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Fetal and maternal hemodynamics in pregnancy: new insights in the cardiovascular adaptation to uncomplicated pregnancy, twin-to-twin transfusion syndrome and congenital diaphragmatic hernia.

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Abstract

The fetal and the maternal cardiovascular compartment undergo dramatic functional changes during pregnancy. In this thesis we examined the heart of fetuses with twin-to-twin transfusion syndrome (TTTS) and congenital diaphragmatic hernia (CDH) using two new ultrasound parameters of ventricular function : the myocardial performance index and speckle tracking derived myocardial strain. Fetal cardiac function was grossly abnormal in recipient fetus of TTTS, yet normalized within 6 weeks after therapy. Ultrasound based cardiac function assessment could not predict short term fetal survival after therapy, nor could it predict eventual further progression to full-blown TTTS in a pre-disease stage. Fetuses with CDH on the other hand, have normal myocardial function, yet smaller left ventricles leading to decreased left ventricular output. We showed that the lower output leads to decreased cerebral perfusion, yet without apparent impact on brain and cranial growth.
On the maternal side, plasma volume strongly increases in pregnancy, in parallel with an increase in insulin-like-growth factor(IGF) II which is secreted at the level of the placenta. Experimental administration of IGF-II by continuous infusion leads to increases in plasma volume whereas decreasing IGF-II by reduction of the feto-placental mass leads to decreased plasma volumes. In contrast to IGF-II, the highly vasoactive peptide apelin decreases near term due to a faster elimination as a consequence of an increase in placental angiotensin-converting-enzyme 2. Our experiments with IGF-II and apelin substantiate an important role for the feto-placental unit in regulating maternal plasma volume expansion and (auto)regulating uterine perfusion and fetal growth.

Keywords : myocardial performance index, fetal surgery, cardiac strain, plasma volume, adipokines, endocrinology

Part 1: Fetal hemodynamics in pregnancy

Background and introduction

While growing from the size of a grain of rice at 12 weeks to the size of a table tennis ball near term, the fetal heart undergoes functional changes: throughout gestation, the fetal myocardium becomes more compliant and making ventricular filling less dependent on atrial contraction. Both the increase in size and the maturational changes lead to a tremendous increase in cardiac output (Van Mieghem *et al*., 2009). Alterations in physiologic growth and maturation can occur in intrinsic cardiac malformations or in response to extra-cardiac fetal anomalies including intra-uterine growth restriction, fetal anemia (Sikkel *et al*. 2005), twin-to-twin transfusion syndrome (Ville, 2007), congenital diaphragmatic hernia

(Allan *et al*., 1996) and sacro-coccygeal teratoma (Wilson *et al*., 2008). As a consequence, fetal medicine specialists have tried to use indicators of fetal cardiac function as outcome predictors in these selected fetal conditions.

Unfortunately however, documentation of fetal cardiac function is difficult due to the surrounding maternal tissues, to the highly variable fetal position, to the high fetal heart rate and to the small cardiac size. In the last decade cardiologists have described two new tools to document cardiac function. The myocardial performance index (MPI, also called 'Tei-index') is a Doppler derived parameter of global ventricular function. The index is calculated as the sum of the isovolumetric contraction and relaxation time divided by the ventricular ejection time. Within the index, the ICT mainly reflects systolic cardiac function and the IRT diastolic function (Tei *et al*., 1995). Following its validation in adult cardiology, the MPI was extrapolated to the pediatric and neonatal setting (Mooradian *et al*., 2000; Ichihashi *et al*., 2005). More recently, it was also introduced into fetal echocardiography where it was first applied using the same methodology as initially described by Tei, ie. by measuring the different time intervals in different cardiac cycles and on different images (Ichizuka *et al*., 2005). This resulted in a large variability between studies. Friedman improved its measurement by defining a different position of the Doppler sample volume, thus allowing the measurement of the different time intervals in the same cardiac cycle (Friedman *et al*., 2003). The method was further fine-tuned by Raboisson *et al*. (2003) and Hernandez-Andrade *et al*. (2005) who proposed a 'modified' MPI. By using the aortic and mitral Doppler valve-clicks as demarcation for the time intervals, they succeeded in reducing the inter- and intra-observer variability, making the test reproducible in fetal medicine.

Speckle tracking on the other hand is a gray scale based tool to assess cardiac ventricular function. The method identifies myocardial speckle patterns on a 2-dimensional B-mode ultrasound image. The speckles are recognized in the subsequent frames of a cine-loop sequence and referenced back to their position in the previous frame. Based on the data obtained, the myocardial displacement can be 'tracked' and velocity vectors can be generated. Comparison of adjacent vectors then allows to calculate the actual displacement, velocity, deformation (strain) and velocity at which deformation occurs (strain rate) in the cardiac wall (Perk *et al*., 2007). The technique has been validated against gold standard methods to assess ventricular function both in animal models (Pirat *et al*., 2008; Li *et al*., 2007) and in humans (Amundsen *et al*., 2006; Cho *et al*., 2006) and its clinical relevance is at present being evaluated in the adult setting for different cardiac pathologies (Dandel and Hetzer, 2009).

Speckle tracking imaging and the MPI have major advantages over the more conventional methods to measure fetal cardiac function: First both methods are relatively angle independent. Second, the methods only the acquisition of a fetal cardiac four-chamber view which does not require extensive fetal cardiology training. Third, images required for the MPI and speckle tracking can be generated with any conventional fetal ultrasound machine and can therefore be 'piggy-backed' on any prenatal ultrasound. Finally, both speckle tracking and the MPI can be used to quantify fetal right ventricular function which can only be partially evaluated with other techniques due to the specific geometry of this ventricle.

The aim of the current thesis was to assess the (clinical) usefulness of the MPI and speckle tracking imaging in 2 fetal conditions, being twin pregnancies complicated by twin-to-twin transfusion syndrome and fetuses with congenital diaphragmatic hernia.

Twin-to-twin transfusion is a pathology exclusively occurring in monochorionic multiples (ie. multiple fetuses sharing a single placenta). As far as the pathology is understood to day, the disorder is due to an imbalance in the ever present intertwin transfusion process which takes place at the level of placental vascular anastomoses (Fisk *et al*., 2009). As a consequence, one fetus becomes hypovolemic (the donor fetus) and the other overfilled (the recipient fetus). This difference in volume status is clinically apparent as overt differences in fetal diuresis, with the donor fetus having anuria and hence oligohydramnios and the recipient fetus having polyuria and polyhydramnios. The hemodynamic differences are also apparent at the level of the fetal heart, with the recipient fetus showing signs of cardiac overload and failure (Barrea *et al*., 2005). The specific aims of the current thesis were to investigate (1) whether cardiac function in uncomplicated monochorionic twins resembles that of singletons, (2) whether abnormal cardiac function can help in predicting which pregnancies will develop TTTS and which not, (3) whether fetal cardiac function is predictive of fetal outcome after causative therapy of the TTTS and (4) whether fetal cardiac function recovers after successful therapy.

In contrast to TTTS fetuses, the heart of singleton fetuses with isolated congenital diaphragmatic hernia is challenged by an anatomical rather than a functional anomaly. In CDH, the abdominal organs herniate through the diaphragmatic defect into the chest and compress the heart and lungs, thereby compromising cardiac and pulmonary growth (Deprest *et al*., 2009; Allan *et al*., 1996). Where the

pulmonary hypoplasia leads to inadequate pulmonary function at birth and neonatal demise, the functional effects of the cardiac compression have never been evaluated. Moreover, the cardiac effects of prenatal therapy (fetoscopic tracheal occlusion) which increases fetal lung size and could lead to even worse cardiac compression, have never been documented. The specific aims of the current thesis were therefore (1) to document fetal cardiac function in untreated CDH fetuses, (2) to document the impact of an altered fetal cardiac function on fetal brain perfusion and (3) to document the cardiac effects of fetoscopic tracheal occlusion.

Experiments in monochorionic diamniotic twin pregnancies

Uncomplicated singleton and monochorionic twin pregnancies

A first experiment was designed to assess feasibility and reproducibility of MPI measurements in uncomplicated singleton and monochorionic twin pregnancies (Van Mieghem *et al*., 2009). We therefore measured the fetal MPI once in 117 singletons at a random time point throughout the second and third trimester of pregnancy and at 4 different occasions (16,20,26 and 30 weeks of gestation) in both fetuses of 23 monochorionic twin pairs. Singletons pregnancies with anatomic fetal cardiac malformations, intra-uterine growth restriction, gestational diabetes mellitus, or infectious diseases of pregnancy were excluded. Moreover, monochorionic twins with twin-to-twin transfusion syndrome (TTTS), severe intertwin growth discordance (sIUGR, defined as a discordance in estimated fetal weight > 20%), single or double intra-uterine fetal death, or cases that delivered prior to 30 weeks of gestation, were excluded. This experiment allowed us to conclude that measurement of the MPI is highly feasible and reproducible, both in uncomplicated singletons and in monochorionic twins and the gathered datasets allowed us to construct nomograms for both patient populations. Interestingly, mean MPI values were highly similar in singletons and healthy monochorionic twins.

In a second experiment, we measured ventricular strain and strain rate using a commercially available speckle tracking algorithm in 59 uncomplicated singleton fetuses (Van Mieghem *et al*., 2010). In contrast to previous reports from other groups, we showed that even in favorable ultrasound conditions, speckle tracking imaging was only feasible in about 80% of cases and that intra- as well as inter-observer variability is high, making this a non-prefered tool for investigating fetal cardiac function.

Monochorionic twin pregnancies complicated by moderately discordant amniotic fluid

In an attempt to identify early predictors of TTTS, and to assess whether cardiac function could predict onset of TTTS in a pre-disease stage, we further recruited a multicentric cohort of 45 monochorionic twin pregnancies complicated by moderate intertwin fluid discordance (ie. obvious fluid discordance not fulfilling the diagnosis of TTTS) and therefore at high risk of further progression to TTTS (Van Mieghem *et al*., 2011). These pregnancies were closely followed with ultrasound to diagnose eventual evolution to TTTS or selective intra-uterine growth restriction (sIUGR). Additionally, fetal cardiac function was assessed using the MPI and other more conventional parameters of fetal cardiac function. Ultimately, thirteen pregnancies (29%) evolved to TTTS, and nineteen pregnancies (40%) were classified as sIUGR without TTTS. The remaining cases had concordant fetal growth and stable or even normalizing amniotic fluid levels. Using multiple regression analysis, we could only identify the gestational age at first presentation and the severity of fluid discordance as independent predictors of TTTS. We further used recursive partitioning to construct flowcharts to predict TTTS and sIUGR (Fig. 1). Although the MPI was (mildly) elevated in the fetuses who later became recipient

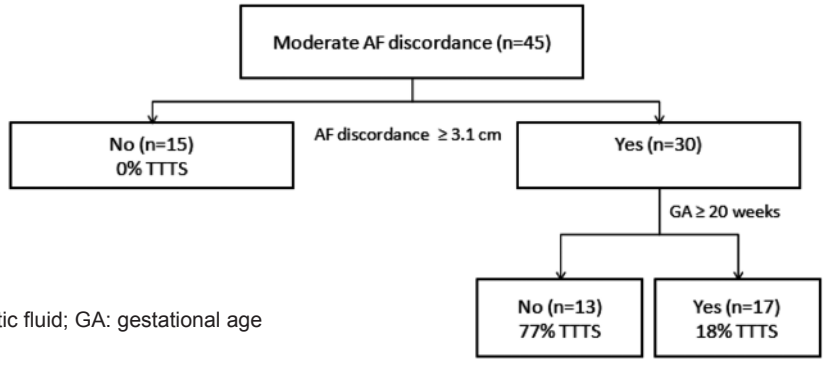

Fig. 1. — Flowchart for risk stratification for twin-to-twin transfusion syndrome (TTTS). Reproduced from Van Mieghem *et al*., 2011.

fetuses of TTTS, the MPI could not predict TTTS accurately as mild to moderate elevation of the MPI was also seen in the larger fetus of pregnancies complicated by sIUGR.

Monochorionic twin pregnancies complicated by twin-to-twin transfusion syndrome

In contrast to healthy monochorionic twins and monochorionic twins with moderate amniotic fluid discordances, recipient fetuses of TTTS show overt alterations in cardiac function. This can be documented both with more conventional functional cardiac ultrasound as with the MPI or speckle tracking derived ventricular strain (Van Mieghem *et al*., 2009; Van Mieghem *et al*., 2010). Measurement of strain however, is only poorly feasible in TTTS due to the adverse imaging conditions which preclude adequate high resolution imaging of the fetal ventricles. Fetal cardiac dysfunction is more common in the higher Quintero stages, yet an elevated MPI is already apparent in 50% of the early (stage I) cases (Van Mieghem *et al*., 2009; Van Mieghem *et al*., 2010). Ultrasound markers also correlate with amniotic fluid derived biomarkers of fetal cardiac damage and dysfunction (brain-type natriuretic peptide and cardiac troponin T) (Van Mieghem *et al*., 2010). Causative therapy, leading to a stop in transfusional imbalance, either by occluding the placental vascular anastomoses through fetoscopic laser coagulation or by selective bipolar cord occlusion of one of the fetuses, cures the TTTS and leads to restoration of normal amniotic fluid levels. We showed in a cohort of fetuses followed longitudinally before and after therapy (n = 39) that the recipients cardiac function improved within 48 hours after the procedure (Van Mieghem *et al*., 2009). The donor on the other hand demonstrated temporary signs of cardiac overload or increased afterload. Longer term follow-up in 14 fetuses from our local population showed a complete normalization of cardiac function in the vast majority of fetuses 6 weeks after the therapy (Fig. 2). Combination of pre-operative functional cardiac ultrasound with amniotic fluid derived cardiac troponin T allowed to identify a group of recipient fetuses at increased risk of postoperative demise after fetoscopic laser coagulation of placental vascular anastomoses. This seems logic in the view that an intervention leading to major fetal hemodynamic changes could be one step too far for an already frail heart. Non-invasive fetal cardiac function assessment alone however, could not predict fetal survival in our hands. As a consequence, the findings of the current study will not influence current management of TTTS nor our way of counseling patients.

Experiments in fetuses with congenital diaphragmatic hernia

In a prospective cohort of 27 fetuses with left sided congenital diaphragmatic hernia, we determined fetal cardiac axis, left ventricular diameter, ejection fraction, shortening fraction, mitral E/A-index and myocardial performance index (Van Mieghem *et al*., 2009). We compared our results in CDH fetuses to a healthy reference population and found that left ventricular diameters were 30 % smaller in CDH than in controls ($p < 0.001$; Fig. 3). All other functional indices were comparable between the 2 groups. This smaller left ventricle leads to a lower left ventricular output (Baumgart *et al*., 1998; Vogel *et al*., 2010).

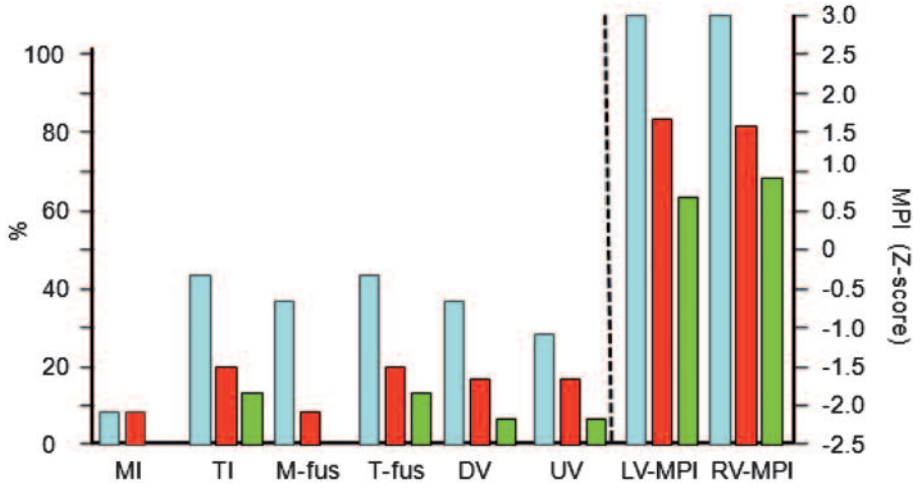

Fig. 2. — Fetal cardiac dysfunction in 14 recipient twins of twin-to-twin transfusion syndrome before (blue bars), 2 weeks (red bars) and 4 weeks after (green bars) fetoscopic laser ablation of the placental vascular anastomoses. Adapted from Van Mieghem *et al*., 2009. Legend: MI: mitral insufficiency, TI: tricuspid insufficiency, M-fus: fusion of mitral valve flows, T-fus: fusion of tricuspid valve flows, DV: reversed a-wave in ductus venosus, UV: pulsatility in umbilical vein, LV-MPI: myocardial performance index of left ventricle, RV-MPI: myocardial performance index of right ventricle.

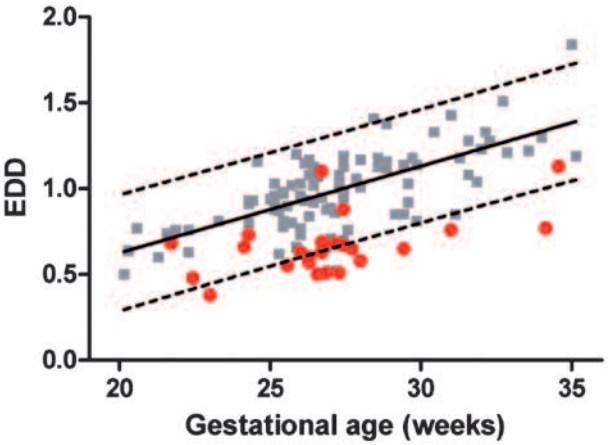

Fig. 3. — Left ventricular end diastolic diameter (EDD) plotted versus gestational age in CDH fetuses (dots) and controls (squares). The regression line and 95% confidence interval for the normals is plotted. Reproduced from Van Mieghem *et al.*, 2009.

In contrast to what is seen in postnatal life, the fetal left and right ventricular circulation are parallel rather than serial circuits, with the right ventricle perfusing the lower body and the placenta and the left ventricle irrigating the brain and upper body. A decrease in left ventricular output could therefore potentially lead to a lower cerebral perfusion and impaired brain and head development. To investigate this hypothesis, we retrospectively analyzed cerebral blood flow patterns, skull size and brain volume in 103 fetuses with CDH (Van Mieghem *et al.*, 2010). We could show that cerebral blood flow was decreased by 20% in CDH fetuses, yet that cranial biometry and brain volumes were maintained. These findings suggest that part of the neurologic morbity observed in long-term CDH survivors may partly be of prenatal origin and research is ongoing to further clarify the clinical impact of our observations.

A final study in a cohort of fetuses with severe isolated left sided CDH undergoing fetal tracheal occlusion to improve lung growth, was designed to assess cardiac function indices before and after the procedure (n = 12) and its reversal (n = 10) (Van Mieghem *et al.*, 2009). We found that tracheal occlusion did not affect cardiac size but reduced the MPI ($p = 0.004$). A trend towards an opposite effect was seen around reversal of the occlusion. All other cardiac functional indices stayed normal around the procedure. As such, this experiment confirmed the cardiac safety of tracheal balloon occlusion. We further speculated from our findings that the decrease in MPI after tracheal occlusion was due to an increased pulmonary flow after tracheal occlusion, leading to an improved right ventricular outflow, decreased shunting over the ductus arteriosus and hence a decreased left ventricular afterload. As such, changes in cardiac function could be a reflection of the therapeutical effect of the balloon and further studies should be designed to investigate whether survival after tracheal occlusion can be predicted based on cardiac function measurements.

Part 2: Maternal hemodynamics in pregnancy

Background and introduction

The maternal cardiovascular system undergoes dramatic changes during pregnancy. Starting off as early as 6 weeks of gestation, the maternal plasma volume expands to reach levels at term which are approximately 40% higher than the non-pregnant values (Salas *et al.*, 2006). In parallel, a drop in total vascular resistance, a 15% increase in maternal heart rate and a 20% increase in stroke volume are consistently observed (Poppas *et al.*, 1997; Bamfo *et al.*, 2007). Consequently, cardiac output is increased with 30% at term (Mabie *et al.*, 1994), which is necessary to sustain uterine demands that can reach up to 600 ml/min in a term singleton pregnancy. As a result of this major hemodynamic challenge, intrinsic systolic cardiac function improves during pregnancy and systolic myocardial velocities increase. The left ventricular mass increases with 52% hence decreasing ventricular compliance slightly (Kametas *et al.*, 2001). As expected therefore, the Tei-index increases during pregnancy and the atrial contraction becomes increasingly important to achieve adequate ventricular filling.

This cardiac adaptation to pregnancy is strongly correlated with fetal weight. Indeed, multiparous women, who generally deliver heavier babies, have a higher plasma volume than primigravid women and stroke volume and cardiac output are 20% higher in twins than in singletons (Kametas *et al.*, 2003). Pregnancies complicated with intra-uterine growth restriction on the other hand, have a much less pronounced plasma volume expansion, show reduced systolic function and cardiac output and have increased total vascular resistance (Salas *et al.*, 2006; Bamfo *et al.*, 2006).

Although it is clear from the above data and generally accepted that adequate maternal hemodynamic adaptation to pregnancy is extremely important to achieve an optimal pregnancy outcome, many essential questions still surround the physiologic pathways regulating the maternal cardiovascular system in gestation. It has been suggested that an early pregnancy arterial vasodilatation, mediated by a nitric oxide dependent pathway, leads to a state of 'relative hypovolemia' which is perceived by the arterial baroreceptors (Lindheimer and August, 2009). Compensatory mechanisms are subsequently activated,

leading to activation of the renin-angiotensin-aldosterone system and to vasopressin secretion with, as a consequence, sodium and water retention despite an initially normal blood volume. However, other theories about volume homeostasis during pregnancy have also been formulated and strong arguments supporting/refuting either theory are lacking (Lindheimer and August, 2009). Moreover, although the renin-angiotenin-aldosterone system is certainly implicated, a good knowledge of the hormonal regulation of the gestational plasma volume expansion is lacking. The aim of the current thesis was to further investigate the role of 2 vasoactive peptides (the insuling like growth factor IGF-II and apelin) in the regulation of maternal plasma volume expansion and fetal growth

The insulin like growth factor II

Insulin like growth factor II is well known as a regulator of intra-uterine growth. Deletion of the fetal *Igf2* gene in mice results in a 40% reduction in birth weight and suppression of the placental *Igf2* gene leads to 30-40% smaller placentas (Fowden, 2003; Constancia *et al*., 2002). In addition, IGF-II infusion during mid- to late gestation in guinea pigs increases fetal weight at term through placental morphologic changes and improves transplacental nutrient transport (Sferruzzi-Perri *et al*., 2006). In the current thesis we hypothesised that, next to the above described pathways, IGF-II can also modulate fetal growth through its vasomotor effects on the maternal circulation.

We investigated maternal plasma volume expansion using a dye dilution method (Evans Blue dye) and measured plasma IGF-II concentrations using radioimmunoassays in a pregnant rat model (Van Mieghem *et al*., 2009). We could show that in normal rat pregnancy maternal term plasma volume was 35% higher than in non-pregnant animals. Circulating IGF-II was raised by 45% in term pregnancy. Going further into the hypothesis that maternal plasma volume is related to maternal weight, we measured plasma volume and plasma IGF-II concentrations in rats with enhanced pre-gestational weight gain provoked by an obesogenic diet. Surprisingly however, we found that plasma volume and IGF-II were unchanged in the obese rats, suggesting that plasma volume is not related to fat mass but to lean body mass. In both above described experiments however, plasma volume was strongly correlated with maternal IGF-II levels. To gain a more mechanistic insight in this relationship, we designed two further experiments in which circulating IGF-II levels were decreased or enhanced and assessed plasma volume as the main outcome variable.

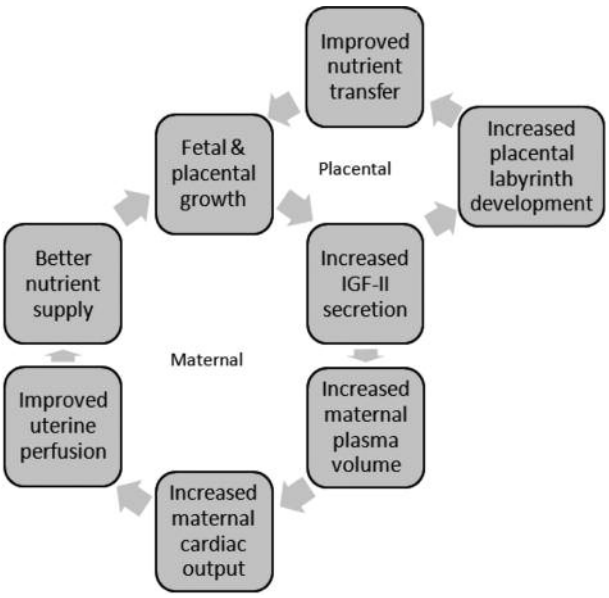

Fig. 4. — Feto-placental regulation of uterine perfusion and placental nutrient transfer.

Surgical removal of half of the gestational sacs at day 16 of rat pregnancy (term is day 22) decreased placental mass and as a consequence we could observe 20% lower maternal IGF-II levels. In response to this alteration, maternal PV was diminished by 14% when compared to sham operated animals. A constant infusion however of IGF-II (1 mg/kg/d) from d16 onward, which raised circulating IGF-II by 38%, increased PV at term by 19%. Moreover, in multivariate analyses, IGF-II was the only significant predictor of PV.

The above experiments shed a new light on the regulation of maternal plasma volume in gestation, and on its importance for fetal growth. Indeed, until now, the widely accepted belief is that maternal plasma volume expansion regulates fetal growth through increased cardiac output, improved uterine perfusion and hence better nutrient delivery. This hypothesis is supported by experiments from Rosso showing that malnourished rats, who do not expand their plasma volume adequately, give birth to smaller pups (Rosso and Streeter, 1979) and by the observation that women with growth restricted babies have lower plasma volumes (Salas *et al*., 2006). Another interpretation of these data however, in line with the current findings, would be that fetoplacental growth autoregulates maternal plasma volume expansion and placental transport through endocrine pathways (in which IGF-II certainly plays an important role), hence creating positive feedback loops (Fig. 4).

Apelin

The second hormone potentially involved in maternal hemodynamics we evaluated in this thesis was

apelin. This 36 aminoacid peptide with short half-life has recently been discovered as being the ligand of the APJ-receptor (Japp and Newby, 2008). The peptide has very potent vaso-active and inotropic effects and has therefore mainly been explored as a potential target for the treatment of heart failure. Apelin plasma levels are increased in acute cardiac decompensation, yet decrease in chronic heart failure (Goetze *et al*., 2006). A biphasic blood pressure response is seen after acute intravascular bolus administration to sheep, with an initial hypotensive reaction, followed by a more sustained hypertensive phase (Charles *et al*., 2006) and chronic elevation of apelin in the brain, as seen in genetically targeted mice overexpressing apelin, leads to hypertension (Zhang *et al*., 2009). Knocking out the peptide on the other hand lowers cardiac performance during exercise and causes age-related heart failure. The body fluid effects of apelin remain unclear at this time: systemic or central administration of apelin to rats causes diuresis by vasorelaxation of the afferent renal vessels and inhibition of the anti-diuretic effect of vasopressin (De Mota *et al*., 2004; Hus Citharel *et al*., 2008). On the other hand, studies in mice lacking the APJ-receptor suggest an anti-diuretic effect of apelin (Roberts *et al*., 2009).

In a pregnant rat model, we measured maternal plasma apelin levels throughout normal gestation using radioimmunoassays and demonstrated that plasma apelin concentrations decrease by half in the last third of gestation (Van Mieghem *et al*., 2010). To further investigate the etiology of this sudden drop in apelin levels, we examined apelin gene expression in the liver, kidney, heart, lung, brain, adipose tissue and breast gland both in virgin and pregnant rats as well as in the placenta. We found that apelin expression did not decrease during pregnancy and therefore hypothesized that the lower plasma levels were due to an increased clearance of apelin. The latter was further confirmed by elimination studies using radiolabeled apelin which showed a significantly shorter elimination time of apelin in pregnancy. Apelin is normally only metabolized by the angiotensin converting enzyme 2 (ACE2), which is also present in the placenta. An experiment in which we surgically reduced half of the feto-placental mass at day 16 of gestation showed that maternal plasma apelin was raised by 23% compared to sham operated dams thereby further confirming the role of the placenta in apelin clearance. Moreover, ACE2 mRNA expression was detectable in late- but not mid-pregnancy placental tissue. Further immunohistochemical localization of the peptide showed that ACE2 was primarily localized in the smooth muscle layer of fetal arterioles in the labyrinth (Fig. 5).

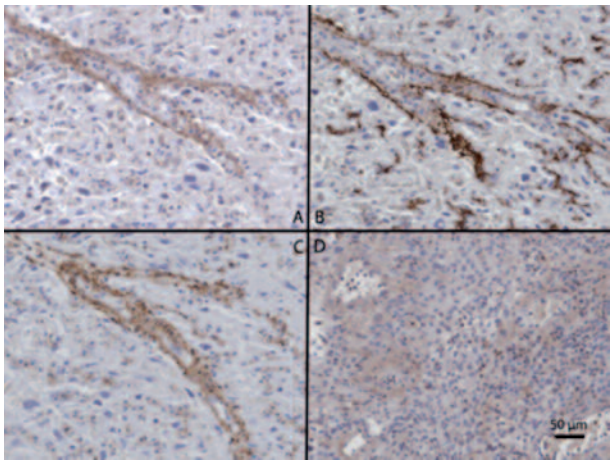

Fig. 5. — Parallel sections through a fetal arteriole running up the labyrinth of a day-22 rat placenta stained for (A) apelin-36, (B) ACE2 and (C) actin, demonstrating the presence of these proteins in the perivascular smooth muscle. (D) Apelin-36 staining of the mesometrial triangle at term demonstrating faint apelin staining in the cytoplasm of stromal cells. Reproduced from Van Mieghem *et al*., 2010.

Taken together, these experiments show that the placenta, through its expression of ACE2, is responsible for the accelerated clearance of apelin in late gestation and hence for the lower maternal plasma apelin concentrations in the last third of pregnancy. Further studies are currently ongoing to evaluate the physiologic and pathophysiologic consequences of this dramatic change in a highly vaso-active peptide. In conclusion, both our experiments with IGF-II as well as with apelin substantiate an important role for the feto-placental unit in regulating maternal plasma volume expansion and (auto)regulating uterine perfusion and fetal growth. They underscore the need for further studies on the (patho)physiology of maternal plasma volume expansion.

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What is already known?

- Several methods for measurement of fetal cardiac function have been described, even more so as novel software makes sophisticated image analysis basically "online" possible. These methods are nicely summarized in chapter 1 of the thesis, which is available as a download from Prenatal Diagnosis.
- Several of these are only performed by a limited number of teams and were not validated in a population at risk for cardiac dysfunction.
- Fetal heart function in twin-to twin transfusion syndrome is often named as a prognostic factor, some even think with therapeutic relevance.
- Fetuses with congenital diaphragmatic hernia pose a difficulty for cardiac assessment. The anatomy is distorted, so that functional assessment is even more difficulty.

What is new from this research?

- This work validates measurement of cardiac function by the myocardial performance index in the second and third trimester of pregnancy. This was done by correlating it to M-mode evaluation as well as cardiac stretch markers.
- ☐ Stretch tracking is a novel method, but when applied to pathologic situations were of no added value. Moreover it is very challenging.
- ☐ Twin-to-transfusion syndrome recipients have an impaired cardiac function: already early in the course of the condition they are functionally compromised. In utero recovery following successful treatment takes more than 4 weeks. Cardiac function measurement in this condition is not used to stratify the population prior to laser, neither when they have TTTS nor when they are growth discordant...
- Fetuses with isolated CDH have a normal heart function but the flow to their brain is somewhat lower. This did not affect brain growth (measured as a volume or another biometric variable)

Which questions will these new findings arise?

- Clinical relevance of cardiac function measurement: does reduced brain perfusion compromise fetal brain development and later function?
- What is the exact contribution of fetal heart function towards predicting outome

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