Feasibility of diffusion weighted imaging (DWI) for murine placenta analysis

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Authors: M. Alison, G. Chalouhi, N. Siauve, G. Autret, D. Balvay, R. Thiam, O. Clement, G. Sebag, C. A. Cuenod; Paris/FR
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Purpose

Placental insufficiency due to perfusion defect leads to decreased placental function with severe materno-fœtal complications such as preeclampsia, fetal growth restriction and preterm birth.

At the moment, no imaging modality is available routinely to analyse direct placental function in vivo.

Uterine and fetal pulse color doppler only make an indirect and delayed diagnosis of placental dysfunction.

Magnetic Resonance Imaging, a non invasive technique without radiation has the potentiality to quantify the microcirculation during pregnancy. These quantitative parameters are in development using either dynamic contrast enhanced MRI (DCE) (1-3) or the diffusion technique (4-8). The diffusion weighted imaging technique (DWI) has only been assessed in very few clinical studies: the Apparent Diffusion Coefficient (ADC) has been recently used to assess placental insufficiency (4). The intra voxel incoherent motion (IVIM) diffusion technique (9) has also been tested in few clinical studies to assess liver disease (10) and also to assess placenta (5-8). The role and physiological significance of the parameters obtained with the DWI techniques have not been established. Further studies, including the use of controlled animal models, are therefore necessary.

The aim of the study was to develop the Diffusion technique, based on both the ADC and the IVIM theory, for the diagnosis of placental insufficiency, on a murine model of vascular fetal growth restriction, on a dedicated small animal 4.7 T scan.

Methods and Materials

Animal model of vascular fetal growth restriction

Gestant sprague Dawley female rats were studied. They have a bicornuate uterus and a short gestation time of 21 days. A left unilateral uterine artery ligation was performed on the 17th embryonic day (E17) (11) under general anaesthesia with Isoflurane gas (4.5% for induction and 1.5% thereafter) (Fig 1). Fetal growth restriction is obtained on the left horn: the most severely growth restricted fetuses are found adjacent to ligation (Fig 2).
Model validation

Validation of the vascular restricted growth model was obtained by measuring the weight of each fetus and each placenta, with comparison of both horns (with and without ligation). This correlation was performed by laparoscopy, at E19, after imaging.

Imaging

MRI was performed on E19, on a 4.7T scan (Biospec 47/40 USR Brucker) with a quadrature transmit/receive body coil with a 7 cm inner diameter, under general anaesthesia with Isoflurane gas (1.5%). Body temperature was controlled with a heated water pad during all study. All sequences were performed with respiratory trigger. The imaging protocol included the following sequences (Fig 3):

1) An anatomic T2-weighted sequence (True Fisp) was performed in the coronal and axial plane:

2) The diffusion weighted imaging (DWI) consisted of a segmented EPI sequence in the coronal plane, in 3 directions: TR=1000; TE=26; FA=90°, BW=250 000; slice thickness=2mm; FOV=10x8cm; Matrix=128x128, spatial resolution= 0.0781x0. 0625 cm/pixel; Nex=1; Segment=4; Dummy scan=5; fat saturation (BW 600Hz); 18 b values=0,10 ,25 ,50 ,75 ,100 ,125, 150, 200, 300, 400, 500, 600, 700, 800, 1000, 1250, 1500 s/mm².

Study design included 2 different analyses: acquisition under air (normal condition) and acquisition under maternal hyperoxygenation, to study if oxygen had an effect on diffusion. The DWI was therefore repeated under those two different states: room air and maternal hyperoxygenation (6L/min).

Data analysis

Anatomic analysis:

Feto-placental units (FPU) were localized, counted and their signal was characterized on the T2-weighted sequence.

Functional analysis:

- Placenta analysis: manual segmentation of the placenta was performed with different region of interest (ROI): the whole placenta (total) and then two layers (fetal and maternal). In each ROI, the signal intensity according to the b factors values was measured, to obtain the SI decay curve.
- DWI analysis: a monoexponential fit of these curves was performed to calculate the Apparent Diffusion Coefficient (ADC), according to the following formula: $S/S_0 = S_0 e^{(-b \cdot \text{ADC})}$.

- IVIM analysis: a biexponential fit was performed to calculate the Diffusion Coefficient ($D_r$), the pseudo perfusion coefficient ($D^*$) and the perfusion fraction ($f$), using the following formula: $S_b/S_0 = f \cdot e^{-b(D_r+D^*)} + (1-f) e^{-b \cdot D_r}$

Curves fitting were obtained with a Matlab program developed in our lab.

**Statistical analysis**

All results are expressed as median and inter quartile.

For each parameter (ADC, $D_r$, $f$, $D^*$):

- left growth restricted placentas were compared to normal right placentas

- fetal layer was compared to maternal layer in each horn, to assess placental structural analysis

- Effect of maternal hyperoxygenation was studied in each horn, and parameters under hyperoxygenation were compared between the two horns.

A multiple regression analysis model was used and a p value of less than 0.05 was considered statistically significant.

**Images for this section:**
Fig. 1: Surgical view of the ligation of the left uterine artery
Fig. 2: Bicornuated uterus of gestant rat. Restricted growth model on the left side obtained with left uterine artery ligation (red cross). Adapted from Wigglesworth (11)
Fig. 3: MRI protocol
Results

1- Validation of the vascular growth restricted model

Ten gestant rats were analyzed. Forty nine viable FPU were found in the right horn and 22 viable FPU in the left horn (Fig 1). Figure 1 shows the mean weight of the fetuses and the placentas in both horns. In the left horn (with ligation) fetal and placental weights were significantly lower than in the right horn (normal horn without ligation) (Fig 1, Fig 2). Only one dead fetus was found in the right horn whereas 17 dead fetuses were found in the left horn. Our study excluded the dead FPUs.

2- Anatomic analysis

On the T2-w sequence (Fig 3), the bicornuate uterus is well visualized with good spatial and contrast resolutions in the fetuses (*) and the placentas (arrow). Two layers of the placentas are differentiated by their different signal intensity: the fetal and the maternal layer. These two layers were also seen on the diffusion sequence.

3- Functional analysis

The figure 4 shows the region of interest on each layer of the placenta. A signal intensity decay curve according to the different b values was obtained for each region of interest. Fig 4 shows an example of a biexponential fit that determines the parameters: f, Dr and D*.

3a) Global analysis of the placenta

Monoexponential analysis: ADC

On normal side (right horn), the median ADC of total placentas was 1.16 mm$^2$/s under room air.

ADC was significantly decreased on growth restricted placentas (median: 1.11 mm$^2$/s) compared to the normal placentas (right) (p <10$^{-4}$). Figure 5 demonstrates the decrease of ADC on growth restricted side compared to normal side. ADC was significantly correlated with fetal weight (p=0.03) (Fig 6).
**Biexponential analysis**

On normal side (right horn), the median values of the parameters for total placentas, under room air, were 0.67 mm$^2$/s for $D_r$, 32.6 % for $f$ and 3.8 mm$^2$/s for $D^*$. 

On growth restricted side, parameters were not statistically different form normal side: 0.59 mm$^2$/s for $D_r$, 33 % for $f$ and 2.8 mm$^2$/s for $D^*$. 

However, $D_r$ (Fig 7), $D^*$ (Fig 8) and $f$ (Fig 9) tended to decrease on growth restricted side (p=0.056, p=0.089, p=0.23 respectively).

**3b) Analysis of the placental layers**

**Monoexponential analysis: ADC**

ADC was significantly higher in fetal than in maternal layer in both horns ($p=10^{-4}$) (Fig 10). In the right horn, median ADC was 1.33 mm$^2$/s for the fetal layer and 1.04 mm$^2$/s for the maternal layer. In the left horn, median ADC was 1.34 mm$^2$/s for the fetal layer and 0.93 mm$^2$/s for the maternal layer.

**Biexponential analysis**

$D_r$ and $f$ were significantly higher in fetal than in maternal layer in both horns ($p=0.015$, $p=0.009$ respectively) (Fig 11). In the right horn, median $D_r$ was 0.76 mm$^2$/s for the fetal layer and 0.56 mm$^2$/s for the maternal layer. In the left horn, median $D_r$ was 0.74 mm$^2$/s for the fetal layer and 0.60 mm$^2$/s for the maternal layer.

$f$ was more decreased in the maternal layer of the growth restricted group (median: 24.3%) compared to the normal side (median: 33.9 %), but this was not statistically significant ($p=0.1$) (Fig 12).

**3c) Maternal hyperoxygenation effect**

**Monoexponential analysis: ADC**

ADC increased significantly with maternal hyperoxygenation in both horn ($p=10^{-4}$) (Fig 13). The median ADC of total placentas was 1.48 mm$^2$/s in the right horn and 1.31 mm$^2$/s in the left horn, under maternal hyperoxygenation.
The increase of ADC was more important on the normal right side, than on the restricted growth left side, therefore the difference of ADC between both horns was more important under maternal hyperoxygenation (Fig 14) than under room air.

**Biexponential analysis**

Dr increased significantly with maternal hyperoxygenation in both horn: 0.94 mm$^2$/s in the right and 0.93 mm$^2$/s in the left horn ($p=10^{-4}$) (Fig 15).

**Images for this section:**

<table>
<thead>
<tr>
<th>Foeto-placental units (FPU)</th>
<th>Right horn</th>
<th>Left horn ligation</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total number</td>
<td>n= 50</td>
<td>n=39</td>
<td></td>
</tr>
<tr>
<td>Dead number</td>
<td>n=1</td>
<td>n=17</td>
<td></td>
</tr>
<tr>
<td>Alive number</td>
<td>n= 49</td>
<td>n=22</td>
<td></td>
</tr>
<tr>
<td>Fetal weight Mean $\pm$ SD</td>
<td>2.8g $\pm$ 0.5</td>
<td>2.2g $\pm$ 0.5</td>
<td>$&lt;10^{-4}$</td>
</tr>
<tr>
<td>Placental weight Mean $\pm$ SD</td>
<td>0.6g $\pm$ 0.1</td>
<td>0.5g $\pm$ 0.1</td>
<td>0.02</td>
</tr>
</tbody>
</table>

**Fig. 1:** Validation of the vascular fetal growth restricted model
**Fig. 2:** Comparison of median and interquartile weights of alive fetuses in both horns

- **Right fetuses:** Median weight = 2.8 g, Interquartile range (IQR) = 2.5 g to 3.1 g, Sample size (n) = 49
- **Left fetuses:** Median weight = 2.4 g, IQR = 2.0 g to 2.8 g, n = 22

**Fig. 3:** MRI of gestant rats at E19 of pregnancy. Anatomic coronal T2-w sequence (True FISP) demonstrates several fetuses (*) in both horns and the related placentas.
(arrows). Diffusion sequence (DWI), at b value= 0. The two layers of the placentas are also differentiated.

**Fig. 4:** Biexponential analysis: Coronal T2-w (True FISP) and DWI at b0 value, focused on a placenta. Regions of interest delineate the fetal layer (higher signal intensity) and the maternal layer (lower signal intensity). The signal intensity decay curve according to the different b values obtained for a region of interest. The red line corresponds to the biexponential fit obtained with a Matlab program.
Fig. 5: The Apparent Diffusion Coefficient (ADC) of the total placentas in both horns. ADC is significantly decreased on growth restricted placentas (left) compared to the normal placentas on the right side (p=0.0001, multiple regression analysis). An important variability of the results was found.
Fig. 6: Linear correlation of the Apparent Diffusion Coefficient (ADC) with fetal weight, including fetuses of both horns (p=0.03).
Fig. 7: The Diffusion Coefficient (Dr) of the total placentas in both horns. Dr tended to decreased on growth restricted placentas (left) compared to the normal side (right), but this was not statistically significant (p=0.056).
**Fig. 8:** The pseudo perfusion coefficient ($D^*$) of the total placentas in both horns. $D^*$ tended to decrease on growth restricted placentas (left) compared to the normal side (right), but this was not statistically significant ($p=0.089$)
Fig. 9: The fraction of perfusion (f %) of the total placentas in both horns. f tended to decrease on growth restricted placentas (left) compared to the normal side (right), but this was not statistically significant (p=0.23)
Fig. 10: ADC in fetal and maternal layers, in both horns: ADC was significantly higher in fetal than in maternal layer (p=0.0001).
**Fig. 11:** Dr and f in fetal and maternal layers, in both horns: both were significantly higher in fetal than in maternal layer (p= 0.015, p=0.009).

![Air: f (fetal vs maternal)](image)

**Fig. 12:** Analysis of the fraction of perfusion (f) of the placental layers: f of the maternal layer was decreased compared to the fetal layer (p=0.01). f was more decreased in the maternal layer of the growth restricted group compared to the normal side, but this was not statistically significant (p=0.1)
**Fig. 13:** ADC of total placentas under air and maternal hyperoxygenation (6L/min): ADC increased significantly with hyperoxygenation (p=0.0001).
**Fig. 14:** ADC under maternal hyperoxygenation (6L/min) Comparison of both horn: ADC is significantly decreased on growth restricted side compared to normal side (p=0.0001).
**Fig. 15:** Dr of total placentas under room air and maternal hyperoxygenation (6 L/min): Dr increased significantly with hyperoxygenation (p=0.0001).
Conclusion

Discussion

DWI and IVIM diffusion techniques are feasible to assess placenta function on a murine model at 4.7 T.

ADC is obtained with a monoexponential fit of the signal intensity decay curve. This parameter is a combination of both perfusion and diffusion component. ADC was significantly decreased on growth restricted placentas and was correlated with fetal weight. These results are consistent with what is expected with this model and what has already been published in clinical study for ADC (4). This restriction of diffusion might be explained both by the areas of infarction and the reduced perfusion. Moreover, this decreased diffusion is correlated with the severity of growth restriction in our study.

The biexponential fit of the signal intensity decay curve gives more parameters: Dr, f and D*. Dr corresponds to the diffusion coefficient, without the perfusion component and reflects the cellular structure of the placenta. The pseudo perfusion coefficient (D*, 10^{-3} mm²/s) and the perfusion fraction (f, %) should represent the perfusion component. These parameters tended to decrease but without significance in our study. However, this tendency is consistent with what is expected in vascular growth restriction: f (%) is supposed to decrease in the growth restricted group. f was more decreased in the maternal layer of the growth restricted group compared to the normal side, which is consistent with previous clinical study (8). This might reflect the fact that the maternal side is the more affected by the reduced perfusion.

Maternal hyperoxygenation modified significantly placental ADC in our study in both horn and increased difference between both horns. Hyperoxygenation could be used to better depict restricted growth with ADC.

Limits and perspective

An important intra and inter animal variability was observed, even if this surgical model was supposed to reduce this inter-animal variability. The standard error of the measured values were therefore important.

Further studies are necessary to improve these first results.

Physiological significance of IVIM parameters remain unclear. Next study will include correlation with dynamic contrast enhanced (DCE) technique (1-3) to determine perfusion components.
Conclusion:

DWI is feasible on a murine model of fetal growth restriction. ADC seems to be the most relevant parameter for the diagnosis of placental insufficiency in this model. Further studies are necessary to determine the potential role of the other parameters (Dr, D* and f).

References


