Mayo Clinic

IRB: 20-000991

Predicting placental pathologies by ultrasound imaging of the human placenta during gestation

Study Chair: Mauro Schenone, M.D.
Obstetrics and Gynecology
Mayo Clinic
200 First Street, SW
Rochester, MN 55905
507-284-0210

Co-Investigator: Shigao Chen PhD
Professor of Radiology
Mayo Clinic
200 First Street, SW
Rochester, MN 55905
507-284-3703

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Version 1.5

11/6/2023

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PARTICIPATING SITES

Mayo Clinic Rochester Only

Protocol Resources

Questions:	Contact Name:
Clinical data submission and record maintenance Protocol document, consent form, regulatory issues	Emily N. Smith, R.N. Research Study Coordinator Phone:507-266-4813 E-mail: smith.emily3@mayo.edu
Adverse Events (AdEERS, MedWatch, Non-AER, AML/MDS)	Emily N. Smith, R.N. Research Study Coordinator Phone:507-266-4813 E-mail: smith.emily3@mayo.edu; lemens.maureen@mayo.edu

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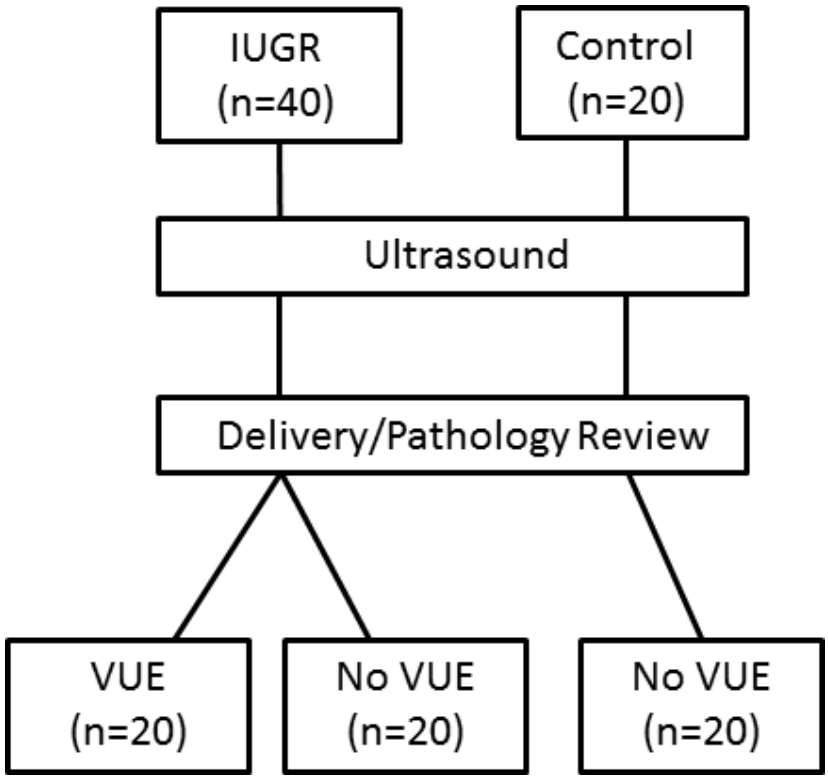
Schema


IUGR
(n=40)
Control
(n=20)
Ultrasound
Delivery/Pathology Review
VUE
(n=20)
No VUE
(n=20)
No VUE
(n=20)

1.0 Background

Many pregnancy complications, including growth restriction, preterm birth and stillbirth, are associated with inflammation in the placenta, which is currently diagnosed after delivery. This non-infectious placental inflammation is known as villitis of unknown etiology (VUE) is characterized by maternal T cells infiltrating into the placenta and causing destruction of the villous architecture. However, it's not known what leads to these maternal T cells becoming activated against fetal cells, nor it is understood whether VUE is a cause or an effect of the associated fetal complications. The main challenge faced by researchers studying VUE is that there are no strategies to predict VUE in utero.

1.1 Villitis of Unknown Etiology

Intrauterine growth restriction (IUGR) appears to be associated with a placental pathology called villitis of unknown etiology (VUE). [1] During VUE, maternal T cells are found at high levels in the chorionic villi, causing destruction of placental architecture without clinical signs and symptoms of maternal (or fetal) infection. [2] VUE is diagnosed in 30% of all placentae. [3] However, exact prevalence and the significance of this diagnosis, along with its potential role in IUGR, remains unknown. As half of a fetus' genetic material is paternally derived, VUE may represent a rejection response by the maternal immune system. Human leukocyte antigens (HLA), which are critical to the success of organ transplantation, may provide novel insights into VUE. Our published work identified that HLA class I and II receptors are upregulated in placentae with VUE compared to unaffected, gestational age-matched controls. [4] Additionally, HLA-typing of VUE affected mothers and their newborns demonstrated increased HLA-mismatch compared to unaffected pairs. [4] Our follow-up data (in review) utilizing T cell receptor spectratyping shows a significant expansion of T cell clones in placentae with VUE compared to controls; however, these clones are unique to each subject further indicating VUE is not a response to a shared antigen (as with an undetected infection).

1.2 Ultrasound Imaging

The scientific premise for this project is supported by strong animal data out of John Hopkins. This group utilized *in utero* Doppler ultrasound to identify intrauterine inflammation in a mouse model. Pregnant mice injected with lipopolysaccharide into the uterine cavity have intrauterine inflammation, which researchers found to cause elevated resistance indices, pulsatility indices and a greater occurrence of absent end-diastolic flow in the umbilical and uterine arteries.[5] Women diagnosed with fetal IUGR during pregnancy will undergo Doppler ultrasound to monitor signs of fetal health. Inflammation is also associated with abnormal growth of microvessels. [6,7] Current Doppler technology is not sensitive enough to detect these microvessels, whereas the new microvessel imaging technology can detect these small changes in blood flow.[8,9] Therefore, as microvessel change is associated with inflammation, and inflammation is related to VUE and its associated fetal complications, we believe combining Doppler imaging with microvessel imaging will provide more power to identify placental villitis, possibly even those cases of mild villitis. Additionally, we will measure the ultrasound attenuation coefficient (UAC) in the placenta [11], which would provide information on fat fraction that may be a useful evaluation of placenta health.

Study Plan: This study will collect:

1. Ultrasound measures during the third trimester (>28 weeks)
2. Clinical placental specimen after birth
3. Patient demographics from the electronic medical record

Studies utilizing these three things will be performed as described in the sections that follow.

2.0 Goals

We aim to develop an ultrasound method to identify VUE induced inflammation in the human placenta during gestation. We hypothesize that hemodynamic changes can also be observed in the human placenta diagnosed with VUE by conventional Doppler ultrasound and microvessel imaging (also called 3D imaging in the consent form).

2.1 Primary

3.11 Our goal is to determine whether Doppler ultrasound measures of blood flow can predict VUE during gestation in a cohort of growth restricted fetuses compared to normal controls.

2.2 Secondary

2.12 We aim to measure differences in microvessel imaging to determine if it can provide stronger evidence for VUE diagnosis.

3.0 Patient Eligibility

3.1 Registration – Inclusion criteria IUGR cohort:

3.11 Age ≥18 and ≤45 years at study entry.

3.12 Diagnosis of IUGR before admission to labor and delivery.

3.14 Ability to provide written informed consent.

3.16 Weight greater than 110 pounds (50 kilograms, a standard requirement in obstetrics studies that include blood draws).

3.17 >28 weeks gestation

3.2 Registration – Inclusion criteria control cohort:

3.21 Age ≥18 and ≤45 years at study entry.

3.22 No known pregnancy complications at obstetrics visit (+/- 1 week gestational age of IUGR cohort)

3.23 Ability to provide written informed consent.

3.24 Weight greater than 110 pounds (50 kilograms, a standard requirement in obstetrics studies that include blood draws).

3.25 >28 weeks gestation

3.3 Registration – Exclusion criteria

3.31 Known immunodeficiency

3.32 Chronic, active viral infections, including HIV-1/2, HTLV-1/2, hepatitis B/C.

3.33 Known autoimmune disease (e.g., rheumatoid arthritis or systemic lupus erythematosus)

3.34 Solid organ or transplant recipient.

3.35 Multiple gestations.

3.36 Not planning on delivering at Mayo Clinic

3.37 Ruptured membranes

3.38 <28 weeks gestation

4.0 Test Schedule

Tests and procedures	At registration (3rd trimester diagnosis of IUGR +\- 1 week for all controls
Maternal and fetal demographics[1]	X
Ultrasound imaging	X
Clinical placenta specimen collection[2]	X

1. Abstracted from the medical record.
2. Placenta pathologic sampling and review per clinical protocol

5.0 Grouping Factor: None.

6.0 Registration/Randomization Procedures

6.1 Participants will be recruited from patients obtaining antenatal care for pregnancy in the obstetrics clinic at Mayo Clinic in Rochester, Minnesota. Women who meet the eligibility criteria (see above) will be offered the opportunity to participate in the study. Informed consent will be obtained prior to enrollment.

7.0 Protocol Intervention

This study will take place in the Rochester Methodist Hospital and will enroll women attending antenatal care through the Department of Obstetrics and Gynecology. Annually, 2500 women receive care and deliver at the Mayo Clinic Family Birth Center. This study will consist of two cohorts: 1) women diagnosed with intrauterine growth restriction (IUGR) and 2) women having uncomplicated pregnancies. Forty women will be enrolled after being diagnosed with IUGR. Based on our clinical data we expect 50% to have a VUE diagnosis. Another cohort of 20 women having uncomplicated pregnancies will be enrolled, matched on gestational age at ultrasound and fetal sex.

7.1 Study Methodology

Following consent, women will undergo a comprehensive Doppler ultrasound of the placenta. Prenatal ultrasound is routinely used in clinical practice and considered safe. The ultrasound output level used in this study will be below the FDA limit for fetal imaging. Ultrasound measures to be collected include uterine artery (UtA) and

umbilical artery (UA), systolic/diastolic (S/D) ratio, resistance index (RI), pulsatility index (PI) and presence of early diastolic notch will be collected for each subject.

Additionally, we will explore the benefit of novel ultra-sensitive Doppler technology for microvessel imaging of the placenta. This technology can substantially improve small vessel imaging on ultrasound. A Verasonics ultrasound scanner will be used for ultra-sensitive Doppler imaging. We will collect data on vessel density, periphery-to-center vessel density ratio (VDR), microvessel morphology and the ultrasound attenuation coefficient (UAC). Relevant informationabout the Verasonics scanner can be found in the Device section.

Maternal and fetal demographics will be collected for all study subjects. Statistical differences between variables in our three groups will be determined ANOVA by Kruskal-Wallis tests if normality is not met. Significance will be defined as a p-value ≤ 0.05.

7.2 Pathologic Review

In order to confirm VUE diagnosis, placentae will be collected after delivery and placed in formalin. A minimum of 6 samples will be obtained, 2 from cord and membranes and 4 from parenchyma. Placental tissue from the control cohort will then be processed and embedded into paraffin blocks (FFPE) for pathological review for the research study. Placental tissue from the IUGR cohort will be processed and embedded into paraffin blocks (FFPE) for pathological review as per standard clinical pathology protocols. Blocks will be prepped and stained with hematoxylin and eosin for histologic examination by our expert pathologist, Dr. Reade Quinton. All diagnoses will be based on established and published criteria from the Amsterdam Placental Workshop Group and Society for Pediatric Pathology.[10] Severity, based on immune cell infiltration into the placenta, will be correlated back to ultrasound findings and pregnancy outcome. We will also share de-identified FFPE blocks collected for research only (not clinical residual tissues) with Alpenglow. Alpenglow Biosciences is located in One Discovery Square – Advanced Diagnostics Laboratory. The team will test whether their 3D histology technique can work for placental vascular evaluation (feasibility study).

7.3 Collection of Clinical Data

Study participants will provide consent for study personnel to access their obstetrical record for information about age, fetal sex, potential confounders (see exclusion criteria) and gestational age at sample collection.

8.0 Intervention Modification Based on Adverse Events: Not applicable

9.0 Ancillary Treatment/Supportive Care: Not applicable.

10.0 Adverse Event (AE) Reporting and Monitoring

10.11 Adverse events will be assessed as a routine part of every clinic visit during pregnancy. These will be reported to the principal investigator. The principal investigator will make a decision on whether the adverse event is expected or unexpected, and if the adverse event is related to the procedure. Based on this information, the PI will determine whether the adverse event should be reported as an expedited report or as part of routine clinical data.

There are no known risks of ultrasound imaging to either the mother or fetus during pregnancy. Therefore, we expect no study related adverse events to occur.

10.12 Expected vs. Unexpected

- The determination of whether an AE is expected is based on whether the event is generally associated with the condition being studied (i.e. IUGR), whether in the research study or in the setting of standard clinical care.
- Unexpected AEs are those events that are generally not associated with the condition being studied and may have a relationship to the procedure or device (i.e. ultrasound) being studied.

10.13 Assessment of Attribution

When assessing whether an adverse event is related to a medical treatment or procedure, the following attribution categories are utilized:

Definite - The adverse event *is clearly related* to the specimen collection.
Probable - The adverse event *is likely related* to the specimen collection.
Possible - The adverse event *may be related* to the specimen collection.
Unlikely - The adverse event *is doubtfully related* to the specimen collection.
Unrelated - The adverse event is clearly *NOT related* to the specimen collection.

11.0 Intervention Evaluation: Not applicable.

12.0 Descriptive Factors: None.

13.0 Follow–up Decision at Evaluation of Patient

13.1 Women who have either (a) developed NO pregnancy complications, or (b) developed IUGR will be **eligible** for the research ultrasound and placental pathology review.

13.2 Women who have developed pregnancy complications including but not limited to those complications listed below will be **ineligible** if:

13.21 They develop a severe infection

13.22 If they have any other complication which is felt by the study participant's obstetrician to preclude her from further participation in the study

13.3 If an eligible participant after signing a consent form refuses to undergo the research collections required, no further data submission is necessary.

13.4 When a participant is found to be *ineligible* (that is, the patient is determined not to have satisfied each and every eligibility criteria for study entry at the time of registration), the participant will discontinue further study participation.

13.5 A participant may discontinue study participation at any time.

14.0 Human Studies Evaluation

14.1 Vulnerable population

Our protocol is in accord with the Code of Federal Regulations, Part 46: Protection of Human Subjects (section 46.204), which states that pregnant women may be involved in research if the following conditions are met: (1) there is a prospect of direct benefit to pregnant women and their fetuses, (2) the risk to the fetus is not greater than minimal, and (3) the purpose of the research is the development of important biomedical knowledge that cannot be obtained by other means.

Fetuses diagnosed with IUGR and/or preterm labor/birth face a significant risk of morbidity and mortality before their first birthday. Currently, we don't know what causes IUGR or spontaneous preterm birth, but many studies indicate the immune system is a significant contributor to their pathobiology. VUE is strongly associated with these diagnoses and has a significant risk of recurrence in a subsequent pregnancy. New animal data suggests that prenatal ultrasound may be a real-time clinical tool for assessing fetal risk of adverse outcomes. Identifying placental inflammation early may allow for effective intervention to minimize placental damage and therefore improve fetal and neonatal outcomes.

15.0 Statistical Considerations and Methodology

15.1 This is a feasibility study; therefore, no formal power analysis was completed

A total of 61women will be accrued; 40 with a diagnosis of IUGR and 20 gestational age matched controls (+/- 1 week) will be analyzed. These women must be undergoing obstetric care for their pregnancy at Mayo Clinic. We are not offering remuneration; however, based on our previous studies we had a high recruitment and retention rate.

All statistical analyses will be performed using JMP version 10.0 (SAS Institute). Data will be examined for normality using the Shapiro-Wilk test, and between-group differences in expression for each ultrasound measure in our three groups will be analyzed with the nonparametric Kruskal-Wallis tests (non-parametric) or ANOVA (parametric). Dunn's method will be utilized to correct for multiple comparison testing. P-values<0.05 will be considered statistically significant.

Principal component analysis (PCA) will be used to assess the ability of the biomarker panel to separate individual data points (score plot) by projection of the original data on the first two principal components (PC1 and PC2), as well as correlations among ultrasound measures (loading plot). This will reduce the complexity of multidimensional data models by linearly combining multiple factors and downsizing them into new variables or principal components while retaining the original information.

15.2 Inclusion of Women and Minorities

This study will be available to all eligible women regardless of race or ethnic group. Based upon the most recent birth data at Rochester Methodist Hospital, where approximately 2,500 babies are born each year, 5% of mothers categorized themselves as Hispanic/Latina, 5% as Asian, 6% as Black/African American, 83% White, and 2% other. In 2019, 235 diagnoses of IUGR were made in Rochester, making this study highly feasible.

16.0 References

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