Thrombosis Research 151, Suppl. 1 (2017) S65–S69

Contents lists available at ScienceDirect

Thrombosis Research

journal homepage: www.elsevier.com/locate/thromres

LMWH in IVF – Biomarkers and benefits

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ARTICLE INFO

Keywords:
In vitro fertilisation
Low-molecular-weight heparin
Annexin A5
Annexin A5M2 haplotype
Placenta mediated pregnancy complications

ABSTRACT

There is biological plausibility that coagulation activation underlies a proportion of in vitro fertilisation IVF failures and recurrent early clinical pregnancy loss (RPL). However, low-molecular-weight heparin (LMWH) use, based upon previous clinical outcome alone, is not effective in preventing RPL. RPL is heterogeneous in mechanism. Identifying those with an underlying thrombotic mechanism would allow stratification for LMWH treatment. Annexin A5 is an anticoagulant protein expressed on the trophoblast surface. The annexin A5 M2 haplotype (ANXA5 M2) is associated with several placenta mediated pregnancy complications (PMPC) and poor IVF outcome. It is transmitted equally by males and females. A pragmatic observational study of IVF couples screened for M2 carriage and treated with LMWH achieved a 37.9% live birth rate, similar to an unscreened and untreated group with fewer adverse risk factors for conception and a better prognosis from assisted conception. This suggests that LMWH may counteract the adverse effects of M2 carriage. Using this biomarker to stratify IVF and PMPC patients for LMWH treatment merits further evaluation.

1. Introduction

Assisted reproduction techniques (ART) are widely used globally with 643 456 ART cycles reported in 2010 and growing at 9% annually. However pregnancy losses (preclinical and clinical) averaged 20.2% for fresh, and 25% for frozen embryo transfer (ET). Pre term delivery (<37 weeks gestation), exacerbated by multiple pregnancies, accounted for 16–18% of preterm deliveries for fresh and frozen ET embryo transfer in the EU [1].

Increasing maternal age is associated with an increasing incidence of embryo aneuploidy, which is the most common cause of implantation failure and first trimester recurrent pregnancy loss (RPL) in ART patients. When transfer of aneuploidy embryos is avoided by pre implantation screening (PGS), clinical pregnancy loss is markedly reduced [2]. Indeed this can be by as much as 65% in women aged over 35 years when PGS is coupled with elective single embryo transfer(eSET) [3]. However, even avoiding aneuploidy does not eliminate pregnancy loss. In 2010, global preimplantation genetic diagnosis (PGD) pregnancy rate per embryo transfer cycle was 39.5% and delivery rate per pregnancy was 77% [1]. One possible mechanism for these losses is activation of the coagulation system [4] with adverse effects on trophoblast; this has led to the use of low-molecular-weight heparin (LMWH) in ART in an attempt to reduce these losses.

2. LMWH as an adjuvant for in-vitro fertilisation (IVF)

Despite the absence of robust clinical evidence, maternal LMWH administration is used in IVF as an intervention that may improve implantation and reduce miscarriage, although clinical practice varies widely between individual clinicians and clinics. A comprehensive systematic review and meta-analysis of published literature on the effect of heparin on the outcome of IVF was conducted by Seshadri et al. in 2012 [5], which included both randomized and observational studies. The randomized studies included only small numbers of women, and had high methodological heterogeneity, and so had significant limitations. These showed no significant difference in implantation rate, clinical pregnancy, clinical miscarriage and live birth rate. Meta analysis of the observational studies showed a significant increase in the clinical pregnancy rate and live birth rate, however the authors concluded that the potential role of heparin during IVF treatment required further evaluation in adequately powered randomized studies as they noted that the observational studies could have exaggerated the value of heparin in IVF due to selection bias.

A retrospective cohort study evaluated the use of aspirin and/or heparin on outcome in 103 women <44 years of age, with a history of one or more unsuccessful IVF cycles [6]. The women received daily low dose aspirin (LDA) (75 mg) and/or LMWH (enoxaparin 20 mg) subcutaneously post embryo transfer until 12 weeks of pregnancy (42 women received LDA/LMWH combined, 43 LDA alone, and 18 LMWH alone). They were compared to 103 control women who received no treatment. There was no significant difference in pregnancy or live birth rates derived with LMWH

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or LDA in this unselected group of subfertile women; however the small numbers and heterogeneous treatment were significant limitations of this study.

A Cochrane review of LMWH for assisted reproduction in 2015 [7] included three randomized controlled studies of 386 women administered LMWH in the peri-implantation period compared to placebo or no treatment. The evidence was limited by inconsistency and imprecision. The participant characteristics varied as did statistical methods. A fixed effect model suggested that peri-implantation LMWH improved live birth outcome but when random effects were used this difference was abolished It was concluded that it remained uncertain whether peri-implantation LMWH improves pregnancy and live birth weights in subfertile women undergoing ART and findings did not justify the use of heparin except in well-conducted research trials

The causes of IVF failure are heterogeneous. Despite this, treatment is often based on clinical pregnancy outcome, such as recurrent miscarriage or preeclampsia, rather than the causative disease mechanism. Clearly if a treatment does not impact on a disease mechanism, it is unlikely to impact on outcome. Placenta mediated pregnancy complications (PMPC) have common and overlapping pathological features including deficient implantation, placental infarction and microvascular thrombosis. Thus there is biological plausibility that coagulation activation may play a role in a proportion of these complications. The use of antithrombotic intervention in heterogeneous conditions such as RPL where coagulation activation may only account for a proportion of losses, limits the ability to detect a treatment effect. Therefore treatment stratified by mechanism rather than outcome may be better able to detect an effect. Without such stratification, treatments may be incorrectly discarded as having no utility [8,9]. Clark noted that if a subset of patients with a particular condition is diluted in a larger group, it is impossible to discriminate a potentially beneficial treatment effect for that specific group [10]. Identification of a biomarker that ascertains an underlying coagulation mechanism for pregnancy complications can better inform the use of antithrombotic treatment.

3. Annexin A5 and the ANXA5 M2 haplotype

Rand et al. [11], using immunostaining, demonstrated that in antiphospholipid syndrome (APS) there is a significant reduction in the naturally occurring anticoagulant protein Annexin A5 (ANXA5) on placental villous surfaces, and that antiphospholipid antibodies can directly decrease levels of villous surface ANXA5 on cultured placental villi. Patients with APS have improved pregnancy outcomes following antithrombotic therapy [8,12]. These data raise the possibility that loss of the anticoagulant ANXA5 may be important in APS, and may be ameliorated by antithrombotic treatment, as has been shown in murine knock-out models [13].

ANXA5 is normally highly expressed on the apical surface of the syncytiotrophoblast layer, the interface between maternal and fetal circulations and is necessary for placental integrity. Essentially, ANXA5 is an anticoagulant protein that crystallizes over negatively charged phospholipid surfaces and thereby blocks them from availability for coagulation reactions. It forms a two-dimensional lattice, preventing coagulation reactions and thereby contributes to the maintenance of blood fluidity in the placenta. This two dimensional lattice can be disrupted by antiphospholipid antibodies [14]. More recently Bouter et al. [15] suggested that ANXA5 is essential for repair and maintenance of the syncytiotrophoblast apical membrane. Without this efficient repair mechanism, coagulation occurs, which adversely effects the embryo/fetus [16]. Burton and Jones [17] also noted that in pre-eclampsia (PE), raised levels of syncytiotrophoblast (ST)-derived syncytial particles (knots and sprouts) are released into maternal blood, triggering the inflammatory response in maternal endothelial cells, which are directly involved in the PE process.

Whilst direct measurement of placental ANXA5 is impractical, the ANXA5 M2 haplotype provides a potential biomarker for reduced ANXA5 in the placenta and in the maternal circulation. It has been shown to be associated with a significant reduction in expression of ANXA5 in placentas from M2 carriers with PE and fetal growth restriction (FGR) [18–20].

The M2 haplotype is associated in natural pregnancies with an increased risk of: recurrent pregnancy loss (RPL) [21–27], FGR [22] small for gestational age infants, [28] and gestational hypertension [22]. Males and females transmit haplotype equally [18–20,24].

Significant reductions both in fetal weight and litter size and has been reported in ANXA5-null mice (ANXA5-KO) [13]. Administration of heparin to ANXA5-KO mice significantly increased litter size, which suggests that in addition to placental ANXA5, maternal expression of ANXA5 in the circulation may be crucial for maintaining normal pregnancy; additionally, compared with wild-type mouse perivascular cells, ANXA5-null cells exhibit a severe membrane repair defect. This repair defect was rescued by addition of ANXA5, which binds only to disrupted membrane areas. [15]

Since some of the key actions of ANXA5 are shared with LMWH e.g. reduced trophoblast apoptosis and platelet aggregation [4], and as ANXA5 is deficient in placentas of APS patients [29], who have an improved outcome with antithrombotic treatment, there is biological plausibility to select patients with PMPC for LMWH treatment using this ANXA5 biomarker.

4. The Annexin M2 haplotype as a biomarker for LMWH treatment in IVF

A pragmatic observational study [30] investigated the potential benefits of using LMWH in IVF patient couples at risk of adverse pregnancy outcome(based on past history and/or maternal age) stratified by testing both partners for the M2 haplotype, observing the timing of pregnancy loss and live birth outcome from embryo transfer onwards, the study design is summarised in the flow chart (Fig. 1).

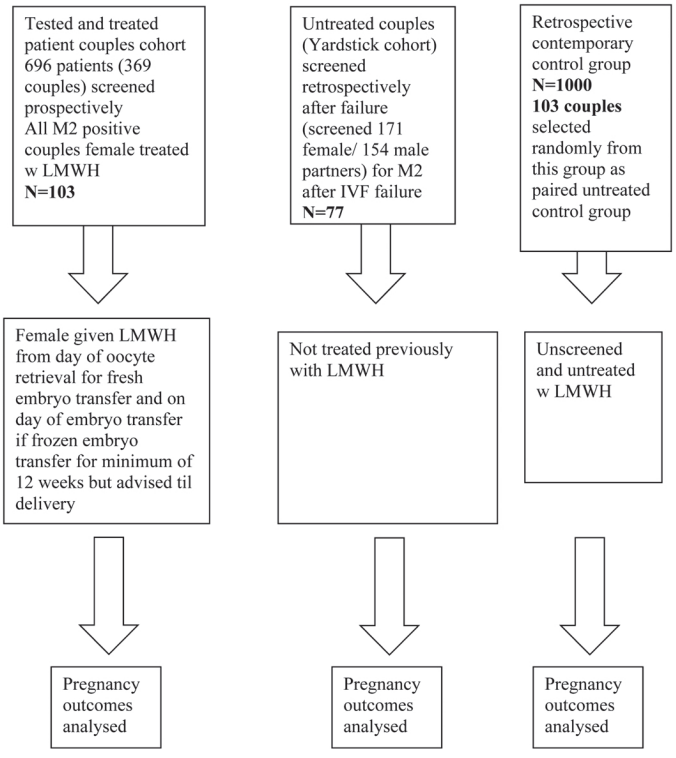

Fig. 1. Flow chart. All patients achieved an embryo transfer.