UNIVERSITY OF NAPLES FEDERICO II

SCHOOL OF MEDICINE

AND SURGERY

Department of

NEUROSCIENCE, REPRODUCTIVE AND ODONTOSTOMATOLOGICAL SCIENCES

PhD Program Neuroscience

XXIX Cycle

Coordinator: Prof. Lucio Annunziato

PhD Thesis

Perinatal preterm brain injury. Risk assessment, antenatal surveillance and managing.

Tutor **Candidate**

Prof. Mariavittoria Locci **Dr. Teresa Palmieri**

Academic Year 2014/2017

CONTENTS

Introduction

Neurological damage in preterm fetus, by intrauterine growth retardation and preterm birth syndrome **pag. 3**

First line research

Fetal and perinatal surveillance of brain injury **pag. 6**

Aim of the research **pag. 14**

Results of the research

"Tenascin expression in human placentas during FGR affected pregnancies and UA Doppler velocimetry correlation" **pag. 15**

"Intrauterine growth restriction and fetal cerebral redistribution. Risk stratification and neurological outcome" **pag. 32**

Second line research

Therapy and prediction of preterm birth in singleton pregnancies at low risk and high-risk twin pregnancies **pag. 41**

Aim of the research **pag. 47**

Results of the research

"Two cycles of Atosiban in preventing preterm birth in twin pregnancies" **pag. 48**

"Role of Vaginal DHA on Uterine Contractions in Patients at low risk for Preterm Birth" **pag.59**

"A-Lipoic Acid As Maintenance Therapy In Patients Treated for Preterm Birth" **pag.71**

"The role of PartoSure Test in predicting imminent preterm birth" **pag. 73**

Future directions **pag. 75**

References **pag. 76**

Introduction

This work reports the results of two different lines of research: 1. On the employment of ultrasound in the management of fetuses with intra-uterine growth restriction (IUGR); 2. On management of preterm birth for prevention of neurological impairment in high-risk pregnancies and low risk pregnancies. Pre-natal flow data and post-natal neurological outcome in IUGR fetuses have been evaluated. Doppler ultrasound in obstetrics has allowed the detection of the early signs of fetal demise, involving the management of pregnancies at risk of fetal hypoxia. The second line of research focuses on the need to identify strategies to predict preterm birth in asymptomatic low-risk women as well as in those presenting with threatened preterm labor (symptomatic high risk women) and underlines the importance to prevent brain injury and long-term neurologic sequelae related to preterm deliveries.

Neurological insults result in significant immediate and longterm physical, emotional, and financial costs. The risks of intraventricular hemorrhage (IVH), cerebral palsy (CP), and neurological impairment are closely related to gestational age of delivery (1); thus, the timing of delivery for FGR (fetal growth restriction) fetuses is crucial as well as the prevention of preterm delivery that are perhaps, the most effective strategy for neonatal neuroprotection (2). The major hypoxic-ischemic lesion of the preterm infant is periventricular leukomalacia (PVL) representing injury to the cerebral white matter. Preterm infants thought to be at greatest risk for PVL are those born between 23 and 32 weeks' gestation. PVL is a spectrum from the classic focal cystic variety to diffuse noncystic white matter injury. Interestingly, PVL with focal macrocysts detectable by sonography has declined and PVL is dominated by the diffuse variety that can be detected by MRI but not sonography. The predominant pathology underlying PVL is an arrest in the lineage of oligodendrocytes at the pre-oligodendrocyte stage and results in reduced brain myelination (3).

For the extreme preterm infant (<29 weeks' gestation), the pre-oligodendrocyte represents most of the oligodendrocyte population and is extremely sensitive to hypoxiaischemia and inflammation, which represent triggers for the initiation of white matter injury.

However, PVL with microcysts (focal necrosis) is associated with remote neuronal loss most commonly in the thalamus, basal ganglia, and cerebellar dentate nucleus compared. The involvement of gray matter lesions with diffuse white matter injury has prompted the term “encephalopathy of prematurity” (4).

Intracranial hemorrhage (ICH) may also represent a hypoxic-ischemic injury occurring primarily in extreme preterm infants (<29 weeks’ gestation). In clinical practice, the terms hypoxia-ischemia and asphyxia are often used interchangeably. Technically there are important differences. Hypoxia is a low content of oxygen in the blood, whereas ischemia represents a reduction in tissue blood flow. Ischemia in turn can be partial or complete in extent, and can be focal or global in distribution; hypoxia and ischemia are often combined because each component may result in the other. In contrast, asphyxia indicates an impairment of gas exchange and is characterized by anoxia and extremes of hypercarbia.

ICH is a hemorrhagic lesion initiated in the periventricular germinal matrix; deranged cerebral hemodynamics may contribute to ICH.

Because it is difficult to identify hypoxia-ischemia/asphyxia among preterm infants at or soon after birth, studies that examined the incidence of these events have focused on more mature gestational ages. Salhab and Perlman reported moderate and severe encephalopathy among infants 31 to 36 weeks’ gestation with severe fetal acidemia; the incidence of encephalopathy was 1.4 per 1000 live births. There are no available data for the incidence of hypoxia-ischemia/asphyxia among extreme preterm infants. (4).

In the IUGR infant, brain injury may be due to a combination of grey matter and white matter disruption and disorganisation in the development of the brain. Clinical imaging studies of preterm IUGR infants have demonstrated significant alterations in white and grey matter volume and structure including decreased cortical thickness, delayed cortical development and altered brain connectivity in comparison to non-IUGR preterm infants. Reduced cerebral cortical grey matter volume in the term IUGR neonate has been shown to correlate with attention disorders (5[a]). A four-to sixfold increase in CP has been shown in IUGR neonates; the IUGR fetus is relatively hypoxic due to chronic placental oxygen deprivation. The chronic IUGR insult leads to a reduction in oxygen delivery to the brain and concomitant reduction in delivery of glucose and aminoacids with potential effects

on immature neurons and neuroglia (5^{b}). When cerebral oxygen is reduced, a cascade of cellular and biochemical events occurs in the fetal brain causing cellular injury that can lead to cell death. Many of these events result in mitochondrial disruption and immediate or delayed cell death (5^{b}). The major putative mechanisms that may underpin the cellular death and injury in IUGR brains are excitotoxicity, oxidative stress, necrotic and apoptotic degeneration and neuroinflammation.

On the other hand, advances in obstetrical and neonatal care have led to survival at earlier gestational ages and consequently increasing numbers of periviable infants who are at significant risk for long-term neurological deficits. Therefore, efforts to decrease and prevent cerebral insults attempt not only to improve neurological outcomes in infants delivered preterm but also primarily to decrease preterm delivery.

I Research line : Fetal and perinatal surveillance of brain injury

Human brain development is a protracted process that begins in the third gestational week (GW) with the differentiation of the neural progenitor cells and extends at least through late adolescence, arguably throughout the lifespan (6). The nine months of intrauterine life are a continuum during which a series of situations and events can occur that result in abnormalities of normal brain growth or injury to the developing brain of the fetus. During this time there is rapid growth and elaboration of both cortical and subcortical structures, including the rudiments of the major fiber pathways.

Both gene expression and environmental input are essential for normal brain development, and disruption of either can fundamentally alter neural outcomes.

By the end of the embryonic period the rudimentary structures of the brain and central nervous system are established and the major compartments of the central and peripheral nervous systems are defined. The early fetal period, which extends to approximately midgestation, is a critical period in the development of the neocortex. Most cortical neurons are generated by that time and many have migrated to their positions in the neocortex and have begun to from essential brain networks for information processing (7). Different pathogenic noxae can damage cerebral tissues in a variety of different ways. The damage that results from a systemic condition of hypoxia / acidosis or from a local ischemic condition is certainly a major cause of perinatal morbidity and mortality. The pattern and consequences of injury depend on the severity and duration of the insult; the neurovascular and anatomical maturity of the brain is primarily a factor related to the gestational age of the fetus, and co-related factors such as the presence or absence of infection or problems with fetal nutrition (8). Hypoxia is central to the genesis of a significant proportion of the brain injury that occurs in the fetus; but the fetus is at risk of brain injury whenever cerebral ischemia occurs as a consequence of impaired cerebral blood flow. It is estimated that the incidence of encephalopathy due to intrapartum asphyxia alone (in the absence of other possible causes antepartum) is 1.6 per 10,000 births (3-20% of all the cerebral palsy); prematurity and infections during pregnancy, however, represent the most common causes of cerebral palsy compared to intrapartum events.

The condition predisposing the neurological damage is cerebral edema; cellular edema is defined as the premorbid cellular process, known as oncotic cell swelling, whereby extracellular Na+ and other cations enter into neurons and astrocytes and accumulate intracellularly, in part due to failure of energy-dependent mechanisms of extrusion. Cells in both gray and white matter are affected initially by cytotoxic edema. Cellular swelling begins within 30 minutes of MCA occlusion, particularly around capillaries, persists for up to 24 hours after reperfusion, and results in an average reduction of extracellular space from the normal 20% down to 4 to 10% (9[a]). The cerebral vascular system has anatomical and physiological barriers that regulate the transfer of compounds into the brain; a damage of this barrier causes a vasogenic cerebral edema, in which extracellular water and other substances passes into cells, resulting in their swelling.

The special autoregulation of fetal cerebral circulation, with more sprayed brain territories during intrauterine life (different Doppler indices) and the physiological subcortical hypoperfusion in fetuses, is a sort of functional reserve of the fetal brain in extreme response to hypoxia.

The concomitant involvement of the cerebral cortex, basal ganglia, thalamus, brain stem and the cerebellum has been associated with poor neurodevelopmental outcome.

Premature neonates who are also of a low birth weight are at the highest risk of periventricular leukomalacia (PVL) and germinal matrix-intraventricular hemorrhage (GM-IVH). PVL is characterized by hemorrhagic necrosis of the white matter surrounding the lateral ventricles. It can be diffuse or focal and commonly occurs in the preterm infant. This “selective vulnerability” (9[b]) governs the subsequent manifestation of injury. Blood vessels within the germinal matrix are fragile, particularly during the period of high cell turnover (24–32 weeks gestation) associated with corticogenesis. IVH implies structural immaturity of blood vessels in the germinal matrix, and is associated with deficient autoregulatory capacity and pressure-passive cerebral perfusion.

The impact of early brain insults depends both on the timing and on the exent of the damage; the developing brain is capable of reorganizing to compensate for injury by creating novel neuronal networs.

So the IUGR fetuses are at risk of intraventricular hemorrhage, because they are often preterm fetuses.

Intrauterine growth restriction (IUGR) refers to a condition in which a fetus is unable to achieve its genetically determined potential size. This functional definition seeks to identify a population of fetuses at risk for modifiable but otherwise poor outcomes. This definition intentionally excludes of fetuses that are small for gestational age (SGA) but are not pathologically small. SGA is defined as growth at the 10th percentile for weight of all fetuses at that gestational age. Not all fetuses that are SGA are pathologically growth restricted and, in fact, may be constitutionally small. Similarly, not all fetuses that have not met their genetic growth potential are in less than the 10th percentile for estimated fetal weight (EFW).

The American College of Obstetricians and Gynecologists (ACOG) defines FGR as an estimated fetal weight less than the 10th centile (10). The Royal College of Obstetricians and Gynaecologists (RCOG) uses fetal abdominal circumference (AC) or estimated fetal weight (EFW) <10th centile to diagnose a FGR fetus (11). Other authors have suggested, as definition of fetal growth restriction, a combination of fetal smallness and umbilical artery Doppler PI >95th percentile (12, 13).

Hovewer, FGR shouldbe referred to fetuses with pathological smallness caused by an underlying functional problem and hence a definition including not only a biometric cut off but also Doppler indices of feto-placental function is currently agreed in most Fetal Medicine Units (12,13, 14).

These fetuses are at risk of developing lifelong disabilities, such as asphyxia, meconium aspiration, haematological disorders and cerebral palsy. Some studies also report an association with several major in adulthood diseases: coronary heart disease, hypertension and type 2 diabetes (15).

Placental insufficiency is the leading cause of FGR and is usually due to poor uteroplacental blood flow and placental infarcts. The reduction of placental supply of nutrients to the fetus has been associated with several adaptive changes taking place in both the placenta and fetus.

Despite numerous approaches to managing FGR, there are no effective treatments to improve the growth pattern of a fetus; howewer prenatal management is aimed

primarily at determining the ideal timing and mode of delivery. This assessment must be individualised, depending on several variables: gestational age of the fetus, maternal health, severity of growth restriction and fetal well-being. Perhaps optimising the delivery time and removing the fetus from a suboptimal environment can prevent the risk of hypoxia and major neurological morbidities (16).

A prerequisite for a correct diagnosis of FGR is accurate dating of the pregnancy, most usually in the first trimester. An accurate prenatal differentiation between FGR and SGA is challenging. Although size is a physical parameter that can be measured at any gestational age, growth is a dynamic process that can be assessed only by repeated measurements; and sequential assessments are necessary to determine whether there is a decrease in the fetal growth rate.

In other words, the term SGA refers to the size, irrespective of the growth velocity in the uterus e these are usually associated with normal placental function and have a normal outcome; FGR is SGA in which circulatory redistribution occurs so that the brain is preferentially perfused and there is a abnormal umbilical artery with pulsatility index above the 95th percentile.

Birth weight alone is no longer considered the key factor, as it poorly reflects intrauterine events and depends on several factors unrelated to developmental programming. Fetal size charts are used to compare the size of a fetus (of known gestational age) with reference data and to compare it on two or more different circumstances; there are several growth charts, but they are very heterogeneous between them.

FGR may also be classified by gestational age at onset as early and late, with an arbitrary cut-off conventionally set at 32 weeks: the extremes of the clinical spectrum of FGR differ not only for gestation at onset, but also for clinical manifestations, patterns of fetal deterioration, association with hypertensive disorders of the pregnancy and severity of placental dysfunction (17, 18). Fetuses with late-onset disease do not present the same sequence of Doppler deterioration described for early-onset FGR (13). Early-onset FGR is associated with high impedance utero placental perfusion which in turn leads to elevated umbilical artery blood flow resistance once villous damage exceeds 30%; late-onset FGR is more common but less severe with absent or mild placental abnormalities;

umbilical artery Doppler may be normal, but fetuses may react with decreased middle cerebral artery (MCA) impedance in response to hypoxemia (19).

The ultrasound examination with Doppler investigation is of great help in the diagnosis and follow-up with IUGR fetuses and in the assessment of a possible brain injury.

The clinical efficacy of umbilical artery Doppler velocimetry in these fetuses IUGR is validated by different randomized studies and, in clinical practice, led to a 29% reduction in perinatal mortality.

The umbilical artery, middle cerebral artery, ductus venosus and uterine artery provide a comprehensive evaluation.

Physiological modification of spiral arteries is required to permit the ten-fold increase in uterine blood flow which is necessary to meet the respiratory and nutritional requirements of the fetus and placenta; impaired trophoblastic invasion of the maternal spiral arteries is associated with increased risk for subsequent development of obstetric complications related to uteroplacental insufficiency. Increased impedance to flow in the uterine arteries in a one-stage color Doppler screening program at 23 weeks can detect a fetus at higher risk of serious complications of impaired placentation and growth restriction and subsequently adverse perinatal outcome. However, this screening test for risk is not an assessment of fetal well-being and has no established place in the management of infants diagnosed with FGR (20).

Umbilical artery Doppler is the only measure that provides both diagnostic and prognostic information for the management of FGR (21); umbilical artery should not be used as a screening tool in healthy pregnancies, as it has not been shown to be of value in this group (22). In terms of monitoring growth-restricted pregnancies, abnormal waveforms in the umbilical artery are an early sign of fetal impairment. With advancing gestation, umbilical arterial Doppler waveforms demonstrate a progressive rise in the end-diastolic velocity and a decrease in the impedance indices. In the presence of placental insufficiency with progressive severity, there is a higher placental resistance, indicated by a high pulsatility index, absent or reversed end-diastolic component of the umbilical artery waveform. Different studies have demonstrated that increased impedance in the umbilical arteries becomes evident only when at least 60% of the placental vascular bed is

obliterated. In pregnancies with reversed or absent end diastolic frequencies in the umbilical artery, mean placental weight is reduced and the cross-sectional diameter of terminal villi is shorter. In addition, significantly more these neonates needed admittance to the neonatal intensive care unit and they had a higher risk of cerebral hemorrhage, anemia or hypoglycemia and increased incidence of long-term permanent neurological damage.

At the lower limits of oxygen availability there is an increase in the blood supply to the brain, myocardium and the adrenal glands and reduction in the perfusion of the kidneys, gastrointestinal tract and the lower extremities.

The middle cerabral artery (MCA) is the vessel of the choice to assess the fetal cerabral circulation because it is easy to identify, has a high reproducibility, and provides an information about the brain sparing effect. Additionaly, it can be studied easily with an angle of zero degrees between an ultrasound beam and the direction of blood flow and, therefore, information on true velocity of the blood flow can be obtained. The MCA is the largest branch of the circle of Willis and it represents the lateral continuation of the internal carotid artery (ICA) into the Sylvian fissure. It has the largest volume of flow of all the vascular branches arising from the circle of Willis, carrying about 80% of the flow to the hemisphere. It consists of four segments: M1 (at the origin- optical chyasma), M2 (the distal tract- the extreme wings of large sfenoid), M3 and M4 (this segment runs temporally and frontally); M1 and M2 supply different parts of the fetal brain, which develop in different periods of fetal life (23), with statistically significant differences between 26 weeks and 36 weeks.

To maintain cerebral homeostasis, there are specialized mechanisms mediate the short-term vasodilatory responses to acute hypoxia and more long-term changes in artery structure and reactivity enable the fetus to adapt to chronic hypoxia.

A condition of chronic hypoxia determines a fetal flow redistribution that manifests as vasodilatation in the brain circulation. Cerebral vasodilatation, easily detectable as a reduction in the PI of the middle cerebral artery (MCA) represents an adaptative mechanism in response to hypoxia; blood flow is centralized and the Brain Sparing Effect appears to be a benign adaptive mechanism preventing severe brain damage (24).

However, compensation through cerebral vasodilatation is limited and a plateau corresponding to a nadir of pulsatility index (PI) in cerebral vessels is reached before the development of the fetus is impaired. In late-onset IUGR, there is observational evidence that MCA vasodilatation is associated with adverse outcome independently of the umbilical artery. This suggests a role of MCA Doppler for fetal monitoring in late-onset IUGR cases, which needs further investigation in randomized trials (25).

In recent years it has increased the interest for the Cerebroplacental ratio (CPR), that quantifies the redistribution of cardiac output by dividing the Doppler indices of the middle cerebral artery (MCA) with that of the umbilical artery. J. Morales-Rosello et al. in their study suggest that low CPR in AGA fetuses is an equally important marker of low neonatal pH secondary to placental underperfusion and this finding may be of value in risk assessment for stillbirth at term and long-term neurodevelopmental disability (26). Of note, the PORTO study demonstrated the association between redistribution, either isolated or associated with umbilical artery PI >95th centile, and adverse perinatal outcome (27). Thus, a reduction of the MCA PI and the relationship between MCA PI and UA PI CPR is an early sign of hypoxia in IUGR fetuses and it is associated with a high risk of brain damage as well as ischemia and cerebral hemorrhage. The finding of a centralization of circulation confirms a state of fetal hypoxia and the need of the study of fetal venous circulation to highlight the deterioration of heart function with cardiovascular changes that can be shown by venous Doppler studies.

The ductus venosus plays a central role in the return of venous blood from the placenta. Well-oxygenated blood flows via this shunt directly towards the heart. Early studies on IUGR fetuses demonstrated a good correlation of abnormal DV waveform with acidemia at cordocentesis and this Doppler sign is considered a surrogate parameter of the fetal base-acid status. Of note longitudinal studies have demonstrated that DV flow waveforms become abnormal only in advanced stages of fetal compromise (28).

All these types of velocimetry evaluations have contributed to the understanding of metabolic and vascular events underlying the hypoxic-ischemic brain.

IUGR is one of the most common pregnancy complications and substantially increases the prospective risk of adverse outcome. Yet according to pregnancy

audits, most instances of IUGR are not detected as such antenatally. Modern obstetric care needs to raise the level of awareness of the importance of this condition, and establish evidence-based protocols for improved surveillance. At present there is no effective intervention for FGR except delivery and importantly, gestational age is the most significant determinant of both survival and intact survival. Thus, the main consideration needs to be appropriate timing, balancing the risk of potential iatrogenic morbidity and continued exposure to an unfavorable intrauterine environment.

Objective

To determine the relationship between prenatal parameters, sonographic and not, with neonatal outcome in pregnancies with IUGR fetuses, it began this research project, that was held in two different time.

The aim of first research article has been to evaluate the expression of some non collagenous extracellular matrix proteins, in particular tenascin, in human placentas of intrauterine growth restricted fetuses with abnormal umbilical Doppler velocimetry. The presence of tenascin might be considered as a placental compensatory mechanism in FGR fetuses with abnormal umbilical artery Doppler velocimetry.

Then the second study has realized an intensive prenatal and postnatal monitoring in IUGR fetuses, in order to correlate fetal Doppler with neurological outcome.

Tenascin Expression in Human Placentas during FGR Affected Pregnancies and Umbilical Doppler Velocimetry Correlation

Abstract

Objective: The aim of this study was to evaluate the expression of some non collagenous extracellular matrix proteins, in particular tenascin, in human placentas of intrauterine growth restricted fetuses with abnormal umbilical Doppler velocimetry.

Study Design: Study group (group A) consisted of 23 pregnant women with intrauterine growth restricted fetuses, with or without preeclampsia. Control group (group B) consisted of 10 pregnant women with appropriate fetal weight for gestational age. Placental specimens were collected from biopsies obtained after cesarean delivery. Umbilical artery Doppler velocimetry was performed within four hours from delivery in all patients. Tenascin expression was studied by immunohistochemistry and western blot techniques.

Results: A difference in birth weight and placental weight was found in the two groups, being lower in the study group. Umbilical artery Doppler velocimetry showed abnormal patterns in the study group and normal findings in the control one. Tenascin was strongly expressed in placentas from growth restricted fetuses, as shown by immunohistochemistry and by RT-PCR, while it was almost absent in placentas from group B.

Conclusion: A relationship between abnormal Doppler patterns and tenascin distribution in growth restricted fetuses has been observed. The presence of tenascin might be considered as a placental compensatory mechanism in FGR fetuses with abnormal umbilical artery Doppler velocimetry.

Introduction

Fetal Growth Restricted (FGR) fetuses are the fetuses in which growth restriction implies a pathological restriction of the genetic growth potential. As a result, FGR fetuses manifest evidence of fetal compromise i.e. abnormal Doppler velocimetry [1]. FGR with or without Preeclampsia (PE) complicates a significant number of pregnancies [2,3]. This condition is an important risk factor for adverse perinatal outcome and contributes to maternal and perinatal morbidity and mortality. Impaired placental perfusion and angiogenesis seem to be the most common causes of FGR, even if, in some cases, both FGR and PE are related to a failure of immunomodulatory placental functions [4,5]. Moreover, excessive levels of placental oxidative stress lead to PE and FGR, and placental hypoxia-reoxygenation is a potential cause of such stress. High levels of Reactive Oxygen Species (ROS) may induce cellular apoptosis. Thus, ROS-scavenging enzymes, such as Superoxide Dismutase (SOD) enzyme family, are important for preserving fetal growth [6].

Several placental histological and morphological abnormalities such as infarcts, terminal villous fibrosis and impaired trophoblastic invasion have been well described in FGR placentas [7-10]. Many studies have reported an association between abnormal Doppler velocimetry changes in umbilical artery and adverse perinatal outcome in growth restricted fetuses. The correlation between placental morphology and umbilical artery Doppler velocimetry, an indicator of placental vascular resistance, shows that substantial changes in the growth of villi and in fetal vasculature can reduce maternal-fetal exchanges of nutrients and oxygen and contribute to fetal hypoxic stress [11].

The human placenta, as a boundary organ between mother and fetus, plays a dynamic role in establishing and maintaining pregnancy through a multifunctional way involving continuous rearrangement in its structure. It is now widely accepted that the Extracellular Matrix (ECM) is a dynamic and highly specialized structure, involved in several signal transduction pathways [12]. For instance, ECM proteins are degraded by Matrix Metalloproteinases (MMPs), endopeptidase capable of processing a number of bioactive molecules. They are released by placental cells during tissue remodeling processes, cell migration and neoangiogenesis: aberrations in MMPs activity in early pregnancy can play a role in the

physiopathology of conditions like FGR [13]. It has been recently suggested that ECM proteins, such as fibronectin, tenascin and laminin, play different roles in cell proliferation, migration and differentiation. Tenascin, in particular, has shown to be able in modulating cellular adhesion by increasing or decreasing it, and seems to be involved in the immunological protection of the embryo during implantation. Moreover, tenascin seems to be involved in villous repairing after placental infarctions and in neoangiogenic mechanisms [14,15]. Also fibronectin, which often co-distributes with tenascin, and laminin, can induce migration of cell populations and metabolites [16]. Cell–matrix interactions represent a so important phenomenon that we can hypothesize that some changes in the structure and in the distribution of these proteins are involved in pregnancies complicated by FGR. Moreover, tenascin, whose expression has been correlated with villous growth, cells proliferation and fibrinoid deposition, may play a critical role in placental homeostasis development throughout pregnancy [14].

Tenascin is a large ECM glycoprotein. Until now five isoforms are known: tenascin-C, or cytotactin, which plays a regulatory role on neuron morphology and adhesion, representing the most abundant form [17], tenascin-X, whose gene is on chromosome 6 and is typically detected in muscles, tenascin-Y distributed on muscletendon junctions, tenascin-J or janusin, exclusive of the nervous tissue and tenascin-R which is synthesized by oligodendrocytes during mielinization [18].

Tenascin-C is mostly expressed in the mesenchymal villi, cell islands and columns. These structures are the proliferating units of the villous trees. Probably this molecule is involved in angiogenesis.

In addition, tenascin separates fibrinoid deposits at the surface of the villous trees from the fetal stroma. This location suggests a role of such protein in placental repair mechanisms and in immunoprotection of fetal tissues [19].

In the present study we have investigated the placental expression of non collage nous ECM components such as fibronectin and tenascins in pregnancies complicated by FGR with or without PE, with abnormal umbilical artery Doppler findings. Our attention was particularly appointed on the expression of tenascin-C, as this protein is involved in various aspects of cell and tissue development and in cell-to-cell and cell-to-substrate adhesion.

Our study includes a control group of healthy pregnancies with appropriate fetal gestational age and normal umbilical artery Doppler waveforms.

Patients and Methods

Patients

A number of 33 Caucasian pregnant women were recruited between January 2010 and December 2013. On the total, 23 patients affected by FGR, with or without PE, were considered for the study group (group A). All patients of the group A were submitted to cesarean section. The control group (group B) consisted of 10 pregnant women with appropriate fetal growth, homogeneous for age, BMI (20-25 kg/m2), socioeconomic status and gestational age to the group A (Table 1). In the group B, abnormal presentation and previous cesarean section were the indications for cesarean section. Gestational age was calculated using the crown-rump length ultrasonographic determination in the first trimester, according to the last menstrual period. Ultrasound criteria were applied to assess fetal growth. Fetuses were considered Appropriate for Gestational Age (AGA) if Abdominal Circumference (AC) was found between 10th and 95th percentile. Fetuses were considered affected by FGR, if abdominal circumference was found below the 10th percentile of our standard population fetal growth curves. At delivery, neonates were consisted Low Birth Weight (LBW) if neonatal weight was less than 2,500 g and Very Low Birth Weight (VLBW) if less than 1,500 g.

PE complicated 11 cases out of 23 of the group A. At delivery, placental tissue was collected with the permission of the local research, Ethics Committee and with the informed consent of all patients. Clinical characteristics of the two groups were compared by the one-way ANOVA test.

Doppler evaluation

The Doppler evaluation of umbilical artery resistance was performed within four hours from delivery in both groups. Doppler signals were obtained using an Echo Color Doppler Samsung Medison A30 and an Echo Color Doppler Samsung WS80 combined with a 3.5 MHz convex probe. The Doppler sample volume was placed on a free-floating tract of the umbilical cord during fetal quiet status. A near zero insonation angle was obtained for every Doppler examination. To analyze the

Doppler flow velocity waveforms, the Pulsatility Index (PI) was automatically calculated (maximum velocity–minimum velocity/mean velocity) and the average of three consecutive measurements was used for analysis to minimize intra-observer variability. Doppler findings were considered as mild if PI values were above the 2SD of the mean for gestational age based reference standards, moderate when the End-Diastolic Flow Was Absent (AEDF), severe when the End Diastolic Flow was Reversed (REDF). All measurements were performed by a single experienced investigator (G. N.) to minimize inter-observer variability.

Immunohistochemistry

Normal and pathological placental tissue samples were randomly harvested from both maternal and fetal side. Specimens were fixed and embedded in paraffin or cryopreserved at – 80°C. 5 μm-thick serial sections were cut, mounted on slides, and immunostained for Tenascin (Sigma, St. Louis, MO, USA, mouse monoclonal IgG) and Fibronectin (Sigma, rabbit polyclonal IgG) as previously described [20]. Briefly, immunohistochemistry was performed with indirect immunofluorescence technique: sections were incubated for 30 minutes with 10% serum derived from the species in which the secondary antibody was raised, and subsequently with primary antibody for 60 minutes at 37oC. After PBS washes, slides were incubated with rhodamine or fluoresce in conjugated anti-rabbit or anti-mouse IgG antibody for 60 minutes at 37oC. Nuclei were labeled with DAPI for 15 minutes at room temperature before the final washes in PBS and sections were then mounted in Vectashield. Signal was visualized with a Leica DMLB fluorescence microscope. Negative controls were included for each staining using an isotype-matched non-specific antibody. Microscopic analysis was performed by three independent observers, using a four point arbitrary scale ranging from 0 (total absence of immunopositivity) to 3 (very strong immunopositivity), and pictures were taken with digital camera connected to the microscope (Leica DC200).

RT-PCR

RT-PCR was used to analyze target gene expression in the present study. Total RNA was isolated by lysing frozen tissue samples (150- 300 mg) in Trizol solution (Life Technologies, GIBCO BRL) according to the supplier's protocol. RNA was

precipitated and quantified by spectroscopy. 1 μg of total RNA of each sample was reversely transcribed using the First-Strand cDNA Synthesis Kit (Amersham Pharmacia Biotech) according to the protocol supplied by the manufacturer. The random hexamer primers provided in the kit were used. The same cDNA product obtained from each sample was used for subsequent PCR amplification with the primer sets prepared for the target gene and GAPDH housekeeping gene. The amplification of the GAPDH gene was used as double internal control. The ratio between the samples and the housekeeping gene was calculated to normalize for initial variations in sample concentration and as a control for reaction efficiency. Primer sequences were designed using the software Primer 3 (developed by Steve Rozen, Helen J Skaletsky) available on-line at http://www-genome.wi.mit.edu. Semiquantitative Polymerase Chain Reaction (PCR) was performed using the following conditions: 95°C, 5 min initial denaturing phase; 95°C, 1 min; 55°C, 1 min; 72°C, 1 min for 35 cycles; 72°C, 10 min, final extension. The reaction was carried out in a total volume of 50 ml containing 3 μl of cDNA, 10-20 pmol of each primer, 200 mM each of dNTP, 1.5 mM $MgCl_2$ and 1 unit of Taq polymerase with the reaction buffer supplied with the kit. In each experiment, possible DNA contamination was determined by a control reaction in which cDNA was omitted from the reaction mixture and replaced by DNAse and RNAse free water.

The amplified products (12 μl of each sample) were analyzed by electrophoresis in a 2% agarose gel containing ethidium bromide, followed by photography under ultraviolet illumination. The levels of mRNA were estimated by densitometric scanning and normalized against GAPDH loading controls. Densitometric analyses of the PCR products were performed using the ImageJ v1.29 software (developed by Wayne Rasband) available on-line at http://rsb.info.nih.gov/ij/. All PCR products were purified using QIAquick PCR purification kit (Qiagen, Santa Clarita, CA, USA) and their identities verified by automated DNA forward and reverse sequencing using adideoxy terminator reaction chemistry for sequence analysis on the Applied biosystem Model 373A DNA sequence.

Results

Patients

Clinical characteristics of the two groups are shown in Table 1. No difference for age, parity and gestational age at delivery was found between the two groups. Both neonatal and placental weight were lower in the study group than in the control group ($p<0.001$). Increased perinatal morbidity was observed in the study group ($p<0.001$). Group A babies were further divided in two groups according to birth weight: 10 LBW and 13 VLBW neonates were found. PE was detected in 5 out of 10 pregnancies with LBW infant and in 6 out of 13 pregnancies with VLBW infant.

Doppler evaluation

Normal umbilical artery Doppler pattern was found in the control group (Figure 1a). Increased umbilical artery PI values above 2DS for our normal curves were found in all LBW fetuses (Figure 1b); AEDF (Figure 1c) or REDF (Figure 1d) were detected in all VLBW fetuses.

Immunohistochemistry

Fibronectin showed a normal distribution without significant differences between control and FGR placentas, with or without Doppler anomalies. Immunopositivity for Tenascin was very weak in healthy placentas (Figure 2), when compared with the FGR ones, where Tenascin was strongly expressed in both maternal and fetal side, with a mosaic-like pattern of distribution. To better relate Doppler findings with immunohistochemistry, Tables 2A and 2B show the Immunohistochemical and Doppler findings in the study group. Table 2A, in particular, regards low birth weight FGR. Table 3 shows Immunohistochemical observations as well as Doppler findings in the control group with appropriate birth weight (AGA).

RT- PCR

RT-PCR analysis allowed the comparison between the two groups of patients in terms of tenascin synthesis (Figure 3). Tenascin-C and tenascin-X were low in placentas from the control group, while they were synthesized in all the placental specimens from the group A. The expression of tenascin subunits was not affected

by individual variability in pathological specimens, but it is reasonable that its amount might be related to different gestational age.

Discussion

Placentation requires extensive vasculogenesis and subsequent angiogenesis with deep ECM involvement in tissue remodeling. Impaired placental perfusion and angiogenesis, resulting in placental pathology, are considered the most important causes of FGR [4,5]. According to umbilical Doppler analyses, FGR fetuses have been subdivided in groups of progressive severity [21]. Correlations between acid-base balance and Doppler findings show that only the most severe FGR fetuses have impaired oxygenation and acid-base balance [22].

Many studies documented severe histological and morphological abnormalities in placentas of affected fetuses [7,8,23,24]. In a previous investigation, we approached some factors involved in the balance between the oxidative stress and the expression of molecules preventing and/or protecting tissues, like SOD, MMP-2 and MMP-9, and some receptors for angiogenic factors, i.e. Vascular Endothelial Growth Factor Receptor (VEGFR)-2 and angiopoietin-1 receptor. We found that extracellular SOD, the main anti-oxidant enzyme in vascular wall, was significantly reduced in FGR placentas. As regards angiogenesis, we observed an increased expression of VEGFR-2 (early marker of neoangiogenesis) and a reduction of angiopoietin-1 receptor (marker of mature vessels) in FGR placentas. Moreover, MMP-2 and MMP-9 were constantly reduced in IUGR placentas. We hypothesized that oxygen levels affect ECM remodeling by MMP and neoangiogenesis as a consequence [25]. In another earlier study, we found extracellular matrix abnormalities more evident in fetuses affected by FGR with AEDF or REDF in umbilical artery [22]. These findings prompted us to hypothesize that the ECM structure plays a critical role in umbilical artery Doppler velocimetry regulation. An increased ECM stiffness and the consequential changes in the pressure gradient between fetus and placenta may exert an effect on the development of FGR and on the onset of abnormalities in Doppler findings in umbilical artery. Tenascin is poorly expressed in adult normal tissues, but it is involved in many wound repair mechanisms [14], being a peculiar ECM component mainly expressed during embryonic and tumor growth, but weakly in term placenta. In human placenta tenascin is expressed in a mosaic-like way in the

mesenchymal villi, cell islands and cell columns during the developmental placental stages [26].

Castellucci et al. [14,19] have investigated the expression of this molecule in the placenta as related to epitelial-mesenchymal interaction, cells proliferation and fibrin deposition. It is particularly interesting to study its expression and its distribution inside an organ like placenta, which has a very high metabolic activity and homes a lot of different cell population. In fact, some authors have hypothesized an immunomodulation and fetal protection function for tenascin; under this point of view, our observation that this protein strongly increases during PE supports the theory of a maternal immune system involvement in this kind of pathology.

Indeed tenascin distribution through gestation seems to suggest that this molecule plays a pivotal role in placental development. Also a positive modulation of cell migration seems to be due to this protein: it is well known, in fact, that it is involved in the detachment of neoplastic cells during breast cancer and in other kinds of neoplasm, as tenascin rich basal lamina are easily crossed by cells and molecules [35]. All these functions are particularly and characteristically expressed during early Placentation, becoming progressively less evident with placental physiological ageing [28]. Since tenascin C and X immunopositivity is very low in term placenta, it was surprising that tenascin X was often highly expressed in FGR specimens we studied, with a “mosaic” distribution within placental tissue.

Tenascin expression was more weak in the FGR fetuses with severe umbilical Doppler findings (ARED); on the contrary, it was very strong in the early gestation as well as in the FGR fetuses with moderate alteration in Doppler findings (PI> 2SD).

The presence of tenascin in the media of larger blood vessels of tumors and in capillary walls might be related with an angiogenic activity of this molecule, as improved tenascin-mediated angiogenesis and wound repair mechanism might be considered a compensatory mechanism in case of growth restriction with LBW.

The weak tenascin immunoreactivity in FGR fetuses with VLBW could be considered as a failed compensative mechanism of placental wound repair and angiogenesis.

Between the 28th and 32nd week of gestation, the reappearance of end diastolic flow in umbilical artery could lead to an improvement of abnormal umbilical artery velocimetry with less severe FGR, or to a persistent ARED flow that lead to the most severe FGR associated with abnormal umbilical artery Doppler velocimetry [29-31]. It is highly suggestive that these fluctuating values depend on wound repair mechanisms which operate during placental development.

Conclusion

Based on our results, strong tenascin immunoreactivity could be considered as a marker of improved wound repair and angiogenesis in these FGR fetuses with better perinatal outcome. Finally, impaired umbilical artery Doppler velocimetry with persistence of end diastolic flow would be possible when the wound repair mechanism in placental tissue does not fail. An ongoing study has been settled to verify if tenascin or related metabolites can be sampled in cervico-vaginal fluid. On these bases, tenascin could be a tailor biomarker of placental angiogenesis in FGR fetuses who have the possibility to respond to chronic hypoxia due to placental insufficiency.

Table 1: Clinical Features of Group A (FGR) and group B (AGA).

	FGR (group A)	AGA (group B)	*p*
Number of cases	23	10	
Age (media +/- DS)	28.8+/−4	21.2 +/− 5	0,525
Parity	0.6+/− 0.3	0.4+/− 0.2	0.338
G. A. (delivery)	35+/− 3.3	36.1+/− 2.1	0.003
Birth weight (g)	1680+/− 530	3002+/− 420	<0.001
Placental weight (g)	298+/− 84	480+/− 95	<0.001
Pre-eclampsia (n)	11/21	-	
Perinatal morbidity (n)	20	-	<0.001
PI > 2 DS (n/%)	9/%	-	<0.001
ARED (n/%)	12/%	-	<0.001

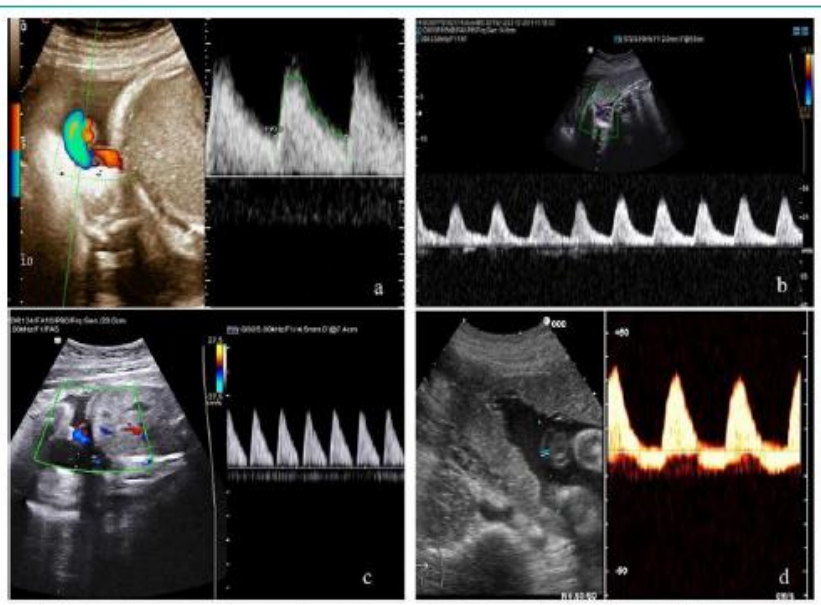

Figure 1: Normal umbilical artery Doppler pattern in the control group (a); increased umbilical artery PI values above 2DS in LBW fetuses (b); AEDF (c) and REDF (d) in VLBW fetuses.

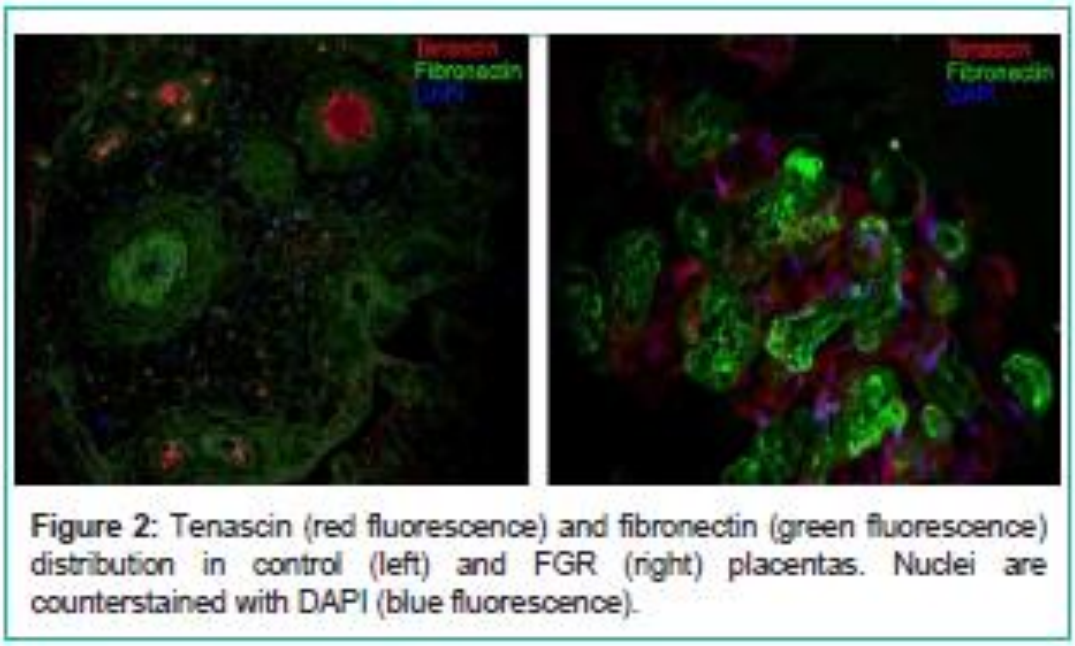

Figure 2: Tenascin (red fluorescence) and fibronectin (green fluorescence) distribution in control (left) and FGR (right) placentas. Nuclei are counterstained with DAPI (blue fluorescence).

Table 2A: Perinatal outcome, Doppler velocimetry and Immunohistochemical features in LBW (Group A) fetuses.

Cases	Birth weight	AG	FGR	FGR+PE	Tenascin	Fibronectin	Velocimetry Doppler
DCB	LBW	III trimester	•		+++	++	P.I.> 2 DS
GC	LBW	III trimester	•		++	+	P.I.> 2 DS
MM	LBW	III trimester		•	+++	+	P.I.> 2 DS
LM	LBW	III trimester	•		+++	++	P.I.> 2 DS
MR	LBW	III trimester		•	++/+++	+	P.I.> 2 DS
AL	LBW	III trimester		•	++/+++	+	P.I.> 2 DS
RA	LBW	III trimester	•		+++	+	P.I.> 2 DS
AT	LBW	III trimester		•	++	+	P.I.> 2 DS
TF	LBW	III trimester	•		+++	+	P.I. >2 DS
LP	LBW	III trimester		•	++/+++	+	P.I. >2 DS

Table 2B: Perinatal outcome, Doppler velocimetry and immunohistochemical features in VLBW fetuses (Group A).

Cases	Birth weight	AG	FGR	FGR+PE	Tenascin	Fibronectin	Velocimetry Doppler
PL	VLBW	III trimester		•	+/-	++	A.R.E.D
MS	VLBW	III trimester	•		+	++	A.R.E.D.
GM	VLBW	III trimester	•		+/-	+++	A.R.E.D.
LN	VLBW	III trimester	•		+	++	A.R.E.D.
AS	VLBW	III trimester		•	++	++	A.R.E.D.
DF	VLBW	III trimester		•	-	++	A.R.E.D.
SB	VLBW	III trimester	•		++	++	A.R.E.D.
AR	VLBW	III trimester		•	+/-	++	A.R.E.D.
RF	VLBW	III trimester	•		+/-	++	A.R.E.D.
MA	VLBW	III trimester		•	+/-	+	A.R.E.D.
LT	VLBW	III trimester	•		+	++	A.R.E.D.
GG	VLBW	III trimester		•	+/-	+	A.R.E.D.
LC	VLBW	III trimester	•		+	++	A.R.E.D.

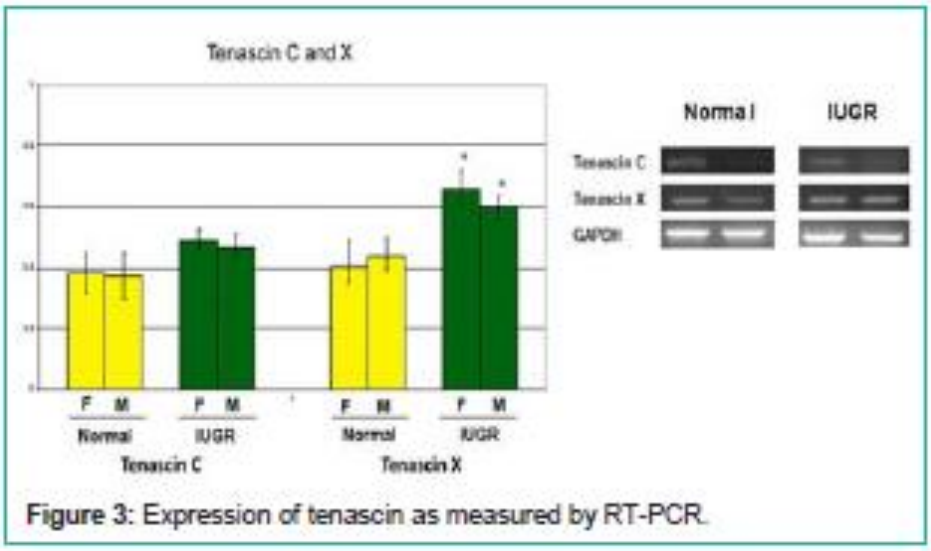

Figure 3: Expression of tenascin as measured by RT-PCR.

Table 3: Perinatal outcome, Doppler velocimetry and immunohistochemical features (Group B).

Cases	Birth weight	AG	Tenascin	Fibronectin	Velocimetry Doppler
PL	AGA	III trimester	+	+++	Regular PI
RT	AGA	III trimester	+/-	++/+++	Regular PI
MG	AGA	III trimester	+/-	++/+++	Regular PI
DA	AGA	III trimester	+	++/+++	Regular PI
MP	AGA	III trimester	+	+++	Regular PI
GG	AGA	III trimester	+/-	++	Regular PI
SR	AGA	III trimester	+/-	+++	Regular PI
ST	AGA	III trimester	+	++	Regular PI
TT	AGA	III trimester	+/-	++/+++	Regular PI
CA	AGA	III trimester	+	+++	Regular PI

References

1. The investigation and management of the small for gestational age fetus. Green–top Guideline No. 31, 2nd Edition. 2013.

2. Intrauterine growth restriction. ACOG Practice Bulletin. 2000; 12.

3. Gaudineau A. [Prevalence, risk factors, maternal and fetal morbidity and mortality of intrauterine growth restriction and small-for-gestational age]. J Gynecol Obstet Biol Reprod (Paris). 2013; 42: 895-910.

4. Alfaidy N, Hoffmann P, Boufettal H, Samouh N, Aboussaouira T, Benharouga M, et al. The multiple roles of EG-VEGF/PROK1 in normal and pathological placental angiogenesis. Biomed Res Int. 2014; 451906.

5. Helske S, Vuorela P, Carpén O, Hornig C, Weich H, Halmesmäki E. Expression of vascular endothelial growth factor receptors 1, 2 and 3 in placentas from normal and complicated pregnancies. Mol Hum Reprod. 2001; 7: 205-210.

6. Nishimura T, Duereh M, Sugita Y, Yoshida Y, Higuchi K, Tomi M, et al. Protective effect of hypotaurine against oxidative stress-induced cytotoxicity in rat placental trophoblasts. Placenta. 2015; 36: 693-698.

7. Madazli R, Somunkiran A, Calay Z, Ilvan S, Aksu MF. Histomorphology of the placenta and the placental bed of growth restricted foetuses and correlation with the Doppler velocimetries of the uterine and umbilical arteries. Placenta. 2003; 24: 510-516.

8. Viscardi RM, Sun CC. Placental lesion multiplicity: risk factor for IUGR and neonatal cranial ultrasound abnormalities. Early Hum Dev. 2001; 62: 1-10.

9. Iskender-Mazman D, Akcoren Z, Yigit S, Kale G, Korkmaz A, Yurdakok M, et al. Placental findings of IUGR and non-IUGR. Turk J Pediatr. 2014; 56: 368-373.

10. Zhang S, Regnault TR, Barker PL, Botting KJ, McMillen IC, McMillan CM, et al. Placental adaptations in growth restriction. Nutrients. 2015; 7: 360-389.

11. Herrera EA, Krause B, Ebensperger G, Reyes RV, Casanello P, Parra- Cordero M, et al. The placental pursuit for an adequate oxidant balance between the mother and the fetus. Front Pharmacol. 2014; 5: 149.

12. Chen CP, Aplin JD. Placental extracellular matrix: gene expression, deposition by placental fibroblasts and the effect of oxygen. Placenta. 2003; 24: 316-325.

13. Zhu J, Zhong M, Pang Z, Yu Y. Dysregulated expression of matrix metalloproteinases and their inhibitors may participate in the pathogenesis of pre-eclampsia and fetal growth restriction. Early Hum Dev. 2014; 90: 657- 664.

14. Castellucci M, Classen-Linke I, Mühlhauser J, Kaufmann P, Zardi L, Chiquet- Ehrismann R. The human placenta: a model for tenascin expression. Histochemistry. 1991; 95: 449-458.

15. Minamitani T, Ikuta T, Saito Y, Takebe G, Sato M, Sawa H, et al. Modulation of collagen fibrillogenesis by tenascin-X and type VI collagen. Exp Cell Res. 2004; 298: 305-315.

16. Korhonen M, Virtanen I. Immunohistochemical localization of laminin and fibronectin isoforms in human placental villi. J Histochem Cytochem. 2001; 49: 313-322.

17. Flück M, Tunc-Civelek V, Chiquet M. Rapid and reciprocal regulation of tenascin-C and tenascin-Y expression by loading of skeletal muscle. J Cell Sci. 2000; 113: 3583-3591.

18. Deckner M, Lindholm T, Cullheim S, Risling M. Differential expression of tenascin-C, tenascin-R, tenascin/J1, and tenascin-X in spinal cord scar tissue and in the olfactory system. Exp Neurol. 2000; 166: 350-362.

19. Castellucci M, Kosanke G, Verdenelli F, Huppertz B, Kaufmann P. Villous sprouting: fundamental mechanisms of human placental development. Hum Reprod Update. 2000; 6: 485-494.

20. Postiglione L, Ladogana P, Montagnani S, di Spigna G, Castaldo C, Turano M, et al. Effect of granulocyte macrophage-colony stimulating factor on extracellular matrix deposition by dermal fibroblasts from patients with scleroderma. J Rheumatol. 2005; 32: 656-664.

21. Pardi G, Cetin I, Marconi AM, Lanfranchi A, Bozzetti P, Ferrazzi E, et al. Diagnostic value of blood sampling in fetuses with growth retardation. N Engl J Med. 1993; 328: 692-696.

22. Locci M, Nazzaro G, De Placido G, Nazzaro A, Colacurci N, Montagnani S, et al. Correlation of Doppler and placental immunohistochemical features in normal and intrauterine growth-retarded fetuses. Ultrasound Obstet Gynecol. 1993; 3: 240-245.

23. Macara L, Kingdom JC, Kaufmann P, Kohnen G, Hair J, More IA, et al. Structural analysis of placental terminal villi from growth-restricted pregnancies with abnormal umbilical artery Doppler waveforms. Placenta. 1996; 17: 37-48.

24. Mayhew TM, Ohadike C, Baker PN, Crocker IP, Mitchell C, Ong SS. Stereological investigation of placental morphology in pregnancies complicated by pre-eclampsia with and without intrauterine growth restriction. Placenta. 2003; 24: 219-226.

25. Guerra G, Calabrese D, Mele V, Tafuri D, D'Anna M, Di Carlo C. et al. Proceeding of 62nd National Congress of Italian Society of Anatomy, 2008 September 14-16, Italy: Italian Journal of Anatomy and Embriology. 2008.

26. Watson AL, Burton GJ. A microscopical study of wound repair in the human placenta. Microsc Res Tech. 1998; 42: 351-368.

27. Yoshida T, Akatsuka T, Imanaka-Yoshida K. Tenascin-C and integrins in cancer. Cell Adh Migr. 2015; 9: 96-104.

28. Chen CP, Aplin JD. Placental extracellular matrix: gene expression, deposition by placental fibroblasts and the effect of oxygen. Placenta. 2003; 24: 316-325.

29. Hanretty KP, Whittle MJ, Rubin PC. Reappearance of end-diastolic velocity in a pregnancy complicated by severe pregnancy-induced hypertension. Am J Obstet Gynecol. 1988; 158: 1123-1124.

30. Brar HS, Platt LD. Antepartum improvement of abnormal umbilical artery velocimetry: does it occur? Am J Obstet Gynecol. 1989; 160: 36-39.

31. Soregaroli M, Bonera R, Danti L, Dinolfo D, Taddei F, Valcamonico A, et al. Prognostic role of umbilical artery Doppler

velocimetry in growth-restricted fetuses. J Matern Fetal Neonatal Med. 2002; 11: 199-203.

Intrauterine growth restriction and fetal cerebral redistribution. Risk stratification and neurological outcome.

The fetal brain is particularly vulnerable to the effects of intrauterine growth restriction (IUGR) (5). Long-term neurological disorders such as cerebral palsy and epilepsy, as well as learning and attention difficulties, neurobehavioural disabilities, and other cognitive and sensory issues have been attributed to restricted growth of the fetus (29). The long-term care of a child with compromised brain development is associated with emotional stress for families and a direct cost on society. Currently there are limited treatments to prevent neurological impairment in the IUGR neonate (5).

Fetal growth restriction is a complex process, and a delayed diagnosis is associated with increased perinatal morbidity with neurological impariment and mortality; IUGR occurs in approximately 5 e 10% of pregnancies. The term refers to poor growth of a fetus while in the mother's womb during pregnancy; is a term used to describe a baby who is smaller than the usual amount for the gestational age due to pathological compromise. Placental insufficiency or utero-placental dysfunction results in insufficient blood flow to the placenta during pregnancy and inadequate supply of nutrients and oxygen to support normal growth of the fetus. Thus, the fetus develops in a chronic hypoxic environment. Placental insufficiency can result in changes in fetal metabolism, hormones, hematology, immunology and cardiovascular function.

The fetus hemodynamically adapts to this pathology (detected by an increase in the umbilical artery pulsatility index—UAPI) through the vasodilation of cerebral circulation (detected by a decrease in the middle cerebral artery pulsatility index—MCAPI). The resulting hyperperfusion is considered pathological (29[b]). This ‘brain-sparing effect’ is associated with an abnormal cerebral/umbilical ratio that quantifies the redistribution of cardiac output by dividing the Doppler

indices of the middle cerebral artery (MCA) with that of the umbilical artery (UA). During pregnancy M1 tract pulsatility's indice (initial landmark), are lower than those from M2 tract (distal part at the extreme wings of large sfenoid), with statistically significant differences. But, when a depletion of the mechanisms of compensation occurs, damage to brain tissue can be seen. The loss of these mechanisms, following the persistence of hypoxia, causes the activation of the cascade of vascular, metabolic and biochemical events, with the releasing of free radicals, typical in case of ischemia with apoptosis and irreversible neuronal necrosis.

Aim of this study was to asses changes in cerebral blood flow in the proxymal (M1) and in the distal tract (M2) of middle cerebral artery in severely growth restricted fetuses in relation to neonatal outcome and neurological morbidity at 2 years of age.

Patients and Methods

This is a prospective observational study. The participants were identified from a cohort of single infants born alive between May 2013 and December 2014 in a single academic center (a level III perinatal center in Naples) and then recruited for the follow-up study at 1-2 years.

Patients were considered to be at risk for fetal growth restriction if they had a fetal abdominal circumference (AC) <5th centile or calculated estimated fetal weight (EFW) <10th centile (13, 30).

Inclusion criteria included gestational age between 24 0/7 and 36 6/7 weeks and an EFW than 500 g or greater; patients were recruited if they had ultrasound examination of the dating of pregnancy and if they had more than one sonographic examination performed at a greater than 2-week interval. Patients were excluded from analysis for the following: fetal death, major fetal defect, presence of twins, lack of a complete Doppler evaluation and lack of a delivery record. Patients with fetal growth restriction were managed according to our institutional protocol, with indications for delivery at gestational age ≥ 34 weeks, a suspected nonreassuring fetal status based on antenatal testing,

progression to reversal of end-diastolic flow, and other obstetric indications such as the development of preeclampsia.

Antenatal surveillance should provide longitudinal assessment that is designed according to the severity of the fetal condition, and directs appropriate intervention to improve outcomes.

All sonographic examinations were performed in a single ultrasound unit. Biometry was performed by measuring the biparietal diameter, head circumference, abdominal circumference, and femur length using the equations of Hadlock et al (31) for calculation of the estimated fetal weight. The umbilical artery (UA) and middle cerebral artery Doppler waveforms were recorded using color Doppler and the pulsatility index (PI) was calculated according to a standard protocol; umbilical artery Doppler findings were obtained and the umbilical artery was considered abnormal if the pulsatility index was above the 95th percentile, if there was absent end-diastolic flow, or if there was reversal of end-diastolic flow (32); we evalueted the occurence of brain sparing effect in middle cerebral artery (circle's centralization).

After birth and before the clamp, has been evalueted a determination of pH and acid-base balance; the infants were then evaluated at 1 and 2 years of life for evaluation of neurological outcomes.

The children included in the study were followed up in the first 24 months of life through dedicated pediatric outpatient clinic. For neurological evaluation was used Amiel-Tison neurological examination – ATNE; we considered mild psychomotor retardation for QS = 71-85. The Griffiths Mental Development Scales is used to measure the rate of development of young children; the six areas of development measured by the scales include: Locomotor, Personal-Social, Hearing and Language, Eye and Hand Co-ordination, Performance and Practical Reasoning.

10 children were lost to follow up at 24 months.

The birthweight values were converted into centiles and Doppler parameters converted into multiples of median (MoM), adjusting for gestational age using reference ranges.

Adverse perinatal outcomes evaluated included cesarean delivery for fetal bradycardia, umbilical artery pH less than 7.0, 5-minute Apgar scores less than 7.0, respiratory distress syndrome and major complications: perinatal death or irreversible damage to organs.

The demographic characteristics and gestational age at the initial dating sonography were similar between our study groups.

Data were analized by ANOVA test, using the SPSS statistical software.

Results

The patients were classified according to middle cerebral artery Doppler into two groups: A group (28 fetuses) presented "abnormal ACM" (loss of BSE in M2), and abnormal UAPI (>95th percentile, absent or reversed umbilical artery end diastolic velocity); B group (36 fetuses) with brain sparing effect in both M1 and M2 and abnormal UAPI.

The average weight was 1075.62±264.06 SD (gr); the mean gestational age at birth was 32.61±2.37 SD (weeks).

The A group show a greater number of days admission to the NICU (26.1±15.92 days), gestational age was lower and cesarean delivery own to suspected fetal distress were more frequent (17.8% vs 8.3%). There were no differences in acidosis at birth (umbilical artery pH). None of the infants had a 5-min Apgar score < 7. We found a significantly reduced growth rate in fetuses with an abnormal ACM (56% birth weight <3th percentile), as opposed to those with normal umbilical artery Doppler findings.

The fetuses of B group had demonstrated a better neonatal outcome than A group.

Two cases of cerebral palsy have been described in group A (momoplegia and diplegia associated with strabismus).

It was found an inverse relationship between umbilical artery pulsatility index and percentile at birthweight; also the estimated fetal weight below the 3th percentile for gestational age has been shown to be associated with the loss of brain sparing effect in M2 tract.

Birth weight less than 1000 g showed a worse neurological outcome at one and two years of life (p 0.0); weight <3th centile is correlated with a delayed psychomotor development at 12 months (p 0.03) (we do not observe a correlation with the neurological examination at 24 months probably for a loss to follow up). Psychomotor development shows a positive trend in correlation with birth weight percentiles, at 12 months (p 0.06).

By analizing all infants, QS 12 mean was 100.05±7.106; QS 24 mean was 98.75±10.953.

Discussion

These results suggest that fetal cerebral redistribution is a risk factor for IUGR infants and that loss of compensation's mechanism identifies those experiencing greatest weakness.

On the other hand, the persistence of an adaptive response like BSE allows a behavior of waiting to implement the preventive therapy for prematurity / immaturity, for example; but it is also, possible that delayed childbirth results in a worsening of prognosis.

Longitudinal studies suggest that fetal Doppler parameters change progressively during fetal deterioration: ductus venosus becomes abnormal at later stages of fetal compromise. Their combined use in clinical algorithms, might provide opportunities for the assessment of fetal wellbeing.

Concerning brain Doppler, the multicenter study of Baschat et al. reported an relative risk of 3.3 for neonatal morbidity in fetuses with abnormal MCA Doppler. Of note, IUGR fetuses with normal MCA Doppler were similarly at risk of detected cranial abnormalities when compared to normal preterm newborns, reflecting its high negative predictive value.

The middle cerebral arteries supply the majority of the lateral surface of the hemisphere, except the superior portion of the parietal lobe and the inferior portion of the temporal lobe and occipital lobe. In addition, they supply part of the internal capsule and basal ganglia. In its territory

lie the motor and sensory areas (distal part) excluding leg and perineum and auditory and speech areas. It is not a coincidence that in advanced stages of fetal compromise we witness the worst changes in biophysical profile with reduced fetal movements too (33).

Since in our managing protocol, MCA Doppler was used as additional criterion for elective delivery, the results of A group might substantiate the current use of the MCA Doppler as a sonographic marker for quantification of placental insufficiency and centralization of fetal blood flow, including as indicator of poor outcome and reduced fetal growth rates. Lower growth rates have been correlated with increased perinatal morbidity (33) although an absolute cutoff at which this risk increased has yet to be determined.

Delivery at lower gestational ages results in risk of significant complications of prematurity. This information is clinically useful in that it would distinguish those patients most likely to benefit from aggressive measures, such as maternal hospitalization, maternal transfer to a tertiary care center, aggressive fetal monitoring, administration of corticosteroids to promote fetal maturation and magnesium for neuroprotection, when indicated.

Our findings suggest that IUGR infants with an abnormal MCA are at increased risk for poorer outcomes and could develop deficits in cognitive functioning in childhood.

We conclude that the MCA Doppler assessment, in addition to the UA Doppler, may be a clinical tool to differentiate patients with FGR that may progress more rapidly towards the delivery to earlier gestational age and therefore more likely will benefit from an increased level of surveillance.

From a clinical perspective, the prediction of neurological morbidity is a major challenge in modern obstetrics and lays the basis for timely delivery and future preventive interventions.

Pending submission

		Birth Weight	Gestational Age
N	Valid	73	73
	Missing	0	0
Mean		1075,62	32,612329
Median		1090,00	33,000000
Std. Deviation		264,060	2,3791306

Birth Weight Percentiles		Frequency	Percent
Valid	1	41	56,2
	2	13	17,8
	3	19	26,0
	Total	73	100,0

Neurological Examination 12 months		Frequency	Percentile
Valid	1	66	90,4
	2	4	5,5
	3	1	1,4
	5	1	1,4
	Total	72	98,6
Missing	System	1	1,4
Total		73	100,0

Neurological Examination 24 months		Frequency	Percent
Valid	1	59	80,8
	2	3	4,1
	5	1	1,4
	Total	63	86,3
Missing	System	10	13,7
Total		73	100,0

QS 12 months		Frequency	Percentile
Valid	1	64	87,7
	2	2	2,7
	3	1	1,4
	6	5	6,8
	Total	72	98,6
Missing	System	1	1,4
Total		73	100,0

QS 24 months		Frequency	Percentile
Valid	1	54	74,0
	2	3	4,1
	3	2	2,7
	4	1	1,4
	5	2	2,7
	6	2	2,7
	Total	64	87,7
Missing	System	9	12,3
Total		73	100,0

	NE 12 months Birth weight	NE 24 months Birth weight	QS 12 months Birth Weight Percentiles
P value	0.0	0.0	0.03

NE neurological esamination
QS psychomotor development

OUTCOME	A Group (28)	B Group (36)	P value
pH	7,25±0,12	7,28±0,09	0.838
acid-base balance	-5,40±3,89	-4,76±3,30	0.64
5-min Apgar score	0	0	1.000
	1	10	1.000
Cesarean delivery			
Admission NICU days	26.1±15.92	8.81±11.54	0.08

II Research line : Therapy and prediction of preterm birth in singleton pregnancies at low risk and high-risk twin pregnancies

Definitions of 'preterm', 'term' and 'post-term' birth have been specified by the American Academy of Pediatrics, American College of Obstetricians and Gynecologists, and the World Health Organization (34, 35). Accordingly, preterm birth occurs ≤ the last day of the 37th week (day 259) and is defined by the presence of regular uterine contractions and progressive dilation of the cervix.

Preterm birth can be the result of three obstetrical circumstances: 1) preterm labor with intact membranes (50%); 2) preterm prelabor rupture of membranes (PROM) (30%); and 3) "indicated" preterm birth, which occurs when maternal or fetal indications require delivery before 37 weeks of gestation (20%). The most common indications are preeclampsia and small for gestational age (SGA).

The increased vulnerability of moderate preterm infants (moderate PT) ($32^{0/7}$-$33^{6/7}$ weeks of gestation) and late preterm infants (late PT) ($34^{0/7}$–$36^{6/7}$) in the neonatal period is now widely accepted (36, 37) and undoubtedly these infants have both excess morbidity and mortality.

A recent executive summary of proceedings from a joint workshop sponsored by the Society for Maternal–Fetal Medicine, the National Institute of Child Health and Human Development (NICHD), the Section on Perinatal Pediatrics of the American Academy of Pediatrics (AAP), and the American College of Obstetricians and Gynecologists (ACOG), in which a diverse group of experts were invited to participate, defined *periviable birth* as delivery occurring from 20 0/7 weeks to 25 6/7 weeks of gestation; the survival varies per week from 6%, 26%, 55% to 72% (Raju 2014).

15 million babies are born too soon every year; more than 1 in 10 babies are born preterm, affecting families all around the world. Over 1 million children die each year due to complications of preterm birth. Many survivors face a lifetime of disability, including learning disabilities and visual and hearing problems. There is a dramatic

survival gap for premature babies depending on where they are born. For example, over 90% of extremely preterm babies (<28 weeks) born in low-income countries die within the first few days of life; yet less than 10% of babies of this gestation die in high-income settings, a 10:90 survival gap (38). Furthermore, mortality for extremely preterm infants (22 – 26 weeks) is reduced in centres offering the highest level of intensive care (tertiary centres), compared with less specialist centres (OR 0.73 (95% CI 0.59 to 0.9)), supporting the recommendation that care is centralised. Transport of these infants during the first 48 hours is, however, associated with increased rates of severe IVH (39) and babies born in tertiary centres have significantly better morbidity free survival than infants transferred there after birth (OR 1.92 (95% CI 1.02 to 3.6)) (40). While the difference in morbidity is likely multifactorial, the findings emphasise the importance of coordinated neonatal and obstetric network strategies for safe antenatal centralisation and improving neurodevelopmental outcome for preterm infants is an important challenge for neonatal medicine. The disruption of normal brain growth and neurological development is a significant consequence of preterm birth and can result in physical and cognitive impairments. While advances in neonatal medicine have led to progressively better survival rates for preterm infants, there has only been a modest improvement in the proportion of surviving infants without neurological impairment, and no change in the proportion with severe disability (39). The overall number of children with neurodisability due to prematurity is increasing.

In the preterm infant, defining hypoxic–ischemic injury (HII), its clinical course, monitoring, and outcomes remains complex. Few studies examine preterm HIE, and these are heterogeneous, with variable inclusion criteria and outcomes reported.

Although many preterm births remain unexplained, in high-income countries, their rise was associated with increasing maternal age at delivery, the greater use of assisted reproductive technologies resulting in higher frequency of twin pregnancies and in some countries, cesarean sections performed before the 39th week of gestation. By

contrast, the causes most frequently associated with preterm delivery in low-income countries are infections, malaria, HIV and the increased frequency of teenage pregnancy along with social deprivation and the lack or insufficiency of care at preconception period, pregnancy and childbirth.

Given the high incidence of preterm birth and its sequelae, it is necessary to investigate the multiple pathogenic mechanisms underlying this syndrome, so as to implement an etiological therapy to prevent neurological injury. Considering, therefore, the multifactorial etiology, is not justifiable or recommended a single treatment for all pregnant women. It is necessary, however, to recognize, change and delete the risk factors and identify and treat early high-risk patients.

The common pathway of parturition has been defined as the anatomical, physiological, biochemical, endocrinological, immunological and clinical events that occur in the mother and/or fetus at the time of parturition regardless of whether this occurs at term or preterm (41). The most well-known components of the common pathway of parturition are the uterine components because they are clinically apparent to obstetricians and patients. Such components include: 1) increased myometrial contractility; 2) cervical ripening/dilatation and effacement; and 3) membrane/decidual activation. The onset of spontaneous labour at term is the result of physiological signals and in most cases there is synchronous activation of the common pathway; whereas preterm parturition is the consequence of pathological signals that activate the common pathway of parturition and the activation may be asynchronous.

It is usually difficult to identify all causes of preterm labor. However, we can refer two groups:

1. 1. related to inflammatory-infectious disease

   Account for about 25-40% of the cases as documented by microbiological and histological studies on amniotic fluid, placenta and membranes.

   The mechanisms by which intrauterine infections lead to preterm labour are related to activation of the innate immune system (42).

Liggins was the first to liken cervical ripening to an inflammatory response. Microorganisms can gain access to the amniotic cavity by: (1) ascending from the vagina and the cervix (the most common pathway); (2) haematogenous dissemination through the placenta; (3) accidental introduction at the time of invasive procedures; and (4) by retrograde spread through the fallopian tubes. Microbial endotoxins and proinflammatory cytokines stimulate the production of prostaglandins, other inflammatory mediators, and matrix-degrading enzymes. Prostaglandins stimulate uterine contractility, whereas degradation of extracellular matrix in the fetal membranes leads to PPROM.

2. On a non-inflammatory basis: uterine ischaemia, uterine overdistension, abnormal allogenic recognition, allergic-like reaction, cervical disease, endocrine disorders.

Most of these mechanisms of disease operate in non-pregnant women. It is also possible that preterm parturition may be caused by mechanisms of disease that are unique to the maternal–fetal relationship (43), because of its unique anatomy, physiology, immunology and metabolic demands. Consequently, we remain open to the discovery of undescribed mechanisms of disease during pregnancy.

For an optimal prevention of PTB, risk stratification should be based on a combination of risk factors, obstetric history, and screening tools. Defining risk factors for prediction of preterm birth is a reasonable goal for several reasons. There are many maternal or fetal characteristics that have been associated with preterm birth, including maternal characteristics, pregnancy history, present pregnancy characteristics, psychological characteristics, adverse behaviours, infection, uterine contractions and cervical length, and biological and genetic markers. The recurrence risk in women with a previous preterm delivery ranges from 15% to more than 50% and this risk is inversely related to the gestional age of the previous preterm birth (44).

These risk factors, however, are neither sensitive nor specific, so most of the women who give birth preterm are not identified on a risk basis and most women considered at high risk do not give birth preterm. In addition, its accuracy is particularly low among primiparous mother.

Delaying childbirth can reduce long-term morbidity, promoting the development of organs and systems. Corticosteroid administration before anticipated preterm birth is one of the most important antenatal therapies available to improve newborn outcomes.

The need for early treatment leads often to unnecessary hospitalization of patients with considerable engagement of human and financial resources and the use of drugs with potential side effects for the mother and the fetus. For this reason new predictive markers have been explored.

Evaluation of the gravid cervix uteri is an important part of prenatal care, especially in the patient at risk for preterm birth. Seeking a method of cervical length measurement that could be used easily regardless of patient habitus, location of the cervix, and gestational age, it's used ultrasound cervical length measurements that can help identify women at risk; another sonographic finding in pregnancies at risk of preterm delivery is 'funneling' or dilatation of the internal os.

Early identification of women at risk allows early detection of threatened preterm delivery and this is one of the main goals in obstetric care.

The therapeutic approach to the prevention and care of preterm delivery is based on the use of different tocolytic agents with the aim to stop labour for at least 48 hours, to accelerate the pulmonary maturity of the fetus and reduce the incidence of respiratory distress syndrome and intraventricular hemorrhage. The second leading objective is to reduce perinatal mortality and morbidity related to severe prematurity (45); *t*ocolytic agents are recommended by 24 + 0 to 33 + 6 weeks. Several drugs at same time is not recommended for the significant increase of maternal and fetal adverse events; *t*ocolysis without the concomitant use of corticosteroids and maintenance therapy for a period longer than 48 hours are not supported by evidence of effectiveness. The tocolytic agents used are: magnesium sulphate, prostaglandin-synthetase inhibitors, calcium-antagonists, progesterone, b-mimetics and oxytocin-antagonists. In early gestational age of 22 to 28 weeks gestation to

delay childbirth than a day causes an increase in neonatal survival of 3%.

The PTB rates have increased over the last 30 years; a delay in delivery means not only decrease the psychological and social implications related to the problem, but also reduce the economic costs of intensive and protracted neonatal care. The "cost" of a preterm birth should be evaluated both in a psycho-social context is purely economic zone.

It should be remembered, in fact, the strong impact that premature birth can have both on the child, with the beginning of learning difficulties or disabilities, either on the parents when these complications cause much discomfort and emotional distress often long term.

Romero R has argued that obstetrical disorders are really syndromes, as well as preterm delivery, and refers to them as “The Great Obstetrical Syndromes” (46). The features of “The Great Obstetrical Syndromes” are the following: 1) multiple aetiology; 2) long preclinical stage; 3) frequent fetal involvement; 4) clinical manifestations which are often adaptive in nature; and 5) predisposition to a particular syndrome is influenced by gene–environment interaction and/or complex gene–gene interactions involving maternal and/or fetal genotypes.

Advancing the research agenda is a critical need to reduce the burden of preterm birth, requiring innovations for both prevention and care; providing quality care means doing the right thing at the right time.

Objective

In this study, we evaluated the effectiveness of two innovative treatments: the repetition of the cycle with antagonists of oxytocin in twin pregnancies with persistent risk of preterm delivery and the use of 'docosahexaenoic acid' (DHA) endovaginal in symptomatic women at low risk with contractile activity uterine acute. Also we assessed the PartoSure Test that is a rapid, non-invasive strip test for the detection of placental alpha microglobulin-1 (PAMG-1) in patients presenting with signs and symptoms of preterm labour; this test is most useful in cases where cervical length measurement (CL) is between 15 and 30 mm and the predictive value of CL is lowest for prediction of spontaneous preterm delivery within 7 days in patients with threatened preterm labour.

Two cycles of Atosiban in preventing preterm birth in twin pregnancies

Locci Mariavittoria, Nazzaro Giovanni, Miranda Marilena, Iazzetta Raffaella, Salzano Emilia, **Palmieri Teresa**, De Placido Giuseppe

Clinical Obstetrics, Gynecology and Reproductive Medicine, 2016
doi:10.15761 / COGRM. 1000155 Volume 2(4): 221-224

Abstract

Twin gestation contributes significantly to perinatal morbidity and mortality related to high occurrence of preterm birth. We evaluated 202 women consecutively selected with twin pregnancies and threatened preterm labor. 98 women were threatened with a single cycle of Atosiban; 34% of them delivered before 34 weeks. The study group consisted of 104 patients submitted to a second cycle of Atosiban because of regular uterine contractions and or cervical length modifications occurred from 48 hours to 7 days and gestational age was prior to 32 week of gestation (group A). After the second cycle of Atosiban, 49 out of 104 patients received a treatment

with a vaginal tablet of lactoferrin (group B). After the second cycle of Atosiban, 84% of patients of group A and 90% of patients of group B delivered after 34 weeks.

The overall rate of delivery before 34 weeks in the studies groups was of 16%. In our experience, repeated cycles of Atosiban have shown effectiveness in delaying delivery in twin pregnancies. It seems logical to use an oxytocin receptor antagonist as first line drugs in twin pregnancies because of the increased risk of pulmonary edema.

Introduction

Twin gestation contributes significantly to perinatal morbidity and mortality related to high occurrence of preterm birth. Over the last decade, the number of assisted-reproduction twins have been increasing, doubling the twining rates in Western countries [1]. In addition, twin gestations imposed financial and psychological burdens on the family and society [2]. In Campania, a Southern region of Italy, the birth rate from assisted reproduction increased from 6% in 2007 to 16,5% in 2012. Overall, the increased risk of maternal and fetal morbidity associated with twin compared with singleton pregnancies is nowadays the strongest argument for single embriotransfer in the debate of assisted reproduction. At least 60% of all twins are born before the 37th week of gestation. In the last seven years twin gestation in our department increased from 3.5% to 7.7% in 2013. In several double blind trials Atosiban has demonstrated a tocolytic effectiveness similar to beta agonists, with the advantages of no side effects due to its specific effect on uterine tissue [3,4].

Moreover, women with multiple pregnancy have a higher risk of pulmonary edema then women with singleton pregnancy. Pulmonary edema occurs in approximately 1/400 women treated with beta-agonists [5]. In twin pregnancies, the levels of urinary aldosterone are increased. This secondary hyperaldosteronism may be further increased by betaagonists, which rise both aldosterone and renin levels. This condition induces fluids retention and plasma volume expansion, leading to a greater risk of pulmonary edema [5].

Prevention of preterm labor is the goal of long-term tocolysis. Women with recurrent episodes of recurrent preterm labor are at risk for preterm premature rupture of membrane (pPROM), further

cervical changes and preterm delivery [6]. Atosiban has been used in Reproductive Medicine Unit of the University of Naples Federico II from 2003 to date, as the first line tocolytic drug in women with threatened preterm delivery in twin pregnancy. We have previously reported our experience in a small series of twin pregnancies submitted

to a second treatment with Atosiban because the presence of symptoms and signs of preterm labor within seven days from the first treatment. This therapeutic approach showed to be relevant for those patients. On the basis of this preliminary experience, we conducted a retrospective cohort study, with the aim to evaluate the effectiveness of repeated Atosiban administration in twin pregnancies over the last ten years in our single centre.

Material and methods

This was a retrospective study of twin pregnancies with 276 consecutive women from April 2003 to December 2013 conducted at the Reproductive Medicine Unit of the University of Naples Federico II. All women with twin pregnancies with threatened preterm labor with or without contractions and reduced cervical length were eligible for the study. Exclusion criteria were maternal age <18 years, fetal death, major fetal defect, severe twin-to-twin transfusion syndrome or selective fetal growth restriction, and cervical length >20mm. On the basis of this criteria 74 patients were excluded.

Diagnosis at admission were: 113 preterm labors, 50 preterm premature ruptures of membranes, 21 incontinent cervices cerclage, 18 vaginal bleedings (Figure 1). On the total, 172 twin pregnancies derived from reproductive assisted techniques. Preterm labor was defined as ≥4 uterine contractions / 30 min and or cervical length <20 mm with or without uterine contractions. pPROM was proven by a sterile speculum examination (RCOG guidelines N° 44/2006). Atosiban was administered according to the recommended protocol with an initial bolus of 6.75 mg in 1 minute followed by a high rate infusion of 18 mg/hour over 3 hours and a lower concentration of 6 mg/hour over a period of 45 hours. During the first cycle of Atosiban, all women received corticosteroids prophylaxis (antenatal administration of 12 mg of betamethasone, followed by a second dose 24 hours later) to enhance lung maturation. The second cycle of Atosiban was administered when regular uterine contractions and or cervical length modifications occurred from 48 hours to 7 days after the first cycle and when

gestational age was prior to 32 weeks of gestation (group A) (Figure 2). Bacterial vaginosis was detected in 22% of women and was treated with antibiotic therapy; erythromycin (250 mg orally 6 hourly) was given for 10 days following the diagnosis of pPROM in all patients. After the second cycle of atosiban, 49 out 104 patients received a treatment with a vaginal tablet

of lactoferrin (Lf) (300 mg) for 21 days (group B) (Figure 3). 98 pregnant women received a single dose of Atosiban just to perform the corticosteroids prophilaxis in 48 hours. This group of patients (the control group) refers to patients evaluated before our preliminary reported experience.

Statistical analysis was performed using GRAPH Pad Prism 4.0 software (Graph-pad Software Inc, La Jolla, CA). The distribution of variables was compared with Student' s Test for paired data. Differences associated with p values lower than 0.05 were considered statistically significant. The results are reported as mean +/- standard deviation.

Results

After 48 hours from the end of the first cycle of Atosiban, 98 women didn't show uterine contractions and/or cervical length modifications; 34% of them delivered before 34 weeks. While 104 women had regular uterine contractions and/or cervical length modifications (group A). 22% of those patients had recurrent symptoms of preterm labor from 48 hours, whereas the remaining 78%

showed symptoms within 7 days (Figure 3). After the second cycle, 84% of patients of group A and 90% of patients of group B delivered after 34 weeks (Figure 4). No difference was detected between spontaneous twin pregnancies and assisted reproduction ones. The overall mean number of days gained after the start of tocolysis was 38 in the group of preterm labor, whereas in the subgroup of women with pPROM the interval until delivery was 19 days; in the group B the days gained were 52. After initial bolus administration, only 5% of women

presented drug related side effects such as flushing for 1-5 minutes. During infusion, 3% of women experienced side effects
possibly related to Atosiban such as nausea and vertigo. The overall rate of delivery before 34 weeks in the studies groups was of 16%.

Discussion

In this retrospective study repeated Atosiban administration resulted effective in prolonging twin pregnancies. In multiple pregnancies, over distension of the uterus with stretching of fetal membranes and or cervical ripening is a more likely mechanism for its release than infection or inflammation. The uterine over distension in twin pregnancies may cause a relative decidua ischemia with release of lysosomal enzymes. These enzymes include several phospholipases which initiate the prostaglandins cascade by releasing arachidonic acid, the critical substrate for their synthesis. The increased release of prostaglandins affects collagen integrity altering the consistence and distensibility of the cervix [7]. There is an inverse relationship between cervical length and the
likelihood ratio for preterm birth. Atosiban acts by blocking oxytocinreceptors. Oxytocin seems to initiate myometrial contractility by both a direct and an indirect mechanism. It directly interacts with membrane receptors leading to an increase in intracellular calcium. The indirect effect of oxytocin is the stimulation of prostaglandins release from decidua and fetal membranes; the prostaglandins are strictly involved in cervical ripening [8].

Recently a Cochrane Database Systematic Review on oxytocin receptor antagonists for inhibiting preterm labor underlines the importance of well studies about different tocolytic strategies at different gestational ages in order to optimize safety and efficacy [9].

Furthermore, Atosiban determined fewer maternal side effects in comparison with other tocolytics [9]. As we previously demonstrated in a selected group of pregnant from ART, the use of oxytocin receptor antagonist in the mid second trimester would appear to be an useful tool to delay preterm labour when prostaglandins release activates

oxytocin receptors [3]. Multiple pregnancies are also associated with a major maternal plasma volume expansion and secondary hyperaldosteronism [5]. This conditions increase the risk of pulmonary edema, particularly when tocolytic therapy with beta agonists combined with fluid therapy is administered. Beta mimetics increase both aldosterone and renin levels in twin pregnancies and may potentiate the risk of pulmonary edema [5,10].

Moreover, same studies revealed that Atosiban is cost-saving versus beta mimetics, due to its superior safety profile [4,11]. In our experience, repeated cycles of Atosiban have shown effectiveness in delaying the interval to delivery, in twin pregnancies. The rate of preterm birth < 34week of gestation with single cycle of Atosiban was 34% (n=33) compared with the overall rate of the studies groups of 16%. It seems logical to use an oxytocin receptor antagonist as first line drugs in twin pregnancies because of the increased risk of pulmonary edema. In addition, the use of vaginal lactoferrin (Lf), an approximately 80-KDa iron-binding glycoprotein of the transferrin family with bacteriostatic and bactericidal properties, was usefull in the maintenance therapy, in our experience. Several studies [8,12,13] suggest that Lf plays an important role against cervicovaginal inflammation and or infection by decreasing levels of interleukin (IL)-6 and increasing cervical length. Both drugs, Atosiban and Lf, seem able in preventing preterm delivery caused by cervical ripening, blocking prostaglandins release. Twin pregnancies are at an increased risk of stillbirth, antenatal morbidity and infant death. This is largely due to preterm delivery and complications of prematurity and low birth weight are the primary risk factors for infant mortality. The NICU admission rate was lowest following delivery at 38 weeks' gestation, and the NICU admission rate

decreased significantly with each additional week of pregnancy from 32 through 38 weeks' gestational age. We found an increased risk of mortality associated with delivery at 34 weeks' [14-16]. We hope that these data provide information to assist providers for the management of threatened preterm labor in twin pregnancies. Based on this

consideration and the safety profile of Atosiban, two cycles are better than one in symptomatic patients before 32 weeks of gestation, in our experience, even if corticosteroid prophylaxis is already performed This management is used to reduce perinatal morbidity/mortality due to extremely prematurity in those twin pregnancies with no additional complications that may indicate earlier delivery. In addition maintenance therapy using vaginal Lf improve gestational age delivery as showed in the study group B.

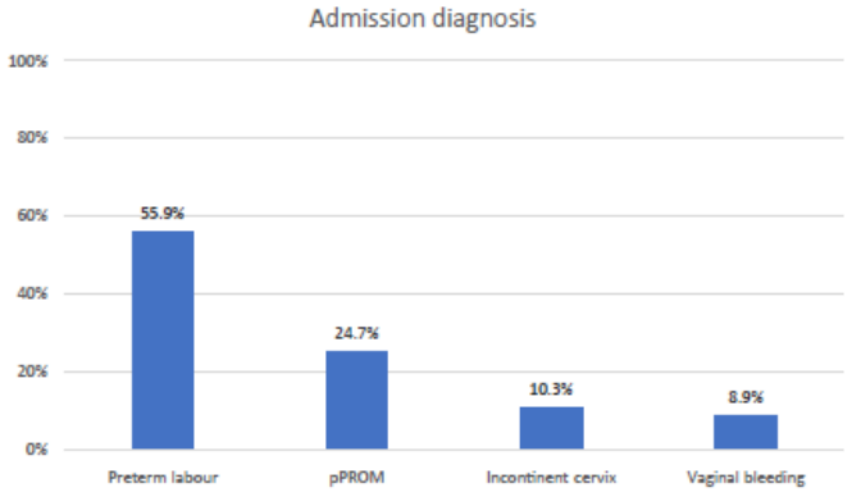

Figure 1. Study population characteristics at admission.

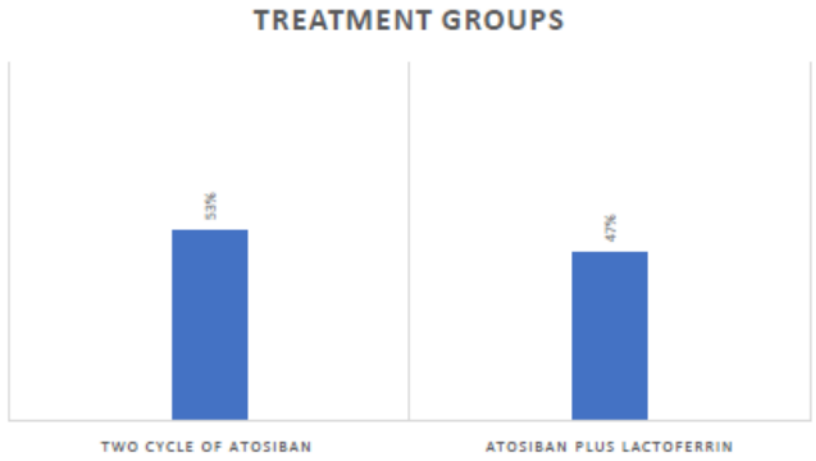

Figure 2. Two studies groups.

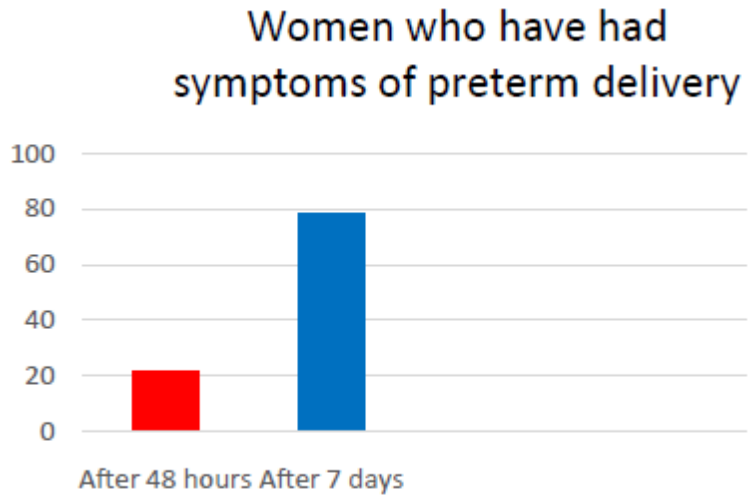

Figure 3. Percentage of patients that respectively showed symptoms of preterm delivery after the first treatment with atosiban.

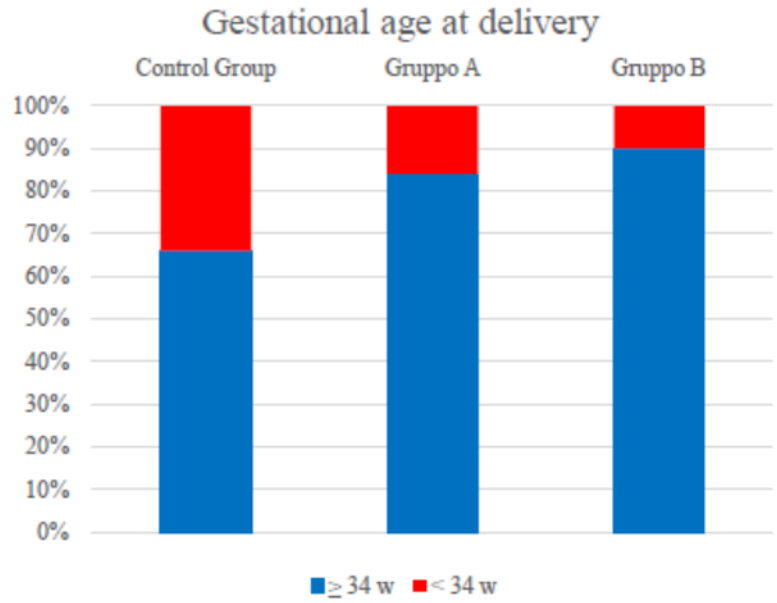

Figure 4. Percentage of patients who delivered <34 weeks in the study groups.

References

1. Ananth CV, Chauhan SP (2012) Epidemiology of twinning in developed countries. *SeminPerinatol*36: 156-161.

2. Luke B, Brown MB, Alexandre PK, Kinoshi T, O'Sullivan MJ, et al. (2005) The cost of twin pregnancy: maternal and neonatal factors. *Am J Obstet Gynecol* 192: 909-915.

3. Locci M, Nazzaro G, Merenda A, Pisaturo ML, Laviscio P, et al. (2006) Atobisan versus ritodrine used prophylactically in ICSI pregnancy to prevent pre-term birth in women identified as being at high risk on the basis of transvaginal ultrasound scan. *J Obstet Gynecol* 26: 396-401.

4. Shim JY, Park YW, Yoon BH, Cho YK, Yang JH, et al. (2006) Multicentre, parallel group, randomized, single blind study of the safety and efficacy of atosiban versus ritidrine in the treatment of acute preterm labour in Korean women. *BJOG* 113: 1228-1234.

5. Lamont RF (2000) The pathophysiology of pulmonary oedema with the use of betaagonists. *BJOG* 107: 439-444.[Crossref]

6. Wu MY, Chen SU, Lee CN, Ho HN, Yang YS (2010) Use of atosiban in a twin pregnancy with extremely preterm premature rupture in the membrane of one twin: a case report and literature review. *Taiwan J Obstet Gynecol* 49: 495-499.

7. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, et al. (2008) The preterm parturition syndrome. *BJOG* 113: 17-42.

8. Locci M, Nazzaro G, Miranda M, Salzano E, Montagnani S, et al. (2013) Vaginal lactoferrin in asymptomatic patients at low risk for pre-term labour for shortened cervix: cervical length and interleukin-6 changes. *J Obstet Gynaecol* 33: 144-148.

9. Flenady V, Reinebrant HE, Liley HG, Tambimuttu EG, Papatsonis DN (2014) Oxytocin receptor antagonists for inhibiting preterm labour. *Cochrane Database Syst Rev:*CD004452.

10. Dudenhausen J (2006) 'Normal' pregnancy with adverse events on initial tocolytic treatment. *BJOG* 113 Suppl 3: 116-117.

11. Wex J, Abou-Setta AM, Clerici G, Di Renzo GC (2011) Atosiban versus betamimetics in the treatment of preterm labour in Italy: clinical and economic importance of sideeffects. *Eur J Obstet Gynecol Reprod Biol* 157: 128-135.

12. Giunta G, Giuffrida L, Mangano K, Fagone P, Cianci A (2012) Influence of lactoferrin in preventing preterm delivery: a pilot study. *Mol Med Rep* 5: 162-166.

13. Poppiti R, Locci M, Nazzaro G (2009) Prevention of preterm delivery in twin pregnancies with atosiban. *J Matern Fetal Neonatal Med* 92: 61.

14. Page JM, Pilliod RA, Snowden JM, Caughey AB (2015) The risk of stillbirth and infant death by each additional week of expectant management in twin pregnancies. *Am J Obstet Gynecol* 212: 630.

15. Conde-Agudelo A, Romero R (2014) Prediction of preterm birth in twin gestations using biophysical and biochemical tests. Am J Obstet Gynecol 211: 583-595.

16. Liem SM, van de Mheen L, Bekedam DJ, van Pampus MG, Opmeer BC, et al. (2013) Cervical length measurement of the prediction of pre-term birth in symptomatic women with a twin pregnancy: a systematic review and metanalysis. Obstet Gynecol Int:125897.

Role of Vaginal DHA on Uterine Contractions in Patients at Low Risk for Preterm Birth

Locci Mariavittoria, Nazzaro Giovanni, Iazzetta Raffaella, **Palmieri Teresa**, Miranda Marilena, Tirone Monica, Salzano Emilia, De Placido Giuseppe

Journal of Women's Health and Gynecology 2016 | Vol 3: 102

Summary

Preterm labor is a public health problem and to this day, it's the great challenge of modern obstetrics. We conducted a randomized double-blind controlled study. At the admission, all patients were submitted to cardiotocography. Patients with regular contractions but without decreasing of cervical length or funneling at transvaginal sonography were admitted. 90 patients at 24-34 weeks of gestation were finally recruited. Patients were randomly divided in two groups: A group (45 patients) received a single transvaginal dose of docosahexaenoic acid (1 gr of DHA); B group (45 patients) received placebo. At 120 minutes, cardiotocographic examination was performed in all patients. A group showed a significant decrease in number and intensity of contractions ($p<0.05$) as compared to B group. The results of our study show that vaginally administered DHA during pregnancy is effective on uterine contractions providing a significant decrease of spontaneous contractions and probably reducing the risk of preterm delivery.

Introduction

Preterm labor is defined as regular uterine contractions resulting in changes in the cervix that start between 24 weeks of pregnancy and 37 weeks of pregnancy. The preterm birth is the leading cause of perinatal mortality and morbidity, accounting for 28% of neonatal mortality worldwide. Although the cause is often unknown, a variety of factors may play a role in preterm birth; inflammation is the most common and most important. Vaginal infections seem to play an important role. Infections result in the activation of the inflammatory cascade, which precedes and causes the shortening of the cervix. On this basis, we focused our studies on inflammatory mediators, including cytokines and chemokines, in women's cervix [2]. The fatty acids are precursors of molecules such as prostaglandins, leukotrienes and thromboxanes: all these inflammatory mediators play a role in the pathophysiology of preterm birth [7]. The human body can produce the saturated and monounsaturated fatty acids but cannot synthesize omega-3 and omega-6 polyunsaturated fatty acids (PUFA). The precursors of these two families- alpha linolenic acid (ALA) and linoleic acid (LA)- are called "essential"; LA and ALA are required in the diet [3,4]. Foods with a higher content of omega- fatty acids include salmon, mackerel, sardines, herring, tuna, anchovies (blue fish). Dietary LA serves as the precursor for the n-6 series of polyunsaturated fatty acids (PUFAs) and dietary ALA is the precursor for the n-3 PUFA series. ALA is converted to eicosapentaenoic acid (EPA) and then in docosahexaenoic acid (DHA), while LA is converted to arachidonic acid (AA). DHA is an essential component of cell membranes, where it modulates, together with the EPA, the right degree of fluidity and permeability. The optimal omega-3/omega-6 fatty acids ratio is 1:5 whereas the western diet ranges from 10:1 to 25:1, fueling concern that this diet, characterized by low fish consumption, leads to a decline of omega-3 PUFAs intake in favour of omega-6 PUFAs and to an altered omega-6/omega-3 ratio from 1:10 to 1:20, a so called pro-inflammatory ratio. In fact, omega-3 fatty acids are essential and can only be obtained from

the diet [8]. The increased intake of omega-3 in proportion to other fats would

result in the down-regulation of the synthesis of omega-6 and consequently the decreased production of pro-inflammatory cytokines [7]. The American Dietetic Association (ADA) with the Dietitians of Canada [2] recommend at least 500 mg/day of PUFAs for all healthy adults including pregnant and lactating women. The European Commission with the International Society for the Study of Fatty Acids and Lipids (ISSFAL) specifically recommends that pregnant and lactating women consume a minimum of 200 mg DHA per day [7,8]. These recommendations could be met by consuming 1 to 2 portions per week of fish with high content of omega-3 fatty acids, which is the recommendation by Health Canada [8] and the United States Dietary Guidelines Advisory Committee for all women [4]. Since this considerations we evaluate the role of docosahexaenoic acid on uterine contractions to show if this molecule can be of use in reducing the early symptoms and signs of preterm delivery.

Materials and Methods

We conducted a randomized double-blind controlled study by February 2014 to November 2014. The study was conducted at the Reproductive Medicine Unit of the University of Naples Federico II. We enrolled 90 symptomatic patients between 24-34 week of gestation, for threatened preterm labour. The study was approved by the Ethics Committee of the University Federico II (Protocol's number 268/13). The patients underwent informed consent. The patients were divided into 2 groups according to random criteria, 45 patients in the treatment group (A group) and 45 to the placebo group (B group). 140 pregnant patients with pelvic pain at 24-34 weeks were evaluated. At the

admission patients were submitted to digital vaginal examination, transvaginal ultrasonography and cardiotocography (CTG). Only patients with regular contractions at CTG (4 in 20 minutes or 8 in 60 minutes, lasting at least 30 seconds) (1) and without clinical or sonographic modifications of the cervix were considered for the study.

The inclusion criteria were the following:

1. Caucasian women between 18 and 38 years of age
2. Singleton pregnancy
3. Uterine contractions reported
4. Regular uterine activity demonstrated to cardiotocography pattern (> 4 contractions in 20 minutes)
5. Cervical length > 25 mm and absence of funneling at transvaginal ultrasound
6. Intact membranes
7. Obstetric history negative for preterm labor.

Patients with the following characteristics were excluded:

1. Maternal age < 18 and > 38
2. Multiple pregnancy
3. Cervical length < 25 mm and / or presence of funneling at transvaginal ultrasound
4. Infections of the genito-urinary tract (positive vaginal/urine culture)
5. Intra-amniotic infections, based on clinical and biochemical parameters: maternal temperature > 38°C, malodorous vaginal discharge, maternal leucocytosis (>15000 cell/mm3), maternal tachycardia (>100 beats/min), uterine tenderness
6. Pre-pregnancy or gestation pathologies (such as maternal autoimmune diseases, antiphospholipid syndrome, gestational hypertension, preeclampsia)

Finally, 90 patients were enrolled in the study. Patients were randomly allocated in the 2 group, the treatment group (A group) and the placebo group (B group) using computer-generated numbers in sealed envelopes (Figure 1). The patients were randomized to receive a single vaginal capsule of docosahexaenoic acid (-Metra-medical device CE Class III produced by Pharmarte, registration number CE 0373 containing fish oil rich in DHA - 1g) or vaginal placebo capsule. The placebo capsules were specially manufactured to look identical to the DHA capsules. The capsules were placed in sacs and then stored in envelopes numbered from 1 to 90. The envelopes were numbered and

randomized according to computer-generated randomization tables to ensure an equal number of patients in each arm. Access to the randomization code was available only to the pharmacist who manufactured the placebo and packed the envelops and was not available to any of the treating physicians or patients. Patients were revalued by cardiotocography pattern after 120 minutes after administration of the preparation. The primary endpoint was to determine the number of uterine
contractions (<4 in 20 minutes or < 8 in 60 minutes) by tocography after 120 minutes from vaginal administration of one gram of DHA.

Statistical analysis

Statistical analysis was performed using Graph Pad Prism 4.0 software (Graph Pad Software Inc., La Jolla, CA). The t test was used for assessing the significance of the difference between continuous variables. The X2 test or the Fisher exact test was used to assess the statistical significance of categorical varables. Differences associated with p values lower than 0.05 were considered statistically significant. The results are reported as mean ± standard deviation (SD). A significant difference was found in terms of number of uterine contractions at CTG between the two groups ($p< 0.05$).

Results

In A group the mean number of contractions was significantly lower after the treatment (6.11±1.30 vs 3.13±3.33, p=0.0001) (Figure 2) while in B group the difference was not significant (5.28±1.32 vs 5.55±1.45, p=0.36) (Figure 3). Patients in A group showed a significant reduction in the mean number of contractions at cardiotocography after 120 minutes of docosahexaenoic acid somministration than in B group (3.13±3.33 vs 5.55±1.45, p=0.0001) (Figure 4). The number of patients with a reduction of uterine contractions was significantly higher in A group. In particular, in A group, 20 patients (44%) had no contractions at the post-treatment CTG, 11 patients (24%) had a reduction in the number of contractions (< 6 in 60 minutes); in 9 patients (20%) the

number of contractions was unchanged and in 5 patients (11%) was detected worsening of symptoms. In B group, no patients showed improvement of symptoms, 28 patients (62%) presented an unchanged cardiotocographic pattern before and after the placebo, 15 patients (33%) showed worsening of the symptomatology and only 2 patients (5%) had a reduction in the number of contractions (Table 1).

Discussion

Variation of dietary intake plays a key role in reducing some risks associated with pregnancy, such as risk of fetal and infant mortality, low birth weight [12,13] and premature births [2]. In this study, we observed that vaginally administered DHA during pregnancy acts on uterine contractions, in symptomatic women resulting in muscle relaxation and induces disappearance of spontaneous contractions in many cases. Several randomized controlled trials have shown that supplementation

with omega-3 may influence the birth process by delaying the onset of labor, reducing the risk of recurrent preterm birth and in animal studies it seems to have a tocolytic effect [2,15]. The exact DHA's mechanisms of action are not well understood, the eicosanoid-mediated changes on myometrial contractions and the connective tissue remodeling [5,12,15] seem involved. A high omega-6/omega-3 fatty acid ratio will result in increasing proinflammatory eicosanoid production; these metabolites have been associated with labor at term and preterm.

A higher intake of omega 3 fatty acids leads to a down-regulation of the synthesis of omega-6 fatty acids and consequently reduces the production of prostaglandins responsible for cervical changes in early labor. In additional, several studies have shown that DHA significantly reduces the secretion of IL-8 and IL-6 from the amnion [2,7]. These cytokines are strongly associated with the onset of labor and this activity may contribute to the ability of DHA, introduced by diet supplements, to prolong gestation and to decrease the risk of preterm delivery before 34 weeks. Animals with n-6 fatty acid deficiency and those fed with high dose of n-3 fatty acids have depressed

prostaglandins synthesis and increased length of gestation [8]. Another possible effect on the duration of pregnancy could be linked to the disorganization of the electrical myometrial activity: this could determine a delay in the onset of rhythmic and regular myometrial contractions [4]. Several randomized controlled trials showed a reduction of preterm delivery with DHA supplementation during pregnancy [6] n−3 supplementation was associated, compared to no supplementation in control subjects, with significantly greater duration of pregnancy [8]. The supplementation with marine
oil in pregnancy is associated with a mean gestational age at delivery 2.6 days longer than women allocated placebo or no treatment and did have a lower risk of giving birth before 34 completed weeks' gestation compared with placebo [7]. Studies performed until today are based on the effects of oral administration of 200 mg omega-3 fatty acids, however, since oral administration is subject to hepatic metabolism and rapid inactivation, we hypothesized that the use of a vaginal formulation of DHA may provide a better bioavailability due to uterine-first-pass-effect, bypassing liver metabolism and directly reaching the cervix. Therefore, we evaluated the efficacy of intravaginal formulation of 1gr of DHA, which, in fact, resulted in a more effective activity on myometrium than the oral one. Avoiding the first-pass effect, vaginal administration could have a more potent cervical action, with a reduction of omega 6 synthesis and consequent lower production of PGE2
and PGF2α.

Conclusion

Maternal long-chain polyunsaturated fatty acids status, particularly docosahexaenoic acid (DHA), during pregnancy may influence maternal and infant outcomes [2,13]. Maternal lifestyle and nutrition have long been recognized as important factors for both perinatal health and for the long-term health of the infant. Fish oil capsules are nearly devoid of mercury and other harmful compounds and can serve to augment omega-3 fatty acids in the diet. The implications of DHA

supplementation on fetal development, maternal outcomes and later infant growth is worth being elucidated and is promising in its potential for a positive impact on fetal and maternal outcomes [6].

The vaginal administration allows the use of dosage five times higher than the oral one, similar to the dosage tested in animal studies [3]. Furthermore, by passing the gastrointestinal absorption, DHA acts directly on the cervix and on the feto placental unit, contrasting hyperoxia [2]. Placental hyperoxia contributes to a persistent imbalance between pro-inflammatory and anti-inflammatory mechanisms that leads to preterm birth and neonatal injuries [17]. The potential benefits of DHA intake (1 gr) in patients at risk of preterm birth should be confirmed and further studies are needed to evaluate the effects and effectiveness of long-term treatment with DHA.

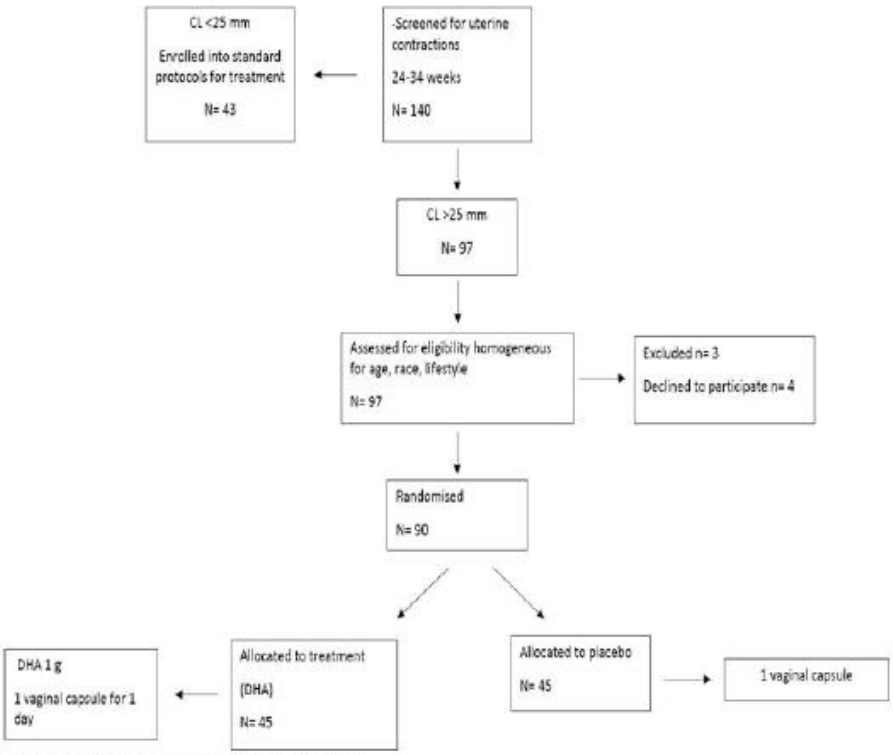

Figure 1: Flow chart for the allocation of the patients.

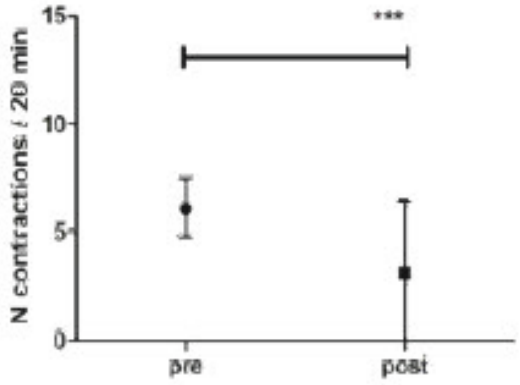

Figure 2: Mean number of contractions before and after treatment with DHA (A group).

***** p 0.0001**

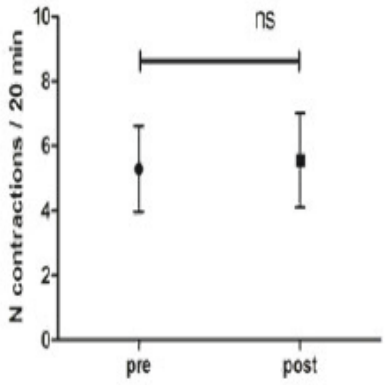

Figure 3: Mean number of contractions before and after placebo (B group).

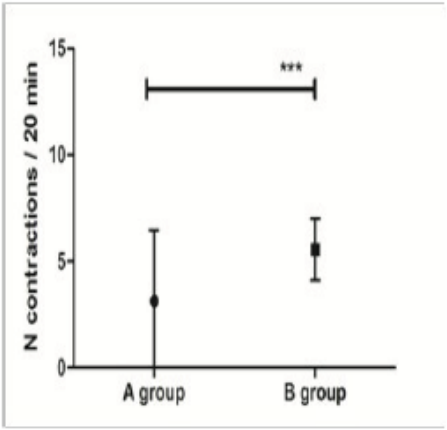

Figure 4: Mean number of contractions after treatment in A group e B group.
*** p 0.0001

Table 1: Effect of treatment on uterine contractions.

Frequency of uterine contractions	No contractions	Decreasing	Unchanged	Increasing
A group n=45	20 (44)	11 (24)	9 (20)	5 (11)
B group n=45	0 (0)	2 (5)	28 (62)	15 (33)
P value	< 0.001	< 0.05	<0.001	< 0.05

Data are presented as number of cases and percentage (%).

References

1) AAP & ACOG (1997) Guidelines for Perinatal Care. (4th edn) 100. Livello VI (ACOG III).

2) Baguma-Nibasheka M, Brenna JT, Nathanielsz PW (1999) Delay of preterm delivery in sheep by omega-3 long-chain polyunsaturates. Biol Reprod 60: 698-701.

3) Churc MW, Jen KL, Dowhan LM, Adams BR, Hotra JW (2008) Excess and deficient omega-3 fatty acid during pregnancy and lactation cause impaired neural transmission in rat pups. Neurotoxicol Teratol 30: 107-117.

4) Dietary Guidelines for Americans (2005) Report of the Dietary Guidelines Advisory Committee on Dietary Guidelines for Americans.

5) Giorlandino C, Giannarelli D (2013) Effect of vaginally administered DHA fatty acids on pregnancy outcome in high risk pregnancies for preterm delivery: a double blinded randomised controlled trial. Journal of Prenatal Medicine 7: 42-45.

6) Hanebutt FL, Demmelmair H, Schiessl B, Larque E, Koletzko B (2008) Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. Clin Nutr 27: 685-693.

7) Hansen HS, Olsen SF (1988) Dietary (n-3)-fatty acids, prostaglandins, and prolonged gestation in humans. Prog Clin Biol Res 282:305-317.

8) Health Canada (2002) Prenatal Nutrition. Health Canada, Ottawa, Ont, Canada.

9) Holmar NT (1998) The slow discovery of the importance of omega 3 essential fatty acids in human health. J Nutr. 128: 427S-433S.

10) Hornstra G (2000) Essential fatty acids in mothers and their neonates. Am J Clin Nutr 71:1262S-1269S.

11) Koletzko, B., Cetin, I., and Brenna, J.T (2007) Dietary fat intakes for pregnant and lactating women. Br. J. Nutr. 98: 873-877.

12) Koletzko B, Lien E, Agostoni C, Bohles H, Campoy C, et al. (2008) The roles of long-chain polyunsaturated fatty acids in

pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. J. Perinat. Med 36: 5-14.

13) Kris-Etherton PM, Innis S (2007) American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. J. Am. Diet. Assoc. 107:1599-1611.

14) Locci M, Nazzaro G, Miranda M, Salzano E, Montagnani S, et al. (2013) Vaginal lactoferrin in asymptomatic patients at low risk for pre-term labour for shortened cervix: cervical length and interleukin-6 changes. J Obstet Gynaecol. 33: 144-148.

15) Makrides M, Duley L, Olsen SF (2006) Marine oil, and other prostaglandin precursor, supplementation for pregnancy uncomplicated by pre-eclampsia or intrauterine growth restriction. Cochrane Database Syst Rev. 19.

16) Olsen SF, Hansen HS, Sorensen TI, et al. (1986) Intake of marine fat, rich in (n-3)-polyunsaturated fatty acids, may increase birthweight by prolonging gestation. Lancet 2:367-369.

17) Sharma D, Nkembi AS, Aubry E, Houeijeh A (2015) Maternal PUFA ω-3 Supplementation Prevents Neonatal Lung Injuries Induced by Hyperoxia in Newborn Rats. Int J Mol Sci 14: 22081-22093.

18) Szajewska H, Horvath A, Koletzko B (2006) Effect of n-3 long chain polyunsaturated fatty acid supplementation of women with low-risk pregnancies on pregnancy outcomes and growth measures
at birth: a meta-analysis of randomized controlled trials. Am J Clin Nutr 83: 1337-1344.

α-LIPOIC ACID AS MAINTENANCE THERAPY IN PATIENTS TREATED FOR PRETERM BIRTH

E. Salzano, R. Iazzetta, **T. Palmieri,** G. Nazzaro, M. Miranda, M. Locci

Journal of Pediatric and Neonatal Individualized Medicine 2017;6(1):e060125

INTRODUCTION

Preterm birth is the leading cause of perinatal and neonatal morbidity and mortality in developed countries contributing to 60-80% of all neonatal deaths. However, despite the intense work done by researchers in recent decades, the incidence of preterm birth remained virtually unchanged over the past 40 years. The etiology of preterm birth is multifactorial. Preterm birth would represent an uterine inflammatory response characterized by the production of prostaglandins by the amnion-chorion membranes and the decidua, with subsequent triggering uterine contractions and cervical modifications. α-lipoic acid (ALA) has antioxidant, as well as anti-inflammatory and immunomodulatory activity and presents no toxicity even at doses much higher than the therapeutic dose. The use of ALA in pregnancy is spreading due to its safety, tolerability and documented anti-inflammatory action on the placenta. The vaginal administration of ALA is a new approach that can provide a direct effect on vaginal and uterine level, also in view of the low bioavailability after oral administration.

METHODS

The purpose of this study was to compare the therapeutic efficacy of ALA versus no treatment and versus progesterone (Pg) vaginally administered, in a selected group of patients with threatened preterm delivery after successful acute tocolytic therapy, considered for high risk due to cervical length < 20 mm. 125 patients were initially managed according to our clinical protocol after admission:

Prophylactic corticosteroid betamethasone i.m. 12 mg/day for 2 days and primary tocolysis (Atosiban or beta-mimetics for 48 hours). Patients who responded successfully to tocolytic therapy were still considered at high risk for cervicometry values < 20 mm. The patients were divided into three groups based on treatment modalities after primary tocolysis: group A (n = 43), vaginal Pg; Group B (n = 42), ALA; Group C (n = 40), no treatment.

RESULTS

We observed a reduction in preterm births (Group A = 23%, Group B = 16% versus Group C = 50%), in recurrence of uterine contractile activity (Group A = 16%, Group B = 9% versus Group C = 32%), and in number of admissions to the Neonatal Intensive Care (Group A = 18%, Group B = 14% versus Group C = 42%), in groups A and B. We found no differences between the group of patients treated with Pg and the group treated with ALA, except for a greater compliance to local therapy with ALA due to a lower frequency of adverse reactions ($p < 0.05$).

CONCLUSIONS

The results of our study seem to support the role of progesterone as maintenance therapy, although in contrast to most of the scientific evidence. ALA appears to be a potential new treatment for the prevention of preterm delivery, allowing to prolong pregnancy in at risk patients, representing a valid alternative to progesterone. As its ability in reaching its site of action, the vaginal route seems to be favourite. ALA is safe, well tolerated, and vaginal administration has a good compliance and therefore it can be considered a viable integration to support tocolysis, even considering that maintenance therapy with progesterone is not supported.

The role of partosure™ test in predicting imminent preterm birth

E. Salzano, G. Nazzaro, M. Miranda, **T. Palmieri**, R. Iazzetta, M. Locci

Journal of Pediatric and Neonatal Individualized Medicine 2017;6(1):e060125

INTRODUCTION

An accurate risk assessment of preterm birth is clinically important in pregnancies with threatened preterm labor. This is particulary true with respect to both the administration of corticosteroids, as well as the transfer of patients to a tertiary care center capable of caring for the birth of premature infants. Clinical evaluation alone, with the measurement of cervical length and dilatation, are not sufficiently predictive of imminent delivery. Currently available biomarker tests, such as the detection of fetal fibronectin, have extramely poor predictive value form imminent delivery. The PartoSure™ test is a rapid, qualitative immunochromatographic test for the *in vivo* detection of placental alpha microglobulin-1 (PAMG-1) in vaginal secretions of pregnant women. PAMG-1 is a protein found in high concentrations in the amniotic fluid.

METHODS

We conducted a prospective observational study from March to June 2016. We enrolled 20 symptomatic patients between 24-34 week of gestation with singleton pregnancy, irregular uterine activity and/or lower abdominal pain and pelvic pressure, intact membranes, cervical length < 20 mm and funneling. Patients were initially managed according to the internal protocol: prophylactic corticosteroid betamethasone i.m. 12 mg/day for 2 days and primary tocolysis for 48 hours. 7 days after the therapy, we evaluated all the patients: 2 patients had delivered and 3 patients were excluded for premature rupture of membranes. In the final analysis, we included 15 patients. The PartoSure™ test was performed for these patients. The result was interpreted once two lines were visible, or after 5 min elapsed since the

insertion of the test strip into the sample vial. The patients were divided in two groups: the test was positive for two patients (Group A) and was negative for 13 patients (Group B). All patients had been revaluated after 7 and 14 days from the execution of the test.

RESULTS

In group A, a patient delivered within 7 days, while the others delivered within 14 days from presentation. In group B, a patient delivered within 7 days, while 12 patients were still pregnant after 14 days.

CONCLUSIONS

In our study, the positive and negative predictive value of the PartoSure™ test seems to be high within 7 and 14 days (PPV 100%, NPV 92%). However, our conclusions are based on a small sample, so further studies are needed. If our results will be confirmed, the device could be considered an excellent test to rapidly assess the risk of preterm delivery within 7 or 14 days from time of collection of cervicovaginal sample in pregnant women with signs and symptoms of early preterm delivery, intact membranes and minimal expansion. A positive PartoSure™ test in these patients indicates with a high degree of accuracy that spontaneous preterm delivery will occur within 7 days. A negative result indicates that spontaneous preterm delivery within 14 days is highly unlikely.

Future directions

Epidemiological data show the association between neurological impairment and several factors such as preterm birth and intrauterine growth retardation. Unfortunately, these associations may not be simple and our understanding of biological mechanisms is often imperfect. However, several areas can be defined where intervention to prevent neurological injury could be valuable.

Brains do not develop normally in the absence of critical genetic signaling and they do not develop normally in the absence of essential environmental input. At all levels of the neural system, progressive differentiation of specific elements and structures coupled with progressive commitment of those elements to functional systems appear to be the governing principles of brain development (47).

The best chance of success will come from an integrated and multi-disciplinary implementation strategy. This process begins with awareness across the medical and general communities that perinatal brain injury is one of modern health care’s greatest challenges, but that prevention in many cases is now possible.

References

1. Stoll BJ, Hansen NI, Bell EF, Shankaran S, Laptook AR et al. Neonatal outcomes of extremely preterm infants from the NICHD Neonatal Research Network. Pediatrics 2010 Sep; 126(3):443-56.
2. Angie C. Jelin , Kirsten Salmeen , Dawn Gano , Irina Burd , Mari-Paule Thiet. Perinatal neuroprotection update. F1000Research 2016, 5(F1000 Faculty Rev):1939 Last updated: 09 AUG 2016
3. Volpe J.J, Kinney H.C, Jensen F.E, et al. The developing oligodendrocyte: key cellular target in brain injury in the premature infant. Int J Dev Neurosci 2011; 29:423-440
4. Di Beena D. Kamath-Rayne, Alan H. Jobe. Birth Asphyxia, An Issue of Clinics in Perinatology. September 2016 ISSN 0095-5108, ISBN-13: 978-0-323-46263-1
5. [a-b]Julie A. Wixey, Kirat K. Chand, Paul B. Colditz, S. Tracey Bjorkman. Neuroinflammation in intrauterine growth restriction. Placenta xxx (2016) 1e8.

   S. Rees, R. Harding, D. Walker, The biological basis of injury and neuroprotection in the fetal and neonatal brain, Int. J. Dev. Neurosci. Off. J. Int. Soc. Dev. Neurosci. 29 (6) (2011) 551e563.
6. Joan Stiles1 and Terry L. Jernigan. The Basics of Brain Development Neuropsychol Rev. 2010 Dec; 20(4): 327–348.
7. Joan Stiles1 and Terry L. Jernigan. The Basics of Brain Development Neuropsychol. Rev. 2010 Dec; 20(4): 327–348.
8. The Etiology and Evolution of Fetal Brain Injury– Bridging Between Basic Research and Clinics Andrew Macnab
9. [a-b]Danny Liang, M.D.,1 Sergei Bhatta et al. Cytotoxic edema: mechanisms of pathological cell swelling. Neurosurg Focus. 2007 May 15; 22(5). Joseph J Volpe. Brain injury in premature infants: a complex amalgam of destructive and developmental disturbances. Lancet Neurol. 2009 January ; 8(1): 110–124.

10. American College of Obstetricians and Gynecologists. ACOG Practice bulletin no. 134: fetal growth restriction. Obstet Gynecol. 2013;121:1122–33.
11. RCOG Green Top Guidline No.31. The Investigation and Management of the Small-for-Gestational Age Fetus. January 2014.
12. Lees C, Marlow N, Arabin B, Bilardo CM, Brezinka C, Derks JB, et al. Perinatal morbidity and mortality in early-onset fetal growth restriction: cohort outcomes of the trial of randomized umbilical and fetal flow in Europe (TRUFFLE). Ultrasound Obstet Gynecol. 2013;42:400–8.
13. Unterscheider J, Daly S, Geary MP, Kennelly MM, McAuliffe FM, O'Donoghue K, Hunter A, Morrison JJ, Burke G, Dicker P, Tully EC, Malone FD. Optmizing the definition of intrauterine growth restriction: the multicenter prospective PORTO Study. Am J Obstet Gynecol. 2013;208(4):290. e1-290 e6.
14. Gordijn SJ, Beune IM, Thilaganathan B, Papageorghiou A, Baschat AA, Baker PN, Silver RM, Wynia K, Ganzevoort W. Consensus definition of fetal growth restriction: a Delphi procedure. Ultrasound Obstet Gynecol. 2016;48(3):333–9.
15. Barker DJ, Eriksson JG, Forsén T, Osmond C. Fetal origins of adult disease: strength of effects and biological basis. Int J Epidemiol. 2002 Dec;31(6):1235-9.
16. M Alberry and P Soothill Arch Dis Child Fetal Management of fetal growth restriction Neonatal Ed. 2007 Jan; 92(1).
17. Baschat AA. Fetal growth restriction – from observation to intervention. J Perinat Med. 2010;38:239–46.
18. Savchev S, Figueras F, Sanz-Cortes M, Cruz-Lemini M, Triunfo S, Botet F, Gratacos E. Evaluation of an optimal gestational age cut-off for the definition of early- and late-onset fetal growth restriction. Fetal Diagn Ther. 2014;36(2):99–105.
19. Andrea Dall'Asta, Valentina Brunelli, Federico Prefumo, Tiziana Frusca and Christoph C Lees. Early onset fetal growth restriction. Maternal Health, Neonatology, and Perinatology (2017) 3:2).

20. M Alberry, P Soothill. Guidelines Management of fetal growth restriction. Arch Dis Child Fetal Neonatal Ed 2007;92:F62–F67.
21. Figueras F, Gratacos E. Update on the Diagnosis and Classification of Fetal Growth Restriction and Proposal of a Stage-Based Management Protocol. Fetal Diagn Ther. 2014;36:86–98).
22. SOGC clinical practice guidelines no. 130, july 2003 the use of fetal doppler in obstetrics.
23. Locci M, Nazzaro G, De Placido G, Montemagno U. Fetal cerebral haemodynamic adaptation: a progressive mechanism? Pulsed and color Doppler evaluation. J Perinat Med. 1992;20(5):337-43.
24. Hypoxic regulation of the fetal cerebral circulation William Pearce, J Appl Physiol 100: 731–738, 2006.
25. Severi FM, Bocchi C, Visentin A, et al. Uterine and fetal cerebral Doppler predict the outcome of third-trimester small-for-gestational age fetuses with normal umbilical artery Doppler. Ultrasound Obstet Gynecol 2002;19:225-8.
26. J. Morales-Rosell´O, A. Khalil, M. Morlando, A. Bhide, A. Papageorghiou And B. Thilaganathan. Poor Neonatal Acid–Base Status In Term Fetuses With Lowcerebroplacental Ratio. Ultrasound Obstet Gynecol 2015; 45: 156–161).
27. Flood K, Unterscheider J, Daly S, Geary MP, Kennelly MM, McAuliffe FM, O'Donoghue K, Hunter A, Morrison JJ, Burke G, Dicker P, Tully EC, Malone FD. The role of brain sparing in the prediction of adverse outcomes in intrauterine growth restriction: results of the multicenter PORTO study. Am J Obstet Gynecol. 2014;211:288. e1-5.
28. Francesc Figueras, Jason Gardosi. Intrauterine growth restriction: new concepts in antenatal surveillance, diagnosis, and management Review. FRCOG 2010 American Journal of Obstetrics & Gynecology.
29. [a-b]G. Freire, M. Shevell, M. Oskoui, Cerebral palsy: phenotypes and risk factors in term singletons born small for gestational age, Eur. J. Paediatr. Neurol. EJPN Off. J. Eur. Paediatr. Neurol. Soc. 19 (2) (2015) 218e225.

Shahina Bano, Vikas Chaudhary,1 Sanjay Pande,2 VL Mehta,2 and AK Sharma. Color doppler evaluation of cerebral-umbilical pulsatility ratio and its usefulness in the diagnosis of intrauterine growth retardation and prediction of adverse perinatal outcome. Indian J Radiol Imaging. 2010 Feb; 20(1): 20–25

30. [a-b]Malin GL, Morris RK, Riley R, Teune MJ, Khan KS. When is birthweight at term abnormally low? A systematic review and meta-analysis of the association and predictive ability of current birthweight standards for neonatal outcomes. BJOG 2014; 121: 515-526.

    Karen Flood, Julia Unterscheider, Sean Daly, Michael P. Geary, et al. The role of brain sparing in the prediction of adverse outcomes in intrauterine growth restriction: results of the multicenter PORTO Study. American Journal of Obstetrics & Gynecology September 2014 Volume 211, Issue 3, Pages 288.e1–288.e5.

31. [a-b]Hadlock FP, Harrist RB, Sharman RS, Deter RL, Park SK. Estimation of fetal weight with the use of head, body, and femur measurements: a prospective study. Am J Obstet Gynecol 1985; 151:333–337.

    Mercedes Bellido-Gonzalez, Psy Miguel, Angel Lopez, Setefilla Lopez-Criadoand Jose´ Maldonado-Lozano. Cognitive Functioning and Academic Achievement in Children Aged 6–8 Years, Born at Term After Intrauterine Growth Restriction and Fetal Cerebral Redistribution. Journal of Pediatric Psychology, 2016, 1–10.

32. Acharya G, Wilsgaard T, Berntsen GK, Maltau JM, Kiserud T. Reference ranges for serial measurements of the umbilical artery Doppler indices in the second half of pregnancy. Am J Obstet Gynecol 2005; 192:937–944.

33. de Jong CL, Francis A, van Geijn HP, Gardosi J. Fetal growth rate and adverse perinatal events. Ultrasound Obstet Gynecol 1999; 13:86–89.

    Locci M, Nazzaro G, De Placido G, Montemagno U. Fetal cerebral haemodynamic adaptation: a progressive mechanism? Pulsed and color Doppler evaluation. J Perinat Med. 1992;20(5):337-43.

34. American Academy of Pediatrics and American College of Obstetricians and Gynecologists 2005. Guidelines of Perinatal Care.

5th Ed. American Academy of Pediatrics. Elk Grove Village. 2005, 211–220. 2.

35. World Health Organization (WHO) International Statistical Classification of Diseases and Related Health Problems, Geneva 1992, rev. 10, vol. 1 and 2.
36. Ananth CV, Friedman AM, Gyamfi-Bannerman C. Epidemiology of moderate preterm, late preterm and early term delivery. Clin Perinatol. 2013;40(4):601–10.
37. Gouyon JB, Vintejoux A, Sagot P, Burguet A, Quantin C, Ferdynus C, et al. Neonatal outcome associated with singleton birth at 34 – 41 weeks' gestation. Int J Epidemiol. 2010;39:769–76.
38. Boorn too soon The Global Action Report on Preterm Birth. World Health Organization 2012.
39. Charlotte L Lea, Adam Smith-Collins, Karen Luyt. Protecting the premature brain: current evidencebased strategies for minimising perinatal brain injury in preterm infants. Arch Dis Child Fetal Neonatal Ed 2016;0:F1–F7.
40. Chien LY, Whyte R & Aziz K et al. Improved outcome of preterm infants when delivered in tertiary care centers. Obstet Gynecol 2001; 98: 247−252.
41. R Romero, J Espinoza, JP Kusanovic, F Gotsch, S Hassan, O Erez, T Chaiworapongsa, M Mazor. The preterm parturition syndrome. BJOG 2006;113(Suppl. 3):17–42.
42. Brenda Timmons, Meredith Akins, and Mala Mahendroo. Cervical Remodeling during Pregnancy and Parturition. Trends Endocrinol Metab. 2010 Jun; 21(6): 353–361.
43. Roberto Romero,1,2,3 Sudhansu K. Dey,4 and Susan J. Fisher. Preterm Labor: One Syndrome, Many Causes. Science. 2014 August 15; 345(6198): 760–765.
44. Robert L Goldenberg, Jennifer F Culhane, Jay D Iams, Roberto Romero Epidemiology and causes of preterm birth. Lancet 2008; 371: 75–84.

45. Gerard H.A. Visser, Angela Kayser. Uterine contraction agents, tocolytics, vaginal therapeutics and local contraceptives. in Drugs During Pregnancy and Lactation (Third Edition), 2015.
46. Romero R. Prenatal medicine: the child is the father of the man. 1996.J Matern Fetal Neonatal Med. 2009 Aug;22(8):636-9.
47. Joan Stiles & Terry L. Jernigan. The Basics of Brain Development. Neuropsychol Rev (2010) 20:327–348.