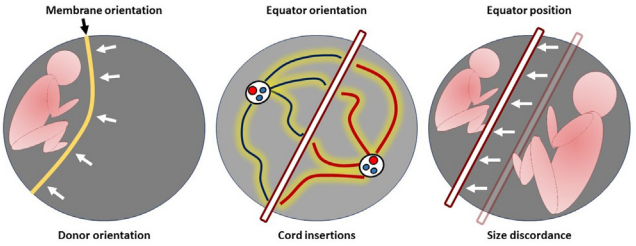
can predict the orientation and position of the intertwin membrane and VE (Figure) to allow successful FLOC.




**STUDY DESIGN:** The orientation of the key landmarks was prospectively and independently documented by 3 surgeons prior to FLOC. Following FLOC the intraoperative findings were compared to the preoperative prediction. Correct identification of basic and specific membrane, VE, VE to membrane orientation and inadvertent anterior septostomy was computed and related to case characteristics, surgeon experience and intraoperative outcome.

**RESULTS:** In a 3 month period 59 assessments were performed prior to 24 FLOC surgeries (10 Quintero stages 1&2, 14 stages 3&4; median gestational age 18.8 weeks (16.3-25.6); median fluid pockets 10 cm (8.1-22) and 1 cm (0-2) in recipient and donor, respectively). The maternal body mass index was 28 kg/m$^2$ (17.6-62.7). Basic membrane and equator orientation were correctly predicted in 52 (88.1%) and 51 (86.4%) of assessments and specific prediction was correct in 40 (66%) and 31 (52.5%), respectively. The basic relationship between the membrane and equator was correctly predicted in 45 (76.3%) of assessments but their specific relationship prediction was correct in only 19 (32.2%). There were 2 anterior septostomies of the intertwin membrane. The predicted entry provided adequate visualization of the vascular equator allowing complete equatorial dichorionization in 87.5% (n=21). Incomplete equator visualization was due to extensive anterior placenta which would not have been circumvented by a different entry site. The prediction accuracy was independent of surgeon experience, placental location, amniotic fluid volume. High body mass index ($r^2$ 0.36, p=0.001) was the only factor that negatively impacted optimal preoperative assessment.

**CONCLUSION:** We present a simple ultrasound technique that allows reproducible and consistent preoperative prediction of key anatomic landmarks for successful FLOC treatment of TTTS independent of multiple potential confounders.

## 183 Fetal neuroprotective effects of maternal magnesium for late gestation inflammation: inhibition of apoptosis, neuronal nitric oxide synthase and nf-kb activation

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**OBJECTIVE:** In preterm birth, maternal magnesium sulphate (Mg) has been used as neuroprotective agent in preventing white matter brain injury. At term, chorioamnionitis and funisitis occur commonly and are associated with cerebral injury. Infection activates cell death pathways (apoptosis) and inflammatory responses through induction of caspase 3, oxidative stress and NF-kB pathways. We sought to determine if maternal Mg prevents the activation of apoptosis and inflammatory pathways in response to inflammation at late gestation.

**STUDY DESIGN:** Pregnant rats at 20 days of gestation (24 total: 4 groups, n=6) received injections of i.p. lipopolysaccharide (LPS; 500 ug/kg) or saline (SAL) at time 0. Dams were randomized to treatment with s.c. saline or Mg (270 mg/kg loading followed by 27 mg/kg q20 min) for 2 hours prior to and 2 hours following LPS /saline injections. Rats were sacrificed 4 hours following LPS/ saline injection. Fetal brains were collected from the 4 treatment groups (LPS/SAL, LPS/Mg, SAL/MG, SAL/SAL). We used one fetal brain from each dam, resulting in 6 brains from 6 different dams in each group. Fetal brain caspase 3 active form (af), NF-kB p65, neuronal nitric oxide synthase (phospho-nNos) and protein levels of interleukin (IL)-6, IL-10 and TNF$\alpha$ were determined by western blot analysis.

**RESULTS:** Maternal LPS (LPS/SAL) at e20 significantly ($p<0.01$) increased fetal brain caspase 3 af (0.27 $\pm$ 0.02 vs. 0.15 $\pm$ 0.06 u), NFkB p65 (0.23 $\pm$ 0.01 vs. 0.13 $\pm$ 0.01 u), and phospho-nNOS (0.22 $\pm$ 0.01 vs. 0.12 $\pm$ 0.01 u) as well as fetal brain pro-inflammatory cytokines (IL-6 0.21 $\pm$ 0.01 vs. 0.11 $\pm$ 0.01 u; TNF$\alpha$ 0.29 $\pm$ 0.01 vs. 0.15 $\pm$ 0.01 u) as compared to control fetuses (SAL/SAL). Maternal LPS did not alter fetal brain IL-10 levels. Mg treatment to LPS dams (LPS/Mg) significantly ($p < 0.05$) reduced fetal brain caspase 3 af (0.16 $\pm$ 0.01 u), NFkB p65 (0.11 $\pm$ 0.01 u) and phospho-Nnos (0.1 $\pm$ 0.01 u) as well as brain pro-inflammatory cytokines (IL-6 0.07 $\pm$ 0.01 u; TNF$\alpha$ 0.15 $\pm$ 0.01 u) to levels similar to Controls (SAL/SAL).

**CONCLUSION:** Maternal and/or fetal inflammation-induced fetal brain injury may be mediated via activation of inflammation, oxidative stress and apoptosis pathways. Maternal Mg may prevent inflammation-induced brain injury at term via inhibition of these putative pathways.

## 184 Fetal inflammatory response in the context of funisitis, but not acute histologic chorioamnionitis without funisitis, decreases with increasing gestational age

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**OBJECTIVE:** Recent study demonstrated that the intensity of amniotic fluid inflammatory response decreases in the setting of acute histologic chorioamnionitis(acute-HCA) with increasing gestational age(GA). However, there is no information about whether the intensity of fetal inflammatory response(FIR) in the same setting of placental inflammatory condition decreases with GA. We hypothesized that the intensity of FIR would decrease with increasing GA in the setting of funisitis, but not acute-HCA without funisitis.

**STUDY DESIGN:** FIR was examined in 209 singleton preterm-pregnancies(23.1< GA at delivery <36wks) with acute-HCA or funisitis and with preterm labor or preterm-PROM. Study population was divided into GA at delivery $\leq$30wks(n=61), 30-34wks(n=87), and 34-36wks(n=61). Acute-HCA was diagnosed in the presence of inflammation in chorio-decidua(CD), amnion or chorionic plate, and funisitis was defined as inflammation in umbilical cord. FIR was determined by umbilical cord plasma(UCP) CRP concentration at birth.

**RESULTS:** UCP CRP concentrations at birth were less intense at higher GA in patients with acute-HCA or funisitis(P<.005). Median UCP CRP concentration(ng/ml) at birth decreased in patients with funisitis (840.6,[7.6-10897.4] vs. 368.8,[4.9-5555.0] vs. 128.2,[2.2-4533.9]; P<.05), but not acute-HCA without funisitis(36.1,[1.9-7401.8] vs. 23.8,[3.0-2702.4] vs. 15.2,[3.9-1295.5]; P>.1), with increasing GA. Moreover, median UCP CRP concentration at birth

decreased in patients with inflammation of the compartment beyond CD(i.e., amnionitis, chorionic plate inflammation or funisitis)(711.9,[7.6-10897.4] vs. 321.1,[3.0-5555.0] vs. 124.9,[2.2-4533.9]; P<.05), but not inflammation restricted to the chorio-decidua(19.0,[1.9-2586.7] vs. 22.1,[4.0-1257.4] vs. 12.5,[3.9-1075.3]; P>.1), with GA. Furthermore, there was a significant inverse relationship between GA and UCP CRP concentrations at birth in patients with funisitis(r=-0.280, P<.0005) and inflammation of the compartment beyond CD(r=-0.253, P=.005).

**CONCLUSION:** UCP CRP concentrations at birth in the context of funisitis, but not acute-HCA without funisitis, distinctly decrease throughout preterm-gestation. This finding suggests that FIR decreases even in the same setting of funisitis with increasing GA.

## 185 The impact of copy number analysis in expanded carrier screening

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**OBJECTIVE:** To examine the impact of copy number variants (CNVs) on detection rates in expanded carrier screening (ECS) panels.

**STUDY DESIGN:** ECS panels typically include analysis of copy number variation (CNV) for only a small subset of genes and exons, often to the detriment of detection rate. To assess the impact of panel-wide CNV analysis, a preliminary analysis was performed on 56,267 de-identified ECS tests performed by full-exon next-generation sequencing at Counsyl's laboratory. A 93 gene subset (consisting of severe and profound diseases) of Counsyl's ECS panel was analyzed to estimate the additional benefit of copy number analysis. The disease risk, i.e., the probability that a hypothetical child from randomly selected parents is affected by disease, was used as a metric for detection rate. No variant curation was performed on the detected CNVs and all detected deletions/duplications were assumed pathogenic. To calculate the disease risk, historical NGS data at Counsyl was used to estimate deleterious allele frequencies (AFs). The AFs were then used to calculate the disease risk with CNV estimates either excluded or included.

**RESULTS:** Without CNV analysis, the disease risk of the 93 gene subpanel was 123 affected children per 100,000. The addition of CNV analysis increased the detection rate by 2.0%, to 125 affected children per 100,000. This change in disease risk is roughly equivalent to calling non-CNV variants via NGS in the 54 least prevalent genes, which contribute 2.0% of the observed disease risk.

**CONCLUSION:** Panel-wide CNV analysis increases detection rate and provides more value than the addition of low-prevalence genes. CNV calling may be important to further reduce a patient's residual risks for specific genes due to certain scenarios, e.g., family history of a genetic disease and partner testing of known carriers for recessive diseases. For example, in HBB, CFTR, and ATM, CNVs account for up to 9% of the total detection rate. Further work to assess the clinical impact of the discovered variants is ongoing.

## 186 Non-invasive prenatal testing (NIPT) versus diagnostic testing for evaluation of fetal structural anomalies

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**OBJECTIVE:** Fetal structural anomalies are associated with whole chromosome aneuploidy and sub-chromosomal copy number variants (CNVs) in approximately 3-6% of cases. Although the recommended evaluation of a fetus with an anomaly is diagnostic testing with microarray analysis, many physicians are offering non-invasive prenatal testing (NIPT) in cases where patients wish to avoid an invasive diagnostic procedure. This analysis evaluates the hypothetical performance of NIPT in this scenario.

**STUDY DESIGN:** This is a secondary analysis of 2 NICHD funded prospective studies evaluating the frequency of aneuploidy and CNVs diagnosed in utero. We determined the frequency of cytogenetic abnormalities in fetuses with structural anomalies (including NT ≥3.5mm). We calculated the ability of either single nucleotide polymorphism (SNP) based NIPT or massively parallel sequencing (MPS) based NIPT to identify these abnormalities. We assumed that SNP-based NIPT could identify all cases of trisomies 13, 18, 21, sex chromosome abnormalities, triploidy and deletions of 1p36, 4p, 15q11, and 22q11.2. MPS-based expanded NIPT was assumed to detect all trisomies and CNVs ≥7Mb as well as deletions of 1p36, 4p, 5p, 8q24, 11q23, 15q11.2, 17p11.2, and 22q11.2.

**RESULTS:** There were 1,724 fetuses with structural anomalies, 317 (18.4%) of which had an abnormal karyotype, and 106 (3.5%) of which had a pathogenic CNV. Among these, 1,294 had an isolated structural anomaly and 430 had multiple anomalies. Table 1 shows the cytogenetic abnormalities. SNP-based NIPT would identify 79.1% of the abnormalities and MPS-based would identify 87.4%. Detection rates vary by specific organ system, as shown in Table 2.

**CONCLUSION:** An expanded MPS-based and a SNP-based NIPT could potentially identify 87.4% and 79.1%, respecitively, of cytogenetic abnormalities among fetuses with structural anomalies. However, this assumes that NIPT has 100% detection of all intended anomalies. Accordingly, its detection in clinical practice will be less. Patients choosing NIPT as their primary genetic test for a structural abnormality must be informed of his limitations.

Table 1: Cytogenetic abnormalities and abnormalities identifiable by NIPT

| | N | All cytogenetic abnormalities, n (%) | Common aneuploidies, n (%)* | Triploidy, n (%) | Pathogenic CNVs, n (%) | | | | Abnormalities identifiable by NIPT, n (%) | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | >7Mb** | ≤7Mb | 22q11.2 del | Other Targeted Deletions*** | SNP-based | MPS-based |
| All anomalies | 1724 | 436 (25.3) | 317 (18.4) | 13 (0.7) | 49 (2.8) | 57 (3.3) | 14 (0.8) | 1 (0.0) | 345 (79.1) | 381 (87.4) |
| Multiple anomalies | 430 | 161 (37.4) | 109 (25.3) | 8 (1.9) | 18 (4.2) | 26 (6.0) | 8 (1.9) | 0 (0.0) | 125 (77.6) | 135 (83.9) |
| Single anomaly | 1294 | 275 (21.2) | 208 (16.1) | 5 (0.4) | 31 (2.4) | 31 (2.4) | 6 (1.4) | 1 (0.0) | 220 (80.0) | 246 (89.5) |

* Includes trisomy 21,18,13, sex chromosome abnormalities
** Includes rare aneuploidies, CNVs > 7mb and 23 mosaic rare aneuploidies
***15q11, 11q23, 8q24, 1p36, 4p-, 5p- (detectable by both SNP and MPSS)

Table 2: Cytogenetic abnormalities by organ system anomaly and potential NIPT detection

| Organ System Anomalies | N | Any abnormality by microarray (%) | Abnormality identifiable by NIPT (%) | |
|---|---|---|---|---|
| | | | SNP-based | MPS-based |
| Cardiac | 369 | 29.3 | 75.9 | 80.5 |
| CNS | 264 | 26.1 | 65.2 | 75.4 |
| Renal | 139 | 25.9 | 66.7 | 72.2 |
| Skeletal | 266 | 27.1 | 77.8 | 83.3 |
| Nuchal translucency, cystic hygroma | 516 | 41.5 | 79.7 | 97.2 |