MicroRNA-148b-3p and MicroRNA-25-3p are overexpressed in fetuses with late-onset fetal growth restriction.

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Word Count: Word count: 3389, **Table count**: 3, **Figure count**: 2 (+1 supplemental figure).

Short title: miRNA and late-onset FGR

ABSTRACT

Objective: to describe a miRNA profile characteristic of late-onset growth restriction (FGR) and to investigate those pathways involved in their biochemical action.

Material and methods: In a prospective study, 25 fetuses: 16 normal and 9 with FGR (estimated fetal weight <10th centile plus cerebroplacental ratio <0,6765 MoM) were evaluated with Doppler ultrasound after 36 weeks. Afterwards, for every fetus, plasma from umbilical vein blood was collected at birth, miRNA was extracted, and full miRNA sequencing was performed. Subsequently, comparisons were done in order to obtain those miRNA that were differentially expressed.

Result: FGR fetuses expressed upregulation of two miRNA: miR-25-3p and especially miR-148b-3p, a miRNA directly involved in Schwann cell migration, neuronal plasticity and energy metabolism (p=0.0072, p=0.0013).

Conclusions: FGR fetuses express a different miRNA profile, which includes overexpression of miR-miR-25-3p and miR-148b-3p. This information might improve our understanding of the pathophysiological processes involved in late-onset FGR. Future validation and feasibility studies will be required to propose miRNAs as a valid tool in the diagnosis and management of FGR.

Key Words: Doppler ultrasound, Late-onset fetal growth restriction, Micro-RNA, miR-148b-3p, miR-25-3p, Schwann cell.

INTRODUCTION

Fetal growth restriction (FGR) occurs when a fetus fails to reach its growth potential[1]. Its importance lies in its association with a higher probability of perinatal morbidity and mortality and the subsequent long-term neurologic and cardiovascular consequences in adult life[2,3]. FGR comprises two varieties: early-onset FGR (<34 weeks) is less frequent and is characterized by the existence of placental disease, deceleration of fetal growth and progressive hemodynamic dysfunction, typically affecting in its onset the uterine and umbilical Doppler, while late-onset FGR (>34 weeks) is more frequent and is defined by the unbalance between fetal demands and placental supply, resulting in the detection of a characteristic low cerebroplacental ratio (CPR) regardless of the estimated fetal weight (EFW)[4]. Late-onset FGR tends to be subtle. However, despite what might be thought, it is specially harmful, as it leads to frequently undiagnosed suboptimal arborization and brain underdevelopment[5,6].

Unfortunately, adverse perinatal outcome (APO) in fetuses with late-onset FGR is difficult to predict. Clinical protocols may use CPR or a combination of CPR and EFW for its identification. However, this methodology presents a poor accuracy and cannot be applied clinically yet[7-9]. Hopefully, this prediction could be theoretically improved using diverse biochemical markers, a search that has become of crucial importance.

Micro-RNAs (miRNA) are small RNA sequences, on average 22 nucleotides in length[11] with the ability to regulate gene expression in different organisms. Their action is mediated thought the inhibition of translation or the promotion of mRNA degradation[12]. Their genes are encoded within the genome, suggesting that their transcription might be coordinated with the transcription of other genes. In summary, generation of the mature miRNA molecule involves the processing of a primary miRNA transcript in the nucleus to obtain the final product in the cell cytosol, a small single RNA strand which participates in a variety of cellular processes (development, proliferation, function and differentiation) and in the pathogenesis of many human diseases[13]. miRNAs can target genes with relative specificity. To date, about to 2500 miRNA sequences are known in humans (miRBase v21)[14], and it was predicted that 30-80% of human genes may be influenced by at least one miRNA[15,16]. Interestingly, recent studies have shown that miRNAs are also expressed in the placenta suggesting a potential regulatory role in its development[17]. In addition, some miRNAs have been described to be hypoxia-regulated and associated with FGR[18].

The purpose of the current study was to define a miRNA profile characteristic of late-onset FGR investigating those pathways involved in their biochemical action.

MATERIAL AND METHODS

Patient recruitment and Doppler examination

This was a prospective study of 25 fetuses attending at the public tertiary maternity of La Fe hospital (Institution Review Board and Hospital Ethics Committee permission number 2016/053). These fetuses were performed an ultrasound examination between 36 and 40 weeks which included a biometry and EFW calculation plus a Doppler evaluation of the umbilical (UA) and middle cerebral arteries (MCA) pulsatility indices (PI). The UA and MCA were recorded using color and pulse Doppler according to earlier descriptions[19-20] and the CPR was calculated as the simple ratio between the MCA PI and the UA PI[21]. All pregnancies were delivered in 15 days or less after the scan and only the last examination per fetus was included in the analysis. In order to adjust for the effect of the GA, EFW, and birth weight (BW) values were converted into local reference centiles[22] adjusted only for fetal gender. Also, CPR values were converted into multiples of median (MoM) dividing each value by the 50th centile at each gestational age as earlier described[19]. CPR medians (50th centile) were represented by the equation:

$$\text{CPR 50th centile} = -3.814786276 + 0.36363249 \times \text{GA} - 0.005646672 \times \text{GA}^2$$

Where GA was gestational age in weeks with decimals.

All Doppler examinations were performed by the first author (JMR), a certified teaching expert in obstetric ultrasound by the Spanish Society of Obstetrics and Gynecology, using General Electric Voluson® (E8/E6/730) ultrasound machines (General Electric Healthcare, Spain) with 2-8 MHz convex probes, during fetal quiescence, in the absence of fetal tachycardia, and keeping the insonation angle with the examined vessels as small as possible and always below 30°.

GA was determined according to the crown-rump length in the first trimester. Multiple pregnancies and those complicated by congenital fetal abnormalities or aneuploidies were excluded. Gestational characteristics including parity, number of gestations and maternal ethnicity, age, weight and height, were collected at examination, together with the indicated ultrasound parameters. Labor outcome data including BW, BW centile, mode of delivery, 5 minutes Apgar score, cord arterial pH and admission to the neonatal care unit were also collected at birth.

Ponderal and hemodynamic characteristics of the groups studied

For comparison purposes, the study included two different types of fetuses: late-onset FGR fetuses, with an abnormal EFW (<10th centile) and an abnormal CPR (<0,6765 MoM), and normal fetuses, with a normal EFW (>10th centile) and a normal CPR (>0,6765 MoM)[19]. Other fetuses with intermediate features (abnormal CPR with normal EFW or normal CPR with abnormal EFW) were not considered.

Sample collection and small RNA extraction and quantification

After birth, plasma samples from fetal umbilical vein and maternal peripheral blood were collected in EDTA tubes and centrifuged at 3500 rpm for 10-15 minutes. Once plasma was obtained, each sample was stored at −80 °C until small RNA extraction. While maternal plasma was stored for future research, 500 µL of fetal blood plasma were used to isolate cell-free total RNA (including miRNAs) using the miRNeasy Serum/Plasma kit (Qiagen, Valencia, CA, USA) following the manufacturer's protocol. The RNA was eluted with 25 µL of RNase-free water. The concentration of cell-free total RNA (including miRNAs) was quantified using NanoDrop ND 2000 UV-spectrophotometer (Thermo Scientific, Wilmington, DE, USA).

Library preparation and next-generation sequencing

Small RNA libraries were generated and indexed using a modified Illumina TruSeq small RNA protocol. In this modified protocol the libraries were size selected (range 90-170pb) using Blue Pippin instrument (Sage Science, Beverly, MA, USA). A positive RNA control was included (Thermo Fisher Scientific Human Brain Total RNA catalog #AM7962). Single-end sequencing was performed on Illumina NextSeq platform on High Output 1x50pb RUN (NextSeq 500/550 High Output v2 75 cycles kit, FC-404-2005).

Differential expression analysis

The first step was to assess the quality of the Illumina raw sequences with the FastQC software (https://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Based on the results obtained, the sequence reads were trimmed to remove sequencing adapters and low-quality bases using the software Cutadapt (http://cutadapt.readthedocs.org/en/stable/). Once the data were deemed of sufficient quality, they were mapped against the human GRCh38 build reference sequence, taken from Ensembl. After that, the intersection between the aligned position of reads and the miRNA coordinates taken from miRBase v21 was performed. The alignment and quantification steps were performed using Subread and Rsubread Packages[23,24].

A multi-dimensional scaling (MDS) plot was used to get a closer look at how samples were distributed according to the miRNAs expression values. MiRNAs with very low counts across all libraries provide little evidence for differential expression. We filtered

out these miRNAs prior to further analysis. Subsequently, trimmed mean of M-values normalization method TMM normalization[25] was performed to eliminate composition biases between libraries. We also estimated the specific dispersions per gene with a negative binomial distribution[26,27].

Groups Comparison

After miRNA was extracted and sequencing was performed, comparisons were done between the group of late-onset FGR fetuses and the group of normal fetuses (control group). As earlier indicated, late-onset FGR was considered when abnormal values of CPR[19,28] plus abnormal values of BW[22] were present. Conversely, normality was considered if both parameters were within normal range. As per local protocol, all fetuses were subsequently managed according to their progression in labor, including intrapartum fetal heart rate, which was interpreted according to the FIGO guidelines[29]. All study comparisons were performed using Mann Whitney test for continuous data and Fisher´s exact test for categorical data. Significance was considered with a $p<0.05$.

Prediction of miRNA targets and over-representation analysis

We first used DIANA-microT-CDS accessed from DIANA web server[30]. This tool shows whether the target was also predicted by miRanda or TargetScan or was experimentally validated in TarBase v7.0. We used the DIANA-miRPath v3.0 functional analysis online suite to identify miRNAs controlling significant molecular pathways annotated on Kyoto Encyclopaedia of Genes and Genomes (KEGG), using as default parameters: experimentally supported interactions from DIANA TarBase v.7.0; a p-value threshold of 0.001; and a microT threshold of 0.8. To reduce the number of false positive miRNA targets, we applied a false discovery rate (FDR) correction to selected KEGG pathways. The algorithm used in this analysis was a one-tailed Fisher's exact test[31].

RESULTS

Descriptive statistics of the study population

The study included 25 fetuses, of which 14 (56%) were male and 11 (44%) female, being all of Spanish Caucasian origin. Supplemental figure 1 shows the distribution of measurements according to the CPR MoM and BW centile[8,19,28]. 9 patients presented late-onset FGR (abnormal CPR plus abnormal BW values) while 16 were fetuses with normal hemodynamic and ponderal features (normal CPR plus normal BW).

In table 1 we compare normal versus FGR pregnancies. In summary, mothers in the FGR group were thinner than those in the normal group and fetuses had a lower CPR MoM, EFW, EFW centile, BW and BW centile ($p<0.001$). In addition, when compared with the fetuses with normal outcome, FGR fetuses were delivered earlier ($p=0.04$).

Identification of differentially expressed miRNAs by smallRNA-seq

Table 2 shows the initial comparison between normal and FGR fetuses regarding the differential expression analysis of up-regulated and down-regulated miRNAs. For accuracy and selection purposes, only miRNAs with the criteria of FDR < 0.05 were included. FGR fetuses showed, in comparison with normal fetuses, a total of 4 initial differentially expressed miRNAs: miR-148b-3p, miR-25-3p and miR-16-5p, which were up-regulated and miR-1910-5p, which was down regulated. miR-148b-3p presented by far the highest significance. As matter of caution, we discarded miR-16-5p due to its relation with hemolysis, which might be always present in blood samples in a minor degree[32,33]. Figure 1 shows the heatmap of miRNA expression profile with the miRNA selected in table 2. The cluster was done on the basis of log2 (expression level in treatment/expression level in control). Yellow shows down-regulation of miRNAs and red shows up-regulation of miRNAs in blood samples from neonatal cord.

Logarithm counts per million of reads comparison

Figure 2 shows the box and whiskers plots representing the logarithm counts per million of reads (logCPM) of those miRNA that presented significant differences: miR-148b-3p and miR-25-3p (Mann Whitney $p<0.05$). It is noteworthy to underline that a difference existed between the differential expression analysis of up-regulated and down-regulated miRNAs and the individual statistical analysis performed between normal and FGR fetuses concerning the Logarithm counts per million of reads. When the analysis of differential expression was done, 4 miRNAs were significant with a FDR <0.05 (table 2). However, when the logarithm counts per million of reads comparison was performed in an independent way and without considering the expression of all those miRNAs that contributed to the differential expression analysis, only miR-148b-3p and miR-25-3p

turned out to be finally statistically significant (figure 2). Therefore, only these were finally selected to explore relevant pathways related with FGR.

Analysis of miRNA targets and biochemistry pathways in the context of FGR

All miRNAs had a large number of potential target sites, so we explored those with relevance for FGR. Interestingly and in agreement with figure 2, miR-1910-5p did not release any pathway. Therefore, miR-16-5p and miR-1910-5p where finally removed from the analysis. In order to clarify the role of the remaining miR-148b-3p and miR-25-3p in relation with FGR, we analyzed the biochemical networks in which they participated. We carried out a DIANA-miRPath v3.0 analysis and KEGG pathway analysis to look for any significantly enriched pathway. A total of 29 pathways with an FDR below 0.05 were retrieved (table 3). Some of the them related with lipid metabolism, like the biosynthesis of fatty acids[31] and sphingolipids[34], crucial for neuronal tissue development, while, other related with protein processing in the endoplasmic reticulum[35] or with protein metabolism, like the biosynthesis of branched chain amino acids (BBCA) Valine, leucine and isoleucine[36,37].

DISCUSSION

1-Principal findings

Analyzing circulating miRNAs from neonatal cord blood and using next-generation sequencing we found that miR-148b-3p and miR-25-3p were up-regulated in late-onset FGR fetuses. This different miRNA profile is a novel finding that might improve our understanding of late-onset growth compromise.

2-Research implications

CPR MoM has emerged as the best marker of APO at the end of pregnancy[38-41], however its accuracy as a single parameter or in association with EFW centile and other clinical parameters is not enough to obtain clinically reliable results[8]. As a consequence, new biochemical markers are being investigated in order to obtain accurate predictions of APO and neurocognitive dysfunction.

In the recent year the focus has shifted to the miRNAs. These molecules behave as fine tuners in the regulation of gene expression and are crucial for many biological processes[42]. A large number of the more than 1000 miRNAs discovered in humans relate with pregnancy and are produced by the placenta and the uterus in normal and pathological conditions. They exert their action locally in a paracrine fashion or distally released as exosomes in maternal blood, regulating fetal and maternal homeostasis[42]. Moreover, several studies showed that altered expression of the miRNome in maternal circulation or in placental tissue may reflect gestational disorders, such as preeclampsia, spontaneous abortion, preterm birth, low BW, or macrosomia[43].

2A-Role in neuronal plasticity

miR-148b seems to have special relevance in diverse molecular mechanisms related to neuronal hypoxia, neurogenesis and neuronal metabolism and development. Particularly, miR-148b-3p up-regulation promoted Schwann cell (SC) migration, whereas silencing of miR-148b-3p inhibited SC migration *in vitro*[44]. The molecular background of miR-148b-3p is in fact very interesting. It belongs to the miR-148/152 family[45], which includes miR-148a, miR-148b and miR-152 and is considered a placental associated miRNA, which means it is expressed ubiquitously[44], not only in the placenta, but also in other tissues. However, as indicated, the most interesting issue concerning its role in fetal medicine is its ability to promote the growth of SC, responsible of myelin formation. miR-148-3p plays a role in the regeneration of peripheral nerves by regulating SC migration via targeting Cullin-associated NEDD8-dissociated protein 1 (Cand1). Overexpression of miR-148-3p enhanced the migratory ability of SC, while inhibition attenuated SC migration in vitro[46]. These effects are done at unison with other miRNAs like miR-132,

miR-210, miRNA sc-3, miR-221, and miR-222, which also increase the migratory ability of SC, and miRNA sc-8, miR-9, miR-98, miR-1, and miR-182, which diminish due ability[47].

A parallelism may therefore be done between peripheral nerve repair and axonal development (arborization) in the central nervous system (CNS). A good example of this is miR-132, which apart from promoting peripheral nerve repair mediated by SC as indicated, has been found to protect the central nervous system; miR-132 controls dendritic plasticity[48] and is required for normal dendrite maturation in newborn neurons[49]. Therefore, miRNA-132 functions as a key activity-dependent regulator of cognition, whose expression must be maintained within a limited range to ensure normal learning and memory formation[50]. In fact, miR-132 has been considered as a master regulator of neuronal health[51] and its supplementation is being evaluated for the treatment of diseases like Tau-associated neurodegenerative disorders[52]. Therefore, in an analogous way, miR-148b-3p might also play a role in the protection of the CNS. In theory, as brain tissue depends on myelination, miR-148b-3p might contribute to the protection of brain tissue under different circumstances, like chronic hypoxia[53].

2B-Role in energy production

The possible role of miR-148b-3p in the protection of SC might have a relationship with a number of biochemical functions. Regarding carbohydrates, miR-148b inhibits hypoxia-induced elevation of lactate production and hypoxia-induced increase of glucose consumption, therefore reducing cellular growth[54]. Regarding amino-acid and proteins, 148b-3p and miR-25-3p behave as key regulators of the biosynthesis of valine, leucine and isoleucine and also regulate protein processing at the endoplasmic reticulum, being both pathways of special relevance for fetal growth during the last trimester of pregnancy[35-37] and in periods of nutritional deprivation[55]. Finally, regarding fatty acid metabolism, both miR-25-3p and miR-148b-3p control the biosynthesis of fatty acids and sphingolipids[31,34,56], essential molecules for stem cell differentiation morphogenesis and embryo development[56] that are also related with preeclampsia and FGR[57,58].

3-Clinical implications

A practical resultant of the differential expression of miR-25-3p and miR-148b-3p in fetal blood would be the possibility to detect them also in maternal blood in order to develop clinical diagnostic tests. Hypoxia-related miRNA produced in the placenta have been detected in maternal blood[59]. In this regard, If miRNAs are able to cross the placental barrier and circulate between the mother and the fetus[40], miR-148b-3p and miR-25-3p might also be detected in maternal serum and become markers of APO in an isolated or combined way, consequently improving the diagnostic accuracy of the fetus with late-

onset FGR.

4-Strengths and limitations

The strengths of this study are first its novelty, as we have been the first investigators to perform a full sequencing of all miRNA in fetal blood, and second the finding of a miRNA profile directly related with neuronal development. A shortcoming however might be the absence of validation in a different population, the paucity of follow-up and the absence of data related with neurocognitive evolution in childhood.

5-Conclusion

FGR fetuses express a different miRNA profile, which includes overexpression of miR-25-3p and especially miR-148b-3p, miRNAs related with cellular metabolism and neuronal plasticity. Future work is needed to assess the levels of miR-148b-3p and miR-25-3p in maternal serum in order to evaluate if they could improve the understanding and management of late-onset FGR, helping in the prediction of neurocognitive disability.

STATEMENTS

Aknowledgements:

We thank the midwifes and the personnel of the maternity operating rooms for their contribution during the collection of blood samples.

Funding sources:

Funds were obtained from several sources: 1-iPlacenta project, European Union's Horizon 2020 Research and Innovation Programme, MarieSkłodowska-Curie grant agreement Number 765274, 2- VLC-Biomed Grant, Universidad de Valencia & Universidad Politécnica de Valencia, 3- Clinical Study Protocol X213220 Xoma, Hospital Universitario y Politécnico La Fe, 4- Charity Run "9 km 9 meses", Instituto de Investigación Sanitaria La Fe and Ayuntamiento de Torrente, Valencia, Spain.

Statement of ethics:

Institutional Review Board permission was obtained for this study (Reference 2016/053).

Authors' contributions:

José Morales-Roselló designed the study, performed the ultrasound examinations and wrote the manuscript.

José Luis García Gimenez, Llucia Martinez Priego, Daymé González-Rodríguez, Salvador Mena-Mollá and Angel Maquieira Catalá made the genetic analysis, supervised the final manuscript and suggested valuable inputs to the text.

Gabriela Loscalzo, Silvia Buongiorno and Vaidile Jakaite, performed data search and made notable contributions to the final text.

Antonio José Cañada Martínez performed part of the statistical analysis.

Alfredo Perales Marín supervised the manuscript and suggested valuable inputs to the text.

Conflict of interests:

José Luis García-Giménez and Salvador Mena-Mollá own stocks in EpiDisease SL, an epigenetics company focused to the development of epigenetic biomarkers. Other authors report no conflict of interests.

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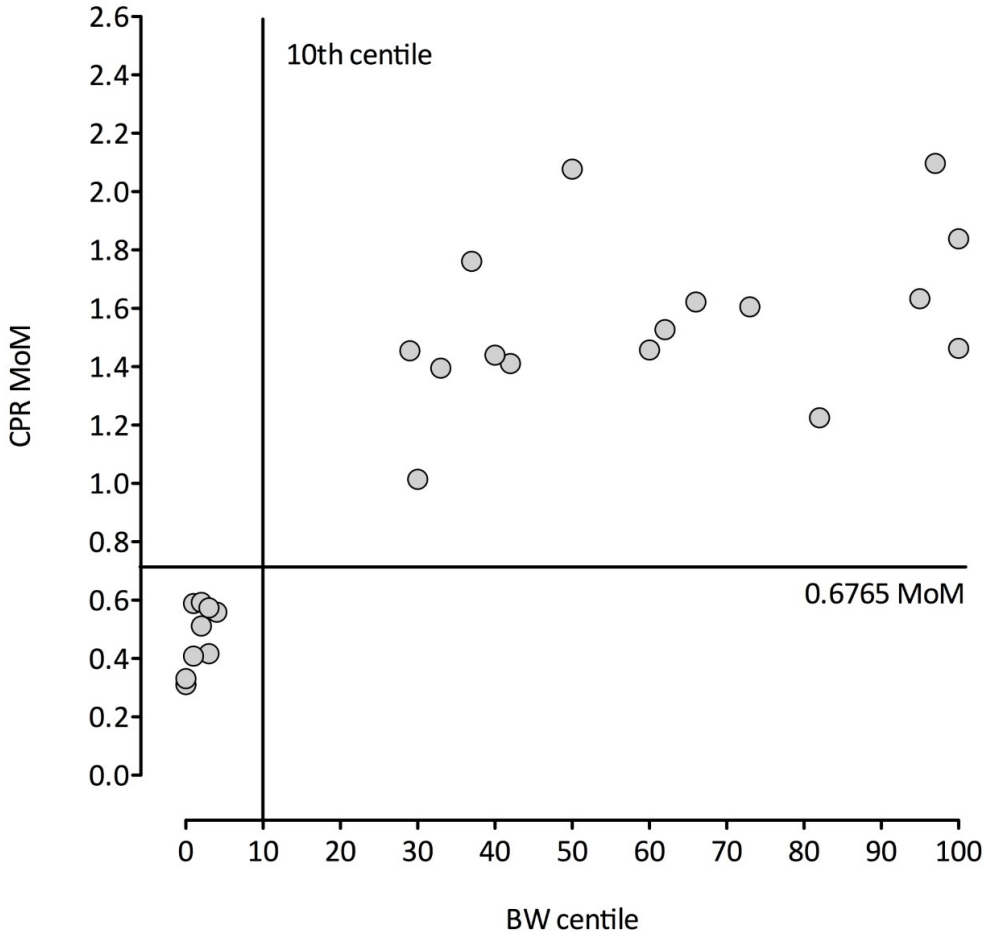


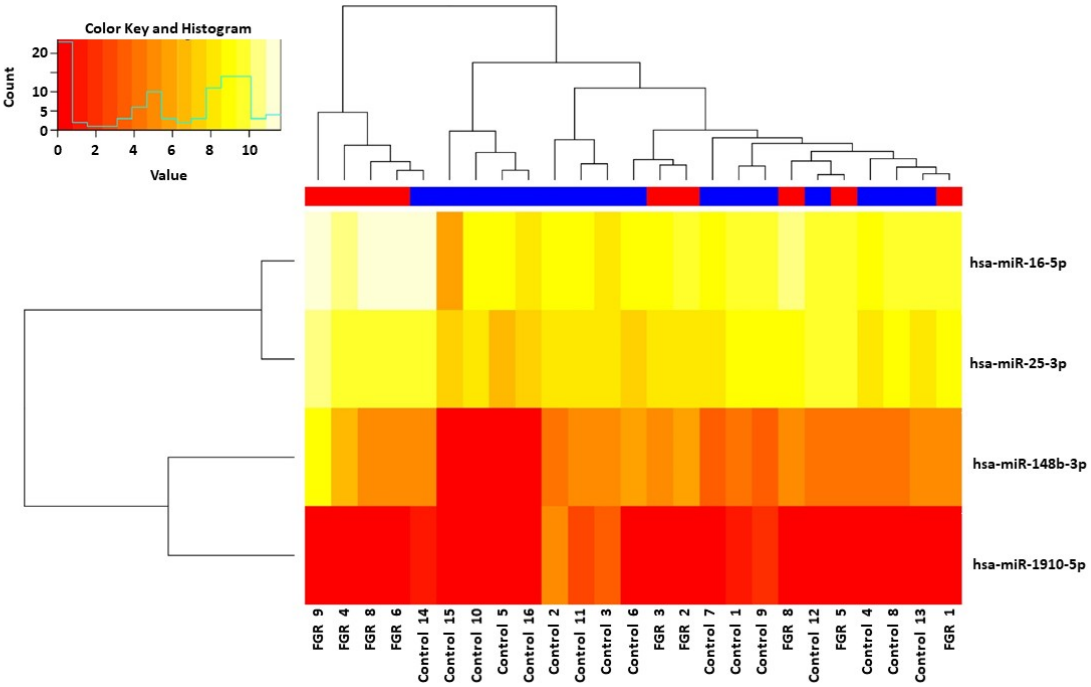
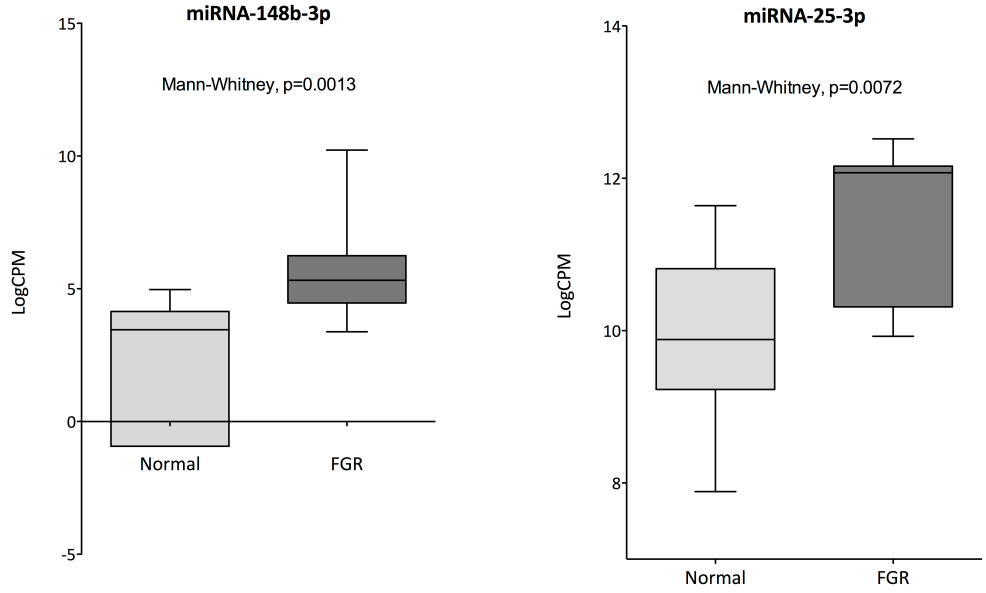


Figure 1.

Heatmap with the hierarchical clustering of differentially expressed miRNAs in late-onset FGR (red) versus normal fetuses (blue) according the expression levels of miRNAS (miR-1910-5p, miR-148b-3p, miR-16-5p and miR-25-3p).

Figure 2.



Box plots representing the logarithm counts per million of reads (logCPM) of circulating miRNAs (miR-148b-3p, and miR-25-3p) in late-onset FGR fetuses compared to normal fetuses. Whiskers represent the 10th and 90th centiles.

Table 1.

	Normal fetuses, N=16		FGR fetuses, N=9		
	Mean (SD)	**Median (1st, 3rd Q), range**	**Mean (SD)**	**Median (1st, 3rd Q), range**	**P-value**
Maternal age	33.4 (4.6)	33.5 (29.25, 36), 27-41	34.2 (5.04)	34 (29.5, 38.5), 27-42	0.73
Gestations	1.87 (1.1)	2 (1, 2), 1-5	1.89 (1.36)	1 (1, 2.5), 1-5	0.78
Parity	0.62 (0.72)	0.5 (0, 1), 0-2	0.67 (0.87)	0 (0, 1.5), 0-2	0.98
Maternal weight (kg)*	65.56 (11.35)	67 (53.5, 73), 52-86	51.7 (8.2)	51 (48, 55), 40-67	0.01
Maternal Height (cm)**	163.8 (10.5)	168 (156, 171), 144-176	161.7 (2.5)	162 (160, 164), 158-165	0.38
GA at examination (weeks)	39.4 (1)	39.6 (38.7, 40.2), 36.7-40.4	38.3 (1.4)	38.1 (37.0, 39.4), 36.7-40.7	0.08
EFW hadlock-4 (g)	3547 (441.5)	3505 (3161, 4013), 2830-4246	2491 (456)	2500 (2134, 2763), 1810-3373	<0.001
EFW Local Pop. Ref. centiles	70.44 (27.2)	78 (47.25, 95.25), 21-99	7.2 (10.3)	4 (0.5, 11), 0-32	<0.001
CPR MoM	1.56 (0.28)	1.49 (1.42, 1.73), 1.01-2.1	0.48 (0.11)	0.51 (0.37, 0.58), 0.31-0.59	<0.001
GA at labor (weeks)	40.14 (1.08)	40.6 (39.3, 41), 37.9-41.4	38.75 (1.5)	38.86 (37.4, 40.1), 36.9-41	0.04
Interval exam-labor (days)	5.44 (3.6)	4.5 (3, 8), 1-15	3 (2.34)	2 (1, 5), 0-7	0.87
Birth weight (g)	3610 (467.9)	3500 (3320, 3823), 3000-4700	2381 (324.7)	2350 (2118, 2665), 1845-2800	<0.001
BW Local Pop. Ref. centiles	62.25 (26.3)	61 (37.7, 91.7), 29-100	1.78 (1.39)	2 (0.5, 3), 0-4	<0.001
	N (%)		**N (%)**		**P-value**
Gender					0.22
Male	7 (43.7%)		7 (77.8%)		
Female	9 (56.3%)		2 (22.2%)		
BW <10th Local Pop. Ref. centiles	0 (0%)		9 (100%)		-
Apgar 5 min <7	0 (0%)		0 (0%)		-
Arterial cord pH <7.20***	3 (18.7%)		2 (22.2%)		0.78
Ethnicity					-
Caucasian	16 (100%)		9 (100%)		
Non Caucasian	0 (0%)		0 (0%)		
Smokers****	0 (0%)		1 (11.1%)		-
Onset of labor					0.93
Induction of labor	6 (37.5%)		8 (88.9%)		
Spontaneous onset of labor	6 (37.5%)		0 (0%)		
Cesarean section (abnormal CTG)	0 (0%)		0 (0%)		
Cesarean section (elective)	4 (25%)		1 (11.1%)		
Mode of delivery					0.95
Spontaneous vaginal delivery	9 (56.3%)		4 (44.4%)		
Assisted vaginal delivery	1 (6.25%)		1 (11.1%)		

Cesarean section (abnormal CTG)	1 (6.25%)	3 (33.3%)	
Cesarean section (dystocia)	5 (31.2%)	1 (11.1%)	
Admission to neonates unit			0.054
No	16 (100%)	7 (77.8%)	
Yes	0 (0%)	2 (22.2 %)	

Descriptive statistics of the two groups studied: normal and FGR fetuses.

Notes: FGR: fetal growth restriction, SD: standard deviation, Q: quartiles, GA: Gestational age, EFW: estimated fetal weight, MCA PI middle cerebral artery pulsatility index, UA PI: umbilical artery pulsatility index, CPR: cerebroplacental ratio, MoM: multiples of the median, Mean UtA PI: mean uterine arteries Doppler pulsatility index, Local Pop. Ref. centiles: centiles according to local population references (Hospital Clinic de Barcelona, Spain population references), CTG: cardiotocography,, SGA: small for gestational age (<p10), *data was missing in 7 and 2 patients respectively, **data was missing in 6 and 2 patients respectively, ***data was missing in 1 patient in each group, ****data was missing in 7 and 1 patients respectively.

Table 2.

Differential miRNA expression between FGR and normal fetuses.

miRNA	LogFC	logCPM	F	p-value	FDR
hsa-miR-148b-3p	2.913109	6.392020	23.88636	7.813586e-06	0.003484859
hsa-miR-16-5p	1.639640	12.972890	18.11788	7.311848e-05	0.016305421
hsa-miR-1910-5p	-4.069241	0.916709	15.22769	2.465907e-04	0.036659814
hsa-miR-25-3p	1.339374	11.323960	14.20868	3.726486e-04	0.041550317

logFC: logarithm with the base of 2 of the fold change. A negative logFC value corresponds to a downregulated miRNA and a positive logFC value means that the miRNA is upregulated relative to the reference condition. logCPM: logCPM: logarithm in base 2 of the counts per million reads obtained by the miRNA; F: the value of the statistic test, FDR: p corrected value with false discovery rate.

Table 3.

Selected KEGG pathways regulated by differentially expressed miRNAs miR-25-3p and miR-148b-3p in FGR vs. normal fetuses.

KEGG pathway	p-value	Genes	miRNAs
Prion diseases	7.31985499212e-15	5	miR-148b-3p and miR-25-3p
Fatty acid biosynthesis	3.52242071263e-13	2	miR-148b-3p
Oocyte meiosis	1.27759516409e-05	31	miR-148b-3p and miR-25-3p
Cell cycle	1.7071934491e-05	42	miR-148b-3p and miR-25-3p
Viral carcinogenesis	1.7071934491e-05	45	miR-148b-3p and miR-25-3p
Lysine degradation	3.01715187799e-05	14	miR-148b-3p and miR-25-3p
Estrogen signaling pathway	0.00028055720575	26	miR-148b-3p and miR-25-3p
P53 signaling pathway	0.000316939071553	24	miR-148b-3p and miR-25-3p
FoxO signaling pathway	0.000929050869461	37	miR-148b-3p and miR-25-3p
Protein processing in endoplasmic reticulum	0.000929050869461	42	miR-148b-3p and miR-25-3p
Adherens junction	0.00183745694845	19	miR-148b-3p and miR-25-3p
Lon-term depression	0.00183745694845	15	miR-148b-3p and miR-25-3p
Proteoglycans in cancer	0.0019678042498	42	miR-148b-3p and miR-25-3p
Steroid biosynthesis	0.00292457111324	4	miR-148b-3p
Hippo signaling pathway	0.00292457111324	34	miR-148b-3p and miR-25-3p
Valine, leucine and isoleucine biosynthesis	0.00360593689391	2	miR-25-3p
Hepatitis B	0.00373563003963	35	miR-148b-3p and miR-25-3p
Progesterone-mediated oocyte maduration	0.00574829839216	26	miR-148b-3p and miR-25-3p
cGMP-PKG signaling pathway	0.00675142184669	40	miR-148b-3p and miR-25-3p
Prostate cancer	0.0079060270266	25	miR-148b-3p and miR-25-3p
Endometrial cancer	0.0100040376492	15	miR-148b-3p and miR-25-3p
Non-small cell lung cancer	0.0100700510791	15	miR-148b-3p and miR-25-3p
Chronic myeloid leukemia	0.0129054369882	21	miR-148b-3p and miR-25-3p
Sphingolipid signaling pathway	0.0131352576976	27	miR-148b-3p and miR-25-3p
Colorectal cancer	0.015375047919	16	miR-148b-3p and miR-25-3p
Glioma	0.015375047919	17	miR-148b-3p and miR-25-3p
Sulfur metabolism	0.0155260139399	2	miR-148b-3p
Thyroid cancer	0.0263000781158	9	miR-148b-3p and miR-25-3p
RNA degradation	0.0440095764865	21	miR-148b-3p and miR-25-3p