetus in utero using real-time and colour velocity imaging (CVI) ultrasound techniques. Twenty human fetal postmortem specimen between 8 and 19 weeks of gestation from the Leiden collection were examined with microscopic reconstruction and macroscopic dissection. The angle between the ductus arteriosus and the aortic isthmus (upstream) and the ductus arteriosus and the descending aorta (downstream) was determined. The upstream angle was always less than 90°, the downstream angle was always 80° or more with no correlation with gestational age for the microscopic and macroscopic preparations. In 52 normally developing fetuses between 14 and 27 weeks the angle between the fetal ductus arteriosus and the thoracic spine as visualized in real-time ultrasound was determined. In

a further 19 normally developing fetuses between 14 and 25 weeks ductal blood flow was visualized by colour velocity imaging (CVI). Using both these ultrasound techniques the angle between ductus arteriosus and thoracic spine was approximately 90°. On CVI highest blood flow velocities were shown to be at the point of ductal insertion into the aorta.

Ultrasound operators attempting to examine the fetal ductus arteriosus using Doppler ultrasound may assume an angle of approximately 90°between the ductus arteriosus and the thoracic spine.

## Chapter 6

Previous studies have demonstrated rest-activity dependent changes in ductus arteriosus blood flow velocity waveforms at term and rest-activity dependent changes of flow velocity waveforms in the descending aorta at 27-29 weeks. In this chapter we investigated whether the changes seen in the descending aorta in the late second/early third trimester of gestation could also be seen in the ductus arteriosus at that time of gestation. Doppler ultrasound was used to obtain blood flow velocity waveforms in the fetal ductus arteriosus in 13 normal pregnancies at 27-29 weeks of gestation. Simultaneous CTG-recordings were made to see if low fetal heart rate variability patterns (FHRP-A) and high fetal heart rate variability patterns (FHRP-B) would correlate with changes in flow velocities of the ductus arteriosus.

There was no statistically significant difference between FHRP-A and FHRP-B pattern for all ductal flow velocity parameters, indicating rest-activity state independency in late second and early third trimester pregnancy.

# Zusammenfassung

## 1. Kapitel

Der fetale *ductus arteriosus* wurde nicht von Leonardo Botallio im 16.Jahrhundert entdeckt sondern bereits im 2.Jahrhundert n.Chr. von Galen beschrieben. Die Einzigartigkeit der Funktion dieses Gefäßes konnte aber erst nach Harveys Entdeckung des Blutkreislaufes und nach Walaeus' Entdeckung der Kapillaren wirklich gewürdigt werden. Der *ductus arteriosus* des Feten und des Neugeborenen sind ausführlich untersucht worden: mit morphologischen Studien im lichtmikroskopischen und elektronenmikroskopischen Bereich und in Kombination mit biochemischen und histochemischen Verfahren gelang der Nachweis, daß das Gewebe der Gefäßwand des *ductus arteriosus* sich vom Gewebe der anderen großen Herzgefäße deutlich unterscheidet wodurch sich das Gefäß nach der Geburt rasch schließen und in der Folge obliterieren kann. Die Einführung des Ultraschalls mit B-Bild, pulsiertem Doppler, Farbdoppler und zuletzt CVI (Color Velocity Imaging) hat neue Möglichkeiten nicht-invasiver Untersuchungen des *ductus arteriosus* des lebenden menschlichen Fetus eröffnet. In diesem Kapitel werden die Ziele der Studie definiert.

## 2. Kapitel

In einer Literaturübersicht werden morphologische, histologische, ultrastrukturelle, biochemische und klinische Studien zur pränatalen Entwicklung des *ductus arteriosus* besprochen. Der *ductus arteriosus* unterscheidet sich von den großen Arterien in seiner Umgebung dadurch, daß er eine muskuläre Arterie ist. Die Aorta und die Pulmonalarterien sind im Gegensatz dazu elastische Arterien. Die intrauterine Entwicklung des ductus arteriosus ist eine langsame Vorbereitung der postpartalen Schließung, die durch einen schrittweisen Reifungsprozess erreicht wird. Im Laufe dieses

Reifungsprozesses bilden sich Endothelkissen und "Mucus-Seen" in der Gefäßwand.

Für das Offenbleiben des *ductus arteriosus* sind Prostaglandine verantwortlich, das wichtigste ist davon ist $PGE_2$, das sowohl durch den ductus selbst produziert wird (intrinsisches $PGE_2$) als auch durch die fetale Lunge.

Wenn Indomethacin, ein Prostaglandin-Synthetase-Hemmer, als Tokolytikum eingesetzt wird, kann dies zu Konstriktion des *ductus arteriosus* führen, die wiederum zu trikuspidaler Regurgitation führt und schließlich zu fetalem Hydrops. Bereits ab der 27 SSW kann Indomethacin den fetalen *ductus arteriosus* verengen.

Indomethacin führt außerdem zu einer mäßigen und manchmal starken Reduktion des Fruchtwasservolumens, was bei Polyhydramnie therapeutisch ausgenutzt werden kann. Wenn eine Schwangere Indomethacin erhält muß routinemäßig der fetale *ductus arteriosus* mit Doppler-Ultraschall überwacht werden um rechtzeitig Anzeichen einer drohenden Verengung oder Schließung des Gefässes zu erkennen.

## 3. Kapitel

Die Reproduzierbarkeit der Doppler-Messungen des fetalen *ductus arteriosus* wurde bei 52 gesunden Schwangeren zwischen der 11 und 25 SSW untersucht. Die systolische Spitzengeschwindigkeit, die mittlere Geschwindigkeit, die enddiastolische Geschwindigkeit und das Integral der Flußkurve wurden zwischen zwei jeweils 15 Minuten auseinanderliegenden Messungen verglichen. Für die einzelnen Parameter wurde eine akzeptable Reproduzierbarkeit festgestellt. Beim Vergleich der Reproduzierbarkeit der Messungen, die vor der 18 SSW gemacht wurden mit jenen nach der 18 SSW, zeigte sich kein statistisch signifikanter Unterschied.

## 4. Kapitel

Die Flußmuster des *ductus arteriosus* wurden bei 295 Frauen zwischen der 9. und 25 SSW mit transvaginalem und abdominellem Ultraschall untersucht. Erst ab der 11 SSW ließen sich akzeptable Flußkurven gewinnen, so daß die Daten von 231 Frauen verarbeitet werden konnten. Im späten ersten Trimenon der Schwangerschaft weist der Fluß des *ductus arteriosus* noch keine Diastole auf, end-diastolischer Fluß kann in manchen Feten ab der 14/15 SSW beobachtet werden und ist in allen Feten ab der 17 SSW nachweisbar. Die systolische Spitzengeschwindigkeit zeigt im Laufe der Schwangerschaft einen signifikanten Anstieg während der Pulsatilitäts-Index während der gesamten Beobachtungszeit gleich bleibt.

In einer weiteren Studie wurden die Flußmuster des fetalen *ductus arteriosus* mit denen des *truncus pulmonalis* mittles Dopplerultraschall verglichen. 133 Frauen zwischen der 9. und 25. SSW nahmen an dieser Studie teil. Da technisch akzeptable Messungen in beiden Gefäßen sehr schwierig zu bekommen waren, konnten nur die Daten von 78 Schwangeren weiter ausgewertet werden. In beiden Gefäßen stieg die systolische Spitzengeschwindigkeit signifikant mit der Schwangerschaftsdauer an, wobei die Geschwindigkeiten im *ductus arteriosus* wesentlich rasche anstiegen als im *truncus pulmonalis*. Blut wird in der Passage vom *truncus pulmonalis* in den *ductus arteriosus* beschleunigt und nach Erreichen der *aorta descendens* wieder verlangsamt.

## 5. Kapitel

Durch Kombination und Vergleich verschiedener anatomischer und sonographischer Techniken wurde untersucht, in wie weit der Ausflußtrakt des rechten Herzens im konservierten anatomischen Präparat mit dem des lebenden Feten, wie er sich mit B-Bild und CVI-Bild darstellt, verglichen werden kann. Zwanzig menschliche Feten zwischen der 8 und 19 SSW aus der Sammlung des

Anatomischen Institutes Leiden wurden mit mikroskopische Rekonstruktion und makroskopischer Präparation untersucht. Der Winkel zwischen dem *ductus arteriosus* und dem *isthmus aortae* ("stromaufwärts") und dem *ductus arteriosus* und der *aorta descendends* ("stromabwärts") wurde bestimmt. Der Winkel stromaufwärts betrug immer weniger als 90°, der Winkel stromabwärts betrug immer 80° oder mehr, ohne daß sich eine Korrelation mit dem Gestationsalter feststellen ließ. Dies gilt für die mikroskopischen Rekonstruktionen genauso wie für die Makro-Präparationen. Bei 52 gesunden Einlingsschwangerschaften zwischen der 14 und der 27 SSW wurde der Winkel zwischen dem *ductus arteriosus* und der fetalen Wirbelsäule im Ultraschall B-Bild bestimmt. Bei weiteren 19 Feten zwischen der 14 und 25 SSW wurde der Blutfluß durch den *ductus arteriosus* mit Hilfe der Colour Velocity Imaging-Technik (CVI) sichtbar gemacht. Bei beiden Ultraschalltechniken ließ sich ein mitllerer Winkel von ca. 90° zwischen fetalem *ductus arteriosus* und fetaler Wirbelsäule feststellen. Beim CVI zeigte sich, daß die hoechsten Flußgeschwindigkeiten an der "Innenbahn" der Insertion des ductus arteriosus in die aorta descendes zu messen waren.

Bei Ultraschallmessungen des fetalen *ductus arteriosus* kann ein Winkel von 90° zwischen dem untersuchten Gefäß und der fetalen Wirbelsäule angenommen werden.

## 6.Kapitel

In früheren Studien war festgestellt worden, daß sich in der Nähe des Geburtstermin die Blutflußgeschwindigkeiten des *ductus arteriosus* abhängig von Ruhe-Aktivitätsstadien des Feten veränderten. Ähnliche Ruhe-Aktivitäts-abhängige Veränderungen fanden sich in der fetalen Aorta zwischen der 27 und 29 SSW. In dieser Untersuchung gingen wir der Frage nach, ob die Ruhe-Aktivitätsabhängigkeit der Blutflußveränderungen, die in der Aorta beobachtet worden waren, auch im ductus arteriosus gegen Ende des zweiten und Beginn des dritten Trimenon der Schwangerschaft feststellbar waren. Bei 13 normalen Schwangerschaften zwischen der 27 und 29 SSW wurde der

fetale *ductus arteriosus* mit Dopplerultraschall gemessen und gleichzeitig CTG-Aufzeichnungen gemacht. Die CTG-Streifen wurden in niedrige Variabilität der Herzaktion (FHRP-A) und hohe Variabilität der Herzaktion (FHRP-B) eingeteilt und es wurde überprüft, ob diese Variabilitätsveränderungen mit Veränderungen in den Flußgeschwindigkeiten des *ductus arteriosus* zusammenhingen.

Es wurden keine statistisch signifikanten Zusammenhänge zwischen FHRP-A und FHRP-B im CTG mit Flußgeschwindigkeiten des *ductus arteriosus* gefunden, was darauf hinweist, daß Flußgeschwindigkeiten im *ductus arteriosus* gegen Ende des zweiten und Beginn des dritten Schwangerschaftstrimenon unabhängig von Ruhe- und Aktivitätszuständen des Feten sind.

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Zonder de logistieke en psychische ondersteuning, de menselijke warmte, het smakelijke eten en het efficiente wassen ten huize van Veronika, Kees en Christopher was mijn verblijf in Nederland lang niet zo aangenaam geweest. Verder dank ik mijn ouders die mij op een afstand hebben gesteund.

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Aan mijn huisbaas de heer Lachman en zijn grote familie heb ik te danken dat ik tussen de Heemraadsingel en de Beukelsdijk in het Rotterdamse Nieuwe Westen een multiculturele tweede *Heimat* heb gevonden.

Prof.Dr.Paelinck en zijn vrouw hebben het met hun geregelde uitnodigingen in hun gastvrije huis mogelijk gemaakt in ongedwongen atmosfeer vele interessante "Erasmus Visitors" te leren kennen.

De heer Baron van de *Verein der Österreicher in den Niederlanden* in Den Haag is erin geslaagd van elke feestdag op de kalender een verband met Oostenrijk te leggen, die met een *Heurigen* gevierd wordt.

Mijn vrienden Monica en Gheorghe Pop, Aldo Ferraresi en Boris Drozdek maakten ook an de Maas een vertrouwd Middeneuropees gevoel mogelijk.

Dankzij de vlijtige zoektocht van Dr.Pietro Marsilli in de

Universiteits-Bibliotheek te Bologna kan op de omslag van dit proefschrift de oudste bekende afbelding van de ductus arteriosus verschijnen.

Hilary Marland van de vakgroep voor medische Geschiedenis in Rotterdam en Prof.Harm Beukers in Leiden hebben mij in staat gesteld mijn medisch-historische studie af te ronden en te publiceren.

Tenslotte ben ik de meer dan 400 patienten bijzonder dankbaar voor hun enthousiaste medewerking aan alle onderzoeken, waardoor uiteindelijk dit proefschrift tot stand is gekomen.

## Curriculum Vitae

| | |
|---|---|
| 1956 | born in Salzburg(Austria) on 10 Nov |
| 1962-1966 | primary school in Innsbruck |
| 1967-1976 | secondary school in Innsbruck, Dublin and Konstanz |
| 1976 | "Abitur" at Ellenrieder-Gymnasium, Konstanz |
| 1976-1977 | Military service in Salzburg |
| 1977-1984 | Medical studies at Innsbruck University<br>President of the Medical Students´Union, Director of International Federation of Medical Students´Associations (IFMSA)<br>Pre-graduate practice in hospitals in Berlin, Reykjavik, Köln, Lausanne, Kiel, Innichen/San Candido and Feldkirch |
| 1984 | graduation as "doctor medicinae universae" in Innsbruck<br>Resident at Dept.of Obstetrics and Gynaecology of InnsbruckUniversity(Head:Prof.Dr.Otto Dapunt) |
| 1989 | one year complementary training in pediatric surgery, 1st Dept. of Surgery, Innsbruck University (Head: Prof.Dr.Franz Gschnitzer; Doz.Dr.Gesine Menardi)) |
| 1990 | 6 months complementary training at Dept. of Internal Medicine,Innsbruck(Head:Prof.Dr.Herbert Braunsteiner) |
| 1990 | Specialist (Facharzt) in obstetrics and gynaecology |
| 1991 - 1993 | research fellow at the Dept. of Obstetrics and Gynaecology (Head: Prof.Dr.A.C.Drogendijk), Division of Prenatal Diagnosis (Prof.Dr.J.W.Wladimiroff) Dijkzigt Hospital, Erasmus University, Rotterdam |
| 1993 | return to Dept. of Obstetrics and Gynaecology, Innsbruck |