HEparin for the PRevention of complications related to placental Insufficiency

The HEPRIN Randomized Controlled Trial.

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Version Date: 16 September 2009
TITLE

Does heparin improve pregnancy outcomes for women with evidence of placental dysfunction? A randomized controlled trial.

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AIMS OF THE TRIAL

Placental dysfunction, as evidenced by ultrasonographic anomalies in placental morphology, abnormal uterine artery Doppler studies, or elevations in biochemical markers associated with first trimester maternal serum screening, have been associated with the occurrence of adverse pregnancy outcomes, including preterm birth, pre-eclampsia, abruption, and perinatal death. These adverse outcomes are potentially mediated via placental ischaemic-thrombotic lesions. While there is interest in investigating the role of heparin for women who screen positive for a thrombophilic condition, most women with adverse pregnancy outcome are ultimately shown to be thrombophilia negative. It is uncertain whether heparin has a therapeutic role to play in improving pregnancy outcomes for this group of women.

SPECIFIC AIMS

The aims of this randomized controlled trial are to assess whether the use of subcutaneous heparin in women with identified placental dysfunction during pregnancy will reduce the risk of intrauterine fetal death and other adverse pregnancy outcomes, improve maternal and infant health, without increasing maternal risks.

TRIAL HYPOTHESES

The primary hypothesis of this randomized trial is that the administration of heparin to women with identified placental dysfunction will
- reduce the risk of intra-uterine fetal death.

The secondary hypotheses of this randomized trial are that the administration of heparin to women with identified placental dysfunction will
- reduce the risk of maternal morbidity from pregnancy complications without adverse effects of therapy;
- reduce the risk of infant morbidity from adverse outcomes;
- improve maternal psychological well-being and satisfaction with care; and
- reduce the occurrence of pathologically identifiable placental thrombotic-ischaemic lesions.

BACKGROUND
**Placental Dysfunction and Pregnancy Outcome:**

The optimal development of the placenta allows for physiological exchange of oxygen and nutrients between the mother and the fetus, to facilitate maintenance of a normal, healthy pregnancy. During early placental development, infiltration of the trophoblast into the placental bed results in transformation of small calibre, high resistance spiral arteries, into large calibre, low resistance uteroplacental vessels. Alterations to this physiological transformation, in addition to the presence of other vascular processes, prevents the establishment of a low resistance placental circulation, with subsequent reduction in uteroplacental blood flow, and the development of pregnancy complications such as pre-eclampsia, intrauterine growth restriction, preterm birth and perinatal death.

The Society for Pediatric Pathology has developed guidelines to assist in placental pathological examination, and to consider markers of maternal vascular perfusion. Placental reaction patterns associated with maternal hypoperfusion were reviewed to determine their value in the creation of a diagnostic framework. Lesions affecting villi and intervillous spaces (such as syncytial knots, increased intervillous fibrin deposition, and distal villous hypoplasia), as well as those affecting maternal vessels at the site of placental implantation (such as acute atherosis, mural hypertrophy of arterioles, muscularisation of basal plate arteries, and increased placental site giant cells) were found to be reproducible, and correlated with a diagnosis of maternal underperfusion. Use of these diagnostic criteria has been used subsequently to correlate placental pathological findings with adverse pregnancy outcome.

Disorders of severe placental insufficiency are related to aberrations in the underlying placental development process. Contributing to this process are both alterations in function of the extravillous trophoblast, resulting in reduced uterine artery blood flow, and alterations in growth of the placental parenchyma, due to defects in villous tree development. Pathological examination of the placenta following delivery for early-onset pre-eclampsia or early-onset severe IUGR reveals that ischemic thrombotic lesions, including villous infarction, are very common. Moreover, these placentas are typically small, often with eccentric cords. Uteroplacental vascular insufficiency was present in a large proportion of these women, with over 85% of severe IUGR pregnancies demonstrating abnormal uterine artery Doppler.

**Identification of Placental Dysfunction during Pregnancy:**

Ultrasound assessment of placental shape and texture, in addition to uterine artery Doppler waveforms have been used to identify women considered to be ‘at risk’ of adverse pregnancy outcome due to placental insufficiency, both alone and in combination with second trimester maternal serum biochemistry markers, particularly alpha-fetoprotein (AFP) and human chorionic gonadotrophin (hCG). The presence of abnormal uterine artery Doppler waveforms prior to 23 weeks gestation, and ultrasound assessment of placental morphology have both been reported as reliable predictors of adverse pregnancy outcome. In a prospective study evaluating the use of second trimester placental volume measurements, Dombrowski and colleagues determined that rates of perinatal mortality, placental abruption, neonatal intensive care unit admission, fetal anomaly and low birth weight infants were all markedly increased in pregnancies with a thick placenta. Similar findings
relating to infant birth weight and perinatal mortality have been subsequently reported\textsuperscript{19}. Both ultrasound assessment of placental morphology and the use of uterine artery Doppler studies may be important screening tools in women considered to be at increased risk of adverse pregnancy outcome.

**The likelihood of Adverse Pregnancy Outcome:**

In our recent cohort study, we identified that women with two or more abnormal tests of placental function were at increased risk of adverse pregnancy outcome, when compared with women with normal tests of placental function\textsuperscript{20}. The risk of adverse outcome increased further when all three tests of placental function were abnormal. The odds ratios for each outcome are presented in the table below.

<table>
<thead>
<tr>
<th>Outcome</th>
<th>OR if 2 tests abnormal</th>
<th>95% CI</th>
<th>OR if 3 tests abnormal</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>PE/HELLP</td>
<td>7.9</td>
<td>2.7-23.6</td>
<td>8.6</td>
<td>2.6-28.7</td>
</tr>
<tr>
<td>PTB &lt;34 wks</td>
<td>8.6</td>
<td>3.1-23.9</td>
<td>61.2</td>
<td>13.4-infinity</td>
</tr>
<tr>
<td>SGA</td>
<td>8.5</td>
<td>3.2-22.7</td>
<td>12.7</td>
<td>4.0-40.1</td>
</tr>
<tr>
<td>IUFD</td>
<td>30.7</td>
<td>6.4-infinity</td>
<td>70.3</td>
<td>13.7-infinity</td>
</tr>
</tbody>
</table>

**The role of Heparin in Mediation of Placental Lesions:**

It has been suggested that the mechanism whereby trophoblast invasion into the placental bed is limited in early onset pre-eclampsia and intrauterine growth restriction relates to increased trophoblast apoptosis, resulting in narrower spiral arteries\textsuperscript{21}. Of interest, Bose and colleagues reported an in-vivo study, where heparin and aspirin modulated the effects of trophoblast apoptosis through villous protein expression\textsuperscript{22}, a potential mechanism that may explain the beneficial effects of both heparin and aspirin in women with recurrent pregnancy loss.

While there have been observational studies reporting the use of low-molecular weight heparin in the antenatal period in women who have placental anomalies detected ultrasonographically\textsuperscript{10}, it is unclear if treatment can reverse the underlying pathological process and improve pregnancy outcomes for these women.

**The use of heparin in pregnancy – potential benefits and harms:**

Unfractionated heparin has been used extensively in pregnancy as treatment for women with venous thromboembolic disease\textsuperscript{23, 24}. While the use of heparin therapy may improve placental function and therefore reduce the risk of adverse pregnancy outcome, there are potential maternal adverse effects.

**Maternal complications:** The most common side effect associated with heparin administration in pregnancy is minor skin bruising. The literature indicates that the more serious maternal complications are uncommon with relatively short-term therapy as seen during pregnancy. Potential side effects include the development of osteopenia and subsequent osteoporotic bone fractures. However, these complications occur in less than 1% of patients exposed to long-term heparin therapy (rather than the short duration associated with use during pregnancy). Heparin induced thrombocytopenia is estimated to occur in less than 1% of women exposed to long-term heparin therapy, and is reversible with cessation of medication. Placental
bleeding or abruption has been reported to occur in 0.04% of patients using long-term heparin therapy during pregnancy\textsuperscript{25}.

**Fetal complications:** Heparin does not cross the placenta, and is therefore safe for the fetus when used during pregnancy\textsuperscript{25}.

<table>
<thead>
<tr>
<th>Is there clinical evidence to suggest a role for heparin in reducing the risk of adverse pregnancy outcome associated with placental dysfunction?</th>
</tr>
</thead>
</table>

**Appraising the evidence:**

We have conducted a systematic review of the literature to assess the use of heparin in pregnancy.

Using the defined search terms, there were no randomized controlled trials identified, evaluating heparin therapy in pregnant women with evidence of placental dysfunction who are negative on thrombophilia testing.

Two ongoing randomized controlled trials are evaluating heparin in women with a known thrombophilic state (TIPPS: ISRCTN 87441504; FRUIT Study: ISRCTN 87325378).

**METHODOLOGY**

**Types of Studies:** Published RCTs

**Participants:** Pregnant women with evidence of placental dysfunction

**Interventions:** Heparin therapy

**Databases** Cochrane Controlled Trials Register (CENTRAL) and MEDLINE (last searched August 2006)

**Search Terms:** heparin, pregnancy, placental dysfunction, pregnancy outcome, and randomized trial

**Limitations of the current evidence on the use of heparin to improve pregnancy outcome in women with evidence of placental dysfunction**

- No randomized controlled trials – evidence of effect limited to cohort studies with inherent bias
- No reporting of any infant outcomes
- No reporting of any maternal outcomes
- Lack of information about potential harmful effects for the woman and fetus from prolonged heparin therapy
- Therefore, there is insufficient information to make recommendations about the benefits and harms of heparin therapy

We propose that a randomized controlled trial to assess the use of subcutaneous heparin therapy for women with evidence of placental dysfunction, to improve maternal and infant health outcomes, is needed and timely for the following reasons:

1. Placental dysfunction mediated by placental thrombotic-ischaemic lesions is a major cause of adverse pregnancy outcome.
2. Heparin may have a role in mediating placental vascular damage, thereby potentially improving pregnancy outcomes.
3. There is insufficient evidence on the benefits and harms of subcutaneous heparin therapy given to women who have evidence of placental dysfunction.

Our literature review highlights that there is no information reported from
randomized controlled trials on heparin in improving pregnancy outcomes and other outcomes of relevance for women and their infants.

4. The benefits and harms of subcutaneous heparin in this clinical setting have been under-investigated to date.

5. Women with evidence of placental dysfunction and their caregivers need unbiased information about the risks and benefits of subcutaneous heparin therapy to enable informed healthcare choices.

RESEARCH PLAN

Study Design:
Multi-centre, randomized controlled trial.

Inclusion Criteria:
Women with a singleton pregnancy, at 18\(^{0}\)-23\(^{6}\) weeks’ gestation, with evidence of placental dysfunction in their current pregnancy as determined by two or more of the following: abnormal ultrasonographic placental morphology; abnormal uterine artery Doppler waveforms; abnormal biochemical markers on first or second trimester maternal serum screening.

Placental Ultrasound
Real-time imaging of the placenta will utilize our previously published approach\(^ {26, 27}\), with abnormal placental morphology defined either by shape, texture or both. Maximal placental length and thickness will be measured in centimetres, with placental thickness >4cm, or >50% of length, defining an abnormal shape. Abnormal placental length is defined as a maximum length of <10cm, or in the presence of an eccentric cord insertion, a length of <10cm measured in the place perpendicular to the maximum placental diameter. Placental texture will be categorized as normal (homogeneously granular), or abnormal when the placenta contains one or more echogenic cystic lesions (ECL)\(^ {6}\), or assumed a “jelly-like” appearance with turbulent uteroplacental flow visible due to lack of normal villous development\(^ {6}\). Minor abnormalities will not constitute abnormal placental morphology (and include: placental cord root insertion >2cm from the placental disc margin, lateral (within 2cm of the margin), marginal (on the margin) or velamentous (inserting into the surrounding membranes); placental lakes; and 2 vessel cord\(^ {26}\).

Doppler Ultrasound
The proximal uterine arteries will be located at their cross-over point with the external iliac arteries using colour flow mapping to obtain waveforms by pulsed Doppler. Uterine artery Doppler will be considered abnormal between 18 and 23 weeks where either a mean pulsatility index (PI) value exceeds 1.45 or there are obvious bilateral early diastolic notches.

Biochemical Markers
Women will have first and second trimester maternal serum screening reported in multiples of the median (MoM) for PAPP-A, AFP, inhibin, hCG and estriol. Values considered abnormal include PAPP-A <0.35 MoM, AFP >2.0 MoM, inhibin >3.0 MoM, and hCG >4.0 MoM.
Serial Serum Samples
Blood samples (5-10cc) at 22, 26, 30 and 34 weeks’ gestation, at delivery (both maternal and cord blood) and 6 weeks post partum will be obtained. These samples, in addition to leftover samples from the maternal serum screen, will be stored for future evaluation or analysis of biomarkers or genetic risks of placental dysfunction.

Exclusion Criteria
Women with known positive thrombophilic screening; known lethal fetal anomaly; the presence of early onset fetal growth restriction prior to trial entry (defined as absent or reversed end diastolic flow on umbilical artery Doppler and fetal growth parameters more than 2 weeks smaller than predicted by gestational age); any contraindication to heparin therapy or continuation of the pregnancy (eg. chorioamnionitis requiring delivery); clinical need for heparin therapy during pregnancy (eg. previous venous thrombo-embolic episode). Women who present at greater than 23+6 weeks gestation will not be eligible for participation in this study.

Trial Entry
Eligible women identified in the Placental or Maternal-Fetal Medicine Clinics of Mt Sinai, Women’s College, and St Michael’s Hospitals will be given the trial information sheet and counselled by the study co-ordinator, before obtaining informed written consent. Randomization will occur between 18°6 and 23°6 weeks gestation by contacting the central telephone randomization service of Perinatal Clinical Trials Unit, The University of Adelaide, Discipline of Obstetrics and Gynaecology. During a short telephone call, information will be given to check eligibility, describe the characteristics of the woman, enable stratification at randomization so that similar types of women are allocated to the treatment groups and to assist in follow-up. At the completion of the telephone call, a study number will be allocated to the woman and the group to which she has been randomized will be stated (Heparin Group or Standard Care Group). Additional information will be obtained regarding baseline demographic characteristics and previous birth outcomes. The randomization schedule will use balanced variable blocks, and will be prepared by an investigator not involved with recruitment or clinical care. There will be stratification of women according to centre. Eligible women will be randomized to either receive Heparin or Standard Care.

Study Medication & Treatment Schedules:
After randomization, the woman will be allocated a study number. The woman, her caregivers and research staff assessing the trial outcomes will not be blinded to treatment allocation.

Heparin Group
Women randomized to the heparin group will receive specific instruction in the self-administration of subcutaneous heparin, and will receive a month’s supply of subcutaneous heparin and syringes. Women will be asked to self-administer 7500IU of un-fractionated heparin subcutaneously twice a day from randomization until 34 weeks gestation or birth (whichever occurs first). Un-fractionated heparin preparations are available in Canada, and will be provided to women at no cost. Women will receive further medication on a monthly basis, and will be asked about the occurrence of any side effects experienced and compliance with the treatment protocol. Ongoing antenatal surveillance will be provided through the
multidisciplinary Placenta Clinic or Maternal-Fetal Medicine Clinics of the collaborating hospitals, according to local standard practice.

**Standard Care Group**
Women randomized to the standard care group will receive ongoing antenatal surveillance provided through the multidisciplinary Placenta Clinic or Maternal-Fetal Medicine Clinics of the collaborating hospitals, but will not be administered medication.

**Follow-up of women in both treatment groups**
Women will be reviewed in the multidisciplinary Placenta Clinic or Maternal-Fetal Medicine Clinics of the collaborating hospitals, according to the recommendations of the practitioner responsible for their care. All women will have Maternal Serum Screening (MSS) reported in multiples of the median (MoM) for PAPP-A, AFP, hCG, inhibin and estriol during the first and second trimester. All women will have an 18-20 week fetal anatomical ultrasound, with uterine artery Doppler and placental morphology either included in this examination or subsequently performed by 23 weeks.

At the time of enrolment in the study, women will be asked to complete a short questionnaire related to their emotional health and wellbeing. At a routine prenatal visit each month, the study co-ordinator will ask women about their experience of any side effects, and provide a further month’s supply of medication. After birth, the placenta will be submitted for pathological examination by a dedicated perinatal pathologist, blinded to the treatment group the woman was allocated to. Information will be obtained relating to birth and infant outcomes from the woman and infant’s case notes by the research assistant and the delivery form will be completed. Similarly, the postnatal and neonatal forms will be completed for each live born infant after discharge from hospital, or at six weeks postpartum if the infant is still in hospital. Data collection forms will be checked and signed by the local clinical coordinator. Women will be asked to complete a postal questionnaire at four months postpartum relating to their emotional wellbeing and infant development.

**Primary Study Endpoints**
The primary study outcomes are
- **Intrauterine fetal death** (defined as fetal death prior to birth and after trial entry).

**Secondary Study Endpoints**
The secondary study endpoints are

1. **Other adverse outcomes for the infant** defined as one or more of the following: neonatal death (defined as death of a live born infant prior to hospital discharge, and excluding lethal congenital anomalies); infant birth weight less than the 10th centile for gestational age and infant sex; Apgar score ≤4 at 5 minutes of age; seizures; cord pH ≤7.18; intraventricular hemorrhage on early cranial ultrasound; periventricular leukomalacia on later cranial ultrasound; inotropic support for the treatment of patent ductus arteriosis; proven necrotizing enterocolitis; proven systemic infection within 48 hours of birth and treated with antibiotics; retinopathy of prematurity; altered level of
consciousness (stupor, decreased response to pain or coma); need for tube feeding ≥4 days; admission to the neonatal intensive care unit ≥ 4 days).

2. **Other adverse outcomes for the woman** including ultrasound diagnosis of intrauterine growth restriction with abnormal Doppler study (defined as absent or reversed end diastolic flow); preterm birth at less than 32 weeks gestation; length of antenatal hospital stay; antenatal corticosteroid therapy; side effects of heparin injection (including bruising, minor hemorrhage, thrombocytopenia, osteopenia, bone fractures); antepartum hemorrhage after 20 weeks’ gestation requiring admission to hospital; pre-eclampsia, eclampsia, or HELLP syndrome; postpartum hemorrhage; anaesthetic complications; antibiotic use after birth; length of postnatal hospital stay; maternal death; not breast feeding at four months postpartum.

3. **Maternal emotional wellbeing** as measured by postal questionnaires completed by the woman at four months postpartum relating to quality of life, anxiety, postnatal depression (as measured using the SF36 Health Survey Questionnaire, Edinburgh Depression Scale, and Short Form Spielberger State Trait Inventory), and preference for treatment and satisfaction with care.

4. **Placental histopathology** including the presence of lesions affecting villi and intervillous spaces (such as syncytial knots, increased intervillous fibrin deposition, and distal villous hypoplasia), and those affecting maternal vessels at the site of placental implantation (such as acute atherosis, mural hypertrophy of arterioles, muscularisation of basal plate arteries, and increased placental site giant cells).

**Sample Size**
The primary trial outcome of intrauterine fetal death has been estimated to occur with a frequency of 41.7% from our prospective cohort data in women with a similar eligibility profile as planned in this trial. A sample size of 272 women (136 women in each group) will be able to show a 40% reduction in the risk of intrauterine fetal death from 41.7% to 25.0% (5% level of significance, two-tailed alpha, 80% power).

**Proposed Timetable and Committee Structure**
REB approval from Mount Sinai Hospital was received in February 2007 and recruitment to the trial commenced July 2007. A multidisciplinary adverse events committee blinded to treatment allocation will review the cause of death for all maternal and perinatal deaths. These data will be made available to the independent Data Monitoring Committee, who will conduct an interim analysis with established terms of reference when 50% of women (136 women) have been recruited to the trial. Recruitment will continue until February 2010, with completion of data collection and final analyses after the last woman has given birth and completed the postnatal questionnaire.

**Analysis and Reporting of Results**
The initial analysis will examine baseline characteristics of all randomized women, as an indication of comparable treatment groups, and include maternal age, race, height, weight, smoking history, past obstetric history (including previous preterm birth or
perinatal loss), and markers of placental dysfunction (placental morphology, uterine artery Doppler anomalies, or elevated maternal biochemical screening during the first trimester).

Outcome comparisons for women and infants will be analysed for the primary and secondary outcomes on an “intention to treat” basis, according to treatment allocation at randomization to either heparin or standard care. The relative risks and 95% confidence intervals will be reported for the major outcomes, and the number needed to treat to prevent one adverse outcome will be calculated. Regression techniques will be used to examine the influence of prognostic factors on the major outcomes.

Confidentiality and Data Security
Information relating to the trial will be kept in a locked secure filing cabinet, and on password-protected database, accessible only to members of the coordinating committee.

OUTCOME AND SIGNIFICANCE

Placental dysfunction, as evidenced by ultrasonographic anomalies in placental morphology, abnormal uterine artery Doppler studies, or abnormalities in biochemical markers associated with first trimester maternal serum screening, have been associated with the occurrence of adverse pregnancy outcomes, including preterm birth, pre-eclampsia, abruption, and perinatal death. There is currently no effective treatment for women with identified placental dysfunction to improve health outcomes for both women and their infants.

Adverse pregnancy outcomes such as those described above are potentially mediated via placental ischaemic-thrombotic lesions. It is biologically plausible that heparin therapy may alter placental pathology and improve pregnancy outcomes for this group of women and their infants.

Systematic review of the literature has highlighted an absence of randomized controlled trials assessing the role of heparin therapy in treating women with identified placental dysfunction to improve health outcomes for women and their infants\(^{28}\). Clearly, high quality trials assessing maternal and infant health outcomes are required before evidence-based clinical practice recommendations can be made.

If heparin therapy during pregnancy for women with identified placental dysfunction is an effective way of reducing the risk of adverse pregnancy outcomes, this would be a highly beneficial, cost effective and worthwhile treatment to reduce the burden of a major cause of maternal and infant morbidity.
REFERENCES