Objective
The aim of our study was to use prenatal high frequency ultrasound (HFUS) to assess fetal cerebellar and cerebral Doppler velocimetry through the superior cerebellar artery (SCA) and the middle cerebral artery (MCA) at three different embryonic stages in a spina bifida (SB) rat model and to investigate brain and cerebellum development.

Methods
We conducted an experimental, controlled and blind study using HFUS (30-70 MHz) to image rat fetuses with spina bifida aperta (SBA), occulta (SBO) and without any neural tube defects (NTD). Fourteen pregnant female Wistar rats were gavaged, with olive oil (sham) to three rats (Control group), and retinoic acid (RA) to the remaining eleven rats, at day 10 of gestation (E10) to induce spina bifida aperta, occulta or no-NTD (three experimental fetal groups). Ultrasound imaging was performed at three different embryonic stages: at E15 (40 fetuses), E17 (43 fetuses) and E20 (70 fetuses). We serially examined the different rat fetuses identifying the cerebral lateral ventricles and determining the SCA and MCA pulsatility indices. Histology was used to confirm ultrasound findings.

Results
HFUS allowed in utero detection of spina bifida (aperta and occulta), hindbrain herniation, and ventriculomegaly. Serial Doppler ultrasound allowed individual fetuses to be analyzed at successive gestational time-points. The average MCA PI and SCA PI in the SBA group at E20 were significantly higher than all other groups (SBO, RA No NTD, and Control). At all other embryonic stages, the difference in the MCA PI among the groups was not significant. At E17, the average SCA PI in the SBA group was also significantly higher than all other groups; and at E15, a slight but not statistically significant increase in the SBA group was found.

Conclusion
Cerebral and cerebellar Doppler velocimetry were detectable in rat fetuses at different embryonic stages suggesting that HFUS is an effective tool to study the anatomic and functional neurological impact of spina bifida in the central nervous system development. This study confirms our hypothesis that herniation of the cerebellum due to the open NTD produces a mechanical resistance to the blood flow in the posterior fossa, creating unfavorable conditions for the cerebellar oxygenation which manifests on ultrasound as a higher resistance (PI) in the SCA. Further studies in human fetuses are needed and now in progress, to establish if Doppler MCA and SCA PI could be relevant ultrasound markers in prenatal spina bifida evaluation.