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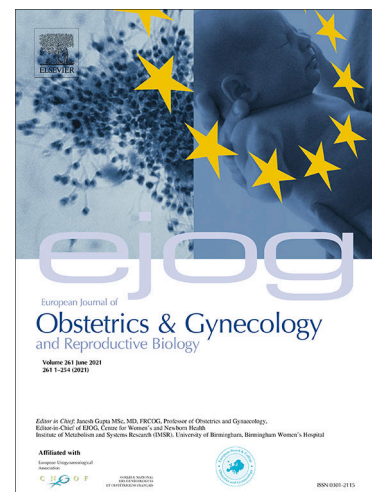
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Use of anticoagulants to improve pregnancy outcomes in couples positive for M2 haplotype: A systematic review

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Abstract

Background: Placental mediated pregnancy complications (PMPC) are common, often recurring, and pose a significant health risk to mother and fetus. Evidence suggests that the hypercoagulable state associated with many PMPC, could reflect reduced expression of Annexin 5 (ANXA5), a naturally occurring anticoagulant protein in placental tissue. The ANXA5 M2 haplotype is a genetic variant, which results in reduced expression of ANXA5 protein. M2 haplotype carrier couples may therefore be at increased risk of PMPC. Evidence regarding the effectiveness of anticoagulation to prevent PMPC is inconsistent. Furthermore, studies have not selected or stratified for M2 haplotype carriers, in whom there is a predisposition to hypercoagulability, to assess the effectiveness of anticoagulation, which may vary from those without the M2 haplotype.

Objectives and rationale: The aim of this study was to systematically review the current evidence to assess whether anticoagulant treatment improves pregnancy outcomes in couples positive for M2 haplotype.

Search methods: The review was registered on PROSPERO (CRD42022343943). A comprehensive literature search was performed using MEDLINE, Embase and Cochrane collaboration databases from inception to January 2023. Two reviewers assessed the articles for eligibility and extracted the data simultaneously. Primary outcome was successful pregnancy and live birth. Secondary outcomes included PMPC (implantation failure, miscarriage, pre-eclampsia, preterm birth and fetal growth restriction).

Outcomes: From a pool of 410 references, 10 were selected for full text review, of which three studies (a post hoc analysis of a randomised controlled trial, cohort study and a case report) were included in this review. Included studies comprised of 223 individuals, 129 of whom who received anticoagulation treatment after testing positive for M2 haplotype. The studies collectively showed an improvement in pregnancy outcomes in M2 haplotype positive individuals however, given the heterogeneity of studies, it was not possible to conduct a meta-analysis and draw firm conclusions.

Wider implications: Current evidence is limited, such that the value of screening couples for the M2 haplotype to select or stratify for treatment with prophylactic anticoagulation remains unknown. Thus, further studies including well designed, large, multi-centre randomised controlled trials are required to assess whether anticoagulation treatment will be effective in improving pregnancy outcomes in M2 haplotype couples.

Introduction

Placenta mediated pregnancy complications (PMPC) include implantation failure, recurrent or late pregnancy loss, pre-eclampsia, placental abruption, fetal growth restriction and premature birth. PMPC represent a significant cause of physical, psychological and emotional morbidity, fetal loss, and maternal and neonatal mortality (Ananth et al. 1999; Christiansen, Elklit, and Olff 2013; Huppertz 2011; Oyelese and Ananth 2006; Pallotto and Kilbride 2006; Tikkanen 2011). Pregnancy loss alone costs the National Health Service in the United Kingdom more than £400 million per year, highlighting the additional economic burden on health systems from PMPC (Quenby et al. 2021). Women experiencing PMPC are also more likely to suffer PMPC in subsequent pregnancies, and in later life have an increased risk of cardiovascular disease and death (Ananth et al. 2007; Mongraw-Chaffin, Cirillo, and Cohn 2010; Mosca et al. 2011; van Rijn et al. 2006). In women undergoing fertility treatment, such as in-vitro fertilisation (IVF), only half of all implanted embryos result in an ongoing pregnancy secondary to PMPC (Chen et al. 2019). In at least some cases, this may be related to a hypercoagulable state, brought about by a range of factors such as underlying thrombophilia and hormonal treatment during IVF. This hypercoagulability may predispose women to implantation failure (Aune et al. 1991).

Although PMPC are a varied group of disorders, it is highly likely they are linked through common mechanisms. A large body of evidence has identified micro-thrombosis at the level of the maternal and fetal circulatory interface, particularly the intervillous space, which causes placental dysfunction and subsequent placental failure, leading to PMPC (P Clark, Greer, and Walker 1999; Demetriou et al. 2015; Gourvas et al. 2014; Greer, Brenner, and Gris 2014; Matsubayashi et al. 2001; Miyamura et al. 2012; Ota et al. 2013; Rand et al. 1994; Rogenhofer et al. 2012; Hock et al. 2015; Tiscia et al. 2012; Tiscia et al. 2009; Tüttelmann et al. 2013). This is supported by histological evidence showing the reduction in levels of a naturally occurring anti-coagulant protein, Annexin-5 (ANXA5), on placental trophoblasts of women with PMPC (Chinni et al. 2009; Markoff et al. 2010; Shu et al. 2000). ANXA5 is thought to not only provide an 'anti-thrombotic shield' at the maternal-fetal circulatory interface but also support embryonic implantation and the repair and maintenance of the placenta, key factors in ensuring a successful pregnancy outcome (Bogdanova et al. 2007; Krikun et al. 1994). Moreover, women with decreased levels of ANXA5 are over-represented in populations suffering with PMPC, specifically those possessing the M2 haplotype (Rogenhofer et al. 2018). First reported more than a decade ago, the M2 haplotype consists of four nucleotide substitutions within the promoter region of the ANXA5 gene, which results in reduced ANXA5 anti-coagulant protein expression and thus presents an increased risk of PMPC (Ang et al. 2019; Aranda et al. 2018; Bogdanova et al. 2007). Furthermore, there is also evidence to suggest that the risk of PMPC is similar whether there is maternal or paternal carriage of the M2 haplotype (Rogenhofer et al. 2021; Rogenhofer et al. 2012).

The combination of Low Molecular Weight Heparin (LMWH) and aspirin is currently recommended by the Royal College of Obstetrics and Gynaecology in those with antiphospholipid syndrome (a hypercoagulable condition) and history of recurrent miscarriages, with similar recommendations in other clinical guidelines (RCOG 2011; Tektonidou et al. 2019). In addition to its anticoagulant properties, heparin has been shown to enhance endometrial receptivity and increase implantation success- similar to the functions of ANXA5 (Fluhr et al. 2010; Wilcox, Baird, and Weinberg 1999). Nevertheless, there is conflicting evidence as to whether exogenous anticoagulation is effective in reducing rates of PMPC. Rodger et al. 2016 in their meta-analysis, consisting of 963 women, did not show any significant difference in rates of PMPC between women receiving LMWH and those who did not. Although Greer

2016 commenting on this meta-analysis, suggests that the lack of biomarkers in PMPC made it difficult to determine whether LMWH treatment could offer benefit in patients selected on the basis of potential pathogenic mechanisms rather than pregnancy outcome. In this respect, the reproductive field lags behind the precision medicine approach now adopted widely, for example, in oncology where treatment is allocated based on disease characteristics that predict effectiveness of treatment. More recently, however, Dias et al in their meta-analysis of 1855 women demonstrated a significant benefit of LMWH in preventing late pregnancy loss (pregnancy loss beyond 20 weeks gestation), albeit there was no significant difference amongst other PMPC outcomes (Dias, Modesto, and Oliveira 2021). Of note, however, is that carriage of the M2 haplotype has not been found to be associated with late pregnancy loss. Crucially, there was no identification of couples with the M2 haplotype in the analysed trials. Therefore, it is unknown whether this population could differentially benefit from anticoagulation.

This systematic review aims to explore the effectiveness of anticoagulation in preventing PMPC in M2 haplotype carriers. In doing so, we aim to inform the need for, and support the development of novel trials to guide treatments and inform clinical guidelines.

Methods:

Literature Search

Online searches were conducted within the MEDLINE, Embase and Cochrane collaboration databases from inception to January 2023. This was supplemented with searches in Clinical trials registry databases (International clinical trials registry platform and Clinicaltrials.gov) and Google Scholar. No language restrictions were placed on the database searches. Reference lists of relevant articles were screened for studies not found during initial database searches. The search strategy is attached as supplementary data (Appendix 1).

Study eligibility criteria

All types of studies were included (randomised controlled trials, observational and cohort studies, and case reports). Included studies reported on the outcome of anticoagulation in patients with M2 haplotype both in assisted reproduction techniques and in those who had natural pregnancies. Studies (n=392) that did not assess the effect of anticoagulation in M2 positive couples, were excluded, along with those ambiguous regarding which outcomes were related to M2 haplotype carriers. Two reviewers (HK and SH) independently conducted the study selection and data extraction process. All studies, including abstracts, which met the pre-defined inclusion and outcomes criteria, were included. Disagreement was resolved through a third reviewer DB. Population, intervention, comparator and outcome data for each full text study was extracted into a pre-designed proforma using Microsoft Excel.

Data extraction

For each of the included studies, information was extracted on the participants (number, assisted/natural reproduction), the anticoagulation (type, frequency, duration) and outcomes (pregnancy, live birth, PMPC).

Study quality assessment

Quality comparison amongst included studies can be seen in supplementary table 1. Risk of bias assessments were performed using ROBINS-I, Cochrane Risk of Bias v2.0 and Oxford Centre of Evidence

Based Medicine levels, as appropriate (OCEBM Levels of Evidence Working Group.; Sterne et al. 2016; Sterne et al. 2019).

Outcome measures

Primary outcome was successful pregnancy and live birth. Secondary measures were PMPC (implantation failure, miscarriage, pre-eclampsia, preterm birth and fetal growth restriction).

Search results

In accordance with PRISMA guidance, relevant studies were selected and reported. From a pool of 410 references, 10 were selected for full text review of which three studies (post hoc analysis of an RCT, cohort study and a case report) were included in this review (see Figure 1).

Results

Three studies were included in this review, comprising of; 129 M2 patients treated with anticoagulation (103 in Fishel et al, 25 in Rogenhofer et al and 1 individual in Ang et al.) and 19 M2 carrier controls (Rogenhofer et al.) plus 75 “yardsticks” which were tested M2 positive after an IVF cycle failure(s) (Ang et al. 2017; Fishel et al. 2016; Rogenhofer et al. 2017). Study characteristics are included in table 1.

Differences were noted amongst the included studies in terms of population, ethnicity, IVF versus natural conception and type of heparin used in treatment. Fishel et al. 2016 evaluated a cohort of IVF patients which were 75% Caucasian (77% male, 75% female), 8% Indian and Pakistani and the rest mixed race and diverse ethnicities. Rogenhofer et al. 2017 included patients who were all derived from a German Caucasian population whilst Ang et al. 2017 was only composed of one Malay couple. The latter two studies involved patients who underwent natural conception. Fishel et al. utilised enoxaparin and Rogenhofer et al. reported genotyping from the patients in the EthigII study where dalteparin was administered. Ang et al. used a combination of aspirin and Fondaparinux (synthetic derivative of LMWH). All studies reported live births and miscarriages but did not report on other placenta mediated pregnancy complications.

The Cochrane risk of bias in non-randomised interventions (ROBINS-I) tool showed Fishel et al. overall possessed a moderate risk of bias, mostly due to significant differences in participants baseline characteristics. Those in the treated group were significantly older ($p=0.0001$) with 67% of the cohort >35 years old and also had a significantly longer ($p<0.0001$) period of infertility compared to the control group. Therefore, it is possible the small, although significant, positive pregnancy outcomes with LMWH in the treatment group are in effect larger than reported in this study.

The post-hoc analysis of an RCT (EthigII trial) presented by Rogenhofer et al. was assessed using the Cochrane risk of bias (RoB) tool (version 2.0) and determined to have ‘some concerns’ with regards to bias. RoB was determined based on the manuscript detailing post-hoc analyses used for meta-analysis and not on the original RCT which did not perform sub-analysis for effect of LMWH on M2 haplotypes. Bias is likely in the selection of participants for analysis, since participants who consented to this were not re-randomised, but instead derived from both the treatment and control groups, ultimately leading to fewer control patients in the final analysis. Other issues with the study included the lack of blinding which could have been overcome with placebo injections, although it is unlikely this affected the objective pregnancy outcome data. This was an ethical consideration in terms of not requiring women

self-inject throughout pregnancy with saline. This open label approach has been adopted in recent thromboprophylaxis trials, for example in the ALIFE2 study. (de Jong et al. 2015; Quenby et al. 2022)

Ang et al. presented a case-study involving one couple who were M2 haplotype carriers managed with LMWH and aspirin. The Oxford Centre for Evidence Based Medicine determines this to be 'level 4' (low quality) evidence and due to the study design, any conclusions regarding causality are not appropriate.

Pregnancy outcomes

Miscarriage

Fishel et al. 2016, reported a 51% reduced risk of clinical miscarriage within the 'tested and treated' M2 cohort versus matched paired controls- who were taken from a pool of unscreened and untreated couples undergoing IVF, although this was not significant (OR=0.49 (0.13, 1.85)). Implantation incidence was similar in the treated (33.1%) and the controls (30.2%). Biochemical pregnancy loss rate was reportedly nearly two times greater in the treatment group versus control group, although not significant (OR=1.63 (0.59,4.56)).

Rogenhofer et al. (2017) reported that although the odds of having a miscarriage were not significantly different depending on M2 carrier status, LMWH did appear to have a greater protective effect in M2 carriers versus non-carriers, reducing the risk by 84% (OR=0.16 (0.016-1.5)) and 40% (OR=0.60 (0.19,1.9)), respectively.

Live births

Accounting for the poorer prognostic factors of women within the 'tested and treated' group reported by Fishel et al (2016), this group still achieved comparable live birth rates (per patient) to the more fertile comparison group and to the matched controls; 37.9%, 38.5% and 33.0%, respectively. There was a significantly greater number of live births in treated couples in which the male was the lone carrier of the M2 haplotype compared to if only the female was the M2 haplotype carrier (p-value=0.0452). In their post-hoc analysis of genotype data, Rogenhofer et al. 2017 did not explicitly report live birth rates amongst M2 carriers and controls, other than to mention that those experiencing three or more pregnancy losses appeared to have higher live birth rates, although this was not a significant difference. Ang et al. 2017 reported a successful live birth in one woman (with four previous miscarriages) following treatment with low dose aspirin and Fondaparinux from 6 weeks gestation (the point at which fetal heartbeat was detected) till delivery.

Discussion

This systematic review assessed whether the use of anticoagulants in couples positive of the M2 haplotype gene improved pregnancy outcomes. Three study were included (129 individuals). The included study in IVF population allowed observation of where pregnancy losses were occurring from implantation to preclinical pregnancy, to early miscarriage in both treated and untreated patients (Fishel et al. 2016). This was the first time that M2 associated losses were able to be observed pre-clinically. As LMWH was administered at or shortly before embryo transfer, the timing of treatment in relation to

development of pregnancy was consistent. Additionally, as this was an IVF study, male partners were able to be screened and compliance with LMWH treatment and follow up was high. The outcome, while not significant, indicated that even in these older and infertile patients an approximately 10% improvement in live births was noted. The study by Rogenhofer et al. 2017 reported on a *post hoc* of a multicentre RCT where LMWH was administered only from fetal heartbeat detection at around 5-8 weeks gestation (which may be sub-optimal as ANXA5 appears critically important from the very earliest stages of embryo development) and continued only until 24 weeks. Furthermore, the male partners were not tested, and the number of women positive for M2 was too small for statistical analysis. Nevertheless, for patients who conceived spontaneously, and had two or more early pregnancy losses, an improvement in live birth rate of approximately 25% was noted (if women and their partners were screened for M2 and LMWH treatment commenced before 7 gestational weeks).

There is a significant body of studies, in several different ethnicities, providing evidence for the association between the ANXA5 biomarker with a range of PMPC conditions, from implantation failure to premature birth (Clark, Greer, and Walker 1999; Demetriou et al. 2015; Gourvas et al. 2014; Greer, Brenner, and Gris 2014; Matsubayashi et al. 2001; Miyamura et al. 2012; Ota et al. 2013; Rand et al. 1994; Rogenhofer et al. 2012; Hock et al. 2015; Tiscia et al. 2012; Tiscia et al. 2009; Tüttelmann et al. 2013). There is also a substantial body of work elucidating the potential role of ANXA5 in placental pathology of PMPC (Bouter et al. 2015; Degrelle et al. 2017). In addition, the potential contribution of male partners has not been assessed because the knowledge that males carry and transmit the M2 haplotype to the fetus equally to the female had not been established. Furthermore, there have been extensive reviews of the potential mechanisms, both in terms of its anticoagulant actions, and non-anticoagulant actions on trophoblast (Nelson and Greer 2008) through which LMWH may reduce PMPC (Tersigni et al. 2012). More recently the molecular actions of heparin and its potential role in reducing placental damage have become available (Wat et al. 2018; Jovian et al. 2020). Trials of antithrombotic interventions in unselected women at risk of PMPC, based on outcome of previous pregnancies rather than disease mechanism, have reported no benefit from antithrombotic treatment (Clark 2013; Peter Clark et al. 2010; Ian A Greer, Brenner, and Gris 2014; Kaandorp et al. 2010; Martinelli et al. 2012; Pasquier et al. 2015; Schleussner et al. 2015).

To the best of our knowledge, this is the first systematic review that assessed the effects of anticoagulants on M2 haplotype positive individuals.

The primary limitations of this study are its small sample size and the high likelihood of bias in the included research. Furthermore due to heterogeneity in the outcomes, it was not possible to conduct a meta-analysis to be able to give an accurate account of the effects of anticoagulants on M2 haplotype positive individuals. Nevertheless, there is biological plausibility for the M2 haplotype being a biomarker for increased risk of PMPC, and a potential for treatment with LMWH, but there has been no adequate assessment of this by clinical trial.

A randomised trial is therefore required to determine whether M2 haplotype screening provides an effective method of stratifying women at risk of PMPC for treatment with LMWH. As treatment should start early in pregnancy, such a trial would require to be done in women undergoing assisted conception where screening for the M2 haplotype can be carried out before embryo transfer or in women with previous PMPC screened following an affected pregnancy in anticipation of them conceiving again. This would allow treatment to be initiated early in pregnancy.

Conclusion

Low Molecular Weight Heparin may be of benefit in M2 carriers, however, due to the heterogeneity of studies, it was not possible to conduct a meta-analysis and draw firm conclusions. Although LMWH should not be used routinely in patients at risk of miscarriage and placental mediated pregnancy complications, it may be useful in certain subgroups of patients. The recent understanding of the role of ANXA5 in placental development and maintenance, coupled with knowledge of the actions of heparin on placental function, suggests that a targeted therapy approach with stratification of patients is appropriate.

Authors' roles

H.K designed the study and developed the protocol for the review. H.K. designed the search strategy. S.A.H. ran the literature search. H.K. identified relevant studies and conducted data extraction. S.A.H extracted the data as the second reviewer and helped draft the manuscript. D.B. and I.G. contributed to study design and critical analysis of the manuscript. All authors approved the final version of the manuscript.

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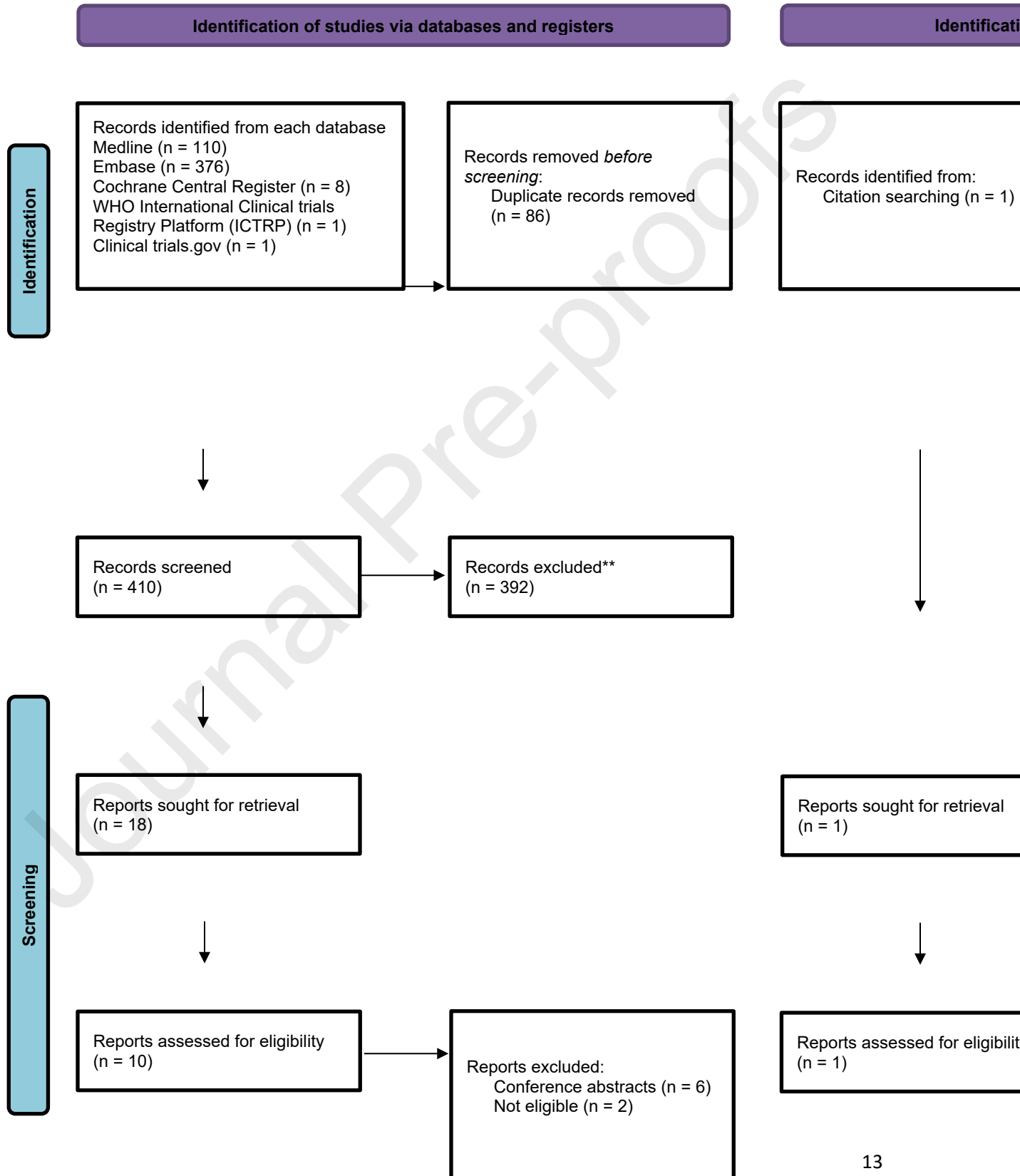
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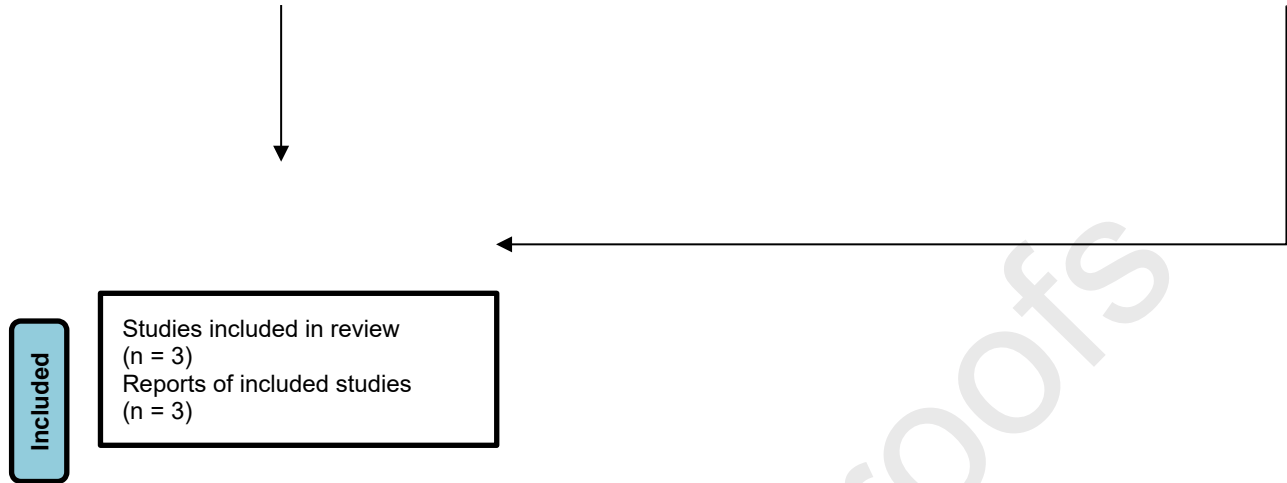
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Table 1. Study characteristics table

Study	Type of study	Number of patients in treatment group	Number of patients in control group	Anticoagulant used	Live birth	Miscarriage	Pregnancy complications
Ang et al. 2017	Case report	1	nil	Nadroparin calcium	Live birth achieved	Not provided	Not provided
Fishel et al. 2016	Prospective cohort study	103	103 (77 untreated)	LMWH	The tested and treated cohort of ANXA5 M2 carriers achieved a similar live birth rate	Miscarriage was noted to be 4% in treatment group and 21% in control group	Not provided

					(37.9%) per embryo transfer (ET) cycle compared to both the more fertile comparison group (38.5%), and to the 103 matched controls (33.0%)		
Rogenhofer et al. 2017	Randomised Controlled trial	25	19	LMWH	Not provided	Miscarriages noted to be 4% in carriers and 21.1% in control group (OR 0.16, CI 0.016-1.5)	Not provided

Highlights

- Our comprehensive review reveals potential benefits of anticoagulants in M2 haplotype positive couples for enhancing pregnancy outcomes.
- Studies included in the review underline the significant association between ANXA5 biomarker and various placental mediated pregnancy complications (PMPC), offering insights for targeted interventions.
- Growing understanding of the role of ANXA5 in placental pathology and mechanism of Low Molecular Weight Heparin (LMWH) in PMPC reduction pave the way for optimistic prospects in tailored treatment approaches.

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