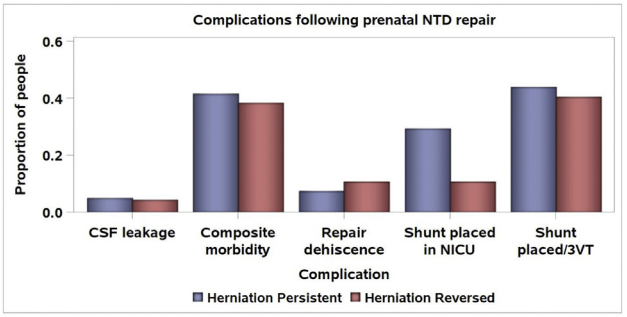

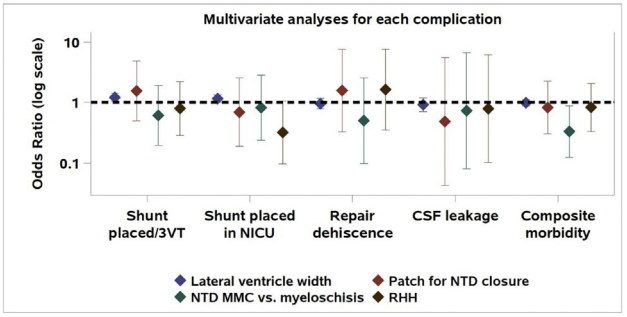

65 Magnetic resonance neuroimaging after laser for Twin-Twin Transfusion Syndrome complicated by single fetal demise

Juliana S. Gebb[1], Rosa Hwang[1], Christina Paidas Teefey[1], Shelly Soni[1], Deborah M. Zarnow[2], Beverly G. Coleman[2], Julie S. Moldenhauer[1], Nahla Khalek[1]

[1]Richard D. Wood Jr. Center for Fetal Diagnosis & Treatment, Children's Hospital of Philadelphia, Philadelphia, PA, [2]Department of Radiology, Children's Hospital of Philadelphia, Philadelphia, PA

OBJECTIVE: To describe fetal magnetic resonance (MR) neuroimaging findings in pregnancies complicated by single fetal demise after laser for Twin-Twin Transfusion Syndrome (TTTS).

STUDY DESIGN: Single-center retrospective review of prospective registry cohort patients who had laser for TTTS with single fetal demise at follow-up. MR neuroimaging was offered 4 weeks after the demise to assess for potential neurologic sequela. MRIs were interpreted by two board certified neuroradiologists and classified as normal, mildly abnormal, or severely abnormal (Table 1). Groups were compared based on recipient vs donor demise using Fisher's exact test and Mann Whitney U test. Multivariate logistic regression was performed to determine risk factors for abnormal MR neuroimaging.

RESULTS: In 378 lasers, 64 (16.9%) cases of single demise were identified (36 donor and 28 recipient). Six patients had rupture of membranes/delivery (3 from each group). Thirty-eight (65.5%) patients underwent MRI. Abnormal neuroimaging was seen in 3/20 (15%) patients after donor demise and 9/18 (50%) patients after recipient demise (Table 1, $p< 0.04$). There was no difference in postoperative interval at the time of diagnosis of demise ($p=0.2$) or rate of Twin Anemia Polycythemia Sequence (TAPS) between groups ($p=0.4$). Logistic regression revealed that recipient vs. donor demise was an independent risk factor for abnormal MRI (Table 2). Two pregnancies with severe MRI findings had complicated perioperative courses. Maternal supraventricular tachycardia with hypotension occurred in the donor demise while the recipient loss was complicated by post-laser Stage V TAPS.

CONCLUSION: Mildly abnormal MR neuroimaging findings are common after laser for TTTS complicated by single fetal demise. They are more common in cases of recipient demise than donor demise. Most mild findings are likely to resolve, but long-term follow-up is warranted. Clinically significant MR neuroimaging findings appear rare and occur in patients with complicated perioperative courses.

	Recipient Demise (n=18)	Donor Demise (n=20)	P value
Post-op interval in days (median, IQR)	7 [1-20]	1 [1-7]	0.16
TAPS	5 (27%)	3 (15%)	0.44
Normal MRI	9	17	
Mild MRI changes in survivor			
CP hemorrhage	2	0	
Grade I hemorrhage	5	2	
Grade 2 hemorrhage	1	0	
Total	8	2	
Severe MRI changes in survivor	1	1	
Total Abnormal MRI	9 (50%)	3 (15%)	0.04

Table 1: Clinical and MRI findings in pregnancies complicated by single fetal demise after laser for TTTS based on type of demised fetus (CP=choroid plexus; IQR=interquartile; MRI=magnetic resonance imaging; TAPS=Twin Anemia Polycythemia Sequence). MRI changes: Mild: choroid plexus or grade 1-2 germinal matrix hemorrhage, mild ventriculomegaly; Severe: Grade 3-4 germinal matrix hemorrhage, severe ventriculomegaly, parenchymal abnormalities

	Odds ratio (95% CI)	p-value
Demised fetus (recipient vs donor)	6.90 (1.35-35.32)	0.02
TAPS	0.35 (0.05-2.73)	0.32
Post-op day of demise	1.01 (0.98-1.05)	0.49

Table 2: Logistic regression of factors associated with abnormal MRI findings (TAPS=Twin Anemia Polycythemia Sequence)

66 Profiling in utero fetal cerebrospinal fluid (CSF) cell populations by single-cell RNA-sequencing (scRNA-seq)

Enrico R. Barrozo[1], Maxim D. Seferovic[1], Maryshe S. Zietsman[1], Trang Do[1], Ellen Tillery[1], Alireza A. Shamshirsaz[2], Michael A. Belfort[3], Kjersti M. Aagaard[4]

[1]Baylor College of Medicine and Texas Children's Hospital, Department of Obstetrics & Gynecology, Division of Maternal-Fetal Medicine, Houston, TX, [2]Baylor College of Medicine/Texas Children's Hospital, Houston, TX, [3]Texas Children's Hospital, Houston, TX, [4]Texas Children's and Baylor College of Medicine, Houston, TX

OBJECTIVE: Previously, the MOMS trial and later fetoscopic approaches demonstrated mid-gestation *in utero* repair of myelomeningocele (or MMC, the most common form of spina bifida) decreased the need for postnatal shunts and improved early motor function. During repair, the protruding MMC sac filled with cerebrospinal fluid (CSF) must be surgically reduced to enable closure. We hypothesized in-depth molecular characterization of fetal CSF by single-cell RNA-sequencing (scRNA-seq) would yield unparalleled and comprehensive insights into the composition and functions of CSF cell populations.

STUDY DESIGN: Fetoscopic neural tube deficit (NTD) surgeries in live fetuses enabled collection of CSF (1-5 mL) and amniotic fluid (AF; 45-60 mL). Cells were prepared for scRNA-seq using the 10x Genomics Chromium 3' Gene Expression platform ($n=6$). 1.5B reads

were aligned with CellRanger and Seurat was used for downstream analyses. Cell clusters and marker transcripts were identified by PCA dimension reduction and differential expression. CSF and AF cells were distinguished based on known cell barcodes and maternal-fetal origin was assigned based on SNPs.

RESULTS: This is the first snapshot of fetal single-cell transcriptomes obtained from ongoing 2nd-trimester pregnancies. Unexpectedly, we observed impressive functional cellular heterogeneity and diversity, including clusters of cells comprised of unique amniocyte subpopulations, microglia subtypes, and Hofbauer-like macrophages (Fig. 1). Comparisons of paired CSF/AF samples revealed unique transcription profiles inferring functional immune infiltration or leakage (Fig. 2).

CONCLUSION: In the first scRNA-seq study of fetal CSF among ongoing gestations, our novel approach revealed impressive diversity in transcription programs consistent with higher than anticipated functional cellular heterogeneity. Appreciating that the fetal BBB in unaffected gestations is similarly "leaky" at the mid-gestation, further analyses will determine if marker mRNAs identified in these analyses correlate with NTD screening, severity, and outcomes.

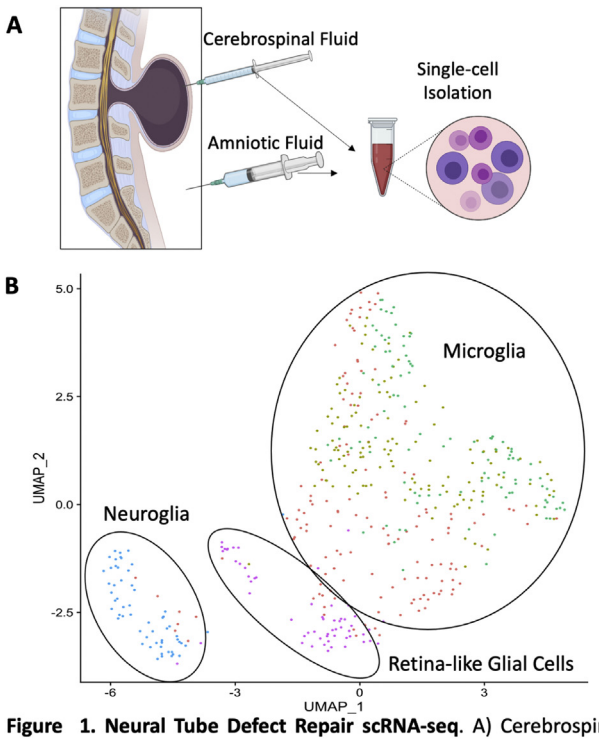

Figure 1. Neural Tube Defect Repair scRNA-seq. A) Cerebrospinal fluid and amniotic fluid single-cell suspensions were subject to scRNA-seq. B) Reads were aligned and pre-processed using CellRanger. Further quality control and clustering were done using Seurat. Cell clusters are annotated based differential expression of known marker transcripts.

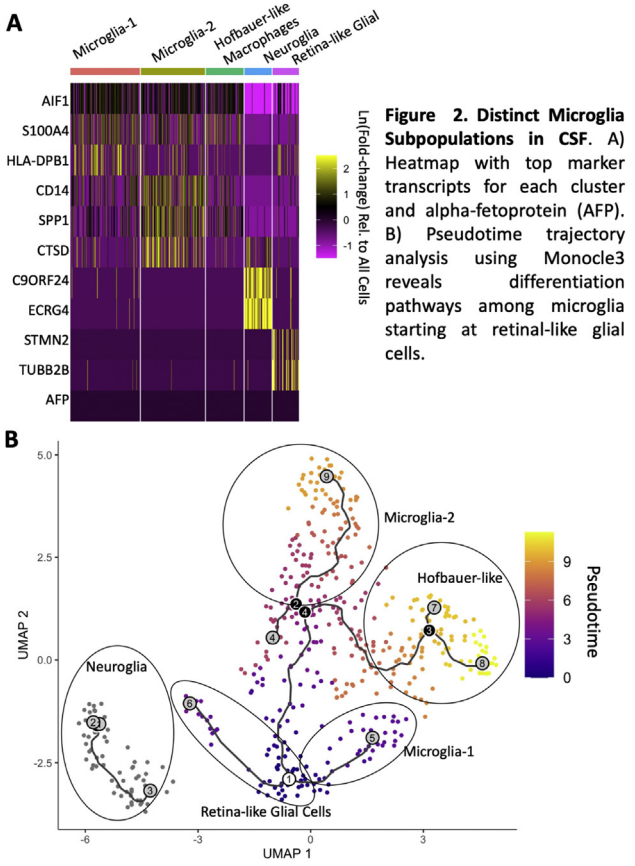

Figure 2. Distinct Microglia Subpopulations in CSF. A) Heatmap with top marker transcripts for each cluster and alpha-fetoprotein (AFP). B) Pseudotime trajectory analysis using Monocle3 reveals differentiation pathways among microglia starting at retinal-like glial cells.

67 Melanin nanoparticles for safe and effective iron chelation therapy for Beta-thalassemia during pregnancy

Nahla Bakhamis, Helen Townley
University of Oxford, Oxford, England

OBJECTIVE: Iron toxicity is a major contributor to adverse pregnancy outcomes in women with transfusion-dependent thalassemia. Currently used chelators are not recommended during pregnancy, as they can cross the placenta causing potential risk to the foetus. Ceasing medication may adversely affect the mother's health even long after delivery.

Previous experiments in iron-overloaded mice have shown that melanin nanoparticles (MNPs) can effectively chelate iron. The aim of this study was to determine whether a nanoparticle can be restricted from movement across the placenta knowing that interaction between nanoparticles with cells and tissues is determined by factors that can be readily manipulated at the synthesis stage.

STUDY DESIGN: A library of 50, 200 and 500 nm MNPs were synthesized and coated with Polyethylene Glycol to improve their stability in circulation. Particles were then tested for their efficacy in chelating iron and for cellular and blood toxicity (n=6). An *in-vitro* model using Bewo cell lines (n=3) followed by an *ex-vivo* human placenta perfusion (n=4) was used to determine if any of the particles can pass through the placental barrier alongside Antipyrine and FITC-dextran as positive and negative controls, respectively.

RESULTS: MNPs of all sizes were able to chelate iron with maximum absorption of 14 mM of iron/g of material; significantly higher than DFO of the same concentration (figure 1). We also found that MNPs with appropriate size (cut off 200nm) can be restricted from moving