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Prediction of miscarriage in women with threatened miscarriage using a combination of biochemical and ultrasound scan markers - A prospective cohort study

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ABSTRACT:

Background: Studies have been previously conducted to investigate biomarkers and ultrasound markers to predict miscarriage in women with threatened miscarriage with varying results. This project reviewed the existing literature and conducted a prospective study to investigate the role of various markers to predict miscarriage in women with threatened miscarriage and develop a prediction model.

Methodology: Three systematic review and meta-analysis were conducted to investigate the role of biomarkers and ultrasound markers to predict miscarriage in women with threatened miscarriage and to investigate the perinatal outcomes in them. Subsequently, a prospective cohort study was conducted (N=296) including women presenting with bleeding and/or pain and had a confirmed fetal heartbeat from 6^{+0} to 11^{+6} weeks of gestation. An extensive exclusion criterion was used.

Results: Comparison of the miscarried women and those who continued pregnancy had shown that, the two groups of women were different in their bleeding score (P-value 0.03), hCG (P-value 0.04), progesterone (P-value 0.03), inhibin A (P-value 0.02), MGSD (P-value 0.04), CRL (P-value 0.03) and FHR (P-value 0.01) . A regression model composed of the variables of age, hCG, inhibin and FHR gave the best sensitivity (57%) and specificity (96%) to predict miscarriage (P-value 0.0003); diagnostic odds ratio (95% CI) of 1.01 (1.01 – 1.02). The study has demonstrated an increased risk of adverse perinatal outcomes including preterm labour, IUGR, LBW and neonatal asphyxia in women experiencing threatened miscarriage in the early pregnancy.

Conclusions: A prediction model was developed to predict miscarriage in threatened miscarriage population using makers including age, hCG, inhibin and FHR. Future studies focussing on developing markers of adverse perinatal outcomes in women experiencing bleeding in early pregnancy will help to plan the antenatal care of high-risk women with bleeding in early pregnancy.

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Author's declaration

I state that this thesis represents my own original unaided work and has not been submitted previously in consideration of a degree in this University or any other University.

30/06/2020

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Dedication

To my husband Dr Manjith Narayanan for the sacrifices he made, the motivation and guidance he gave and for the steadfast support, love and understanding.

Table of Contents

List of tables

List of Figures

Index of abbreviations

A

AFP – Alpha feto protein

APH – Antepartum Hemorrhage

ASV – Amniotic Sac Volume

AUC – Area under the curve

B

BAI – Beck Anxiety Inventory

BDI – Beck Depression Inventory

BMI – Body Mass Index

C

Ca 125 – Cancer antigen 125

CEBM- Centre for Evidence Based Medicine

CI - Confidence interval

CMW- Community Midwife

CRF- Clinical Research Fellow

CRL – Crown Rump Length

D

DOR – Diagnostic Odds Ratio

E

EPAU – Early Pregnancy Assessment Unit

F

FHR- Fetal Heart Rate

FSH – Follicle Stimulating Hormone

G

GA – Gestational Age

GP – General Practitioner

GS – Gestational sac

GSV - Gestational Sac Volume

H

hCG – Human Chorionic Gonadotrophin

HPL – Human Placental Lactogen

hs-CRP – High sensitive C- Reactive Protein

HSROC – Hierarchical Summary Receiver Operating Curve

I

IGF – Insulin- Like Growth Factor

IGFBP – Insulin Growth Factor Binding Protein

IUGR – Intra Uterine Growth Restriction

IUH – Intra Uterine Hematoma

IVF – In Vitro Fertilisation

L

LH – Luteinizing hormone

LMP – Last Menstrual Period

M

MeSH – Medical Subject Heading

MGSD- Mean Gestational Sac Diameter

mRNA- Messenger RiboNucleic Acid

N

NICE – National Institute of Clinical Excellence

NPV – Negative Predictive Value

P

PAPP-A

PET – Pre-Eclamptic Toxaemia

PI – Pulsatility Index

PID – Pelvic inflammatory disease

PIH – Pregnancy Induced Hypertension

PPROM- Preterm Prelabour Rupture of Membrane

PPV – Positive Predictive Value

PSV – Peak Systolic Volume

PTD- Preterm delivery

PUL- Pregnancy of Unknown Location

PV - Per Vaginum

R

RR – Risk ratio

S

SCH – Subchorionic Hematoma

SGA – Small for Gestational Age

SD – Standard deviation

SHBG – Serum Hormone Binding Globulin

T

TSH – Thyroid Stimulating Hormone

TV – Trans Vaginal

U

UK – United Kingdom

USS – Ultrasound scan

W

WHO – World Health Organisation

Y

YS – Yolk sac

Publications and presentations arising out this thesis

Publications

- Pillai RN, Konje JC, Richardson M, Tincello DG, Potdar N. Prediction of miscarriage in women with viable intrauterine pregnancy-a systematic review and diagnostic accuracy meta-analysis. European Journal of Obstetrics & Gynecology and Reproductive Biology. 2017 Nov 4. PubMed PMID: 29207325
- Pillai RN, Konje JC, Tincello DG, Potdar N. Role of serum biomarkers in the prediction of outcome in women with threatened miscarriage: a systematic review and diagnostic accuracy meta-analysis. Human reproduction update. 2016 Mar 1; 22 (2):228-39. PubMed PMID: 26663220

Oral Presentations

- July 2020 - ESHRE 36th annual meeting, Virtual meeting - Prediction of miscarriage in women presenting with threatened miscarriage in their first trimester of pregnancy using biomarkers, ultrasound markers and demographic variables (Abstract of the 36th Annual Meeting of the ESHRE, 5 to 8 July 2020; i114)
- November 2017- Second Annual conference of Egyptian society of Maternal and Fetal Medicine, Cairo, Egypt- Utility of biomarkers in predicting outcome of threatened miscarriage
- July 2016- ESHRE 32nd annual meeting, Helsinki, Finland - Late pregnancy complications and outcomes in women with threatened miscarriage: A systematic review and Meta-analysis. (Abstracts of the 32nd Annual Meeting of ESHRE, Helsinki, Finland 3-6 July 2016; i29)
- June 2016- RCOG world congress 2016, Brimingham, UK- Role of ultrasound scan markers in prediction of miscarriage for women with confirmed viable intrauterine

pregnancy- A systematic review and diagnostic accuracy meta-analysis (Pillai R, Konje J, Richardson M, Tincello D, Potdar N. Role of ultrasound scan markers in prediction of miscarriage for women with confirmed viable intrauterine pregnancy: A systematic review and diagnostic accuracy meta-analysis. BJOG 2016 Jun 1 (Vol. 123, pp. 19-20)

Poster presentations

- Role of ultrasound scan markers in the prediction of outcome for women with or without vaginal bleeding and viable intrauterine pregnancy- A systematic review and diagnostic accuracy meta-analysis- Cochrane Gynaecology and Fertility Society meeting, Oxford. April 2016
- Accuracy of serum biomarkers in predicting outcome of threatened miscarriage- A systematic review and meta-analysis- ESHRE 2015, Lisbon. June 2015 (Pillai R, Potdar N. Accuracy of serum biochemical markers in predicting outcome for women with threatened miscarriage-a systematic review and meta-analysis. In Human Reproduction 2015 Jun 1 (Vol. 30, pp. 187-187).

1 Introduction

Abstract: This chapter aims to summarise and critically appraise the existing literature on the prediction of miscarriage in women with threatened miscarriage. This chapter also lays the background on the importance of miscarriage prediction by exploring the existing evidence on psychological morbidities associated with threatened miscarriage. It also explores the existing evidence on perinatal morbidity associated with threatened miscarriage.

Critical appraisal of the existing literature highlighted the significant variations in the existing literature on prediction markers for miscarriage in the threatened miscarriage population. This discrepancy in the existing literature mandated conducting a systematic review of the existing literature.

1.1 Miscarriage

Miscarriage is defined as a spontaneous loss of pregnancy before the fetus reaches the age of viability. The term includes all pregnancy losses from the time of conception up to 23 weeks of pregnancy and weighing up to 500gms(World Health Organization, 2000) . Miscarriage is the most common complication of early pregnancy. Approximately 20% of pregnancies miscarry and early pregnancy loss accounts for over 50,000 admissions in the UK annually (Ananth and Savitz, 1994, National Collaborating Centre for Women's and Children's Health (UK), 2012). This incidence is only the tip of an iceberg and the magnitude of pregnancy loss before implantation and clinical identification of pregnancy is in the region of 22% (Wilcox *et al.*, 1988). The vast majority of these miscarriages occur before 12 weeks of pregnancy. Sporadic miscarriages after 12 weeks complicate only 1-2 % of pregnancies (Regan and Rai, 2000).

Fetal chromosome abnormalities account for about 50% of first-trimester pregnancy losses. Most of these abnormalities are numerical abnormalities where a whole chromosome is either missing or present as an extra (86%). A low percentage is caused by structural abnormalities where a part of an individual chromosome is missing, present as an extra, switched to another chromosome or turned upside down (6%) or other genetic mechanisms, including chromosome mosaicism (8%) (Goddijn and Leschot, 2000). Multiple environmental and occupational factors have been described in the literature as possible aetiologies behind foetal chromosomal abnormalities. Exposure to a potentially teratogenic and mutagenic agent can cause chromosomal damage and furthermore, maternal exposure to organic solvents, consumption of coffee, alcohol and cocaine have been found to be associated with miscarriage (Regan and Rai, 2000). Maternal health conditions such as endocrine diseases, autoimmune conditions, thrombotic factors and infections in the first trimester can also contribute to miscarriage (Regan and Rai, 2000). Inadequate placentation was thought to be another major factor behind miscarriages. A study by Hustin et al (Hustin *et al.*, 1990)) have demonstrated that the normal physiological process of trophoblastic penetration into the decidua and the spiral arteries was limited or absent in miscarriages. This results in early initiation of blood flow in the inter-villous space which in turn is associated with

arrest of pregnancy and eventual expulsion of the foetus (Hustin *et al.*, 1990). Normally, a physiological hypoxia is created until the end of first trimester to protect the rapidly dividing embryo from the impact of oxygen free radicals in the maternal blood. In normal pregnancies, this is accomplished by plugging maternal decidual spiral arteries with extra-villous trophoblast from the developing embryo.

A Doppler imaging study by Jauniaux in 1994 in missed miscarriage has shown that the trophoblastic shell was fragmented or absent in 53% and trophoblastic infiltration and physiological changes in the spiral arteries were reduced or absent in 43% of the study population. Extended dislocation of the trophoblastic shell and massive infiltration of the inter-villous space and placental bed by maternal blood was also found in cases presenting with a continuous inter- villous blood flow before 12 weeks of gestation. These findings suggest that abnormal flow velocity waveforms in early pregnancies complicated by embryonic death are related to deficient placentation and dislocation of the trophoblastic shell that follows embryonic demise. The premature entry of maternal blood into the inter-villous space disrupts the maternal-embryonic interface and is probably the final mechanism causing miscarriage (Jauniaux, E. *et al.*, 1994).

There can be lasting psychological impact due to miscarriage and women experience anxiety, increased level of post-traumatic stress disorder and obsessive compulsive disorder following a miscarriage (Brier, 2004). Women with a history of miscarriage were more likely to experience pregnancy specific anxiety in their future pregnancies than women who did not have any previous history of miscarriage (Bergner *et al.*, 2008). Pain and bleeding in early pregnancy are also distressing if it brings anxiety about the health and viability of the pregnancy even if it doesn't end in miscarriage (National Collaborating Centre for Women's and Children's Health (UK), 2012). Healthcare professionals caring for women undergoing miscarriage also experience a significant level of subjective distress due to the negative affect experienced at the time of care, negative appraisal of care given to the family and due to maladaptive ways of coping (Wallbank and Robertson, 2013). The healthcare professionals also can't give a definitive

answer regarding the prognosis of threatened miscarriage due to the lack of a robust predictive tool for miscarriage (Pillai *et al.*, 2016).

Various trials have recently been published or are currently taking place, evaluating interventions to prevent the occurrence of miscarriage. The PRISM trial (https://www.miscarriageassociation.org.uk/research/) investigated the role of progesterone in preventing miscarriage in women with early pregnancy bleeding (Coomarasamy *et al.*, 2019a). The Alife2 trial (https://warwick.ac.uk/fac/sci/med/research/ctu/trials/alife2) is investigating whether anti-coagulant (blood–thinning) treatment reduces the risk of miscarriage in women with two or more (unexplained) miscarriages and inherited thrombophilia. The TABLET trial is an important research study which is looked at the role of thyroid antibodies in women with unexplained miscarriage (https://www.birmingham.ac.uk/research/activity/mds/trials/bctu/trials/womens/tablet/index.aspx).

Table 1 Ongoing and recently published trials to prevent miscarriage

Trial (Trial Registration Number)	**Type of study**	**Sample size**	**Planned intervention**	**Current status**
The PRISM Trial (ISRCTN14163439)	Randomised double blind placebo-controlled trial	4150	Comparing progesterone versus placebo treatment for threatened miscarriage	Results published. Treatment with vaginal micronized progesterone might help.
The TABLET Trial (ISRCTN15948785)	Randomised double blind placebo-controlled trial	952	Comparing Levothyroxine versus placebo for treatment of unexplained miscarriage	Results published. Treatment with levothyroxine will not help
The ALIFE-2 Trial (EudraCT 2015-002357-35)	Multi centre Randomised Controlled Trial	400	Comparing anti-coagulant versus placebo as treatment of recurrent miscarriage	Ongoing recruitment

1.2 Threatened miscarriage

1.2.1 Definition and incidence

Threatened miscarriage is diagnosed when the woman presents in early pregnancy with vaginal bleeding, a closed cervix on clinical examination and a subsequent ultrasound scan demonstrates foetal cardiac activity(National Collaborating Centre for Women's and Children's Health (UK), 2012, Saraswat *et al.*, 2010). It is reported to occur in about

one-fifth of pregnancies (Everett, 1997), but there is considerable disparity in the estimated rate of miscarriage in this population and it is reported to be anywhere between 3-16% (Hill *et al.*, 1991, Makrydimas *et al.*, 2003, Siddiqi *et al.*, 1988). A study by Hill *et al.* has demonstrated that the miscarriage rate was 4.2% in a subgroup of women without bleeding compared with 12.7% in a subgroup with bleeding (Hill *et al.*, 1991) after successful demonstration of foetal cardiac activity on scan. A community-based cohort study in pregnant women has demonstrated that heavy bleeding in the first trimester particularly when there is pain, is associated with a higher risk of miscarriage compared to spotting or mild vaginal bleeding (Hasan *et al.*, 2009). This difference observed in the occurrence of miscarriage based on the variation in the severity of symptoms explains the observed disparity in the incidence of miscarriage between studies on threatened miscarriage population.

1.2.2 Pathophysiology behind threatened miscarriage

Various theories have been suggested behind early pregnancy loss in threatened miscarriage. Histopathological examination of first-trimester pregnancy loss has shown that two third of early pregnancy losses have a thinner and fragmented trophoblast shell and reduced cytotrophoblast invasion of the spiral arteries. This causes incomplete plugging of the spiral arteries resulting in the early onset of maternal circulation through the placenta. The excessive entry of maternal blood into the inter-villous space creates a direct mechanical effect and indirect oxidative stress effect that contributes to cellular dysfunction and damage (Jauniaux, E. and Burton, 2005).

Another theory is, in the event of an intra-uterine bleed, it has been suggested that weakening of the decidual membrane happens due to thrombin formation and subsequent proteolytic process leads to membrane weakness and eventual preterm rupture (Elovitz *et al.*, 2001). Sub-chorionic bleed will result in an increase in the amount of free iron available, catalysing the generation of extremely toxic hydroxyl radical and subsequent free radical damage to the membranes causing disruption to the maternal-foetal interface.

In a normally developing pregnancy, two third of the developing placenta degenerates to form the chorion laeve. It has been shown that in the peripheral developing placenta, there is increased oxygen free radical formation, correlating with the increase in maternal blood flow between 8 and 12 weeks of gestation and it has been suggested that it is a normal physiological process required for villous regression and the formation of the chorion laeve (Jauniaux, Eric *et al.*, 2003). In threatened miscarriage, it is possible that maladaptation to this process results in free radical damage to the developing placenta and membrane, resulting in an increase in placental hormone production and subsequent release into the maternal circulation.

1.2.3 Psychological impact

Women experiencing bleeding in early pregnancy experience significant psychological distress, including anxiety, fear of loss and depression (National Collaborating Centre for Women's and Children's Health (UK), 2012). There are only a limited number of studies that have investigated the emotional aspect of women when they experience threatened miscarriage. A prospective cohort study by Aksoy *et al.* (Aksoy *et al.*, 2008) has indicated a potential link between threatened miscarriage and anxiety and depression disorders. The study was conducted between September 2013 and August 2014 comparing the anxiety score between 94 women with threatened miscarriage and 120 healthy pregnant women. The study used the Beck anxiety inventory (BAI) and Beck depression inventory (BDI) to measure the level of anxiety and depression in the study participants. The mean BAI score was 18.9+- 10.52 in the threatened miscarriage group and 8.24+- 5.24 in the normal healthy women. The mean BDI score was 18.7 +- 8.49 in the threatened miscarriage population and 7.47+-6.22 in the healthy cohort group. They have suggested that patients with threatened miscarriage should be evaluated for anxiety and depression alongside their medical condition and medical professionals should be sensitive to the psychological consequences of threatened miscarriage.

1.2.4 Prognosis of threatened miscarriage

Threatened miscarriage is associated with both short term and long-term morbidities. Miscarriage is the most common immediate complication of threatened miscarriage (Hill *et al.*, 1991, Makrydimas *et al.*, 2003, Siddiqi *et al.*, 1988). Similarly, women who have suffered from threatened miscarriage are at a higher risk for late maternal and perinatal complications (Saraswat *et al.*, 2010). A systematic review and meta- analysis had shown a significantly higher incidence of antepartum haemorrhage due to placenta praevia in women with threatened miscarriage (odds ratio (OR) 1.62, 95% CI 1.52, 4.02) when compared with those without first trimester bleeding. They were more likely to experience preterm prelabour rupture of membrane (PPROM) (OR 1.78, 95% CI 1.28, 2.48), preterm delivery (OR 2.05, 95% CI 1.76, 2.4) and to have babies with intrauterine growth restriction (OR 1.54, 95% CI 1.18, 2.00). First trimester bleeding was associated with significantly higher rates of perinatal mortality (OR 2.15, 95% CI 1.41, 3.27) and low birth weight babies (OR 1.83, 95% CI 1.48, 2.28) (Saraswat *et al.*, 2010). However, these results were based on a combination of retrospective, case control and prospective studies. According to the Oxford CEBM level of evidence (https://www.cebm.net), a systematic review combining retrospective studies and case-control studies brings down the level of evidence to 2a compared to doing a systematic review of cohort studies validated in different populations, where the level of evidence would have been 1a. Hence, we could conclude that the systematic review by Saraswat *et al.* was not of the best evidence available.

Currently, women experiencing threatened miscarriage in early pregnancy are not recognised as high risk by the NICE guideline on antenatal care (http://www.nice.org.uk/Guidance/CG62). Women presenting with threatened miscarriage are often extremely distressed and at least half of them can suffer from moderate or severe anxiety and depression (Aksoy *et al.*, 2008). Healthcare professionals can often find it challenging in providing information to the women on the prognosis of threatened miscarriage and the women can often resort to internet forums to seek hope and support that they are not receiving from the healthcare professionals (Betts *et al.*, 2014). These women end up with repeated scans in early pregnancy units

to allay their anxieties, which in turn adds to the increase in waiting times and costs (National Collaborating Centre for Women's and Children's Health (UK), 2012).

1.2.5 Prediction of the outcome of threatened miscarriage

Prediction of outcome of threatened miscarriage can be better examined by having a detailed knowledge of the physiology and embryology of early pregnancy formation and placentation.

1.2.5.1 Normal early pregnancy and placentation

Fertilization between the sperm and ovum happens in the peritoneal cavity or the fimbrial end of the fallopian tube and the fertilised egg travels into the uterine cavity in 48 hours from fertilisation. From the fertilised egg or zygote, 10% of its cells develop into the embryo and the rest of the cells become trophoblast. The zygote divides to form a blastocyst which starts implanting to the endometrium by day 5 of ovulation and it gets completely embedded by day 12 of ovulation. Initially the decidua which is the modified mucosal lining of the uterus formed in preparation for a pregnancy, starts forming in the local site on implantation which then spreads into the entire endometrium. The trophoblast develops into the extra embryonic tissue and the embryo detaches from the trophoblast by day 4 of the embryogenic phase. The trophoblast undergoes rapid proliferation and fusion to form the syncytiotrophoblast which is multi nucleated and invades the uterine stroma. The syncytiotrophoblast separates into the villous trophoblast and extra villous trophoblast. The villous trophoblast forms the villous membranes and becomes responsible for absorption, exchange and hormone production. The cells of the extra villous trophoblast undergo interstitial invasion and invade the arterial walls of the decidua and form intra-arterial plugs (endovascular invasion). These intra-arterial plugs prevent the maternal blood from entering the inter-villous space until the end of the first trimester and thus protect the rapidly dividing embryo from high oxygen levels. Although a placental structure forms as early as 3rd week of gestation, it is not until 12 weeks the placenta becomes fully functional (Sadler, T. W., & Langman, J., 2012).

1.2.5.2 Biomarkers of early pregnancy

Studies have been done previously to try and predict the outcome of threatened miscarriage with various biomarkers being investigated (Johns, Jemma *et al.*, 2007, Maged and Mostafa, 2013, Phupong and Hanprasertpong, 2011, Reljic, 2001, Ruge *et al.*, 1990). The markers that have been studied can be broadly classified as a) biochemical markers b) ultrasound markers (USS) c) demographic variables and d) different combinations of markers.

1.2.5.2.1 Biochemical markers

Villous trophoblast cells are organized into arborizing chorionic villi with an outer layer of syncytiotrophoblast covering mononucleated cytotrophoblast. The syncytiotrophoblast is in direct contact with maternal blood in the inter-villous space and is the major source of protein and steroid hormone production (Sadler, T. W., & Langman, J., 2012). The placenta has maternal circulation, fetal circulation (active) and amniotic fluid (more or less static). The syncytiotrophoblast is in direct contact with maternal blood which facilitates the hormones produced by the syncytiotrophoblast to directly enter the maternal blood. The syncytiotrophoblast is responsible for the production of hormones, proteins and growth factors needed for early pregnancy. The syncytiotrophoblast produces hCG which helps the corpus luteum to produce the hormones and growth factors needed for sustaining the early pregnancy. By around the end of seven weeks, this role is shifted from the corpus luteum to the trophoblast and the decidua (Elson, 2005). The placenta produces placental proteins, growth factors, cytokines, hormones and inflammatory markers (Staun-Ram and Shalev, 2005).

1.2.5.2.1.1 HCG

HCG is a heterodimeric glycoprotein secreted by the syncytiotrophoblast. It has an alpha subunit with 92 amino acids and is similar to the pituitary hormones LH, FSH and TSH and a unique beta subunit with 145 aminoacids which distinguishes it from other glycoprotein hormones (Canfield *et al.*, 1987). HCG enters the blood on the day of implantation of the embryo and it doubles every 1.4-1.6 days until the 35th day of the pregnancy. Thereafter it doubles every 2-2.7 days until the 42nd day of pregnancy

(Pittaway *et al.*, 1985). The level of hCG is around 1000 IU/L at four weeks of gestational age and then it increases to 50,000 to 100,000 IU/L at ten weeks of gestational age. By 20 weeks of gestational age, the level plateaus from 10,000 IU/L to 20,000 IU/L and subsequently the level plateau until the end of the pregnancy (OZTURK *et al.*, 1987).

The hCG is currently used in the follow up care after miscarriage and in the diagnosis, treatment and follow up of ectopic pregnancy. A urine pregnancy test using hCG is used for following up of patients following medical management of miscarriage and a negative urine pregnancy test confirms treatment success. The serum hCG levels along with the transvaginal USS, are used to differentiate between an ongoing early pregnancy, miscarriage and ectopic pregnancy in cases of an initial diagnosis of pregnancy of unknown location on ultrasound scan. A cut-off of 1500 IU/L of serum hCG is used to decide between the treatment options (medical versus surgical) for diagnosed tubal ectopic pregnancies. The serum hCG levels are also used to assess the treatment success following medical management of ectopic pregnancy and the dropping hCG levels shows the successful response to medical treatment (National Collaborating Centre for Women's and Children's Health (UK), 2012). The hCG levels can be 2-20 times higher in multiple pregnancy and molar pregnancy compared to normal singleton pregnancies. HCG levels can also be raised in pregnancies with chromosomal anomalies (Bidus *et al.*, 2002).

1.2.5.2.1.2 Progesterone

Progesterone is an endogenous steroid involved in the menstrual cycle, pregnancy and embryogenesis (King TL, 2010). During the pre-ovulatory period, progesterone levels are low and start rising during ovulation and stay elevated during the luteal phase of the menstrual cycle. The levels are <2ng/ml before ovulation and are >5ng/ml after ovulation. If pregnancy occurs, the hCG released from the syncytiotrophoblast maintains the corpus luteum allowing it to secrete progesterone. The concentration of progesterone remains as in the luteal phase until about the 8th week of pregnancy. Between 7-9 weeks, the developing placenta starts producing progesterone and this is called luteal-placental shift (Al *et al.*, 1973). After the luteal-placental shift the

progesterone level starts to rise and reaches a level of 100-200ng/ml at term (Tulchinsky and Hobel, 1973). Progesterone promotes proliferation and differentiation of endometrial stromal and epithelial cells, creating an environment conducive for fetal survival and it also suppresses the contractility of uterine muscles (Bowen R, 06/08/2000, Norwitz *et al.*, 2001) .

1.2.5.2.1.3 Oestradiol

Oestradiol is the primary female sex hormone and is responsible for the development and maintenance of the female reproductive tissue such as breast, uterus and vaginal during puberty, adulthood and pregnancy. As the placenta develops in early pregnancy, it starts producing oestrogen. This oestrogen stimulates the growth of the myometrium and mammary glands and as the gestation advances, the oestradiol level steadily increases. (Bowen R, 06/08/2000).

1.2.5.2.1.4 Ca 125

Ca 125 or cancer antigen 125 is a glycoprotein in humans encoded by the MUC 16 gene and is used as a tumour marker for certain cancers and benign conditions (Yin and Lloyd, 2001). Ca 125 is the most frequently used biomarker for ovarian cancer detection (Suh *et al.*, 2010). The Ca 125 is produced by amnion and derivatives of coelomic epithelium like peritoneum, pleura and pericardium. Normal endometrium also produces ca 125 and this can be proved by the significant rise in the circulating levels of Ca 125 during menstruation. Any cause for peritoneal irritation like hyperstimulation, salpingitis, ruptured ectopic and threatened miscarriage releases the Ca 125 into maternal circulation giving elevated Ca 125 levels (Bischof, 1993).

1.2.5.2.1.5 Inhibin

Inhibin is a glycoprotein that belongs to the transforming growth factor-beta family and participates in “fine-tuning” the hypothalamic-pituitary-gonadal secretion of gonadotropins. Inhibin is a dimer composed of an alpha subunit and a beta A or beta B subunit (Yin and Lloyd, 2001). Inhibin A is composed of an alpha subunit and a beta A subunit and inhibin B is composed of an alpha subunit and a beta B subunit (Burger,

1988). The inhibins were initially discovered in the gonads and shown to have an inhibitory effect on pituitary follicle-stimulating hormone (FSH) release (Zonneveld *et al.*, 2003). Specific mRNAs for the inhibins are also expressed in the human placenta, and the respective immunoreactive proteins are localized in small amounts within cytotrophoblastic cells and abundantly within the syncytiotrophoblast (Riley *et al.*, 2000). Inhibin secreted by the placenta does play a role in modulating maternal pituitary FSH secretion during pregnancy, participating in the suppression of this gonadotropin and thus follicular development. Inhibin has also been postulated to have a role in governing trophoblast function. In early pregnancy, Inhibin A level first rises up to 12 weeks, decile to a nadir and then remains low in the second trimester (Muttukrlshna *et al.*, 1995). Inhibin A is currently used as a part of QUAD screen in pregnancy and an elevated level of Inhibin A along with increased β HCG and decreased AFP and oestradiol is suggestive of Down's syndrome (Aitken *et al.*, 1996)

1.2.5.2.1.6 PAPP-A

PAPP-A is also known as pregnancy associated plasma protein A is produced primarily by the syncytiotrophoblast and cytotrophoblast. PAPP-A helps in mediating the trophoblast invasion and modulates glucose and amino acid transport in the placenta by increasing the bioavailability of insulin-like growth factor. PAPP-A is also produced by ovarian granulosa cells and in non-reproductive tissues, such as fibroblasts, osteoblasts and vascular smooth muscle cells. PAPP-A levels rise progressively with gestational age and increase exponentially with a doubling time of 3-4 days during the first trimester and then the levels continue to rise throughout pregnancy until delivery (Shiefa *et al.*, 2013). Decreased levels of PAPP-A are found to be associated with an abnormal placental function (Shiefa *et al.*, 2013). Low plasma levels of PAAP-A have been suggested as a biomarker for aneuploidy in the fetus and may also predict issues with the placenta resulting in complications like IUGR, pre-eclampsia, premature birth, placental abruption or fetal death (Breathnach and Malone, 2007).

1.2.5.2.1.7 High Sensitivity C- Reactive Protein (hs-CRP)

CRP is an acute phase protein secreted by the liver in response to inflammation and it can now be measured using a highly sensitive assay and is called hs-CRP (Vashist *et al.*, 2014). A raised CRP level is noted not only with infection but also with inflammation and malignancy. Measurement of hs-CRP has been used for more than a decade in the screening, diagnosis and management of coronary artery disease (Koenig, 2013). Its use in reproductive medicine has been so far limited to the prediction of pre-eclampsia (Kashanian *et al.*, 2013). The human placenta releases hs-CRP predominantly into the maternal blood and a raised amount of hs-CRP directly relates to poor pregnancy outcome (Malek *et al.*, 2006). However, it is still not very clear about the real source of hs-CRP in the amniotic fluid and its function during pregnancy (Kashanian *et al.*, 2013).

1.2.5.2.2 Studies on Biochemical markers

The list of biochemical markers that have been studied in the past for the prediction of the outcome of threatened miscarriage is extensive. Some of them include serum hCG, progesterone, oestradiol, pregnancy associated plasma protein A (PAPP-A), ca 125, human placental lactogen (HPL), alpha feto-protein (AFP), inhibin A, follistatin and activin A (Johns, Jemma *et al.*, 2007, Maged and Mostafa, 2013, Ruge *et al.*, 1990, Scarpellini *et al.*, 1995, Vavilis *et al.*, 2001, Westergaard *et al.*, 1985). The existing literature gives very conflicting evidence on various biochemical markers and their use in predicting miscarriage.

1.2.5.2.2.1 Studies on HCG

Table 2 summarises the studies done on hCG. Studies done on HCG shows a wide variation in its results. The study by Jouppila *et al*. (Jouppila, Penttl *et al.*, 1980) showed a sensitivity for predicting miscarriage using hCG as low as 10% (no cut-off value used), whereas the study by Stoppelli (Stoppelli *et al.*, 1981a) demonstrated a sensitivity of 85% for hCG. Moreover, except for two studies, others did not check the diagnostic accuracy against a cut off value, making them inadequate to extrapolate for clinical use.

Table 2 Studies on hCG to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Duff et al., 1980(Duff *et al.*, 1980)	69	sensitivity 55%; specificity 96%	Not used
Jouppila et al., 1980	103	sensitivity 10%; specificity 94%	Not used
Stoppelli et al., 1981 (Stoppelli *et al.*, 1981a)	62	sensitivity 85%; specificity 89%	Not used
Dessaive et al., 1982	49	sensitivity 11%; specificity 90%	Not used
Simes et al., 1983	74	sensitivity 79%; specificity 77%	Not used
Westergaard et al., 1985	77	sensitivity 0%; specificity 94%	<10th centile
Scarpellini et al., 1995	48	sensitivity 58% specificity 86%	Not used
Leylek et al., 1997	40	sensitivity 80% specificity 72%	20000miu/L
Maged and Mostafa et al., 2013	150	sensitivity 66% specificity 81%	Not used

1.2.5.2.2.2 Studies on progesterone

Table 3 summarises the studies on progesterone. There is wide variation in the results of the studies with Jouppila *et al*.(Jouppila, Penttl *et al.*, 1980) reporting a sensitivity of 0% while Maged and Mostafa *et al*. (Maged and Mostafa, 2013) reported a sensitivity of 89%. Except for two studies, others did not investigate the predictive cut off value for serum progesterone.

Table 3 Studies on progesterone to predict miscarriage in threatened miscarriage population

Study	Sample size	Result	Cut off value
Jouppila et al., 1980	103	sensitivity 0%; specificity 94%	Not used
Stopelli et al., 1981	62	sensitivity 83%; specificity 92%	Not used
Dessaive et al., 1982	49	sensitivity 0%; specificity 71%	Not used
Westergaard et al., 1985	77	sensitivity 18%; specificity 89%	<10th centile
Leylek et al., 1997	40	sensitivity 87% specificity 72%	21ng/ml
Maged and Mostafa et al., 2013	150	sensitivity 89% specificity 87%	Not used

1.2.5.2.2.3 Studies on oestradiol

Table 4 summarises the studies on oestradiol. Significant disparity noted in the results among the studies done on oestradiol. Stopelli *et al*. (Stoppelli *et al.*, 1981a) reported a sensitivity of 88% while Westergaard *et al.* (Westergaard *et al.*, 1985) reported a sensitivity of 0% for the prediction of miscarriage. Among all the studies on oestradiol, only one used a cut off value for the prediction of miscarriage.

Table 4 Studies on oestradiol to predict miscarriage in threatened miscarriage population

Study	Sample size	Result	Cut off value
Stopelli et al., 1981	62	sensitivity 88%; specificity 79%	Not used
Dessaive et al., 1982	49	sensitivity 28%; specificity 94%	Not used
Simes et al., 1983	74	sensitivity 83%; specificity 93%	Not used
Westergaard et al., 1985	77	sensitivity 0%; specificity 88%	<10th centile

1.2.5.2.2.4 Studies on Ca 125

Table 5 summarises the studies done on Ca 125. Their variation in the results is not as extensive as in the studies for other marker and more studies on Ca 125 used a cut off value. However, there is significant variation in the cut off value levels being used, making it difficult to pick up a single cut off value for Ca 125.

Table 5 Studies on Ca 125 to predict miscarriage in threatened miscarriage population

Study	Sample size	Result	Cut off value
Ocer et al., 1992	25	sensitivity 100%; specificity 95%	>65 U/ml
Scarpellini et al., 1995	48	sensitivity 79%; specificity 76%	>120 IU/ml
Leylek et al., 1997	40	sensitivity 87%; specificity 96%	125 IU/ml
Sherif et al., 2000	100	sensitivity 95%; specificity 98%	>21 IU/ml
Feigler et al., 2003	200	sensitivity 93% specificity 86%	>43 IU/ml
Maged and Mostafa et al., 2013	150	sensitivity 80% specificity 78%	Not used
Xie et al., 2014	135	sensitivity 91% specificity 84%	Not used

1.2.5.2.2.5 Studies on PAPP-A

Table 6 summarises the studies done on PAPP- A and the studies have shown significant variation in the results with Jandial *et al.* (Jandial *et al.*, 1978) showing a sensitivity of 14% and Kuntz *et al*. (Kunz and Keller, 1976) showing a sensitivity of 79%. None of the studies on PAPP-A defined a cut off value for the prediction of miscarriage.

Table 6 Studies on PAPP- A to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Kuntz et al., 1976	65	sensitivity 79%; specificity 63%	Not used
Jandial et al., 1978	33	sensitivity 14%; specificity 100%	Not used
Duff et al., 1980	69	sensitivity 48%; specificity 92%	Not used
Hertz et al., 1983	109	sensitivity 51%; specificity 95%	Not used

1.2.5.2.2.6 Studies on AFP

Table 7 summarises the studies done on AFP and there is a significant variation in the reported results. Kuntz *et al.* (Kunz and Keller, 1976) reported a sensitivity of 7% for the prediction of miscarriage and Hertz et al. (Hertz and Schultz-Larsen, 1983) reported a sensitivity of 94% for prediction of miscarriage. Both studies did not use a cut off value for the prediction of miscarriage.

Table 7 Studies on AFP to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Kuntz et al., 1976	65	sensitivity 7%; specificity 77%	Not used
Hertz et al., 1983	109	sensitivity 33%; specificity 94%	Not used

1.2.5.2.2.7 Studies on Inhibin

There was only one study done on inhibin that presented its results using sensitivity and specificity. The study by Johns *et al*. presented their results using mean and standard deviation and in women who experienced miscarriage, the mean levels of inhibin were significantly lower than those who achieved term pregnancy.

Table 8 Studies on Inhibin to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Phupong et al., 2012	30	sensitivity 33%; specificity 92%	Not used
Johns et al., 2007	122	Term pregnancy 252+-161 pg/ml and miscarriage 148+-153 pg/ml	Not used

1.2.5.2.2.8 Studies on SP1

Two studies (Table 9) looked into SP1 for prediction of miscarriage, and there was variation in the results with Hertz *et al*. (Hertz and Schultz-Larsen, 1983) showing a sensitivity of 61% and Masson *et al*. (Masson *et al.*, 1983) showing a sensitivity of 90%. Instead of using a definitive cut off value, Masson *et al*., 1983 used less than 2 SD as its cut off value and Hertz did not use any cut off value.

Table 9 Studies on SP1 to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Hertz et al., 1983	109	sensitivity 61%; specificity 84%	Not used
Masson et al., 1983	110	sensitivity 90%; specificity 96%	Less than 2 SD

The tables above (Table 2-9) give an overview of the results of studies conducted on biochemical markers and their diagnostic accuracies. As demonstrated in the above tables, there is a significant variation in the results between studies with the sensitivities and specificities reported from 0% to 100%. One explanation for this variation can be the differences in the selection criteria of the studies. Some studies had very coherent selection criteria, and others had not. For example, bleeding not from the uterus cannot be treated as a threatened miscarriage, and unless we do a pelvic examination using a speculum, this fact cannot be ascertained. This can contribute to variation in results. Only a few studies have specified that they have done a local examination and excluded those with local cause for bleeding (Dessaive *et al.*, 1982, Jouppila, Penttl *et al.*, 1980). Similarly, coexisting medical conditions can contribute to variation in the level of biomarkers. For example, existing endometriosis, pelvic inflammatory disease (PID), ovarian masses can contribute to a raised Ca 125 level. A molar pregnancy can cause raised hCG levels. Multiple pregnancies also contribute to elevated levels of all biomarkers. A concurrent chromosomal abnormality can derange PAPP-A, alpha-fetoprotein, oestradiol and hCG levels. Those participants with systemic diseases were excluded by Stopelli *et al*. and Westergaard *et al.* (Stoppelli *et al.*, 1981b, Westergaard *et al.*, 1983). However, they have not specified which systemic diseases they have excluded. Azogui *et al*. specifically excluded women with endometriosis in their study (Azogui *et al.*, 1996). Women who are taking hormonal medications can contribute to spurious results — a typical example is progesterone supplementation in assisted conception patients. Azogui *et al.* and Scarpellini *et al*. have excluded patients conceived through fertility treatment in their studies (Azogui *et al.*, 1996, Scarpellini *et al.*, 1995).

Duration and method of follow up of participants can have an impact on the results. Follow up duration was not adequate and the method of follow up was not robust in many studies. Some studies did not follow up until 24 weeks, which is the WHO defined gestational age of viability. For example, Hanita *et al*. followed up women only up to 22 weeks (Hanita *et al.*, 2012). Follow up duration was not mentioned in Azogui *et al*., Leylek *et al*., Phupong *et al*. and Vavilis *et al*. (Azogui *et al.*, 1996, Leylek *et al.*, 1997, Phupong and Hanprasertpong, 2011, Vavilis *et al.*, 2001). A robust form of follow up

would have been to follow every single participant up to 24 weeks of gestation clinically and ultrasonographically to determine the outcome. However, many studies have followed up women based on their hospital admissions or phone calls (Azogui *et al.*, 1996, Vavilis *et al.*, 2001). Method of follow up was not specified by Leylek *et al.* and Phupong and Hanprasertpong *et al*. (Leylek *et al.*, 1997, Phupong and Hanprasertpong, 2011)

Many studies, especially the older studies, have a poor reporting format and have not mentioned the methodology of the study to assess its validity (Leylek *et al.*, 1997, Phupong and Hanprasertpong, 2011, Stoppelli *et al.*, 1981b). All these factors might have contributed to the variation in the results between studies.

1.2.5.3 USS markers of early pregnancy

Ultrasound features diagnostic of anembryonic pregnancies, a pregnancy where a gestational sac was seen on USS but no signs of fetus seen inside(Robinson, Hugh P., 1975), were described as early as 1972 (Donald *et al.*, 1972) and since then, various studies have used USS markers to diagnose and predict miscarriage. These markers include the presence of the gestational sac (GS) or yolk sac (YS), crown rump length (CRL), fetal heart rate (FHR) and doppler indices of the uteroplacental circulation (Achiron *et al.*, 1991, Alcázar and Ruiz-Perez, 2000, Laboda *et al.*, 1989, Merchiers *et al.*, 1991, Reljic, 2001, Stampone *et al.*, 1996). Some of these markers such as GS and CRL are currently used in the diagnosis of viable intra uterine pregnancy (National Collaborating Centre for Women's and Children's Health (UK), 2012). Studies have also been reported where USS markers have been used to predict the outcome of medical management of a miscarriage (Elson, 2005) and for the prediction of aneuploidy in early pregnancy (Spencer *et al.*, 2006).

1.2.5.3.1 Ultrasound appearance of normal early pregnancy

Transvaginal ultrasound is the procedure of choice in assessing viability in early pregnancy. Viability can be assessed accurately when the exact gestational age is known or when the findings are correlated with the beta hCG levels. A study by Bree *et al.* demonstrated that when the hCG level had reached 1000 mIU/ml, a gestational sac was

seen sonographically in each patient. When the hCG level had reached 7200 mIU/ml, a YS was seen in each patient. Every patient with hCG greater than 10,800 mIU/ml had a visible embryo with a heartbeat. A GS can be normally seen by 32 days of the last menstrual period. A YS was seen in every patient between 36 and 40 days. Every patient with an accurate date greater than 40 days had an embryo with a heartbeat identified. When correlating sac size with structures within the sac, a yolk sac was first seen in a gestational sac between 6 and 9 mm and a heartbeat was seen in every patient with a 9 mm or greater gestational sac diameter (Bree *et al.*, 1989).

1.2.5.3.1.1 Gestational sac

A gestational sac is the first sign of early pregnancy and can be seen with a TV scan as early as 3-5 weeks of gestational age when the mean sac diameter is as small as 2-3mm in diameter. A true gestational sac can be differentiated from a pseudo gestational sac by its eccentric location in the uterine cavity, double decidual sign and presence of a YS (Nyberg *et al.*, 1985). A gestational sac of more than or equal to 25mm mean sac diameter without an embryo and a distorted sac have been reported as distinguishing criteria between an abnormal gestation and a normal gestation(Nyberg *et al.*, 1986).

1.2.5.3.1.2 Yolk sac

The yolk sac is the structure that develops within the gestational sac. It provides nutrients such as glucose and fatty acids to the developing embryo. It is also involved in initial haematopoiesis and serves endocrine, metabolic and immunological functions (Jauniaux, Eric *et al.*, 1991). It also contributes to the development of fetal gastrointestinal and reproductive systems. The size of the YS progressively increases from the 5^{th} to the 10^{th} gestational week, after which the YS gradually gets compressed and disappear by the 14^{th} to 20^{th} week of pregnancy (Callen, 2011). A normal YS is less than 6mm and near spherical in shape. During USS, a YS appears as a circular thick-walled echogenic structure with an anechoic area within it and it is located inside the gestational sac but outside the amniotic membrane. An absent YS is considered to be associated with subsequent embryonic death. An irregular YS has high sensitivity and low specificity (sensitivity of 29% and specificity of 95%) to correlate with pregnancy

failure (Küçük *et al.*, 1999a, Tan, S. *et al.*, 2011). A calcified YS is a yolk sac with abnormally increased echogenicity of the rim with posterior acoustic shadowing and possibly comet tail artefact and is reported to be associated with early embryonic demise (Harris *et al.*, 1988). An echogenic YS differs from a calcified YS in the fact that it is echogenic throughout and not just at the rim and there is a controversy that it may be associated with fetal demise (Tan, Sinan *et al.*, 2014). Berdahl et al. has reported that it can be associated with fetal demise (Berdahl *et al.*, 2010). However, a study by Tan et al. reported 6 cases with echogenic YS and all of them reverted to normal YS before the 10th week of pregnancy. Only one study followed the pregnancy with echogenic sac alone, and it has shown that of the 39 cases, 19 cases had raised nuchal translucency and echogenic YS and all those pregnancies were chromosomally abnormal. Whereas twenty cases were echogenic YS was the only abnormal finding, all those pregnancies were chromosomally normal (Szabo *et al.*, 1996). A small yolk sac is a nonspecific feature. A small yolk sac may be a normal feature during early embryonic development. A study by Varelas *et al*. in 2005 has demonstrated that YS diameter is significantly small in the miscarried group compared to the group of women who continued their pregnancy (P-value 0.001). Some authors consider a large yolk sac more than 5mm between 5-10 weeks of gestational a normal feature of early pregnancy, but others consider it as a feature of spontaneous miscarriage (Cepni *et al.*, 1997, Cho *et al.*, 2006, Tan, Sinan *et al.*, 2012).

1.2.5.3.1.3 Fetal Pole

A fetal pole measured as a crown rump length (CRL) in the USS is seen as a thickening along the margin of the yolk sac. It is usually identified around 6.5 weeks on a transabdominal scan and at around six weeks on a trans-vaginal scan (Merz and Bahlmann, 2005). According to the NICE guideline on Ectopic pregnancy and miscarriage: Diagnosis and initial management in early pregnancy of ectopic pregnancy and miscarriage (National Collaborating Centre for Women's and Children's Health (UK), 2012), when the CRL measures 7mm and if there is no FHR, confirms the diagnosis of a non-viable pregnancy. A study by Reljic has reported a significant association between the deficit in CRL for gestational age and the incidence of subsequent miscarriage. The

study has shown that if the CRL is less than 18mm and the deficit is more than 2 SD, the incidence of miscarriage was 13.7%, whereas if the CRL is between the mean and 2 SD, the incidence of miscarriage was only 8.5% (P value 0.025) (Reljic, 2001).

1.2.5.3.1.4 Fetal heart rate

In the first trimester, the fetal heartbeat rises from an average of 100bpm at 5-6 weeks to an average of 140bpm at eight weeks (Laboda *et al.*, 1989) . It then increases to around 170 bpm at ten weeks and then slowly decreases to 130 bpm at term. A fetal heartbeat can be measured using M mode or doppler ultrasound scan. There is evidence to suggest that a low heartbeat (100 bpm) at the 7th week of pregnancy has high sensitivity and positive predictive value of 90.8 % and 83.4% respectively, in predicting miscarriage (Merchiers *et al.*, 1991).

1.2.5.3.1.5 Chorion

The embryonic membranous structures than encloses both the fetus as well as the amnion is called the chorion. The chorionic villi arising from the outer surface of the chorion provides nutrition to the developing embryo. Later, the placenta is formed from the chorion frondosum which is formed by the arborisation of a part of the chorionic villi (Bourne, 1962).

1.2.5.3.1.6 Amnion

A membranous structure that forms inside the chorion which covers and protect the embryo is called the amnion. The amnion usually fuses with the chorion around 14 weeks of gestational age. On ultrasound scan this appears as a thin membrane that separates the amniotic cavity which contains the fetus from the extraembryonic coelom and the secondary yolk sac (Jeanty *et al.*, 1982). The amnion is first seen at 6.5 weeks when the CRL corresponds to seven mm. All embryos of CRL seven mm or higher should have cardiac activity. A study by Ikegawa suggested that an enlarged amniotic sac surrounding an embryo of less than 7mm that failed to show a cardiac activity suggested pregnancy failure. This is called 'The expanded amnion sign' (Ikegawa, 1997). The expanded amnion sign has been reported in the literature as a sign of early embryonic

demise. A study by Yegul in 2009, investing the role of "The expanded amnion sign" had 116 patients who had a visible amnion with no heartbeat had a CRL less than 5mm. Patients who had a visible amnion with no heartbeat had a CRL less than 5mm. Eight of these patients lost to follow up, and the remaining 108 patients subsequently miscarried (Yegul and Filly, 2009).

1.2.5.3.1.7 The Chorio-decidual plate

The Chorio-decidual plate has the developing placenta and it consists of a chorionic plate and basal plate. The chorionic plate contains branches of the umbilical artery, cytotrophoblast and syncytiotrophoblast. The stem villi arise from the plate and forms the inner boundary of the chorio-decidual space. The basal plate consists of a compact layer of decidua basalis, a layer of fibrinoid degeneration of outer syncytiotrophoblast at the junction of cytotrophoblastic cell and decidua, cytotrophoblastic shell and syncytiotrophoblast (Konar, 2015).

A study by Bajo *et al*. highlighted the significance of trophoblast thickness in predicting miscarriage (Bajo *et al.*, 2000). A thinning of the trophoblast was defined as when the numerical difference between the gestational age in weeks and the thickness of the trophoblast in millimetres is more than 3 mm. The study has demonstrated that when the difference is more than or equal to 3 mm the sensitivity for prediction of spontaneous abortion was 82% and specificity was 93% (Bajo *et al.*, 2000)

1.2.5.3.1.8 Corpus luteum

The corpus luteum is a hormone producing structure that develops in the ovary after ovulation and supports early pregnancy. The remnants of the follicle in the ovary that is left over after ovulation becomes corpus luteum and it ranges from 2-5cm in size. It produces oestrogen and progesterone and maintains an optimum condition for implantation of the embryo if the ovum fertilises. The corpus luteum reaches a maximum size at around ten weeks and gradually regresses around 16-20 weeks. If the ovum is not fertilised, the corpus luteum involutes into a structure called the corpus albicans by around two weeks. On ultrasound scan, the corpus luteum is seen as a thick-

walled cyst with a “ring of fire” vascular appearance on doppler scan (Morgan and Jones, 2016). Glock et al. demonstrated an association with a decrease in corpus luteum volume before eight weeks of pregnancy and early pregnancy loss (Glock *et al.*, 1995).

1.2.5.3.2 Studies on ultrasound markers

When women undergo early pregnancy ultrasound, looking for markers that can predict ongoing pregnancy can offer extra reassurance to the women. Hence over the years, clinicians were searching for ultrasound markers that can predict miscarriage. Henceforth, several studies have been already published in this topic investigating the role of a variety of ultrasound markers (Achiron *et al.*, 1991, Alcázar and Ruiz-Perez, 2000, Laboda *et al.*, 1989, Merchiers *et al.*, 1991, Reljic, 2001, Stampone *et al.*, 1996).

1.2.5.3.2.1 Studies on FHR

Six studies on FHR are summarised in table 10. Most of the studies except one (Phupong and Hanprasertpong, 2011) had a good sample size, and all the studies used a cut off value to predict miscarriage. There were some differences in the results reported with Falco *et al*. (Falco *et al.*, 1996) reporting a sensitivity of 30% for prediction of miscarriage when Phupong and Hanprasertpong (Phupong and Hanprasertpong, 2011) reported a sensitivity of 100%.

Table 10 Studies on FHR to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Chittacharoen and Herabutya, 2004	240	sensitivity 54%; specificity 95%	< 120 bpm
Dede *et al*., 2010	202	sensitivity 81%; specificity 85%	Not used
Falco *et al*., 1996	270	sensitivity 30%; specificity 93%	< 1 SD
Maged and Mostafa, 2013	150	sensitivity 96%; specificity 99%	110bpm
Phupong and Hanprasertpong, 2011	30	sensitivity 100%; specificity 100%	<2 SD

1.2.5.3.2.2 Studies on CRL

Three studies on CRL have been summarised in table 11. Except for the study by Maged and Mostafa (Maged and Mostafa, 2013), the other two studies used a cut off value to predict miscarriage. The specificity of all the studies to predict miscarriage were low.

Table 11 Studies on CRL to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Falco *et al*., 1996	270	sensitivity 74%; specificity 52%	<14 mm
Maged and Mostafa, 2013	103	sensitivity 46%; specificity 40%	Not used
Reljic *et al*., 2001	310	sensitivity 75%; specificity 39%	≤18mm

1.2.5.3.2.3 Studies on IUH

There are five studies summarised in table 12 on IUH. There is a significant difference in the sensitivity of the markers between studies. Falco *et al.* (Falco *et al.*, 1996) reported a sensitivity of 17% for IUH, whereas Goldstein (Goldstein, S. R. *et al.*, 1983) reported a sensitivity of 100%. Only one study used a cut off value to predict miscarriage (Pedersen and Mantoni, 1990).

Table 12 Studies on IUH to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Alcazer and Ruiz-perez, 2000	49	sensitivity 92%; specificity 17%	IUH +/-
Borlum *et al*., 1989	380	sensitivity 45%; specificity 80%	IUH +/-
Falco *et al*., 1995	270	sensitivity 17%; specificity 83%	Not used
Goldstein *et al*., 1983	50	sensitivity 100%; specificity 83%	Not used
Pedersen and Mantoni, 1990	342	sensitivity 21%; specificity 82%	2ml

1.2.5.3.2.4 Studies on YS

Three studies done on YS has been summarised in table 13. Two studies looked into the size of the YS (Stampone *et al.*, 1996, Tan, Sinan, Tangal *et al.*, 2014, Tan, S. *et al.*, 2011) and the third study looked into the shape of the YS (Tan, S. *et al.*, 2011). The specificity varied from 78% in Tan *et al.* (Tan, Sinan, Tangal *et al.*, 2014) to 99% in Stampone *et al*. (Stampone *et al.*, 1996) and the sensitivity differed from 16% in Tan *et al.* (Tan, S. *et al.*, 2011)to 69% in Stampone *et al*. (Stampone *et al.*, 1996).

Table 13 Studies on YS to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Stampone *et al*., 1996	117	sensitivity 69%; specificity 99%	+/- 2 SD
Tan *et al*., 2011	183	sensitivity 16%; specificity 83%	Irregular YS
Tan *et al*., 2014	305	sensitivity 33%; specificity 78%	≥ 5mm

1.2.5.3.2.5 Studies on MGSD-CRL

Only one study has reported MGSD-CRL as a marker for predicting miscarriage and is summarised in table 14. The study showed 30% sensitivity and 88% specificity in predicting miscarriage using MGSD-CRL as a USS marker.

Table 14 Studies on MGSD-CRL to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Result**	**Cut off value**
Falco *et al.*, 1996	270	sensitivity 39%; specificity 88%	≤ 0.5 SD

1.2.5.3.2.6 Studies on miscellaneous USS markers

Various other isolated USS markers had been studied, and all those markers not described above are summarised in table 15. Those markers include the difference in

gestational sac volume (GSV) and amniotic sac volume (ASV), the difference between gestational sac size (GSS) and CRL, the deficit in the CRL in relation to the gestational age (CRL deficit), the difference between menstrual age and sonographic age and cervical length and their predictive accuracy described in sensitivity and specificity has been outlined in table 14.

Table 15 Studies on miscellaneous USS markers to predict miscarriage in threatened miscarriage population

Study	**Sample size**	**Marker studied**	**Result**	**Cut off value**
Odeh *et al.,* 2012	90	GSV- ASV	sensitivity 84%; specificity 43%	AUC for ROC< 0.364
Bromley *et al*., 1990	68	GSS-CRL	sensitivity 78.9%; specificity 82.7%	5mm
Reljic *et al.,* 2001	310	CRL deficit	sensitivity 46.6%; specificity 68.3%	>2SD below mean
Falco *et al.,* 1996	270	Menstrual age - sonographic age	sensitivity 34.7%; specificity 85%	1 week
Dede *et al*., 2010	202	Cervical length	sensitivity 81.5%; specificity 51%	<40mm

Similar to the previous studies on biomarkers, studies on ultrasound markers also showed wide variation in its results. This variation can be explained by variation in the selection criteria used by individual studies, inter and intra observer variability of USS measurements, quality of individual studies and accuracy and adequacy of study reporting. Uterine myomas can interfere with the accuracy of ultrasound measurements. The study by Alcazar *et al*. and Tan *et al*. excluded participants with uterine myomas/malformations that can influence the USS measurements (Alcázar and Ruiz-Perez, 2000, Tan, Sinan, Tangal *et al.*, 2014). Intra-observer and inter-observer variability can account for variation in the results. Chittacharoen *et al*. and Alcazar *et al.*

have ensured the same sonographer scanned all the participants (Alcázar and Ruiz-Perez, 2000, Chittacharoen and Herabutya, 2004), and they made corrections for intra-observer variability. Borlum *et al*. used multiple sonographers (Borlum *et al.*, 1989), and many studies have not reported about the sonographers.

Some studies did not have adequate follow up. An example is Falco *et al*. and Dede *et al*., who followed up participants only up to 22 weeks and 20 weeks respectively (Dede *et al.*, 2010, Falco *et al.*, 1996). The mode of follow up was not adequate for Tan *et al*., where the women were followed by telephone interviews or retrospective case notes review (Tan, Sinan, Tangal *et al.*, 2014). Many studies were not clear about their follow up criteria.

There was inadequate reporting noted in many studies on USS markers, especially with the selection criteria used, precautions taken to avoid intra and inter observer variability on USS and the follow-up criteria used. These reasons account for the variation observed in the results of the study on ultrasound markers.

1.2.5.4 Demographic variables

Some of the described risk factors for miscarriage in the literature are high maternal age, previous miscarriage, history of termination and infertility, assisted conception, low pre pregnancy BMI, regular or high alcohol consumption, feeling stressed, high paternal age and changing partner (Maconochie *et al.*, 2007).

A study by Gitau 2009 demonstrated an increased incidence of pregnancy loss at maternal age >35 years (17% compared to 4% for less than 35 years of age) (Mbugua Gitau *et al.*, 2009). Another study by O'Dwyer, 2012 had demonstrated that the miscarriage rate was higher (11.3%) in moderate to severe obesity, but there was no increase in the normal BMI group (O'Dwyer *et al.*, 2012). However, a study published by Turner in 2010 had shown no evidence of increased miscarriage in women with BMI >30 compared to normal BMI (Turner *et al.*, 2010). Apart from these few studies,

demographic variables have not been widely investigated in the context of the outcome of threatened miscarriage.

1.2.5.5 Markers in combination

In the literature, various combinations of markers have been studied with improved results, but still, there is a lack of consensus among the studies or the combination markers have not been tested again to reproduce and validate the results of the primary studies. Table 16 shows the list of studies, which had used combination markers to predict the outcome of threatened miscarriage.

Table 16 Studies on combination markers to predict miscarriage in threatened miscarriage population

Study	Prediction model used	Sensitivity	Specificity	PPV	NPV
Jun *et al.*, 1992	Discriminant analysis using mean GS size, CRL and FHR	94.1%	96.%		
Scarpellini *et al.*,1995	Beta hCG+ Ca 125	78.9%	96.5%		
Varelas *et al.*, 2008	GA+FHR GA+YSD	91% 76.8%	100% 91.7%		
Altay *et al.*, 2009	Logistic regression model using maternal age, MGSD, MGSD-CRL, FHR and Progesterone level			50%	98.9%
Maged *et al.*, 2013	FHR+ progesterone	100%	100%		
Oates *et al.*, 2013	Log model using GA by LMP, presence of PV bleeding, presence of PV clots, GA by USS, menstrual dates, mean GS size, mean YS size and previous caesarean sections	82%	79%		

1.3 Summary

A threatened miscarriage is associated with a 3-16% risk of miscarriage (Hill *et al.*, 1991, Makrydimas *et al.*, 2003, Siddiqi *et al.*, 1988) and the risk profile vary according to the severity of symptoms of the women(Hasan *et al.*, 2009). Numerous studies have been conducted to predict miscarriage in women with threatened miscarriage. Various markers, including biochemical markers, ultrasound markers, demographic variables and several combinations of markers have been studied over the years. Huge variation in results has been noted between studies in the predictive accuracy of various markers. Biomarkers have been extensively investigated in the past to predict miscarriage. They include beta hCG, progesterone, PAPP-A, inhibin A and B, oestradiol, alpha-fetoprotein, Ca 125, SP1 and vascular endothelial growth factors. A newer biomarker that has been studied with a promising result is hs- CRP (Jauniaux, Eric *et al.*, 2015). Variation in results have been noticed between studies for individual biomarkers, and there are many studies making it difficult to summarise the results. Another critical barrier in summarising the results is the quality of the existing studies.

There is significant variation noted in the quality of the studies on biomarkers, particularly with the selection criteria used, follow up of the participants and the laboratory tests used to measure the biomarkers. The biological levels of biomarkers such as Ca 125, hCG, progesterone and PAPP-A, can be influenced by other co-existing conditions like endometriosis, chromosomal anomalies, molar pregnancy, PID or any cause of peritoneal irritation (Scarpellini *et al.*, 1995) and external factors such as using prescription-based progesterone in early pregnancy. The results can also be influenced if local causes for bleeding such as bleeding from the cervix, vagina and vulva have not been excluded before analysis. Other factors that can cause miscarriages such as co-existing medical conditions of maternal diabetes, PCOS, thyroid disorders, thrombophilia, uterine malformations and subfertility can also influence the results.

A considerable drawback of the existing literature of biomarkers is that many of them have not used a cut off value for the biomarkers for the prediction of miscarriage. Biomarker level varies according to the gestational age, and this gestational age

variation of biomarkers have not been taken into consideration while interpreting the results.

A significant number of studies also investigated ultrasound markers to predict miscarriage. The markers studied are GS size and shape, YS size and shape, FHR, CRL, trophoblast thickness, doppler flow of umbilical artery, presence, size and shape of corpus luteum, presence and size of hematoma and amniotic sac volume (ASV). Similar to biomarkers, significant variation has been observed between the studies on ultrasound markers. This variation can be attributed to the difference in the studies selection criteria, studies follow up criteria, inter and intra-observer variability of the sonographer and difference in the scan machines used.

Study selection criteria are important as various factors like the presence of uterine myomas, uterine malformations, and multiple pregnancies can influence the measurement of the ultrasound markers. The ultrasound machine used can also influence the measurements. Some studies used trans-abdominal technique, and others have used trans-vaginal modality of scanning. Though the machine should adjust the measurement based on the route of scanning, variations can happen to the measurements due to the limitations with the view. Sonographers can play a huge role in the accuracy of the measurement. There can be inter-observer variability and intra-observer variability between the sonographers. These limitations can be solved by having a single person performing all the scans in the same machine using the same technique of scanning. However, still there can be intra-observer variability and corrections can be made during analysis for intra-observer variability.

Duration and method of follow up was another major limitation of all the studies on ultrasound markers. Some studies have not followed up patients up to the age of viability, and some studies have not used a robust method of follow up such as following the participants clinically and ultrasonographically. Studies not using a predetermined cut off value also impacted the quality of the existing literature on ultrasound markers.

Combinations of markers were also previously looked into in the existing literature, and some of the combinations looked promising especially those combinations with FHR (Altay *et al.*, 2009, Maged and Mostafa, 2013, Varelas *et al.*, 2008). Demographic variables are useful markers to be used. However, it is less explored so far in the literature. The combination of maternal age and the presence of bleeding has been investigated previously in the studies conducted by Oates *et al.* and Altay *et al.* (Altay *et al.*, 2009, Oates *et al.*, 2013). This is an area that needs to be further explored in future research for the prediction of miscarriage.

A large number of studies have been done on the prediction of miscarriage in women presenting with threatened miscarriage using a variety of markers, with massive variation in the selection criteria, index test and duration and method of follow up used making it extremely difficult to summarise the results. Hence the way forward will be to conduct a systematic review and meta-analysis to systematically summarise the results of existing literature and to come up with coherent conclusions, taking into consideration of the quality of the individual studies. This will help to design prognostic marker research as a first step in developing a miscarriage prediction model.

2 Systematic Reviews

Abstract: This chapter narrates three systematic reviews and meta-analysis to summarise the existing literature by addressing the quality of the eligible papers. The systematic review and meta-analysis one summarises the existing literature on biochemical markers in predicting miscarriage in the threatened miscarriage population. Systematic review and meta-analysis two summarise the existing literature of the ultrasound markers in predicting miscarriage in the threatened miscarriage population. Finally, the systematic review and meta-analysis three investigates the perinatal morbidity associates with threatened miscarriage.

The systematic reviews demonstrated that the biomarker CA 125 and the ultrasound marker FHR have the highest sensitivity and specificity in predicting miscarriage. They also demonstrated that women experiencing threatened miscarriage have a higher relative risk of suffering from i perinatal morbidity later in pregnancy or delivery.

The systematic reviews have highlighted the inadequacies in the existing literature, including the quality and reporting of individual studies, as well as helped to highlight areas that can be improved in future research on the topic.

2.1 Introduction

The literature search which was discussed in the introduction chapter identified numerous studies done on biochemical, ultrasound and on various combinations of markers in predicting the outcome of women with threatened miscarriage. However, there were significant differences in the results of the existing literature, quality of the previously published literature and reporting format of the previously published studies. Hence the best way forward was to proceed with a systematic review of the existing literature to summarize the available evidence and to find out the methodological soundness of the existing studies. If the pregnancy continues beyond 23 completed weeks, evidence has shown that threatened miscarriage can be associated with perinatal complications (Saraswat *et al.*, 2010). Therefore, another systematic review was done to investigate the evidence on perinatal morbidity in women who have experienced a threatened miscarriage in early pregnancy.

Various markers have been investigated in the past to predict miscarriage in women who experienced threatened miscarriage in early pregnancy. The commonly studied biomarkers include hCG, progesterone, alpha-fetoprotein, Ca 125, oestradiol, inhibin, activin, hs-CRP, PAPP-A and SP 1(Hertz and Schultz-Larsen, 1983, Jauniaux, Eric *et al.*, 2015, Johns, Jemma *et al.*, 2007, Jouppila, Penttl *et al.*, 1980, Phupong and Hanprasertpong, 2011, Westergaard *et al.*, 1985, Xie *et al.*, 2014). The commonly studied ultrasound markers include gestational sac size, yolk sac size and shape, fetal heart rate and crown-rump length (Falco *et al.*, 1996, Maged and Mostafa, 2013, Stampone *et al.*, 1996, Tan, Sinan, Tangal *et al.*, 2014). This systematic review and diagnostic accuracy meta-analysis aimed to investigate the biomarker and ultrasound markers with the highest diagnostic accuracy in predicting miscarriage or ongoing pregnancy in women with threatened miscarriage. It also aimed to investigate the existing evidence on perinatal morbidity experienced by women with threatened miscarriage.

2.2 Objectives

Systematic review 1: To assess the existing evidence on the accuracy of biochemical markers in predicting miscarriage in women with threatened miscarriage.

Systematic review 2: To assess the existing evidence on the accuracy of ultrasound markers in predicting miscarriage in women with threatened miscarriage.

Systematic review 3: To evaluate the existing literature on the perinatal outcomes experienced by women with a history of threatened miscarriage in early pregnancy.

2.3 Methodology

The protocol for systematic review one and three were not registered in a database. The protocol for systematic review two was registered in PROSPERO International Prospective Register of Systematic Reviews (CRD42016046470).

2.3.1 Eligibility criteria

Only prospective studies including women with threatened miscarriage were included in the systematic reviews. Threatened miscarriage was defined as patients presenting with bleeding PV at less than 24 weeks gestation with or without lower abdominal pain, closed internal os on cervical examination and subsequent ultrasound scan confirming a viable intrauterine pregnancy (National Collaborating Centre for Women's and Children's Health (UK), 2012, Saraswat *et al.*, 2010). We excluded all retrospective studies, case reports, case series, letters, reviews and studies not in English where no translated version was available.

2.3.1.1 Eligibility criteria and outcomes studied for systematic review 1

Studies that used biochemical markers to determine outcomes for women with threatened miscarriage and gestational age between 6 -23 weeks were included in the review. We excluded studies involving women with infertility, recurrent miscarriage or pregnancy of unknown location (PUL) or where women had ovulation induction medications, exogenous hormones or any form of treatment for prevention of miscarriage. The primary outcome studied in the review was the occurrence of miscarriage.

2.3.1.2 Eligibility criteria and outcomes studied for systematic review 2

Those studies which used ultrasound scan markers to predict miscarriage in women from six weeks of gestational age till 23 complete weeks of gestational age were included in the systematic review. We excluded studies involving women who had multiple pregnancies and intrauterine pregnancy of uncertain viability, women who were offered treatment for miscarriage and studies that investigated Doppler ultrasound scan for prediction of miscarriage. The primary outcome of interest for the review was the occurrence of miscarriage.

2.3.1.3 Eligibility criteria and outcomes studied for systematic review 3

Those studies that investigated maternal and perinatal outcomes in women who experienced a threatened miscarriage at less than 24 weeks of gestational age were included in the systematic review and meta-analysis. Exclusion criteria were studies with asymptomatic intrauterine haematomas, those with multiple pregnancies, who had fertility treatment or had medical problems that can have an impact on the course of pregnancies and studies that used any form of treatment for threatened miscarriage.

The primary outcomes of interest were stillbirth/intrauterine fetal death, intrauterine growth restriction, low birth weight, preterm delivery, preterm pre-labour rupture of membranes, placental abruption, pre-eclampsia, eclampsia, and HELLP syndrome. Secondary outcomes of placenta praevia, pregnancy-induced hypertension, retained placenta, postpartum haemorrhage, neonatal asphyxia, and congenital malformations were also investigated.

2.3.2 Information sources and search strategy

Electronic databases searched included Medline (1946 to June 2015), Embase (1980 to June 2015), Cochrane library, ClinicalTrials.gov, World Health Organization international clinical trials registry, LILAC database and OpenGrey (System for Information on grey literature from Europe). Two authors did an independent literature search, and the

reference lists of all recent reviews and original articles were reviewed to identify any articles not captured by the search by two independent authors (RP and NP). Disagreements between the authors in selecting the papers and data extraction were resolved by consensus.

2.3.2.1 Search strategy for systematic review 1

The MeSH terms used for the literature search were (1) miscarriage (abortion, pregnancy loss, early pregnancy outcome) (2) biochemical markers (biomarkers, biological markers, hormonal markers, progesterone, β hCG, hCG, human chorionic gonadotrophin, progesterone, follistatin, Ca 125, PAPP-A, activin, activin- A, inhibin, inhibin-A, oestradiol, estriol, hydroxyprogesterone, human placental lactogen, HPL, alpha-fetoprotein (AFP), schwangersshaft protein (SP1), pregnancy specific beta 1 glycoprotein, pregnancy zone protein (PZP)). The two subsets were combined using the Boolean term 'AND' to obtain the citations relevant to our research question.

2.3.2.2 Search strategy for systematic review 2

The MeSH terms used for the search were (1) miscarriage (abortion, early pregnancy loss, early pregnancy outcome) (2) USS markers (gestational sac, amniotic sac, yolk sac, crown-rump length, fetal heart, fetal heart rate, embryonic heart rate, chorio-decidual plate thickness, corpus luteum, endometrial thickness, trophoblastic thickness, uteroplacental thickness, subchorionic hematoma, fetal growth delay, fetal motion, chorionic bump). The two subsets were combined using the Boolean term 'AND' to obtain a set of citations relevant to our research question.

2.3.2.3 Search strategy for systematic review 3

The MeSH terms used for search were (1) Threatened miscarriage ("early pregnancy bleeding", "Threatened abortion", "bleeding and pain in early pregnancy", "Threatened pregnancy loss", "Threatened f*etal loss", "Threatened f*etal death", "First trimester bleeding") (2) pregnancy complications ("stillbirth", "intra uterine death", perinatal complication*", "maternal complication*", "perinatal morbidity", "maternal morbidity", "preterm labour", "preterm delivery" , "PTD", "preterm birth" "preterm prelabour

rupture of membrane", PPROM, "pregnancy induced hypertension", PIH, "pre-eclampsia", PET, eclampsia, "HELLP syndrome", "antepartum haemorrhage", APH, "placental abruption", "placenta praevia", "retained placenta", "postpartum haemorrhage", "caesarean section", "instrumental delivery", "forceps delivery", "ventouse delivery", "vacuum delivery", "small for gestational age", SGA, "intra uterine growth restriction", IUGR, "low birth weight", "birth weight", "neonatal asphyxia", "low Apgar score", "congenital anomal*", "congenital malformation*"). The two subsets were combined using the Boolean term 'AND' to obtain a set of citations relevant to our research question.

2.3.3 Data extraction

RP and NP independently extracted the data, and pre-determined forms were used. The outcome data were extracted using 2×2 tables or mean and standard deviation. The outcome data for systematic review three were extracted as the number of cases and controls that experienced each complication and the total number of cases and controls.

2.3.4 Quality assessment

The quality of the individual studies was assessed by RP using QUADAS-2 (Quality Assessment for Diagnostic Accuracy Studies-2: A Revised Tool) (Whiting *et al.*, 2011) for systematic reviews 1 and 2. There are four key domains in the tool covering patient selection, index test(s), reference standard and the flow and timing for evaluating the studies. The first three domains were assessed for concerns regarding applicability and risk of bias, and the other domains were assessed only for the risk of bias. Signalling questions were included in the tool to help judge the risk of bias. The index test(s) for the included studies were the biomarkers, and the reference standard was the occurrence of miscarriage. The miscarriage was confirmed clinically or by ultrasound scan or by histopathological examination. The study quality assessment for the third systematic review was performed using the Newcastle Ottawa scale (Wells *et al.*, 2000) as it was a systematic review of prospective cohort studies.

2.3.4.1 Statistical analysis

The statistical analysis was performed using the Cochrane systematic review software (The Cochrane Collaboration, 2014). The meta-analysis of the eligible studies for the systematic reviews one and two were completed using the diagnostic test accuracy review stream of the software. The 2 x 2 tables were used to summarize the test results and forest plots were constructed showing within-study estimates and confidence interval for sensitivity and specificity of each biomarker and ultrasound marker. The meta-analysis of diagnostic accuracy studies is very different from the meta-analysis of therapeutic/interventional studies. The meta-analysis of diagnostic accuracy studies has to account for the correlation between the sensitivity and specificity of a study. Also, the meta-analysis has to incorporate the cut off value used to define positive and negative results and this needs to be incorporated into the data synthesis. Hence the meta-analysis of diagnostic accuracy studies requires sophisticated statistical models such as the bivariate model or hierarchical model (Kim *et al.*, 2015). Therefore, the meta-analysis data summary were presented using coupled forest plots and the hierarchical summary receiver operator characteristic model (HSROC) were used for further statistical modelling (Harbord *et al.*, 2007, Rutter and Gatsonis, 2001) for biomarkers and ultrasound markers with four or more studies. The graph demonstrated a summary receiver operating characteristic (SROC) curve and the prediction region, the summary point and the confidence region. The between-study heterogeneity was accounted for in the HSROC model. Posterior predictions (empirical Bayes estimates) of the sensitivity and specificity in each study were obtained and plotted since the empirical Bayes estimates give the best estimate of the true sensitivity and specificity in each study. The sensitivity, specificity, the positive and negative likelihood ratio for each biomarker were also tabulated.

For systematic review 3, the data were collected as raw numbers and the crude risk ratio (RR) for individual studies was calculated with the 95% confidence interval (CI) before pooling the data. A combined risk ratio (RR) and confidence interval (CI) for each outcome were calculated after pooling the data. The risk ratio was used to assess the association instead of the odds ratio as this is a better measure in cohort studies, and it

is easier to interpret the risk ratio compared to the odds ratio (Bhopal, 2016). Forest plots were created for individual outcomes and test of heterogeneity performed. Heterogeneity between studies was calculated using I^2. The I^2 statistic describes the percentage of variation across studies that is due to heterogeneity rather than chance. I^2 is an intuitive and simple expression of the inconsistency of studies' results. It does not depend on the number of studies considered. An I^2 value of less than 50% was considered low heterogeneity and therefore, a fixed effect model was used, whereas an I^2 value of more than 50% was considered moderate heterogeneity and hence a random effect model was used. If there is little variation between trials, then I^2 will be low and a fixed effects model might be appropriate. With fixed effects, all of the studies that you are trying to examine as a whole are considered to have been conducted under similar conditions with similar subjects, in other words, the only difference between studies is their power to detect the outcome of interest. An alternative approach, 'random effects', allows the study outcomes to vary in a normal distribution between studies (Higgins, Julian PT and Thompson, 2002, Higgins, J. P. *et al.*, 2003).

2.4 Results

2.4.1 Results of systematic review 1: Use of biomarkers for predicting miscarriage in women with threatened miscarriage

2.4.1.1 Study selection

There was a total of 6,727 articles identified in the electronic database searches, and after reviewing reference lists of individual manuscripts, a further 93 articles were found. From these articles, after reviewing the titles and removing the duplicates, 154 manuscripts were identified. From these 154 manuscripts, after reading the abstract, another 119 articles were excluded. Full manuscripts of the remaining 35 articles were obtained and reviewed in detail and of these 16 studies were excluded (patient population was different in 11 studies (Garoff and Seppälä, 1975, Hertz and Schultz-Larsen, 1983, Kunz and Keller, 1976, Masson *et al.*, 1983, Osmanağaoğlu *et al.*, 2010,

Salem *et al.*, 1984, Schmitt *et al.*, 2012, Sugita *et al.*, 1983, Taylor *et al.*, 2011, Tong *et al.*, 2012, Tu'uhevaha *et al.*, 2012), four studies were excluded for retrospective study design (Duff *et al.*, 1980, Jandial *et al.*, 1978, Muttukrishna, S. *et al.*, 2002)and one excluded due to data duplication (Westergaard *et al.*, 1983)). Nineteen studies were used for qualitative data synthesis. A further four studies (Azogui *et al.*, 1996, Jauniaux, Eric *et al.*, 2015, Johns, Jemma *et al.*, 2007, Vavilis *et al.*, 2001) were excluded in the quantitative meta-analysis since the data could not be obtained in the form of 2 x 2 tables. Overall, 1253 women were included in the quantitative meta-analysis from 15 studies (Figure 1) (Dessaive *et al.*, 1982, Fiegler *et al.*, 2003, Hanita *et al.*, 2012, Jouppila, Penttl *et al.*, 1980, Leylek *et al.*, 1997, Maged and Mostafa, 2013, Öçer *et al.*, 1992, Phupong and Hanprasertpong, 2011, Ruge *et al.*, 1990, S. Sherif, AG El-Metwaly, H. Shalan, AM Badawy E., Abu-Hashem, L, 2000, Scarpellini *et al.*, 1995, Siimes *et al.*, 1983, Stoppelli *et al.*, 1981b, Westergaard *et al.*, 1985, Xie *et al.*, 2014). Among the included studies, only one study had studied a combination of biomarkers (Scarpellini *et al.*, 1995) and all other studies, which used combination markers, could not be included in the review because they did not meet the predefined inclusion criteria (Hertz and Schultz-Larsen, 1983, Kunz and Keller, 1976, Osmanağaoğlu *et al.*, 2010)

PRISMA 2009 Flow Diagram

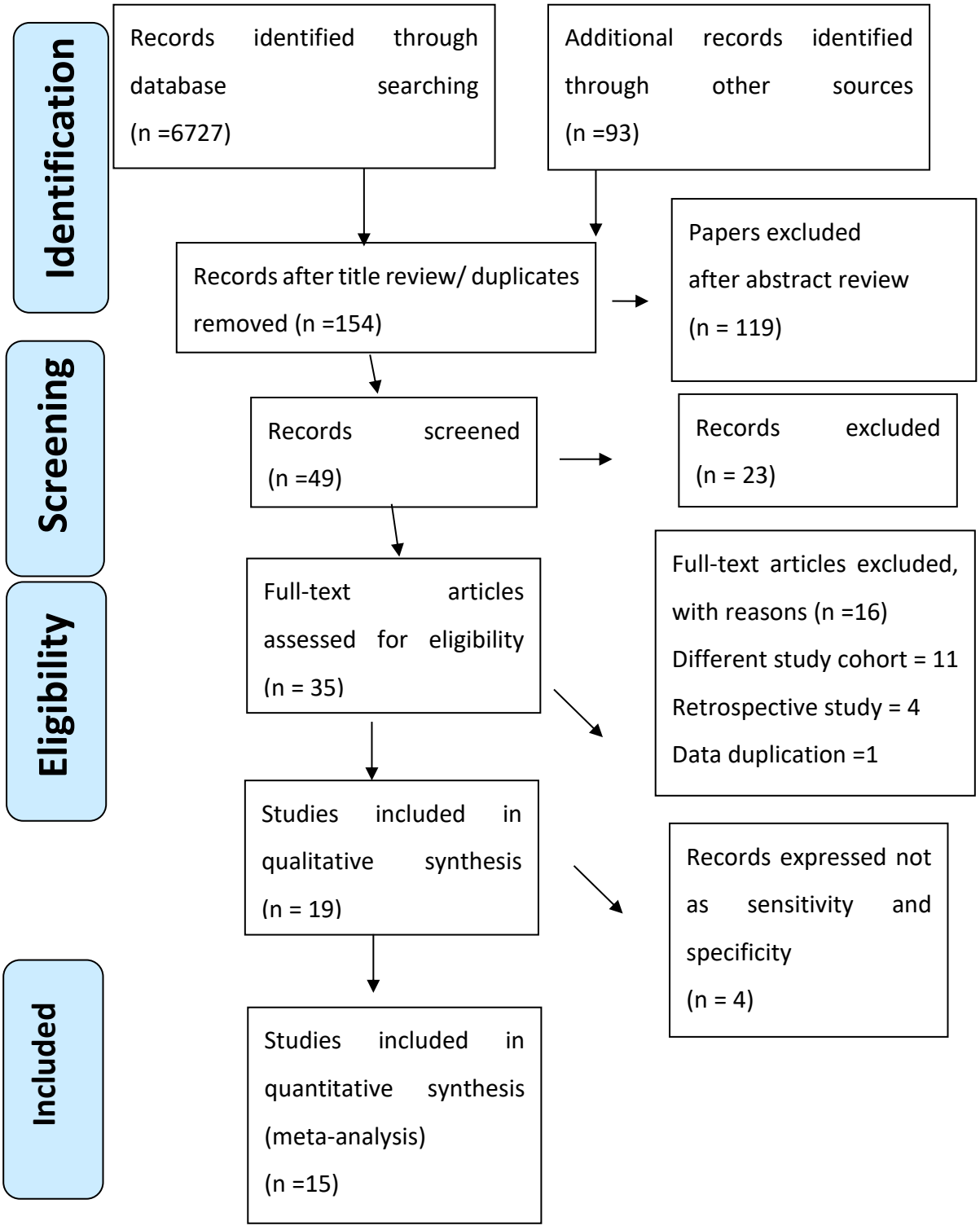


Figure 1 PRISMA flow chart showing study selection process for systematic review and meta-analysis investigating the role of biomarkers in predicting miscarriage in the threatened miscarriage population (Moher *et al.*, 2009)

2.4.1.2 Study characteristic

All included studies except four (Hanita *et al.*, 2012, Jouppila, Penttl *et al.*, 1980, Ruge *et al.*, 1990, Westergaard *et al.*, 1985) studied women of gestational age less than 14 weeks. The characteristics of the included studies are summarized in Table 17 and the excluded studies in Table 18.

Table 17 Characteristics of the included studies in the systematic review and meta-analysis investigating the role of biomarkers in predicting miscarriage in the threatened miscarriage population

Authors and publication year	**Country**	**Patient characteristics**	**Index tests (biomarkers)**	**Index test cut off**	**Miscarriage diagnosis**	**Follow up duration**
Jouppila *et al.*, 1980	Finland	N=103, 6-20 weeks, excluded cervical causes of bleeding	β hCG, progesterone and oestradiol	Not pre specified	USS	Not specified
Stoppelli *et al.*, 1981	Italy	N=62, 5-13 weeks, excluded uterine malformations and systemic diseases	hCG, progesterone, oestradiol	Not pre specified	Not specified	Not specified
Dessaive *et al.*, 1982	Belgium	N=49, 4-12 weeks, excluded missed and incomplete miscarriage, ectopic and molar	β hCG, progesterone, oestradiol and estriol	Not pre specified	USS/histology/ clinical history	Not specified
Siimes et al., 1983	Finland	N=74, <14 weeks, no exclusion criteria mentioned	hCG, oestradiol, plasma renin substrate, sex-hormone binding globulin, plasma renin activity	Not pre specified	Hospital record /histology	Not specified

Authors and publication year	Country	Patient characteristics	Index tests (biomarkers)	Index test cut off	Miscarriage diagnosis	Follow up duration
Westergaard *et al.,* 1985	Denmark	N=77, 7-20 weeks, excluded blighted ovum, missed abortion, molar and ectopic pregnancy	hCG, progesterone, oestradiol, PAPP-A, AFP, HPL, schwangerschafts protein 1(SP1), pregnancy zone protein (PZP)	Not pre specified	USS	Not specified
Ruge et al., 1990	Denmark	N=128, 6-19 weeks, no exclusion criteria mentioned	PAPP-A	Not pre specified	USS	End of pregnancy
Ocer et al., 1992	Turkey	N= 25, 7-12 weeks, excluded vaginitis, cervicitis, history of recurrent miscarriage and smoking	Ca 125	Not pre specified	Not specified	20 weeks
Scarpellini *et al.,* 1995	Italy	N=48, 6-11 weeks, excluded blighted ovum, ectopic, multiple pregnancy, assisted conception and those who could not be contacted or followed up	β hCG, Ca 125, Ca 125 + β hCG	Ca 125 > 120 IU/ml	USS	24 weeks

Authors and publication year	Country	Patient characteristics	Index tests (biomarkers)	Index test cut off	Miscarriage diagnosis	Follow up duration
Leylek et al., 1997	Turkey	N= 40, 6-12 weeks, no exclusion criteria mentioned	β hCG, progesterone, oestradiol, estriol, Ca 125	β hCG 25 IU/ml, progesterone 21ng/ml, Ca 125 120 IU/ml	Not specified	Not specified
Sherif et al., 2000	Egypt	N=100, 6-13weeks. No exclusion criteria mentioned.	Ca 125	> 21 U/ml	USS	Not specified
Fiegler *et al.*, 2003	Poland	N=200, 5-12 weeks, excluded multiple pregnancy, cervical insufficiency, cervical surgery, assisted conception, history of endometriosis, ovarian abnormality and inability to detect or examine one or both ovaries by USS	β hCG, Ca 125	Not pre specified	Hospital record	Until 4 weeks of discharge from hospital

Authors and publication year	Country	Patient characteristics	Index tests (biomarkers)	Index test cut off	Miscarriage diagnosis	Follow up duration
Phupong and Hanprasertpong, 2011	Thailand	N= 30, 6-14^{+6} weeks, excluded multiple pregnancy, diabetes, hypertension, fetal and chromosomal anomaly	Inhibin A	Not pre specified	Not specified	Not specified
Hanita *et al.*, 2012	Malaysia	N=42, 6-22 weeks, excluded missed abortion, local cause of vaginal bleeding, confirmed congenital anomalies, twin pregnancies and pregnant women who smoked	PAPP-A	Not pre specified	USS	Up to 22 weeks
Maged *et al.*, 2013	Egypt	N=150, 5-12 weeks, excluded those with multiple pregnancy, missed/ inevitable / incomplete miscarriage, ectopic and molar pregnancy	β hCG progesterone, Ca 125	Not pre specified	Not specified	Not specified

Authors and publication year	Country	Patient characteristics	Index tests (biomarkers)	Index test cut off	Miscarriage diagnosis	Follow up duration
Xie *et al.*, 2013	China	N= 135, first trimester, excluded multiple pregnancy, pregnancy by artificial insemination, abnormal uterine development, smoking, diabetes, hypertension	Ca 125	Not pre specified	USS/ telephone interview	Up to 28 weeks

Table 18 Characteristics of the excluded studies in the systematic review and meta-analysis investigating the role of biomarkers in predicting miscarriage in the threatened miscarriage population

Author and publication year	**Study Design**	**Patient characteristics**	**Index test studied**	**Exclusion criteria**
Garoff and Seppala, 1975	Prospective cohort	N= 112, first and second trimester, included women with PV bleed, no USS	HPL, AFP	Different study population
Kunz and Keller, 1976	Prospective cohort	N=65, 6-20 weeks, excluded extra uterine and molar pregnancies and missed abortion	hCG, progesterone, oestradiol, AFP and HPL	Different study population (patients treated with progesterone, benzodiazepenes and bed rest).
Jandial *et al.,* 1978	Retrospective	N=64, 6-18 weeks	Pregnancy specific beta 1 glycoprotein, HPL	Retrospective study design
Duff *et al*., 1980	Retrospective	N=66, <20 weeks, women with threatened miscarriage	hCG, progesterone, oestradiol, HPL, AFP, beta 1 glycoprotein and cystyl amino peptidase	Retrospective study design

Author and publication year	Study Design	Patient characteristics	Index test studied	Exclusion criteria
Hertz *et al*., 1983	Prospective cohort	N= 109, 6-19 weeks, included pregnant women with PV bleed and on examination uterus enlarged and cervix closed, no USS	HPL, SP1 and AFP	Different study population (no USS done to check fetal viability)
Masson *et al*., 1983	Design not clear	N=54, 7-14 weeks, included symptomatic patients after clinical examination, no USS	hCG, PAPP-A, HPL, SP1	Different study population (no USS scan at recruitment to confirm viability) and study design not clear
Sugita *et al*., 1983	Prospective cohort	N=214, 4-20 weeks, included mixed population of normal, threatened and missed miscarriage	hCG, HPL and progesterone	Different study population and difficult to interpret results (results not clearly presented; levels not clearly specified)

Author and publication year	Study Design	Patient characteristics	Index test studied	Exclusion criteria
Westergaard *et al.,* 1983	Prospective cohort	N=51, 6-16 weeks, excluded pregnancies with missed miscarriage, molar, anembryonic and ectopic.	PAPP-A	Duplication of data (Same data used in Westergaard *et al.,* 1985)
Salem *et al*., 1984	Prospective cohort	N=67, 6-18 weeks, included women with PV bleed in ≤ 48hrs, no USS	hCG, progesterone, SP 1 and placental protein 5	Different study population (no USS done to check fetal viability)
Azogui *et al.,* 1996	Prospective cohort	N=25, 7-12 weeks, excluded those with history of infertility/ endometriosis	β hCG, oestradiol, Ca 125	Data could not be obtained in 2x2 table
Lamarca *et al.,* 1998	Retrospective	N=45, 6-10 weeks, excluded women with missed miscarriage, anembryonic pregnancy, history of miscarriage, thyroid disorder, and infertility	hCG, TSH, Free T3, FreeT4, Immunogloblin G, Immunoglobulin M, neutrophil and lymphocyte	Retrospective study design

Author and publication year	Study Design	Patient characteristics	Index test studied	Exclusion criteria
Schmidt *et al*., 2001	Prospective cohort	N=236, 6-12 weeks, excluded women with acute/ chronic infection, impaired hepatic/ renal or other organ dysfunction, trophoblastic disease, or neoplasia. Study wing 2: threatened miscarriage patients treated with oral magnesium and IM injection of oestradiol caproate and progesterone Study wing 1: mixed population of patients with missed miscarriage, incomplete miscarriage, threatened miscarriage and ectopic pregnancy.	Ca 125 and beta hCG	Different study population
Vavilis *et al*., 2001	Prospective cohort	N=39, 7-11 weeks, no exclusion criteria mentioned.	Ca 125	Data could not be obtained in 2x2 table

Author and publication year	Study design	Patient characteristics	Index test studied	Exclusion criteria
Johns *et al*., 2007	Prospective cohort	N=122, < 14 weeks, excluded multiple gestations, congenital anomalies and presence of large fibroid distorting the cavity	β hCG, progesterone, oestradiol, PAPP- A, inhibin A, activin A, follistatin	Data could not be obtained in 2x2 table
Osmanagaoglu *et al.*, 2010	Prospective cohort	N= 140, 5-13 weeks, excluded multiple pregnancies, ectopic, missed miscarriage, blighted ovum, threatened miscarriage, pregnant women with prior treatment with progesterone or smokers or with diabetes mellitus, renal, trophoblastic or thrombophilic disease	β hCG, progesterone and Ca 125	Different study population (threatened miscarriage population was excluded from the study)
Muttukrishna *et al.*, 2011	Retrospective	N=40, first trimester	Soluble vascular endothelial growth factor receptor 1, soluble endoglin, placental growth factor	Retrospective study design

Author and publication year	Study design	Patient characteristics	Index test studied	Exclusion criteria
Taylor *et al*., 2011	Prospective cohort	N= 45, 6-12 weeks, included asymptomatic women, no USS	β hCG, progesterone, PAPP-A and AEA (endocannabinoid anathamide)	Different study population (asymptomatic women with no USS were recruited)
Tong *et al.,* 2012	Prospective cohort	N= 782, 6- 10 weeks, included asymptomatic women, USS FH+	β hCG, PAPP-A, anandamide and macrophage inhibitory cytokine 1	Different study population (asymptomatic women were included in the study)
Tu'uhevaha *et al*., 2012	Retrospective	N= 181, 6-12 weeks, included asymptomatic women	Soluble FMS like tyrosine kinase -1, placental growth factor and soluble endoglin	Different study population (asymptomatic women were included) and retrospective study design.

Author and publication year	**Study design**	**Patient characteristics**	**Index test studied**	**Exclusion criteria**
Jauniaux *et al*., 2015	Prospective cohort	N= 71, 6-8 weeks, excluded multiple pregnancies, extra uterine pregnancies, hydatidiform mole, recurrent miscarriage, infertility treatment or endocrinological disorders	hCG, progesterone, PAPP-A, hs-CRP	Data could not be obtained in 2x2 table

2.4.1.3 Risk of bias assessment

The risk of bias was assessed in four main domains of patient selection, index test, reference standard and flow and timing using the ‘QUADAS-2: A Revised Tool' (Figure 2). Four studies reviewed scored ‘high risk' for patient selection as they did not specify their exclusion criteria. For the index test, 12 studies have not either used a cut off value or have not pre specified it prior to the start of the project. The outcome we have assessed for the review is the occurrence of miscarriage. The outcome was diagnosed using USS or clinical history, followed by histopathological examination of the products of conception. However, three used telephone interviews or retrospective review of case notes to determine the outcome, which might have contributed to bias and five studies have not specified the method of follow up. Although it was difficult to predict a specific time interval from the index test to the occurrence of miscarriage, in the flow and timing section of the QUADAS-2 tool, we used the sampling question to see whether the patients were followed up until at least 23 weeks. WHO(World Health Organization, 2000) has defined miscarriage as the premature loss of a fetus up to 23 completed weeks of pregnancy and weighing up to 500 grams. Nine studies have not specified their follow up duration and three studies failed to follow up the participants till 24 completed weeks of pregnancy. In summary, quality concerns exist for the diagnostic accuracy studies included for the prediction of miscarriage. Figure 2 summarises the result of the quality assessment on the included studies.

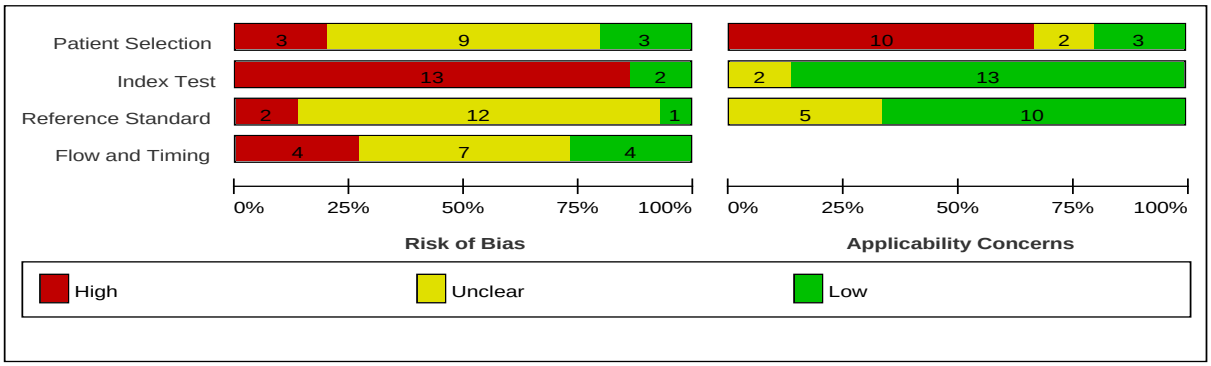


Figure 2 Figure showing the risk of bias assessment using the QUADAS tool in the systematic review investigating the role of biomarkers in predicting miscarriage in the threatened miscarriage population

2.4.1.4 Quantitative data summary and synthesis of results

The data for the biomarker's serum hCG, progesterone, oestradiol, PAPP-A and Ca 125 were summarised using 2x2 tables, and forest plots were constructed for the sensitivity and specificity of the biomarker with their confidence intervals. Only one study was available for the biomarkers HPL, AFP, Schwangerschafts Protein 1 (SP1) and Pregnancy Zone Protein (PZP) (Westergaard *et al.*, 1985)); Plasma Renin Activity (PRA), Plasma Renin Substrate (PRS) and Sex-Hormone Binding Globulin (SHBG)(Siimes *et al.*, 1983), inhibin A, activin A, follistatin (Johns, Jemma *et al.*, 2007, Phupong and Hanprasertpong, 2011) and estriol (Dessaive *et al.*, 1982) and therefore it was not possible to do a meta-analysis.

2.4.1.4.1 Serum human chorionic gonadotrophin

There were eight studies with a total of 584 women that investigated either intact hCG (International Federation of Clinical Chemistry denotes intact hCG as 'HCG') (Stenman *et al.*, 2006)or beta hCG to predict the outcome in women with threatened miscarriage. Of these, three studies used intact hCG (Siimes *et al.*, 1983, Stoppelli *et al.*, 1981b, Westergaard *et al.*, 1985) and five used β hCG (Dessaive *et al.*, 1982, Jouppila, Penttl *et al.*, 1980, Leylek *et al.*, 1997, Maged and Mostafa, 2013, Scarpellini *et al.*, 1995). The forest plots were plotted separately for studies that used β hCG and intact hCG (Figure 3 and 4). Figure 3 summarises the studies done on β hCG alone. The figure demonstrates that there is variation in the sensitivities and specificities across the studies with older studies having lower sensitivities and higher specificities compared to the newer studies. Also, the variation in the sensitivities and specificities across the studies can be attributed to the difference in the cut-off values used between studies. Hence it was not possible to summarise the sensitivities and specificities across the studies. Figure 4 summarises the studies done on intact hCG. Similar to studies on β hCG, these studies show variation among the results, mainly for sensitivity. This is also attributable to the difference in cut-off values.

Further analysis using HSROC (β hCG and intact hCG studies combined) showed a summary sensitivity of 44% (95% CI 17-75%), a specificity of 86% (95% CI 80-91%), a

positive likelihood ratio of 3.37(95% CI 1.98-5.74%) and a negative likelihood ratio of 0.63(95% CI 0.36-1.11) (Figure 5). Since the positive likelihood ratio was neither greater than five nor the negative likelihood ratio is less than 0.2, the results don't even show moderate diagnostic evidence (McGee, 2002).

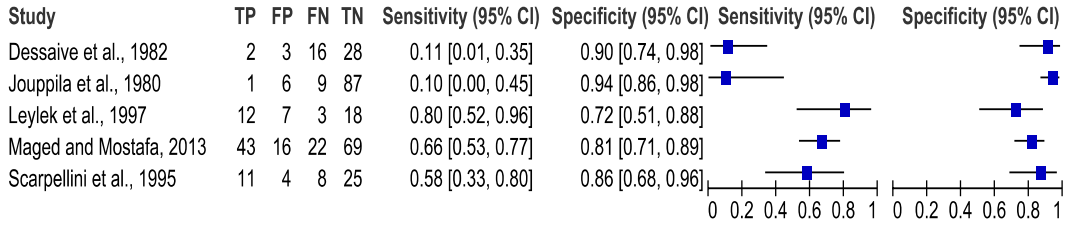


Figure 3 Forest plot of studies investigating the role of serum β hCG in predicting miscarriage in women with threatened miscarriage (N = 415). FN=false negative; FP=false positive; TN=true negative; TP=true positive

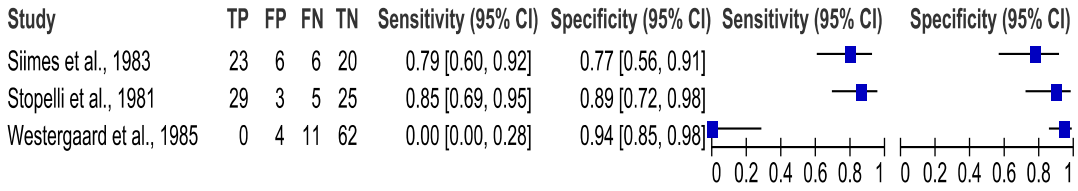


Figure 4 Forest plot of studies investigating the role of serum intact hCG in predicting miscarriage in women with threatened miscarriage (N = 194). FN=false negative; FP=false positive; TN=true negative; TP=true positive

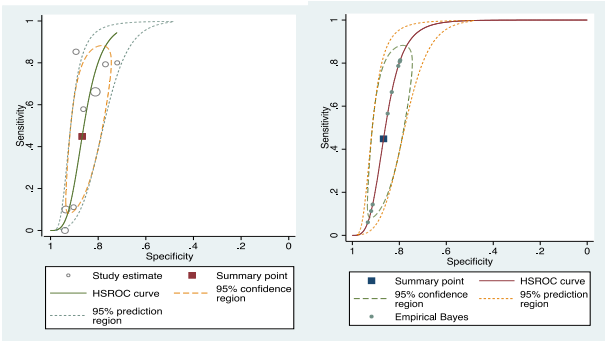


Figure 5 Summary receiver operating curve investigating the role of hCG (intact and β hCG) in predicting miscarriage in women with threatened miscarriage

2.4.1.4.2 Serum Progesterone

Serum progesterone was used to predict the outcome of threatened miscarriage in six studies (n=481 women) (Dessaive *et al.*, 1982, Jouppila, Penttl *et al.*, 1980, Leylek *et al.*, 1997, Maged and Mostafa, 2013, Stoppelli *et al.*, 1981b, Westergaard *et al.*, 1985). Figure 6 demonstrates the couple forest plot for serum progesterone. Variation in the results were noted, especially with the sensitivity results. Earlier studies showed lower sensitivity compared to relatively newer studies. Due to the variation in the results, an HSROC curve was used to summarise the results. HSROC analysis showed a sensitivity of 30% (95% CI 2-87%), a specificity of 86% (95% CI 78-91%), a positive likelihood ratio of 2.24 (95% CI 0.32-15.8%) and a negative likelihood ratio of 0.81 (95% CI 0.35-1.86) (Figure 7). The likelihood ratios demonstrate that serum progesterone is not a strong diagnostic marker to predict miscarriage.

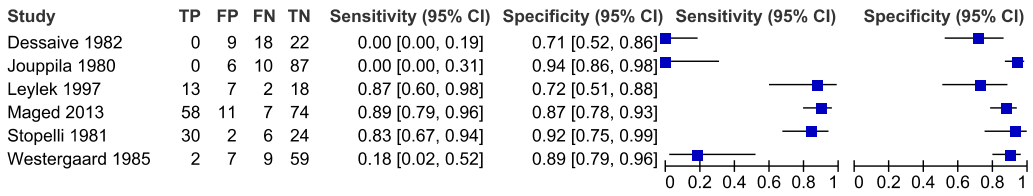


Figure 6 Forest plot of studies investigating the role of serum progesterone (N = 481) in predicting miscarriage in women with threatened miscarriage (N=481). FN=false negative; FP=false positive; TN=true negative; TP=true positive

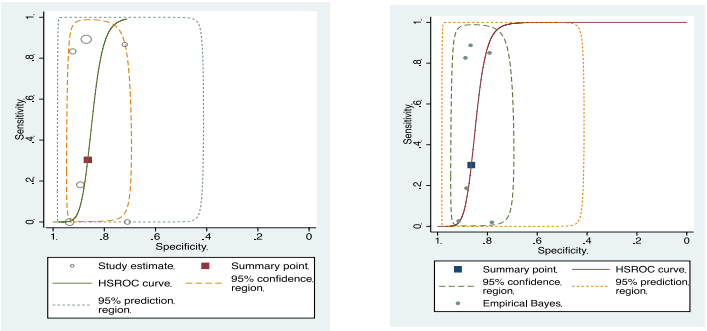


Figure 7 Summary receiver operating curve investigating the role of serum progesterone in predicting miscarriage in women with threatened miscarriage

2.4.1.4.3 Serum Oestradiol

Serum oestradiol was studied by four studies, with 244 women (Dessaive *et al.*, 1982, Siimes *et al.*, 1983, Stoppelli *et al.*, 1981b, Westergaard *et al.*, 1985). Figure 8 demonstrates the coupled forest plot for serum oestradiol. Variation in the result was noted, especially with the sensitivity results. Due to the variation in the results and due to the variation in the threshold values used, an HSROC curve was used to summarise the results. The HSROC analysis showed a sensitivity of 45% (95% CI 6-90%), a specificity of 87% (95% CI 81-92%), a positive likelihood ratio of 3.72 (95% CI 1.01-13.71) and a negative likelihood ratio of 0.62 (95% CI 0.20-1.84) (Figure 9). The likelihood ratios demonstrate that serum oestradiol was not a strong diagnostic marker to predict miscarriage.

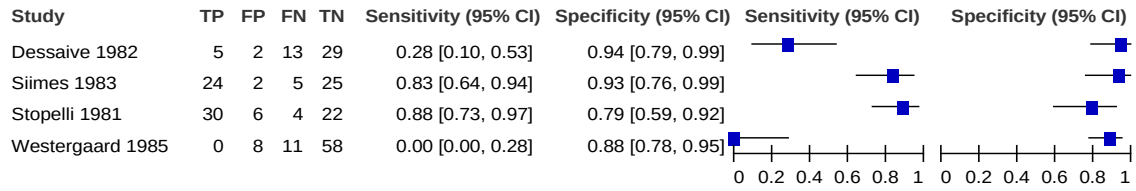


Figure 8 Forest plot of studies investigating the role of serum oestradiol in predicting miscarriage in women with threatened miscarriage (N = 244). FN=false negative; FP=false positive; TN=true negative; TP=true positive

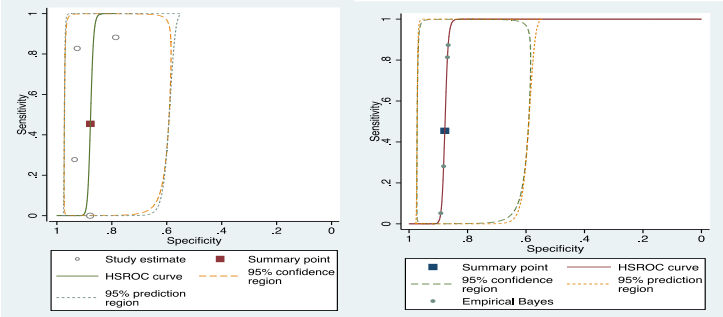


Figure 9 Summary receiver operating curve investigating the role of serum oestradiol in predicting miscarriage in women with threatened miscarriage

2.4.1.4.4 Serum PAPP-A

Serum PAPP-A was studied by three studies with 236 women to predict miscarriage (Hanita *et al.*, 2012, Ruge *et al.*, 1990, Westergaard *et al.*, 1985) The three studies that met the qualifying criteria for meta-analysis were summarised in the coupled forest plot, and the plot demonstrates variation in the results, especially for the sensitivity. PAPP- A had a poor and wide sensitivity that ranged from 25-64% but a high specificity ranging from 88-94% (Figure 10). As there were only three studies on PAPP-A, an HSROC curve was not created.

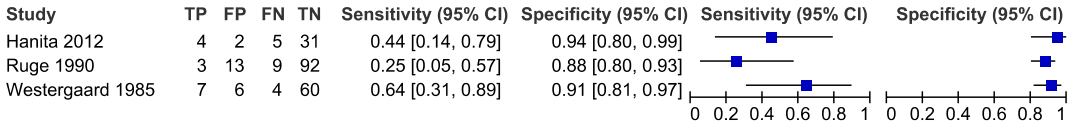


Figure 10 Forest plot of studies investigating the role of serum PAPP-A in predicting miscarriage in women with threatened miscarriage (N = 236). FN=false negative; FP=false positive; TN=true negative; TP=true positive

2.4.1.4.5 Serum CA 125

Seven studies with 648 women investigated the accuracy of CA 125 in predicting miscarriage in women with threatened miscarriage (Fiegler *et al.*, 2003, Leylek *et al.*, 1997, Maged and Mostafa, 2013, Öçer *et al.*, 1992, S. Sherif, AG El-Metwaly, H. Shalan, AM Badawy E., Abu-Hashem, L, 2000, Scarpellini *et al.*, 1995, Xie *et al.*, 2014). Figure 11 summarises seven studies investigating CA 125 and the forest plot does not show much variation in sensitivities and specificities between the studies. Further analysis using HSROC showed a sensitivity of 90% (95% CI 83-94%), a specificity of 88% (95% CI 79-93%), a positive likelihood ratio of 7.85 (95% CI 4.23-14.6) and a negative likelihood ratio of 0.10 (95% CI 0.05-0.20). The inverse of the negative likelihood ratio was 9.31 (95% CI 5-17.1) indicating that a negative test is likely to identify those who are likely to continue with the pregnancy. Empirical Bayes estimate gives the best estimate of the true sensitivity and specificity in each study, and the estimates are shrunk towards the summary point compared to the study-specific estimates. With the positive likelihood

ratio more than and five and negative likelihood ratio, less than 0.2 provide moderate diagnostic evidence on the use of CA 125 to predict miscarriage (McGee, 2002) (Figure 12).

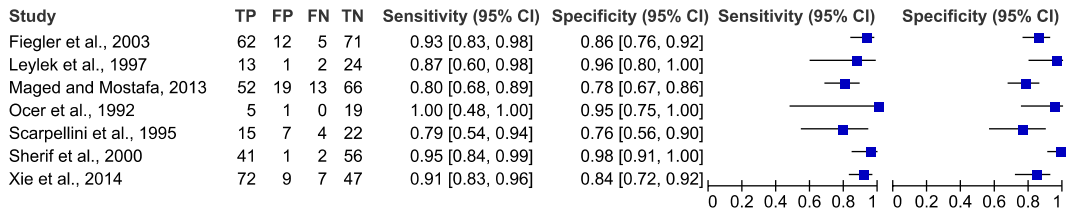


Figure 11 Forest plot of studies investigating the role of serum CA 125 in predicting miscarriage in women with threatened miscarriage (N = 648). FN=false negative; FP=false positive; TN=true negative; TP=true positive

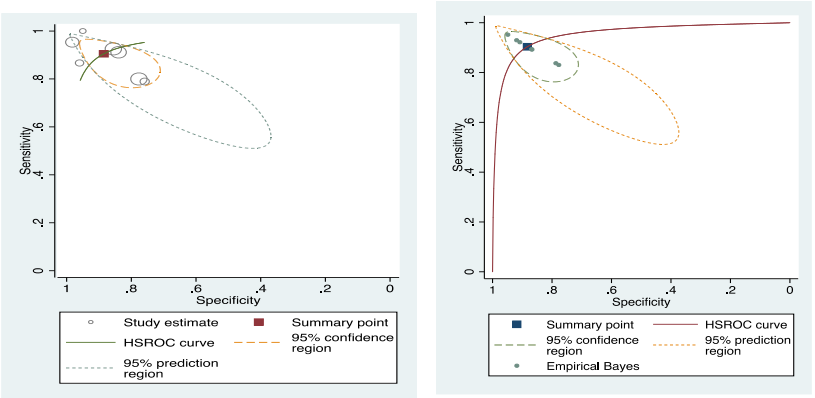


Figure 12 Summary receiver operating curve investigating the role of serum CA 125 in predicting miscarriage in women with threatened miscarriage

Further sensitivity analysis was done after excluding the study with a higher miscarriage rate (Scarpellini *et al.*, 1995, Stoppelli *et al.*, 1981b). However, there were no significant differences noted in the prediction parameters for the biomarkers of hCG, serum progesterone and oestradiol. The shape of the prediction region on the SROC plots indicates between study heterogeneity, which was considerable.

2.4.2 Results of systematic review 2: Use of ultrasound markers to predict miscarriage in threatened miscarriage population

2.4.2.1 Study selection

The electronic database search identified 4,094 articles and a further 46 articles were found from other sources and review of reference lists of individual manuscripts. After reviewing the titles and removing duplicates, 200 manuscripts were identified, of which 159 were excluded after reading the abstract. Full manuscripts of 41 articles were reviewed in detail and of these 12 studies were excluded (different patient population [n=5] (CHO *et al.*, 2006, Glock *et al.*, 1995, Lindsay *et al.*, 1992, Mantoni, 1985, Stern and Coulam, 1992), difficult to ascertain study population [n=2] (Chama *et al.*, 2005, Küçük *et al.*, 1999b), retrospective study design [n=2] (Doubilet, P. M. and Benson, 1995, Reus *et al.*, 2013) and case control study design [n=3] (Benson and Doubilet, 1994, Bromley *et al.*, 1991, Kurjak *et al.*, 1996)). A total of 29 studies were included in the qualitative data synthesis. Table 19 summarises the characteristic of included studies and table 20 summarises the characteristics of excluded studies. Nine studies (Achiron *et al.*, 1991, Bajo *et al.*, 2000, Doubilet, Peter M. *et al.*, 2000, Jun *et al.*, 1992, Mukri *et al.*, 2008, Oates *et al.*, 2013, Odeh *et al.*, 2012, Tadmor *et al.*, 1994, Varelas *et al.*, 2008)were further excluded from the quantitative meta-analysis since these were single studies for the given USS marker. Another study (Phupong and Hanprasertpong, 2011) was excluded in the quantitative meta- analysis because the data could not be obtained for the 2 x 2 table (data were expressed as mean and standard deviation). Overall, 19 studies were eligible for the quantitative meta-analysis and included 5684 women (Figure 13) (Abuelghar *et al.*, 2013, Alcázar and Ruiz-Perez, 2000, Altay *et al.*, 2009, Borlum *et al.*, 1989, Chittacharoen and Herabutya, 2004, Dede *et al.*, 2010, El-Mekkawi *et al.*, 2015, Falco *et al.*, 1996, Goldstein, S. R. *et al.*, 1983, Laboda *et al.*, 1989, Maged and Mostafa, 2013, Merchiers *et al.*, 1991, Pedersen and Mantoni, 1990, Qasim *et al.*, 1997, Reljic, 2001, Stampone *et al.*, 1996, Stefos *et al.*, 1998, Tan, Sinan, Tangal *et al.*, 2014, Tan, S. *et al.*, 2011).

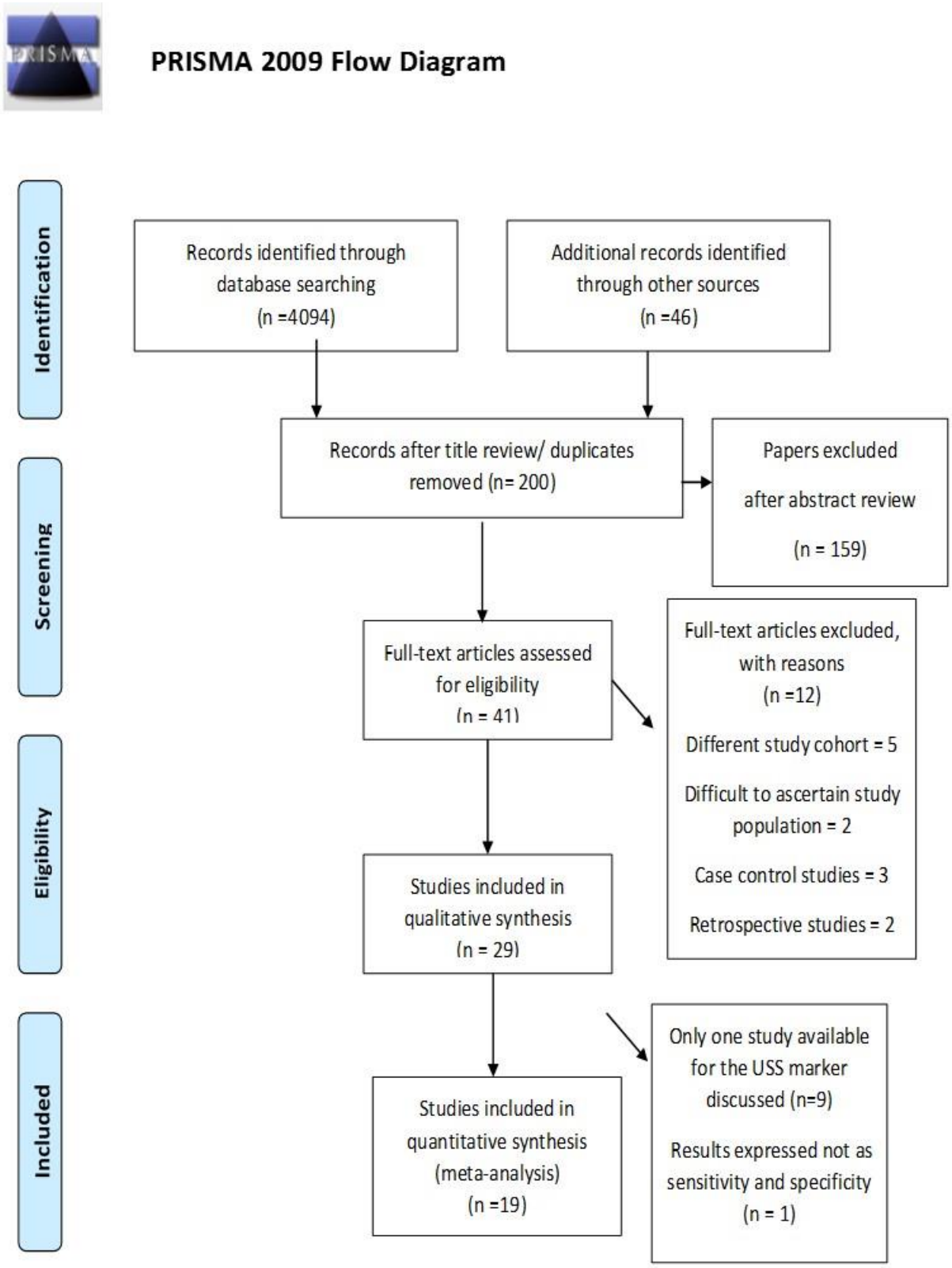


Figure 13 Flow chart for identification and selection of studies in the systematic review and meta-analysis investigating the role of ultrasound markers in predicting miscarriage in the threatened miscarriage population (Moher *et al.*, 2009)

2.4.2.2 Study characteristics

All included studies were prospective cohorts (N=29) that investigated USS markers for the prediction of miscarriage in women with or without vaginal bleeding and viable intrauterine pregnancy. Of these, 11 studies were on women with vaginal bleeding and viable intrauterine pregnancy; eight studies were on asymptomatic women with confirmed fetal viability and 10 studies were on a mixed population of women with and without vaginal bleeding. The characteristics of the included studies are summarized in Table 19 and of excluded studies in Table 20.

Table 19 Characteristics of the included studies in a systematic review and diagnostic accuracy meta-analysis of ultrasound markers used in the prediction of outcome in women with threatened miscarriage.

Authors and publication year	**Country**	**Patient characteristic**	**Index tests (USS markers)**	**Index test cut off**	**Miscarriage diagnosis**	**Follow-up duration**
Goldstein *et al.,* 1983	United States of America	N=50, 9-16 weeks, PV bleed +	SCH TA or TV scan not specified	Not specified	USS	Until the outcome or delivery
Borlum *et al.*, 1989	Denmark	N= 380, >8 weeks till second trimester, PV bleed +	IUH TA scan	IUH +	Individual follow up on an ambulatory basis	Until miscarriage or delivery
Laboda *et al.,* 1989	United States of America	N= 65, 5-8 weeks, symptom not specified	FHR Both TA and TV scan	<90 bpm	USS or clinic review	Not clear
Pedersen and Mantoni, 1990	Denmark	N= 342, 9-20 weeks, PV bleed +	IUH TA or TV scan not specified	2mls	USS	Until hematoma was resolved

Authors and publication year	Country	Patient characteristic	Index tests (USS markers)	Index test cut off	Miscarriage diagnosis	Follow-up duration
Merchiers *et al.*, 1991	Belgium	N= 170, 5-12 weeks, symptom not specified	FHR TA or TV scan not specified	100 bpm	Not specified	Beyond first trimester
Achiron *et al*., 1991	Israel	N= 603, first trimester, PV bleed +	FHR TV scan	FHR outside the 95% confidence interval	Telephone, mail and USS	Beyond 13 weeks
Jun *et al.*, 1992	Korea	N= 111, 6-9 weeks, both symptomatic and asymptomatic women	Mean Gestational Sac size, CRL, FHR TA scan	Not specified	Medical notes, USS	Until delivery or miscarriage
Tadmor *et al*., 1994	Israel	N= 603, first trimester, both symptomatic and asymptomatic women	Gestational sac diameter / crown rump length (GSD/CRL) TV scan	Outside 95% CI	Telephone, mail survey and USS	Up to 13 weeks

Authors and publication year	Country	Patient characteristic	Index tests (USS markers)	Index test cut off	Miscarriage diagnosis	Follow-up duration
Falco *et al.*, 1996	Italy	N=270, 5-12 weeks, PV bleed +	MGSD-CRL, CRL, SCH, FHR and menstrual age – sonographic age TV Scan	<14 mm (CRL), ≤ 0.5 SD (MGSD-CRL), <1 SD (FHR), >1 week (menstrual age-sonographic age)	Clinics follow up	Up to 20 weeks
Stampone *et al.*, 1996	Italy	N=117, first trimester, PV bleed +	Size and shape of YS TV scan	+/- 2 SD	Not clear	Not clear
Qasim *et al.*, 1997	United States of America	N= 116, 5.5-9.5 weeks, both symptomatic and asymptomatic women	FHR TV scan	>2 SD	Not clear	24 weeks
Stefos *et al.*, 1998	Greece	N= 2164, 6-8 weeks, symptom status not known	FHR TA and TV scan	≤ 85 bpm	USS	12 weeks

Authors and publication year	Country	Patient characteristic	Index tests (USS markers)	Index test cut off	Miscarriage diagnosis	Follow-up duration
Alcazar and Ruiz-Perez, 2000	Spain	N= 49, 5-12^{+6} weeks, PV bleed+	PSV and PI of uterine and spiral artery, Retro chorionic hematoma TV Scan	Present or absent	Not clear	End of pregnancy
Bajo *et al*., 2000	Spain	N= 592, 5-12 weeks, PV bleed -	Trophoblast thickness TV Scan	>3mm	USS	12 weeks
Doubilet *et al*., 2000	United States of America	N= 2817, <7 weeks, PV bleed +	Rapid heart rate TA or TV scan not specified	134bpm before 6.3 weeks and 154 bpm 6.3 to 7 weeks	USS or delivery of the baby	At least 13 weeks
Reljic, 2001	Slovenia	N= 310, up to 13 weeks, PV bleed +	CRL TV Scan	≤ 18mm	Hospital records and patient interview	Not clear

Authors and publication year	Country	Patient characteristic	Index tests (USS markers)	Index test cut off	Miscarriage diagnosis	Follow-up duration
Chittacharoen and Herabutya, 2004	Thailand	N= 240, 6-12^{+6} weeks, PV bleed last 24 hour +	FHR TV Scan	<120 bpm	Until delivery or outcome	Not clear
Mukri *et al.*, 2008	United Kingdom	N= 292, 5-10 weeks, both symptomatic and asymptomatic women	CRL deficit TV Scan	>2 SD	USS or by contacting women or GP	12-14 weeks
Varelas *et al.*, 2008	Greece	N= 219, 6-12 weeks, PV bleed -	GA+ FHR GA+ Yolk sac diameter (YSD) TV Scan	ROC cut off > 0.948 (GA+FHR) ROC cut off > 0.939 (GA+YSD)	USS	12 weeks

Authors and publication year	Country	Patient characteristic	Index tests (USS markers)	Index test cut off	Miscarriage diagnosis	Follow-up duration
Altay *et al.*, 2009	Turkey	N=99, 10 weeks, PV bleed +	MGSD, FHR, MGSD-FHR TV Scan	No cut off specified	USS	20 weeks
Dede *et al.*, 2010	Turkey	N= 202, 5-14 weeks, PV bleed +	CRL Cervical length FHR TV Scan	<40 mm (cervical length) <130bpm (FHR)	Not clear	Up to 20 weeks
Tan *et al.*, 2011	Turkey	N= 183, 6-8^{+6} weeks, PV bleed -	Irregular YS TV Scan	Irregular YS present or absent	USS	20 weeks

Authors and publication year	Country	Patient characteristic	Index tests (USS markers)	Index test cut off	Miscarriage diagnosis	Follow-up duration
Phupong and Hanprasertpong, 2011	Thailand	N= 30, 6-14^{+6} weeks, PV bleed +	FHR Both TA and TV scan	<2 SD	USS	Not clear
Odeh *et al.*, 2012	Israel	N=90, 6-12 weeks, PV bleed +	Amniotic sac volume (ASV), Gestational sac volume (GSV), GSV-ASV TV scan	≤ 1.8 cm^3 (GSV-ASV)	Not mentioned	24 weeks
Abuelghar *et al.*, 2013	Egypt	N= 341, 6-13 weeks, PV bleed -	Smaller than expected CRL TV scan	<2 SD	USS	Not clear
Maged and Mostafa, 2013	Egypt	N=150, 5-12 weeks, PV bleed+	GSD CRL FHR YSD TV Scan	21mm (CRL) 110 bpm (FHR)	Not clear	Not clear

Authors and publication year	**Country**	**Patient characteristic**	**Index tests (USS markers)**	**Index test cut off**	**Miscarriage diagnosis**	**Follow-up duration**
Oates *et al*., 2013	Australia	N= 443, first trimester, both symptomatic and asymptomatic women	Log model using mean gestational sac size and mean yolk sac size. TV scan	AUC of 0.55	Obstetrics database	12 weeks
Tan *et al*., 2014	Turkey	N=305, 6-9 weeks, PV bleed-	Size, shape and echogenicity of yolk sac TV Scan	YSD ≥ 5mm	Medical records and telephone interview	Until delivery
El-Mekkawi *et al*., 2015	Egypt	N=200. 7weeks, PV bleed-	MGSD CRL FHR MGSD-CRL TV Scan	14mm (MGSD) 5.5mm (CRL)	USS and clinical symptoms	20 weeks

Table 20 Characteristics of the studies excluded from the systematic review and diagnostic accuracy meta-analysis of ultrasound markers used in the prediction of outcome in women with threatened miscarriage.

Author and publication year	**Study design**	**Patient characteristics**	**Index test studied**	**Exclusion criteria**
Mantoni *et al.*, 1985	Prospective cohort	N= 260, ≤ 20weeks, included women with both FH+ and FH- and PV bleed	FHR	Mixed population of women with both FH+ and FH-
Bromley *et al.*, 1991	Case control study	N=68, 5.5- 9 weeks, FH +	GSS-CRL	Case control study
Lindsay *et al.*, 1992	Prospective Cohort	N= 486, < 10 weeks, included mixed population of women with FH+ and FH-, Symptom status not specified	YSD	Mixed population
Stern and Coulam, 1992	Prospective cohort study	N= 83, 4-12 weeks, included women with both FH+ and FH-, symptom status of the participants not specified	CRL	Mixed population

Author and publication year	Study design	Patient characteristics	Index test studied	Exclusion criteria
Benson and Doubilet, 1994	Case control study	N= 40, < 8 weeks, FHR ≤ 90 bpm, included both asymptomatic and symptomatic women	FHR	Case control study
Doubilet and Benson, 1995	Retrospective study	N= 809, ≤ 8 weeks, included singleton pregnancies with FH+, Symptom status of the participants not specified	FHR	Retrospective study
Glock *et al.,* 1995	Prospective cohort	N=55, 4-8 weeks, included symptomatic and asymptomatic women with both FH+ and –	Size and shape of corpus luteum	Mixed population of women with both FH+ and FH -
Kurjak *et al*., 1996	Case control study	N= 59, 6-14 weeks, included women with PV bleed and FH+	IUH	Case control study
Kucuk *et al.,* 1999	Prospective cohort	N= 250, 6-11 weeks, not mentioned about the FH and symptom status	Size and shape of YS	FH status not specified
Chama *et al*., 2005	Prospective cohort	N= 105, < 12 weeks, FH or symptom status not specified	Size and shape of YS	FH status not specified

Author and publication year	Study design	Patient characteristics	Index test studied	Exclusion criteria
Cho *et al*., 2006	Case control study	N= 154, 6-10 weeks, Included asymptomatic women with both FH+ and FH-	YS	Case control study and mixed population
Reus *et al*., 2013	Retrospective study	N= 168, 6-8 weeks, included a mixed population of symptomatic and asymptomatic women with both FH+ and FH-	3D USS measurement of trophoblastic thickness	Retrospective study and mixed population

2.4.2.3 Risk of bias assessment

The risk of bias was assessed in four main domains using the 'QUADAS-2: A Revised Tool' for patient selection, index test, reference standard and flow and timing (Figure 14). Strict exclusion criteria for the systematic review were followed as described above (section of eligibility criteria). In the QUADAS-2: A Revised Tool, under the patient selection, if the study did not specify about their exclusion criteria, it was considered unclear risk for bias. For the index tests, three studies had not specified a cut off level to differentiate between ongoing pregnancies and miscarriage or they had not specified it prior to starting the study. This was an area of bias for the included studies. Similarly, if the same sonographer did not perform the USS, then there was a potential for inter observer bias. Four studies did not specify the mode of scanning and three studies had scanned patient either trans- abdominally or trans-vaginally. This can also contribute to bias due to the variation in the mode of scanning technique. Occurrence of miscarriage can be best diagnosed using USS, clinical history or histopathological examination of the products of conception. However, in 14 studies the occurrence of miscarriage data was collected by telephone interview of the patients or retrospective collection of data from medical records rather than a clinical follow up or USS review. In seven studies it was not clear on the method of collection of the outcome data. This could have contributed to recall bias. In all the studies it was not clearly stated whether the person who collected the outcome data was blinded to the results of the index test. However, this is unlikely to affect applicability of the studies since miscarriage is an objective diagnosis and is not prone to subjective interpretation. In the flow and timing section of the QUADAS-2 tool, although it was difficult to predict a specific time interval from the index test to the occurrence of miscarriage. We used the sampling question to determine whether the patients were followed up until at least completed 23 weeks. The World Health Organisation (World Health Organization, 2000) has defined miscarriage as premature loss of a fetus up to 23 weeks of pregnancy or below 500 grams of weight. Out of the 29 studies that were used for the qualitative data synthesis, 15 studies have not followed up patients till completed 23 weeks and seven studies were not clear about

their duration of follow up. Therefore, some quality concerns exist for the diagnostic accuracy studies included in the review.

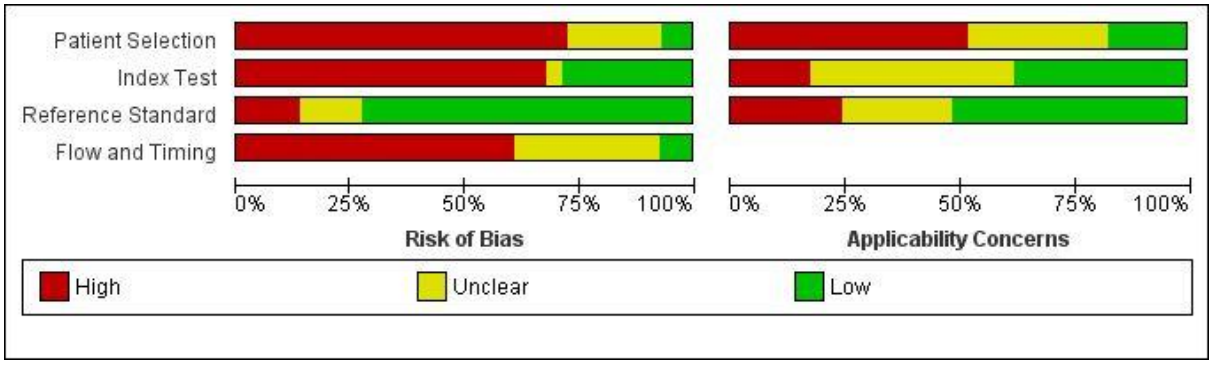


Figure 14 Summary of quality assessment of the included studies for meta-analysis using the QUADAS-2: A Revised Tool for the systematic review and meta-analysis of ultrasound markers used in the prediction of outcome in women with threatened miscarriage.

2.4.2.4 Quantitative data summary and synthesis of results

Data were summarized for the USS markers of FHR, CRL, mean gestational sac diameter (MGSD) minus CRL, YS and intra uterine haematoma (IUH). Test results were tabulated in a 2 x 2 table and forest plots constructed for the sensitivity and specificity of the USS marker with their confidence intervals. Further subgroup analysis was done for women with viable intra uterine pregnancy and bleeding PV (threatened miscarriage population) for the USS markers FHR, CRL and IUH. Sensitivity analysis was performed for the year of the publication (pre year 2000 and after 2000) since USS technology has significantly advanced over the years resulting in better quality imaging and therefore more accurate evaluation of these markers. For CRL and FH, the majority of the selected studies were after the year 2000 and for IUH, the majority of the selected studies were before the year 2000. A sensitivity analysis based on the GA (≤ 14 weeks and >14 weeks) and mode of scanning (TAS vs TVS) was also performed. Studies that were single studies for the specific USS markers could not be included in the meta-analysis. These single USS markers were mean sac diameter/CRL (Tadmor *et al.*, 1994) difference between the observed and expected CRL for the gestational age (Mukri *et al.*, 2008), trophoblast thickness (Bajo *et al.*, 2000), amniotic sac volume and GS volume – amniotic sac volume (Odeh *et al.*, 2012), rapid heart rate (Doubilet, P. M. and Benson, 1995), discriminant

analysis using GS, CRL and FHR (Jun *et al.*, 1992), GA + FHR and GA + YS diameter (Varelas *et al.*, 2008), FHR outside 95% CI (Achiron *et al.*, 1991) and log model including mean GS size and YS size (Oates *et al.*, 2013).

2.4.2.4.1 Fetal Bradycardia

There were ten studies (Chittacharoen and Herabutya, 2004, Dede *et al.*, 2010, El-Mekkawi *et al.*, 2015, Falco *et al.*, 1996, Laboda *et al.*, 1989, Maged and Mostafa, 2013, Merchiers *et al.*, 1991, Phupong and Hanprasertpong, 2011, Qasim *et al.*, 1997, Stefos *et al.*, 1998) including both symptomatic and asymptomatic women (N=1762) that investigated the ability of fetal bradycardia in predicting miscarriage (Figure 15). The couple forest plot showed variation in the sensitivities between studies. The HSROC plot showed a summary sensitivity of 68.41% (95% CI 43.62- 85.84%), specificity of 97.84% (95% CI 94.50-99.17%), positive likelihood ratio of 31.73 (95% CI 12.78- 78.75) and negative likelihood ratio of 0.32 (95% CI 0.16-0.65) (Figure 16). The high positive likelihood ratio signifies that there is a high likelihood of miscarriage when there is low FHR (i.e., in presence of fetal bradycardia). On the other hand, the negative likelihood ratio of 0.32 suggests weaker association between absence of fetal bradycardia and absence of miscarriage.

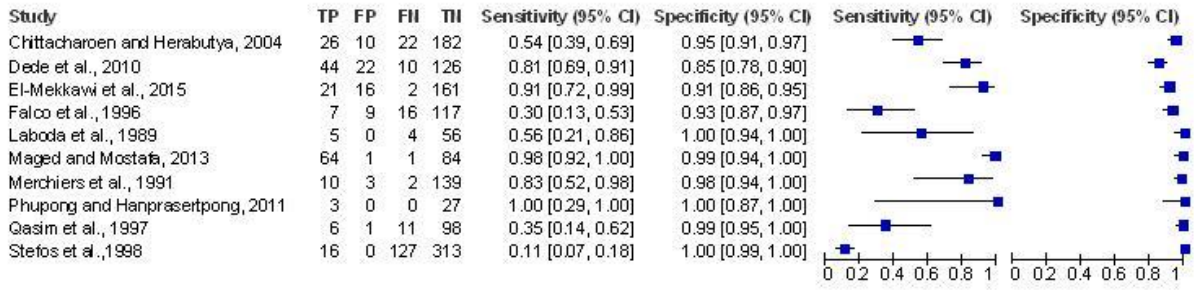


Figure 15 Forest plot of studies investigating the use of FHR to predict miscarriage in women with viable intrauterine pregnancy (N = 1762). FN=false negative; FP=false positive; TN=true negative; TP=true positive.

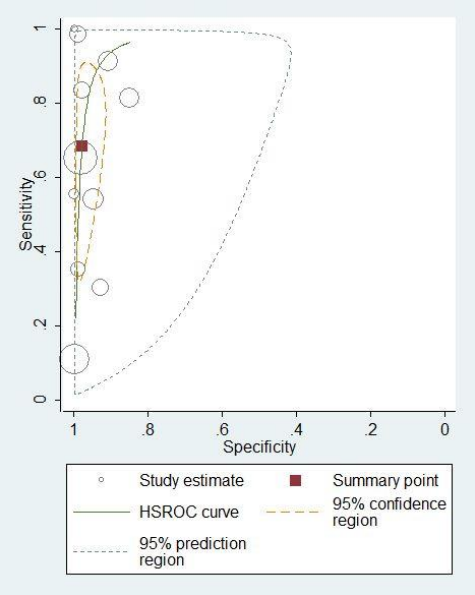


Figure 16 HSROC curve investigating the use for FHR in predicting miscarriage in women with viable intra uterine pregnancy.

Further subgroup analysis was performed for only the symptomatic women, which included five studies (N=771) (Figure 17) (Chittacharoen and Herabutya, 2004, Dede *et al.*, 2010, Falco *et al.*, 1996, Maged and Mostafa, 2013, Phupong and Hanprasertpong, 2011). The HSROC analysis showed a significant increase in the sensitivity of FHR to predict miscarriage from 68.41% to 84.18% (95% CI 42.02% - 97.50%), specificity of 95.68% (95% CI 87.76% - 98.56%), positive likelihood ratio of 19.51 (95% CI 5.44-69.84) and negative likelihood ratio of 0.16 (95% CI 0.03- 0.91)) (Figure 18). This means that in threatened miscarriage population, fetal bradycardia is a better predictor of miscarriage as the positive likelihood ratio is high, and the negative likelihood ratio is low.

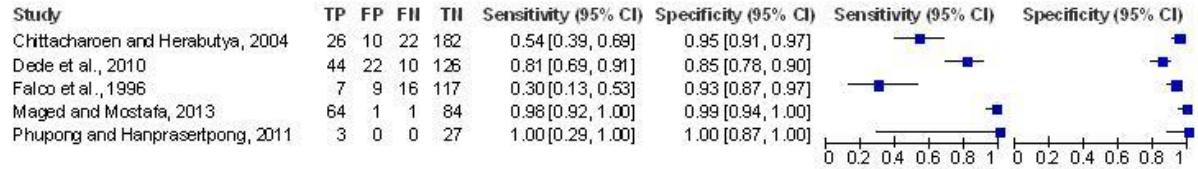


Figure 17 Forest plot of studies investigating the use of FHR in predicting miscarriage in women with threatened miscarriage (N = 771). FN=false negative; FP=false positive; TN=true negative; TP=true positive.

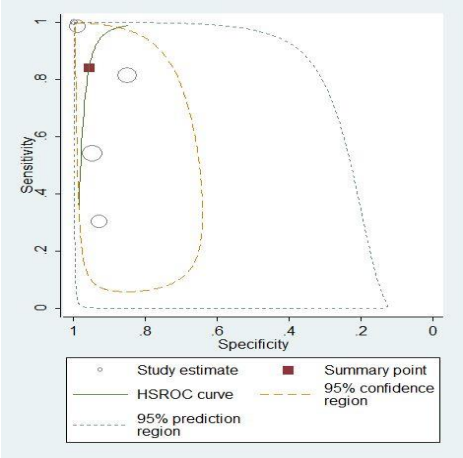


Figure 18 HSROC curve investigating the use of FHR in predicting miscarriage in women with threatened miscarriage.

A sensitivity analysis based on the year of the study (before and after the year 2000 AD) showed a significant increase in the sensitivity for the studies performed after year 2000 (sensitivity of 90.70% (95% CI 65.75- 98.02%), specificity of 95.20% (95% CI 87.08- 98.31%), positive likelihood ratio of 18.91 (95% CI 6.25- 57.21) and a negative likelihood ratio of 0.09 (95% CI 0.02-0.43)). This improvement in the diagnostic accuracy of FHR to predict miscarriage in studies done after the year 2000 can be attributed to the availability of more sophisticated USS machine and better training of the sonographers. Most of the studies for FHR had women with gestational age ≤ 14 weeks and were undertaken with TVS.

In order to use FHR as a predictive marker a cut off value is needed but only seven studies (Chittacharoen and Herabutya, 2004, Dede *et al.*, 2010, El-Mekkawi *et al.*, 2015, Laboda *et al.*, 1989, Maged and Mostafa, 2013, Merchiers *et al.*, 1991, Stefos *et al.*, 1998) specified a cut of value of FHR for the prediction of miscarriage. However, different studies used different cut off values. Having a single summary cut off value from these seven studies will be more beneficial clinically. In order to find a single summary cut of value, we plotted the log diagnostic odds ratio of all these seven studies against the cut off levels of FHR from all the seven studies. The graph showed that a cut-off of 110 beats per minute (bpm) predicts miscarriage best. Above 110 bpm the diagnostic power of FHR diminishes. (Figure 19). Only two (Laboda *et al.*, 1989, Stefos *et*

al., 1998) of these seven studies investigated fetal heart rate based on the gestational age and a meta-regression model showed a FHR of more than 134bpm at seven weeks gestation and 158 bpm at eight weeks gestation was predictive of an on-going pregnancy (i.e. did not miscarry).

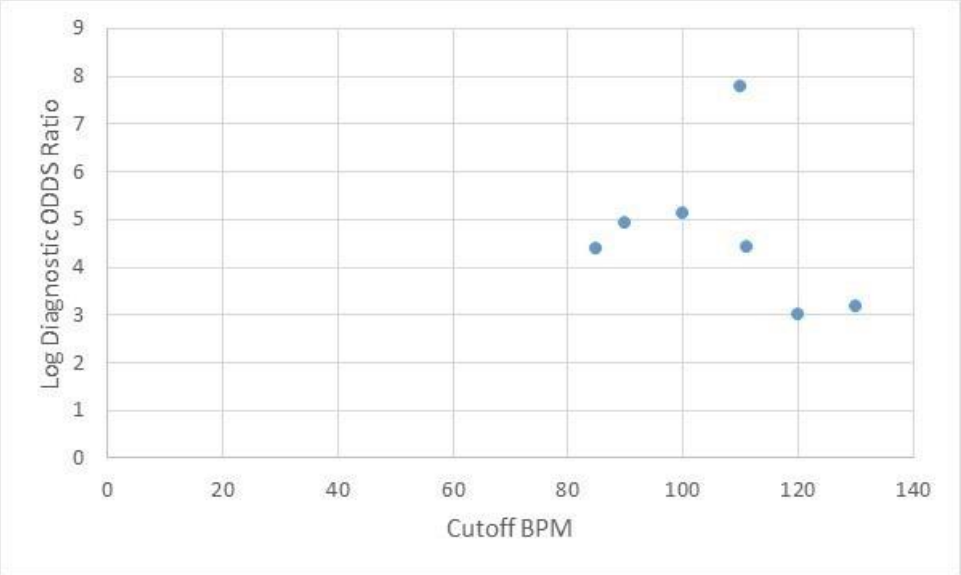


Figure 19 Plot of cut off value for heart rate versus Log Diagnostic Odds of FHR in women with viable intrauterine pregnancy demonstrating the optimum cut-off value for FHR in predicting miscarriage for women with threatened miscarriage

2.4.2.4.2 CRL

Five studies (Abuelghar *et al.*, 2013, El-Mekkawi *et al.*, 2015, Falco *et al.*, 1996, Maged and Mostafa, 2013, Reljic, 2001) with 1136 women investigated the use of CRL for the prediction of miscarriage (Figure 20). The couple forest plot showed huge variation in the sensitivities and specificities across the included studies. An HSROC plot showed a sensitivity of 59.81% (95% CI 48.78-69.93%), specificity of 55.68% (95% CI 39.95-70.35%), positive likelihood ratio of 1.34 (95% CI 0.91-2.00) and negative likelihood ratio of 0.72 (95% CI 0.49-1.06) (Figure 21) with very wide prediction region and confidence region. The results of HSROC plot highlight that CRL is not a strong predictive marker for miscarriage.

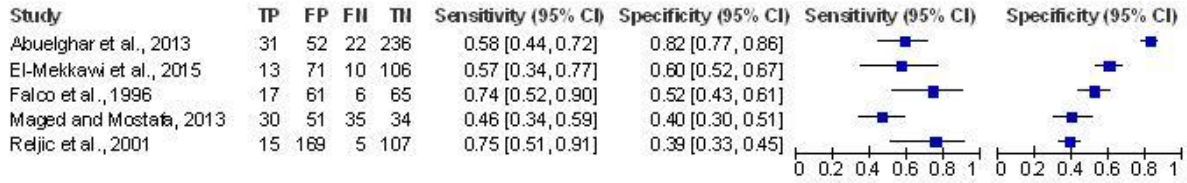


Figure 20 Forest plot of studies investigating the use of CRL in predicting miscarriage in women with viable intrauterine pregnancy (N = 1136). FN=false negative; FP=false positive; TN=true negative; TP=true positive.

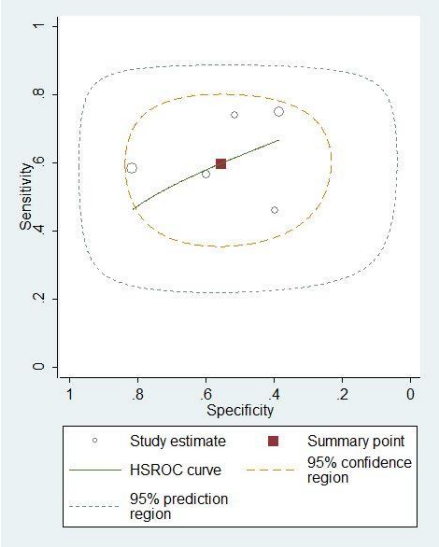


Figure 21 HSROC curve investigating the use of CRL in predicting miscarriage for studies with viable intra uterine pregnancies

A subgroup analysis was performed on symptomatic women with three studies (N= 595) (Falco *et al.*, 1996, Maged and Mostafa, 2013, Reljic, 2001) and no significant difference in the sensitivity and specificity was noted between the miscarried group and those who continued their pregnancy

Sensitivity analysis based on the year of the study (studies after the year 2000 AD) did not show any significant difference in the results (sensitivity of 55.40% (95% CI 44.41-65.90%), specificity of 56.57% (95% CI 37.13-74.17%), positive likelihood ratio of 1.27 (95% CI 0.75-2.14) and negative likelihood ratio of 0.78 (95% CI 0.50-1.24)).

2.4.2.4.3 IUH

Five studies on 956 symptomatic women used IUH to predict miscarriage (Figure 22) (Alcázar and Ruiz-Perez, 2000, Borlum *et al.*, 1989, Falco *et al.*, 1996, Goldstein, Steven R., 1992, Pedersen and Mantoni, 1990). The forest plot demonstrated huge variation in the sensitivities of the studies and this variation can be attributed to the variation between studies in the way it was conducted. The HSROC plot showed a sensitivity of 57.37% (95% CI 21.41- 86.92%), a specificity of 71.02% (95% CI 46.51- 87.34%), a positive likelihood ratio of 1.98 (95% CI 1.17- 3.33) and a negative likelihood ratio of 0.60 (95% CI 0.29-1.24) with wide prediction region and confidence region (Figure 23) making it a not useful marker to predict miscarriage. A subgroup analysis for studies with women >14 weeks of gestation failed to show any increase or decrease in accuracy for predicting miscarriage. Four out of the five studies (Borlum *et al.*, 1989, Falco *et al.*, 1996, Goldstein, S. R. *et al.*, 1983, Pedersen and Mantoni, 1990) were done before the year 2000. Two studies used only TVS (Alcázar and Ruiz-Perez, 2000, Falco *et al.*, 1996) and one study used TAS (Borlum *et al.*, 1989) and the other two studies (Goldstein, S. R. *et al.*, 1983, Pedersen and Mantoni, 1990) did not specify their scanning modality.

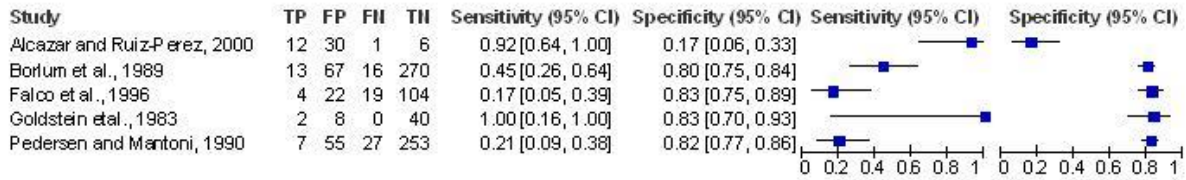


Figure 22 Forest plot of studies investigating the use of IUH in predicting miscarriage in women with viable intrauterine pregnancy (N = 956). FN=false negative; FP=false positive; TN=true negative; TP=true positive.

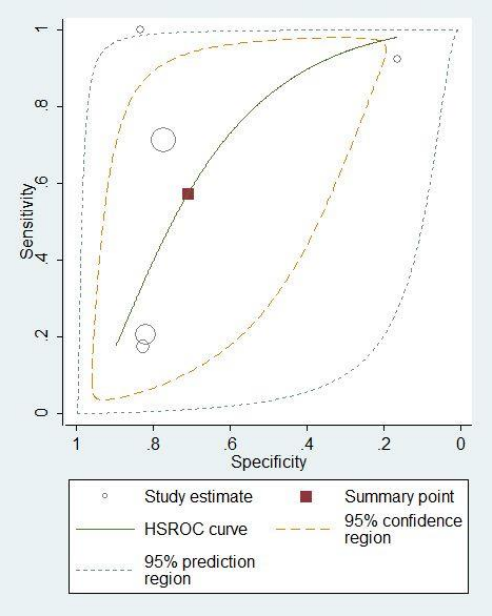


Figure 23 HSROC curve investigating the use of IUH in predicting miscarriage for studies with viable intra uterine pregnancy

2.4.2.4.4 Difference between the mean gestational sac diameter and crown rump length (MGSD- CRL)

Two studies (N=349 women) (El-Mekkawi *et al.*, 2015, Falco *et al.*, 1996) evaluated the MGSD minus CRL difference (MGSD-CRL) in the prediction of miscarriage in women with confirmed fetal viability. These had a sensitivity range of 39% -96% and a specificity range of 73% - 88 % (Figure 24). Since there were only two studies a HSROC plot was not created.

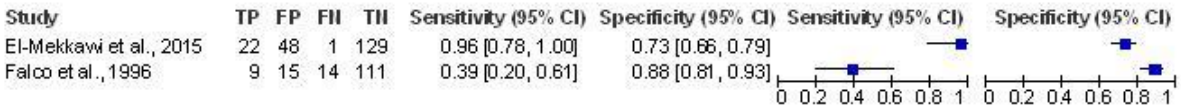


Figure 24 Forest plot of studies investigating the use of MGSD-CRL in predicting miscarriage in women with viable intrauterine pregnancy (N = 349). FN=false negative; FP=false positive; TN=true negative; TP=true positive

2.4.2.4.5 Yolk Sac

Three studies (N= 605 women) investigated YS (abnormal shape, size, echogenicity or absent YS) for the prediction of miscarriage (Figure 25) (Stampone *et al.*, 1996, Tan, Sinan *et al.*, 2014, Tan, S. *et al.*, 2011) All the studies that investigated YS in miscarriage prediction were on normal asymptomatic women. The studies demonstrated a wide variation in sensitivity ranging from 17%- 69% and specificity ranging from 79%- 99%. Since there were only three studies a HSROC plot was not created.

Figure 25 Forest plot of studies investigating the use of abnormal YS in predicting miscarriage for women with viable intrauterine pregnancy (N = 605). FN=false negative; FP=false positive; TN=true negative; TP=true positive

2.4.2.4.6 Combination of USS markers

There were three studies (Jun *et al.*, 1992, Oates *et al.*, 2013, Varelas *et al.*, 2008) that looked into a combination of ultrasound markers for prediction of miscarriage (Table 21). All studies used different combination markers; therefore, it was not possible to perform a meta-analysis.

Table 21 Studies using combination markers for prediction of miscarriage in women with confirmed fetal viability

Study	**Prediction model used**	**Sensitivity**	**Specificity**
Jun *et al*., 1992	Discriminant analysis using mean GS size, CRL and FHR	94.1%	96.%
Varelas *et al.,* 2008	GA+FHR GA+YSD	91% 76.8%	100% 91.7%
Oates *et al*., 2013	Log model using GA by LMP, presence of PV bleeding, presence of PV clots, GA by USS, consistency with menstrual dates, mean GS size, mean YS size and number of previous caesarean sections	82%	79%

2.4.3 Results of systematic review 3: Perinatal outcome of women with threatened miscarriage

2.4.3.1.1 Study selection

The electronic searches identified 570 articles and a further 17 were found from other sources and review of the reference lists of individual manuscripts. After reviewing the titles and removing the duplicates, 53 manuscripts were identified, of which 30 were

excluded after reading the abstract. Full manuscripts of 23 articles were reviewed in detail and of these nine studies were excluded (no control group were used in six studies (Asanti and Vesanto, 1963, Basama and Crosfill, 2004, Calleja-Agius *et al.*, 2011, Dadkhah *et al.*, 2010, Dongol *et al.*, 2012, Nielson *et al.*, 1991) two studies used medications for the treatment of threatened miscarriage (Jouppila, P. and Koivisto, 1974, Verma *et al.*, 1994) and data were not clearly presented in one (Turnbull and Walker, 1956) A total of 14 studies (Akhter *et al.*, , Calleja-Agius *et al.*, 2011, Dadkhah *et al.*, 2010, Das *et al.*, 1996, Davari-Tanha *et al.*, 2008, Evans and Beischer, 1971, Hertz and Heisterberg, 1985, Johns, J. and Jauniaux, 2006, Konje *et al.*, 1992, Sipilä *et al.*, 1992, Strobino and Pantel-Silverman, 1987, Tikreeti and Alsaadi, 1990, Tongsong *et al.*, 2008, Weiss *et al.*, 2004) were included in the quantitative data synthesis which included a total of 36601 women (Figure 26).

PRISMA 2009 Flow Diagram

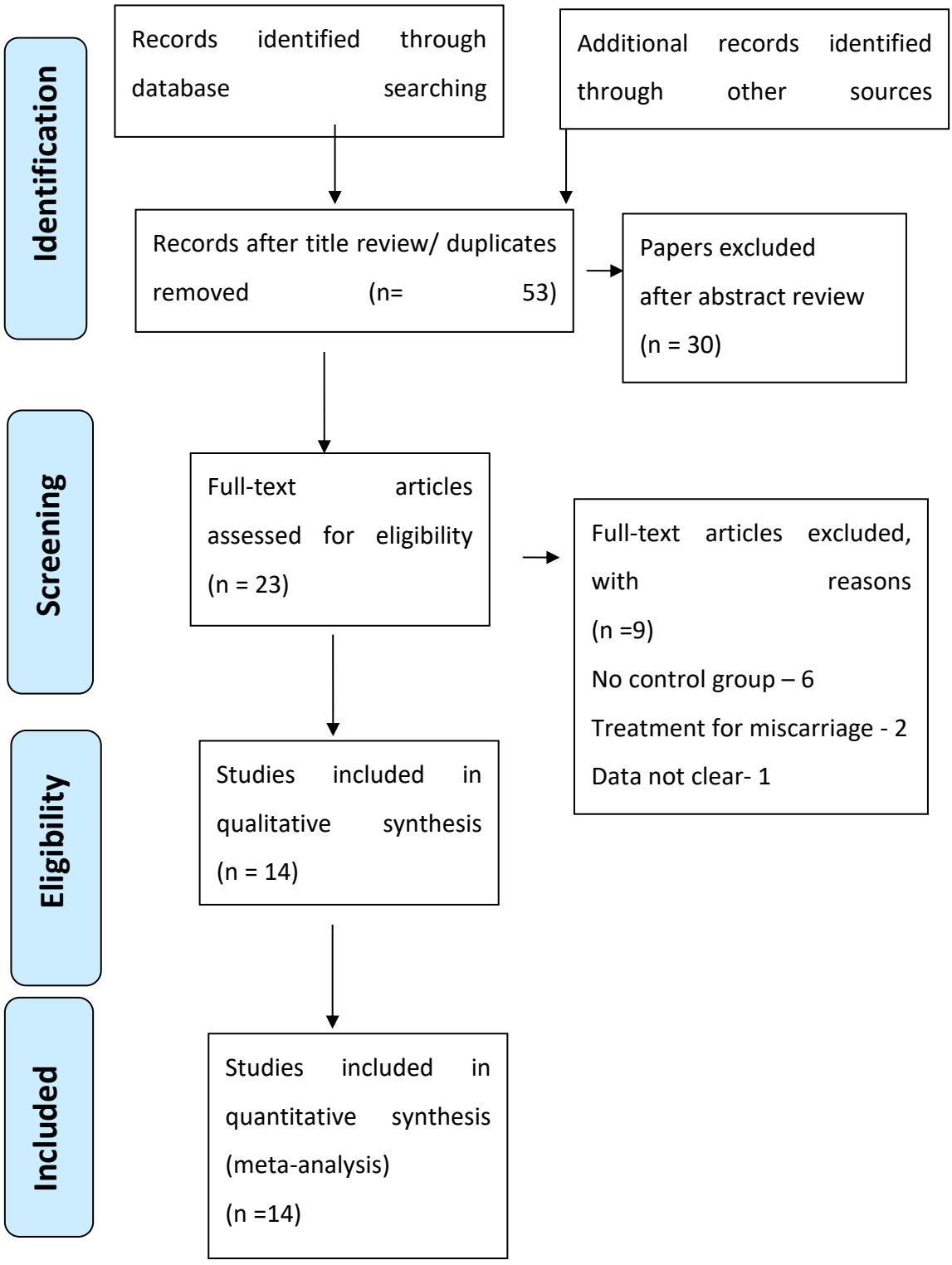


Figure 26 Flow chart for identification and selection of studies in the systematic review and meta-analysis investigating the perinatal morbidity in women with threatened miscarriage (Moher *et al.*, 2009)

2.4.3.2 Study characteristics

All included studies were prospective cohort studies (n=14) of women who had experienced threatened miscarriage at less than 24 weeks gestation and followed-up until the end of the pregnancy for maternal and perinatal complications. The characteristics of the included studies are summarized in (Table 22) and of excluded studies in (Table 23).

Table 22 Characteristics of the included studies in the systematic review and meta-analysis investigating the perinatal morbidity in women with threatened miscarriage

Authors and publication year	**Country**	**Patient characteristic**	**Pregnancy outcome**
Evans and Beischer, 1970	Australia	N=326 cases and 2909 controls, bleeding episode <13 weeks, included 3 groups with incidental haemorrhage, haemorrhage with and without hospitalisation	Prematurity, stillbirth and neonatal death
Hertz *et al.*, 1985	Denmark	N=93 cases and 282 controls, bleeding episode <20 weeks, included singleton pregnancies continued until at least 28 weeks	Preterm labour, preterm delivery, preterm prelabour rupture of membrane, caesarean section, retention of placenta, birth weight <2.5kg, neonatal asphyxia, malformations and hyperbilirubinemia
Tikreeti and Al-Saadi, 1990	Iraq	N=200 cases and 415 controls, bleeding episode <20 weeks, included singleton pregnancies continued until at least 28weeks, non-smoker, no significant medical or obstetric history	Preterm labour, preterm delivery, placental abruption, preterm prelabour rupture of membrane, low birth weight, perinatal mortality, perinatal mortality excluding lethal anomaly, birth weight< 2.5kg, small for gestational age, respiratory distress syndrome, pregnancy induced hypertension, anaemia, placenta praevia, malpresentations, postpartum haemorrhage, congenital anomalies and caesarean section

Authors and publication year	Country	Patient characteristic	Pregnancy outcome
Strobino and Pantel-Silverman, 1989	USA	N=3531, bleeding in first trimester, included singleton pregnancies continued until at least 28 weeks with both heavy and light bleeding.	Preterm delivery, placental abruption and placenta praevia, low birth weight<2.5kg, small for gestational age<2.5kg at 36 weeks, stillbirth with no malformations, chromosomal anomalies, all malformations, major and minor malformations
Konje *et al.*, 1992	Nigeria	N=120 cases and 150 controls, bleeding <28 weeks, excluded smokers. Included only 72 cases with bleeding in the first trimester out of the 120 cases for analysis in this systematic review	Preterm labour, preterm prelabour rupture of membrane, antepartum haemorrhage, pregnancy induced hypertension, intrauterine death, birth weight <2.5kg, neonatal asphyxia, still birth, Immediate neonatal death and congenital anomalies
Sipila *et al.*, 1992	Finland	N=601 cases and 7911 controls, bleeding in first trimester, excluded multiple pregnancies	Preterm delivery, birth weight <2.5kg, small for gestational age < 2 standard deviation, admission to neonatal unit, congenital malformations, stillbirth and perinatal mortality < 7days
Tongsong *et al.*, 1995	Thailand	N=255 cases and 265 controls, bleeding in first trimester, included singleton pregnancies with no serious medical or gynaecological condition.	Preterm labour, preterm prelabour rupture of membrane, antepartum haemorrhage, pregnancy induced hypertension, chorioamnionitis, intrauterine growth retardation, stillbirth, low birth weight, congenital anomalies, apgar<7 at 5-minute, mean birth weight and caesarean section

Authors and publication year	Country	Patient characteristic	Pregnancy outcome
Das *et al.,* 1996	India	N = 55 cases and 55 controls, bleeding < 20 weeks, excluded cervical incompetence, uterine anomaly, uterine fibroid, recurrent miscarriage, hydatidiform mole, multiple pregnancy, chronic hypertension, diabetes mellitus, syphilis and termination of pregnancy <28 weeks	Preterm delivery, placenta praevia, low birth weight, mean birth weight, caesarean section
Weiss *et al.,* 2004	United States	N = 2346 cases and 14160 controls, bleeding <10-14 weeks, Included singleton pregnancies. 2094 women with light bleeding were included in the analysis of this systematic review	Preterm delivery, preterm prelabour rupture of membrane, pregnancy induced hypertension, pre-eclampsia, placental abruption, placenta praevia, intrauterine growth retardation and caesarean section
Johns *et al.,* 2006	United Kingdom	N = 214 cases and 214 controls, bleeding < 14 weeks, excluded women who reported only spotting, twin pregnancy, congenital uterine anomaly, leiomyomata, known thrombophilia and women presenting to the unit from outside catchment area	Preterm delivery, preterm prelabour rupture of membrane, mean birth weight and still birth

Authors and publication year	Country	Patient characteristic	Pregnancy outcome
Davari-Tanha *et al.*, 2008	Iran	N = 150 cases and 450 controls, bleeding in first trimester	Preterm delivery, preterm prelabour rupture of membrane, pre-eclampsia, placental abruption, placenta praevia, anaemia, intrauterine growth retardation, low birth weight, intrauterine fetal death and caesarean delivery
Dadkhah *et al.*, 2010	Iran	N = 500 cases and 500 controls, bleeding < 20 weeks, included women with singleton pregnancy without previous miscarriage, normal cervix on examination and reliable gestational age; excluded chronic hypertension, diabetes, systemic disorders, drug use, multiple pregnancy, smoker, previous congenital anomalies, consanguinity, any try for termination of pregnancy, surgery during present pregnancy, placenta praevia and history of trauma	Preterm delivery, preterm prelabour rupture of membrane, placental abruption, pre-eclampsia, small for gestational age, neonatal weight and caesarean section

Authors and publication year	Country	Patient characteristic	Pregnancy outcome
Calleja-Agius *et al.,* 2011	Malta	N = 69 cases and 564 controls, bleeding < 24 weeks, excluded twin pregnancies, hydatidiform mole, congenital uterine anomaly, cervical incompetence, large fibroid distorting uterine cavity and known thrombophilia	Preterm delivery, premature labour, pregnancy induced hypertension, pre-eclampisa, antepartum haemorrhage, gestational diabetes mellitus, intrauterine growth retardation, low birth weight, stillbirth, neonatal death, emergency and elective caesarean section, instrumental delivery, retained placenta and postpartum haemorrhage
Akhter *et al.,* 2014	Pakistan	N = 133 cases and controls, bleeding in first trimester, limited inclusion and exclusion criteria	Preterm delivery and preterm prelabour rupture of membrane

Table 23 Excluded studies and reason for exclusion in the systematic review and meta-analysis investigating the perinatal morbidity in women with threatened miscarriage

Study	**Reason for exclusion**
Turnbull and Walker, 1956	Data not clear
Thompson and Lein, 1961	No control group
Asanti and Vesanto, 1963	No control group
Johannsen, 1970	No control group
Jouppila and Koivisto, 1974	Treatment given for threatened miscarriage
Neilson *et al.,* 1991	No control group
Verma *et al.,* 1993	Treatment given for threatened miscarriage
Basama and Crosfill, 2004	No control group
Dongol *et al.,* 2011	No control group

2.4.3.3 Risk of bias assessment

The risk of bias and quality assessment was performed using the Newcastle-Ottawa scale for observational studies. In this quality assessment tool, a star system is used and a study is judged on three broad perspectives: the selection of the study groups (representativeness of exposed cohort, selection of non-exposed cohort, ascertainment of exposure, demonstration that outcome of interest was not present at start of study); the comparability of the groups and the ascertainment of outcome of interest (assessment of outcome, adequacy of follow up, and duration of follow up). A study can be awarded a maximum of one star for each numbered item within the 'selection' and 'exposure' categories. A maximum of two stars can be given for 'comparability'. If the studies were not clear in their inclusion, and exclusion criteria, they scored less on the 'selection of study' groups. The most important factor we chose for comparability of the cohort was age and the additional factor chosen was parity. All other studies except one (Strobino and Pantel-Silverman, 1987), scored five or more on quality assessment out of a total score of nine (Table 24).

Table 24 Newcastle- Ottawa scale for assessment of quality of included studies in the systematic review and meta-analysis investigating the perinatal morbidity in women with threatened miscarriage

Study	Selection	Comparability	Outcome	Total score
Evans and Beischer, 1970	**		***	5
Hertz *et al.,* 1985	****		***	7
Tikreeti and Al-Saadi, 1990	****	**	***	9
Strobino and Pantel-Silverman, 1989	**		**	4
Konje *et al.,* 1992	**	**	***	7
Sipila *et al.,* 1992	**	**	***	7
Tongsong *et al.,* 1995	****	**	**	8
Das *et al.,* 1996	****	**	**	8
Weiss *et al.,* 2004	**	**	***	7
Johns *et al.,* 2006	**	**	***	7
Davari-Tanha *et al.,*2008	**		***	5
Dadkhah *et al.,* 2010	****	**	***	9
Calleja-Agius *et al.,* 2011	***	**	***	8
Akther *et al*., 2014	**	**	***	7

2.4.3.4 Quantitative data summary and synthesis of results

Data were summarised for the pregnancy outcomes described above. A quantitative data summary was done for the outcomes where more than two papers were present; this was not possible for the outcomes of postpartum haemorrhage, adherent placenta, eclampsia, and HELLP syndrome as these were reported only in single studies. A further sensitivity analysis was done for studies published after the year 2000 to assess whether

the improvements in perinatal care and newer ultrasound technology influenced the outcomes.

2.4.3.5 Association with late pregnancy complications

2.4.3.5.1 Stillbirth/Intrauterine fetal death

There were eight studies (n=17131 women) that described stillbirth/intrauterine fetal death as a complication of threatened miscarriage (Calleja-Agius *et al.*, 2011, Davari-Tanha *et al.*, 2008, Evans and Beischer, 1971, Johns, Jemma *et al.*, 2003, Konje *et al.*, 1992, Sipilä *et al.*, 1992, Strobino and Pantel-Silverman, 1987, Tongsong *et al.*, 1995). The meta-analysis showed a risk of 2.14 (95% CI: 1.43 to 3.23) times in women with threatened miscarriage compared to the control group, with low heterogeneity between studies (I^2 = 48%) (Figure 27).

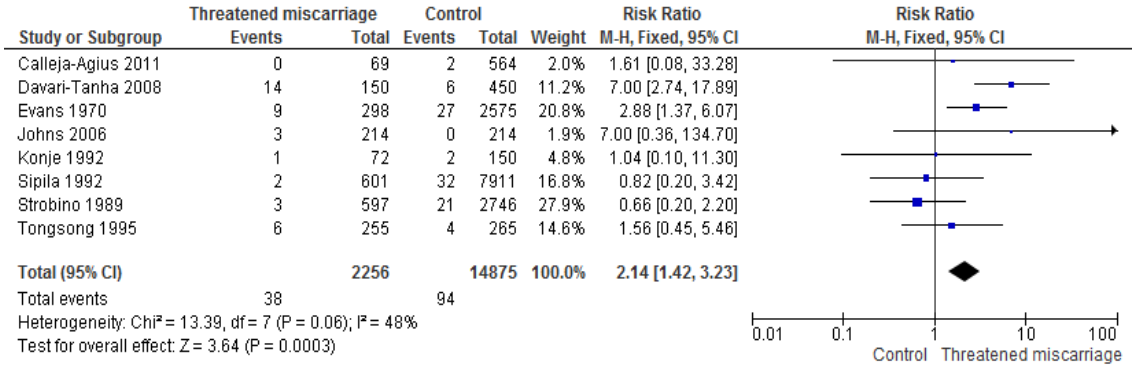


Figure 27 Forest plot summarising the results of the studies investigating the outcome of stillbirth/intrauterine fetal death in women with and without threatened miscarriage (N = 17131)

The subgroup analysis for studies conducted after the year 2000 (Calleja-Agius *et al.*, 2011, Davari-Tanha *et al.*, 2008, Johns, Jemma *et al.*, 2003) showed a significantly increased risk of stillbirth/ intrauterine fetal death in women with threatened miscarriage (RR =6.37, 95% CI: 2.71 to 14.98) (Figure 28). There was no heterogeneity noted between the included studies (I^2= 0%).

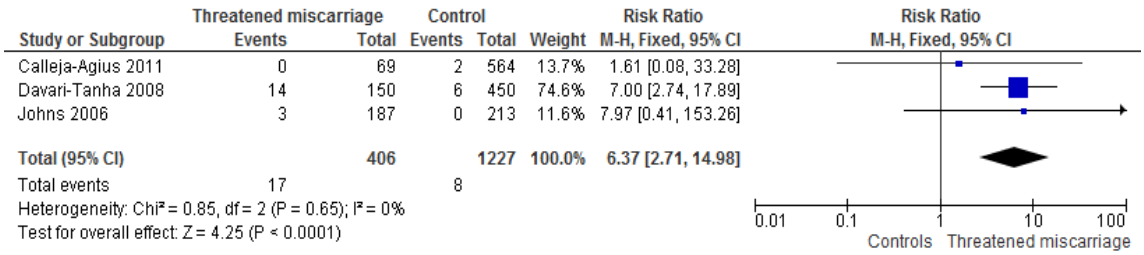


Figure 28 Forest plot on subgroup analysis for the outcome of SB/ IUD on studies performed after the year 2000 in women with and without threatened miscarriage (N = 1633)

2.4.3.5.2 Intrauterine growth restriction/small for gestational age

There were eight studies with 29434 women that studied intrauterine growth restriction/ small for gestational age (Calleja-Agius *et al.*, 2011, Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Hertz and Heisterberg, 1985, Konje *et al.*, 1992, Sipilä *et al.*, 1992, Strobino and Pantel-Silverman, 1987, Tikreeti and Alsaadi, 1990, Tongsong *et al.*, 1995, Weiss *et al.*, 2004). The meta-analysis showed a RR of 1.53 (95% CI: 0.92 to 2.54) and there was substantial heterogeneity noted between the studies (I^2 = 73%) (Figure 29).

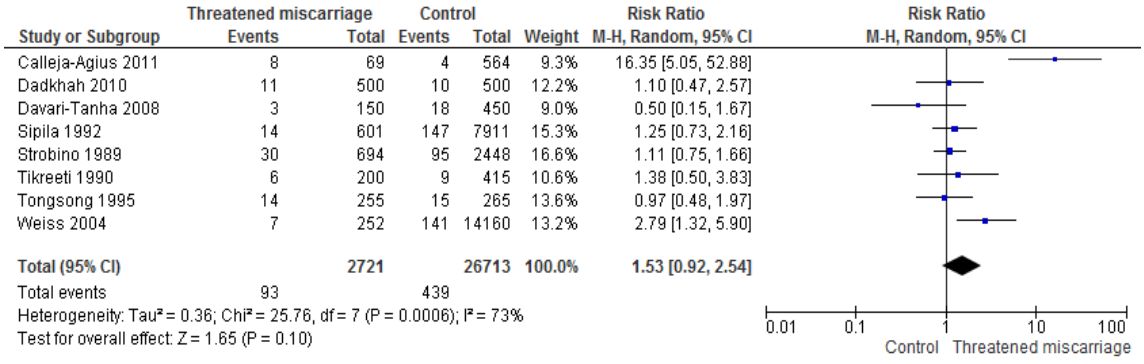


Figure 29 Forest plot summarising the studies on the outcome of IUGR/SGA in in women with and without threatened miscarriage (N = 29434)

The subgroup analysis of studies after the year 2000 (Calleja-Agius *et al.*, 2011, Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Weiss *et al.*, 2004)) showed a RR of 2.22 (95% CI: 0.63 to 7.90) (Figure 30) indicating that although the risk was increased in two out of four of the studies, it was not statistically significant.

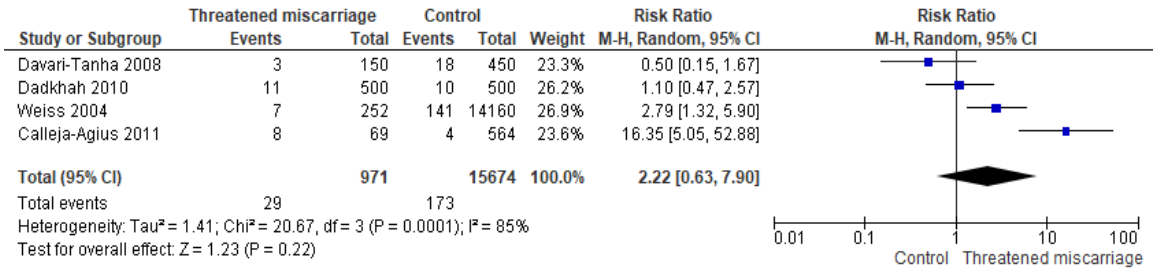


Figure 30 Forest plot on subgroup analysis for the outcome of IUGR on studies performed after the year 2000 in women with and without threatened miscarriage (N = 16645)

2.4.3.5.3 Low birth weight

There were nine studies (n=15023 women) that reported low birth weight as a complication of threatened miscarriage (Calleja-Agius *et al.*, 2011, Das *et al.*, 1996, Davari-Tanha *et al.*, 2008, Hertz and Heisterberg, 1985, Konje *et al.*, 1992, Sipilä *et al.*, 1992, Strobino and Pantel-Silverman, 1987, Tikreeti and Alsaadi, 1990, Tongsong *et al.*, 1995) The meta-analysis showed a RR of 1.64 (95% CI: 1.26 to 2.15) (Figure 31) with moderate heterogeneity. A subgroup analysis was not done for studies after 2000 as there were only two studies available.

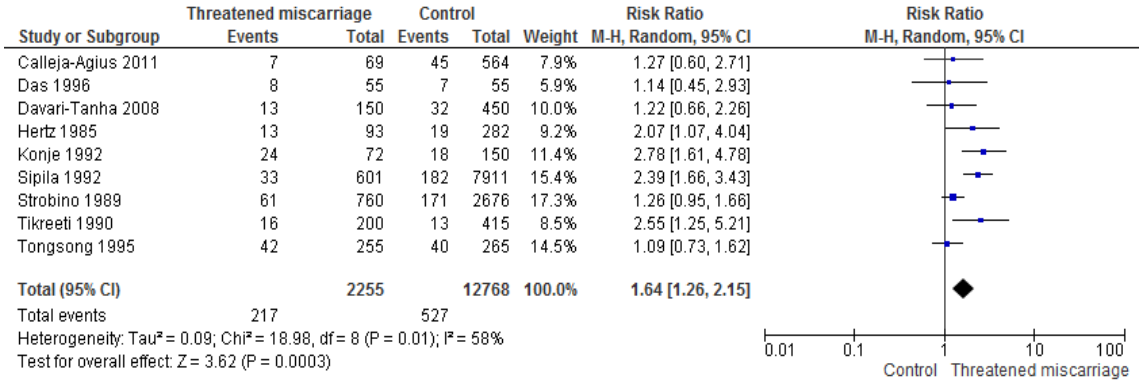


Figure 31 Forest plot summarising the studies on the outcome of LBW in women with and without threatened miscarriage (N = 15023)

2.4.3.5.4 Preterm delivery

There were 14 studies (n= 36116 women) that studied PTD as a complication of threatened miscarriage (Akhter *et al.*, , Calleja-Agius *et al.*, 2011, Dadkhah *et al.*, 2010,

Das *et al.*, 1996, Davari-Tanha *et al.*, 2008, Evans and Beischer, 1971, Hertz and Heisterberg, 1985, Johns, Jemma *et al.*, 2003, Sipilä *et al.*, 1992, Strobino and Pantel-Silverman, 1987, Tikreeti and Alsaadi, 1990, Tongsong *et al.*, 1995) The forest plot shows a combined risk of 2.35 times (95% CI: 1.70 to 3.26) in the threatened miscarriage group compared to the controls; there was substantial heterogeneity noted between the studies (I^2 =88%) (Figure 32).

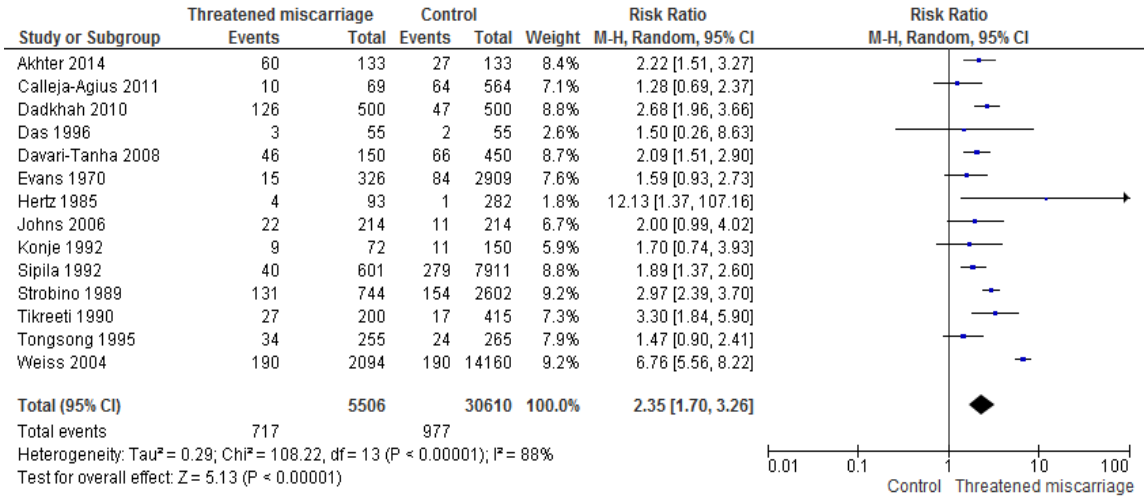


Figure 32 Forest plot summarising the studies on the outcome of PTD in women with and without threatened miscarriage (N = 36116)

Subgroup analysis of the studies after year 2000 (Akhter *et al.*, , Calleja-Agius *et al.*, 2011, Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Johns, Jemma *et al.*, 2003, Weiss *et al.*, 2004) showed a RR of 3.00 (95% CI: 1.73 to 5.20) (Figure 33).

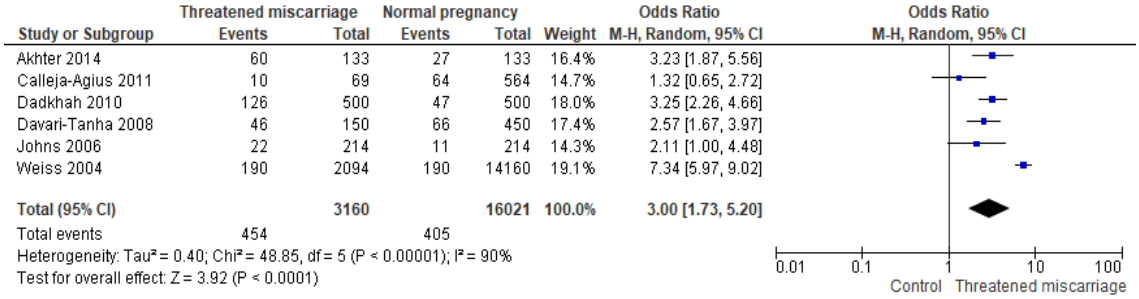


Figure 33 Forest plot on subgroup analysis for the outcome of PTD on studies performed after the year 2000 in women with and without threatened miscarriage (N = 19181)

2.4.3.5.5 Preterm prelabour rupture of membrane

There were nine studies (n= 20280 women) that described preterm prelabour rupture of membrane as a complication of threatened miscarriage (Akhter *et al.*, , Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Hertz and Heisterberg, 1985, Johns, Jemma *et al.*, 2003, Konje *et al.*, 1992, Tikreeti and Alsaadi, 1990, Tongsong *et al.*, 1995, Weiss *et al.*, 2004). The meta-analysis showed a risk of 2.80 times (95% CI: 1.65 to 4.75) in the threatened miscarriage group compared to the control group; significant heterogeneity was noted among the studies (I^2 =82%) (Figure 34).

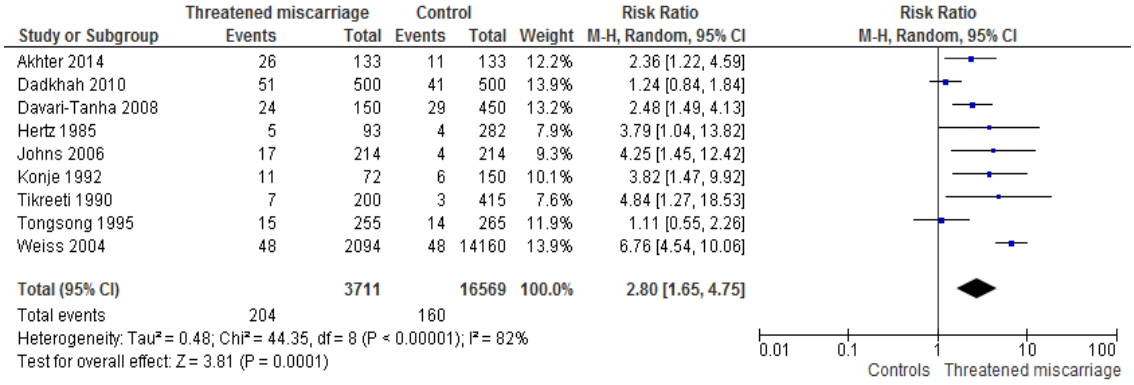


Figure 34 Forest plot summarising the studies on the outcome of PPROM in women with and without threatened miscarriage (N = 20280)

Subgroup analysis of the studies after year 2000 (Akhter *et al.*, , Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Johns, Jemma *et al.*, 2003, Weiss *et al.*, 2004)) showed a RR of 2.86 (95% CI: 1.39 to 5.90) (Figure 35).

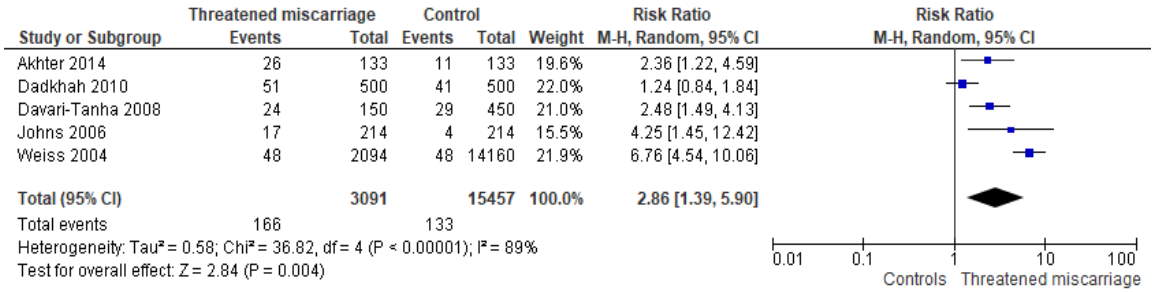


Figure 35 Forest plot on subgroup analysis for the outcome of PPROM on studies performed after the year 2000 in women with and without threatened miscarriage (N = 18548)

2.4.3.5.6 Placental abruption

There were four studies with 18469 women that investigated placental abruption (Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Tikreeti and Alsaadi, 1990, Weiss *et al.*, 2004) The meta-analysis showed a RR of 2.89 (95% CI: 1.29 to 6.47), with significant heterogeneity (I^2 =72%) (Figure 36).

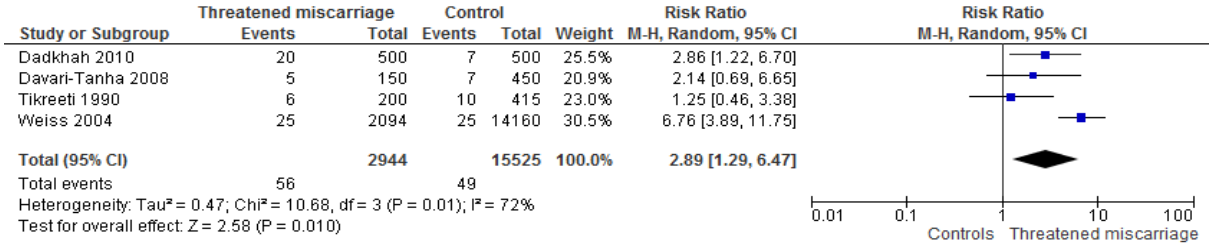


Figure 36 Forest plot summarising the studies on r the outcome of placental abruption in women with and without threatened miscarriage (N = 18469)

Subgroup analysis after year 2000 (Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Weiss *et al.*, 2004) showed a RR of 3.96 (95% CI: 1.89 to 8.30) (Figure 37).

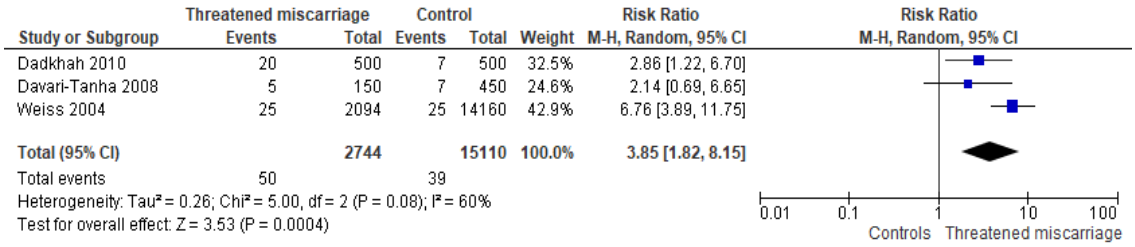


Figure 37 Forest plot on subgroup analysis for the outcome of placental abruption on studies performed after the year 2000 in women with and without threatened miscarriage (N = 17854)

2.4.3.5.7 Pre-eclampsia

There were four studies with 18487 women that described pre-eclampsia as a complication (Calleja-Agius *et al.*, 2011, Dadkhah *et al.*, 2010, Davari-Tanha *et al.*, 2008, Weiss *et al.*, 2004) The meta-analysis showed a RR of 1.45 (95% CI: 0.33 to 6.27) (Figure 38), indicating no significant association between threatened miscarriage and pre-eclampsia.

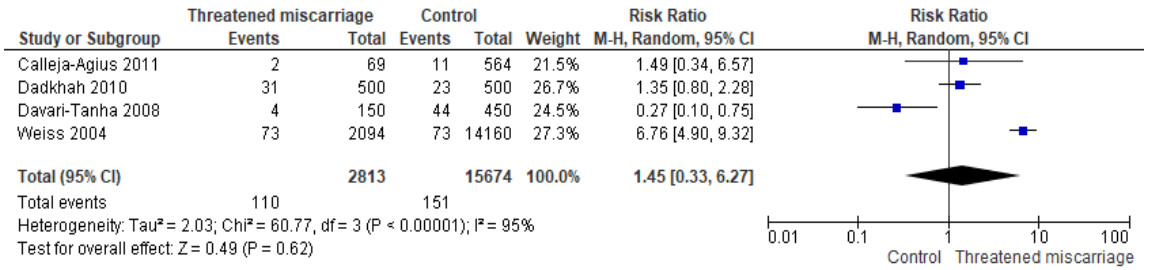


Figure 38 Forest plot summarising the studies on the outcome of PET in women with and without threatened miscarriage (N = 18487)

There were no studies after 2000 for subgroup analysis.

2.4.3.5.8 Placenta praevia

There were four studies with 17579 women that described placenta praevia (Das *et al.*, 1996, Davari-Tanha *et al.*, 2008, Tikreeti and Alsaadi, 1990, Weiss *et al.*, 2004). The meta-analysis showed a RR of 4.13 (95% CI: 2.22 to 7.68) and the heterogeneity was low (I^2 = 12%) (Figure 39), indicating a significant association with placenta praevia in women with threatened miscarriage. A subgroup analysis was not done for studies after 2000 as there were only two studies available.

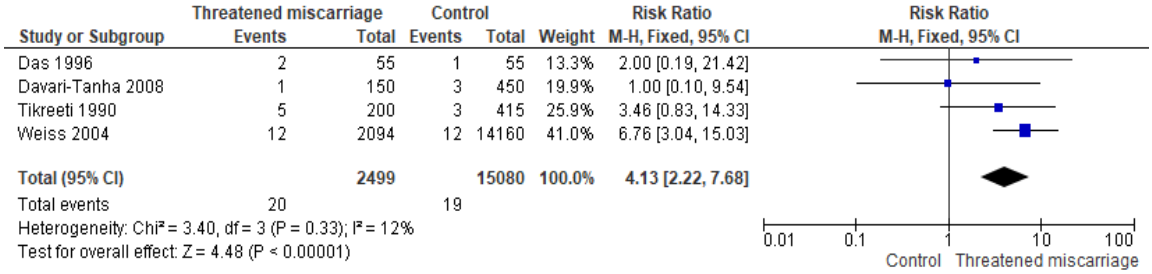


Figure 39 Forest plot on the studies summarising the outcome of Placenta praevia in women with and without threatened miscarriage (N = 17579)

2.4.3.5.9 Pregnancy induced hypertension

There were five studies with 18244 women that studied pregnancy induced hypertension as a complication of threatened miscarriage (Calleja-Agius *et al.*, 2011, Konje *et al.*, 1992, Tikreeti and Alsaadi, 1990, Tongsong *et al.*, 1995, Weiss *et al.*, 2004) The meta-analysis showed a RR of 3.10 (95% CI: 0.96 to 9.97) with heterogeneity among the studies (I^2 =96%) (Figure 40) and indicating no significant association. A subgroup analysis was not done as there were only two studies available after the year 2000.

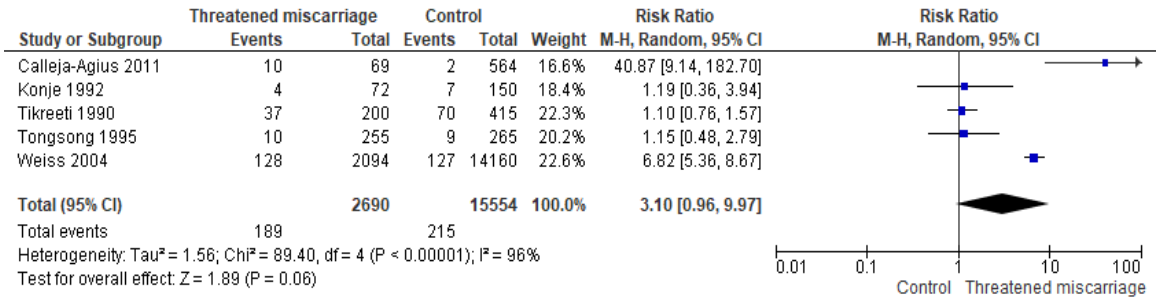


Figure 40 Forest plot summarising the studies on the outcome of PIH in women with and without threatened miscarriage (N = 18244)

2.4.3.5.10 Congenital anomalies/ malformations

There were seven studies (n = 16496 women) that described congenital anomalies/malformations as a complication of threatened miscarriage (Evans and Beischer, 1971, Hertz and Heisterberg, 1985, Konje *et al.*, 1992, Sipilä *et al.*, 1992, Strobino and Pantel-Silverman, 1987, Tikreeti and Alsaadi, 1990, Tongsong *et al.*, 1995) with no significant heterogeneity between the studies (I^2 = 0%) (Figure 41). There were no studies after 2000 for subgroup analysis.

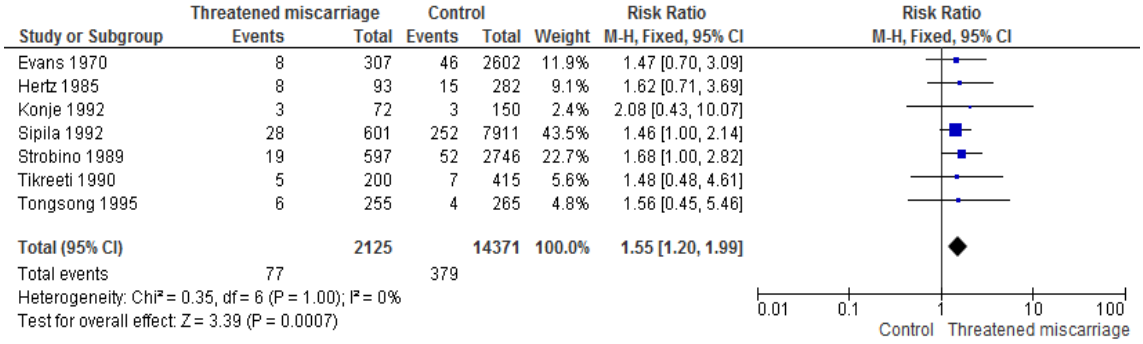


Figure 41 Forest plot summarising the studies on the outcome of congenital anomalies in women with and without threatened miscarriage (N = 16496)

2.4.3.5.11 Neonatal asphyxia

There were three studies (n = 1117 women) that described neonatal asphyxia as a complication of threatened miscarriage (Hertz and Heisterberg, 1985, Konje *et al.*, 1992, Tongsong *et al.*, 1995) The meta-analysis showed a risk ratio of 1.67 (95% CI: 1.10 to 2.56) and there was no significant heterogeneity between the studies (I^2 =0%) (Figure 42). There were no studies after 2000 for subgroup analysis

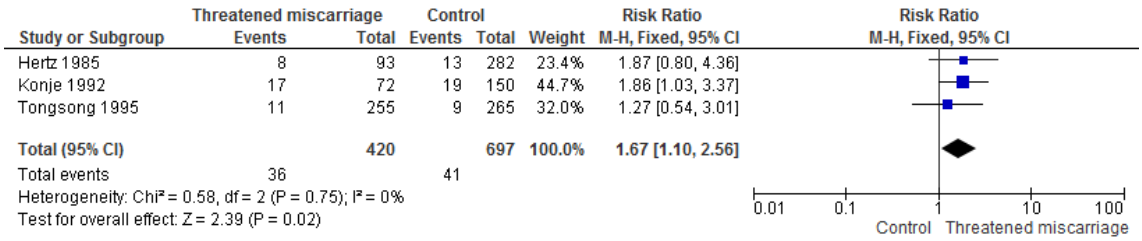


Figure 42 Forest plot summarising the studies on the outcome of neonatal asphyxia in women with and without threatened miscarriage (N = 1117)

2.5 Discussion

2.5.1 Discussion of systematic review 1: Use of biomarkers for predicting miscarriage in women with threatened miscarriage

The systematic review shows that among biomarkers, CA 125 has the highest sensitivity and specificity in predicting miscarriage followed by hCG, oestradiol and serum progesterone. A summary analysis using the HSROC curve was not done for PAPP-A, as the number of studies on PAPP-A were few. It was not possible to do a meta-analysis for the biomarkers HPL, AFP, Schwangerschafts Protein 1 (SP1) and Pregnancy Zone Protein (PZP) (Westergaard *et al.*, 1985); Plasma Renin Activity (PRA), Plasma Renin Substrate (PRS) and Sex-Hormone Binding Globulin (SHBG) (Siimes *et al.*, 1983), inhibin A, activin A, follistatin (Johns, Jemma *et al.*, 2003, Phupong and Hanprasertpong, 2011) and estriol (Dessaive *et al.*, 1982) as there was only a single study available for each of these biomarkers . In the quantitative meta-analysis, a further four studies (Azogui *et al.*, 1996, Jauniaux, Eric *et al.*, 2015, Johns, Jemma *et al.*, 2003, Vavilis *et al.*, 2001) were excluded since the data could not be obtained in the 2 x 2 tables.

There is an extensive list of biomarkers that have been investigated for the prediction of early pregnancy outcome, but these were not included in this systematic review and meta-analysis as the studies did not meet the eligibility criteria. Some of these are activin A (Florio *et al.*, 2007, Kirk *et al.*, 2009, Muttukrishna, S. *et al.*, 2002, Warrick *et al.*, 2012), maternal serum angiogenic factors like placental growth factor, vascular endothelial

growth factor and soluble endoglin (Muttukrishna, Shanthi *et al.*, 2011, Ugurlu *et al.*, 2009), macrophage inhibitory growth factor (Tong *et al.*, 2012), endocannabinoids (Taylor *et al.*, 2011) and cytokine and chemokines (Hannan *et al.*, 2014). The majority of these studies have used a different study population than threatened miscarriage like PUL, missed miscarriage or ectopic pregnancy (Florio *et al.*, 2007, Kirk *et al.*, 2009, Muttukrishna, S. *et al.*, 2002, Taylor *et al.*, 2011, Tong *et al.*, 2012, Warrick *et al.*, 2012). Two studies were of retrospective design with a small sample size (Hannan *et al.*, 2014, Muttukrishna, Shanthi *et al.*, 2011). Hence it is unlikely that not including these studies in the meta-analysis would have affected the results of the meta-analysis.

In the review, CA 125 had shown moderate diagnostic accuracy in predicting miscarriage. Studies have shown that the chorio-decidual plate produces a large amount of CA 125 in early pregnancy and it is released into the blood stream with the trophо-decidual detachment at the time of miscarriage (Check *et al.*, 1990, Hornstein *et al.*, 1995, Scarpellini *et al.*, 1995). However, CA 125 is a very non-specific biochemical marker of cellular activation of mesothelial derived tissues (Scarpellini *et al.*, 1995). It can show false positive results with concomitant clinical conditions like endometriosis, pelvic inflammatory disease, liver disease, uterine fibroids and cancers of the ovary, endometrium and fallopian tube. Therefore, it should be used with caution as a predictor of miscarriage. The only negatively reported study for CA 125 (Vavilis *et al.*, 2001) was not included in the meta-analysis and this was because the results were presented using mean and standard deviation.

The biomarker, hCG is found to have the second-best predictive accuracy in this systematic review. hCG is the earliest detectable marker and is still the mainstay of modern pregnancy diagnosis. Serum hCG can be detected as early as 8-11 days following ovulation (i.e., shortly after implantation) (Carmona *et al.*, 2003). The level of hCG in the blood increases rapidly with a maximum level of 50, 000-1, 00,000 IU/ml attained at about 8-10 weeks of gestation. The advantage of hCG is that it shows a consistent nature in the pattern in which it rises which makes the quantitative determination of hCG easier and hence even now hCG remains as a valuable tool in the clinical assessment of early

pregnancy abnormalities (Duan *et al.*, 2011) Although a combination of biomarkers may give higher predictive value, all other studies that used combination markers except one (Scarpellini *et al.*, 1995)) did not meet the inclusion criteria for the review (Hertz and Schultz-Larsen, 1983, Kunz and Keller, 1976, Osmanağaoğlu *et al.*, 2010).

One of the common limitations of the studies included in this systematic review was that they did not specify a cut-off value for the respective biomarker in the prediction of miscarriage. Because of this drawback, we could not determine a useful 'cut off level' but could only comment on the utility of each biochemical marker in predicting miscarriage. Despite the well-established evidence that the levels of serum progesterone, hCG, CA 125, oestradiol and serum PAPP-A change with each week of gestation, most of the included studies did not take this into consideration. Ideally, gestation specific normal values should be used to compare the levels of these biochemical markers.

Another pitfall is the quality and reporting of the included studies. To improve the reporting of the diagnostic accuracy studies, the STARD checklist (Bossuyt, P. M. *et al.*, 2015) was published in 2003. Most of the included studies in this review were published before 2003 except for four (Hanita *et al.*, 2012, Maged and Mostafa, 2013, Phupong and Hanprasertpong, 2011, Xie *et al.*, 2014). There was a large amount of missing information in the older studies and many had an inadequate reporting format. However, even the recently published studies have drawbacks in their reporting format. Some of these studies could not be included in the meta-analysis (Azogui *et al.*, 1996, Johns, Jemma *et al.*, 2007, Vavilis *et al.*, 2001) due to the difference in their reporting statistics.

In conclusion, biochemical markers are a useful tool to predict the outcome of threatened miscarriage, particularly serum CA125. Recently, high-sensitivity C-reactive protein (hs-CRP) has been highlighted as a useful predictive marker in threatened miscarriage (Jauniaux, Eric *et al.*, 2015) and its role needs to be further studied in larger studies. In order to reliably interpret the biochemical markers in early pregnancy, pre

specified cut-off values and gestational age specific nomograms are required. To reduce patient anxiety and to be cost-effective, it is important to emphasise on biomarkers that can reliably predict an ongoing pregnancy rather than predicting miscarriage. Large well-designed prospective cohort studies are needed in the future with rigorous quality control and reporting methodology to accurately predict miscarriage outcome.

2.5.2 Discussion of systematic review 2: Use of ultrasound markers to predict miscarriage in threatened miscarriage population

Among all the USS markers, FHR was found to have the highest diagnostic accuracy in predicting miscarriage in women with threatened miscarriage (sensitivity of 84.18 and specificity of 95.68%). Above a cut off level of 110bpm, the predictive accuracy of FHR diminishes.

Based on the analysis of seven studies including 1467 women, our review has determined a FHR cut off of 110 bpm between 6-14 weeks gestation which had the best diagnostic accuracy for predicting miscarriage. However, in view of the small number of studies these results need to be interpreted with caution. It has been reported that if the transducer is pressed firmly against the vagina during USS scan can result in an increase in the intra uterine pressure which can be transmitted to the uterine cavity and to the embryo including the umbilical vessels. This can cause extreme transient fetal bradycardia in an eventually normal pregnancy (Mendoza *et al.*, 1989). Therefore, a single observation cannot be considered as a predictive marker unless an experienced operator conducts the ultrasound scan. Pregnancies with chromosomal anomalies or poor development of the conduction system can also show an association with fetal bradycardia (Shenker *et al.*, 1986).

Other ultrasound markers investigated such as IUH, CRL, and MGSD-CRL were noted to have lower predictive values. Theoretically, a pressure effect from the IUH can cause irritation to the uterus and affect pregnancy outcome and this depends on its

size/volume and location in relation to the placenta (Johns, Jemma *et al.*, 2003, Kurjak *et al.*, 1996, Mantoni, 1985). The existing literature shows variable evidence on the impact of IUH on the occurrence of miscarriage with some studies supporting an increased miscarriage rate (Borlum *et al.*, 1989, Mantoni, 1985, Sauerbrei and Pham, 1986) and some studies not supporting an association with miscarriage (Johns, Jemma *et al.*, 2003, Pedersen and Mantoni, 1990, Stabile *et al.*, 1987). This variability within the existing literature is reflected in the current meta- analysis and it demonstrated that the presence of an IUH on USS is not a useful tool in miscarriage prediction.

The meta-analysis also showed that CRL has a lower predictive value than FHR for miscarriage (sensitivity of 59.81% for CRL versus sensitivity of 68.41% for FHR and specificity of 55.68% for CRL versus specificity of 97.84% for FHR) and this might be because few of the included studies did not take into consideration the gestational age variation in CRL (Maged and Mostafa, 2013, Reljic, 2001) and might have contributed to the disparity in the results.

The existing literature has shown abnormal YS size and appearance also to be useful markers for miscarriage prediction before the demonstration of fetal viability (Chama *et al.*, 2005, Küçük *et al.*, 1999b), but in women with viable intra uterine pregnancy with a demonstrable heartbeat, we found its usefulness was limited. This might be due to the fact that once the FHR is observed, the yolk sac becomes less significant and therefore not reported in the included studies and review, in the symptomatic women with viable intra uterine pregnancy, there were no studies identified on yolk sac.

Various models have been developed using other markers and patient demographics to improve the diagnostic accuracy of USS biomarkers in the prediction of miscarriage. A logistic regression model developed by Oates *et al.* (Oates *et al.*, 2013) combined demographic variables of the last menstrual period, vaginal bleeding and ultrasound markers of mean GS size and mean YS size. The model had a sensitivity of 82% and a specificity of 79%. The drawback of the model was it had too many variables and performed less well than the single ultrasound parameter of FHR in the current meta-

analysis (fetal bradycardia has a sensitivity of 68.41% and specificity of 97.84%). The other probable reason for the poor performance of the model was the use of GSD and YSD. These markers have shown to be poor predictors of miscarriage in our meta-analysis. The study by Jun *et al*. (Jun *et al.*, 1992) showed that a model using GS and CRL had a sensitivity of 76.5% and a specificity of 96.8% and with a further addition of FHR in the model, the prediction of miscarriages improved to 94.1%. A model combining gestational age and FHR (Varelas *et al.*, 2008) showed a sensitivity of 91% and a specificity of 100%. This study further indicates that FHR is a good predictor of poor pregnancy outcome in the first trimester.

One of the limitations of this systematic review and meta-analysis was both TA and TV modalities of scanning were used in the included studies to measure ultrasound markers, which could contribute to measurement bias. A study by Kaur et al. investigating the merits and demerits of TV scan over TA scan in early pregnancy has shown that TV scan provided 78.3% additional information compared to TV scan in detection gestational sac, yolk sac and in the visualisation of embryonic anatomy (Kaur and Kaur, 2011). A sensitivity analysis based on the scanning approach was not possible due to the low number of studies. The quality of some of the included studies as determined by the 'QUADAS-2: A Revised Tool' was another major limitation of this systematic review and meta-analysis.

In conclusion, FHR with a cut off level of less than 110bpm was found to be the single most useful marker for the prediction of miscarriage. Other markers of CRL, IUH, GS and YS had a lower predictive value. In the future, large well-designed prospective cohort studies are needed on this topic with rigorous quality control and reporting methodology, specifically looking into a combination of markers.

2.5.3 Discussion of systematic review 3: Perinatal outcome of women with threatened miscarriage

This meta-analysis demonstrates a strong association of threatened miscarriage with perinatal complications. The results showed that the relative risk of

stillbirth/intrauterine fetal death in those who have had threatened miscarriage was 2.14 times compared to the controls. For studies after the year 2000, the relative risk was 6.37 times in the threatened miscarriage group; although the confidence interval was wide, the observed increase in the risk could be related to the better reporting of stillbirth/intrauterine fetal death after the year 2000. Furthermore, the results showed a two-fold increased risk of preterm delivery and an almost three-fold increased risk of preterm pre-labour rupture of membrane and placental abruption. There was a smaller but significant increased risk of low birth weight. Other outcomes including placenta praevia, neonatal asphyxia and congenital anomalies were also more likely to occur in women who had suffered from a threatened miscarriage in early pregnancy. The sensitivity analysis of studies after the year 2000 demonstrated that despite advances in maternity care, the risk of adverse outcomes for women with threatened miscarriage remained high.

It is known that vaginal bleeding in threatened miscarriage originates from the spiral arteries in the deciduo-placental interface and the uterine wall during the formation of the placenta and the membranes (Johns, Jemma *et al.*, 2003). Subsequently, this leads to chronic inflammatory reaction and further disruption of the chorio-amniotic space (Johns, J. and Jauniaux, 2006). This chronic inflammatory reaction can impair utero-placental circulation resulting in an increased risk of stillbirth, low birth weight and placental abruption. In addition, this can lead to increased uterine irritability and the risk of preterm pre-labour rupture of membrane and preterm delivery. Preterm labour and preterm pre-labour rupture of the membrane could further contribute to the increased risk of stillbirth/intrauterine fetal death. Although placenta praevia was an outcome in only four studies, it was 4.13 times higher in the threatened miscarriage group compared to the control. This could be due to abnormal placentation with repeated episodes of bleeding during the phase of placental development. As expected, there was no significant association between threatened miscarriage and pre-eclampsia; this is likely due to the completely different aetiopathogenesis of pre-eclampisa. Intra uterine growth retardation did not show a significant association with threatened miscarriage. This could be due to the lack of a standard definition in the included studies contributing

to the underestimation of intra uterine growth retardation. One study defined intra uterine growth retardation as estimated fetal weight by ultrasound of less than 10th percentile or birthweight of less than 10th percentile for the gestational age (Davari-Tanha *et al.*, 2008). Whereas another study defined intra uterine growth retardation as a documented fall off in growth velocity on ultrasound scan. In contrast, for low birth weight, a standardised definition was used among the studies and the results showed increased risk in women with threatened miscarriage. The increased risk of low birth weight could also be due to the higher occurrence of preterm delivery.

In conclusion, this updated systematic review focussing on well-characterised prospective studies has demonstrated a clear and significant increased risk of perinatal complications after threatened miscarriage.

2.6 Summary

The systematic reviews were able to summarise the large volume of existing literature and point towards the most useful markers both biochemical and ultrasound, which can be used in predicting miscarriage. The systematic reviews also helped in highlighting the inadequacies of existing literature. The existing literature, especially the older studies, have an inadequate reporting format making it difficult to extract the data (Stoppelli *et al.*, 1981b). In order to address this pitfall, various reporting guidelines have been developed, like STROBE statement for reporting of observational studies (Von Elm *et al.*, 2007). Henceforth, the recent studies have been more transparent in their reporting. Despite this, there were still studies that were not very explicit in reporting (Maged and Mostafa, 2013).

The systematic reviews only included prospective studies and the main reason behind this was to maintain the quality of the systematic reviews. In the Oxford CEBM (Centre for Evidence Based Medicine) levels of evidence (https://www.cebm.net), a systematic review of retrospective studies will come under level two evidence. However, systematic review of prospective prognostic studies will be classified as level 1 evidence. But it is be noted that miscarriage is a significant event in life and is unlikely to be

affected by re-calling bias which is one of the important concerning features of retrospective studies.

Another drawback was the reporting statistics. Studies did not follow a uniform measure to report the results which made it impossible to use their data in the meta-analysis. For example, some studies have used mean and standard deviation (Johns, Jemma *et al.*, 2007), whereas others have used median and lower/upper quartile (Jauniaux, Eric *et al.*, 2015). Most studies have used sensitivity and specificity as a statistic tool to report the results. The meta-analyses carried out above show, in most cases, a consistently high specificity but variable sensitivity. This may be because the cut-off levels used in the studies were biased to higher specificity. Unfortunately, the precise cut off levels used was not reported in the majority of the studies, and hence the review failed to develop a cut off value for the biomarkers and ultrasound markers that can be used in practice, limiting the practicality of these markers.

Another major limitation to be addressed is the gestational age-specific variation of the biomarker which was not considered in the majority of the pre-existing literature. As the biomarker levels vary based on the gestational age, unless it is taken into account, it can be a limiting factor in estimating the normal and abnormal values of the biomarkers. A significant drawback of the systematic review on USS markers was that the studies on USS markers have used both transabdominal (TA) and transvaginal (TV) scan methods for the study. However, there were not enough studies to do a subgroup analysis based on the scanning method. Also, intra-observer and inter-observer variability of ultrasound scan measurements can contribute to bias in the results. There were studies conducted on combination of markers. However, there were not enough studies to do a meta-analysis on combination markers, and this is partly due to the variety of combinations being investigated.

Henceforth in the future, well defined prospective cohort studies are required with rigorous quality control and reporting methodology to investigate further the role of biomarkers, ultrasound markers and demographic variables to improve the prediction

of miscarriage. In order to reliably interpret the results, it is crucial to define pre-specified values for the markers and account for the gestational age variation in the level of biomarkers and ultrasound markers.

3 Methodology

Abstract: This chapter describes the rationale and methodology of the study 'Prediction of miscarriage in women with threatened miscarriage using a combination of biochemical and ultrasound scan markers.' The study aimed to answer the hypothesis that the outcome of threatened miscarriage can be predicted by using biochemical markers (serum progesterone, hCG, PAPP-A, inhibin A, oestradiol, hs-CRP and CA 125) and USS markers (FHR, gestational sac size, CRL, size of YS, trophoblast thickness/volume and presence of corpus luteum and hematoma) and demographic variables either alone or in combination. To answer this hypothesis, a prospective study design was selected with two study cohorts, women experiencing threatened miscarriage in early pregnancy (cohort A) and an asymptomatic cohort of women in the first trimester of pregnancy (cohort B). This chapter elaborates on the inclusion, exclusion criteria, recruitment, index tests, outcomes measures and timing and follow up of the study. The chapter also touches on data collection and the statistical techniques used to analyse the data.

3.1 Introduction

The systematic reviews highlighted that large, well-designed prospective cohort studies, with rigorous quality control and reporting methodology, are needed to accurately predict the miscarriage outcome in women with threatened miscarriage. Because different biochemical and USS parameters have been shown to be predictors of outcome for patients with threatened miscarriage, it follows that using a combination of parameters may improve the diagnostic accuracy. Combination of markers or prediction models have been tested before (Jun *et al.*, 1992, Oates *et al.*, 2013, Scarpellini *et al.*, 1995, Varelas *et al.*, 2008) but those combinations were selected randomly and not from well conducted studies showing good evidence on its utility. We hypothesised that a combination of biochemical, USS markers and demographic variables which demonstrates the highest diagnostic accuracy in the proposed study, may have a better predictive value. The predictive value will be increased with strict adherence to diagnostic study quality criteria and using a gestational age-specific cut off levels for the markers. Moreover, the current study aims to follow the STARD 2015 checklist for conducting and reporting diagnostic accuracy studies (Bossuyt, P. M. *et al.*, 2015).

From the systematic review on biochemical markers, it was evident that CA 125 was the best available biochemical marker in predicting miscarriage, followed by serum oestradiol. Johns *et al.,* 2007 which scored high on quality assessment, studied inhibin A, activin A, hCG, PAPP-A and follistatin in a threatened miscarriage population. They showed significantly lower concentrations of inhibin A, PAPP-A and hCG in those who had first trimester miscarriage compared to those who had term pregnancies. We could not include this study in our meta-analysis as the results were expressed as mean and standard deviation. Therefore, we decided to investigate the value of inhibin A and PAPP-A in our study. Also, a recently published paper (Jauniaux, Eric *et al.*, 2015) explained a new marker for the prediction of pregnancy loss, high-sensitivity C-reactive protein (hs-CRP). The paper described that women whose serum hs-CRP levels are greater than 75% percentile had a decreased odds ratio for pregnancy loss. This prompted us to add hs-CRP as one of the biomarkers to be investigated. Existing

evidence regarding serum beta hCG and progesterone for predicting ongoing pregnancy in threatened miscarriage is of poorer quality. Therefore, beta hCG and serum progesterone were added to the list of biomarkers to be investigated further.

The systematic review completed on ultrasound scan markers had shown FHR as the single best marker in predicting miscarriage, followed by crown rump length. All the studies that investigated YS in miscarriage prediction (Stampone *et al.*, 1996, Tan, Sinan, Tangal *et al.*, 2014, Tan, S. *et al.*, 2011) were asymptomatic pregnancies. Our systematic review had shown a wide variation in sensitivity for YS (17% -69%) in predicting the outcome of threatened miscarriage and there were quality concerns in the included studies. Therefore, we decided to investigate it further in our cohort study. Two studies (N=349 women) evaluated the MGSD minus CRL difference (MGSD-CRL) in the prediction of miscarriage in women with confirmed fetal viability. These had a sensitivity range of 39% -96% and a specificity range of 73% - 88% (El-Mekkawi *et al.*, 2015, Falco *et al.*, 1996). Again, the studies in the review showed a wide heterogeneity in the results and therefore we decided to include gestational sac also in our cohort study. There is evidence to suggest that trophoblastic thickness variation has a sensitivity of 82% and specificity of 93% in predicting miscarriage (Bajo *et al.*, 2000), but this didn't qualify for the meta-analysis due to the lack of ample studies. Though there are not many studies investigating the relationship between corpus luteum and miscarriage, it was demonstrated that a decreasing corpus luteum volume before eight weeks is associated with a higher probability of early pregnancy loss (Glock *et al.*, 1995).

Therefore, we hypothesised that the outcome of threatened miscarriage can be predicted by using biochemical (serum progesterone, hCG, PAPP-A, inhibin A, oestradiol, hs-CRP and CA 125) and USS markers (FHR, gestational sac size, CRL, size of YS, trophoblast thickness/volume and presence of corpus luteum and hematoma) either alone or in combination. This hypothesis was investigated by undertaking a prospective study to test the objectives below.

3.2 Primary objectives

1. To investigate whether the value of serum markers such as hCG, progesterone, PAPP-A, inhibin A, oestradiol, hs-CRP and CA 125 are significantly different in women who experience miscarriage from those women who continue their pregnancy, in women diagnosed with threatened miscarriage from 6+0 to 11+6 weeks of gestational age.
2. To investigate whether the value of USS markers like FHR, MGSD, CRL, YSD, trophoblast thickness, trophoblast volume, presence of hematoma and presence of the corpus luteum are significantly different in women who experience miscarriage from those women who continue their pregnancy, in women diagnosed with threatened miscarriage from 6+0 to 11+6 weeks of gestational age.
3. To create a prediction model for miscarriage in women presenting with threatened miscarriage using the serum biochemical markers (serum progesterone, hCG, Inhibin A, oestradiol, PAPP-A, hs-CRP and CA 125), USS markers (FHR, MGSD, CRL, YSD, trophoblast thickness, trophoblast volume, presence of hematoma and presence of corpus luteum) and maternal demographics (age, partner's age, parity, body mass index, ethnicity, smoking status, alcohol status, caffeine intake, the intensity of bleeding and history of previous miscarriage).

3.3 Secondary objective

To investigate any association between threatened miscarriage and adverse outcomes of pregnancy such as still birth/ intra uterine death, pre-eclampsia, pregnancy induced hypertension, intra uterine growth retardation, low birth weight, placental abruption, preterm delivery and preterm prelabour rupture of membrane.

3.4 Study design

In order to address the study objectives, we designed a prospective diagnostic accuracy cohort study called Prediction of Miscarriage (POM) study. All eligible participants who presented to the Early Pregnancy Assessment Unit (EPAU) at the University Hospitals of Leicester NHS Trust (UHL) during the study period were approached. The unit serves an ethnically diverse population, and it is the tertiary referral centre for the whole of

Leicestershire. This was a nurse led unit with around 7800 appointments per year. Women with early pregnancy complications are referred to the unit by their General practitioner (GP), Midwives, Urgent care centre or from the Gynaecology assessment unit. All the referrals were initially assessed by a specialist gynaecology nurse followed by an ultrasound scan. After the scan, the nurses reviewed them and discussed further management. Management offered can be either reassurance, treatment for miscarriage or ectopic pregnancy, further evaluation with beta hCG or referral to a medical doctor for further advice. A normal asymptomatic cohort of women was recruited from the pregnancy booking clinics in the community through the community midwives (CMW) and from the EPAU. There are approximately 150 community midwives working in 10 teams covering Leicestershire and Rutland. They book approximately 10,000 women per year to deliver at UHL and up to 2000 more to deliver at neighbouring units.

3.5 Participants

There were two cohorts for the research study. Cohort A were women presenting with threatened miscarriage and gestational age of 6^{+0} to 11^{+6} weeks gestation and cohort B were pregnant women presenting to the EPAU without symptoms of threatened miscarriage between 6^{+0} to 11^{+6} weeks gestation or pregnant women without symptoms of threatened miscarriage booked with CMW between 6^{+0} to 11^{+6} weeks gestation.

3.5.1 Exclusion criteria

Women with known uterine anomalies/uterine myomas, multiple pregnancy, extra uterine pregnancy or suspected trophoblastic disease, women underwent fertility treatment, women having known endometriosis or adnexal masses, women taking or having taken exogenous hormones peri-conceptionally, women with type 1 and 2 diabetes or other medical disorders that can increase the risk of miscarriage, women less than 16 years of age and differently abled women were excluded from the study.

3.6 Recruitment

3.6.1 Cohort A (Threatened miscarriage cohort)

Women who presented to the EPAU, and met the eligibility criteria for the study, were given a patient information leaflet (PIL) about the study by the EPAU nurses. If they volunteered to participate, they were offered an appointment with the Clinical Research Fellow (CRF) for an hour. Also, pregnant women who were found to be eligible for the study on reviewing the EPAU attendance list were contacted through telephone by the CRF. Study information was given by telephone and if they agreed to participate, an appointment was made with the CRF. At this appointment, a patient information leaflet was given and the study was discussed again if it was not given before, written consent was obtained, a questionnaire was filled in by the participant regarding the amount of bleeding (using a pictorial chart to semi-quantify the blood loss) (Warrilow *et al.*, 2004), number of days of bleeding, associated abdominal pain, age, BMI, parity, medical history, the quantity of daily caffeine intake, number of cigarettes smoked per day, estimated daily alcohol and recreational drug intake. A blood test and USS followed this. The participants were advised to return to the EPAU on the occurrence of any further PV bleeding.

3.6.2 Cohort B (Asymptomatic Cohort)

Pregnant women who attended EPAU without symptoms or threatened miscarriage, if they were willing to participate and met the eligibility criteria were given an appointment with the CRF for an hour. Also, pregnant women without the symptoms of threatened miscarriage who were found to be eligible for the study on reviewing the EPAU attendance list were contacted through telephone by the CRF. Study information was given by telephone and if they agreed to participate, an appointment was made with the CRF. At this appointment, the patient information leaflet was given if it was not given before and the study was discussed again, a written consent was obtained, study proforma filled in, followed by a blood test and USS.

The CMW provided information of women who met the inclusion criteria for cohort B of the study and wished to know more about the study to the CRF. The CRF contacted these women and gave them the detailed information of the study. If they were willing to participate, they were given an appointment at the hospital with the CRF. On their visit, a patient information leaflet was given, and the study was discussed again. A written consent was obtained, the study questionnaire was filled in, followed by a blood test and USS. Alternatively, eligible participants were identified from the maternity notes when they arrived for booking in the hospital by the clinical research fellow alongside the midwives who were screening these notes. Those eligible participants were sent a patient information leaflet and were contacted through telephone. Study information was given again through telephone and if they were willing to participate in the study, they were given an appointment in the hospital with the clinical research fellow. The women were advised to attend EPAU if they experienced any PV bleed later in the pregnancy.

3.7 Index tests

3.7.1 Biochemistry

20 ml blood sample was collected from each volunteer and was used for quantification of biomarkers. Four biomarkers were analysed (hCG, progesterone, oestradiol and hs-CRP) according to the hospital's Standard Operating Policy using an automated analyser ADVIA centaur XP Immunoassay system. The machine works on the principle of direct chemiluminescence immunoassay. Inhibin and PAPP-A were analysed using the PerkinElmer AutoDELFIA automatic immunoassay system which is a solid-phase, two-site fluoroimmunometric assay based on the direct sandwich technique in which two monoclonal antibodies are directed against two separate antigenic determinants on the dimeric inhibin A molecule. Biomarker hs-CRP was analysed using a Behring Nephelometer II (BNII) from Siemens Healthcare Diagnostics Ltd. Polystyrene particles coated with monoclonal antibodies specific to human CRP were aggregated when mixed with samples containing CRP. These aggregates scattered a beam of light passed through the sample. The intensity of the scattered light is proportional to the

concentration of CRP in the sample. The result was evaluated by comparison with a standard of known concentration.

UHL lab is a Clinical Pathology Accreditation UK Ltd (CPA) accredited lab (Reference number: 3040) and was assessed to be in conformance with standards for the medical laboratory.

3.7.2 Ultrasound scans

To avoid inter observer variability, the principal investigator (RP) did all the scans using the same USS machine for all the patients. A GE Voluson S6 (GE Voluson^{TM} E8 BT16) machine was used for all the ultrasound scans using a 5MHZ transvaginal probe. The mechanical index was kept less than one and the thermal index was always kept less than 0.2 throughout the scanning.

All scans were done with the patient lying in the lithotomy position using a trans-vaginal probe. The probe was gently inserted into the vagina and the uterus and the cervix was visualised in the sagittal plane and then, the probe was gently moved from one edge of the uterus to the other to screen for multiple pregnancy or fibroid uterus. Once the uterus and the cervix were completely examined in the sagittal plane, the probe was rotated ninety degrees to the coronal plane. The uterus and the cervix were visualised from the fundus to the tip of the cervix in the coronal plane. While in the coronal plane, the tip of the probe was tilted to the right and the right adnexa and right ovary was screened in the coronal and sagittal plane and then this was repeated in the left adnexa as well. Finally, the pouch of Douglas was screened. The measurements were done using frozen sections of the images using callipers.

Gestational sac

Gestational sac measurements were performed from the inner edge to the inner edge and the outer edge to the outer edge of the gestational sac in three planes. The inner and outer volume of the gestational sac was calculated. The volume is calculated using the formula for the ellipsoid (Volume=AXBXCX0.523). The Mean Gestational Sac

Diameter was calculated by calculating the mean of the three inner-to-inner edge measurements in three planes.

Yolk sac diameter

The inner-to-inner diameter of the yolk sac was measured. The inner diameter of the yolk sac was measured by placing the callipers on the inner limits of the longest diameter of the yolk sac.

Crown rump length

The most accurate and reproducible measurement in early pregnancy is the crown rump length. The measurement was taken along the midline sagittal section of the fetus or the embryo oriented horizontally on the screen. The screen was magnified to fill most of the ultrasound screen so that the measuring calliper was horizontal to the ultrasound beam. The fetus was measured in the neutral position with care taken to avoid any structures such as the yolk sac. In very early pregnancy, it was very difficult to differentiate between the cephalic and caudal end and in those situations, the greatest diameter of the embryo was measured (Salomon *et al.*, 2013). From six to nine weeks, the fetal pole grows at the rate of 1mm per day.

Fetal heart rate

A fetal heart can be seen to flicker even before a fetal pole is identified. A cardiac activity can be seen from as early as 35 days of gestational age (Jurkovic *et al.*, 1995). Except in a small proportion of embryo measuring 2-4mm on CRL, cardiac activity is usually seen from a CRL of 2mm or more (Goldstein, Steven R., 1992, Levi *et al.*, 1990, Tezuka *et al.*, 1991). The M mode was used for measuring the fetal heart rate. Due to its limited acoustic output, M mode appears to be safe for all stages of pregnancy (Abramowicz *et al.*, 2000, Torloni *et al.*, 2009). A Doppler ultrasound was not used for measuring fetal heart rate due to the possibility of potential bioeffects arising from greater energy output, especially when applied to a small region of interest (Hershkovitz *et al.*, 2002).

Trophoblast thickness

Trophoblast plate (CD) thickness was measured from the point of attachment of the umbilical cord to the outer edge of the gestational sac. Early on in pregnancy, when the umbilical cord was not yet formed, the trophoblast thickness was measured opposite to the location of the yolk sac. Also, the thickness of the gestational sac exactly opposite to the first measurement was done. This measurement was subtracted from the previous measurement to obtain the trophoblast thickness. The trophoblast volume was calculated by subtracting the inner gestational sac volume from the outer gestational sac volume.

Presence of hematoma

The intrauterine pregnancies were screened during the scan for any presence of hematoma and noted down if a hematoma was seen.

Corpus luteum

Both ovaries were screened for corpus luteum and noted down whether a corpus luteum was seen or not.

3.8 Outcomes

The primary outcome investigated for the study was the occurrence of miscarriage/ successful continuation of pregnancy beyond 23 completed weeks of pregnancy. We also looked into the long term pregnancy outcomes such as pre-eclampsia, pregnancy induced hypertension, intra uterine growth retardation, low birth weight babies (<2.5kg at birth), placental abruption, preterm pre-labour rupture of membrane, preterm delivery, neonatal asphyxia, still birth and Intrauterine death.

3.9 Blinding

The outcome measures (miscarriage or pregnancy complications) could not be influenced by the investigator. Therefore, blinding of the measurements from the investigator was not required. This was because both the blood tests and the USS measurements (index test) were done on the same day and before the outcome of pregnancy was known. The occurrence of miscarriage was not a subjective outcome that can alter depending on the prior knowledge of the result of the index test to the data collector.

3.10 Timing and follow up

The participants were advised to come to the EPAU if they developed any further episodes of vaginal bleeding. They were followed up as per the local protocol for women attending the EPAU. The follow up details for the study subjects were collected from the patients' medical records. The primary outcome for the study was the continuation of pregnancy beyond 24 weeks and the details of this outcome was collected from E3 – an online robust and up-to-date obstetric database maintained by the University Hospitals of Leicester. Any missing data from the records were obtained by contacting the participant's GP or CMW.

3.11 Data collection

Data were entered into the case record file (CRF) and to the Microsoft excel sheet. Data screening was conducted three times to look for missing data, outlying values and to transform it to make it suitable for analysis. Data checks were conducted regularly to correct data errors and a data cleansing was done on the final work sheet.

3.12 Statistical Analysis

A sample size calculation for the study was performed using power calculation for diagnostic accuracy studies (Jones *et al.*, 2003). We chose to identify a test with high specificity (high capability to predict those without miscarriage) since this information

would be meaningful clinically and would be helpful in reassuring and counselling women. Therefore, we used the formula based on specificity to calculate power calculation.

$$FP+TN = z^2 \times \frac{SP\,(1-SP)}{w^2} \qquad N\,(SP) = \frac{FP+TN}{(1-P)}$$

Where, confidence interval (z) =1.96, specificity (SP) =80 (98% sensitivity), accuracy (W) = 0.05, prevalence (P) =10%, false positive (FP), true negative (TN). Taking into account for a 10% drop out rate, the sample size was calculated as 300.

STATA IC 15(Texas, USA) software was used for statistical analysis. The comparison was made between the threatened miscarriage (cohort A) and asymptomatic group (cohort B) to see the demographic variables, biomarkers and ultrasound markers were different between them. In the threatened miscarriage cohort, a further comparison was made between the miscarried women and women who continued their pregnancy.

The descriptive variables were checked whether they were normally distributed or not by creating histograms (Appendix 1) and by doing Shapiro-Wilks test for normality. All the descriptive variables studied showed non normal distribution. Hence all other continuous variables (age, partner's age, BMI, gestational age, bleeding score, number of days of bleeding, number of pads used, all biomarkers and USS markers like MGSD, YS diameter, CRL, FHR, trophoblast thickness and trophoblast volume) were compared using Mann Whitney U test. All the categorical variables (history of a previous miscarriage, smoking, alcohol intake, caffeine intake, nulliparous status, relevant medical history, ethnicity, the intensity of bleeding and presence of corpus luteum) were compared using chi-square test. For all the continuous, not normally distributed variables, data were presented as median (95% confidence interval). For categorical variables, the data were presented as number (percentage).

A multivariate analysis was done to determine the effect of multiple variables on the outcome. The logistic regression analysis was used to determine the significant

variables. A stepwise regression using backward elimination was done and a regression model was developed. The ROC plot for the model was created and from the plot, the sensitivity, specificity and the diagnostic odds ratio for the model were calculated.

For the secondary perinatal outcomes, the chi-square test was used to find the significantly different outcomes between the threatened miscarriage cohort and the asymptomatic cohort. A P-value of <0.05 was considered significant. Whenever there was an occurrence of the outcome in both cohorts, the relative risk of the occurrence of the outcome was calculated.

3.13 Ethics statement and Study Registration

Ethics approval for the study was obtained on 30/10/2015 from the East Midlands - Leicester Central Research Ethics Committee (REC reference number: 15/EM/0439). Two successful amendments to the original ethics application were obtained on 15/09/2016 and 06/12/201.

The study protocol was reviewed and accepted by the University of Leicester and the study sponsor was the University Hospitals of Leicester NHS Trust Research and Innovation Team. The study was registered in the EDGE research management system (EDGE ID: 71146).

4 Results

Abstract: This chapter presents the results of the current study. The results were analysed from 278 participants in the threatened miscarriage cohort and 107 participants from the asymptomatic control. The study demonstrated that women with threatened miscarriage were associated with a higher rate of miscarriage (5.34%) compared to the asymptomatic population (2.8%).

Comparing the biomarkers and the ultrasound markers between women who experienced bleeding in early pregnancy versus asymptomatic women showed that progesterone, PAPP-A, trophoblast volume and presence of hematoma were different between the two populations.

Comparison of the miscarried women and those who continued pregnancy in the threatened miscarriage group had shown that the two groups of women were different in their age, bleeding score, the biomarkers hCG, progesterone, inhibin A and ultrasound markers MGSD, CRL and FHR. A multivariate regression model including the variables hCG, inhibin A, age and FHR gave the highest sensitivity and specificity to predict miscarriage with a DOR (95% CI) of 1.01 (1.01 – 1.02).

The secondary outcome analysis established that women who experienced threatened miscarriage are more likely to have adverse perinatal outcomes such as preterm labour, IUGR, LBW and neonatal asphyxia.

4.1 Study recruitment and incidence of miscarriage

The study recruited 296 participants in the threatened miscarriage cohort and 117 participants in the asymptomatic control cohort. During the follow-up period, 18 participants were lost to follow up in the threatened miscarriage cohort, and 10 participants were lost to follow up in the asymptomatic control cohort. Outcome data were collected from 278 participants in the threatened miscarriage cohort and 107 participants from the asymptomatic control cohort for the final analysis. Figure 43 and 44 demonstrate the recruitment flow chart for the threatened miscarriage and the asymptomatic control cohorts.

In the threatened miscarriage cohort, on follow up, 15 women miscarried, and 263 women continued with their pregnancy. Hence, 5.34% of women who experienced threatened miscarriage between 6 and 12 weeks of pregnancy eventually miscarried. On follow up, three women miscarried in the asymptomatic control cohort, and 104 women continued with their pregnancy. Therefore, 2.80 % of asymptomatic women who had a definite heartbeat seen on scan between 6 and 12 weeks of their pregnancy eventually miscarried later in their pregnancy.

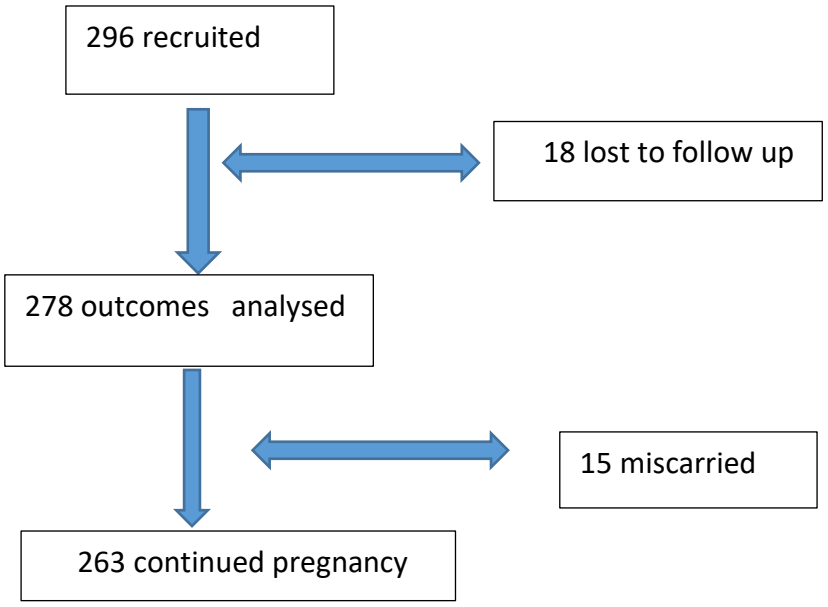


Figure 43 Flowchart showing recruitment for the threatened miscarriage cohort in the POM study

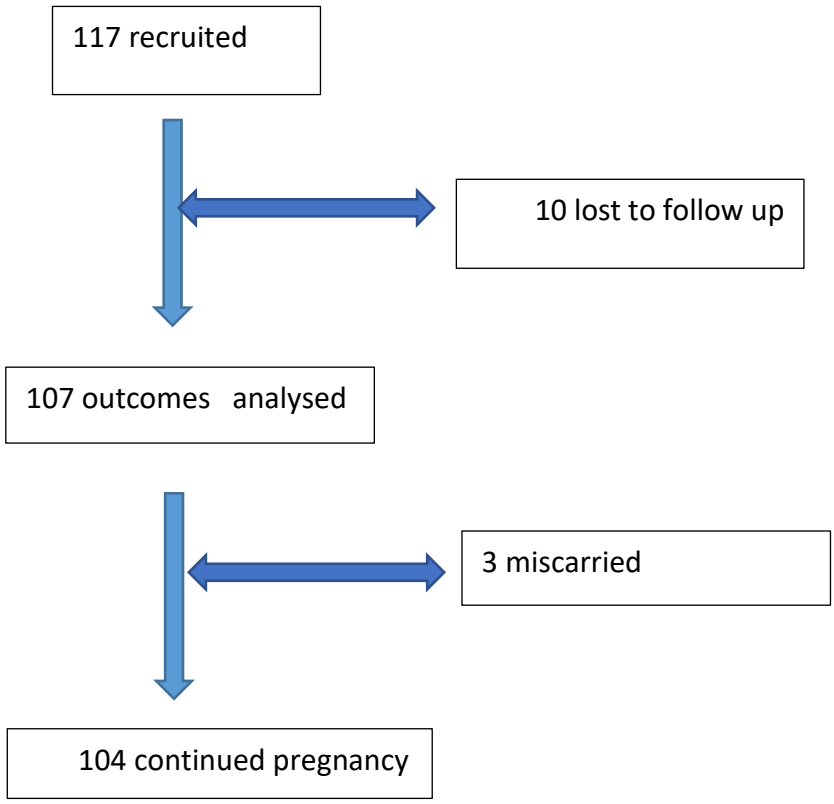


Figure 44 Flowchart showing recruitment for asymptomatic control cohort in the POM study

All continuously distributed variables were checked for normality by doing shapiro wilks test. All the descriptive continuous variables (age, partner's age, BMI), biomarkers and continuously distributed ultrasound markers studied showed a non-normal distribution. Hence all the continuous variables were compared using Mann-Whitney U test. All the categorical variables were compared using chi-square test. For all the not normally distributed variables, data were presented as median (95% confidence interval). For categorical variables, the data were presented as number (percentage).

In the next section, the threatened miscarriage cohort was compared with the asymptomatic normal cohort using demographic variables, biomarkers and ultrasound markers.

4.2 Comparing women who had threatened miscarriage with the asymptomatic cohort

4.2.1 Comparing the demographic variables in women who had threatened miscarriage with the asymptomatic cohort

The demographic variables were compared between both cohorts to see whether they were different, and the analysis showed that both cohorts were different for age, partner's age and parity (nulliparous or not). Table 25 summarises the demographic variables, comparing between the two cohorts.

Table 25 Comparison of the demographic variables between the threatened miscarriage cohort and asymptomatic control cohorts in the POM study

Variable		**Threatened miscarriage (N= 278)**	**Asymptomatic control (N= 107)**	**P-value**
Age (years) *		30 (29 – 30)	27 (26 - 29)	0.0008
Partners age (years) *		32 (31-33)	30 (28-32)	0.02
BMI *		25 (24-26.72)	25 (24-26)	0.32
Nulliparous n (%)		103 (37.18)	53 (50)	0.02
Previous miscarriage n (%)		121 (43.53)	37 (34.58)	0.11
Smokers n (%)		39 (14.08)	19 (17.76)	0.36
Alcohol intake n (%)		5 (1.82)	1 (0.93)	0.53
Caffeine intake n (%)		151 (67.41)	59 (68.60)	0.84
Ethnicity n (%)	Caucasian	202 (72.92)	86 (80.37)	0.22
	Mixed race	10 (3.61)	3 (2.80)	
	Asian	56 (20.22)	12 (11.21)	
	Afro-Caribbean	7 (2.53)	4 (3.74)	
	Chinese	2 (0.72)	2 (1.87)	

** Results presented as median (95% confidence interval)*

4.2.2 Comparing the biomarkers in women who had threatened miscarriage with the asymptomatic control cohort

Seven biomarkers were compared between the threatened miscarriage cohort and the asymptomatic control cohort and the results showed that women who experienced threatened miscarriage have lower median progesterone levels (P-value of 0.02) with a median value of 58.10 nmol/L than women who were asymptomatic (median value 68.3 nmol/L). Also, those women who experienced threatened miscarriage had significantly lower PAPP-A levels (median value 251) compared to the asymptomatic control cohort (median value 488.5). The P-value for PAPP-A was 0.002. Other biomarkers including hCG, Ca 125, inhibin A, oestradiol and hs-CRP did not show a statistically significant difference between the two groups. Table 26 summarises the results of comparison of biomarkers between women who experienced threatened miscarriage and the asymptomatic control cohort.

Table 26 Comparison of the biomarkers between the threatened miscarriage cohort and the asymptomatic control cohorts in the POM study

Biomarker	**Threatened Miscarriage (N= 278)**	**Asymptomatic control (N = 107)**	**P-value**
HCG (iU/L) *	103,300 (97,590-110,575)	10,940 (101,753- 122,737)	0.28
Progesterone (nmol/L) *	58.10 (54.30-65.27)	68.30 (63.23-74.38)	0.02
Ca 125 (kU/L) *	26 (24-28)	23 (20-27)	0.42
PAPP-A (mU/L) *	251 (219.16-322.68)	488.50 (359.63- 597.63)	0.002
Inhibin A (pg/mL) *	353.70 (323.75-399.43)	359.30 (311.58- 399.80)	0.74
Oestradiol (pmol/L) *	3,693 (3,351- 4,026)	4,124 (3,623- 4,857)	0.22
hs-CRP (mg/L) *	2.91 (2.13-3.46)	2.23 (1.89-3.00)	0.54

**Results presented as median (95% confidence interval)*

4.2.3 Comparing the ultrasound markers in women who had threatened miscarriage with the asymptomatic control cohort

Out of 12 ultrasound markers studied, only two markers showed statistically significant difference between the women who experienced bleeding in early pregnancy and women who were asymptomatic. One of those markers was trophoblast volume where women who had bleeding in early pregnancy had a lower trophoblast volume (median value 42,701 mm^3) compared to asymptomatic women (median value 49,760 mm^3) (P-value of 0.03). The presence of hematoma also showed statistically significant difference (27% in threatened miscarriage population Vs 3.6% in the asymptomatic population) (P-value 0.00). Table 27 summarises the comparison of the ultrasound markers between women with threatened miscarriage and asymptomatic women.

Table 27 Comparison of ultrasound markers between the threatened miscarriage cohort and asymptomatic control cohorts in the POM study

Ultrasound marker	**Threatened miscarriage (N= 278)**	**Asymptomatic control (N=107)**	**P-value**
MGSD (mm) *	34.70 (33.20- 36.19)	37.83 (34.41-40.28)	0.08
YSD (mm) *	5.90 (5.80-6.00)	5.75 (5.57-6.00)	0.35
CRL (mm) *	22.20 (20.49-24.30)	24.60 (22.83- 27.40)	0.14
FHR (bpm) *	169 (167-169)	167 (164-167)	0.33
Trophoblast thickness (mm) *	8.00 (6.70- 8.79)	8.05 (7.16- 9.36)	0.34
Trophoblast volume (mm^3) *	42,701 (36,157-49,295)	49,760 (44,471- 59,466)	0.03
Hematoma present n (%)	62 (27.07)	3 (3.61)	0.00
CL present n (%)	108 (41.2)	43 (40.95)	0.95

**Results presented as median (95% confidence interval)*

In summary, the threatened miscarriage cohort population was significantly older and multiparous than the asymptomatic control group. The threatened miscarriage cohort had significantly lower progesterone levels and PAPP-A compared to the asymptomatic controls. Also, they had lower trophoblast volume and more chance of having hematoma on scan compared to the asymptomatic controls.

In the next section, further comparisons were made between the miscarried women and those who continued with their pregnancy in the threatened miscarriage cohort.

4.3 Comparing the miscarried women with those who continued their pregnancy in the threatened miscarriage population

4.3.1 Comparing the demographic variables between the miscarried women with those who continued their pregnancy in the threatened miscarriage population

Table 28 shows the comparison of the demographic variables between those women who miscarried and those women who continued with their pregnancy in the threatened miscarriage population. There was no significant difference in the demographic variables between the two groups except for the maternal age. In the threatened miscarriage cohort, the miscarried women were older compared to those who continued their pregnancy and the P-value was nearing significance (P-value 0.05).

Table 28 Comparison of demographic variables between the miscarried group and the ongoing pregnancy group in the threatened miscarriage cohort (N=278) in the POM study

Variable		**Miscarried pregnancy (N=15)**	**Ongoing pregnancy (N=263)**	**P-value**
Age (years) *		31 (29 -36)	30 (28 – 30)	0.05
Partners age (years) *		32.50 (29.83-40.33)	32 (31-33)	0.33
BMI *		29 (22.17-34.28)	25 (24-26)	0.23
Nulliparous n (%)		7 (46.67)	96 (36.67)	0.43
History of previous miscarriages n (%)		8 (53.33)	113 (42.97)	0.43
Smoking n (%)		3 (20)	36 (13.74)	0.49
Alcohol intake n (%)		0 (0.00)	5 (1.92)	0.60
Caffeine intake n (%)		10 (66.67)	141 (67.46)	0.94
Ethnicity n (%)	White (%)	10 (66.67)	192 (73.48)	0.84
	Mixed ethnic group (%)	1 (6.67)	9 (3.44)	
	Asian (%)	4 (26.67)	52 (19.85)	
	Black/African/ Caribbean (%)	0 (0)	7 (2.07)	
	Chinese (%)	0 (0)	3 (0.76)	

** Results presented as median (95% confidence interval)*

4.3.2 Comparing the intensity of the bleeding between the miscarried women with those who continued their pregnancy in the threatened miscarriage population

A comparison of the objective and subjective assessment of bleeding was done between the women who miscarried and those who continued their pregnancy. In the objective

assessment, the comparison of bleeding score, the number of days of bleeding and number of pads used showed a statistically significant difference between the miscarried population and women with ongoing pregnancy (Table 29). However, the comparison of the subjective assessment of the bleeding did not show any significant difference between the two groups.

Table 29 Comparison of the intensity of bleeding between the miscarried group and ongoing pregnancy group in the threatened miscarriage cohort in the POM study

Bleeding history	**Miscarried pregnancy (N=15)**	**Ongoing pregnancy (N=263)**	**P-value**
Intensity of bleeding (Self-assessment)			
Mild n (%)	8 (53.33)	187 (71.92)	0.45
Moderate n (%)	5 (33.33)	55 (21.15)	
Heavy n (%)	2 (13.34)	18 (6.92)	
Bleeding score *	5 (2-8.82)	2 (2-3)	0.03
Number of days of bleeding *	4 (2.17-19.75)	3 (2-3)	0.05
Number of pads used/day *	1 (1-4.64)	1 (1-1)	0.01

** Results presented as median* (95% confidence interval)

4.3.3 Comparing the biomarkers between the miscarried women with those who continued their pregnancy in the threatened miscarriage population

On univariate analysis, out of the seven biomarkers studied, hCG, progesterone and inhibin A showed a significantly lower levels in the miscarried women compared to the women who continued with their pregnancy. The summary table comparing the biomarker levels between the miscarried group and the ongoing pregnancy is set out in Table 30.

Table 30 Comparison of the biomarkers between the miscarried group and the ongoing pregnancy group in the threatened miscarriage cohort in the POM study

Biomarker	**Miscarried pregnancy (N=15)**	**Ongoing pregnancy (N=263)**	**P-value**
HCG (iU/L)	75,973 (39,449.13–121,202.50)	103,674 (98,220.35– 111,527.70)	0.04
Progesterone (nmol/L)	50.2 (37.38 – 67.46)	60.25 (55.50-65. 89)	0.03
Ca 125 (kU/L)	21 (11.89 -34.64)	26 (24-29)	0.25
PAPP-A (mU/L)	187.5 (62.17 -264.44)	260 (220.58 -339.83)	0.06
Inhibin-A (pg/mL)	210.05 (120.48 -468.99)	363.95 (332.41 -401.37)	0.02
Oestradiol (pmol/L)	3,261 (376.65- 5,204.98)	3,708 (3,386.01- 4,101.66)	0.09
hs-CRP (mg/L)	4.16 (1.07 -6.59)	2.82 (2.12 -3.43)	0.92

Results presented as median (95% confidence interval)

In the next section, in order to explore the gestational age variation of the biomarkers further and to assess the difference of those statistically significant biomarkers between the miscarried group and pregnancy continued group, scatter plots were created.

4.3.3.1 Scatter plots to show the gestational age variation of the biomarkers in those women who miscarried and those who continued their pregnancies for those biomarkers which were found to be significant in the univariate analysis

4.3.3.1.1 Scatter plot to show the gestational age variation of the hCG in those women who miscarried and those who continued their pregnancies

The below plot (Figure 45) demonstrated the week to week variation of the hCG levels in women who miscarried and those who continued their pregnancies. The plot demonstrated that the hCG levels were lower in the earlier gestation and that variation gradually diminishes as the pregnancy advances the end of first trimester.

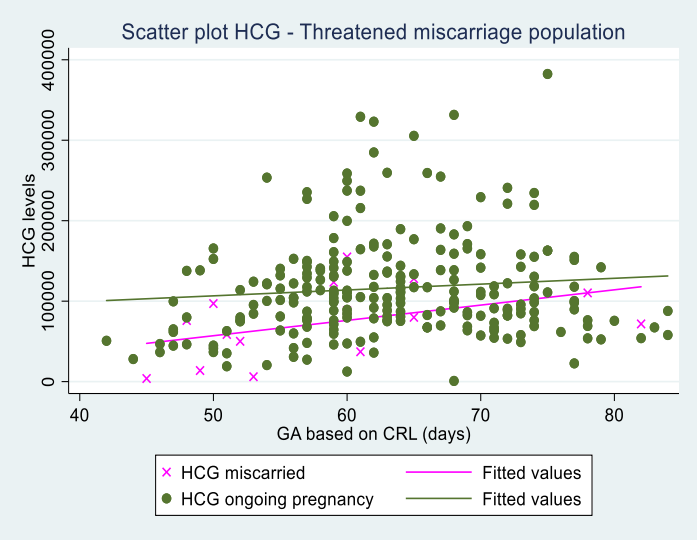


Figure 45 Scatter plot demonstrating the week to week variation of the hCG levels between the miscarried women and those women who continued their pregnancies in the threatened miscarriage cohort in the POM study

4.3.3.1.2 Scatter plot to show the gestational age variation of the progesterone in those women who miscarried and those who continued their pregnancies

The plot below (Figure 46) demonstrated the week to week variation of the progesterone levels in women who miscarried versus those women who continued their pregnancies. The difference in the progesterone levels were lower in the early part of the first trimester and that difference increased towards the latter part of the first trimester.

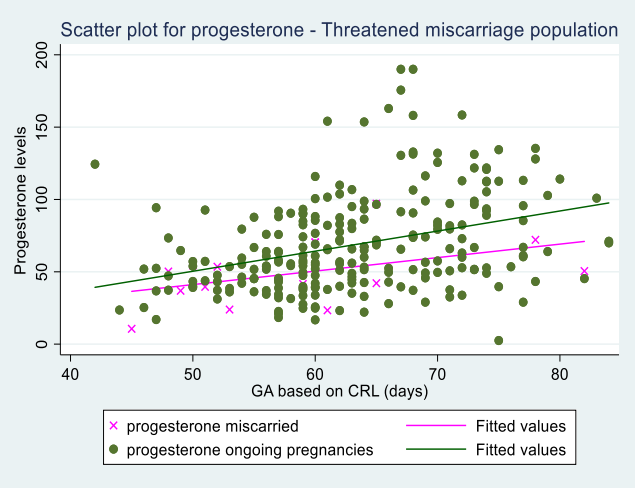


Figure 46 Scatter plot demonstrating the week to week variation of the progesterone levels between the miscarried women and those women who continued their pregnancies in the threatened miscarriage cohort in the POM study

4.3.3.1.3 Scatter plot to show the gestational age variation of the inhibin A in those women who miscarried and those who continued their pregnancies

The plot (Figure 47) demonstrated that the inhibin A levels were slightly different between the miscarried women and those who continued their pregnancy in early first and that difference became insignificant as pregnancy advances towards 9 weeks of gestation.

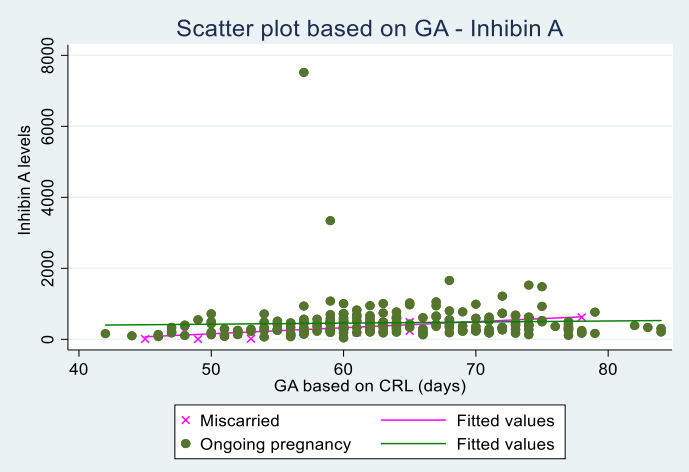


Figure 47 Scatter plot demonstrating the week to week variation of the inhibin A levels between the miscarried women and those women who continued their pregnancies in the threatened miscarriage cohort in the POM study

4.3.4 Comparing the ultrasound markers between the miscarried women with those who continued their pregnancy in the threatened miscarriage population

Comparison of ultrasound markers between the miscarried group and the continued group showed that the mean gestational sac diameter, abnormal yolk sac, crown rump length, fetal heart rate and the trophoblast thickness were significantly different between the two groups. Table 31 summarises the value of USS markers between the two groups.

Table 31 Comparison of the ultrasound markers between the miscarried group and the ongoing pregnancy group in the threatened miscarriage cohort in the POM study

Ultrasound marker	**Miscarried pregnancy (N=15)**	**Ongoing pregnancy (N=263)**	**P-value**
MGSD (mm) *	27.80 (20.38-36.95)	35.06 (33.37-36.20)	0.04
YSD (mm) *	6 (5.23–7.95)	5.9 (5.8-6)	0.86
CRL (mm) *	18.30 (11.44–24.15)	22.70 (20.91-24.51)	0.03
FHR (bpm) *	156 (138-169)	169 (167-169)	0.01
Trophoblast thickness (mm) *	3.15 (1.13- 9.21)	8.10 (6.74-8.85)	0.05
Trophoblast volume (mm^3) *	20,950 (5417-78,328)	43,110 (36,966-49,548)	0.07
Hematoma present (%)	3 (21.43)	59 (27.44)	0.62
CL present (%)	4 (26.67)	97 (39.27)	0.37

**Results presented as median (95% confidence interval)*

In the next section, in order to explore the gestational age variation of the ultrasound markers further and to assess the difference of those statistically significant ultrasound markers between the miscarried group and pregnancy continued group, scatter plots were created.

4.3.4.1 Scatter plots to show the gestational age variation of the ultrasound markers in those women who miscarried and those who continued their pregnancies for those ultrasound markers which were found to be significant in the univariate analysis

4.3.4.1.1 Scatter plot to show the gestational age variation of the FHR in those women who miscarried and those who continued their pregnancies

Scatter plot below (Figure 48) demonstrated the weekly variation of FHR in women who miscarried their pregnancies versus the variation of FHR in those women who continued their pregnancies. The FHR was significantly lower in women who miscarried compared to women who continued their pregnancies and the variation slowly diminished as the pregnancy advanced in the gestational age.

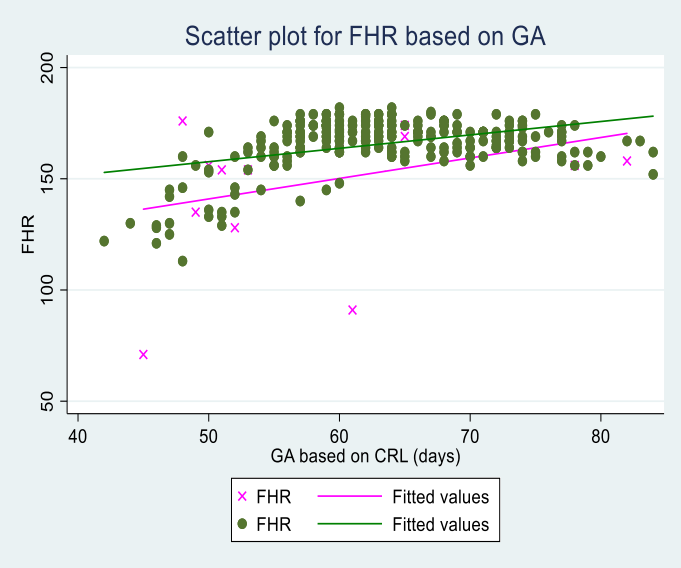


Figure 48 Scatter plot demonstrating the week to week variation of the FHR levels between the miscarried women and those women who continued their pregnancies in the threatened miscarriage cohort in the POM study

4.3.4.1.2 Scatter plots to show the gestational age variation of the CRL in those women who miscarried and those who continued their pregnancies

The plot (Figure 49) demonstrated that on splitting the CRL based on GA there was no significant difference between the CRL in women who miscarried and, in those women, who continued their pregnancies.

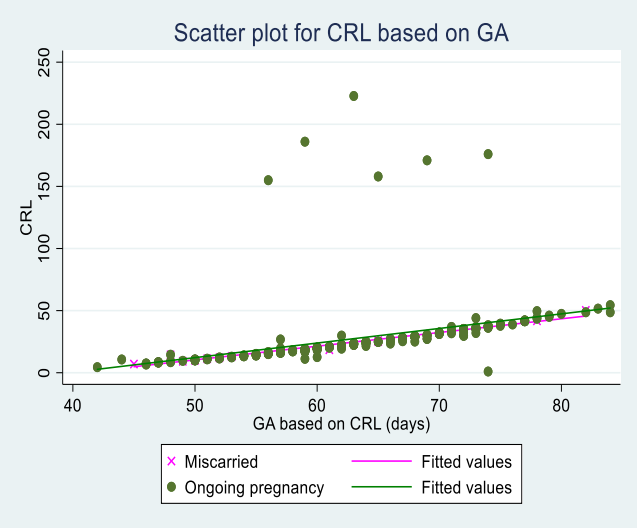


Figure 49 Scatter plot demonstrating the week-to-week variation of the CRL levels between the miscarried women and those women who continued their pregnancies in the threatened miscarriage cohort in the POM study

4.3.4.1.3 Scatter plots to show the gestational age variation of the MGSD in those women who miscarried and those who continued their pregnancies

The scatter plot (Figure 50) demonstrated that the MGSD was lower in the miscarried women in the early part of first trimester and as the pregnancy advanced the difference was diminishing.

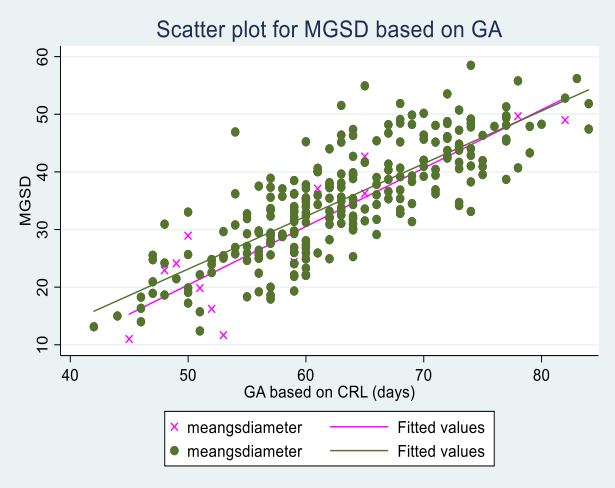


Figure 50 Scatter plot demonstrating the week to week variation of the MGSD levels between the miscarried women and those women who continued their pregnancies in the threatened miscarriage cohort in the POM study

Thus far, the results have shown the significance of individual markers between the two groups of women who miscarried versus continued their pregnancy in the threatened miscarriage cohort. Next section shows the results of the multivariate analysis considering all the significant demographic, biochemical and ultrasound markers using logistic regression model.

4.4 Multivariate analysis using demographic, biomarkers and ultrasound markers to predict miscarriage

A multivariate analysis was done using variables which reached significance ($p \leq 0.05$). Age was included in the multivariate analysis as maternal age is a proven risk factor for miscarriage (de La Rochebrochard and Thonneau, 2002). The other variables used for the multivariate analysis were bleeding score, number of days of bleeding, number of pads used while bleeding, hCG, progesterone, inhibin, MGSD, YS diameter, CRL, FHR and trophoblast thickness. A stepwise regression using backward elimination was used for doing multivariate regression. On stepwise multivariate regression analysis, a regression model composed of the variables of age, hCG, inhibin and FHR demonstrated the best prediction probability (P-value 0.0003) (Table 32).

Table 32 Multivariate regression with the outcome variable as miscarriage and using predictor variables age, hCG, inhibin A and FHR in the threatened miscarriage cohort in the POM study

Miscarried	Odds ratio	95% CI	Std. Err	P- value
Age	1.11	0.99 – 1.25	0.0588323	0.06
HCG	1.00	0.99 – 1.00	3.24e-06	0.06
Inhibin A	0.99	0.99 – 1.00	0.0018353	0.12
FHR	0.95	0.92 – 0.99	0.0164946	0.01
Constant (_cons)	2.05	0.003 – 1.69.6	3.19588	0.82

The prediction model based on the multivariate regression analysis above gives the log (odds) which is equal to log of the probability of miscarriage ÷ (1- probability of miscarriage) is expressed in terms of the following equation:

Log (odds) = 0.704+0.108xage+5.88e-06xhCG-0.003xinhibin A-0.41xFHR

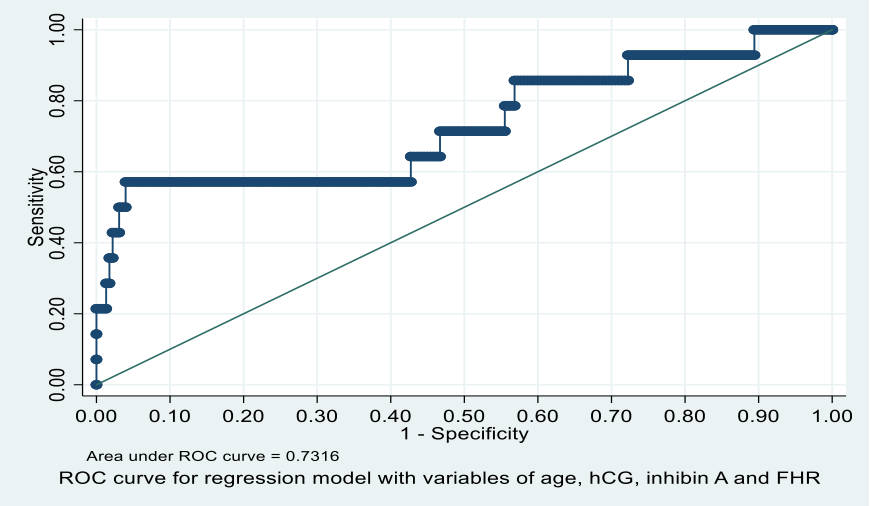


Figure 51 Receiver Operating Curve (ROC) curve for the regression model comprising the variables of age, hCG, inhibin A and FHR for predicting miscarriage in the threatened miscarriage cohort in the POM study

The ROC curve (Figure 51) shows the area under the curve of 0.73 with a sensitivity for the model of 57% and specificity for the model of 96%. The model gives a diagnostic odds ratio (DOR) (95% CI) of 1.01 (1.01 – 1.02) to predict miscarriage.

In the next section, the perinatal outcomes of those pregnancies that continued beyond 24 weeks in the threatened miscarriage population were compared with the perinatal outcomes of those pregnancies that continued beyond 24 weeks in the asymptomatic control group.

4.5 Perinatal outcomes of women who continued their pregnancies

As a secondary outcome, the perinatal outcomes of women who experienced bleeding in early pregnancy and continued their pregnancy beyond 24 weeks were compared with asymptomatic control population who continued their pregnancy beyond 24 weeks. The analysis showed that women who had bleeding in early pregnancy were more likely to have preterm delivery (RR 95% CI; 2.98 (1.07 – 8.27)), IUGR (unable to calculate the RR, as none of the women who continued their pregnancies beyond 24 weeks of gestation, develop IUGR in the asymptomatic control cohort. Nonetheless, IUGR occurred more frequently in the threatened miscarriage cohort than the asymptomatic cohort (P-value 0.02)), LBW (RR 95% CI; 6.14 (1.49 – 25.19), neonatal asphyxia (unable to calculate the RR, as none of the babies who were born to women in the asymptomatic control cohort develop neonatal asphyxia. Nonetheless, neonatal asphyxia occurred more frequently in the threatened miscarriage cohort than the asymptomatic cohort (P-value 0.02)). Table 33 summarises the comparison of perinatal outcome between the threatened miscarriage cohort and the asymptomatic control population.

Table 33 Perinatal outcomes in women who experienced threatened miscarriage in early pregnancy compared with the asymptomatic control population in the POM study

Perinatal outcome	Threatened miscarriage n/N (%)	Asymptomatic n/N (%)	Risk ratio	P-value
Preterm labour	30/261 (11.40)	4/104 (3.80)	2.98 (1.07 – 8.27)	0.02
PPROM	13/263 (4.94)	1/104 (0.96)	5.14 (0.68 – 38.80)	0.07
Still birth	0/ 263 (0)	1/104 (0.96)	0	0.11
IUGR	13/ 263 (4.94)	0/104 (0)	Indeterminate	0.02
LBW	31/260 (11.92)	2/103 (1.94)	6.14 (1.49 – 25.19)	0.003
PIH	6/263 (2.28)	2/104 (1.92)	1.18 (0.24 – 5.78)	0.83
PET	7/263 (2.65)	0/104 (0)	Indeterminate	0.09
HELLP	1/263 (0.38)	0/104 (0)	Indeterminate	0.52
APH	7/263 (2.66)	1/104 (0.96)	2.76 (0.34 – 22.22)	0.31
Neonatal asphyxia	12/246 (4.87)	0/97 (0)	Indeterminate	0.02
Congenital anomalies	4/264 (1.51)	1/104 (0.96)	1.57 (0.18 – 13.93)	0.68
Caesarean section	76/259 (29.34)	22/92 (23.91)	1.22 (0.82 – 1.85)	0.31

4.6 Conclusions

Women who experienced threatened miscarriage in early pregnancy had a miscarriage rate of 5.34% and asymptomatic women had a miscarriage rate of 2.80%. On comparing the biomarkers and the ultrasound markers between women who experienced bleeding in early pregnancy versus women who were asymptomatic showed that progesterone, PAPP-A, trophoblast volume and presence of hematoma were different between the two populations.

Comparison of the miscarried women and those who continued pregnancy in the threatened miscarriage group had shown that, the two groups of women were different in their age, bleeding score, the biomarkers hCG, progesterone, inhibin A and ultrasound markers MGSD, CRL and FHR. A multi variate regression model including the variables hCG, inhibin A, age and FHR gave the highest sensitivity and specificity to predict miscarriage with a DOR (95% CI) of 1.01 (1.01 – 1.02).

The secondary outcome analysis established that women who experienced threatened miscarriage are more likely to have adverse perinatal outcomes such as preterm labour, IUGR, LBW and neonatal asphyxia.

In summary, women with symptoms of threatened miscarriage in early pregnancy experience a high risk of miscarriage and a high incidence of perinatal complications compared to the asymptomatic control group. Prediction of miscarriage in women presenting with threatened miscarriage in early pregnancy is possible using a log regression model incorporating the parameters maternal age, FHR, hCG and inhibin A with a DOR of 1.01.

5 Discussion

Abstract: This chapter discusses the results of the POM study, compare, and analyse the findings with the result of the systematic reviews and existing literature. Serum biomarkers such as hCG, inhibin A and ultrasound markers of FHR can be used to predict miscarriage in the threatened miscarriage population. A regression model using maternal age, FHR, inhibin and hCG had a sensitivity of 57% and specificity of 96% with a diagnostic odds ratio (95% CI) of 1.01(1.01 – 1.02) in predicting miscarriage in women who experienced threatened miscarriage in early pregnancy. However, the model is not clinically useful due to its low sensitivity and diagnostic odds ratio. Bleeding in early pregnancy was associated with an increased risk of adverse perinatal outcomes such as preterm labour, low birth weight, IUGR and neonatal asphyxia and hence women with threatened miscarriage should be managed as high risk group and monitored closely in the antenatal period.

5.1 Incidence of miscarriage

The results show that the percentage of women who miscarried in the threatened miscarriage population was 5.34 %, and the percentage of women who miscarried in the asymptomatic control population was 2.80%. There is considerable variation in the reported miscarriage rates in women with threatened miscarriage in the existing literature. A study conducted in Egypt, El-Mekkawi *et al*., 2015 reported a miscarriage rate of 15% (El-Mekkawi *et al.*, 2015). A study by Dede *et al*.,2010 conducted in Turkey reported a miscarriage rate of 26.7%(Dede *et al.*, 2010), whereas another study conducted in Sydney reported a miscarriage rate of (Oates *et al.*, 2013) 7.9% and a study by Hill *et al*.,1991 (Hill *et al.*, 1991) from the United States reported a miscarriage rate of 6.1%. This variation in the miscarriage rate can be due to the ethnic and socioeconomic variation in the study population and the variation in the healthcare systems in these countries. Ethnic differences in the incidence of miscarriage had been previously reported in the literature. A retrospective observational study conducted in London in 196,040 women concluded that the miscarriage rates were higher in Black and South Asian women (Oliver-Williams and Steer, 2015). A cross-sectional population-based study conducted in China from 84531 women summarised that women with lower socioeconomic status are at higher risk of spontaneous miscarriage (Zheng *et al.*, 2017).

In England, the women wait for their scan appointment in the EPAU, which can be after one to two weeks, after they have experienced bleeding in early pregnancy. While they wait, a significant proportion of the miscarriages happen at home, or they present to the emergency department. Hence it can be assumed that the miscarriage rate might have been under- reported in our study population. However, in countries such as Turkey or Egypt, where the population more predominantly access private healthcare, women might present earlier as soon as they experience bleeding. This may explain why the miscarriage rate was higher in studies reported from Egypt or Turkey.

This study recruited women who presented to the EPAU for an early pregnancy scan after experiencing bleeding in early pregnancy. These women were referred to the EPAU

by the GP or the community midwife. Therefore, women who miscarried at home or presented to the emergency department with miscarriage were not included in this study cohort. It is worth observing that the miscarriage rate was almost similar in another United Kingdom-based study. The study by Johns *et al*., 2007 reported a rate of 6.6% (Johns, Jemma *et al.*, 2007). This further suggests that the reason for the variation in the reported miscarriage rate might be due to the variation in the healthcare systems in each country.

The reported variation in the miscarriage rate could also be explained by the variations in the study designs, especially the gestational age of recruiting women into the study. Hill *et al.* (Hill *et al.*, 1991) looked into the variation in the miscarriage rate based on the gestational age and demonstrated two peaks in the occurrence of miscarriage. The first peak at seven weeks of the gestational age, with the miscarriage rate of 11.1% and the second peak at 12-13 weeks of gestational age, with the miscarriage rate of 10.8%, compared to an average miscarriage rate of 6.1%. Early miscarriages can explain the first peak due to the chromosomal anomalies (Check *et al.*, 1990, Ljunger *et al.*, 2005) and the second peak can be explained by the miscarriages due to the abnormal trophoblast invasion (Jauniaux, E. and Burton, 2005). In our study, we did not have enough numbers of miscarriages to stratify the miscarriage rate according to the gestational age.

Furthermore, a study by Fiegler *et al*., 2003 (Fiegler *et al.*, 2003) stratified the occurrence of miscarriage based on the symptoms experienced by women. In those group of women who experienced only abdominal pain, the miscarriage rate was 10%; in those who had abdominal pain and vaginal bleeding for less than three days, the miscarriage rate was 12.5%, while in those women who had abdominal pain and vaginal bleeding for more than three days the miscarriage rate was 81.4%. Again, in the current study, there were not enough numbers of miscarriages to stratify the miscarriage rate according to the symptoms.

In summary, this study showed that in those women who attend EPAU with threatened miscarriage, the miscarriage rate is 5.4% and in those who are pregnant with no pain or bleeding the miscarriage rate is 2.8%.

5.2 Comparison of the threatened miscarriage cohort with the asymptomatic cohort

5.2.1 Demographic profile

The current study compared the demographic profile of women who experienced threatened miscarriage with asymptomatic women. It showed that asymptomatic women were more likely to be younger and nulliparous compared to women who experienced threatened miscarriage in early pregnancy. Pre-existing literature did not show any significant difference with age and parity between women with threatened miscarriage and asymptomatic women. This difference is probably because previous studies (Johns, Jemma *et al.*, 2007) have used a matched control group and in this study, the two cohorts were not matched. Hence the difference became apparent.

5.2.2 Serum Biomarkers

Progesterone and PAPP-A were significantly low in women who experienced threatened miscarriage in early pregnancy compared to asymptomatic women. In the current study, PAPP-A levels were lower in the threatened miscarriage cohort with a median value of 251.0 mU/L compared to the asymptomatic control cohort (median value of 488.50 mU/L) (P-value 0.002). Abnormally low levels of PAPP-A was reported in women with threatened miscarriage in previously reported studies by Westergaard and Masson (Masson *et al.*, 1983, Ruge *et al.*, 1990, Westergaard *et al.*, 1983). PAPP-A is a selective proteinase enzyme which is responsible for cleavage of Insulin Growth Factor binding protein (IGFBP) and therefore enhances the bioavailability of Insulin-Like Growth Factor (IGF). IGF mediates trophoblast invasion and glucose and amino acid transportation to the placenta. Though PAPP-A is seen in many other tissues, placental production exceeds than others. Antsaklis has demonstrated in his review that placental dysfunction can be associated with low PAPP-A. (Antsaklis *et al.*, 2019). Since threatened

miscarriage is associated with placental dysfunction, it is reasonable to expect a lower level of PAPP-A in threatened miscarriage. The study by Hanita *et al*., 2012 showed that there was no significant difference in the PAPP-A levels between the women who experienced threatened miscarriage and the asymptomatic women (Hanita *et al.*, 2012). One potential reason for this finding was that the study had a very low sample size and hence failed to demonstrate the difference. Similarly, Hanita (Hanita *et al.*, 2012) used a different test (ECLIA technology) compared to the very sensitive radioimmune assay technique for PAPP-A measurement. This can also be a possible contributing factor for the disparity in the results.

Previously published literature on progesterone has demonstrated lower progesterone levels in the threatened miscarriage women compared to the asymptomatic women (Leylek *et al.*, 1997, Maged and Mostafa, 2013). The study by Leylek *et al.,* 1997 was done on a small sample size of 55 women. The study reporting was incomplete and therefore, it was not possible to assess the study design and quality. The study by Maged and Mostafa was a prospective study conducted on 250 women with sound methodological quality. The current study confirmed a similar finding with threatened miscarriage women having a significantly lower median progesterone value of 58.10 nmol/L than asymptomatic women (median value 68.30 nmol/L) and adds on to the existing volume of evidence (P-value 0.02).

5.2.3 Ultrasound markers

Among the ultrasound markers, the trophoblast volume was significantly lower in women with threatened miscarriage (median value 42,701 mm^3) compared to asymptomatic women (median value 49,760 mm^3). There was no pre-existing literature investigating the difference in the trophoblast volume between women who experienced bleeding in early pregnancy and asymptomatic women. The current study also showed that the percentage of women with intra-uterine hematoma was significantly higher in the threatened miscarriage women (27.07%) compared to the asymptomatic women (3.61%). Alcázar and Ruiz-Perez in 2000 reported an 85.7% incidence of intra-uterine hematoma in women with threatened miscarriage (Alcázar

and Ruiz-Perez, 2000). However, the study by Alcázar and Ruiz-Perez did not give a comparison of the incidence of an intra-uterine hematoma between the threatened miscarriage and asymptomatic women.

5.3 Comparing the miscarried pregnancies and ongoing pregnancies in the threatened miscarriage cohort

5.3.1 Demographic profile

In the threatened miscarriage group, the women who miscarried had a similar demographic profile to the women who continued their pregnancy except for maternal age. In the threatened miscarriage cohort, the miscarried women were older compared to those who continued their pregnancis and the P-value was nearing significance (P-value 0.05). Maternal age is a recognised risk factor for miscarriage. A prospective register-based Norwegian study has reported the lowest risk of miscarriage in the maternal age group of 25 to 29 years (10%), the risk increased rapidly after 30 years of age, and it reached 53% in women aged 45 years or over(Magnus *et al.*, 2019).

5.3.2 Intensity of blood loss

On objective analysis of bleeding using a pictogram, the current study has shown that the women who eventually miscarried had a higher bleeding score (P-value 0.03) and the number of days of bleeding (P-value 0.05) than the women who continued their pregnancy. However, the subjective assessment of bleeding by the participants as mild, moderate and heavy bleeding did not show significant association with miscarriage.

Studies have previously been reported on the association of intensity of bleeding with miscarriage. A study by Hasan et al. in 2009 reported that heavy bleeding particularly accompanied by pain is associated with an increased risk of miscarriage (Hasan *et al.*, 2009). However, the data for the study were collected by telephone interview (subjective assessment) as part of another ongoing cohort study. There were no reported studies comparing the objective and subjective assessment of miscarriage blood loss.

Since there was no specific miscarriage blood loss pictogram currently available to quantify the miscarriage blood loss, and it was not in the scope of the current study to develop a validated miscarriage blood loss pictogram, we extrapolated the menstrual blood loss pictogram for our study (Warrilow *et al.*, 2004). This was done on the assumption that objectively assessing early pregnancy blood loss using the menstrual pictogram should give a reasonably accurate objective assessment of miscarriage blood loss. Warrilow et al. in 2004 have demonstrated that the subjective and objective assessment of menstrual blood loss does not correlate (Warrilow *et al.*, 2004). Similarly, Chimbira et al. in 1980 demonstrated that women are not good judges of the actual blood loss (Chimbira *et al.*, 1980). In the current study, the disparity noted between the results of subjective and objective assessment of bleeding further support this pre-existing data and emphasize that objective assessment of blood loss is a more accurate method for assessing miscarriage blood loss.

5.3.3 Serum Biomarkers

Among the biomarkers studied to predict miscarriage in the threatened miscarriage population, hCG, progesterone and inhibin A showed a significant difference between the miscarried and the ongoing pregnancy group. Serum hCG had a low median value of 75973 iU/L (39449.13 – 121202.50) in the miscarried group compared to the ongoing pregnancy group (103674 iU/L (98220.35 – 111527.70)) (P-value 0.04). Similarly, serum progesterone had a low median value of 50.2 nmol/L (37.38 – 67.46) in the miscarried population compared to the ongoing pregnancy group (60.25 nmol/L (55.50-65. 89)) (P-value 0.03). Serum inhibin A also had a low median value of 196.05 pg/mL (128.48 - 379.88) in the miscarried population and had a higher median value of 320.90 pg/mL (277.6 -346.06) in the ongoing pregnancy group (P-value 0.02).

Serum hCG is the earliest easily detectable biomarker in early pregnancy and it remains the mainstay of modern early pregnancy diagnosis, as explained in the introduction chapter. The hCG is a glycoprotein with a non-specific α subunit which is similar to LH and FSH and a specific β subunit which is unique to hCG (Stenman *et al.*, 2006). Hence some studies have used β hCG subunit for early pregnancy investigations and prognosis

(Dessaive *et al.*, 1982, Jouppila, Penttl *et al.*, 1980, Leylek *et al.*, 1997, Maged and Mostafa, 2013, Scarpellini *et al.*, 1995)and others have used intact hCG for early pregnancy investigations and prognosis (Siimes *et al.*, 1983, Stoppelli *et al.*, 1981b, Westergaard *et al.*, 1985). However, it is proven that the measurement of free β hCG subunits offers no clinical utility over the measurement of intact hCG during the first half of pregnancy (Thomas *et al.*, 2012). Hence in the current study, complete hCG was used for the prediction of miscarriage. Studies have suggested a single hCG value to be used as a marker of implantation success as it reflects the quality of implantation of the pregnancy (Chen *et al.*, 1997, Glatstein *et al.*, 1995). Hence in our study, we used a single measurement of total hCG for the prediction of miscarriage as a marker of implantation failure. The systematic review conducted as part of this research summarised the existing literature and reiterated the usefulness of hCG in predicting miscarriage with a positive predictive value of 3.37 (95% CI 1.98 – 5.74) and a negative predictive value of 0.63 (95% CI 0.36 – 1.11).

Johansson *et al*., 1969 first demonstrated that abnormal early gestations have lower serum progesterone level than viable intrauterine pregnancies. Due to the significant variation in the biological level for serum progesterone, choosing a discriminatory cut off level has been difficult (Williams *et al.*, 1992). Verhaegen *et al.,* 2012 concluded that, among women with symptoms of pain and bleeding in early pregnancy and inconclusive ultrasound assessment (PUL), the progesterone test predicted a non-viable pregnancy with a pooled sensitivity of 74.6%, a specificity of 98.4%, the positive likelihood ratio of 45 and negative likelihood ratio of 0.26, for a cut off value of 3.2- 6 ng/ml (Verhaegen *et al.*, 2012). The study population in Verheagen *et al.,* 2012 was any women with pain and bleeding in early pregnancy without an ultrasound diagnosis of viability. The systematic review for this research was performed specifically for the threatened miscarriage population (viability of the pregnancy was demonstrated using an USS scan) and demonstrated that progesterone is not a useful tool in predicting miscarriage. The positive predictive value was 2.24 (95% CI 0.32‑15.80) and the negative predictive value was 0.81 (95% CI 0.35‑1.86). In the current study on the threatened miscarriage population, although on univariate analysis progesterone was demonstrated to be a

useful tool in predicting miscarriage (P-value 0.03), it failed to show significance in the multivariate logistic regression analysis. The study findings agree with the systematic review outcome and progesterone does not seem like a useful biomarker in predicting miscarriage in the threatened miscarriage population.

Decreased concentrations of inhibin A have been found in women who subsequently miscarry in the recurrent miscarriage population (Al-Azemi *et al.*, 2003). Also, significantly low levels of inhibin were found in women who subsequently miscarry in the IVF population (Hauzman *et al.*, 2004). Inhibin, as a marker of viable trophoblast, was found to be useful in differentiating between incomplete and complete miscarriage (Luisi *et al.*, 2003). Johns *et al*., 2007 noticed significantly lower levels of inhibin A in women who subsequently miscarried in a threatened miscarriage population and they demonstrated inhibin A on its own could be used to predict the pregnancy outcome(Johns, Jemma *et al.*, 2007). In comparison to other markers such as hCG, inhibin A levels were found to be significantly lower as early as six weeks in women destined to miscarry in a population of women with recurrent miscarriage(Al-Azemi *et al.*, 2003). This means that a single measurement of inhibin A even before clinical confirmation of pregnancy can be used to predict pregnancy outcome (Muttukrishna, S. *et al.*, 2002). For our systematic review, only two studies qualified for the qualitative review (Johns, Jemma *et al.*, 2007, Phupong and Hanprasertpong, 2011). However, the study by Johns *et al.* expressed their results in means and standard deviation, which limited the study from including in the quantitative analysis of the systematic review. Conversely, Phupong *et al.* have reported that Inhibin A is not a sensitive biomarker in predicting miscarriages. Although they had noted a lower mean inhibin A level in the miscarried group compared to those women who continued their pregnancy, the results did not reach statistical significance. This could be due to the smaller study sample size (N = 30). This study has shown that inhibin A levels can be significantly low in those women who subsequently miscarry, and it was found to be a useful predictive marker both in the univariate analysis (P-value 0.02) and in the multivariate prediction model for predicting miscarriage.

The systematic review, which included seven studies on Ca 125, showed Ca 125 as the most predictive marker for miscarriage in the threatened miscarriage population. However, with the current cohort study, Ca 125 did not show significance as a predictive marker. One possible reason for this disparity can be due to the variation in the point at which the blood test was done (nearer to the bleeding episode or few days after the bleeding episode). Also, out of the seven studies on Ca 125, six studies (Leylek *et al.*, 1997, Maged and Mostafa, 2013, Öçer *et al.*, 1992, S. Sherif, AG El-Metwaly, H. Shalan, AM Badawy E., Abu-Hashem, L, 2000, Scarpellini *et al.*, 1995, Xie *et al.*, 2014) did not take into the consideration the other possible causes for the non-specific elevation of Ca 125 such as endometriosis, adnexal masses, women who underwent fertility treatment and women with other medical disorders like liver disease. In our study, we followed strict exclusion criteria and excluded all women with possible causes for mesothelial activation and non-specific elevation of Ca 125. This could explain the difference in the results of the current study from the systematic review.

5.3.4 Ultrasound markers

In the threatened miscarriage cohort, a comparison of ultrasound markers (univariate analysis) between the miscarried and the ongoing pregnancy groups showed that the MGSD (P-value 0.04), CRL (P-value 0.03) and the FHR (P-value 0.01) were significantly different. However, on doing the multivariate logistic regression analysis, including all covariates, FHR was the single most predictive marker (P-value 0.01).

The study used M mode to measure the FHR. It was established that the mean FHR progressively increases from 6 to 8 weeks gestation with a rate of 111±14 bpm at 42- 45 days, 125±15 bpm at 46-49 days, 145±14 bpm at 50-52 days and 157±13 bpm at 53-56 days (Stefos *et al.*, 1998). In our study, on scatter plot analysis, the mean fetal heart rate both in the miscarried and the ongoing pregnancy group at six weeks of gestation was lower than 12 weeks of pregnancy, and the FHR value gradually increased towards 12 weeks of pregnancy. On the scatter plot for FHR, the difference in the FHR levels between the miscarried group and the ongoing pregnancy group appeared to be gradually diminishing towards 12 weeks of gestation. The caveat is that we were not

able to statistically establish this difference due to the small number of miscarriages in the study group.

The bioeffects of ultrasound scanning include thermal effects due to a rise in tissue temperature and mechanical effects like cavitation and tissue streaming. Cavitation is the development of gas bubbles in an acoustic field at high negative pressure, and acoustic streaming is pushing target tissue away from the transducer by disseminating ultrasound waves. There is no proven evidence of side effects in humans, but evidence of side effects from animal and laboratory studies have pushed the authorities to issue advice on precautionary measures especially on the use of Doppler ultrasound imaging (Joy *et al.*, 2006). Therefore, doppler examinations should only be used in the first trimester if clinically indicated (Salomon *et al.*, 2013). M mode was used for measuring the fetal heart rate in the current study and due to its limited acoustic output, M mode is considered to be safe (Abramowicz *et al.*, 2000, Torloni *et al.*, 2009). Some studies have used manual counting (Laboda *et al.*, 1989, Merchiers *et al.*, 1991) which make the results less reliable and some others used Doppler to assess fetal bradycardia (Achiron *et al.*, 1991).

Some of the previously conducted studies switched between TA and TV scans to assess fetal heart rate which could add to inconsistency while counting fetal heart rate (Dede *et al.*, 2010, Laboda *et al.*, 1989). In the current study, transvaginal scanning was used to maintain consistency. Our study is in agreement with previously reported literature that fetal bradycardia is a useful marker to predict miscarriage (Dede *et al.*, 2010, El-Mekkawi *et al.*, 2015, Maged and Mostafa, 2013, Merchiers *et al.*, 1991, Phupong and Hanprasertpong, 2011). The meta-analysis we conducted also demonstrated high predictive accuracy for fetal bradycardia in women with threatened miscarriage with a positive predictive value of 19.51 (95% CI 5.44–69.84) and a negative predictive value of 0.16 (95% CI 0.03–0.91). In summary, the current study along with the previously published literature, highlights the usefulness of FHR in predicting miscarriage in women with threatened miscarriage.

Few previous studies have established a cut off level for fetal bradycardia (Maged and Mostafa, 2013, Stefos *et al.*, 1998) and the meta-analysis we have conducted on those studies, suggest a cut off level of 110 bpm below which the risk of miscarriage increases. However, the current study was not able to define a cut off level, which is best in predicting miscarriage, and this was mainly due to the smaller number of miscarriages in the study population.

The trophoblast thickness has not been investigated extensively in the past for the prediction of miscarriage. The chorio-decidual plate, which is the developing trophoblastic tissue, is the predecessor for the developing placenta and is essential to maintain pregnancy. Hence, it can be hypothesised that a thinner chorio-decidual plate can be associated with miscarriage. A previously reported study on trophoblastic thickness investigated the thinning of trophoblast as a sign to predict miscarriage (Bajo *et al.*, 2000). The study showed that the thinning of the trophoblast is a sensitive marker, with a sensitivity of 82% and specificity of 93% in predicting miscarriage for women with threatened miscarriage. Bajo *et al*., 2000 described the thinning of trophoblast as when the numerical difference between the gestational age in weeks and the trophoblastic thickness in millimetres is more than three millimetres. However, the drawback of this measurement was that it was assumed that the trophoblast thickness was uniform in each pregnancy for each gestational age which was not established before. The current study demonstrated that trophoblast thickness as a marker was nearing significance in predicting miscarriage with a P-value of 0.05 on univariate analysis. However, on multivariate analysis, the trophoblast thickness did not show significance. In the present study and Bajo *et al.,* 2000 the trophoblast thickness was measured at the site of embryonic implantation which is located by identifying the omphalomesenteric canal at 5-6 weeks and later by identifying the insertion point of the umbilical cord. However, there is no established association between the point of insertion of the umbilical cord and the chorio-decidual plate thickness. Hence it can be presumed that the lack of consensus between both study's results could be due to the wrong point of measurement chosen to measure the trophoblast thickness. Similarly, both studies included participants less than eight weeks of gestational age and the chorio-decidual

plate is not well developed before that stage. This could have also contributed to the disparity in the results between the current study and Bajo *et* al., 2000. Therefore, more studies are required, adjusting for these limitations to look for the role of trophoblast thickness in predicting miscarriage.

CRL had been described extensively in the literature as a predictive marker of miscarriage. Some studies had chosen an arbitrary cut off value for CRL to predict miscarriage. Maged *et al*., 2013 had chosen a cut off value of 21mm to predict miscarriage (Maged and Mostafa, 2013). Reljic *et al*., 2001 had chosen a cut off value of 18mm, and they compared it with a deficit in the CRL for the gestation age. In comparison, those fetuses with CRL less than 18 mm showed a positive correlation with the CRL deficit for the gestational age and subsequent spontaneous miscarriage (Reljic, 2001). However, CRL is a variable marker and its value increases with gestational age. The possible value of CRL for each gestational age had been previously reported in the literature and is currently extensively used in clinical practice to assess the gestational age in early pregnancy (Robinson, HP and Fleming, 1975). Hence it is logical to look for the deviation of the observed and expected CRL. A study by Abuelghar *et al*., 2013 used the difference in the observed and expected CRL and a CRL of two standard deviations or less from the expected showed a sensitivity of 56.6% and specificity of 81.9% to predict miscarriage. The expected CRL depends on the last menstrual period and it can be inaccurate as the periods can be irregular in 80% of women (Karout *et al.*, 2012). In order to overcome this, for the current study, the GA was calculated using the measured CRL on the ultrasound scan. The current study only investigated the median value of CRL between the miscarried and the continuing pregnancy group and showed that a low median value of CRL was predictive of miscarriage (P-value 0.03) on univariate analysis. The study did not investigate the CRL deficit and was not able to suggest a cut off value. Also, in the current study, CRL failed to show significance on multivariate regression analysis and this might be because a CRL deficit for the gestational age was not calculated.

Mean gestational sac size varies as gestational age increases and hence some of the studies that used it as a marker used the difference between the gestational sac and CRL to predict miscarriage (El-Mekkawi *et al.*, 2015). El-Mekkawi *et al*., 2015 reported a significantly lower MGSD and MGSD – CRL in the miscarried group (El-Mekkawi *et al.*, 2015). Falco *et al*., 2015 reported that a gestational sac less than 1.34 SD of the mean was associated with a higher risk of miscarriage (Falco *et al.*, 1996). The current study showed a significant association for smaller MGSD with miscarriage (P-value 0.04) on univariate analysis. However, it failed to show significance on multivariate regression analysis. This might be because the current study did not use the gestational specific values of MGSD for comparison. It was not possible to compare with a gestational age specific values of MGSD from the current study data due to the fewer number of the outcome variable.

5.3.5 Prediction model

In the POM study, a multivariate regression model was developed to predict miscarriage in the threatened miscarriage population. The model composed of the variables of age, hCG, inhibin and FHR gave a sensitivity of 57%, a specificity of 96% (P-value 0.0003). The ROC curve demonstrated an area under the curve of 0.73. A model with an AUC of 1.0 represents a model with 100% sensitivity and specificity, whereas a model with an AUC of 0.5 represents an uninformative model (Bossuyt, Patrick *et al.*, 2008).

Previous studies have presented models to predict miscarriage in women with threatened miscarriage (Altay *et al.*, 2009, Oates *et al.*, 2013, Varelas *et al.*, 2008). Varelas *et al.* in 2008 (Varelas *et al.*, 2008) looked at USS markers only and reported that a combination model of GA and YSD can predict miscarriage in the threatened miscarriage population with a sensitivity of 76% and specificity of 91%. However, there was no prediction model described in the paper that can be used clinically. Altay *et al.,* 2009 (Altay *et al.*, 2009), suggested that a combination of progesterone levels and FHR can predict miscarriage with a PPV of 50% and a NPV of 98.9%. Again, in their study, the prediction model was not described in the paper. In 2013, Oates *et al.* (Oates *et al.*, 2013) used multiple variables in the prediction model which included GA by LMP, the

presence of PV bleeding, the presence of PV clots, GA by ultrasound, consistency with menstrual dates (defined as gestational age by ultrasound <7 days difference to the gestational age by dates), mean GS size, mean YS size and number of previous caesarean sections. The model gave a sensitivity of 83% and a specificity of 79%. The major limitation was a large number of variables were used in the model. Compared to this study by Oates *et al*., 2013, the prediction model proposed by the current study showed higher specificity and the aim of the current study is to predict women with threatened miscarriage who are likely to continue their pregnancy as opposed to predicting a miscarriage. This has an advantage in the clinical setting where women are keener to know whether their pregnancy is likely to continue despite the pain and bleeding.

The prediction model for the current study is a simple model with a limited but relevant number of variables such as age, hCG, inhibin A and FHR. The strength of the model is that it has a higher specificity of 96%, but the limitation is the lower sensitivity to predict miscarriage. This could be because the study has a smaller number of women with the miscarriage outcome. The study did not recruit those women who miscarried at home and those women who presented to the emergency department with miscarriage.

Furthermore, using a traditional approach of stepwise backward regression for selecting the predictor variables tends to overfit the data. Ideally, the prediction model should be externally validated in other independent data sets (Pescatore *et al.*, 2014) to evaluate further the predictive performance of the model. However, the model cannot be used in clinical practice due to its poor sensitivity (57%) and poor diagnostic odd ratio (1.01) and therefore, further steps in model building which includes internal validation, external validation and assessing the model's impact in clinical practice were not undertaken.

Prediction models can be useful in identifying those women who would benefit from treatment. Critical evaluation of two large multicentre trials by Coomarasamy *et al.* with subgroup analysis demonstrated that in women with a history of miscarriage and early pregnancy bleed, the use of vaginal micronized progesterone 400mg twice daily might

benefit from improving the chance of live birth rate (Coomarasamy *et al.*, 2020). Hence, a prediction model can be a useful tool in clinical practice to reassure and treat women.

5.4 Perinatal outcomes for women with threatened miscarriage

The data from the current study has shown that women with threatened miscarriage have a high incidence of preterm labour (P-value 0.02), IUGR (P-value 0.02), low birth weight (P-value 0.003) and neonatal asphyxia (P-value 0.02) compared to the asymptomatic cohort. These results agree with the previously exiting literature and with the updated systematic review conducted as part of the current study. A systematic review published by Saraswat *et al.* in 2009 which included retrospective studies along with prospective studies, demonstrated that women with threatened miscarriage were at high risk of preterm labour, PPROM, IUGR and low birth weight babies. An updated systematic review on prospective studies conducted as part of this research has also shown that threatened miscarriage was associated with a high incidence of still birth, preterm labour, PPROM, low birth weight, placental abruption, neonatal asphyxia and congenital anomalies.

The increased perinatal morbidities of preterm labour, IUGR and low birth weight in women with threatened miscarriage could be due to the chronic inflammation of the decidua in early pregnancy. There is evidence of defective placentation with thinning and fragmentation of trophoblast and reduced cytotrophoblastic invasion of the spiral arteries (Johns, J. and Jauniaux, 2006) in women with threatened miscarriage. The defective placentation explains the increased risk of IUGR and low birth weight seen in women with threatened miscarriage.

There is a greater likelihood of having a difference in the occurrence of the outcome based on the intensity of bleeding. Though the current study was able to quantify the bleeding objectively, it was not possible to segregate the secondary outcomes based on the intensity of bleeding. This was due to the fewer number of reported perinatal morbidity outcomes in the study groups. There is a possibility that outcomes such as preterm labour, PPROM and low birth weight can be interrelated. Since the pathology

for these events initiates from placentation affecting circulation and hormonal milieu, it is difficult to isolate the individual outcomes. Nevertheless, so far, the evidence from the current study as well as from systematic reviews has consistently shown an association between threatened miscarriage and perinatal morbidities, especially preterm labour, low birth weight, IUGR and neonatal asphyxia.

Future research with a large prospective cohort study is required to confirm further the above findings, which can have a significant impact on the care provided to women with threatened miscarriage.

5.5 Conclusions

From the current study, we can conclude that women with threatened miscarriage were associated with a higher rate of miscarriage compared to the asymptomatic population. Serum biomarkers such as hCG, inhibin A and ultrasound markers of FHR can be used to predict miscarriage in the threatened miscarriage population. A regression model using maternal age, FHR, inhibin and hCG had a sensitivity of 57% and specificity of 96% with a diagnostic odds ratio (95% CI) of 1.01(1.01 – 1.02) in predicting miscarriage in women who experienced threatened miscarriage in early pregnancy. Bleeding in early pregnancy was associated with an increased risk of adverse perinatal outcomes such as preterm labour, low birth weight, IUGR and neonatal asphyxia. Hence women with threatened miscarriage should be managed as high-risk group and monitored closely in the antenatal period.

6 Summary

Abstract: This chapter summarises the results of systematic reviews and the POM study, discuss its implications, and provides directions for future research. The work that presented in the thesis concluded that biomarkers and ultrasound markers can be used to predict miscarriage. However, the prediction model developed from the POM study had too low a diagnostic odds ratio to be of use in clinical practice. It also concluded that threatened miscarriage is associated with an increased risk of perinatal complications. Future research focussing on developing markers to predict the adverse perinatal outcomes can help to plan the antenatal care of women experiencing bleeding in early pregnancy.

6.1 Summary

This chapter aims to summarise the results from the systematic reviews of previously published studies on outcomes and prediction of miscarriage in women with threatened miscarriage. It also seeks to summarise the current prospective study on the same topic, compare and critically analyse the results, highlight the strengths, limitations and clinical applications of the studies and give directions for future research.

Serum Biomarkers:

The systematic review from the current study summarised that among all the serum biomarkers so far researched, Ca 125 had the highest sensitivity and specificity in predicting miscarriage. In contrast, the cohort study concluded that hCG and inhibin A were significantly associated with the outcome of threatened miscarriage, but not Ca 125.

It is to be noted that there was only one study that met the inclusion criteria for the systematic review on inhibin A and hence a meta-analysis was not possible. Nevertheless, the single study on inhibin A had shown that inhibin A levels were significantly lower in women who miscarried compared to those who continued their pregnancies.

Ca 125 level is known to increase with cellular activation of any mesothelial tissue. While the studies included in the systematic review did not rigorously exclude women with other causes of mesothelial activation (e.g. women with endometriosis, PID, adnexal masses), we excluded such subjects. In the QUADAS tool (section 1.4.1.4), this is shown as an applicability concern under the 'patient selection' category. One of the past studies on Ca 125 (which showed no significant predictive value for Ca 125) was not included in the systematic review as the results were presented as mean and standard deviation.

Ultrasound Markers:

In the systematic review from the current study, among all the ultrasound markers so far researched, FHR had the highest sensitivity and specificity in predicting miscarriage with a cut off value of 110 bpm. The current cohort study showed results similar to the systematic review, with FHR being the best predictive marker.

Prediction Model:

The current study presented a prediction model composed of variables such as age, inhibin A, hCG and FHR. The only previous study (Oates *et al.*, 2013) which has presented a model that used eight variables in the model, which makes it a complicated model to use. The current model had a high specificity of 96%. A model with higher specificity is useful in reassuring the women that they have a high chance of not having a miscarriage. However, the sensitivity and diagnostic odds ratio of the current model is low to consider it to use in clinical practice. Therefore, further steps in model creation which includes internal validation, external validation and testing the model's utility in clinical practice were not undertaken in this study.

Perinatal outcomes:

The systematic review that investigated the perinatal outcomes in women with threatened miscarriage showed that women who experienced threatened miscarriage are at an increased risk of stillbirth, preterm labour, PPROM and placental abruption. There was a small but increased risk for low birth weight babies. Also, placenta previa, neonatal asphyxia and congenital abnormalities were more likely to occur in women who suffered from a threatened miscarriage in early pregnancy. The current cohort study showed that threatened miscarriage was associated with an increased risk of preterm labour, IUGR, low birth weight and neonatal asphyxia. The results of the current study strengthen the evidence of the association of threatened miscarriage with increased perinatal morbidity.

Managing women with threatened miscarriage to prevent adverse perinatal outcomes can be challenging. The results show that women with threatened miscarriage are at

increased risk of adverse pregnancy outcomes. Currently, women diagnosed with threatened miscarriage are not treated as high risk for antenatal care and do not receive any extra surveillance in pregnancy. Further prospective studies are required to establish the perinatal morbidity associated with threatened miscarriage. Any future studies should allow the researchers to adjust for all the confounding variables and conduct multivariate analysis to adjust for interrelated outcomes. Various biomarkers have been identified to predict the occurrence of maternal morbidities such as IUGR and pre-eclampsia. The review by Carty *et al.* had examined a list of biomarkers for early prediction and diagnosis of pre-eclampsia (Carty *et al.*, 2008). A review by Albu *et al.* in 2014 highlighted the role of various predictive factors for IUGR (Albu *et al.*, 2014). Future research focussing on identifying biomarkers or prediction models with a combination of markers will help in the early identification of women who are at risk of these adverse perinatal outcomes in women with threatened miscarriage.

6.2 Strengths and limitations of the project

The study had a prospective design with a clearly defined study population and exclusion criteria. The prospective study designs are expensive and can take a longer time to get the outcome data. However, a prospective study design helps to control the nature and quality of the study. A prospective study is a longitudinal study done over time. Hence it helps the researcher to plan and conduct the study in a way that can ensure strict quality control in deciding the selection criteria, conducting the index tests and in timing and follow up of the participants.

We initially conducted the systematic reviews and based on the findings of these, planned the study protocol and designed the study methodology. Pitfalls from the existing literature were identified and addressed before the conduct of the current study. One of the major drawbacks of the existing literature was poor methodology. We designed and reported the study according to the STARD 2015 guideline for diagnostic accuracy studies (Cohen *et al.*, 2016). In our study, we used clearly defined selection criteria. The ultrasound scans were done by the same sonographer in the same ultrasound machine trans-vaginally to ensure consistency. All the index tests were done

in the same lab with the same technique with strict standard operating policies. Two main drawbacks highlighted in the systematic reviews which our study also failed to address were defining a cut off value for the markers and following gestational age specific values for comparing the markers.

Due to the lower number of miscarriages in the study data, it was not possible to accurately define a cut off value for the markers. This was the case with comparing the markers with gestational age specific values. Due to the sparsity in the number of miscarriages, accurately developing gestational age specific values for the markers were not possible. The ideal way to address this is by doing a longitudinal study in a cohort of women to measure the week to week variation of the biomarkers and ultrasound markers. This data can then be used to compare the median value of biomarkers and ultrasound markers from the miscarried population.

The study succeeded in meeting the sample size as suggested by the power calculation. However, the sample size was calculated from the data available from the literature. There was considerable variation in the reported miscarriage rate in the literature (3-16%). For sample size calculation, we used the mean miscarriage rate (10%) from what was reported in the existing literature. However, in the current study, the miscarriage rate in the threatened miscarriage population was only 5.34%. This resulted in having a fewer number of the primary outcome variable (miscarriage) in the current study. Due to the fewer number of the primary outcome variable, i.e. miscarriage, we were unable to calculate a cut off value of individual sensitive markers. We were also not able to develop gestational age-specific values of the markers to compare.

The study used a pictogram to quantify the blood loss objectively. The study by the Menorrhagia Research Group had highlighted that an objective assessment of the blood loss is more reliable in reflecting the actual blood loss than a subjective assessment of blood loss (Warrilow *et al.*, 2004). The pictogram used for the objective assessment of miscarriage blood loss was the menstrual pictogram, and it was validated for menstrual blood loss by the Menorrhagia Research Group. The pictogram was not validated for the

miscarriage population. Though the amount of blood lost in a miscarriage is different from a menstrual period, the pictogram should be able to objectively quantify the relative amount of blood lost during the miscarriage. Hence using a non-validated pictogram should not have had a huge impact on the results of the study.

One of the limitations of the conduct of the study was not doing a speculum examination to exclude the local causes of bleeding while recruiting the participants. One of the causes for spotting in early pregnancy can be due to local causes like bleeding from the perianal area, vulva, vagina and cervix. A prospective study conducted to assess the extent to which a speculum examination can alter the subsequent management plan in women presenting with bleeding in early pregnancy has shown that out of the 236 women included in the study, only three women (1.3%) had a change of diagnosis and management plan after the speculum examination (Hoey and Allan, 2004). The study concluded that the speculum examination contributed to only a minority of management decision. Henceforth, it is safe to assume that not doing a speculum examination would have had a big impact on the results of the current study.

In the threatened miscarriage group, 18 women were lost to follow up. There was a high possibility that the women who lost to follow up could have a higher representation of women who miscarried compared to women with ongoing pregnancies. This might be because miscarriages could happen at home and in case of a complete miscarriage without heavy bleeding, women might not always attend hospital. However, every attempt was made to find the outcome of these women by contacting the GP and community midwives.

Both Intra and inter-observer variations have been reported in the literature for fetal measurements (Sarris *et al.*, 2012). In the current study, in order to avoid inter-observer variations, the same researcher had done all the scans. Nevertheless, no corrections have been taken for intra-observer variations of the measurements, which is not a huge but a limitation of the study.

6.3 Future research and directions

We need future research to test the proposed prediction model. The model needs to be tested and externally validated in a prospective research setting to assess the generalizability of the model.

Though we aimed to correct for the weekly variation of biomarkers, we were unable to do that due to the lower number of miscarriages. The appropriate method to correct for the weekly variation of biomarkers would be to follow up a group of women with threatened miscarriage weekly and to test the biomarker levels in them serially. This will help us to estimate the weekly variation of biomarkers and ultrasound markers accurately. Though we had intended to do that in the current study, we failed to recruit patients to come every week to do the blood test and the scan due to the lack of personnel and resources. This needs to be conducted as a separate research on its own.

The adverse perinatal outcomes of women with threatened miscarriage is an area that can be researched further. It would be ideal to have predictive markers for adverse perinatal outcomes. This will help the obstetrician to identify those women who are at this specific risk and provide them with personalised high-risk care.

6.4 Studies on treatment for threatened miscarriage and its relevance

Another existing challenge is the lack of definitive treatment for threatened miscarriage. Recently there was immense interest in the use of progesterone for treating threatened miscarriage. A Cochrane systematic review (Wahabi *et al.*, 2018) and meta-analysis published in 2018 combined the available evidence from seven RCT and concluded that treatment of threatened miscarriage with progesterone reduces the miscarriage rate compared to placebo or no treatment (risk ratio (RR) 0.64, 95% confidence interval (CI) 0.47 to 0.87; 7 trials; 696 women). The PRISM trial (Coomarasamy *et al.*, 2019b), which was a multicentre, randomised, double-blind, placebo-controlled trial looked at the use of progesterone for improving pregnancy outcome in women who experienced bleeding

in early pregnancy, has shown a trend towards a benefit on the use of progesterone (live birth rate of 75% in the progesterone treated group versus a live birth rate of 72% in the placebo group). On Subgroup analysis, the use of progesterone showed a definite benefit in those women who had one or more miscarriages. The relative rate of miscarriage in women with one or two miscarriage was 1.05; 95% CI (1-1.12) and the relative rate of miscarriage in women with three or more previous miscarriage was 1.28; 95% CI (1.08-1.51). With the emerging evidence on therapeutic interventions to treat miscarriage, prediction of miscarriage in women with threatened miscarriage became relevant. The current study and from the systematic reviews and meta-analysis on available evidence on predicting miscarriage in the threatened miscarriage so far shows promising results. However, larger studies with a greater number of miscarriages are required to give conclusive answers.

6.5 Conclusions

The study concluded that biomarkers and ultrasound markers could be used to predict miscarriage in women presenting with threatened miscarriage. The study developed a log regression model using demographic variables, biomarkers and ultrasound markers to predict miscarriage in women with threatened miscarriage.

The study also concluded that women with bleeding in early pregnancy are associated with a higher risk for perinatal complications. In order to reduce the morbidity and mortality associated with these adverse perinatal outcomes, future studies are required focussing on developing markers of adverse perinatal outcomes in women experiencing bleeding in early pregnancy. This will help to plan the antenatal care of those high-risk women with bleeding in early pregnancy.

Appendix 1 – Study questionnaire

Study no:	DOB:	Initials:

CONFIDENTIAL

The POM Study

Structured Interview Questionnaire

This study is about prediction of miscarriage using biochemical markers, ultrasound parameters and demographic variables. This questionnaire is designed to find out more about you. There are questions about your age, weight, ethnicity, employment, previous and current pregnancy, family history and your diet. Please answer every question.

The answers will be treated as strictly confidential and will be used only for medical research.

SECTION A INFORMATION ABOUT YOU

Please tick the most appropriate answer or fill out the details required on both sides of each page.

1. What is your date of birth?

d	d	m	m	y	y	Y	y

2. What is your height? ……. Feet …….. inch or …… cms

3. What is your weight? …… Stones or …….. Kg

4. What is your partner's age? ……..

5. What is your ethnic origin? (Please tick one box only)

White ☐	White British ☐	White Irish ☐	White Other ☐	
Mixed Race ☐	White & Black Caribbean ☐	White & Black African ☐	White & Asian ☐	Other mixed background ☐
Asian or Asian British ☐	Indian ☐	Bangladeshi ☐	Pakistani ☐	Other Asian background ☐
Black or Black British ☐	Caribbean ☐	African ☐	Black Other ☐	
Chinese or other ethnicity☐	Chinese ☐	Other ☐ (please specify)		

6. What is your occupation? …….

7. How would you describe your job?

Mostly sitting ☐
Mostly standing ☐
Mostly moving about ☐
Mixture of above ☐

8. Were you recently very stressed out?

None ☐ Mild ☐ Moderate ☐ High ☐

SECTION B GENERAL HEALTH

9. Has a doctor ever told you that you have or have had any of the following condition? Please tick all that apply and if known, give the year at which each condition was first diagnosed?

	Yes	No	Year of diagnosis
Raised blood pressure (Hypertension)	☐	☐	☐☐☐☐
Diabetes Mellitus	☐	☐	☐☐☐☐
Underactive thyroid (Hypothyroidism)	☐	☐	☐☐☐☐
Overactive thyroid (Hyperthyroidism)	☐	☐	☐☐☐☐
Eating disorder (Anorexia nervosa)	☐	☐	☐☐☐☐
Polycystic ovaries (PCOS)	☐	☐	☐☐☐☐
Any other illness	☐	☐	☐☐☐☐

If yes, please write the type of illness here: ……

10. Are you following any of these diet?

Vegetarian ☐

Vegan ☐

Low calorie ☐

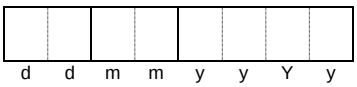
Other special diet eg: diabetic ☐

Please describe: ……..

11. Do you currently smoke? Yes ☐ No ☐

12. If yes, how many cigarettes/tobacco/e-cigarettes do you smoke per day? (Circle the one which is applicable)
    1) 0-5 2) 5-10 3) 10-20 4)>20 or if you use nicotine patches- how many in a week ……..

13. If no, when did you stop smoking?

    1)

    2) Never smoked

14. How many were you smoking previously/day (cigarettes/tobacco/e-cigarettes)? (Circle the one which is applicable)
    1) 0-5 2) 5-10 3) 10-20 4)>20 or if you use nicotine patches- how many in a week? ……..

15. Are you exposed to passive smoking at home or work i.e. does your partner or other family or work colleagues smoke? ☐ Yes ☐ No

16. Do you drink alcohol in the current pregnancy? Yes ☐ No ☐

17. If yes, how many units of alcohol do you drink per week? (Please use the pictogram below to calculate the number of alcohol units) …….

18. If no, did you use to drink alcohol before? Yes ☐ No ☐

19. If yes, how many units of alcohol do you drink before per week? (Please use the pictogram below to calculate the number of alcohol units) ……..

20. If yes to question number 18, when did you stop drinking alcohol? ……

21. If you drink coffee/tea/coke/Lucozade/Red bull in the current pregnancy, how many cups/ cans you drink per week? …….

22. How many chocolates do you eat per week? .....

Brand name …….

23. If you use any recreational drugs in the current pregnancy, what do you use? …….

If yes, how often do you use recreational drug/s? ……

*Gin, rum, vodka, whisky, tequila, sambuca. Large (35ml) single measures of spirits are 1.4 units.

Picture 1: Reference http://www.nhs.uk/Livewell/alcohol/Pages/alcohol-units.aspx

OBSTETRIC HISTORY

24. If this is not your first pregnancy, please record details of previous pregnancies in the table below:

Pregnancy number	Weeks of pregnancy	Pregnancy outcome (miscarriage, ectopic pregnancy, termination of pregnancy, live birth)	Pregnancy complication/s (Pre-eclampsia, small baby, gestational diabetes, Pre labour rupture of membrane, Preterm labour, placental abruption)	Mode of delivery
1.				
2				
3.				
4.				
5.				
6.				
7.				
8.				
9.				
10.				
11.				
12.				
13.				

FAMILY HISTORY

25) Did your mother suffer from miscarriage? Yes ☐ No ☐ Don't Know ☐

If yes, how many miscarriages she had? ……

26) If you have sister/s, did she/ they suffer from miscarriage?
Yes ☐ No ☐ Don't know ☐

If yes, how many miscarriages did she or them had? ……

CURRENT PREGNANCY

27) If you have used any contraception before getting pregnant, what contraception did you use? ……..

If you have used contraception, when did you stop using it? ……..

28) What was the first day of your last menstrual period (dd/mm/yyyy)?

d	d	m	m	y	y	Y	y

29) Have you experienced any of these following symptoms in this pregnancy?

Vaginal Bleeding ☐
Lower abdominal pain ☐
Vaginal bleeding and lower abdominal pain ☐

30) If you had any of the above symptoms, how many days you are having it?

1) <1 ☐
2) 1-3 ☐
3) 3- 7 ☐
4) > 7 ☐

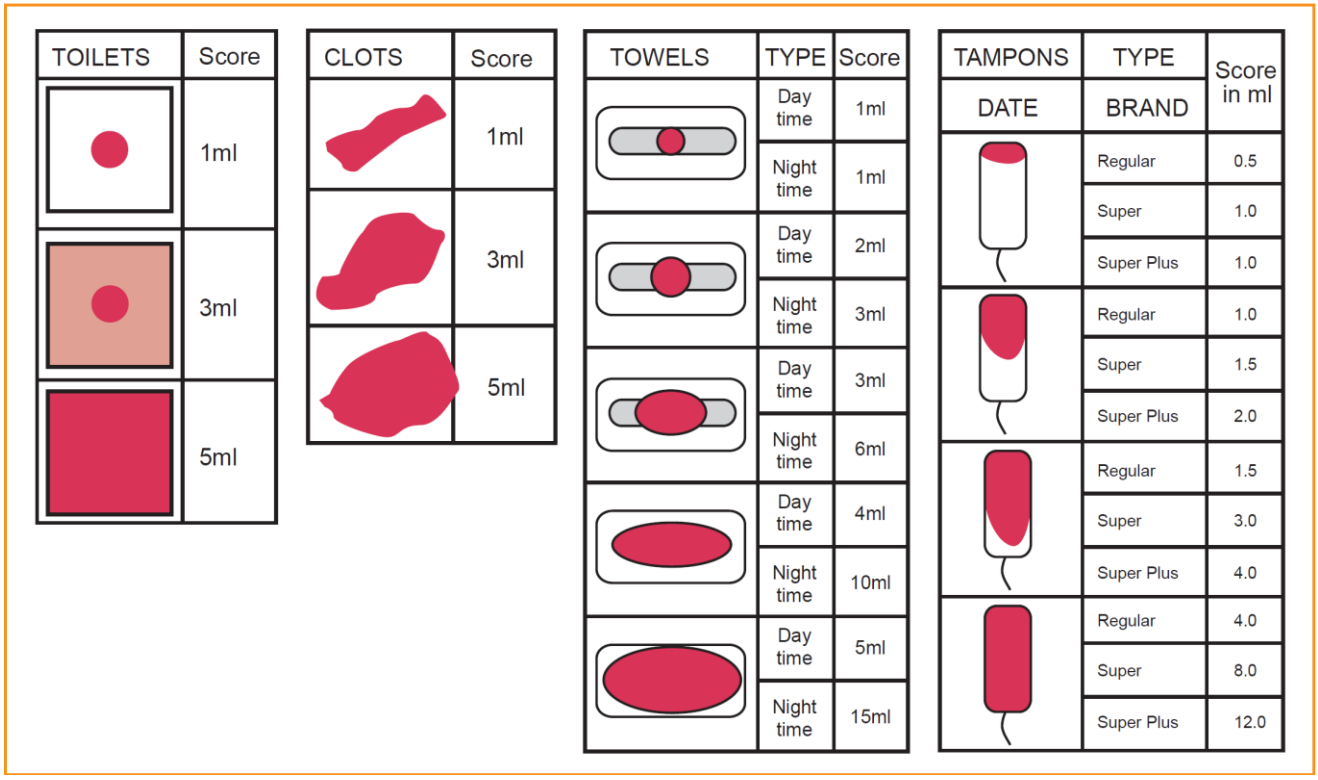
AMOUNT OF BLOOD LOST

(Please use the pictogram below to quantify your blood loss.)

31) Please give your score for blood loss looking into the pictogram given at the end of this questionnaire …….
32) How many pads/ tampons you had to use/ day? ……
33) How many days you had bleeding? ……
34) How do you best describe the blood loss you experienced?

Mild ☐
Moderate ☐
Heavy but not causing physical symptoms ☐
Heavy causing physical symptoms of dizziness, fast heartbeat, tiredness or collapse ☐

Figure 1: Pictogram to quantify vaginal blood loss



Picture 2: Reference: The menorrhagia research group

Assessment of menstrual blood loss using the menstrual pictogram. The scores (in milliliters) associated with each icon are given. Warrilow, G., Kirkham, C., Ismail, K. M., Wyatt, K., Dimmock, P., & O'Brien, S. (2004). Quantification of menstrual blood loss. The Obstetrician & Gynaecologist, 6(2), 88-92.

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