Placenta 163 (2025) 8–15

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Vaginal miR-210-3p as a potential biomarker for pregnancies complicated by early fetal growth restriction: A proof-of-concept case-control study

Elisabetta Bigagli [a,*], Elisa Spataro [b,c], Lucia Pasquini [d], Lorenzo Cinci [a,1], Mario D'Ambrosio [a,2], Chiara De Blasi [b,c], Chiara Bartolini [b,c], Felice Petraglia [b,c], Cristina Luceri [a,**]

[a] Department of Neuroscience, Psychology, Drug Research and Child Health (NEUROFARBA), Section of Pharmacology and Toxicology, University of Florence, Florence, Italy
[b] Department of Experimental and Clinical Biomedical Sciences, Obstetrics and Gynecology, University of Florence, Florence, Italy
[c] Obstetrics and Gynecology, Department of Maternal and Child Health, University of Florence, Careggi University Hospital, Florence, Italy
[d] Fetal Medicine Unit, Department for Woman and Child Health, Careggi University Hospital, Florence, Italy

ARTICLE INFO

Keywords:
Fetal growth restriction
Pregnancy
miR-210-3p
Vaginal fluid
Biomarkers
Preeclampsia

ABSTRACT

Introduction: Fetal growth restriction (FGR) is associated with increased risk of neonatal morbidity and mortality or long-term adverse outcomes. We investigated the ability of hypoxia and angiogenesis-related miR-210-3p and miR-126-5p to identify early FGR cases and their correlations with neonatal outcomes.
Methods: Twenty-nine women with pregnancies complicated by early FGR diagnosis and 25 controls matched for gestational age (GA) were enrolled and their vaginal fluid (VF) and plasma were collected. MiR-210-3p and miR-126-5p were measured by RT-qPCR and their targets were identified by in-silico analysis limited only to those already experimentally validated in other contexts.
Results: Overall, VF levels of miR-210-3p were lower in early FGR cases compared to controls ($p < 0.05$). miR-210-3p was lower in severe cases and in women who later developed preeclampsia ($p < 0.05$). VF miR-210-3p levels correlated with lower birth weight, premature birth and severe complications at birth ($p < 0.05$). miR-210-3p was not detected in plasma and no correlations were observed between miR-126-5p and FGR or neonatal outcomes. In silico analyses identified HIF-1α, HIF-3α, BDNF, IGFBP3, RAD52 and TWIST-1 as experimentally validated targets of miR-210-3p. Among the predicted biological pathways controlled by miR-210-3p, we found hypoxia-responsive signaling such as autophagy, oxidative stress and metabolic pathways.
Discussion: Although validation is needed, these findings suggest that VF levels of miR-210-3p may potentially serve as biomarker for the diagnosis of early FGR; future mechanistic studies are also advisable to investigate whether pharmacological strategies based on miR-210-3p, or its downstream targets may be useful for FGR.

1. Introduction

Fetal growth restriction (FGR), also known as intrauterine growth restriction, is a condition in which biometric and functional parameters of the fetus pathologically deviate from the expected growth trajectory, resulting in low birth weight and impaired organ function [1]. FGR globally affects 10–15 % of all pregnancies, reaching 30 % in low-income countries, and it is associated with increased risk of stillbirth, neonatal morbidity and mortality as well as long-term multiorgan adverse consequences [2–4]. Early onset FGR (diagnosed <32 weeks gestation) in particular, represents the most severe phenotype and is associated with worse neonatal outcomes [3]. Moreover, despite the

* Corresponding author.
** Corresponding author.
E-mail addresses: elisabetta.bigagli@unifi.it (E. Bigagli), cristina.luceri@unifi.it (C. Luceri).
[1] Present address: Department of Experimental and Clinical Biomedical Sciences, University of Florence, Radiodiagnostic Unit no. 2, Careggi University Hospital, Florence, Italy.
[2] Present address: Enteric Neuroscience Program, Department of Medicine, Section of Gastroenterology and Hepatology, Mayo Clinic, Rochester, 55905, MN, United States.

https://doi.org/10.1016/j.placenta.2025.02.015
Received 14 July 2024; Received in revised form 14 February 2025; Accepted 24 February 2025
Available online 25 February 2025

advances in obstetric monitoring, a significant proportion of FGR fetuses are diagnosed only late in pregnancy or at birth, thus increasing the risk of perinatal death of five-fold compared to those diagnosed prenatally [5].

In addition to maternal and fetal factors, placental insufficiency caused by inadequate remodeling of spiral arteries, excessive apoptosis, autophagy, oxidative damage and deregulation of angiogenesis, is a well-established cause of FGR and of early onset cases, in particular [6].

There is currently no treatment for placental dysfunction and preventive prenatal pharmacotherapy has not been clearly established, but the use of low-dose aspirin for women with major risk factors for placental insufficiency has been recommended by several international guidelines [7,8]. Furthermore, two metanalyses provided evidence that prophylactic aspirin can halve the incidence of preeclampsia (PE) and FGR if treatment starts before 16 weeks of gestation [9,10]. Therefore, the identification of FGR markers could not only aid in the monitoring of pregnancies but also support the identification of patients eligible for prophylactic pharmacotherapy.

MicroRNAs (miRNAs) are small, non-coding RNAs which regulate gene expression at post-transcriptional level and play an important role in regulating uteroplacental vascular function, placental and fetal development [11,12]. More than 600 miRNAs have been detected in human placenta and a series of differentially expressed miRNAs have been observed in the placentas from pregnancies complicated by PE and FGR [12–15]. Among those, miR-210 is a well-known hypoxia-inducible miRNA expressed in the villous and extra villous trophoblasts of the placenta where it regulates trophoblasts invasion and migration and mitochondrial activity [16]; miR-210 is also crucial for endothelial cell proliferation, migration and angiogenic response to hypoxia [17]. MiR-126, a master regulator of endothelial function, angiogenesis, and vascular integrity, is also expressed in syncytiotrophoblasts and villous endothelial cells in normal placentas and its reduction was associated to increased inflammation and oxidative stress in PE placentas [18,19]. A remarkable role of these miRNAs in defective placentation has been described in two *in vivo* studies: Sharma et al. [20] demonstrated that the loss of miR-126 function reduced placental volume and caused FGR or even embryonic death while mice lacking miR-210 showed defective placental development and severe FGR due to maternal exposure to hypoxia in early pregnancy [21].

Besides offering mechanistic insights into pathophysiology and new drug targets and treatments for FGR, an attractive feature of miRNAs is that they circulate stably in biological fluids where they can serve as mediators of intercellular communication and non-invasive biomarkers [22–24].

Vaginal fluid (VF) might be ideal for detecting biomarkers associated with pregnancy complications by means of a minimally invasive procedure, but so far, it has never been used to analyze placental miRNAs and their association with FGR.

In this proof-of-concept study, we hypothesized that defective placentation might lead to altered miRNA release from gestational tissues into VF. Accordingly, we explored whether the levels of VF miR-210-3p and miR-126-5p can identify early FGR cases, compared their performance with plasma levels, and investigated their correlations with neonatal outcomes.

2. Materials and methods

2.1. Study participants

We conducted an observational, prospective study at the Fetal Medicine Center of the Careggi University Hospital (Florence, Italy) after approval from the local ethics committee (CEAVC code 19152). Twenty-nine consecutive women with singleton pregnancies complicated by early FGR diagnosis, defined according to Delphi criteria [1] were enrolled. Since this definition includes FGR cases with different levels of severity, they were subdivided into different groups according to both prenatal Doppler abnormalities, and postnatal neonatal weight. Prenatally, FGR cases were divided in 3 groups according to their Doppler ultrasound findings: 1) fetuses with a CA/EFW below the 3rd percentile and abnormal Doppler waveforms in the umbilical and/or uterine arteries (severity criterion 1); 2) fetuses with a CA/EFW below the 3rd percentile and abnormal Doppler waveforms in the umbilical artery only (severity criterion 2); and 3) those without any Doppler abnormalities (non-severe cases). After birth, cases were divided into 3 groups based on neonatal weight: Low birth weight if < 2500gr; Very Low birth weight if < 1500 gr; extremely low birth weight if < 1000gr.

GA was calculated from the last menstrual period and was confirmed by fetal crown–rump length measurement at the first trimester ultrasound.

Patients with comorbidities that could lead to an increase in inflammatory indices (e.g., autoimmune diseases) or with vaginitis/flu-like symptoms at the time of enrollment were excluded. Patients taking immunomodulatory drugs or showing evidence of infections during pregnancy were also excluded. Healthy pregnant women with uncomplicated pregnancies and normally grown fetuses, without comorbidities and of a comparable gestational age ($\pm$7 days), were included as controls (n = 25). Informed written consent was obtained from each patient.

PE was defined as systolic blood pressure at $\geq$ 140 mm Hg and/or diastolic blood pressure at $\geq$ 90 mm Hg on at least two occasions measured 4 h apart in previously normotensive women and accompanied by one or more of the following new-onset conditions at or after 20 weeks of gestation.

1. Proteinuria (i.e. $\geq$ 30 mg/mmol protein: creatinine ratio; $\geq$300 mg/24 h);
2. Evidence of other maternal organ dysfunction, including: acute kidney injury (creatinine $\geq$1 mg/dL); liver involvement (elevated transaminases, e.g. alanine aminotransferase or aspartate aminotransferase $\geq$40 IU/L) with or without right upper quadrant or epigastric abdominal pain; neurological complications (e.g. eclampsia, altered mental status, blindness, stroke, clonus, severe headaches, and persistent visual scotoma); or hematological complications (thrombocytopenia–platelet count <150 000/μL, disseminated intravascular coagulation, hemolysis);
3. Signs of utero-placental dysfunction (such as fetal growth restriction, abnormal umbilical or uterine artery Doppler, or stillbirth).

2.2. Neonatal outcomes

Data on pregnancy outcomes were collected from the electronic hospital obstetric and neonatal records or by telephone interviews. The perinatal outcome variables investigated were GA at delivery, mode of delivery (vaginal or cesarean section (CS), elective CS, emergency CS), birthweight (g), Apgar Score at 5th minutes, umbilical cord pH, admission to neonatal intensive care unit (NICU), postnatal morbidities and average hospital days at NICU. Neonates weighing less than 2500 g were defined as low birth weight (LBW); very low birth weight (VLBW) was defined as a birth weight of less than 1500 g and extremely low birth weight (ELBW) as a birth weight of less than 1000 g.

2.3. Blood and vaginal fluid collection

All samples were collected at enrollment. Peripheral blood was collected in an EDTA containing tube and plasma was separated by centrifugation at 2000 rpm for 10 min and stored at −20 °C until analysis. VF was collected by using a sterile swab with a flocked nylon tip and preserved in a polypropylene tube containing liquid transport medium (Copan Liquid Amies Elution Swab (ESwab®), Brescia, Italy). VF was then centrifuged at 1200 rpm for 5 min to remove any suspended cells and stored at −20 °C until analysis. Pregnancies were stratified according to GA at enrollment into those <32 weeks: (CNTR, n = 20; FGR, n = 23) and those >32 weeks: CNTR, n = 5; FGR, n = 6).

2.4. Real time PCR (qPCR) for mir-210-3p and mir-126-5p expression

Total RNA was isolated from 200 μL VF or plasma using the TRIzol Reagent (Invitrogen) according to the manufacturer's instructions. RNA was quantified by using the NanoPhotometer® (Implen, Munich, Germany) measuring the absorbance at 260 nm. cDNA was synthesized using the miRCURY LNA RT kit (Qiagen, Hilden, Germany). cDNA samples were stored at − 20 °C. RT-quantitative PCR (qPCR) was performed using miRCURY LNA SYBR Green PCR kit (Qiagen) according to the manufacturer's instructions. The following primers from miRCURY LNA miRNA PCR Assays (Qiagen) were used: miR-210-3p, YP00204333; miR-126-5p, YP00206010; RNU6b, YP00203907. The analyses were performed using the Rotor-Gene Q thermal cycler (Qiagen). RNU6b was selected as reference due to its stability across samples. Results were expressed using the $2^{-\Delta\Delta Ct}$ formula [25].

2.5. miRNA-target gene interactions and pathway analysis

The MIENTURNET (http://userver.bio.uniroma1.it/apps/mienturnet/) web tool was used to assess miRNA-target genes interactions and perform network-based analyses [26]. Experimentally validated miRNA-target interactions were queried by using miRTarBase (https://mirtarbase.cuhk.edu.cn). In the functional enrichment analysis, Kyoto Encyclopedia of Genes and Genomes (KEGG) terms with $P < 0.05$ were considered statistically significant.

2.6. Statistical analysis

Statistical analyses were performed using GraphPad Prism 7 and Statgraphics Centurion XVII software. Data distribution was assessed using D'Agostino and Pearson's and Shapiro-Wilk test. Continuous data were expressed by mean ± standard deviation (SD) or median (interquartile ranges, IQR) as appropriate, depending on the distribution. Categorical data was expressed by absolute and relative frequencies. Categorical data were compared by Fischer exact test or chi2 test. Continuous variables were compared by *t*-test or one-way analysis, or by Mann-Whitney test or Kruskal-Wallis's test, when not normally distributed. The diagnostic accuracy of miR-210-3p for discriminating between early FGR and normal pregnancies was evaluated by Receiver Operating Characteristic (ROC) curves. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Characteristics of study participants

A total of 54 women, 25 controls and 29 early FGR cases, were included in this study. The clinical characteristics of pregnancies are described in Table 1. No differences were observed in any of the matching variables such as GA at enrollment, mother's age, Body Mass Index (BMI), previous miscarriages, maternal comorbidities, or fetal sex. Among early FGR pregnancies, 75.9 % met severity criteria 1 and 62.1 % met severity criteria 2.

3.2. Perinatal data and neonatal outcomes of early FGR pregnancies

Table 2 shows perinatal data and neonatal outcomes of 25 out of 29 early FGR pregnancies included in this study, since four cases were lost at follow-up due to miscarriages/terminations of pregnancy. Most of the neonates (72 %) were born prematurely and the caesarean section was the prevalent mode of delivery (76 %). The caesarean section was also performed in seven control pregnancies for the following reasons: previous caesarean section (n = 4), myomectomy (n = 1), cholestasis gravidarum (n = 1), emergency (n = 1). Apart from just a single case, all neonates with early FGR weighted less than 2500 g (96 %) with very low-birth weight (VLBW) and extremely low-birth weight (ELBW), accounting for 28 % and 20 % of the total. Twenty babies (80 % of FGR pregnancies) were admitted to neonatal intensive care units (NICUs) for critical care, either for observation or for treatment of prematurity and associated complications such respiratory distress syndrome (RDS), bronchopulmonary dysplasia (BPD), patent ductus arteriosus (PDA), congenital malformations, and infections (Table 2).

Table 1
Characteristics of the controls and early FGR complicated pregnancies.

	Controls	early FGR	*p*
n	*25*	*29*	
GA at enrollment			>0.9999
<32 weeks	20 (80.0 %)	23 (79.3 %)	
>32 weeks	5 (20.0 %)	6 (20.7 %)	
GA at enrollment	27.3 ± 0.9	27.3 ± 0.7	>0.9999
Maternal age, years	34.56 ± 0.96	33.66 ± 1.19	0.5655
Pre-pregnancy BMI	25.16 ± 0.69	25.23 ± 0.87	0.9498
Previous miscarriages			>0.9999
no	22 (88.0 %)	26 (89.7 %)	
yes	3 (12.0 %)	3 (10.3 %)	
Maternal comorbidities			0.4305
no	23 (92.0 %)	24 (82.8 %)	
yes	2 (8 %)	5 (17.2 %)	
Fetal sex			0.5827
M	12 (48.0 %)	11 (37.9 %)	
F	13 (52.0 %)	18 (62.1 %)	
FGR severity criterion 1			
no		7(24.1 %)	
yes		22 (75.9 %)	
FGR severity criterion 2			
no		11 (37.9 %)	
yes		18 (62.1 %)	

FGR severity criterion 1: CA/EFW <3° centile + pathological umbilical and/or Uterine artery Doppler.
FGR severity criterion 2: CA/EFW <3° centile + pathological umbilical artery Doppler.

Table 2
Perinatal data and immediate neonatal outcomes of early FGR complicated pregnancies.

	CTRL	early FGR
n	*25*	*25*
Birth <37th week	1	18 (72.0 %)
Low birth weight (LBW)	2	24 (96.0 %)
Very Low birth weight (VLBW)	0	7 (28.0 %)
Extremely Low birth weight (ELBW)	0	5 (20.0 %)
Birth weight, g	3248 ± 93	1650 ± 115
Mode of delivery		
Caesarean section	7 (32.0 %)	19 (76.0 %)
Vaginal delivery	18	6 (24.0 %)
5-min Apgar score, ≤ 7	0	2 (8.0 %)
Cord pH, ≥7.2	0	20 (80.0 %)
Admitted to Neonatal Intensive Care Unit (NICU)	0	20 (80.0 %)
Postnatal morbidities (not exclusive)		
Bronchopulmonary dysplasia (BPD-RDS)	0	8 (32.0 %)
Patent ductus arteriosus ligation (PDA)	0	2 (8.0 %)
[a]others fetal distress	0	13 (52.0 %)
Average hospital NICU stay, days	0	45.71 ± 10.6

[a] Congenital malformations, sepsis, hypoglycemia.

3.3. miR-210-3p and mir-126-5p levels in plasma and in VF fluid from controls and early FGR pregnancies

MiR-210-3p was detectable only in VF fluid, while it was not detected in any plasma samples. In VF, miR-210-3p was significantly lower in early FGR cases compared to controls ($p < 0.05$) (Fig. 1A). In addition, to investigate whether GA at the time of VF collection is a factor affecting miR-210-3p levels, both control and early FGR pregnancies were stratified according to GA at enrollment into two groups: those collected before 32 weeks and those collected after 32 weeks;

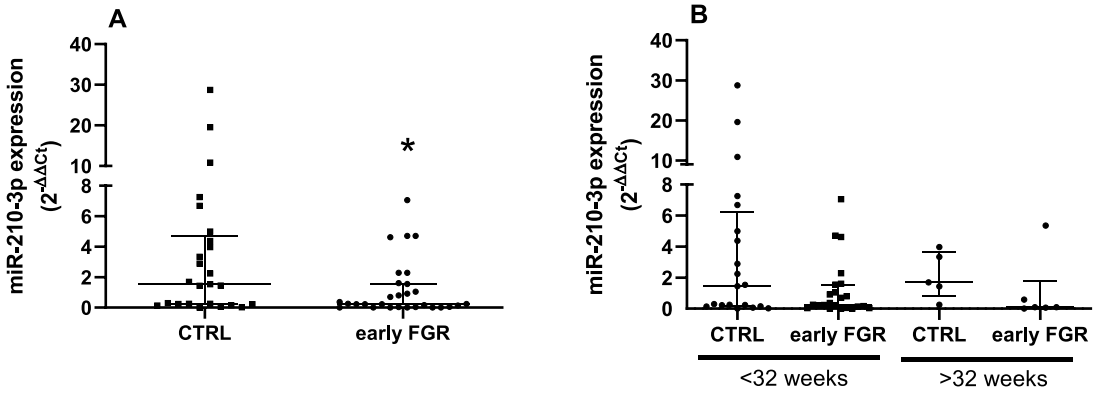

Fig. 1. Panel A: Levels of miR-210-3p in VF from pregnancies complicated by early Fetal Growth Restriction (FGR, n = 29) and in matched controls (CTRL, n = 25). Data is expressed as median and interquartile range (IQR) and compared using Mann-Whitney test; * = $p < 0.05$. Panel B: miR-210-3p levels in pregnancy complicated by early FGR and in matched controls stratified into subgroups based on gestational age at enrollment (<32 weeks: (CNTR, n = 20; FGR, n = 23); >32 weeks: (CNTR, n = 5; FGR, n = 6). Data is expressed as median and IQR and compared Kruskal Wallis test with post-hoc Dunn's multiple comparisons test.

according to this stratification, the reduction of miR-210-3p levels seems independent from GA at enrolment, i.e., at VF sampling (Fig. 1B).

Unlike miR-210-3p, miR-126-5p was detectable in plasma samples but it did not differ between early FGR and control pregnancies (1.74 (IQR: 0.54–3.3) vs 0.54 (IQR: 0.19–7.2)); similarly, no differences were observed in the levels of miR-126-5p in VF from early FGR cases and controls (0.68 (IQR: 0.09–11.18) vs 0.49 (IQR: 0.16–7.10)). Mir-126-5p levels did not also differ between VF from early FGR cases collected before 32 weeks, compared to matched controls (0.68 (IQR: 0.08–17.4) vs 0.52 (IQR: 0.15–8.4); likewise, early FGR cases and controls showed similar levels even when VF sampling was performed at GA over 32 weeks (0.76 (IQR: 0.2–1.7) vs 0.49 (IQR: 0.2–8.2). Neither the VF levels of miR-210-3p nor those of miR-126-5p were associated to maternal characteristics such as age, overweight (BMI<25) or history of previous miscarriages (data not shown).

3.4. miR-210-3p expression in VF fluid of pregnancy complicated by severe early FGR and preeclampsia

Stratifying early FGR cases according to severity criteria 1 and 2, we observed a non-significant downward trend of miR-210-3p in severe FGR cases. However, when restricted to VF samples collected before 32 weeks of gestational age, the analysis showed significantly lower levels of miR-210-3p in FGR cases with severity criterion 1 compared to non-severe cases (Fig. 2). No associations between miR-126-5p and severity criteria were found (Supplementary Table 1).

Among 29 women enrolled with early FGR, four cases were lost at follow-up due to miscarriages/termination of pregnancy. Out of the 25 remaining FGR, the six who also developed PE later in pregnancy showed lower levels of miR-210-3p compared to pregnancy complicated

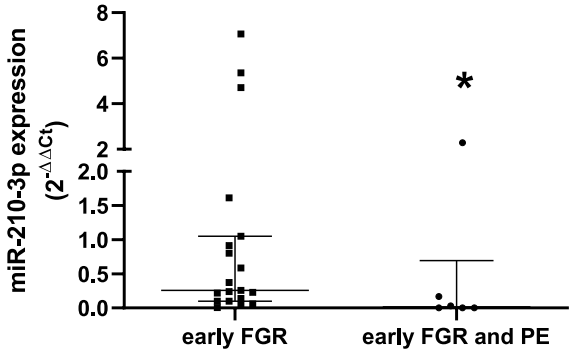

Fig. 3. VF levels of miR-210-3p in pregnancy complicated by early FGR alone (n = 19) and in those with early FGR who later developed preeclampsia (PE) (n = 6). Data is expressed as median and IQR; * = $p < 0.05$ by Mann-Whitney test.

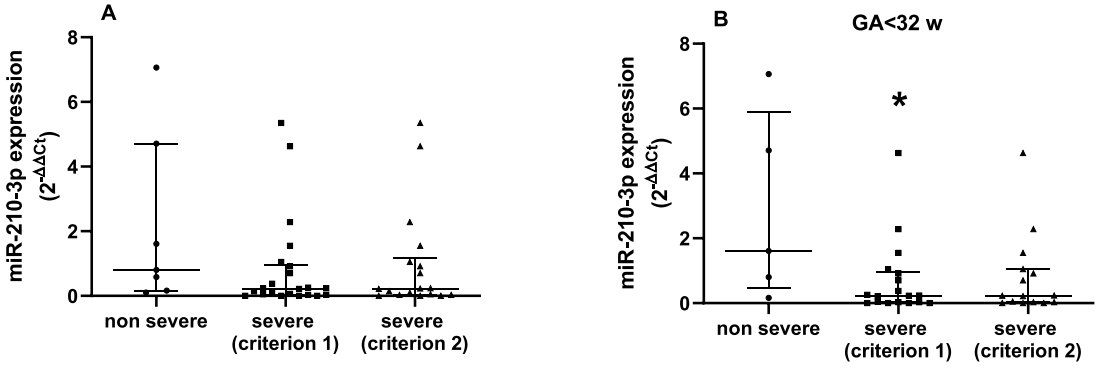

Fig. 2. Panel A: VF levels of miR-210-3p in pregnancy complicated by early FGR stratified according to severity criteria (no = 7, criterion 1 = 22, criterion 2 = 18). Panel B: miR-210-3p levels in VF of early FGR cases collected before 32 weeks gestational age (GA) and stratified into subgroups according to severity criteria (no = 5, criterion 1 = 18, criterion 2 = 15). Data is expressed as median and IQR. * = $p < 0.05$ by Kruskal-Wallis and Dunn's multiple comparisons test.

by early FGR only ($p < 0.05$) (Fig. 3). MiR-126-5p levels in VF or plasma did not differ between these two groups (Supplementary Table 1).

3.5. Associations of miR-210-3p levels in VF with perinatal data and postnatal morbidities of early FGR pregnancies

Considering fetal outcomes of early FGR pregnancies with no further stratification according to FGR severity criteria, GA at enrolment or future development of PE, miR-210-3p levels were lower in VLBW neonates compared to LBW ($p < 0.05$) and in those born prematurely compared to babies born at term according to GA ($p < 0.05$). miR-210-3p was significantly lower in FGR cases with severe postnatal morbidities such as patent ductus arteriosus (PDA), respiratory distress syndrome (RDS) and bronchopulmonary dysplasia (BPD), compared to those without complications and those with non-severe complications ($p < 0.05$) (Fig. 4). No significant differences in the VF levels of miR-210-3p were observed according to the mode of delivery (cesarean vs vaginal, delivery in urgency or emergence vs non-urgent). No associations were found between miR-210-3p, z-score or admission to NICU. Neither plasma levels nor VF levels of miR-126-5p were associated with any of the considered perinatal data or neonatal outcomes (Supplementary Table 1).

3.6. miR-210-3p for the detection of early FGR and for the prediction of PE and severe perinatal complications

Receiver operating characteristic curve (ROC) analysis unstratified by GA at enrollment showed that VF miR-210-3p levels had good performances in diagnosing early FGR. As shown in Supplementary Fig. 1 (panel A), VF miR-210-3p levels distinguished early FGR cases from healthy controls, with an AUC value of 0.68 (95 % CI, 0.53–0.82), indicating an acceptable discrimination level ($p < 0.05$). The optimal threshold value was set at <1 that corresponds to the maximum likelihood ratio (LR) of 2.17, with a good specificity (72.4 %) but poor sensibility (60 %). MiR-210-3p demonstrated good predictive ability for the development of PE with an AUC of 0.80 ($p < 0.05$) (panel B). Perinatal severe complications were also well predicted by miR-210-3p levels with an AUC of 0.75 ($p < 0.05$) (panel C).

3.7. miRNA-target gene interactions prediction and predicted pathway analysis

To explore the potential functional consequences of miR-210-3p alteration in the clinical setting of early FGR, miRTarBase, a database of experimentally validated miRNA-target interactions was interrogated. Supplementary Fig. 2 shows the 53 and 11 target genes of miR-210-3p and miR-126-5p respectively, that have been previously validated in other experimental settings. Among miR-210-3p targets it is worth mentioning the brain-derived neurotrophic factor (BDNF), the IGF-binding protein (IGFB-P3) and twist-related protein 1 (TWIST1). miR-210-3p also targets hypoxia-inducible factor 1A and 3A, and the DNA repair gene RAD52.

Supplementary Table 2 shows the functional enrichment analysis performed using KEGG database with input list consisting of the 53 experimentally validated target genes of miR-210-3p. Among the pathways under the regulatory control of miR-210-3p we found hypoxia-responsive signaling such as autophagy (involving genes such as Bcl-2/adenovirus E1B 19-kDa interacting protein 3 (BNIP3) and vacuole membrane protein 1 (VMP1), oxidative stress (involving components of the mitochondrial electron transport chain such as NADH dehydrogenase (ubiquinone) 1 alpha subcomplex 4 (NDUFA4) and succinate dehydrogenase complex subunit D (SDHD) and the negative modulator of leptin signaling, protein tyrosine phosphatase non-receptor type 1 (PTPN1); lactate dehydrogenase (LDH) A and B) were also included in several metabolic pathways modulated by miR-210-3p such as pyruvate and propanoate metabolism.

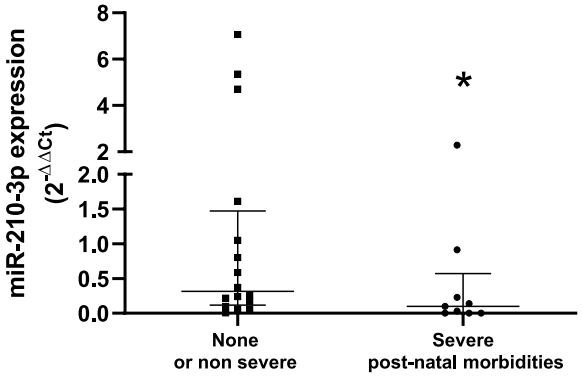

Fig. 4. VF levels of miR-210-3p in early FGR cases with severe post-natal morbidities (n = 9) and in those without or with non-severe complications (n = 16). Data are not stratified according to gestational age at enrolment or severity criteria of the FGR diagnosis or future development of preeclampsia. Data is expressed as median and IQR; * = $p < 0.05$ by Mann-Whitney test.

4. Discussion

The management of growth-restricted fetuses mainly relies on early recognition, careful monitoring and appropriate timing of delivery chosen by balancing the risk of stillbirth and iatrogenic prematurity, a particularly relevant issue for cases of early FGR [27]. To date, there is no approved drug for the treatment of FGR but the prophylactic use of low-dose aspirin in women at increased risk for FGR or PE, is recommended by some international guidelines [7,8,27].

In this proof-of-concept study, we showed for the first time that miR-210-3p is detectable in VF, non-invasively collected during gestation, and that its levels are significantly lower in FGR cases diagnosed <32 weeks gestation, the exact threshold defined by the Delphi consensus panel for the diagnosis of early FGR [1]. We also found that this reduction appears to be independent of gestational age at enrollment, i.e., independent of the gestational age at which VF was collected; however, this data should be taken with caution because approximately 80 % of our controls and FGR cases were enrolled at a gestational age <32 weeks. An interesting study that analyzed miR-210-3p expression in placenta samples during normal gestation, found that it is highest in the first trimester and then steadily decreases, reaching minimum levels between 26 and 40 weeks [28]. Similarly, we observed a downward trend of miR-210-3p in VF samples collected from normal pregnancies after 32 weeks. Postulating a more critical protective role of miR-210-3p at early gestation, our hypothesis is that the physiological reduction of miR-210-3p that occurs later in healthy pregnancies may be anticipated pathologically in early FGR cases due to a maladaptive response to hypoxia. The degree of reduction is also associated with the severity of FGR, suggesting that the lower is placental perfusion, the lower is the abundance of miR-210-3p in VF. Despite being limited by the small sample size, our data also suggest that the reduction of miR-210-3p may be predictive of the development of pre-eclampsia, another obstetric complication characterized by placental insufficiency.

The protective role of miR-210 is supported by a functional study demonstrating that the exposure of dams lacking miR-210 to severe hypoxia in early pregnancy resulted in defective placental development and severe fetal growth restriction [21]. Furthermore, it was shown that miR-210 is involved in endothelial cell response to hypoxia including proliferation, migration, and angiogenesis, but also growth arrest and apoptotic cell death [17].

In contrast with our findings and with the putative protective role of miR-210-3p, the review by Ali et al. [12] reported 15 different studies

showing the upregulation of miR-210 in placenta or in serum of PE women, and 3 studies documenting increased miR-210 expression in placental tissue of FGR. MiR-210 was also upregulated in whole maternal blood from women with severe FGR, compared to gestational age matched controls [29], but downregulated in placenta from patients with mild PE compared to normal pregnancies [30]. In our cohort, miR-210-3p was undetectable in maternal plasma of both FGR and controls. The different biological tissue/fluid examined and gestational age at enrollment, do not allow the comparability of our results with former studies and may explain these conflicting results. Indeed, most of the studies conducted so far explored miR-210 in the blood or in placental tissues collected at delivery but at this time, miR-210 may be increased by acute hypoxia during labour itself [30]. Another probably crucial point is which of the two strands, termed 5p or 3p, has been investigated, since most of the studies conducted to date do not specify this aspect.

Moreover, it is the first time that miR-210-3p is measured in VF of women with FGR but the single study examining the levels of miR-210 in a biological matrix other than placenta or blood, reported an inverse association between miR-210 expression in the amnion and the risk of PE and preterm labor [31].

FGR and especially early FGR, is associated with increased risk of perinatal mortality and morbidity and long-term adverse outcomes including neurodevelopmental, cardiovascular, and metabolic complications [2–4]. To our knowledge, this is the first study associating perinatal outcomes of early FGR based on miRNAs measurement in VF, but others reported that placental miR-210-3p abundance was positively associated with birth weight in babies born small for gestational age [32]. Similarly, we found that reduced miR-210-3p levels were associated with lower birth weight, with prematurity and with the occurrence of severe perinatal complications such as PDA, RDS and BPD that, in most cases, required admission to NICU for a prolonged stay.

By means of our in-silico analysis of mir-210-3p targets experimentally validated in other experimental settings, we found that miR-210-3p targets several genes involved in syncytialization, placental development and fetal growth such as the brain-derived neurotrophic factor (BDNF) and the IGF-binding protein (IGFBP3). Despite these targets being experimentally validated in other experimental settings and not in the current study, they offer useful insight into the potential functional consequences of mir-210-3p alteration in the current study as well.

Consistent with our observed reduction of miR-210-3p in early FGR pregnancies, the expression of BDNF and its functional receptor, the tyrosine kinase receptor B was increased in FGR placentas in rats and humans [33]; similarly, IGFBP3 was increased in amniotic fluid of FGR cases [34].

It is well known that miR-210-3p is a hypoxia-responsive miRNA regulated by the transcription factor HIF-1α, however, a feedback regulation between the two also exists since miR-210-3p also targets HIF1-α, HIF3-α and the cytoplasmic polyadenylation element-binding 2 (CPEB2). An *in vitro* study demonstrated an inhibitory effect of miR-210-3p on trophoblast syncytialization by targeting CPEB2, a translation suppressor of HIF-1α [35]. However, this is in contrast with in *vivo* studies showing that mice lacking miR-210 showed severe FGR due to maternal exposure to hypoxia in early pregnancy [21] and suggests that the *in vivo* feedback mechanism between hypoxia and miR-210 is highly dynamic and dependent on gestational age. During the early stages of pregnancy, before placental vasculature is established, HIF-1α plays a crucial role in facilitating placental adaptation to a low oxygen environment favoring angiogenesis and trophoblast invasion; however, prolonged expression of placental HIF-1α, beyond the first trimester, have been associated to PE and FGR [36]. Other genes under the epigenetic control of miR-210-3p are TWIST1, a downstream molecule of HIF-1α involved in syncytialization of the human placenta that is upregulated in PE placenta as compensatory mechanism against reduced invasion [37]. Mir-210-3p also targets RAD52, a gene involved in the regulation of DNA repair in response to hypoxia whose expression is inversely correlated with fetal growth parameters [38].

Pathway analysis also showed the potential broad impact that miR-210-3p might exert in placental development through altering autophagy and oxidative stress signaling, which are two crucial and interconnected processes in FGR [39]. Zhu et al. (2021) demonstrated that placental BNIP3-dependent mitochondrial autophagy (mitophagy) contributes to environmental stress-induced FGR, and this was rescued by the antioxidant activity of melatonin [40]. In extra villous trophoblasts, Anton et al. (2019) also showed that miR-210 decreases mitochondrial respiration through targeting NDFUA4 and SDHD supporting the role of miR-210 in mitochondrial dysfunction [41]. Moreover, miR-210-3p regulates several metabolic pathways mostly involving two genes, lactate dehydrogenase (LDH) A and B, that is consistent with a glycolytic metabolic shift that occurs frequently, in placental specimens from PE + FGR complicated pregnancies [42].

Although animal data support the role of miR-126 in placental development and FGR [20], we found no association between miR-126-5p and early diagnosis of FGR or perinatal outcomes, indicating that it is measurement in vaginal fluid or plasma is not likely useful in this setting; likewise, Hromadnikova et al. recently found that miR-126-3p has predictive potential for small-for-gestational-age but not for FGR [43].

Overall, this study suggests that vaginal miR-210-3p may represent a new tool to identify women at risk of early FGR and that it may have protective role as its deficiency is associated with the diagnosis of early FGR, its severity and adverse neonatal outcomes.

Since this is a proof-of-concept study, we cannot infer the predictive power of miR-210-3p, but our results provide supportive evidence to assess its potential in a larger cohort of patients and boost the application of this approach to future prospective studies with VF collection starting in the first trimester.

At the molecular level, using appropriate experimental models, it will be possible to explore the role of mir-210-3p in this pathological context, to understand whether the reduction of mir-210-3p is a cause or only a consequence of placental alterations, and whether modulation of hypoxia-responsive pathways could be a target for new pharmacological strategies for FGR.

CRediT authorship contribution statement

Elisabetta Bigagli: Writing – review & editing, Writing – original draft, Validation, Supervision, Methodology, Investigation, Conceptualization. **Elisa Spataro:** Writing – review & editing, Resources, Data curation, Conceptualization. **Lucia Pasquini:** Writing – review & editing, Resources, Data curation. **Lorenzo Cinci:** Writing – review & editing, Investigation. **Mario D'Ambrosio:** Writing – review & editing, Investigation. **Chiara De Blasi:** Resources, Data curation. **Chiara Bartolini:** Resources, Data curation. **Felice Petraglia:** Writing – review & editing, Resources, Data curation, Conceptualization. **Cristina Luceri:** Writing – review & editing, Visualization, Validation, Supervision, Project administration, Methodology, Funding acquisition, Formal analysis, Conceptualization.

Funding

This work was financially supported by the University of Florence.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

The authors would like to sincerely thank the patients for their

willingness to participate in the study.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.placenta.2025.02.015.

References

[1] S.J. Gordijn, I.M. Beune, B. Thilaganathan, A. Papageorghiou, A.A. Baschat, P. N. Baker, R.M. Silver, K. Wynia, W. Ganzevoort, Consensus definition of fetal growth restriction: a Delphi procedure, Ultrasound Obstet. Gynecol. 48 (2016) 333–339, https://doi.org/10.1002/uog.15884.

[2] J.B. Armengaud, C. Yzydorczyk, B. Siddeek, A.C. Peyter, U. Simeoni, Intrauterine growth restriction: clinical consequences on health and disease at adulthood, Reprod. Toxicol. 99 (2021) 168–176, https://doi.org/10.1016/j.reprotox.2020.10.005.

[3] A. Malhotra, B.J. Allison, M. Castillo-Melendez, G. Jenkin, G.R. Polglase, S. L. Miller, Neonatal morbidities of fetal growth restriction: pathophysiology and impact, Front. Endocrinol. 10 (2019) 55, https://doi.org/10.3389/fendo.2019.00055.

[4] A.C. Lee, N. Kozuki, S. Cousens, G.A. Stevens, H. Blencowe, M.F. Silveira, A. Sania, H.E. Rosen, C. Schmiegelow, L.S. Adair, A.H. Baqui, F.C. Barros, Z.A. Bhutta, L. E. Caulfield, P. Christian, S.E. Clarke, W. Fawzi, R. Gonzalez, J. Humphrey, L. Huybregts, S. Kariuki, P. Kolsteren, J. Lusingu, D. Manandhar, A. Mongkolchati, L.C. Mullany, R. Ndyomugyenyi, J.K. Nien, D. Roberfroid, N. Saville, D.J. Terlouw, J.M. Tielsch, C.G. Victora, S.C. Velaphi, D. Watson-Jones, B.A. Willey, M. Ezzati, J. E. Lawn, R.E. Black, J. Katz, CHERG Small-for-Gestational-Age-Preterm Birth Working Group, Estimates of burden and consequences of infants born small for gestational age in low and middle income countries with INTERGROWTH-21st standard: analysis of CHERG datasets, Br. Med. J. 358 (2017) j3677, https://doi.org/10.1136/bmj.j3677.

[5] J. Gardosi, V. Madurasinghe, M. Williams, A. Malik, A. Francis, Maternal and fetal risk factors for stillbirth: population based study, Br. Med. J. 346 (2013) f108, https://doi.org/10.1136/bmj.f108.

[6] H. Wang, P. Xu, X. Luo, M. Hu, Y. Liu, Y. Yang, W. Peng, Y. Bai, X. Chen, B. Tan, Y. Wu, L. Wen, R. Gao, C. Tong, H. Qi, M.D. Kilby, R. Saffery, P.N. Baker, Phosphorylation of Yes-associated protein impairs trophoblast invasion and migration: implications for the pathogenesis of fetal growth restriction, Biol. Reprod. 103 (2020) 866–879, https://doi.org/10.1093/biolre/ioaa112.

[7] N. Melamed, A. Baschat, Y. Yinon, A. Athanasiadis, F. Mecacci, F. Figueras, V. Berghella, A. Nazareth, M. Tahlak, H.D. McIntyre, F. Da Silva Costa, A.B. Kihara, E. Hadar, F. McAuliffe, M. Hanson, R.C. Ma, R. Gooden, E. Sheiner, A. Kapur, H. Divakar, D. Ayres-de-Campos, L. Hiersch, L.C. Poon, J. Kingdom, R. Romero, M. Hod, FIGO (international Federation of Gynecology and obstetrics) initiative on fetal growth: best practice advice for screening, diagnosis, and management of fetal growth restriction, Int. J. Gynaecol. Obstet. 152 (2021) 3–57, https://doi.org/10.1002/ijgo.13522.

[8] L.M. McCowan, F. Figueras, N.H. Anderson, Evidence-based national guidelines for the management of suspected fetal growth restriction: comparison, consensus, and controversy, Am. J. Obstet. Gynecol. 218 (2018) S855–S868, https://doi.org/10.1016/j.ajog.2017.12.004.

[9] S. Roberge, K. Nicolaides, S. Demers, J. Hyett, N. Chaillet, E. Bujold, The role of aspirin dose on the prevention of preeclampsia and fetal growth restriction: systematic review and meta-analysis, Am. J. Obstet. Gynecol. 216 (2017) 110–120.e6, https://doi.org/10.1016/j.ajog.2016.09.076.

[10] S. Meher, L. Duley, K. Hunter, L. Askie, Antiplatelet therapy before or after 16 weeks' gestation for preventing preeclampsia: an individual participant data meta-analysis, Am. J. Obstet. Gynecol. 216 (2017) 121–128.e2, https://doi.org/10.1016/j.ajog.2016.10.016.

[11] X.Q. Hu, L. Zhang, MicroRNAs in uteroplacental vascular dysfunction, Cells 29 (2019) 1344, https://doi.org/10.3390/cells8111344.

[12] A. Ali, F. Hadlich, M.W. Abbas, M.A. Iqbal, D. Tesfaye, G.J. Bouma, Q.A. Winger, S. Ponsuksili, MicroRNA-mRNA networks in pregnancy complications: a comprehensive downstream analysis of potential biomarkers, Int. J. Mol. Sci. 22 (2021) 2313, https://doi.org/10.3390/ijms22052313.

[13] J.F. Mouillet, Y. Ouyang, C.B. Coyne, Y. Sadovsky, MicroRNAs in placental health and disease, Am. J. Obstet. Gynecol. 213 (2015) S163–S172, https://doi.org/10.1016/j.ajog.2015.05.057.

[14] E. Spataro, L. Pasquini, C. Luceri, F. Petraglia, Trophoblast microRNAs, pre-eclampsia and intrauterine growth restriction, Minerva Obstet Gynecol 76 (2024) 43–48, https://doi.org/10.23736/S2724-606X.22.05109-0.

[15] J. Hong, S. Kumar, Circulating biomarkers associated with placental dysfunction and their utility for predicting fetal growth restriction, Clin. Sci. 137 (2023) 579–595, https://doi.org/10.1042/CS20220300.

[16] X. Bian, J. Liu, Q. Yang, Y. Liu, W. Jia, X. Zhang, Y.X. Li, X. Shao, Y.L. Wang, MicroRNA-210 regulates placental adaptation to maternal hypoxic stress during pregnancy, Biol. Reprod. 104 (2021) 418–429, https://doi.org/10.1093/biolre/ioaa187.

[17] P. Fasanaro, Y. D'Alessandra, V. Di Stefano, R. Melchionna, S. Romani, G. Pompilio, M.C. Capogrossi, F. Martelli, MicroRNA-210 modulates endothelial cell response to hypoxia and inhibits the receptor tyrosine kinase ligand Ephrin-A3, J. Biol. Chem. 283 (2008) 15878–15883, https://doi.org/10.1074/jbc.M800731200.

[18] S. Wang, A.B. Aurora, B.A. Johnson, X. Qi, J. McAnally, J.A. Hill, J.A. Richardson, R. Bassel-Duby, E.N. Olson, The endothelial-specific microRNA miR-126 governs vascular integrity and angiogenesis, Dev. Cell 15 (2008) 261–271, https://doi.org/10.1016/j.devcel.2008.07.002.

[19] X. Chu, Y. Gu, W. Sheng, J. Sun, J.A. Morgan, D.F. Lewis, D.B. Cooper, C. E. McCathran, Y. Wang, Downregulation of miR-126-3p expression contributes to increased inflammatory response in placental trophoblasts in preeclampsia, J. Reprod. Immunol. 144 (2021) 103281, https://doi.org/10.1016/j.jri.2021.103281.

[20] A. Sharma, L.A. Lacko, L.B. Argueta, M.D. Glendinning, H. Stuhlmann, miR-126 regulates glycogen trophoblast proliferation and DNA methylation in the murine placenta, Dev. Biol. 449 (2019) 21–34, https://doi.org/10.1016/j.ydbio.2019.01.019.

[21] X. Bian, J. Liu, Q. Yang, Y. Liu, W. Jia, X. Zhang, Y.X. Li, X. Shao, Y.L. Wang, MicroRNA-210 regulates placental adaptation to maternal hypoxic stress during pregnancy, Biol. Reprod. 104 (2021) 418–429, https://doi.org/10.1093/biolre/ioaa187.

[22] E. Bigagli, L.G. Locatello, A. Di Stadio, G. Maggiore, F. Valdarnini, F. Bambi, O. Gallo, C. Luceri, Extracellular vesicles miR-210 as a potential biomarker for diagnosis and survival prediction of oral squamous cell carcinoma patients, J. Oral Pathol. Med. 51 (2022) 350–357, https://doi.org/10.1111/jop.13263.

[23] E. Bigagli, G. Maggiore, L. Cinci, M. D'Ambrosio, L.G. Locatello, C. Nardi, A. Palomba, G. Leopardi, P. Orlando, G. Licci, O. Gallo, C. Luceri, Low levels of miR-34c in nasal washings as a candidate marker of aggressive disease in wood and leather exposed workers with sinonasal intestinal-type adenocarcinomas (ITACs), Transl Oncol 25 (2022) 101507, https://doi.org/10.1016/j.tranon.2022.101507.

[24] E. Bigagli, C. Luceri, D. Guasti, L. Cinci, Exosomes secreted from human colon cancer cells influence the adhesion of neighboring metastatic cells: role of microRNA-210, Cancer Biol. Ther. 17 (2016) 1062–1069, https://doi.org/10.1080/15384047.2016.1219815.

[25] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative C(T) method, Nat. Protoc. 3 (2008) 1101–1108, https://doi.org/10.1038/nprot.2008.73.

[26] V. Licursi, F. Conte, G. Fiscon G, P. Paci, MIENTURNET: an interactive web tool for microRNA-target enrichment and network-based analysis, BMC Bioinf. 20 (2019) 1–10.

[27] S. Giouleka, I. Tsakiridis, A. Mamopoulos, I. Kalogiannidis, A. Athanasiadis, T. Dagklis, Fetal growth restriction: a comprehensive review of major guidelines, Obstet. Gynecol. Surv. 78 (2023) 690–708, https://doi.org/10.1097/OGX.0000000000001203.

[28] H. Hayder, G. Fu, L. Nadeem, J.A. O'Brien, S.J. Lye, C. Peng, Overexpression of miR-210-3p impairs extravillous trophoblast functions associated with uterine spiral artery remodeling, Int. J. Mol. Sci. 22 (2021) 3961, https://doi.org/10.3390/ijms22083961.

[29] X.M. Zhu, T. Han, I.L. Sargent, G.W. Yin, Y.Q. Yao, Differential expression profile of microRNAs in human placentas from preeclamptic pregnancies vs normal pregnancies, Am. J. Obstet. Gynecol. 200 (2009) 661.e1–661.e7, https://doi.org/10.1016/j.ajog.2008.12.045.

[30] C.L. Whitehead, W.T. Teh, S.P. Walker, C. Leung, L. Larmour, S. Tong, Circulating MicroRNAs in maternal blood as potential biomarkers for fetal hypoxia in-utero, PLoS One 8 (2013) e78487, https://doi.org/10.1371/journal.pone.0078487.

[31] D.A. Enquobahrie, M. Hensley, C. Qiu, D.F. Abetew, K. Hevner, M.G. Tadesse, M. A. Williams, Candidate gene and MicroRNA expression in fetal membranes and preterm delivery risk, Reprod. Sci. 23 (2016) 731–737, https://doi.org/10.1177/1933719115612925.

[32] P. Kochhar, P. Dwarkanath, G. Ravikumar, A. Thomas, J. Crasta, T. Thomas, A. V. Kurpad, A. Mukhopadhyay, Placental expression of miR-21-5p, miR-210-3p and miR-141-3p: relation to human fetoplacental growth, Eur. J. Clin. Nutr. 76 (2022) 730–738, https://doi.org/10.1038/s41430-021-01017-x.

[33] S. Mayeur, M. Silhol, E. Moitrot, S. Barbaux, C. Breton, A. Gabory, D. Vaiman, I. Dutriez-Casteloot, I. Fajardy, A. Vambergue, L. Tapia-Arancibia, B. Bastide, L. Storme, C. Junien, D. Vieau, J. Lesage, Placental BDNF/TrkB signaling system is modulated by fetal growth disturbances in rat and human, Placenta 31 (2010) 785–791.

[34] G. Petetin, S. Gremlich, P. Hohlfeld, S. Gerber, Insulin-like growth factor-1 and binding protein 3 profile in second trimester amniotic fluid, as a predictive marker of fetal growth, Am. J. Obstet. Gynecol. 193 (2005) S135, https://doi.org/10.1016/j.ajog.2005.10.487.

[35] H. Wang, Y. Zhao, R. Luo, X. Bian, Y. Wang, X. Shao, Y.X. Li, M. Liu, Y.L. Wang, A positive feedback self-regulatory loop between miR-210 and HIF-1α mediated by CPEB2 is involved in trophoblast syncytialization: implication of trophoblast malfunction in preeclampsia, Biol. Reprod. 102 (2020) 560–570, https://doi.org/10.1093/biolre/ioz196.

[36] R.E. Albers, M.R. Kaufman, B.V. Natale, C. Keoni, K. Kulkarni-Datar, S. Min, C. R. Williams, D.R.C. Natale, T.L. Brown, Trophoblast-Specific expression of hif-1α results in preeclampsia-like symptoms and fetal growth restriction, Sci. Rep. 9 (2019) 2742, https://doi.org/10.1038/s41598-019-39426-5.

[37] R.M. Balahmar, B. Ranganathan, V. Ebegboni, J. Alamir, A. Rajakumar, V. Deepak, Sivasubramaniam, Analyses of selected tumour-associated factors expression in normotensive and preeclamptic placenta, Pregnancy hypertension 29 (2022) 36–45, https://doi.org/10.1016/j.preghy.2022.06.001.

[38] K. Vrijens, M. Tsamou, N. Madhloum, W. Gyselaers, T.S. Nawrot, Placental hypoxia-regulating network in relation to birth weight and ponderal index: the

ENVIRONAGE Birth Cohort Study, J. Transl. Med. 16 (2018) 2, https://doi.org/10.1186/s12967-017-1375-5.

[39] T.H. Hung, T.T. Hsieh, C.P. Wu, M.J. Li, Y.L. Yeh, S.F. Chen, Mammalian target of rapamycin signaling is a mechanistic link between increased endoplasmic reticulum stress and autophagy in the placentas of pregnancies complicated by growth restriction, Placenta 60 (2017) 9–20, https://doi.org/10.1016/j.placenta.2017.10.001.

[40] H. L Zhu, X.T. Shi, X.F. Xu, G.X. Zhou, Y.W. Xiong, S.J. Yi, W. B Liu, L.M. Dai, X. L. Cao, D.X. Xu, H. Wang, Melatonin protects against environmental stress-induced fetal growth restriction via suppressing ROS-mediated GCN2/ATF4/BNIP3-dependent mitophagy in placental trophoblasts, Redox Biol. 40 (2021) 101854, https://doi.org/10.1016/j.redox.2021.101854.

[41] L. Anton, A. DeVine, E. Polyak, A. Olarerin-George, A.G. Brown, M.J. Falk, M. A. Elovitz, HIF-1α stabilization increases miR-210 eliciting first trimester extravillous trophoblast mitochondrial dysfunction, Front. Physiol. 10 (2019) 699.

[42] W.E. Ackerman, C.S. Buhimschi, T.L. Brown, G. Zhao, T.L. Summerfield, I. A. Buhimschi, Transcriptomics-based subphenotyping of the human placenta enabled by weighted correlation network analysis in early-onset preeclampsia with and without fetal growth restriction, Hypertension 80 (2023) 1363–1374, https://doi.org/10.1161/HYPERTENSIONAHA.122.20807.

[43] I. Hromadnikova, K. Kotlabova, L. Krofta, First-trimester screening for fetal growth restriction and small-for-gestational-age pregnancies without preeclampsia using cardiovascular disease-associated MicroRNA biomarkers, Biomedicines 10 (2022) 718, https://doi.org/10.3390/biomedicines10030718.