PREGNANCY OF UNCERTAIN VIABILITY: INVESTIGATING A STRATEGY FOR OUTCOME PREDICTION IN MALTA

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DEDICATION

To my husband Julian and my three beautiful children:
James, Mark and Matthew
Thanking you for being my greatest support

&

To all women who have suffered early pregnancy loss in silence, feeling isolated or misunderstood. May they find solace in the knowledge that they are not forgotten.

Abstract

Purpose: Pregnancy of uncertain viability (PUV) is an intra-uterine pregnancy that does not present the ultrasonographic criteria to confirm viability or miscarriage. Follow-up scans are required until a definitive diagnosis is reached, resulting in patient anxiety and cost to the health services. The purpose of the study was to investigate PUV prediction and ultrasound follow-up strategies, in order to optimise PUV management.

Objectives: The predictive aspect of the study analysed clinical, biochemical and ultrasonographic factors in relation to PUV outcome. Assessment included a bleeding score, ultrasound parameters and baseline Human Chorionic Gonadotropin (HCG) and Progesterone levels. The strategic aspect of the study compared interval ultrasound findings.

Methodology: A prospective, non-experimental, quantitative, correlational design was adopted. Women diagnosed with PUV were followed up weekly until a definitive diagnosis of viability or miscarriage was made. The predictive parameters were analysed individually and in combination. The 7 and 14-day ultrasound findings were compared to identify an optimal follow-up strategy.

Results: 43 women diagnosed with PUV, aged between 18 and 42 years were recruited, of whom 41.9% miscarried. Pregnancy loss was statistically associated with vaginal bleeding ($p=0.003$), presentation at a later gestational age ($p=0.000$) and with lower progesterone levels ($p=0.000$). Viable gestational sacs showed a faster daily MSD growth rate (1.161mm/day) than non-viable ones (0.769mm/day). A model combining bleeding, progesterone level and MSD was devised to predict PUV outcome. It had a sensitivity of 72.2% and a specificity of 88% (AUC=0.801). Ultrasound findings at 7 and at 14 days showed that 79% of the cohort received a definitive diagnosis by 7 days.

Conclusions: A 7-day ultrasound follow-up strategy was seen to provide a definitive diagnosis in the majority of participants, suggesting that this strategy should be further investigated as it may play a role in the management of PUV patients in Malta.

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List of Abbreviations and Acronyms

ACOG: American College of Obstetricans and Gynaecologists

ALARA: As Low As Reasonably Achievable

Ang-2 : Angiopoietin-2

ASUM: Australian Society of Ultrasound in Medicine

AUC: Area Under the Curve

Ca 125: Cancer antigen 125

CI: Confidence Intervals

CRL: Crown Rump Length

Flt-1: Soluble fms-like tyrosine kinase-1

HADS: Hospital anxiety and depression score

HCG: Human Chorionic Gonadotropin

IPUV: intra-uterine pregnancy of uncertain viability

IVF: in-vitro fertilization

LMP: last menstrual period

mmol/l: millimoles per litre

MSD: Mean Sac Diameter

NOIS: National Obstetric Information System

NICE: National Institute for Health and Care Excellence

Ng/ml: nanograms per millilitre

OR: Odds Ratio

PBAC: Pictorial Bleeding Assessment Chart

PUL: Pregnancy of unknown location

PUV: Pregnancy of uncertain viability

RCOG: Royal College of Obstetricians and Gynaecologists

ROC: Receiver Operating Characteristic

SCH: Subchorionic Haematoma

SOGC: Society of Gynaecologists of Canada

SPSS: Statistical Package for the Social Sciences

TNF- alpha: Tumour Necrosis Factor alpha

WHO: World Health Organization

Definition Of Key Concepts

Early viable pregnancy: an intra-uterine pregnancy with a fetal pole having visible heart pulsations on ultrasound**.**

Expectant management of miscarriage: waiting for natural expulsion of products of conception after fetal demise.

First trimester: up to 13 completed weeks of pregnancy.

Miscarriage a miscarriage can be defined as the spontaneous loss of a pregnancy before the fetus has reached viability at 24 weeks (RCOG 2006)

Pregnancy of Uncertain Viability: an intra-uterine pregnancy that does not present the criteria to confirm viability but is still not diagnostic of miscarriage (Bourne 2012)

Pregnancy of unknown location: When a woman has a positive pregnancy test, but no intrauterine or extrauterine pregnancy can be seen with a transvaginal ultrasound scan.

CHAPTER 1: INTRODUCTION

1.1 Introduction

This chapter gives a brief synopsis of pregnancy of uncertain viability in its clinical context (Section 1.2), addresses the local perspective on the subject (section 1.3), setting the scene for the PUV study by stating its aims and objectives in section 1.5.

Miscarriage is not preventable (Norton & Furber, 2018). It is the most common complication experienced during the first trimester of pregnancy. It places a large burden on health services in terms of workload and cost, not to mention the physical and emotional toll on the patients and their families (Farren et al., 2018; Jurkovic, Overton, & Bender-Atik, 2013). Miscarriage incidence rates range from 10-25% (Jurkovic et al., 2013). The American College of Obstetricians and Gynaecologists (hereafter referred to as ACOG) indicates a 10% incidence of miscarriage overall during the first trimester (ACOG, 2018), whilst the National Institute for Clinical Health and Excellence (hereafter referred to as NICE) reports a 20% incidence. A possible reason for this variation is that miscarriage rates increase with age from 25% at age 35, to 40% at age 40, and rises to 80% at 45 years of age (ACOG 2018).

Early pregnancy loss most commonly presents with vaginal bleeding and/or abdominal pain (Norton & Furber, 2018). It may be completely asymptomatic, hence resulting in silent miscarriage diagnosed on a subsequent ultrasound (Jurkovic et al, 2013). Moreover, not all women presenting with symptoms suspicious of early pregnancy loss will actually miscarry. Clinical and

ultrasonographic assessments aim to achieve a diagnosis at the first instance, even if this is not always possible (Bottomley et al., 2009; Preisler et al., 2015). In cases where there is evidence of intrauterine gestation, there are three possible diagnoses. The first is a live gestation with confirmed fetal heart motion, termed viable. Women in this category are reassured and referred for an antenatal booking visit. The second possible diagnosis is a confirmed miscarriage, and consequently, such women are offered appropriate management. The last diagnosis is classified as pregnancy of uncertain viability (hereafter referred to as PUV); also known as an intrauterine pregnancy of uncertain viability (IPUV) (Abdallah, Daemen, Kirk et al., 2011b) or pregnancy of unknown viability (Rodgers, Chang, De Bardeleben, & Horrow, 2015). These women fulfil ultrasonic criteria which are neither diagnostic of miscarriage nor confirmatory of a viable pregnancy. PUV, is common, possibly affecting one in five early pregnancy unit attendees (Hu, M., Poder, & Filly, 2014). This last category of women needs follow-up to confirm their pregnancy outcome. Follow-up may happen between 5 and 14 days from presentation depending on individual hospital protocols and resources (NICE 2019). The uncertainty of the diagnosis in females with PUV has been shown to increase the anxiety levels of these women(Richardson, Raine-Fenning, Deb, Campbell, & Vedhara, 2017). Thus, providing them with a definitive diagnosis in the most efficient way possible is desirable.

1.2 Clinical Background

The management of early pregnancy loss is governed by regularly updated guidelines published by international bodies such NICE and the ACOG.

However, the ultrasonic cut-off measurements for the diagnosis of PUV, recommended by these guidelines, have been challenged over the last 2 decades (Abdallah et al., 2011; Doubilet, Benson, Bourne, & Blaivas, 2014). This stemmed from a report of four mistaken diagnoses of miscarriage published in 1995 (Hately, Case, & Campbell, 1995). In 2012, the "Society of Radiologists in Ultrasound Multi-specialty Panel on Early First Trimester Diagnosis of Miscarriage and Exclusion of a Viable Intrauterine Pregnancy" issued a newer set of recommendations (Doubilet, Benson, Bourne, & Blaivas, 2013, p.1443). These 2012 guidelines were more rigorous than the previous ones issued by the Royal College of Obstetricians and Gynaecologists (2006) (hereafter referred to as RCOG) and allowed for larger inter-observer variation.

Hence, the new recommendations changed the thresholds for PUV versus non-viability. If the measurement of the fetal pole, termed crown-rump length (CRL), was less than 7mm, with no visible heartbeat it would constitute a PUV. If the size of the gestational sac measured as a mean gestational sac diameter (MSD) was less than 25mm without a visible fetal pole, it would classify as a PUV. Previous cut-off values were 6mm for the CRL and 20mm for MSD according to RCOG (2006). The new guidance regarding the follow-up timing for PUV now ranged from 7-13 days (Doubilet et al., 2013). These guidelines were internationally endorsed by the ACOG (Lockwood 2015) as well as NICE (2012) and, remained identical in the recent update (NICE 2019). However, there is still considerable variation in practice between different obstetric units. This is especially true in relation to the timing of follow-up scans and the rescan criteria for the diagnosis of miscarriage (Abdallah et al., 2011b; Preisler et al.,

2015). NICE (2019) suggests reviewing a patient with PUV in at least 7 days following the initial presentation with the possibility of needing other scans. The ACOG (2018) mentions 7-14 days but then questions the rationale behind this guidance.

1.3 The Local Perspective

The local state general hospital caters for circa 4500 deliveries per annum (Gatt & Borg, 2017). Patients having complications of early pregnancy, irrespective of gestation and severity of symptoms, are allowed to refer themselves and are reviewed on the same day in a 24-hour emergency room with a daily departmental morning sonographic service. This is unlike early pregnancy units in other countries such as the UK (NICE 2019), where telephonic advice and pre-set appointments may act as gatekeepers to very early gestational ages under 6 weeks when the chances of diagnosing PUV are higher (Bottomley et al., 2009). They have also been shown to be cost effective (van den Berg 2015). Locally, patients requiring hospital admission, for complications such as bleeding, may be admitted to the gynaecology ward. On the other hand, a patient diagnosed with PUV is asked to return for review, to confirm a diagnosis of viability or miscarriage in a variable time interval of between 7 and 14 days. There is currently no protocol to determine an exact review schedule (Consiglio & Muscat Baron 2017).

The Malta College of Obstetricians and Gynaecologists (MCOG) and the local state general hospital follow the current NICE guidelines (2019), adapted to local hospital protocols (Consiglio & Muscat Baron, 2017). The main difference

is the requirement of a second scan re-confirming the diagnosis of miscarriage, rather than it being optional as suggested by NICE (2019). This aims to reduce mistaken diagnoses of early pregnancy loss. However, there is still marked variation in local practice regarding the management of early pregnancy complications, especially with regard to the timing and repetition of ultrasound scans used to confirm a diagnosis. Multiple visits and ultrasound scans result in increased life disruption for the patients with PUV, coupled with an increase in hospital workload and expense (Bottomley et al., 2009). It is therefore vital to explore a way of balancing accuracy of diagnosis with efficiency. In a detailed audit of 40 cases of miscarriage, carried out by Consiglio and Formosa (2016) at a local state general hospital, 78.9% of cases were diagnosed after 2 scans and the mean duration for diagnosis was 7.76 days. However, 18.4% of cases needed between three and five ultrasound scans to reach a diagnosis.

1.4 Rationale For The Study

The 2012 updated international guidelines (NICE) allowed for more inter-observer variation and hence more certainty in the diagnosis of miscarriage. However, the guidance with respect to timing of the follow-up ultrasound scans was not clear (Bourne, 2016).

Whilst allowing for flexibility, different ultrasound timing strategies may have different clinical implications. A 7-day follow-up schedule may result in a diagnosis of persistent PUV. This would necessitate extra follow-up visits, leading to further uncertainty as well as carrying financial implications (Richardson et al. 2017). On the other hand, a 14-day review, is more likely to

ensure a definitive diagnosis but with the possible drawbacks of delaying “closure” and emergency admissions in the interim period. This may lead to more maternal morbidity (Jurkovic et al., 2013).

There seems to be no consensus in the literature as to the best timing strategy to be used in PUV patients; although there appears to be agreement that a diagnosis is evident after 14 days (ACOG, 2018; Huchon et al., 2016; NICE, 2019). In its recent 2019 update, NICE has retained the 2012 research recommendations in relation to patients with PUV. It specifically refers to “timing and frequency of ultrasound examination” and, the effect that these “different strategies” have on “cost and women’s experience” (NICE 2019 NG 126 Research Recommendation 2). There is ample evidence for the use of clinical (Naji et al., 2010), biochemical (Memtsa, Jurkovic & Jauniaux, 2018; Verhaegen et al., 2012) and sonographic (Lane & Wong-You-Cheong, 2014; Mazzariol et al., 2015) factors to predict PUV outcome. In addition, predictive models (Guha et al., 2013; Model, 2011; Reid et al., 2011b) have been suggested to help guide clinicians in predicting the outcome of PUV. These may be used to help design the optimal ultrasound timing strategy for the management of PUV. Hence, the purpose of the current PUV study is to address this knowledge gap concerning the ultrasound follow-up strategy for patients with PUV.

1.5 Aims And Objectives

The first aim of this PUV study was to investigate the possibility of predicting the outcome of PUV using clinical, biochemical and ultrasonographic markers used either individually or in combination.

The objective was to correlate the variables with the final outcome i.e. viability or miscarriage. These included:

- Clinical variables : maternal age, gestational age, bleeding assessed by a pictorial bleeding assessment chart, past obstetric history.
- Biochemical variables: Progesterone and HCG
- Ultrasonographic variables: MSD, CRL, yolk sac mean diameter.

The second aim was to identify the most effective ultrasound follow-up strategy for PUV patients within the local scenario.

The objective was to compare the participant ultrasound findings at 7 and 14 days using different stratification criteria namely gestational age, MSD size and the presence of the fetal pole.

1.6 Methodology

A prospective, quantitative, non-experimental, correlational study design was employed for this research. Ethical approval from the Faculty Research Ethics Committee, University Research Ethics Committee and consent from the local state hospital authorities was obtained (see Appendix A).

The participants included pregnant women, aged 18-50, in their first trimester, attending the obstetric emergency services of a state general hospital in Malta,

having vaginal bleeding, pain or other symptoms suspicious of miscarriage . Participants had to be clinically stable and carrying a single gestation. Ultrasound inclusion criteria comprised a diagnosis of PUV following a transvaginal scan in accordance with the current NICE guidelines (NICE 2019). This comprised a Mean Gestation Sac Diameter of <25mm with no fetal pole; or a fetal pole with a Crown Rump Length measuring <7mm with no fetal heart motion. Women fulfilling the criteria were invited to participate by the intermediary members of staff aiding in the data collection and, if willing to participate they were then asked to sign a consent form prior to data collection (sample in Appendix B).

A data collecting tool, discussed in Section 3.8, was designed by the researcher, following an extensive literature review on the topic. The tool was used to document the necessary clinical and obstetric information. The patients' amount of bleeding was assessed using an internationally validated Pictorial Blood loss Assessment Chart (PBAC) (El-Nashar, Shazly, & Famuyide, 2015; Higham, O'brien, & Shaw, 1990). Blood tests required to collect the biochemical data, including Human Chorionic Gonadotropin (hereafter referred to as HCG) and Progesterone levels, were taken during the patient's first clinical assessment, termed Day 0 (Pillai, Konje, Tincello, & Potdar, 2016; Puget et al., 2018). The patients were electively followed-up on a weekly basis using ultrasound and clinical assessments until the diagnosis was certain. If these women required extra emergency visits in the interim period, the information from those visits was also collected. In case of miscarriage, the participants were referred for standard management to the same state general hospital; whilst viable pregnancies were referred for

antenatal booking. Pregnant patients were subsequently followed till the end of the first trimester to ascertain that the pregnancy was still viable.

All data collected was analysed using Statistical Package for the Social Sciences (SPSS) version 25 and a statistician was also consulted to perform the following analysis:

- To establish the incidence of miscarriage in this PUV patient cohort
- To analyse the possible clinical predictive factors in the selected patient cohort in terms of age, ethnicity, parity and presenting symptoms.
- Univariate analysis of the Biochemical data, including HCG and progesterone levels, compared to the patient's outcome.
- Univariate analysis of the individual ultrasonic parameters compared to the patient outcome including MSD, CRL and Yolk Sac Size.
- A stepwise multivariate analysis of the different variables to outcome; comparing the performance to previously suggested predictive models.
- The time interval taken to ascertain a diagnosis in the selected patient cohort. This was reviewed with the aim to recommend possible ultrasound strategies to be used locally.

1.7 Overall Structure Of The Study

After this brief introduction, Chapter 2 will examine the literature pertaining to the change in PUV definitions, as well as give an insight into research previously carried out in relation to predicting its outcome. The study design and methodology are detailed in Chapter 3. The Results and Discussion will follow in Chapter 4, while Chapter 5 will conclude the study and make recommendations for further research.

CHAPTER 2: LITERATURE REVIEW

2.1 Introduction

This chapter starts by giving a brief overview of the research strategy adopted in the study (Section 2.2). It then goes onto depicting a normally developing pregnancy to contextualise PUV (Section 2.3). It aims to clarify the difference between a viable and a non-viable pregnancy (Section 2.4) and explores the concept of PUV and why it was redefined (Section 2.7). It also looks at exposing the knowledge gap and literature debates in the area of scan strategies and prediction studies (Section 2.9.-2.13). This gap is what this study is intended to address.

2.2 Research Strategy

An extensive research into health-related databases was carried out using the Portal of the University of Malta (Hydi) together with databases such as Pubmed, Medline, EBSCO (Elton B. Stephens Co), CINAHL (Cumulative Index to Nursing and Allied Health Literature), Cochrane and Proquest. The search was restricted to full-text peer-reviewed articles in the English language. Since the definition of PUV changed over the last 10years, the said research goes back the same number of years. However, some older but pertinent studies were also included.

Different citation subsets were used to address the different aspects of the study. The first includes: miscarriage, pregnancy of uncertain viability, pregnancy of unknown viability, PUV, IPUV, prediction, prediction model, scoring, outcome. Another relates to biochemical markers: HCG, Progesterone. Original studies, as well as meta-analyses, were also included.

Some editorials and opinion pieces of well-known researchers in the field were also referenced since they were considered to amplify the researcher's knowledge.

2.3 Normal Ultrasonographic Appearances

The use of transvaginal ultrasound has improved the visualization of early pregnancy, anticipating abdominal scan findings by a week, therefore becoming the gold standard for early pregnancy (Bickhaus, Perry, & Schust, 2013; Infante et al., 2013).

2.3.1 The gestational sac

At around 4.5 to 5 weeks, a small fluid collection representing the sac may appear within the thickened endometrium (Al-Memar, Kirk, & Bourne, 2015; Bree et al., 1989; Infante et al., 2013). The sac is distinguished from a pseudosac by its eccentric placement in the decidua and the presence of a double decidual or intradecidual signs (Bickhaus et al., 2013; Infante et al., 2013; Mizia et al., 2018).

2.3.2 The Yolk sac

The yolk sac is the first structure to confirm an intrauterine pregnancy at around 5-5.5 weeks (Levi et al., 1990; Moradan & Forouzeshfar, 2012). This antecedes the placenta in providing feto-maternal transport and disappears by the end of the first trimester. Initially, it is visualised as two parallel lines, representing the anterior and posterior walls, later to appear circular and anechoic (Rodgers et al., 2015).

2.3.3 The Fetal Pole

A 1-2 mm fetal pole appears at the edge of the yolk sac at around 6 weeks giving a signet ring appearance (Bottomley & Bourne, 2009). The measurement of the pole or CRL is the accurate dating parameter for early pregnancy (Bottomley & Bourne, 2009). Fetal heart pulsations may be seen early (CRL =1.3mm) (Infante et al., 2013). A heart rate of at least 100 beats per minute is usually visible when the pole measures 4-7mm (Rodgers et al., 2015). However, the heartbeat may not be detectable in around one-third of 5mm fetal poles (Infante et al., 2013). At 6.5-7 weeks, the pole appears kidney-bean shaped and distant from the yolk sac with a heart rate of 120 beats /minute (Bottomley & Bourne, 2009). Concurrently, the amnion becomes visible until it fuses to the chorion at the end of the first trimester (Lane & Wong-You-Cheong, 2014). By the 8th week, the pole becomes differentiated having a cephalic curve and limb buds (Doubilet, 2014; Rodgers et al., 2015). In embryological terms, this should be referred to as an embryo and replaced by the term fetus after 10 weeks. However, these terms are commonly interchanged in early pregnancy literature (Bottomley et al., 2013). The ultrasonographic appearances described are quite consistent such that any differences may raise suspicion of impending miscarriage (Rodgers et al., 2015).

2.4 Viability Versus Non-Viability

In early pregnancy terminology, viability refers to the presence of visible heart pulsations (Al-Memar et al., 2015). This may be considered an imprecise term, since, according to the World Health Organization (WHO), viability actually refers to the possibility of the fetus surviving outside the uterus; that is 22 weeks gestation or a weight of 500g (Salomon et al., 2013; Pignotti & Donzelli, 2008;

Gatt & Borg 2017). Alternative early pregnancy terms include live gestation or ongoing pregnancy. However, the term “viable” has been accepted in early pregnancy parlance (NICE 2019).

On the other hand, non-viability, a confirmation of early pregnancy loss has been contentious since making a false positive diagnosis of miscarriage is totally unacceptable and a 100% specificity and 0% false positive rate is expected (Bourne, 2016; Doubilet et al., 2014). This refers to the lower limit of the Mean Sac Diameter (MSD) devoid of a fetal pole to be defined as non-viable, or the lower limit of fetal pole size,termed Crown Rump Length (CRL), without heart pulsation to be defined as non-viable. In cases where the cut-off limits for MSD and CRL are never reached, time-based criteria for non-viability need to be defined (Doubilet et al., 2014). This refers to the time interval beyond which it is acceptable to confirm a miscarriage.

2.5 Pregnancy Of Uncertain Viability

2.5.1 PUV: a changing definition

PUV refers to those intrauterine pregnancies that do not present the criteria to confirm viability but are still not diagnostic of miscarriage (Rodgers et al., 2015). The definition of this category changes as the definitions for non-viability change. PUV may be considered to be an iatrogenic concept and ultimately, all patients with this interim diagnosis would progress to certainty given sufficient time (Bottomley et al., 2009). There are no pregnant women, in the community, aware of having a diagnosis of PUV. Thus, the PUV population is a clinical one; an important concept in the interpretation of generalisation of research studies

(Hulley, Cummings, Browner, Grady, & Newman, 2013). The condition exists mainly due to sensitive pregnancy tests and widespread access to early obstetric emergency care at a time when the outcome is not yet clear (Bottomley et al., 2009). However, once this phenomenon is known, the clinician is bound to offer care and the researcher to investigate it. It has been emphasized that early scans may increase the chances of finding a PUV, and possibly the risk of making a mistaken diagnosis. Hence, waiting and repeating the ultrasound scan is the safer option in a haemodynamically stable patient (Al-Memar et al., 2015).

2.5.2 PUV: a mixed population

Pregnancy of uncertain viability is a mixed population. It consists of early, healthy pregnancies that have not yet confirmed a live gestation, and pregnancies with arrested growth that will ultimately miscarry (Bottomley et al., 2009). Any predictive studies ultimately differentiate these sub-populations. In a review of the recommendations of the Society of Radiologists, Rodgers et al. (2015) suggested a sonographic sub-classification into two categories to reflect the two sub-populations. The first would include smaller empty gestational sacs or fetal poles <4mm without a visible heartbeat suggestive of the healthy early intrauterine pregnancies. The second category would encompass larger empty sacs >16mm and the larger fetal poles (4-6mm) with no visible heart pulsations suggestive of delayed miscarriage. The latter category would be more "suspicious" of early pregnancy loss and in their opinion more congruent with the term "true PUV". However, this classification is not diagnostic and has not been adopted (Rodgers et al., 2015).

2.5.3. Incidence rates of PUV

The quoted incidence of PUV is variable for multiple reasons. First, it is altered by the changing definition of non-viability (Rodgers et al. 2015). Hence, older studies may be considered outdated. Secondly, sensitive commercially available pregnancy tests increase awareness of pregnancy losses that may have been previously ignored (Jurkovic et al. 2013). Thirdly, some early pregnancy units are more stringent in accepting referrals than others, and this affects the incidence of PUV. According to Bottomley et al. (2009), a definite diagnosis may be given at the first ultrasound visit in 78.6% of cases if women are scanned beyond 7 weeks as opposed to 37.7% when scanned prior to 49 days. NICE (2019) recommends referral after 6 completed weeks unless there is suspicion of ectopic pregnancy. For these reasons, the incidence of PUV varies in different studies. Reid (2011) indicated a 13% incidence whilst other studies quoted incidences of between 25% (Davison, Appiah, Sana, Johns, & Ross, 2014) and 29% (Infante et al., 2013). Interestingly, a 2018 study by Puget et al., (2018) showed a lower incidence of 12.5% despite the new definitions used. One would expect an increased incidence if the definitions of PUV have become broader.

2.5.4 Miscarriage incidence in PUV

The miscarriage risk for PUV is also variable. Abdallah's cohort had an incidence of 46.1% (Abdallah et al., 2011); whilst the Puget cohort (2018) showed a lower pregnancy loss of 38.3%. One reason for this variation is selection bias, as Lautmann et al. (2011) acknowledged for their study. In their case, predictive scoring was optionally offered to early pregnancy unit attendees. Their study population was considered as "higher risk" than those

women who declined the test. They concluded that this factor contributed to their higher miscarriage rate of 50.3%. The outcome rates also depend on the duration of follow-up. It is known that miscarriage may happen after the fetal heart is visualised. Lautmann et al (2011), who followed up their patients till the end of the first trimester, would have underestimated their rate by 5% had they stopped follow-up at 2 weeks, as Elson et al. (2003) did in their study. In addition, outcome rates were shown to depend on the clinical and sonographic findings of the participants involved. In the Reid study (2011), the PUVs with a visible fetal pole fared worse compared to those with an empty sac. The average risk of miscarriage for the whole cohort was 47.7% whilst those with a visible pole was 58% (Reid et al., 2011). In another cohort, miscarriage risk was 12%, but rose to 30% in the presence of bleeding (Bottomley et al., 2009). Interestingly, once PUV pregnancies were confirmed to be viable, their long term outcome was found to be good (Reid et al., 2011).

2.6 Consequences Of PUV: Why Research It?

2.6.1 Zero tolerance to mistakes

In 1995, a public inquiry, known as the Cardiff report, disclosed 4 medico-legal cases of mistaken diagnosis of miscarriage (Hately et al., 1995). This heightened the need for zero tolerance to mistakes which are more likely to occur when the CRL and MSD values are close to the boundary of viability (Bourne, 2016; Datta et al., 2012). Bourne requoted from the Cardiff report: "'the death of an early pregnancy should be regarded as of equal significance to that occurring at a later stage'. This sentence should be printed on the

ultrasound machine in any unit that examines women in early pregnancy". (Bourne 2006, p. 5).Thus, a clear understanding of PUV is essential.

2.6.2 PUV and Psychological Morbidity

Early pregnancy loss is known to be associated with negative psychological sequelae: anxiety, depression and post-traumatic stress disorder (Farren et al., 2018; Jacob, Polly, Kalder, & Kostev, 2017). Being a common event, it runs the risk of being normalized and its impact underestimated (Farren et al., 2016). Women suffer in isolation and find it difficult to share their pain (Bardos, Hercz, Friedenthal, Missmer, & Williams, 2015). Moreover, society encourages privacy in this area (Bolvin & Lancastle, 2010).

A small study explored mental health outcomes in 57 women attending an early pregnancy unit. This study had a high (26%) attrition rate and a possible selection bias towards anxious patients who were more likely to respond. However, the researchers demonstrated that women who had a threatened but viable pregnancy had higher rates of persistent anxiety at follow-up than the ones who actually miscarried (Moscrop et al., 2013). Another study specifically compared the anxiety levels in women following an ultrasound diagnosis related to early pregnancy. There was a significantly increased anxiety score in the women who had an uncertain diagnosis e.g PUV or Pregnancy of unknown location (PUL), as compared to those with a definite diagnosis, even if they were diagnosed with miscarriage (Richardson, Raine-Fenning, Deb, Campbell, & Vedhara, 2017). In an interpretative phenomenological assessment of miscarriage, the main source of anxiety elicited during the interviews was "the waiting game" related to the wait for an ultrasonic

confirmation (Norton & Furber, 2018). The same reasoning applies to another study focusing on In-Vitro Fertilization, where Bolvin and Lancastle (2010) concluded that anxiety was more pronounced if the patient had no objective guide and no control over the expected outcome. They deduced that the uncertainty may interfere with natural coping strategies adopted in negative but definite situations (Bolvin & Lancastle, 2010).

Davison et al. (2014), explored whether having a PUV outcome prediction test would be acceptable, and how it would affect mental health scores. The participants had a hospital anxiety and depression score (HADS) upon recruitment and after one week. They were randomized to having the prediction test or not. Results showed that anxiety levels decreased over time but more so in women randomised to having a prediction test, even if the outcome was a miscarriage. The majority of participants found the test useful irrespective of the outcome and would choose to repeat it in future. This study was not blinded and was prone to expectation and observation bias. However, it reiterates that uncertainty results in increased psychological morbidity and that prediction testing could ameliorate this (Davison et al., 2014).

2.6.3 Workload and Cost

PUV is common, affecting one in every 5 women presenting to an early pregnancy unit (Hu et al., 2014). PUV management inevitably requires repeat visits, resulting in inconvenience to patients, and a delay in returning to a normal lifestyle. Repeat visits also increase costs and workload for the service provider. This has become more relevant since the change of definition of non-viability (discussed in section 2.7). With such new definition, a 12 % increase

in rescans is expected. However, in spite of the increased workload, this is considered to be justified in the interest of pregnancy safety (Hu et al., 2014).

In a large Miscarriage Treatment Trial (MIST), involving 1200 women, expectant management of miscarriage, i.e. waiting for spontaneous passage of the fetus, has been seen to result in increased emergency admissions, unscheduled surgical evacuations of products of conception and increased transfusion rates. Emergency care is known to be more costly and carries more morbidity (Trinder et al., 2006). In addition, a longer follow-up interval is known to increase emergency visits due to increased patient anxiety, thus also impacting workload and cost (Elson et al., 2003). An earlier definite diagnosis could help in this regard.

2.6.4 Healthcare Professional awareness of PUV

In a questionnaire given to 27 health care professionals, (Lawson & Bottomley, 2018), it was concluded that inconsistent information was being imparted to PUV patients with possible impact on their experience. Viability chances quoted to patients were variable (10-80%), with 25% of the staff admitting to being unsure. Follow-up visits were offered at 7-14 days, with 71% not using evidence to determine this. Moreover, 75% of healthcare professionals expressed that a personalized outcome prediction would be helpful. Although the study was small, the conclusions justify the need for more research and awareness of PUV to equip healthcare professionals to manage it better. The possible individualized prognosis and an optimal visit strategy could be useful to patients and health care services alike (Guha et al., 2013). However, this has to be contextualised in the knowledge of an unequivocal diagnosis.

2.7 The PUV Versus Non-Viability Debate

2.7.1 Pre-2012 Definitions

Up to 2011, there was no singular international definition of non-viability. The ACOG and the Society of Gynaecologists of Canada (SOGC), used an MSD of 16mm and a CRL of 5mm (Bickhaus et al., 2013). These recommendations were based on small studies carried out in the late ’80s (Levi et al. 1988; Levi et al. 1990). The RCOG and the Australian Society of Ultrasound in Medicine (ASUM) used an MSD of 20mm and a CRL of 6mm to diagnose early pregnancy loss (Infante et al., 2013; RCOG, 2006; Rodgers et al., 2015).

Goldstein (1992), a respected expert, recommended less stringent cut-offs of 4mm for CRL to be diagnostic of miscarriage based on a study involving only 27 patients. In the said study, a CRL of 3mm would be rescanned in 3-5days. On the other hand, the radiologists would quote the “rule of 5s”. This meant that a CRL measuring 5mm should have a fetal heart, a gestational sac of 10mm should have a yolk sac and a gestational sac of 15mm should have a visible fetal pole (Lane & Wong-You-Cheong, 2014).

2.7.2 Cut-Off Debates

In 2011, there was a drive to reach a diagnosis with absolute certainty having a 0% false positive rate (Infante et al., 2013). The controversy was set off by Jeve (2011), who reviewed the studies defining miscarriage and concluded that there was a “paucity of high quality, prospective data on which to base guidelines for the accurate diagnosis of early pregnancy demise” (Jeve, Rana,

Bhide, & Thangaratinam, 2011,p. 489). The authors indicated that the reviewed studies were small, involving 55 to a maximum of 211 patients; as well as having been carried out between 1986 to 1994. Moreover, a number of them used transabdominal scans. It was later pointed out that none of the reviewed studies included reproducibility of the scans, hence questioning their validity (Infante et al., 2013).

The ACOG definitions of 16mm for MSD, and 5mm for CRL were based on only 2 studies comprising 47 patients, hence considered unsafe (Bourne & Bottomley, 2012). Although the RCOG guidelines (RCOG, 2006) were more conservative than the American ones, using a limit of 20mm for MSD and 6mm for CRL, they were still criticised for not being clear regarding what was expected after 7 days and for being only based on clinical experience (Bourne & Bottomley, 2012; Preisler et al., 2015).

Ultimately what instigated the change in clinical guidance were two studies conducted in 2011. Pexsters et al. (2011) performed a study involving rescanning of 44 women by 2 expert observers who were blinded to each other's findings. The interobserver variation for MSD was 18.7%, and 14.7% for CRL measurements. This meant that an MSD reading of 20mm (RCOG cut-off) could actually be anywhere between 16.8mm and 24.5mm, whilst a CRL reading of 6mm could lie between 5.4 to 6.7mm. All this indicated that it was considered unsafe to retain existent definitions. Their recommendations were to increase the cut-offs to 25mm for MSD and 7mm for CRL, whilst instructing ultrasonographers to observe more caution in the measurements that were close to the boundary cut-offs. Ultrasound is known to be machine and operator

dependent (Sarris et al., 2013), and hence this seemed to be a sensible conclusion, albeit not evidence-based. In the study by Pexsters et al. (2011), the observers were obstetricians with specialist training in early obstetric ultrasound. Thus, it is difficult to generalise their findings to standard hospitals. Moreover, it was not clear which and how many scanned cases were close to the boundary limits. In addition, since the study conducted by Pexsters (2011) included 44 women, these borderline cases must have been few. However, the concept that one must consider the impact of interobserver variation was still a valid one.

The second study published by Abdallah et al. (2011b), was a prospective, multicenter, observational study, involving 1060 participants. The research concluded that raising the MSD threshold from 16mm to 21mm dropped the false positive rate from 4.4% to 0%. Similarly, a CRL cut-off increase from 4-5mm to 5.3mm reduced the false positive diagnosis from 8.3% to 0%. The strength of this study was the fact that it was large and prospective making the results generalisable to standard hospital settings. The viability of the pregnancies was confirmed at 11-14 weeks since up to 14% of women are known to miscarry even after the appearance of a fetal heart (Mukri et al., 2008). Ross and Johns (2012) heavily critiqued Abdallah's results. The authors accused this study of "carrying the hallmarks of a retrospective study or audit" (p. 363). This criticism was fair, in that the data was collected in different units, over different time periods, with different cut-offs. The methodology described in the study by Abdallah et al. was also unclear. Ross and Johns (2012) suspected over-measurement of the fetal pole by perhaps including the yolk sac, because 13.4% of PUV cases with a visible pole and no fetal heart, in their

opinion, appeared to be excessive. They also questioned how the number of cases close to the boundary limits was not specified by Abdallah et al., and how their study seemed to finalise its conclusions through the data collected by Pexsters (2011). Ross and Johns (2012, p.364) stated: “Unfortunately, they have not performed the prospective multicenter observational study needed to address their aims.”

Jurkovic (2012), argued that no matter what cut-off levels are introduced, human error will likely lead to false diagnoses. In the Cardiff report (Hately et al., 1995), the new definitions for non-viability would have still been erroneous. In his opinion, the solution is rescanning at a time interval: “ never in one visit, never by one person” (Jurkovic, 2012, p. 361).

2.7.3 2012 Non-Viability Definitions

Notwithstanding the fact that the studies by Pexsters et al. (2011) and Abdallah et al. (2011) were criticised, these publications resulted in an unprecedented rapid change in guidance. A multidisciplinary team was convened by the Society of Radiologists (Doubilet et al. 2013) and their recommendations were approved by both British and American Colleges.

The NICE (2012) guideline highlighted the preferential use of a transvaginal approach. It emphasized how menstrual age calculation is often mistaken or misleading due to the variation of cycle length. It also stated that in the presence of a fetal heart, only the fetal pole needs to be measured.

According to NICE guidance (2012), non-viability is to be diagnosed when:

- The CRL is 7mm or more with no visible heartbeat. The diagnosis of miscarriage can be confirmed by asking for a second opinion on the day and /or rescanning in 7 days.
- The MSD is 25mm or more with no visible fetal pole. The diagnosis is confirmed by a second observer and /or a rescan in 7 days.

NICE (2012) published this guideline with the revised definitions in 2012. The document was updated in April 2019. The definitions and areas related to PUV have remained unchanged (NICE 2012; NICE 2019).

2.8 Time-Based Criteria Debate

The 2012 NICE definitions for miscarriage were welcomed. However, there was still a lack of clarity in the time-based criteria of PUV (Bourne, 2016).

"If the crown-rump length is less than 7.0 mm with a transvaginal ultrasound scan and there is no visible heartbeat, perform a second scan a minimum of 7 days after the first before making a diagnosis. Further scans may be needed before a diagnosis can be made." (NICE 2019, NG126, 1.4.6)

Preisler et al. (2015), argued that the 2012 definitions offered no detail as to what should constitute non-viability on the review scan. The authors stated that these definitions resulted in considerable variation in practice which was not evidence-based.

The time-based criteria by the Society of Radiologists (Doubilet et al., 2013) recommended that miscarriage may be diagnosed when no fetal heart was visible:

- 14 days after the first scan showed an MSD < 25mm with no visible fetal pole or yolk sac.
- 11 days after the first scan showed an MSD <25mm but a yolk sac was present.

The 2016 French clinical practice guidelines (Collège National des Gynecologues Obstetriciens Français) adopted these recommendations. They also recommended that if the CRL measured less than 7mm, a follow-up scan needed to be performed at least 7 days later. However, they graded this guidance as Grade C, suggesting a low level of evidence (Huchon et al., 2016).

Preisler et al., (2015) criticised Abdallah et al.'s study (2011b), stating that the confidence intervals were wide and that the study did not account for gestational age, or give clear indications for rescan time intervals. Thus, a follow-up study was carried out incorporating the dataset from the study conducted by Abdallah et al.(2011b), with an additional cohort, with the aim of reducing the confidence intervals and focusing on rescan recommendations. This led to a larger, prospective, multi-centre cohort study. This research, conducted by Preisler et al. (2015), reconfirmed the new definitions but also recommended to include the gestational age. Thus, in pregnancies over 70 days, the absence of a heartbeat in a fetal pole measuring 3mm, or an empty sac measuring over 18mm would diagnose pregnancy loss. However, this was

contradictory to existent recommendations to ignore the menstrual gestational age in view of known inaccuracies (Bourne & Bottomley, 2012; NICE, 2019). Al-Memar et al. (2015), also recognized the relevance of considering gestational age but stated that there was insufficient data to support its use in the diagnosis of miscarriage. Regarding rescan intervals, Preisler et al. (2015) suggested that:

- a miscarriage is confirmed if a fetal heartbeat is not visible by 7 days after the fetal pole was first seen.
- in case of an MSD measuring >12mm, then a rescan should be performed in 7 days; if it measured <12mm, a 14-day review was suggested. Doubling of the sac and a live gestation was expected in both cases.

However, this timing strategy has not been universally accepted or practised.

The Australian guidelines (Mizia et al., 2018) adopted most of Preisler's recommendations. They also emphasized the appropriate experience of the observer and the quality of the equipment used, suggesting a rescan when necessary. All of these were lacking in the Cardiff report (Hately et al., 1995; Mizia et al., 2018). In another study, Abdallah et al. (2011a) examined the growth rates in early pregnancies to optimise the rescan schedule. Their findings indicated that both fetal poles and gestational sacs grew by 1mm/day. However, there was considerable overlap in growth rates between viable and non-viable pregnancies. In their cohort, a fetal pole growth of 0.2mm/day, i.e. 1.4mm in 7 days still resulted in a viable pregnancy. Hence, no discriminatory level for fetal pole or sac growth could be advocated. In a thorough review of

the new guidelines recommended by the Society of Radiologists, Bickhaus et al. (2013) upheld the new definitions and recommended that if no growth occurred in 7-10 days, and if no fetal heartbeat was seen in a previously visible fetal pole, a diagnosis of miscarriage was reached. The authors also referred to the overlap in growth rates in the Abdallah study (2011), reinforcing the need of absolute certainty when diagnosing pregnancy loss.

In the recent updated NICE (2019) guideline on early pregnancy, the document reiterated its recommendation to research the area of PUV and PUL, acknowledging the need for more evidence in ultrasound time intervals.

“The evidence base for the timing and frequency of scanning in early pregnancy is limited, and the number of scans is organised by individual units according to capacity and demand. Some healthcare professionals choose to wait 5 days between scans whereas others will wait 10 to 14 days. These decisions are driven by resource availability as well as clinical considerations, but in particular, the effect of different strategies on cost and women's experience is not clear” (NG126, Research Recommendation 2).

This is what has prompted the researcher’s question, targeting the difference in scan findings at 7 and 14 days as NICE (2019) suggests.

2.9 Prognostic And Predictive Factors

Apart from the new definitions, Doubilet et al. (2013), also recommended a list of sonographic features described as “suspicious for” but not diagnostic of miscarriage. These included an absent fetal heart in a CRL of <7mm, a sac devoid of a fetal pole when the MSD measured 16-24mm, an absent fetal pole at 6 weeks gestational age, an absent fetal heartbeat after 7-10 days from visualising a sac with a yolk sac, or 7-13 days after visualising a sac with no yolk sac. Their value was to help guide the clinician and the patient as to what to expect in a PUV (Rodgers et al., 2015).

However, as discussed in the following sections, research went a step further to attempt to predict the outcome of threatened miscarriage as well as pregnancies of uncertain viability. In this literature review, the main emphasis was on studies specific to PUV. The prognostic and predictive studies may be broadly categorized into:

- Clinical features
- Biochemical markers
- Ultrasonographic features
- Prediction models using a combination of factors together.

The researcher’s PUV study explores these features in a similar fashion. Thus, the results presented in in sections 4.3-4.6 follow the same format of the predictive research detailed in sections 2.10-2.13.

2.10 Clinical Predictors

There is ample evidence of positive correlations between certain clinical factors and PUV outcome. Most of the studies, however, were performed in the context of assessing other predictors, mainly ultrasonographic and/or biochemical (Lane & Wong-You-Cheong, 2014). Some studies formed part of a predictor model (Bottomley et al., 2011; Falco et al., 2003; Guha et al., 2013). All the studies, with the exception of the study by Puget et al. (2018), used the pre-2012 definitions that could have impacted their results. All of these clinical predictors were assessed by the researcher in the PUV study.

2.10.1 Maternal Age

It has been long established in PUV cohorts that patients who miscarry have a higher mean maternal age than those with a good outcome (Elson et al., 2003; Falco et al., 2003). This could be due to the fact that the risk of miscarriage increases with maternal age (Jurkovic et al., 2013) (see Section 1.1). Reid et al. (2011b) concurred with this, showing a mean maternal age of 30.7 years for women who miscarried versus a mean of 26.9 years for women whose pregnancy was viable. Similarly, in a retrospective cohort study involving 115 PUV cases, the mean maternal age for a negative outcome was 31.8 years as opposed to 26.4 years for viable outcomes. Maternal age had a significant p-value of <0.001 in this study (Ng et al., 2014).

2.10.2 Gestational Age

Advanced gestational age is another factor that has been associated with miscarriage in PUV (Elson et al., 2003; Falco et al., 2003). Bottomley et al. (2008), showed that PUVs presenting before 42 days had a lower rate of miscarriage. Reid et al. (2011b) found that the mean age for their cohort of viable pregnancies was 41.9 days as opposed to 52.7 days for those who miscarried. Similarly, Ng et al., (2014) found a significant difference in presenting gestational age ($p<0.008$) of 5.7 weeks for miscarriage versus 5.2 weeks for viable PUVs. The interpretation of this finding is that earlier gestations may represent early healthy pregnancies. The advanced gestations could reflect a missed miscarriage.

2.10.3 Bleeding

Bleeding occurs in 27% of pregnancies of which 12% will subsequently miscarry (Lane & Wong-You-Cheong, 2014). In a retrospective study conducted by Hessert and Juliano (2012), which included 117 patients, the results indicated an overall miscarriage rate of 30% in the PUV patients that increased to 35% in cases of bleeding and pain (Hessert & Juliano, 2012). Another prospective cohort study involving 248 participants, showed that bleeding with clots was a significant predictor of miscarriage (Riemke et al., 2010). Similarly, Ng et al. (2014) indicated a significant ($p<0.001$) rise in miscarriage risk from 32.98% to 70.4% associated with bleeding at presentation. Conversely, in a prospective observational study following 78 PUVs for 7-10 days, the presence or amount of bleeding with clots was not predictive of viability. Rather, it was the gestational sac shape that was most

predictive, with a regular sac being 91% sensitive and 41% specific to viability (Naji et al., 2010). In the PUV study, blood loss was assessed using a validated pictorial blood assessment chart (PBAC) (discussed in chapter 3.8.2) (El-Nashar, et al., 2015; Higham et al., 1990).

2.10.4 Previous miscarriage

Previous miscarriage is known to increase the risk of loss of the index pregnancy even when corrected for age. The risk rises as the number of previous miscarriages increases (Magnus, Wilcox, Morken, Weinberg, & Håberg, 2019). Reid et al. (2011), in a prospective PUV study, showed a positive association between previous miscarriage and a negative outcome. Lautmann et al. (2011) indicated that women with no history of previous miscarriage had a significantly better outcome than women with previous pregnancy loss. However, 2 or more previous miscarriages were not significantly associated with increased loss in the same study. This clinical aspect was interrogated by the researcher in the PUV study.

2.11 Biochemical Markers

The clinical use of biochemical markers is established in the management of PUL but not so in miscarriage or PUV (Jurkovic et al., 2013). The literature often includes a mixed population of PUL, PUV and threatened miscarriage (Pillai et al. Konje, 2016). Nevertheless, biomarkers, the most common being HCG and Progesterone, have been investigated with the aim of determining a discriminatory level to confirm or exclude viability of pregnancy. In addition, their prognostic value was interrogated, both as single markers and within

prognostic models (Elson et al., 2003; Lautmann et al., 2011). Consequently, the researcher has included Progesterone and HCG in the PUV study (section 3.8.3).

2.11.1 Progesterone

Progesterone is released by the corpus luteum (Pillai et al., 2016) increasing linearly between the 5th and the 13th week of gestation (Hanita & Hanisah, 2012). Its physiological roles in early pregnancy include: decidualising the endometrium in preparation for implantation, preventing rejection of the newly implanted embryo and, reducing contractility of the uterus. Its presence is important in the maintenance of pregnancy (Hanita & Hanisah, 2012). There may be variations of progesterone levels across populations, and this may be a limiting factor in its use (Hanita & Hanisah, 2012). One must also be aware of the different units in use for progesterone measurement (1 nmol=0.31 ng/mL). There are a myriad of studies showing the role of progesterone in distinguishing viable from non-viable pregnancies. However, there seems to be no consensus regarding the use of discriminatory levels to do this (Hanita & Hanisah, 2012).

Earlier work by Elson et al. (2003), indicated it as being possibly the most useful predictor for viability in the context of PUV without a visible fetal pole. In a six-year retrospective study, Lautmann et al. (2011), continued on Elson's work and suggested a progesterone cut-off level of <16 nmol/l (5.1ng/ml) for non-viability and of <10 nmol/l for PUL, in order to discontinue follow-up. In another publication by Osmanağaoğlu et al., (2010) comparing biomarkers, progesterone was found to be comparable to HCG when using a cut off value

of <15ng/ml and <20ng/ml respectively. In this study, progesterone was preferred due to its widespread availability and low cost. On the other hand, a study by Bignardi et al. (2010) did not show a particular progesterone threshold to be predictive of viability in PUV. The HCG ratio was found to be more useful in this study. A limitation of this research was that it was initially designed to investigate a PUL cohort and therefore utilised a 48-hour serial HCG repeat leading to increased cost and workload. Nevertheless, a meta-analysis conducted by Verhaegen et al. (2012), showed that using a progesterone cut-off level of 16nmol/l may nearly rule out a viable pregnancy, albeit most studies in this meta-analysis mainly targeted PUL. Moreover, exceptions of normal pregnancies with low progesterone levels have also been documented. Therefore, progesterone may not be used in isolation to reach a diagnosis of miscarriage (Jurkovic et al., 2013). In a recent "Scientific Impact Paper on behalf of the RCOG", it was concluded that higher levels of progesterone of 60 nmol/l (19ng/ml) are in keeping with a likely viable pregnancy whilst a lower level of 20 nmol/l (6ng/ml) is suggestive of a failing pregnancy (Memtsa, Jurkovic & Jauniaux , 2018).

The prognostic role of progesterone has been researched retrospectively by Ng et al. (2014), investigating 4,919 gestations over a three-year period. Only 106 cases fulfilled the criteria for PUV classification reflecting the difficulty of retrospective research. In this study, there was a significantly higher ($p<0.001$) progesterone level (mean 67ng/ml) in viable PUVs than in those that miscarried (mean 27ng/ml). The higher progesterone remained significant for viability even after multivariate logistic regression, alongside lower maternal age and lower gestational age.

2.11.2 Human Chorionic Gonadotropin (HCG)

HCG is a hormone released by the syncytiotrophoblast very early on in pregnancy (Pillai et al., 2016; Puget et al., 2018). It is known to double over 1.2-1.6 days as from day 35 of gestation; and thereafter every 2-2.7 days until day 42 of pregnancy (Jurkovic et al., 2013). Although its main clinical use is in PUL (Bobdiwala et al., 2016; Guha et al., 2014), a falling HCG level has been strongly associated with a failing pregnancy. However, the general consensus is that there is no HCG discriminatory value for viability (Memtsa et al., 2018).

A PUV study by Falco (2003), including 50 patients with no visible embryo, showed a correlation between low HCG (<1200 miU/ml) and miscarriage in the univariate analysis that did not remain significant in multivariate analysis. Lautmann et al. (2011), using an HCG cut-off level of <465miU/ml, attained a 100% sensitivity to miscarriage but with only a 14.1% specificity. Meanwhile, a 2014 publication by Ng et al. (2014) found no association at all between HCG and outcome. Guha et al. (2013a) found that it was in the context of serial checks in PUL studies, that this factor was found to be useful. In a large prospective study by Bignardi et al. (2010) involving 1003 cases of PUL, of which 379 evolved to a PUV, a baseline progesterone and 48-hour serial HCG levels were taken. It was concluded that an HCG ratio of 2 was more accurate (72% accuracy) in predicting outcome compared to baseline progesterone (65% accuracy). The authors felt confident enough to refer the pregnancies predicted as viable straight to the dating ultrasound visit (11 to 13+6 weeks). However, at least 8% of these had miscarried by the dating scan (Bignardi et al., 2010).

A similar but more recent publication, by Puget et al. (2018), using updated PUV definitions, compared baseline progesterone to serial HCG levels as predictors of PUV outcome. This study followed 107 women over 7 to 14 days. Progesterone alone diagnosed 10.2% of cases in the study. Combining HCG and Progesterone diagnosed a total of 43.9%. The biomarkers were able to provide a diagnosis within 48 hours as opposed to a mean of 11.2 days by ultrasound. The results confirmed a better prediction by serial HCG ratios (>1.7 for viability; <1.1 for miscarriage), than when using a low progesterone cut-off value of 6.2ng/mol.

Notwithstanding all the evidence, the disadvantage of using serial HCG in PUV management is the inconvenience and cost of repeat visits whilst still not providing an accurate diagnosis (Lautmann et al., 2011). The official stand of the RCOG for HCG use remains restricted to PUL and in the follow-up of molar pregnancies (Memtsa et al., 2018).

2.11.3 Other Biomarkers

A number of inflammatory markers have been investigated, one of them being Cancer antigen 125 (Ca125). According to a meta-analysis, conducted by Pillai et al. (2016), the sensitivity of this biomarker can be as high as 90% with an 88% specificity in threatened miscarriage, but this was not specific to PUV. Another limitation of using Ca125 is its lack of specificity, rising also in cases of endometriosis, fibroids, ovarian hyperstimulation syndrome as well as non-gynaecological conditions. Other inflammatory markers explored included cytokines such as Interleukin IL-6, and Tumour Necrosis Factor-alpha both of which may play a role in predicting outcome (Memtsa et al., 2018).

Other markers have recently stimulated interest for use in PUV prediction. They are based on the knowledge that early pregnancy is a hypoxic process with high oxidative stress (Richardson, Deb, Campbell, & Raine-Fenning, 2018). Lower levels of Angiopoietin-2 (Ang-2) and Soluble fms-like tyrosine kinase-1 (Flt-1) have been identified in viable pregnancies as opposed to impending miscarriages, during a phase 1 exploratory study carried out by Richardson et al. (2018). Another prospective study involving 120 PUVs was carried out by Akkaya et al. (2018). This study indicated that non-viable pregnancies were found to have lower native thiol levels and higher disulphide levels than viable PUVs. This is another new area of research for outcome prediction of PUV. However, since this area of PUV research is still very novel and the commercial availability of the biomarkers is not widespread, it has been decided that it would be premature to include these factors in the PUV study.

2.12 Ultrasound Features

This next section reviews the use of ultrasonographic findings that, using Doubilet's terminology (2013), are considered as suspicious of an impending miscarriage, or may be of prognostic value in PUV outcome. All these features were observed in the PUV study (section 3.9.4).

2.12.1 Gestational Sac features

It has long been established that an irregular or distorted sac is likely to miscarry, as is a sac that is low-set in the uterine cavity (Mazzariol et al., 2015; Rodgers et al., 2015). According to Nyberg , Laing and Filly (1986), the finding of an irregular sac is 100% sensitive and specific for miscarriage. Irregular sacs

were also shown to exhibit a slower daily MSD growth of 0.47mm as opposed to 0.99mm for regular sacs (Abdallah et al. 2011).

An abnormally small gestational sac is thought to reflect a defect in placentation. (Papaioannou, Syngelaki, Maiz, Ross, & Nicolaides, 2011). If the sac is relatively small i.e. less than 5mm larger than the fetal pole, the outcome has also been found to be poor (Al-Memar et al., 2015; Mazzariol et al., 2015; Rodgers et al., 2015). Other findings “suspicious” of miscarriage (Doubilet et al., 2013), are a gestational sac of more than 8mm without a visible yolk sac, and a sac of 16-24 mm without a visible fetal pole.

A normal sac was found to grow at the rate of 1.13mm per day (Nyberg, Mack, Laing, & Patten, 1987). In a study by Abdallah et al. (2011), a significant difference of 1.003mm/day for viable pregnancy sacs versus 0.503mm/day for non-viable sacs was found ($p= 0.001$). A 0.2mm growth rate per day gives a 99% specificity for miscarriage but due to the considerable overlap of growth rates between viable and non-viable sacs, a diagnostic cut-off for non-viability was not possible. Another interesting approach used by Al Darwish (2019), was to assess the growth of sonographic parameters by using centiles. Gestations with MSD, CRL and yolk sacs measurements <10th or >the 90th centiles all fared worse. These findings were predictive of miscarriage within a week. The methodology, however, was not comparable, since abdominal scanning was employed in this study with a CRL cut-off value of 8mm, and only 1 diameter for yolk sac measurement. Similarly, Falco (2003), using 16mm as the cut-off limit, found that if the MSD was -1.34 standard deviations from the mean, there was a 90% association with pregnancy failure.

2.12.2 Fetal pole

An absent fetal pole at 6 weeks gestation is a poor prognostic factor (Doubilet et al., 2013). On the other hand, publications relating to the same research, have shown that in a cohort of 268 PUVs, the miscarriage rate was higher in those cases where a fetal pole was present (67.4%) than in cases of an empty sac (43%) (Riemke et al., 2010b). It was also noted that the mean CRL for viable pregnancies (0.22mm) within the cohort, was significantly less than that for miscarriage (0.95mm) (Reid et al., 2011b; Riemke et al., 2010a). This could be linked to the fact that although the CRL cut-off was reset at 7mm (NICE 2012), the fetal heart is usually visualised much earlier, even before the pole is measurable at 1-2 mm. Hence, an absent fetal heartbeat at around 4 mm is considered suspicious (Doubilet et al., 2013).

The slow growth rate of the fetal pole is thought to represent early growth restriction that results in pregnancy loss (Bottomley & Bourne, 2009). This was confirmed by an Indian study that showed a significant +1 positive correlation of 1.09mm daily growth in the fetal pole for viable PUVs as opposed to a <0.7mm daily growth in non-viable PUV (Deshmukh, Yelikar, & Tibdwal, 2013). This had been similarly indicated in an earlier study carried out by Kumar & Sridevi (1995). Nevertheless, Abdallah et al. (2011), warned about the overlap in growth rates in fetal poles and the need to correct for interobserver variability, meaning that a fetal growth of 1.4mm in 7 days could be still indicative of a viable pregnancy.

2.12.3 Yolk Sac

A normal yolk sac is generally expected to be round and measure 3-6mm (Al Darwish et al., 2019). There is disagreement on how the yolk sac is to be measured. Some studies have used an inner-to-inner measurement (Al Darwish et al., 2019; Kumari, Roychowdhury, & Biswas, 2016); others apply the calipers to the middle of the echogenic wall (Datta & Raut, 2017); whereas others use an outer-to-outer approach (Bickhaus et al., 2013). This could account for some of the discrepancies in different studies (Muralidhar, Patil & Dasar, 2018). In the PUV study, an outer-to-outer measurement was chosen for technical reasons that will be clarified in section 3.9.4.

There are a number of poor prognostic features associated with the yolk sac. There is a long-standing consensus on the fact that an enlarged yolk sac is a bad prognostic factor (Levi et al., 1988; Al-Memar et al., 2015; Doubilet et al., 2014; Mazzariol et al., 2015), although there is no agreement on the upper limit of normality. Doubilet (2014) and Rodgers et al. (2015) quoted 7mm, whilst Lane and Wong-You-Cheong (2014) and Al Darwish (2019) quote 6mm. There is also evidence of an enlarged yolk sac in diabetic mothers (Moradan & Forouzeshfar, 2012). Some researchers consider a yolk sac less than 3mm as being abnormal (Doubilet 2014). Moradan & Forouzeshar (2012) concluded that a yolk sac should be visible by the time the MSD measures 9mm although Tan et al. (2014) quoted 11mm for this same conclusion. It is accepted that a heavily calcified yolk sac may be suggestive of a 2-week fetal demise (Doubilet 2014). An echogenic yolk sac, however, may be normal and therefore care is required at distinguishing it from a calcified yolk sac (Moradan & Forouzeshfar, 2012). There is also some evidence that the yolk sac volume increases linearly

till the 10th week and that if it is >5mm before the 7th week, this is associated with miscarriage (Bagratee, Regan, Khullar, Connolly, & Moodley, 2009).

As regards to prognostic studies using the yolk sac, an enlarged yolk sac of >5mm gives a three-fold increase in miscarriage rates (Berdahl, Blaine, Van Voorhis, & Dokras, 2010). These studies, however, refer to viable pregnancies in a threatened miscarriage scenario and hence would not be comparable to the PUV study. In a prospective controlled cohort study involving one observer assessing 22 cases and 164 controls between 5 and 6.5 weeks of gestation, the yolk sac was categorised as abnormal in terms of appearance and size (<3mm,>6mm). It was shown that there was a 90% miscarriage risk in cases of abnormally sized yolk sac and 50% risk in cases of a distorted yolk sac. The miscarriage rate in the normal yolk sacs was 3.6% but increased to 63% in an abnormal sac. The largest yolk sac seen in the study was of 9.2mm whereas the largest sac in a normal pregnancy was found to be 6.6mm (Moradan & Forouzeshfar, 2012).

2.12.4 Subchorionic Haematoma (SCH)

A SCH is a blood collection between the chorion and the decidua. It is a common finding especially in the presence of bleeding, occurring in 18-22% of cases (Rodgers et al., 2015) up to 39.5% (Alberto et al., 2018). To date, there is no standardized way of measuring SCHs (Heller et al., 2018). There is considerable controversy as to their significance with respect to early pregnancy outcome (Sükür et al., 2014). For this reason, SCH were noted and measured but not included in the prognostic observations in the current research.

2.13 Predictive Models

A number of researchers investigated various factors in a stepwise logistic analysis to create predictive models and scoring systems (Guha et al., 2013). These are presented in Table 2.1. The older definition of PUV was employed in all these studies. However, it was argued that the participant numbers at the boundary of definition were small, such that the results should not be affected (Bottomley et al., 2013). Some of the studies are a continuation of each other, carried out to validate a model proposed in a previous study as explained by Van Belle et al. (2012).

A study by Falco et al. (2003), consisted of 50 participants with empty sacs smaller than 16mm. The term PUV had not been coined yet. In this multivariate analysis, that also included maternal age, gestational age, and a baseline HCG level, only the MSD remained as an independent predictor, with the results concluding that slow sac growth may be a helpful predictor of miscarriage.

A crucial study by Elson et al. (2003), was pivotal to the research that followed. It focused on 200 pregnancies with empty sacs using a cut-off of 20mm. Multivariate analysis indicated advanced maternal age, advanced gestational age and low progesterone as predictors of miscarriage. The researchers devised a formula including these 3 factors. Using a 10% probability of viability, the sensitivity was 99.2% and specificity was 70.7 %. However, using a 1% probability cut-off, the sensitivity was 100% at the expense of a 43.9% specificity. Progesterone was the strongest single predictor in this study but the other factors contributed to its accuracy. They recommended further research in the area including the psychological impact of predictive testing.

Table 2.1 PUV Predictive Studies

AUTHORS	NO	AFTER LOGISTIC REGRESSION	MODEL INCLUDES	RESULTS
Falco 2003	50	MSD Standard deviation score	No model	p=0.0457, ROC 0.908
Elson 2003	200	Maternal age Progesterone MSD	Maternal age Progesterone MSD	Probability viability 10%; AUC 0.9693 99.2% sensitivity 70.7% specificity Probability 1%; 100% sensitivity, 43.9% specificity For progesterone alone at 25mmol/l cut off AUC=0.9493; 100% sensitivity 40.2% specificity.
Lautmann 2011	400	Maternal age Progesterone MSD	Age Progesterone MSD	Using same formula as Elson. AUC= 0.85
Bottomley 2008	481	Gestational age, Bleeding, Presence of Yolk Sac	Gestational age, Bleeding, Presence of yolk sac	AUC 0.82
Bottomley 2011	493	Maternal age, Gestational age, PBAC MSD, Presence of yolk sac	Maternal age, Gestational age PBAC, MSD deviation from 7mm, Presence of Yolk sac	AUC 0.837 for 1st dataset, 0.831 for 2nd dataset
Guha 2012	172	NA since validation study	Validated Bottomley 2011 model	Model AUC 0.8334, Scoring system AUC 0.8259
Guha 2013	400	NA since validation study	Validated Bottomley 2001 model M0 and Score SS0, Developed new model :Mn and score SSn with Maternal age, PBAC, MSD	M0 AUC 0.845 SS0 AUC 0.832 Mn AUC 0.801 SSN AUC 0.773
Model 2011	185	NA since validation study	Maternal age, Gestational age, Previous normal delivery, CRL, MSD	Viability prediction - AUC 0.91, Sensitivity 81.1% Specificity 85.3%
Ng 2014	115	Maternal age Progesterone	NA	Progesterone significant 0.001, Advanced maternal age p=0.001.

Subsequently, Lautmann et al. (2011) published retrospective data of 400 women, with empty sacs <20mm, who voluntarily accepted the predictive test using Elson's model. The viability prediction for the Lautmann dataset using Area Under the Curve (AUC) was 0.85 which was lower than Elson's original cohort with an AUC of 0.9693. The AUC is derived from a Receiver Operating Characteristic (ROC) that plots true positive against false positive results. An AUC close to 1 reflects a good predictive ability for a given model that is not attributable to chance. An AUC of 0.5 is considered a chance prediction, and therefore useless (Hulley, Cummings, Browner, Grady & Newman 2013). The authors debated whether this was a model weakness or caused by selection bias. It was only 9.1% of the clinic attendees seen over six years who accepted the test, and these were found to be at higher risk of miscarriage.

Cecilia Bottomley has researched PUV extensively. A 2008 publication, involving 481 PUV cases, confirmed a number of possible predictors as individually significant i.e. univariate analysis. These included: maternal age, gestational age, bleeding and the presence of a yolk sac. However, after performing multivariate analysis, only the yolk sac ($p=0.0079$), gestational age ($p<0.0001$) and bleeding ($p=0.0018$) remained significant with an AUC of 0.82. This led to the development of a model that used clinical and ultrasonic data with the advantage over Elson's model of avoiding a blood test with the aim at improving acceptability to patients. (Bottomley, et al., 2008). Bottomley et al. (2011) developed the previous research further. In this study, 493 women were included and randomly divided into two subsets. The first was used to construct a model and scoring system, and the second one to validate it. Maternal age ($p=0.0027$), gestational age ($p<0.0001$), PBAC score ($p=0.0458$), MSD

(p=0.0197) and the presence of a yolk sac (p<0.0001) were all found to be significant after multivariate logistic regression. Moreover, a novel predictive scoring system was devised consisting of: maternal age, gestational age (</> 6weeks), MSD deviation from 7mm, PBAC score and the presence/ absence of a yolk sac. The score was ranked from 0-19 giving a probability of viability from 0.94 through 0.01. The AUC values taken at initial diagnosis of viability were 0.837 and 0.821 for the respective datasets and 0.812 for the scoring system. However, 12% of the initially diagnosed viable pregnancies miscarried by the end of the first trimester, decreasing the final predictive value of the model to an AUC of 0.788 and 0.774 for the two datasets and 0.771 for the scoring system. The authors noted a relatively high 12% miscarriage rate between the initial appearance of the fetal heart and the end of the first trimester which they attributed to selection bias at the voluntary recruitment stage. They did not claim the model to be diagnostic but they did consider referring women scored as viable directly to a dating scan visit at 11-13 week. Guha et al. (2012), used the same predictive system from the Bottomley (2011) study, validating it on a new dataset with AUC values of 0.8334 for the model and 0.8259 for the scoring system concluding that it is a potentially useful and validated tool for PUV.

A subsequent study by Guha et al. (2013), recruited another similar cohort that confirmed the calibration of the Bottomley model (now called M0) as well as the scoring system (SS_0) with better AUC values of 0.845 and 0.832 respectively. They also devised a new model (M_N) and scoring system (SS_N) that simply excluded the gestational age which was found to be unknown in 20% of their patients. The performance of the two models in the new cohort

was compared. The new model performed slightly worse with an AUC of 0.801 for the M_N and 0.773 for SS_N. However, the authors concluded that this system offered a comparable predictive model that obviated the need for a blood test, as in the Elson model, and of requiring the knowledge of the last menstrual period. More recently Ng and Tan (2014) studied 115 patients retrospectively and confirmed findings from previous studies. Their results showed that women who miscarried had a significantly increased mean maternal age, were more likely to be bleeding, and more likely to present at a later gestational age. On logistic regression analysis, however, only maternal age and low progesterone levels remained predictive of miscarriage.

A recent interesting study was carried out by Fourie et al. (2018). Six expert gynaecologists were shown ultrasound images of 19 cases of PUV to whose outcome they were blinded. Their ability to predict the outcome correctly by subjective observation was very low but increased after being given clinical information about each case including pain, bleeding, gestational age, maternal age. They could predict a viable PUV in 81.5% and a miscarriage in 43%. Applying the Bottomley model (Bottomley et al., 2011) to the same cases, 100% of Viable PUVs were correctly predicted, whilst miscarriage was predicted in 50% of the cases. This shows that neither experts nor models can predict PUV outcome correctly especially if the outcome is a miscarriage.

2.14 Conclusion

This chapter has explained the change in PUV definition guided by the Hippocratic oath “first do not harm” (Allen, 2013). The literature has revealed prognostic factors and models that could help in the clinical guidance of patients diagnosed with PUV, albeit none of which are diagnostic (Lautmann et al., 2011). However, the evidence related to timing strategies is very limited. (NICE 2019). The PUV study was designed to explore both these aspects with the aim of investigating a strategy for outcome prediction in Malta.

CHAPTER 3: METHODOLOGY

3.1 Introduction

This chapter outlines the research approach taken in order to reach the set aims and objectives of the study introduced in section 1.5. The researcher's first aim was to investigate the prediction of PUV outcome by using clinical, biochemical and ultrasonic variables. The second aim was to identify an optimal ultrasound timing strategy to manage patients presenting with PUV, at a state general hospital in Malta. The research design is described in section 3.3, followed by a definition of the population and sampling methods (section 3.4, 3.5). The subsequent sections outline the inclusion criteria, together with the study protocol, the research tools used as well as the data analysis (sections 3.6- 3.11). The ethical aspects are delineated in section 3.12 followed by the limitations of the study in section 3.13.

3.2 Research Paradigm And Approach

The paradigm or world view (Guba & Lincoln, 1994) from which the current research idea has evolved, was driven mostly by the medical background of the researcher. This research fits into a positivist paradigm, fulfilling the assumption that knowledge is " out there" (Cohen, Manion, & Morrison, 2002, p. 5) and that the information is obtained by observation and measurement (Cohen et al., 2002). This, in turn, substantiates the chosen quantitative approach, required to examine the relationship between variables (Babbie, 2010; Muijs, 2010).

3.3 Research Design

3.3.1 The chosen design

A prospective, quantitative, observational, correlational study was adopted. The study was non-experimental since there was no intervention by the researcher whilst collecting the data; hence, the natural course of events was observed (Labaree, 2016; Setia, 2016).

Inherent to the chosen prospective analytical design, the researcher was not aware of the outcome or diagnosis from the outset. The study comprised a single cohort of patients. The aim of the research was, not to investigate the causality of risk factors for miscarriage, but to explore associations to be able to predict the outcome of PUV (Labaree, 2016). An advantage of using an observational design was the possibility of calculating the incidence of pregnancy viability versus miscarriage in the study dataset. (Setia, 2016). Cohort studies are notoriously inefficient and expensive in cases of rare outcomes or in conditions requiring long follow-up periods (Hulley et al., 2013; Setia, 2016). The advantage, in this case, was that the observed condition is relatively common, affecting one in every five referrals to an early pregnancy unit (Ng et al., 2014), having a dichotomous outcome occurring over a short time period of a few weeks (NICE 2019).

The chosen design was ethically acceptable since no risks were imposed on the patients (Setia, 2016). The participant study protocol chosen, was similar to the routine patient care pathway. It only differed in that a structured follow-

up was adopted rather than offering *ad-hoc* visits. The blood loss was assessed by using a validated method and biochemical markers were taken during the initial patient presentation. In addition, the sonographic data was collected in a standardised fashion using one machine and a designated group of observers. The similarity of the study design to the normal patient care pathway was an important consideration so as to shorten the learning curve of the intermediary members of staff.

The research was carried out prospectively (Euser, Zoccali, Jager, Dekker, 2009). Both the researcher and the participants were blinded to the outcome of the study at the recruitment stage, hence reducing measurement bias (Hulley et al., 2013). The observers were trained to collect the required data and the inter-rater reliability was found to be within acceptable limits. The use of a single ultrasound unit improved the reliability of the data by avoiding variation between machines (Pexsters et al., 2011). Referring to retrospective studies, Hulley, Cummings, Browner, Grady and Newman (2013) explained how case notes are notoriously inaccurate and incomplete. A prospective study was essential in this case, since, specific ultrasound variables e.g. the yolk sac size and appearance together with the hormonal markers (HCG and Progesterone) needed to be collected for every participant, and these were specific to the study.

A disadvantage of prospective studies is the risk of sample attrition in case of long follow-up periods needed for the outcome of interest to occur (Bankhead, Aronson & Nunan., 2017; Setia, 2016). However, as NICE (2019) states, the diagnosis for PUV is usually evident by 14 days. This short study period

significantly reduced sample attrition. Another strategy used to minimise attrition in this PUV study involved consenting the participants for a follow-up phone call in order to confirm ongoing pregnancy viability. This formed part of the overall consenting procedure, for which ethical permission was obtained.

3.3.2 Alternative study designs considered

An additional cohort consisting of completely asymptomatic pregnant women in their first trimester could have been included in this research, to introduce the concept of “non-exposed cohort”, in contrast to the “study” or “exposed” participants who were symptomatic for PUV. Matching the data collected from both cohorts would have reduced confounding variables (Aronson, Bankhead & Nunan, 2018). However, the ethical and logistical issues involved in recruiting asymptomatic women in early stages of pregnancy precluded this alternative dual-cohort design.

Another alternative considered, was a randomized controlled trial, in which participants would have been randomly assigned to groups following different follow-up strategies, with the advantage of reducing bias (Hulley et al., 2013). However, this design is usually more difficult to carry out, requiring a large population sample recruited over a longer time-period. In addition, randomization to the longer 14-day review could have resulted in the participants either refusing to continue participation or seeking help from their own obstetrician in the interim period (Bankhead, Aronson, Nunan, 2017; Webb, 2011). Hence, this design was not considered feasible.

3.4 Population

PUV may be considered an interim diagnosis given to pregnant women in the first trimester. Ultimately all patients with this uncertain condition would progress to a definite diagnosis of either having a viable pregnancy or a miscarriage, within a set time frame. As discussed in section 2.5.2, the condition “exists” mainly due to the widespread awareness of services and ease of access of patients to early obstetric emergency care, at a time when the outcome of the pregnancy is not yet clear (Bottomley et al., 2009). The diagnosis of PUV is not used within the general population of “unscanned” pregnant females. In other words, the target population is a clinical population, not a community one (Hulley et al., 2013).

3.4.1 Target Population

The Target population for this study included all women in the first trimester of pregnancy, having an ultrasound diagnosis of PUV (Hulley et al., 2013). The PUV diagnosis was based on the 2012 NICE definitions, that were reconfirmed in 2019; indicating the following transvaginal parameters for PUV: a Crown rump Length of <7mm with no visible fetal heart pulsations or a gestational sac with a mean diameter of <25mm with no visible fetal pole (NICE 2019). Thus, the target population refers to the larger group of women exhibiting these characteristics to which the study could be eventually generalised (Hulley et al., 2013).

3.4.2 Accessible Population

The Accessible population in this research referred to women with a diagnosis of PUV who attended the emergency obstetric services at a local state general hospital in Malta, during the stipulated time period of data collection; hence a "geographically and temporally defined subset" (Hulley et al., 2013 p. 23)

3.4.3 Intended Population

The intended population (Hulley et al., 2013) included the subset of the accessible population who fulfilled the inclusion and exclusion criteria.

3.4.4 Actual Population or Sample

This refers to those women who actually participated and completed the PUV study (Hulley et al., 2013), therefore, including all the participants of this research.

3.5 Sampling and Sample Size

The sampling concept is especially important when research is addressing a large target population. The selected sample is meant to be large enough to reduce random error (Hulley et al., 2013), and it needs to be representative of the whole population, in terms of its characteristics, to reduce systematic error and bias (Hulley et al., 2013). In this way, inferences drawn from the findings of the study in question may be generalised to the whole target population, determining the external validity of the data collected (Mohajan, 2017; Onwuegbuzie, 2003). An ideal sampling method used to ensure

generalisability, is probability sampling, i.e. includes randomisation (Etikan, Musa & Alkassim et al., 2013). However, it is often the case that, clinical studies use convenience sampling due to the accessibility of participants to the researcher (Hulley et al. 2013). This is a non-probability sampling method which has logistic advantages, however at the expense of representation of the target population in the study cohort (Hulley et al., 2013). Convenience sampling was employed in this PUV study, since the participants were the patients who fulfilled the recruitment criteria at the time and place the study was carried out.

Estimation of the sample size required to power the study, was also very challenging to establish since the local incidence of PUV is unknown. Local birth statistics are available on the National Obstetric Information System (NOIS) (Gatt & Borg 2017), but unfortunately, there are no similar local records for early pregnancy complications. Even the internationally quoted incidence of PUV varies considerably from 12.5% (Reid et al., 2011) to 29% (Infante et al., 2013). From the researcher's experience, whilst auditing all 172 miscarriages in a local state hospital over a 6-month period in 2015 (Consiglio & Formosa, 2016), the accessible PUV population was estimated to be small. Thus, all pregnant females presenting with PUV at the state general hospital within the period of data collection, and falling within the inclusion criteria, were considered for recruitment to increase the representativeness of the sample. In addition, within the local scene, early obstetric care is shared between the private and public sector such that an unquantifiable proportion of women with early pregnancy complications, including PUV, will resort to private sonography and consultations without ever accessing the state emergency obstetric

services. Hence, the target PUV population number was unknown to the researcher; therefore, one must generalise the results with caution.

3.6 Inclusion Criteria

Eligibility for participation in this research was defined using the following criteria.

3.6.1 Clinical inclusion criteria included pregnant women who :

- Were aged 18 to 50, in the first trimester of pregnancy i.e. before 13 weeks, who presented to the emergency obstetric service of a local state general hospital;
- Had symptoms such as bleeding, pain, loss of pregnancy symptoms or any symptoms suspicious of early pregnancy loss;
- Were clinically stable;
- Were able to communicate in Maltese or English, in order to understand instructions and to give informed consent;
- Were residing in Malta for the duration of their pregnancy.

3.6.2 The ultrasonographic inclusion criteria included women having:

- A transvaginal ultrasound (NICE 2019) performed by selected trained sonographer/obstetricians, at a state general hospital;
- An ultrasound performed at the departmental ultrasound service available Monday to Saturdays using the same ultrasound unit;
- A single intrauterine pregnancy with an MSD <25mm with no visible fetal pole; or a visible fetal pole with a CRL <7mm with no visible heart pulsations (NICE 2019).

3.6.3 Exclusion criteria included pregnant females who :

- Declined a transvaginal ultrasound, since trans-abdominal scans are not comparable (NICE 2019);
- Already had a known diagnosis of viability or miscarriage prior to recruitment;
- Were already on progesterone supplementation since the outcome or duration till diagnosis may be affected by treatment;
- Had multiple pregnancies because the physiology and variables measured would be altered.

3.7 Study Protocol

Patients attending the emergency obstetric department in a local state general hospital who were under 13 weeks of gestation were considered for inclusion in this study. The patients presented with symptoms such as bleeding, pain, loss of pregnancy symptoms or any other symptoms suspicious of early pregnancy loss. They were then assessed by the emergency obstetric doctor and referred for a transvaginal ultrasound to evaluate the pregnancy.

3.7.1 Participant Recruitment

If the first ultrasound performed on the above-described patients classified their gestation as Pregnancy of Uncertain Viability, using the 2019 criteria (NICE 2019), and all the inclusion and exclusion criteria (outlined in 3.6) were met, then the patients were invited to participate in this research by the intermediaries. The patients were asked to give their consent and, thereafter given the necessary verbal and written information regarding the study aims

and objectives, in line with the ethical permissions obtained. A brief obstetric and medical history of each participant was also documented, and a bleeding assessment was made using the pictorial bleeding assessment chart (Higham et al., 1990). All the participant information collected was inputted into a proforma sheet by the members of staff aiding in the data collection. This data tool was designed by the researcher as outlined in section 3.8. Serum HCG and Progesterone levels were also taken as a one-time baseline, at the recruitment stage. All the information including the subsequent participant clinical and ultrasound data were documented in the same proforma sheet and passed on to the researcher by the intermediaries.

3.7.2 Follow-up

Follow-up of the participants was electively planned at 7 and at 14 days from recruitment and weekly thereafter if necessary. Follow-up was concluded when the diagnosis was confirmed to be either a viable pregnancy or a miscarriage. In some cases, the participants may have needed to visit the emergency services in between the scheduled visits due to the onset of new symptoms e.g. renewed fresh bleeding, the passage of products of conception or abdominal pain. These additional emergency visits were also documented.

In case of confirmed fetal heart pulsations, a diagnosis of viability was made. The participant was then referred for an antenatal booking visit. Follow-up communication by telephone was organised at the end of the first trimester. This was done to confirm that the pregnancy was still ongoing.

A diagnosis of miscarriage was made following the guidelines of a state general hospital. These included:

- The passage of products of conception;
- Cases where the first ultrasound scan showed an empty sac and, after a 1-week follow-up this remained empty with no yolk sac or fetal pole, and with no growth of the sac;
- Cases where the first ultrasound scan showed a fetal pole measuring <7mm with no heart pulsation and there was no growth of the fetal pole, and no heart pulsation was visible after 1-week follow-up.

A diagnosis of persistent PUV was made if:

- There was growth of the gestational sac but this was still measuring <25mm;
- There was growth of the fetal pole but CRL was still measuring <7mm and there was no visible fetal heartbeat.

The above protocol was used in this research, following local state hospital practices, in order to ascertain 100% specificity of diagnosis of early pregnancy loss (Consiglio & Muscat Baron, 2017). Once the participant diagnosis was ascertained, the patient was redirected to the standard hospital protocol, in line with either a viable pregnancy or a miscarriage.

3.8 Data Collection Sheet

A data collection sheet was designed by the researcher to document the necessary information throughout the study. This was filled in by the emergency staff collecting the patient data after receiving their consent.

The sonographic and clinical assessments were updated at the subsequent 7 and 14-day visits and during any other emergency visits in the interim period.

3.8.1 Clinical History

General clinical information included demographics, medical and surgical history. Relevant obstetric information was also documented including previous pregnancies and miscarriages, the mode of conception i.e. spontaneous or assisted. The date of the last menstrual period (LMP), and also the regularity and duration of the menstrual cycle were noted to calculate the gestational age.

3.8.2 Clinical Assessment

The symptoms of each participant were documented on the data collection sheet such as bleeding and/or pain. In case of bleeding, the amount was assessed using a Pictorial Blood Assessment Chart**:** PBAC Score. This internationally validated objective method of blood loss assessment has been used in research for many years (Higham et al., 1990; El-Nashar et al., 2015). This score was used in a similar predictive PUV study by Guha et al. (2013). The amount of blood lost by the patient was assessed by the clinic nurse or midwife and compared to a pictorial chart, and then graded on a Likert-scale as None, Light, Moderate, Soaks a towel or Flooding with clots. The PBAC score was repeated and documented at each participant encounter.

3.8.3 Biochemical data:

Two biochemical markers were chosen for this study based on previous PUV predictive studies as discussed in chapter 2.11. These were HCG and Progesterone (Bignardi et al., 2010; Jurkovic et al., 2013; Pillai et al., 2018;

Puget et al., 2018). The baseline biochemical markers were taken at the recruitment stage. This was unlike the 48-hour serial HCG protocol that is usually adopted in cases of PUL (NICE, 2019). The researcher chose a one-time baseline due to evidence that one progesterone level is sufficient to determine pregnancy viability and to limit cost and inconvenience to the participants (Lautmann et al., 2011). This was also aimed at improving adherence to the protocol by keeping it as similar as possible to the standard patient care pathway.

3.8.4 Ultrasound data

The sonographic data collected at each participant encounter were documented on the data collection sheet. The MSD, CRL and Yolk Sac Diameter were measured and described (section 3.9.4). In the review scan, if the fetal heart was noted then only the fetal pole required measurement as recommended by NICE (2019). The ultrasonographic diagnosis was reported indicating if the participant had a PUV, a persistent PUV, a miscarriage, or a viable pregnancy.

3.9 Sonographic assessment

3.9.1 The Observers

The researcher involved three observers who, in line with ethical permission obtained, accepted to aid the researcher with the data collection. The observers were running the emergency ultrasound clinic service during the standard working hours at the time of participant recruitment.

This eased the logistics of performing research and worked towards better reproducibility of the results (see section 3.10.5)

3.9.2 The Equipment

All participants were examined using the same machine in use at the emergency obstetric unit during normal working hours (section 3.9.2). The advantage of this was a reduction in measurement bias that would otherwise occur when using units of different specifications and parameters (Hu et al., 2014; Mahtani, Spencer & Brassey, 2017; Morgan, Unipan, & Datta, 2016). Ultrasound is known to be affected by different lighting and ambient conditions, hence reducing reproducibility and precision causing a negative impact on the internal validity of the data collected (Hulley et al., 2013; Onwuegbuzie, 2003).

3.9.3 Technical aspects

Prior to the commencement of the data collection, the observers concurred on the technical aspects and on following the same operational definitions for obtaining the sonographic variables (Hulley et al., 2013). These were documented on an Operations Manual drawn up by the researcher (see Appendix C). This was done to have scanning conformity between the observers, thus aiding to reduce bias.

A transvaginal ultrasound approach was adopted in keeping with contemporary early obstetric recommendations (Bickhaus et al., 2013; Huchon et al., 2016; NICE, 2019). The participants were instructed to empty their bladder to reduce discomfort and to optimise the transvaginal image of the uterus (Moorthy,

2000). The probe was cleansed and covered with a protective sheath, and ultrasonic gel applied to it according to the standard departmental protocol (BMUS, 2016). First trimester transvaginal presets were used based on the operations manual. The image was centralized, enlarged and the sector width adjusted. The Greyscale and Dynamic range were altered as necessary to improve visualisation and facilitate caliper placement. The focal point was to be set just below the area of interest. Image-optimization techniques including Differential Harmonics were used to reduce noise and clutter in the image (Fulgham, 2015).

3.9.4 Ultrasonographic observations

Measurements of the gestation sac and yolk sac in three orthogonal planes were taken to calculate a mean (Hu et al., 2014). Care was taken in measuring the three diameters: anteroposterior, longitudinal and transverse; and to avoid errors in taking the same diameter twice (Datta et al., 2012). The gestational sac was measured using a standard inner-to-inner diameter basis (Lautmann et al., 2011; Davison et al., 2014; Huchon et al., 2016). Measurements were taken in millimetres, to the nearest tenth of a millimetre (Hu et al., 2014). The shape and general appearance were noted e.g. a misshapen or low set sac (Mazzariol et al., 2015). The Yolk sac was measured. Measurements were taken on an outer-to-outer diameter basis (Bickhaus et al., 2013; Bottomley & Bourne, 2009), in view of greater ease of measurement, especially in case of echogenic or calcified yolk sacs (section 2.12.3). The appearance of the yolk sac was described e.g irregular, hyperechoic, calcified (Bamniya, Panchal, Singh, Shah, & Ladola, 2017; Berdahl et al., 2010; Moradan & Forouzeshfar, 2012; Tan, Sinan et al., 2014). The Fetal pole was measured, ideally lying

horizontally on the image (Al Darwish et al., 2019; Salomon et al., 2013). The presence of fetal heart pulsations visible on B-mode were considered to be diagnostic of pregnancy viability (Kolte et al., 2015). Care was taken not to foreshorten the CRL measurement. Conversely, attention was given to avoid including the yolk sac into the fetal pole measurement (Al Darwish et al., 2019; Ross & Johns, 2012). Overestimation of the CRL in the absence of fetal heart pulsations would inadvertently lead to a false diagnosis of miscarriage (Datta et al., 2012). Other abnormalities were noted but were not included in this predictive study. These included the presence of subchorionic haematomas (Mazzariol et al., 2015), chorionic bumps (Lane & Wong-You-Cheong, 2014; Mazzariol et al., 2015).

Health and Safety guidelines were followed throughout the scanning procedures including keeping the Thermal and Mechanical Indices to the recommended limits of <0.7 and <0.3 respectively, whilst always aiming to observe the ALARA principle (As Low As Reasonably Achievable) (Salomon et al., 2013). This is relevant in early obstetric scanning to avoid the theoretical thermal effects on a developing fetus (BMUS, 2012).

3.10 Reliability And Validity Of The Research

From its inception, a research study aims to find answers to a given question with accuracy and reliability, since both contribute to its internal validity (Onwuegbuzie, 2003). Unless the researcher is attentive to these aspects and other facets of validity, from the design phase through to the execution of

his/her work, then the results are, at best, worthless and possibly dangerous if applied to patients and the scientific community at large (Hulley et al., 2013).

Accuracy refers to how a measured variable approximates its true value (Hulley et al., 2013). In the PUV study, this is most relevant in the measurements of the ultrasound parameters: the MSD, Yolk sac diameter and CRL. Reliability or Precision refers to the reproducibility of a measurement when it is taken repeatedly (Hulley et al., 2013). In other words: 'consistency and replicability over time, over instruments and over groups of respondents' (Cohen et al., 2011, p. 119). This was relevant in this study for the assessment of the blood loss as well as the ultrasound measurements. Internal validity refers to whether the study measures what it proposes to measure. As described by Onwuegbuzie (2003, p.62), a study has internal validity "if the results are due only to the manipulated independent variable." In this research, steps were taken to minimize threats to reliability and validity. These strategies, as outlined below, often overlapped in reducing random as well as systematic error or bias.

3.10.1 Data Collection sheet

The data sheet was designed by the researcher specifically for the PUV study. It was reviewed by two experts in the field of ultrasound and obstetrics to ascertain Content Validity. The first was a specialist obstetrician with over 30 years of experience in the field. The second was a specialist obstetrician with 10 years of experience and with a special interest in ultrasound. They were requested to score the different parts of the form for Clarity and Relevance using a 4-point Likert Scale ranging from "Not clear", "Somewhat Clear", "Clear" to "Very Clear" with the same scale being used for relevance.

Both experts scored the data sheet very highly on both clarity and relevance. Minor amendments to the sheet were made based on their recommendations. (See sample of data sheet in appendix D).

3.10.2 PBAC Score

The researcher preferred a staff assessment of participant blood loss rather than adopting **patient self-assessment, to avoid the introduction of observer bias (Mahtani, Spencer, Brassey, 2017). The** members of staff involved in assessing the participants for bleeding were very experienced, such that intra-rater reliability was deemed unnecessary. However, inter-rater reliability was carried out. The staff members were asked to rate different sanitary towels for assessment of blood loss compared to the PBAC pictorial chart to which they were familiarized (El-Nashar et al., 2015; Higham et al., 1990) The bleeding was rated on a Likert-Scale ranging from Nil, Little, Moderate, Soaks a towel or Flooding/clots. These scores were analysed using the Kendall Tau test which is used to assess inter-rater reliability when the evaluations (rating scores) have an ordinal scale (Wagner, 2016). The Correlation Coefficient ranges from -1 to 1 and statistical significance is obtained if the p-value is 0.05. The results obtained indicated satisfactory internal consistency between the raters, with correlation coefficients exceeding 0.88. Moreover, all p-values were <0.05 indicating that all the raters were consistent with each other in performing the PBAC score. The purpose of this statistical test was to ensure that the inter-rater reliability was not a threat to the rigour of the study (Mohajan, 2017).

3.10.3 The Subjects

Variation in uterine size, large degrees of anteversion or retroversion of the uterus, as well as patient's body habitus, may cause difficulty in imaging the uterine cavity. This is a limitation that is acknowledged in this study. Overall, ultrasound as an imaging modality remains user, machine and subject dependent (Morgan et al., 2016).

3.10.4 The Equipment

The ultrasound unit in use was subjected to regular Quality Assurance checks according to standard local policies (Dudley, Russell, Ward, Hoskins, & BMUS QA Working Party, 2014). This included calibration to optimize accuracy and to minimize measurement bias as much as possible. Limiting data collection to one machine also reduced precision issues between different machines (Mahtani et al., 2017).

3.10.5 The Observers

For practical reasons, the observers were limited to three, since these experienced healthcare professionals ran the emergency service at the time of participant recruitment. The disadvantage of not involving all hospital sonographers was that the results would not be as generalisable, hence limiting the external validity (Mohajan, 2017; Onwuegbuzie, 2003). However, this was offset by the advantage of reducing the inter-observer errors which would introduce measurement bias (Mahtani et al., 2017; Pexsters et al., 2011), that could falsely mitigate or augment any significant associations (Hulley et al., 2013). Such a decision was considered a trade-off in favour of reliability and internal validity. The experienced observers did not need a learning curve.

They aided the researcher to optimise the Operations Manual (Appendix C) used for this research in order to standardise the readings (Sarris et al., 2013). The fact that repeated measurements and mean values were used reduced random error, improving accuracy and hence internal validity (Hulley et al., 2013; Sarris et al., 2013).

Prior to commencing the study, the observers underwent inter-rater reliability testing. Intraclass correlation is normally used to assess absolute agreement between raters when the measures have a metric scale. This was relevant to the ultrasound measurements. The intraclass correlation ranges from 0 to 1 where a correlation close to 1 indicates very good agreement, while a correlation close to 0 indicates poor agreement. It was computed as over 0.99 for MSD and CRL measurements. Moreover, the p-value obtained reflects whether the level of agreement is significant or not. The output for the three raters clearly showed satisfactory agreement for the scan variables with a p-value of 0.000.

3.11 Data Collection And Analysis

The data collection took place between 31st October 2018 and 14th March 2019. The first 10 cases were evaluated as part of the pilot phase. The results were analysed with the aid of a statistician.

3.11.1 The pilot study

The first 10 cases recruited were evaluated to assess what aspects needed refining. The researcher received feedback from the intermediaries and

sonographers involved. Following the pilot study, minor changes were made to the data collection sheet. In the initial version, the weight and height were requested to standardise the data. However, it was pointed out to the researcher that no height measure or weighing scales were available in the emergency room. It is known that data given by participants about their own biometrics are often erroneous tending to overestimate height and underestimate weight (Bolton-Smith et al., 2000; Dhaliwal, Howat, Bejoy, & Welborn, 2010. Although it is usually recommended to exclude data from the pilot study from the final results (Cormack, 2000), in this case, it was decided to retain them in view of the limited actual population sample.

3.11.2 Data Analysis

The data collected was inputted into the SPSS (version 25) for analysis. The aid of a statistician was also sought to ascertain which statistical tests should be carried out on the data collected.

The analysis was performed in two main sections. The first was linked to the predictive aim of the study. Hence, the clinical, biochemical and ultrasonographic constructs were analysed individually in comparison to the outcome, that is: viability or miscarriage. The Independent Samples T-test (Wagner, 2016), was used to measure mean values for the ordinal constructs such as maternal age, progesterone, HCG and the ultrasonographic parameters (CRL, yolk sac size and, MSD). The nominal or categorical constructs were compared using Pearson's Chi-Square test (Wagner, 2016). This included the PBAC score, ethnicity and parity. Univariate analysis was performed on each construct to assess if any variable could be an effective

predictor of outcome in cases of PUV. A stepwise logistic regression analysis was performed to analyse associations between the variables measured and miscarriage using Receiver Operator Characteristic (ROC) curves with a significance set at 0.05. The second part of the analysis compared 7 and 14-day ultrasound results and, using different stratification methods, observations were made to identify a possible optimal strategy.

3.12 Ethical Considerations

Ignacio and Taylor (2013), classified ethical issues into three broad areas. One area addresses the relationship between the participant and the researcher. The other two involve informed consent and, the need for anonymity and confidentiality. This concept is also emphasized in the declaration of Helsinki initially developed in 1964 (World Medical Association, 2001).

3.12.1 Addressing ethics in the study design

Research projects involving pregnant women need to have a thorough ethical evaluation at the planning stage (Hulley et al., 2013). The research design of this PUV study was drawn up taking the principles of beneficence and non-maleficence into consideration. Fouka and Mantzorou (2011, p.3) stated that "beneficence relates to the benefits of the research, while non-maleficence relates to the potential risks of participation". The choice of a non-interventional design implied that the participants were not at an increased risk compared to the non-participants. The involvement of the intermediary members of staff in this research, whilst adhering to the requirements posed by the university

ethical committee, avoided the researcher having a dual role as a clinician and an investigator, reducing the potential for any conflict of interest (Hulley et al., 2013) This issue was described by Ignacio et al. (2013) as a misconstrued researcher-to-participant relationship, in which the researcher takes on a dual role. In such cases, the patient might feel obliged to participate in a study for fear that her care would be compromised if she declines. On the other hand, the investigator could face a conflict of interest if the patient's needs would not be in line with the requirements for the study. The role of the intermediaries was important in this regard, by inviting, obtaining consent and imparting the necessary written information required to take part in the research. The intermediaries subsequently guided the participants either through the miscarriage or pregnancy viability pathways.

3.12.2 The need for informed consent

The participants were given a consent form (Appendix B) together with an information sheet (Appendix B) indicating the aims and objectives, as well as the layout of the study. Vulnerable patients under the legal age of consent and mentally infirm patients were excluded from this research, due to their inability to give their own consent for participation. It was made clear to the participants in the information letter given to them, that their participation was voluntary and that they could withdraw from the study at any point with no prejudice to their subsequent care. No financial incentive was offered to the patients for participation reducing again the possibility of any conflicts of interest in this research (Hulley et al., 2013).

3.12.3 Anonymity and Confidentiality

The Declaration of Helsinki (1964), states that “every precaution must be taken to protect the privacy of research subjects and the confidentiality of their personal information” (World Medical Association, 2001, p. 373). In this research, cases were pseudonymized and soft data was retained by the researcher on a password-protected computer system, in order to ensure patient anonymity. The data was discarded on completion of the study to ensure the participant’s confidentiality in accordance with the local General Data Protection Regulations (GDPR, 2018). Moreover, data was collected with the aid of intermediary members of staff to ensure patient anonymity.

3.12.4 Permissions obtained

Permission was granted from all the relevant stakeholders within a local state general hospital, in order to conduct this research project as indicated in this chapter. This included all obstetric consultants, the heads of department of Obstetrics and of Pathology, Midwifery Officers, Radiographers, intermediaries and the hospital authorities including the Chief Executive Officer and Data Protection Officer. The study was subsequently approved by both the Faculty Research Ethics Committee and the University Research Ethics Committee (Reference: FREC-FHS-1718-129).

3.13 PUV Study Strengths And Limitations

Every researcher strives to optimise his/her work. However, awareness of the limitations of a research study is an essential factor in order to improve it. The next section describes the limitations of the study and the steps identified to minimise them.

3.13.1 User-dependence

Diagnostic ultrasound makes the study user-dependent. (Pexsters et al., 2011; Sarris et al., 2013). In this research, care was taken to minimise ultrasound related observation error and other biases. This included the use of a single ultrasound unit, the limited number of observers, and the use of a study operations manual to standardise images and measurements (Pexsters et al., 2011). The choice of ultrasound variables was based on international guidelines, stated also in other studies (Kolte et al., 2015; NICE, 2019; Salomon et al., 2013). In addition, inter-rater reliability checks were successfully carried out prior to commencement of recruitment (section 3.10.5). This conferred good construct validity to the study overall (Hulley et al., 2013).

3.13.2 Bias

Bias is to be considered as a limiting factor during the data collection (Mahtani et al., 2017). Steps were taken in designing the study to minimise this. The choice of PBAC scoring by staff members was implemented to eliminate "recall" bias. The alternative patient self-assessment would have likely overestimated blood loss (Spencer et al., 2017). To further improve this, the inter-observer reliability checks for PBAC scoring by staff members was carried

out (section 3.10.2). Another safeguard to the introduction of bias was the use of blinding when possible. It is known that non-blinding may exaggerate associations by 23% in cases of subjective outcomes (Balk et al., 2002). In this study, the researcher, sonographers, intermediary staff members aiding in the data collection and the participants themselves were all blind to the outcome of the study during the recruitment stage (Nunan et al., 2018).

3.13.3 Participant Sample Size

The sample of participants included in the research was small. However, one must put this in the perspective of the duration over which the study was carried out and the pregnancy population served by the hospital involved in the study. A similar predictive study carried out in England over a 9-month period analysed 172 cases (Guha et al., 2013). Another large British PUV study by Lautmann et al. (2011), analysed 400 cases. However, this was carried out retrospectively, over 6 years in a unit that examined 25,928 pregnancies, with 5163 eligible cases of PUV (Lautmann et al., 2011). The local unit serves a much smaller population than this, considering the national birth rate is around 4500 (Gatt & Borg, 2017). In addition, an unknown proportion of pregnant women seek private health care which would further reduce the accessible population. In order to increase the sample size, the data used to pilot the study protocol was also included in the final data analysis. In addition, recruitment was commenced as soon as ethical approval was confirmed.

3.13.4 Sampling Method

Convenience sampling was used in this research. An advantage, in this case, was that the emergency local state hospital setting, caters for a broad spectrum of patients in terms of nationality and social strata. Nevertheless, this type of sampling may lead to recruitment or sampling bias (Hulley et al., 2013; Nunan et al., 2017), resulting in an actual population which is not representative of the target population posing a threat to external validity (Hulley et al., 2013; Onwuegbuzie, 2003). In this study, patient recruitment took place during standard hospital working hours from Monday to Saturdays. Specific recruitment times were implemented so as to ensure that all participants were scanned using the same equipment, to ameliorate the reliability and internal validity of the data collected. However, the potential for introducing selection bias was recognised and accounted for (Nunan et al., 2017). At the time of recruitment, stable patients attending after-hours were routinely referred for a departmental obstetric scan on the following morning's ultrasound list, thus having an equal chance of being recruited, safeguarding generalisability and thus the external validity of the study (Onwuegbuzie, 2003)

3.13.5 Study Design

The observational design used is a strong point in predictive studies and being non-interventional made it ethically sound (Setia,2016). However, confounders are known to be a limitation associated with observational studies (Aronson, Bankhead & Nunan, 2018). In the PUV study, one of the most important factors to consider was age and ethnicity and these were accounted for (Setia, 2016).

3.13.6 Non-compliance

Non-compliance or failure to follow the study protocol is a possible weakness in prospective research especially when intermediary members of staff are carrying out data collection (Spencer, 2018). In this regard, the study was purposely designed to be similar to the patient care pathway to shorten the learning curve. In addition, the researcher reminded the staff regularly about the PUV study protocol.

3.13.7 Attrition

Another limitation associated with prospective research is sample attrition. An attrition rate of <5% is considered acceptable. A rate above 20% poses a threat to the validity of the study (Bankhead et al., 2017). In this PUV study, the attrition rate was of 4.6%, falling within the acceptable level. Intrinsic factors in the design may justify this. This included the short time period required for follow-up and to obtain a definite diagnosis. In addition, steps were taken to keep loss to follow up as low as possible. These included consenting to a telephone or email for follow-up of viable pregnancies and including only patients residing in Malta as part of the inclusion criteria.

3.14 Conclusion

This chapter has outlined design and methodology aspects of this PUV study. The next chapter will present the results of this study together with the discussion based on the analysed data in comparison to the literature reviewed.

CHAPTER 4:

RESULTS AND DISCUSSION

4.1 Introduction

This chapter presents the results of the PUV study starting with the incidence of miscarriage versus viability (Section 4.2). It then exhibits the clinical, biochemical and ultrasonographic aspects of the results, including a predictive model (Section 4.3-4.6) in keeping with predictive aim of the study. This is followed by a presenation of the ultrasound timing results relating to the strategic aim of this PUV study (Section 4.7). The results obtained are presented by the use of graphs and tables after performing the statistical tests required. A summary of the univariate analysis is shown in Table 4.11.

Forty-seven patients who presented to a state general hospital with a diagnosis of PUV were recruited between 31/10/18 and 14/3/19. After performing the initial assessment, the participants were followed up weekly until a definitive diagnosis of viability or miscarriage was reached. In case of viability, ongoing pregnancy was confirmed at the end of the first trimester.

Complete case analysis was carried out on 43 out of the 47 cases (91.4%). Two women failed to attend the follow-up visit and another two participants had incomplete blood results. In a similar study by Guha et al.,(2013), the data was complete in 78.8% and the outcome was known in 89% of cases. The raw data collected for the current PUV research may be found in Appendix E.

4.2. Incidence Of Miscarriage

The incidence of miscarriage until the end of the first trimester in the PUV cohort was 41.9% (n=18). The incidence rates of miscarriage in PUV populations vary amongst studies discussed in the literature. Comparable figures to those found in this research were identified by Bottomley et al. (2008) of 45% (n=481); Ng Zy et al.,(2014) of 47% (n= 115); and by Riemke al. (2011) of 47.7% (n=268). A miscarriage incidence rate of 49.3% (n=400) was found in the PUV predictive model and scoring study by Guha et al. (2013). A more recent study by Puget et al. (2018) also identified a lower miscarriage rate of 38.1% (n=107).

Whether the redefining of the PUV cut-off points that occured in 2012 (NICE 2012), would have impacted the miscarriage figures of previous studies is unknown. However, researchers publishing work relating to PUV during the definition change-over argued that the number of patients at the boundaries would be small and should not impact the results (Bottomley et al., 2013). It can be debated that the miscarriage incidence rates depend on the referral criteria adopted by the healthcare services. A local Maltese state general hospital offers a 24-hour emergency service that accepts self-referrals from pregnant patients even at very early gestations, under six weeks, where it is more likely to misdiagnose a healthy early pregnancy as PUV (Bottomley 2009). In contrast, as alluded to in section 1.3, in countries such as the United Kingdom (UK), since the 1980s, over 150 early pregnancy units have been set up, with telephone advice services and pre-set appointments complementing

the emergency referrals. Thus, in the UK it is the practice to reassure women with mild symptoms, such as painless spotting, and to review them electively after 6 completed weeks (NICE, 2019). According to Bottomley et al. (2009), the most likely diagnosis at 35-41 days of gestation is a PUV whilst after 42 days the more common outcome is a viable pregnancy. The authors also concluded that by deferring the scans to beyond 7 weeks of pregnancy, they may increase the rate of a certain diagnosis at the first encounter from 37.7% to 78.6%.

4.3 Participant Characteristics

This section describes the results relating to the demographic and clinical characteristics of the study cohort and how they related to the PUV outcome.

4.3.1 Participant Nationality

Twenty four of the 43 participants were Maltese nationals, while 19 were non-Maltese. This means that 44.1% of the cohort was composed of Non-Maltese nationals as shown in Figure 4.1. This contrasts with the 2016 National Obstetric Information System (NOIS) report that established that 20% of parturients were Non-Maltese (Gatt & Borg, 2017). The discrepancy in the national representation may be interpreted in a number of ways.

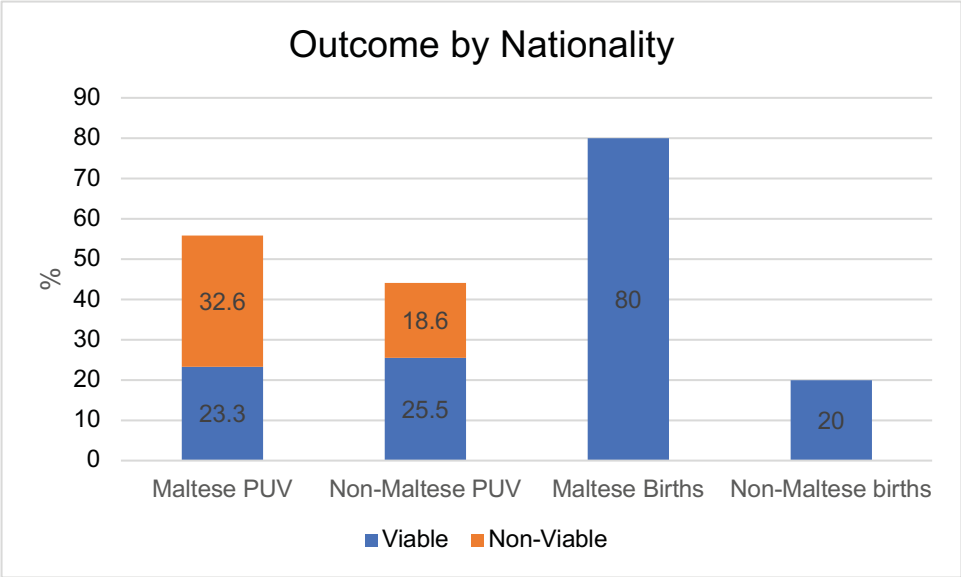

Figure 4.1 PUV outcome as compared to births in Malta in 2016

One could suspect a positive association between ethnicity and miscarriage as had been shown for Asians in a predictive PUV study (p=0.047) (Bottomley et al., 2011). This was not confirmed by Ng Zy et al., (2014) whose study did not find ethnicity to show any difference in miscarriage rates. The Chi-Square test was applied to the current PUV cohort to assess a possible association between the two categorical variables of nationality and outcome. However, there was no statistically significant difference (p=0.977) in the miscarriage rates between the Maltese (41.7%) and the non-Maltese nationals (42.1%). This is further represented in Table 4.1.

The number of non-Maltese nationals in the PUV cohort could be an indication of a selection bias (Nunan et al., 2017). This may be because the cohort is reflecting the women making use of early emergency obstetric services in a Maltese state general hospital only, not accounting for PUV patients presenting in the Maltese private sector. It is not possible to determine the exact reasons for the different proportions of Maltese versus non-Maltese participants from this study, and this was outside the scope of the research aims.

			Final Outcome		
			Miscarriage	Viable	Total
Nationality	Maltese	Count	10	14	24
		Percentage	41.7%	58.3%	100.0%
	Foreigner	Count	8	11	19
		Percentage	42.1%	57.9%	100.0%
Total		Count	18	25	43
		Percentage	41.9%	58.1%	100.0%

$X^2(1) = 0.001$, $p = 0.977$

Table 4.1 Pregnancy outcome compared to participants' nationality

4.3.2 Maternal Age

The age of the participants ranged from 18 to 42 with a mean of 31.4 years. This is similar to the mean age for Maltese parturients indicated by the 2016 NOIS report (Gatt & Borg 2017) which was 30.2years (range 14-47 years). Moreover, the commonest maternal age encountered locally is that of 31 years (Gatt & Borg 2017). The Independent Samples t-test was used to compare the mean age between the two subgroups clustered by final diagnosis (miscarriage versus viable). The mean age of the miscarrying subgroup was 31.72 years which marginally exceeded that of the viable subgroup, that was

31.2 years. This minimal difference of 0.52 years was not found to be significant (p=0.789). This is shown in Table 4.2.

Final Outcome	Sample size	Mean Age	Std. Deviation	P-value
Miscarriage	18	31.72	5.969	0.789
Viable	25	31.20	6.461	

Table 4.2 Independent samples t-test for age and final outcome

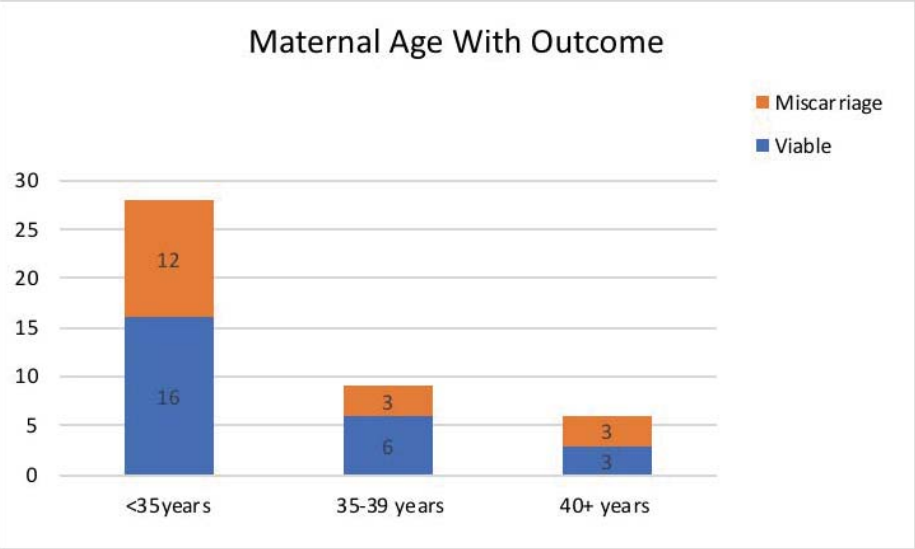

Figure 4.2 PUV outcome based on maternal age categories

In contrast to these findings, as discussed in section 2.10.1, the literature states that advanced maternal age is a known risk factor for early pregnancy loss (Jurkovic 2103, Magnus et al. 2019). The above results are also discordant with the current literature relating specifically to PUV.

A significantly higher mean age for miscarrying PUV patients was found by different researchers including older studies dating back to 2003 (Elson et al., 2003; Falco et al., 2003). At least two studies reported a significant mean age difference of 3.8years (Reid et al., 2011) and 4.6 years respectively (Ng, Ng, & Tan, 2014) between viable and non-viable PUVs. Moreover, the PUV prediction scoring models used by Elson et al. (2003), whose work was developed further by Lautmann et al. (2011), included maternal age. The Bottomley prediction models and scoring systems (Bottomley et al., 2011; Bottomley et al., 2013) that were externally validated by Guha et al. (2013), also incorporated maternal age.

However, even after subdividing this PUV dataset into age groups as indicated by the Bottomley scoring system (<35years, 35-39 years and 40 years or above)(see Figure 4.2), there was still no significant difference in the outcome, with a p-value of 0.801. A possible reason for such incongruent results could be a type-2 random error (Hulley et al., 2013), indicating that the small sample size may have nullified the true correlation between increased maternal age and early pregnancy loss.

4.3.3 Gestational Age

The literature has been consistent in showing that women diagnosed with PUV at a later gestational age are more likely to miscarry.

This has been shown by PUV predictor model studies by Falco et al. (2003), Elson et al. (2003), Lautmann et al., (2011), Bottomley et al. (2011), and Ng et al. (2014). The reason for this association may be explained physiologically. Females presenting in earlier gestation are likely to represent the healthy early pregnancies; whilst the later gestations may indicate a failing pregnancy, in other words, a missed miscarriage. This was detailed in a review by Rodgers et al. (2015). Nevertheless, despite this clear predictive association, the newer model in use by Guha et al. (2013) had opted to exclude this variable from their scoring system. The reason for this was that 20% of their previous cohort was found to have an unknown last period date (Bottomley et al., 2011). Although the model including gestational age performed better than the model without, the latter was recommmended for use in cases of unknown period date or irregular cycles (Guha et al., 2013).

Final Outcome	Sample size	Mean gestational age at presentation	Std. Deviation	P-value
Miscarriage	18	7.12	1.362	0.000
Viable	25	5.74	0.741	

Table 4.3 Independent samples t-test for gestational age and outcome

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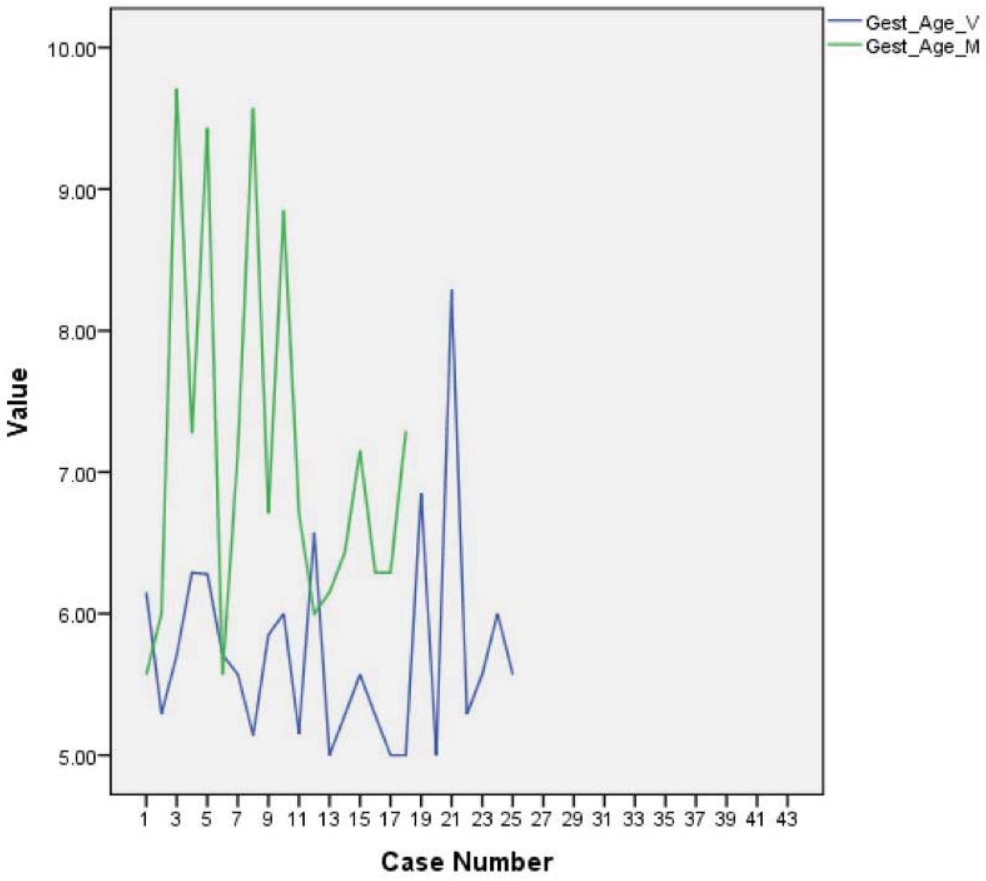

Figure 4.3 Gestational age in weeks and final outcome

In this PUV study, a very strong association was indicated by the Independent samples t-test as shown in Table 4.3. The mean gestational age of PUV participants who miscarried was 7.12 weeks, in contrast with 5.74weeks for PUV participants who went onto viability. The 1.38 week-difference had a significant p-value of 0.000. The gestational ages are represented in Figure 4.3.

4.3.4 Bleeding Scores

The 43 participants were assessed for bleeding using the pictorial blood assessment chart (PBAC) (El-Nashar et al.,2015; Higham et al.,1990) (Section 2.10.3; 3.8.2). This score had also been previously used in devising a model and scoring system for PUV and threatened miscarriage. Hence, it is a comparable construct (Bottomley et al., 2013; Guha et al., 2013).

Results (See Table 4.4) showed that 48.8% (n=21) of the participants had no bleeding at all. The other 52.2% (n=22) had some degree of bleeding, of whom 44.2%(n=19) were classified as light by the clinical assessors. In addition, 4.7% (n=2) had moderate blood loss, whilst 2.7 % (n=1) soaked a towel. There were no patients with flooding or heavy bleeding recruited in this cohort. The results are represented in Figure 4.4. The presence of bleeding was strongly associated with the risk of miscarriage. All three participants, who, at the recruitment stage were classed as having moderate bleeding or more, miscarried. However, an interesting and perhaps less expected finding was, that in the presence of even light bleeding, the miscarriage rate was 66.7% as opposed to only 16.7% miscarriage rate in the total absence of bleeding.

This result was found to be statistically significant when applying the Chi-Square test with a p-value of 0.003. Clinically, this indicates an optimistic 72% viability rate in the absence of bleeding.

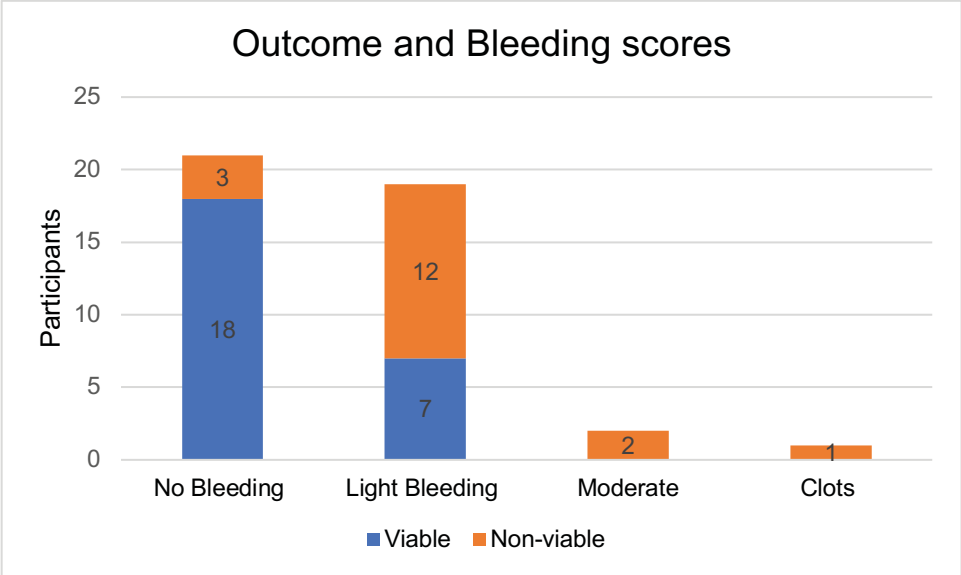

Figure 4.4 PUV outcome classified by bleeding scores

The literature is not in complete agreement on this point. Consistent with this PUV study, Ng et al. (2014) showed an increase in the incidence of miscarriage from 32.8% to 70.4% in the presence of bleeding (n=115). On the other hand, according to a retrospective study by Hessert & Juliano (2012), the presence of bleeding and pain only moderately increased the rate of miscarriage in PUV patients (n=117) from 30% up to 35%. The presence of clots was also found to be significant in another prospective study specific to PUV patients (n=268) by Riemke et al. (2010). In contrast, there was no significant association found between pregnancy outcome and the presence or amount of bleeding in another PUV study (n=78) by Naji et al. (2010).

			Final Diagnosis		
			Miscarriage	Viable	Total
PBAC	Nil	Count	3	18	21
		Percentage	16.7%	72.0%	48.8%
	Light	Count	12	7	19
		Percentage	66.7%	28.0%	44.2%
	Moderate	Count	2	0	2
		Percentage	11.1%	0.0%	4.7%
	Soaks towel	Count	1	0	1
		Percentage	5.6%	0.0%	2.3%
Total		Count	18	25	43
		Percentage	100.0%	100.0%	100.0%

$X^2(3) = 14.269$, $p = 0.003$

Table 4.4 Chi-Square test for PBAC versus final outcome

4.3.5 Previous miscarriage and outcome

Of the 43 recruits, 30.2 % (n=13) were women in their first pregnancy, 34.9% (n=15) were women who had at least one live birth and no previous miscarriages. Furthermore, 27.9% (n=12) of the participants had at least one live child but had suffered at least one pregnancy loss while the remaining 7% (n=3) had only experienced previous pregnancy loss with no live births. (See Figure 4.5).

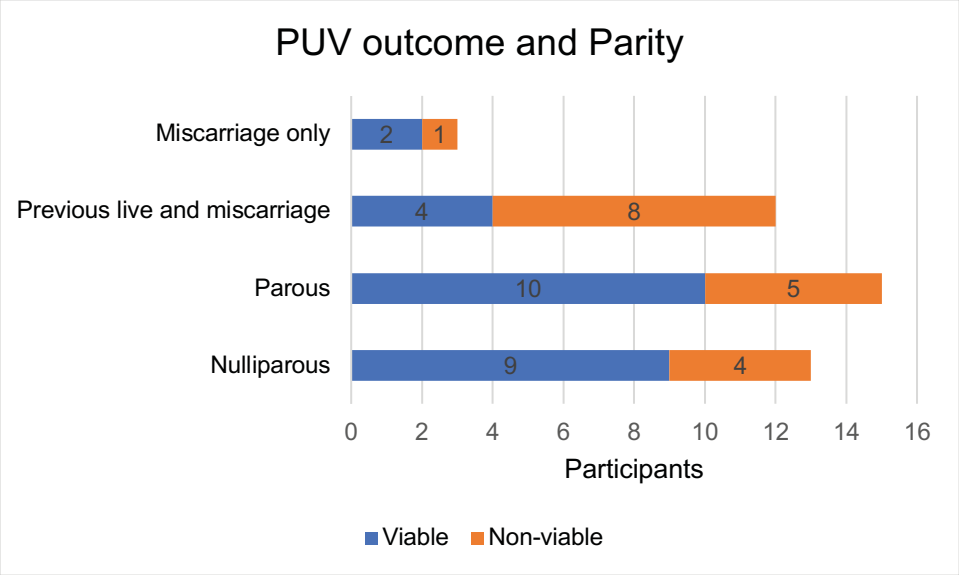

Figure 4.5 PUV outcome compared to the parity distribution

Previous pregnancy loss is a known risk factor for repeat miscarriage (Section 2.10.4). This was demonstrated in a recent Norwegian study, with the results indicating an increased odds ratio for miscarriage as the number of previous miscarriages increased, even after correction for maternal age (Magnus et al., 2019). Previous miscarriage was also noted to be a predictor for future pregnancy loss in a study specific to PUV (Reid et al., 2011). It was interesting to note that in the women who had no history of pregnancy loss, either being in their first gestation or having had at least one live birth, the rate of miscarriage was 32.1% (n=9), in this study. However, in those participants who had at least one miscarriage, with or without a previous live birth, the miscarriage rate was 60% (n=9). (See Figure 4.6, Table 4.5). This result, however, was not found to be statistically significant using Two-population Proportions testing and Chi-Square testing, both yielding a p-value of 0.078.

			Current Pregnancy		Total
			Viable	Miscarriage	
Previous Pregnancy	Loss	Count	6	9	15
		% within Previous Pregnancy	40.0%	60.0%	100.0%
	No loss	Count	19	9	28
		% within Previous Pregnancy	67.9.0%	32.1.0%	100.0%
Total		Count	25	18	43
		% within Previous Pregnancy	58.1%	41.9%	100.0%

Table 4.5. PUV outcome compared with history of miscarriage

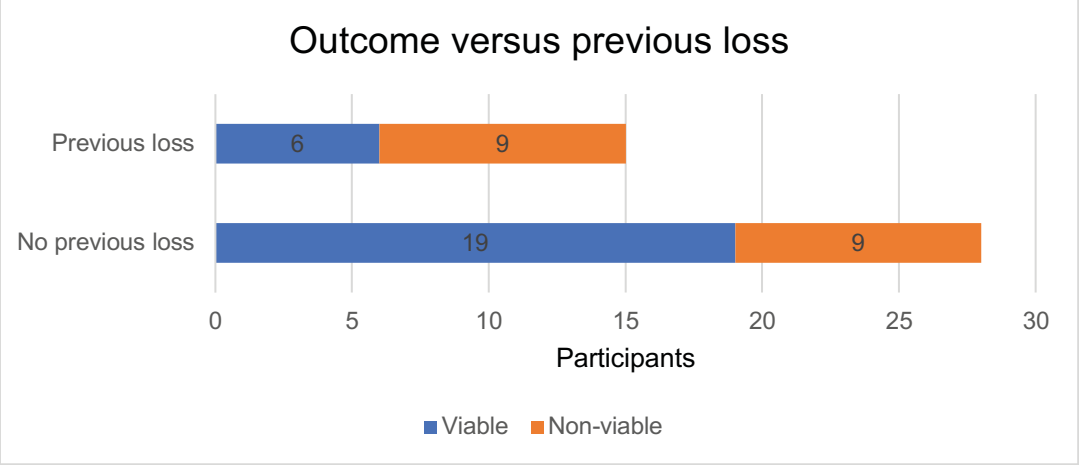

Figure 4.6 PUV outcome versus previous early pregnancy loss

4.4 Biochemical Markers As Predictors Of Outcome

The results of the chosen biochemical markers, HCG and Progesterone, are outlined below.

4.4.1 Progesterone

Progesterone has been shown to be a strong predictor of viability in early pregnancy (Muttalib, 2018) (section 2.11.1). Its use has been investigated in the management of PUL (Guha et al., 2013), PUV (Puget et al., 2018), as well as in cases threatened miscarriage (Ku, Allen, Sze, Tan, & Tan, 2018). A meta-analysis by Verhaegen et al., (2012) has demonstrated that this hormone may be used to distinguish a viable from a non-viable pregnancy. A value of 16nmol/l (5.1ng/ml) has been quoted as a possible cut-off both by Verhaegen et al., (2012) and by Lautmann et al. (2011). However, it has been more challenging to identify a progesterone limit to confirm viability (Hanita & Hanisah, 2012). The RCOG Scientific impact paper, suggested a non-viability cut-off of 20nmol/l (6ng/ml) and 60nmol/l to suggest a viable pregnancy (Memtsa, Jurkovic, & Jauniaux, 2018).

In this PUV study, the mean progesterone level for recruits who miscarried was 18.36nmol/l. This was considerably lower than the mean progesterone level for PUV patients with viable outcomes which was found to be 34.26nmol/l. These values were found to be statistically significant on Independent samples t-test with a p-value of 0.000. This is shown in Table 4.6.

Final Diagnosis	Sample size	Mean Progesterone	Std. Deviation	P-value
Miscarriage	18	18.36	11.945	0.000
Viable	25	34.26	10.151	

Table 4.6 Independent samples t-test for Mean Progesterone levels

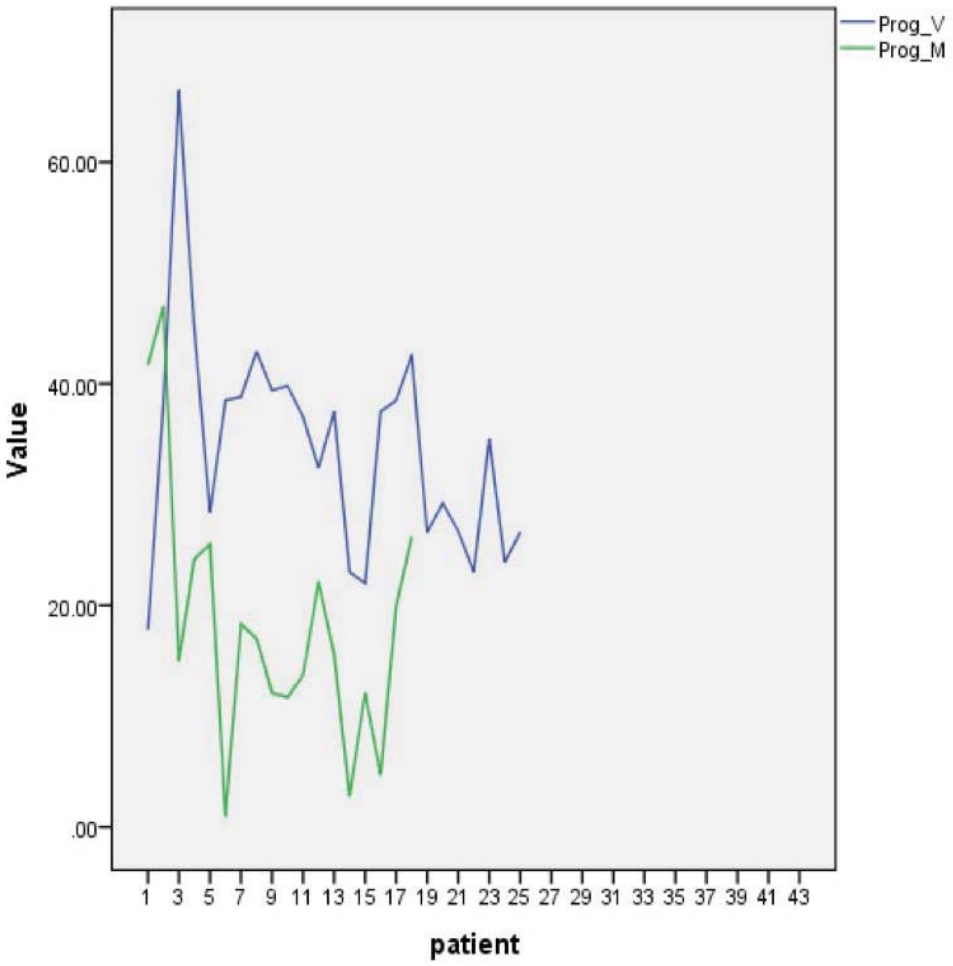

Figure 4.7 Progesterone levels in Viable and Miscarrying PUVs

In this current research, the lowest level of progesterone for a viable PUV was found to be 17.8 nmol/l, at a gestational age of 6weeks+1day; which is in keeping with the recommended 16nmol/l levels by Lautmann et al., (2011) and Verhaegen et al., (2012). The highest level of progesterone found in a miscarrying PUV patient was found to be 47nmol/l at a gestational age at 6 weeks. It is important to mention, though, that a clinician cannot reach a diagnosis of viability or miscarriage by taking a progesterone level in isolation (Jurkovic et al., 2013; Puget et al., 2018). All the progesterone levels are pictorially represented in Figure 4.7.

4.4.2 Human Chorionic Gonadotropin

HCG is in standard clinical use in the management of PUL using 48-hour serial levels, and also in the follow-up of molar pregnancies (RCOG 2010; Memtsa et al., 2018; NICE, 2019). Its clinical use in the management of visualised intrauterine pregnancies or miscarriage has not been established, even if it has been evaluated in the research setting (Whittaker, Schreiber, & Sammel, 2018) (section 2.11.2).

In this PUV study, the mean HCG level of the PUV participants who miscarried was 9,323.56 miU/ml, whilst that of the participants with a viable outcome was 10,343.96miU/ml. Although the hormonal level was lower in those women who eventually had a confirmed miscarriage as compared to the viable PUVs, the p-value indicated no statistical significance (p=0.724) as shown in Table 4.7. In the current dataset, the highest level of HCG in a non-viable pregnancy was 36,233miU/ml, whilst the lowest HCG level for a viable pregnancy was 622miU/ml at 5 weeks +5 days. A particular finding was an HCG level of

6,548miU/ml in a participant who eventually miscarried a pregnancy that was later found to be a molar pregnancy. A higher HCG level is usually associated with gestational trophoblastic disease (RCOG, 2010). The progesterone level in this abnormal pregnancy was 41.7mmol/l, which was higher than the mean progesterone level of miscarried PUVs (18.36nmol/l) found in the current research. Another interesting observation was the large variation of HCG levels for viable PUV participants even at the same gestational age. Four participants, recruited at exactly 5 weeks according to their menstrual age, had HCG levels of: 2980iU/ml , 3106iU/ml, 16,290iU/ml and 22977iU/ml.

Final Diagnosis	Sample size	Mean HCG	Std. Deviation	P-value
Miscarriage	18	9323.56	11127.705	0.724
Viable	25	10343.96	7701.174	

Table 4.7 Independent samples t-test for mean HCG levels in PUV

There has been conflicting evidence in the literature about HCG over the past years. A PUV study (n=50) by Falco (2003), had shown a positive association of low HCG to miscarriage, but this was contradicted later in another PUV study (n=115) in which no association was found (Ng et al., 2014). The current understanding is that a single low HCG level may not be used as a predictor of pregnancy loss and there is no discriminatory value for miscarriage or viability (Whittaker et al., 2018). However, a falling HCG is associated with a failing pregnancy (Guha et al., 2014). Yet, the accepted clinical application is limited to investigating a PUL and to the monitoring of gestational trophoblastic disease (RCOG 2010, NICE, 2019).

4.5 Ultrasonographic Features As Predictors Of PUV Outcome

4.5.1 Gestational Sac Features

The presence of the gestational sac is inherent to the basic definition of an intrauterine pregnancy of uncertain viability (Rodgers et al., 2015) (section 2.12.1). Two well-established suspicious prognostic sonographic features of the gestational sac are: a low-set position of the sac within the uterine cavity, and an irregularly shaped sac (Mazzariol et al., 2015; Nyberg, D. A. & Filly, 2003). In the current PUV cohort, there were no low-set gestational sacs, but there were three cases showing irregular sacs all of which resulted in miscarriage. From the findings of this study, it was not possible to comment about the difference between the sac size and the CRL. It has been indicated that a difference between the MSD and the CRL of less than 5mm is a bad prognostic feature (Rodgers et al., 2015). In this dataset, there were only 11 cases having a gestational sac with a visible fetal pole, all of them showing a difference of more than 5mm.

When assessing the MSD, the largest empty sac measured 20.1mm and this eventually resulted in a non-viable pregnancy. However, the largest MSD with no fetal pole, having a viable outcome was found to be 19.3mm. This result was very close to the pre-2012 recommended 20mm discriminatory value for MSD (RCOG 2006). Thus, this finding is supportive of the NICE 2012 definition changes for MSD cut-off limits from 20mm to 25mm, and reconfirmed in 2019 (NICE 2012, NICE 2019). The current data indicated that the mean value for MSD in the non-viable PUV group was 11.7mm, which exceeded the mean value for MSD measured in viable PUVs found to be 9.38mm.

However, this 1.62mm difference was not statistically significant since on applying the Independent samples t-test, the p-value computed was 0.242. This is shown in Table 4.8.

Final Diagnosis	Sample size	Mean sac diameter	Std. Deviation	P-value
Miscarriage	18	11.00	4.578	0.242
Viable	25	9.38	4.313	

Table 4.8. Independent samples t-test for mean MSD values

The growth rates of the gestational sacs from recruitment (day 0) to Day 7 were also observed. In this case, not all the 43 cases were represented for two reasons. First, if the patient miscarried before day 7 there would be no measurable sac in the miscarriage arm; secondly in the viable group the sonographer was not obliged to measure the sac since measuring the fetal pole was considered sufficient according to the study operator's manual that followed the NICE guidelines (NICE NG126, 2019).

However, when calculating the mean MSD growth rate for the viable PUVs, the rate was found to be 1.161mm per day. In contrast, the mean growth rate for MSD in the PUVs that subsequently miscarried was calculated to be 0.769mm per day. This is in agreement with the findings by Nyberg et al. (1987), who noted a 1.11mm daily growth in a normal sac. Similarly, Abdallah et al. (2011a) showed a 1mm daily growth in viable PUVs and a 5mm daily growth in a non-viable PUV case (see section 2.12.1). The findings in the PUV study were

comparable but did not reach statistical significance with a p-value of 0.159 as shown in table 4.9 and Figure 4.8. This concurs with what Abdallah et al. (2011) had pointed out in their study. The overlap in gestational sac growth rates between viable and non-viable sub-cohorts would make this factor unsafe to be applied clinically as the sole factor determining a viable pregnancy (Abdallah et al., 2011).

	Outcome	Sample Size	Mean	Std. Deviation	P-value
MSD Growth	Viable	20	1.161	.476	0.159
	Miscarriage	7	0.769	.930	

Table 4.9 Independent samples t-test for MSD growth

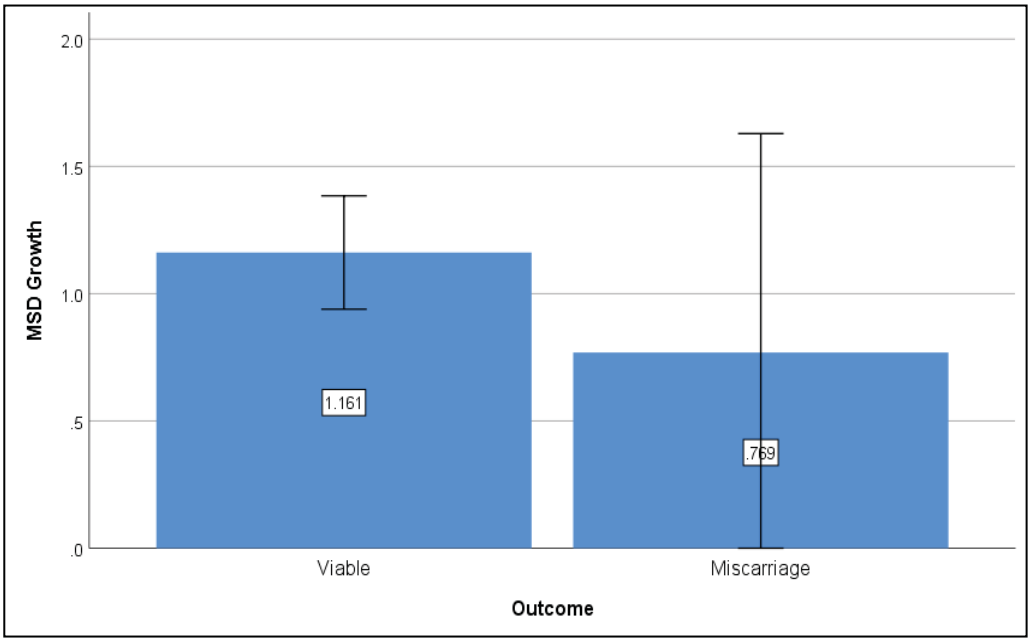

Figure 4.8 MSD growth rate in Viable versus Miscarriage

4.5.2 Fetal Pole features

The literature has described a few aspects of the fetal pole and its growth rate that are suspicious but not diagnostic of poor prognosis (Section 2.12.2). These include an absent fetal pole at 6 weeks gestation, slow growth of the fetal pole, and/or a larger CRL with no visible fetal heart (Doubilet et al., 2014). The diagnostic ultrasonographic features of the fetal pole include an absent fetal heart if the CRL exceeds 7mm and a persistent absent fetal heart after 7 days from the first appearance of a fetal pole (Huchon et al., 2016; NICE, 2019).

In this research, the PUV cohort included only 11 cases having a measurable fetal pole at recruitment. However, there were a number of interesting observations regarding individual cases. The largest CRL in this PUV dataset was 4.6mm which would have been diagnostic of miscarriage when using the criteria by Goldstein (1992). In fact, this participant subsequently miscarried. Another case showed a 4.2mm growth of the fetal pole from a CRL of 2.7mm to 6.9mm in 7 days but with a persistent absent visible heartbeat. Preisler's et al. (2015), the current French college guidelines (Huchon et al., 2016) and the Australian guidelines (Mizia et al., 2018) would all agree that this would classify as a silent miscarriage in view of an absent heartbeat after a week from the appearance of the fetal pole. However, in keeping with the Maltese local hospital practices (Consiglio & Muscat Baron, 2017) and, as stated in this PUV study operator's manual, a diagnosis of persistent PUV would be made if there is growth of the fetal pole, but it is still <7mm with no visible fetal heart (Section 3.4.2). This case too was diagnosed as a miscarriage at 14 days.

Another finding, that Riemke et al. (2010) had pointed out in their study (n=268), was that those PUV cases with no visible fetal pole had a better outcome (57% viability) than those cases where the fetal pole was visible (32.6% viability). In this PUV cohort, 11 cases had a visible fetal pole, of whom 45.5% were viable (n=5). Of the 32 cases without a visible pole, 62.5% were viable (n=20). This finding is represented in figure 4.9. Using Chi Square analysis, as well as Two populations proportion test, the p-value of 0.32 indicated no statistical significance for this factor.

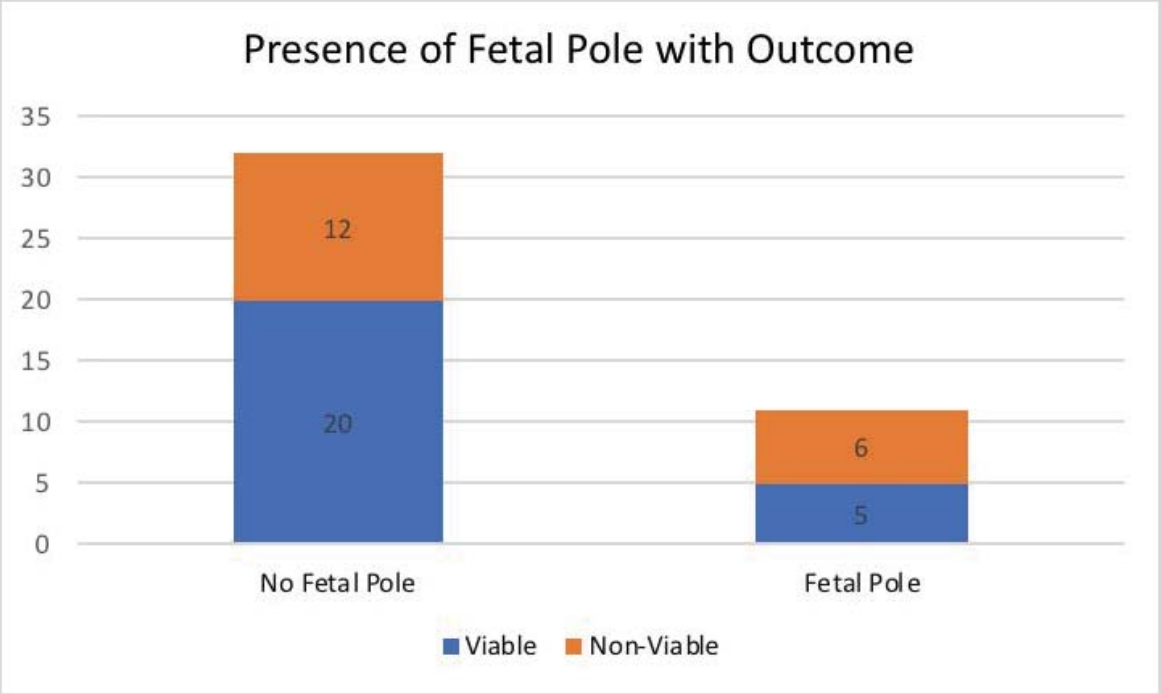

Figure 4.9. Presence of fetal pole and final outcome

4.5.3. Yolk Sac features

Various studies have outlined negative prognostic features associated with the yolk sac including the presence of hyperechoic, irregular or absent yolk sacs (Kumari et al., 2016; Tan et al. 2014). Out of the 43 participants, 27 had a visible yolk sac at recruitment. Only one patient had an abnormal (calcified) yolk sac and this resulted in a negative outcome in keeping with the review by Rodgers et al. (2015). The size of the yolk sac may also be prognostic. A yolk sac smaller than 3mm has been considered abnormal (Doubilet et al., 2014), whilst one larger than 5mm could be predictive of miscarriage (Berdahl et al., 2010).

When evaluating the yolk sac sizes with respect to the final outcome, the cases in this research were sub-grouped into three categories according to the yolk sac size (Figure 4.10), namely the abnormally small ones under 3mm, the normal 3-5mm category and the abnormally large yolk sacs of 5mm and over. Although not found to be statistically significant (p= 0.403), the viability rate was still highest (72.7%) in the 3-5mm yolk sac group (see Table 4.10).

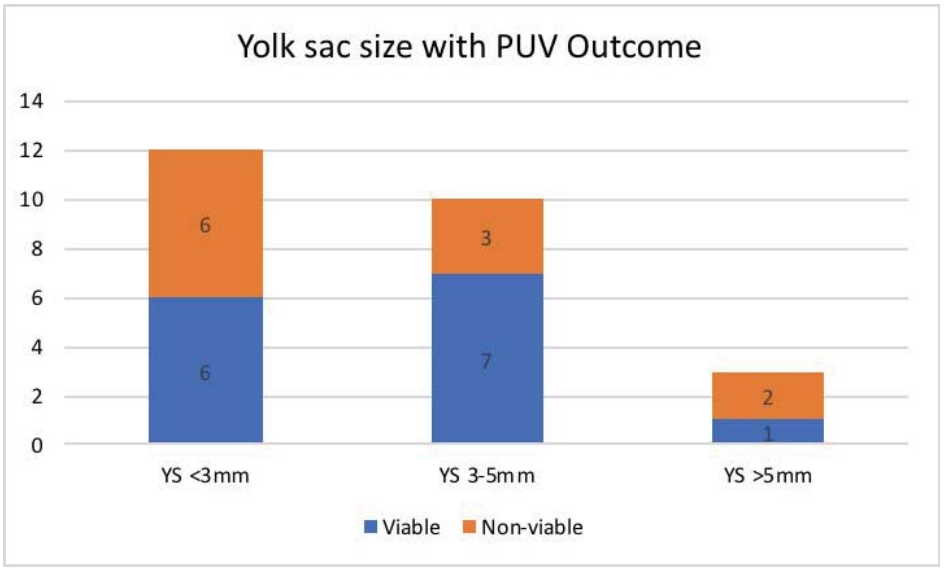

Figure 4.10 PUV outcome compared to yolk sac size categories

			Outcome		Total
			Miscarriage	Viable	
Yolk sac categories	Less than 3mm	Count	6	7	13
		Percentage	46.2%	53.8%	48.2%
	3-5mm	Count	3	8	11
		Percentage	27.3%	72.7	40.7%
	Over 5mm	Count	2	1	3
		Percentage	66.7%	33.3%	11.1%
Total		Count	11	16	27
		Percentage	100.0%	100.0%	100.0%

Table 4.10. Chi- Square test for yolk sac categories and outcome

FACTOR	PUV STUDY FINDINGS	PUV STUDY P VALUE	LITERATURE FINDINGS
NATIONALITY	Maltese 55.8%; Miscarriage rate 41.7% Non-Maltese: 44.2% Miscarriage Rate 42.1%	p=0.977 Not significant	NA
MATERNAL AGE	Mean age for patients who miscarried -31.72y Mean age for patients with Viable pregnancy 31.2years	p=0.789 Not significant	Significant difference in maternal age 3.8years – Reid et al. 2011 5.4years- Ng et al. 2014
MEAN GESTATIONAL AGE	Miscarriage:7.12 weeks Viable: 5.74weeks	**p=0.000**	Concordant with literature Reid et al. 2011, Ng et al. 2014
BLEEDING	No bleeding : 16.7% miscarried Bleeding: 66.7% miscarried	**p=0.003**	Mixed literature findings Increased risk- Ng et al. 2014 No association- Naji et al. 2010
PARITY	No previous loss: 32 % miscarried Previous loss : 60% miscarried	p=0.078 Not significant	Previous miscarriage a significant risk Reid et al. 2011 Magnus et al. 2019
MEAN PROGESTERONE	Miscarriage: 15.9nmol/l Viable: 34.26nmol/l	**p=0.000**	Concordant with literature findings Progesterone higher in viable- Ng et al. 2014 Discriminatory value 16nmol/l- Verhaegen et al 2012
MEAN HCG	Miscarriage:9323.56miU/ml Viable:10,343miU/ml	p=0.724 Not Significant	Concordant with literature findings Whittaker et al. 2019
MEAN MSD	MSD in miscarriage group: 11.7mm MSD in viable group :9.38mm	p=0.42 Not significant	Included in Predictive models Bottomley et al. 2011 Guha et al. 2013
MSD GROWTH	MSD growth rate 0.769mm/day in miscarriage 1.161mm/day in viable	0.159 Not Significant	Significant Growth difference 0.503mm/day in miscarriage 1.003mm/day in viable Abdallah et al. 2011
FETAL POLE	Sample too small	NA	NA
YOLK SAC	Viability : 3-5mm 72.7% <3mm 53.8% >5mm 33.3%	p=0.403 Not Significant	Very small or very large or calcified yolk sac poor prognosis Berdahl et al. 2010, Doubilet et al. 2014

Table 4.11 Summary of univariate analysis

4.6 Predictive Models

During the data analysis of this study, a number of predictive models, similar to those discussed in section 2.13, were explored with the support of a university statistician, aiming at devising a predictive model for PUV outcome. Clinical, biochemical and sonographic variables were selected using likelihood ratio tests to determine the optimal one.

4.6.1 Model 1: Maternal age, PBAC, MSD and Yolk Sac Diameter

The factors in this model were the variables used initially by Guha et al. (2013), a modification of the original Bottomley et al. model (2011). This consisted of maternal age, PBAC scores, MSD and Yolk sac diameters. However, the model did not perform well, with a pseudo R-square of 0.195 (Table 4.12). Hence, other models were explored.

Likelihood Ratio Tests					
	Model Fitting Criteria	Likelihood Ratio Tests			
Effect	-2 Log Likelihood of Reduced Model	Chi-Square	df	P-value	Odds ratio
MSD	32.315	0.036	1	0.850	0.974
Age	33.034	0.754	1	0.385	1.062
Yolk sac diameter	32.307	0.027	1	0.869	0.939
PBAC	35.882	3.602	1	0.058	5.010

Pseudo R-Square value = 0.195

Table 4.12 Model 1: Maternal age, PBAC, MSD and Yolk sac diameter

4.6.2 Model 2: Maternal age, Progesterone and MSD

The second model explored included three variables: maternal age, MSD, and progesterone levels entered in a Nagelkerke logistic regression model in relation to PUV outcome. On multivariate logistic regression, only Progesterone remained a significant predictor. The p-values for age and MSD were not found to be significant. As shown in Table 4.13, the pseudo R-Squared value in this case was 0.530. This means that this 3-point predictor model explains 53% of the total variation in the final diagnosis. This indicates that other factors could affect the patient diagnosis apart from the ones chosen for this model. However, this model raised other valid considerations. The Odds Ratio for Progesterone (0.865) indicated that for each unit increase in progesterone, the odds of miscarriage decreased by 14.4%. Similarly, for each millimetre increase in MSD, the odds of a miscarriage increased by 16.4%. In addition, there was evidence of a small, non-significant, 0.5% increase in miscarriage for each year increase in maternal age.

Likelihood Ratio Tests					
	Model Fitting Criteria	Likelihood Ratio Tests			
Effect	-2 Log Likelihood of Reduced Model	Chi-Square	df	P-value	Odds ratio
MSD	39.543	2.604	1	0.107	1.164
Maternal Age	36.944	0.005	1	0.941	1.005
Progesterone	56.865	19.926	1	**0.000**	0.856

Pseudo R-Square value = 0.530

Table 4.13 Model 2: Maternal Age, Progesterone and MSD

4.6.2 Model 3: Bleeding, Progesterone and MSD

This final model considered bleeding, progesterone levels and MSD. In this case, bleeding was taken as a two-tier variable: absent or present as opposed to the PBAC quantification score. This method was previously used by Elson et al., (2003) and by Ng et al. (2014) in similar PUV prediction studies. As is often the case in multivariate analysis, variables may correlate to each other as well as to the outcome itself. In such cases, the researcher has to exclude or combine variables in order to find the best performing model (Bottomley et al., 2011).

Likelihood Ratio Tests

Effect	Model Fitting Criteria	Likelihood Ratio Tests			
	-2 Log Likelihood of Reduced Model	Chi-Square	df	P-value	Odds ratio
MSD	32.376	1.310	1	0.252	1.131
Progesterone	44.109	13.044	1	**0.000**	0.867
Bleeding	36.944	5.879	1	**0.015**	8.329

Pseudo R-Square value = 0.634

Table 4.14 Mode l 3: Bleeding- Progesterone-MSD

This Bleeding, Progesterone, MSD model explained 63.4% of the total variation in the final diagnosis of this dataset, (shown in Table 4.15). The model identified 2 significant predictors.

Progesterone was the best significant predictor with the lowest p-value of 0.000. The presence of bleeding also remained a significant factor with a p-value of 0.015. The MSD, however, was not found to be statistically significant with a p-value of 0.252. Utilizing this model, the odds ratios indicate that for every 1 mm increase in the MSD, the odds for miscarriage increased by 13.1%. Similarly, for every 1 unit (mmol/l) increase of progesterone, the odds of a PUV case leading to miscarriage decreased by 13.3%. However, in the presence of bleeding, the odds of a miscarriage in this PUV cohort was 8.329 times higher than in the absence of bleeding.

Parameter Estimates

Final Diagnosis		Parameter Estimates	Std. Error	Wald	df	P-value
Miscarriage	Intercept	1.046	1.713	.373	1	.541
	MSD	.123	.110	1.246	1	.264
	Progesterone	-.143	.051	7.930	1	.005
	Bleeding	2.120	.924	5.267	1	.022
	No bleeding	0	.	.	0	.

The reference category is: Viable.

Table 4.15 Model 3: Parameter Estimates

The model devised using the three parameters of Bleeding, Progesterone and MSD would be:

Log_e (P/ 1-P) = 1.046+ 0.123MSD – 0.143progesterone+ 2.12Bleeding

Where P= miscarriage

1-P = No miscarriage

Bleeding =1 in the presence of bleeding

Bleeding= 0 if the patient has no bleeding

The Model 3 prediction results for the whole dataset are found in Appendix F. When applying this predictive model to this PUV cohort, the number of correct diagnoses was 81.4% (35/43); whilst the number of mismatches was 18.6% (8/43). The sensitivity was found to be 72.2% (13/18) and the specificity was 88% (22/25) (Table 4.16). Moreover, the area under the Receiver Operating Characteristic (ROC) curve (sensitivity to 1- specificity) was 0.801; (Figure 4.11). In addition, the p-value was 0.001, (< 0.05 level of significance), implying that the model was predicting outcome not attributable to chance.

This model has demonstrated the best performance of the three models explored. Nevertheless, like other prediction models, it does not offer a foolproof diagnosis. Moreover, the diagnosis and management of PUV revolves around the need of a 0% false positive diagnosis of miscarriage (ACOG, 2018; Mizia et al., 2018; NICE 2019). However, this model may guide the clinician in offering an individual risk assessment for patients presenting with PUV.

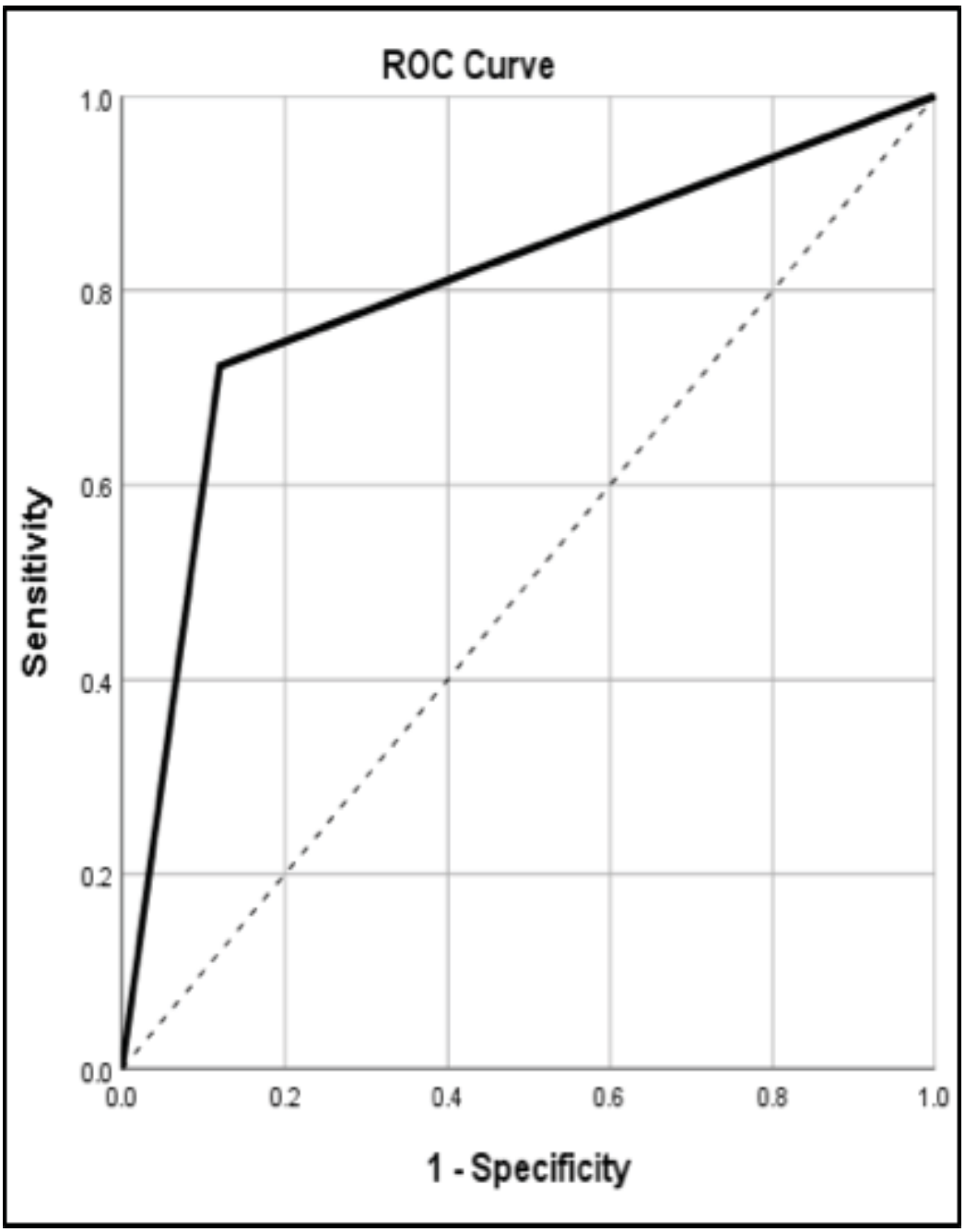

Figure 4.11 ROC Curve for Bleeding-Progesterone-MSD model

		Final Diagnosis		
		Miscarriage	Viable	Total
Predicted Diagnosis	Miscarriage	13	3	16
	Viable	5	22	27
Total		18	25	43

Table 4.16 Model 3: Sensitivity and Specificity

4.7 Ultrasound Timing Strategy

Despite the recent 2019 update to the early pregnancy guidelines by NICE, there is still no clear agreement on the timing strategy to be adopted for a definite diagnosis of PUV. NICE (2019) stated:

> "The literature suggests that there is no clear consensus, but there is general agreement that by 14 days a diagnosis will be clear. To establish the most appropriate time for scans, the efficacy of scans taken after 14 days could be compared with scans taken after 7 days for diagnosis of ectopic pregnancy or viability." (NICE NG126, 2019, Research Recommendation 2).

Following NICE (2019) recommendations, this PUV study compared ultrasound findings at 7 and 14 days, with the objective of possibly finding an optimal timing strategy.

4.7.1 Comparing 7-day to 14-day ultrasound findings

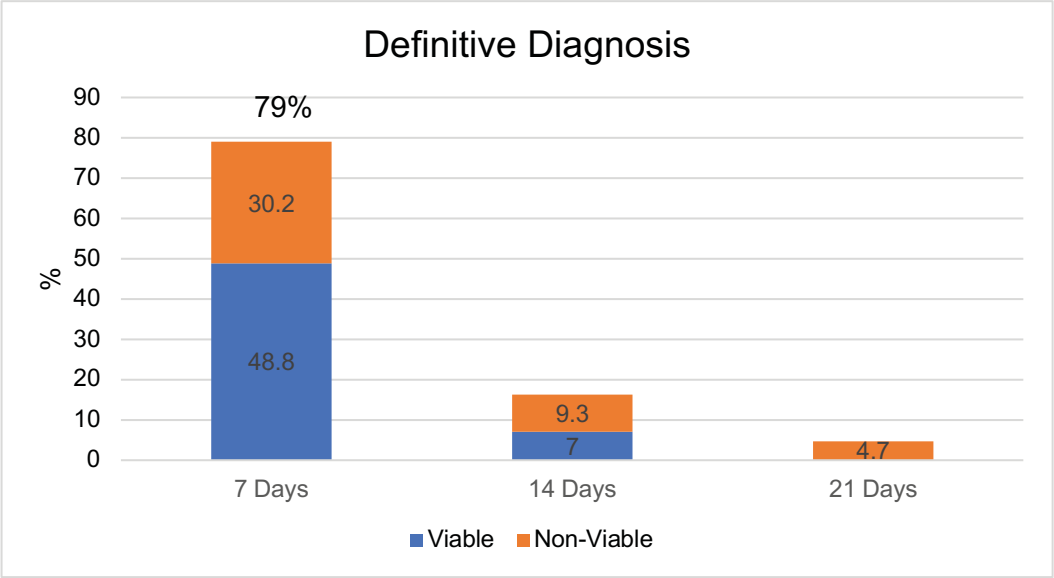

Figure 4.12 Definitive diagnosis stratified by follow-up visits

The results of the PUV study indicated the following findings (Figure 4. 12)

- By 7 days 79% of patients received a definitive ultrasound-based diagnosis (34/43)
- By 14 days, a further 16.3% of patients received a definitive ultrasound-based diagnosed (7/43)
- By 21 days a definitive diagnosis was given to a further 4.7% of cases (2/43).

In this PUV cohort, 79% of cases could be referred to an antenatal booking visit or directed toward the miscarriage management pathway.

4.7.2 Follow-up stratification based on gestational age

NICE (2019), recommends early pregnancy scanning after 6 completed weeks of gestation to reduce the overdiagnosis of PUV and PUL with consequent unnecessary patient recalls (NICE, 2019). One may infer that a

patient presenting with PUV at an earlier gestational age, may need a longer wait for a definite diagnosis to be made. Following the NICE guidelines (2019), PUVs could be stratified by gestational age such that pregnancies under 6 weeks at first encounter would be given a longer follow- up appointment.

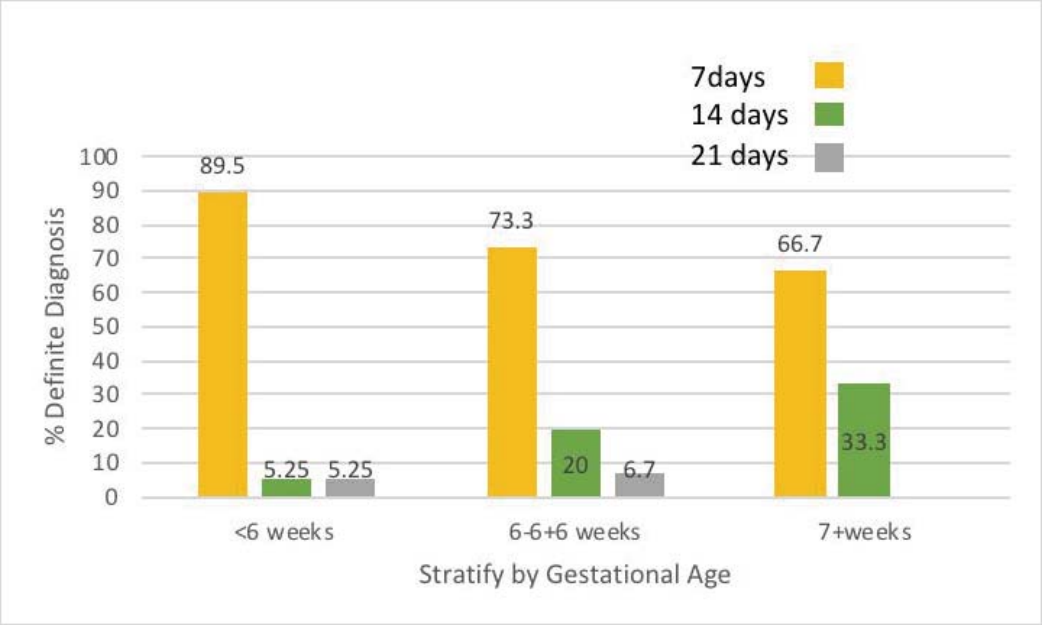

Figure 4.13 Definite outcome stratified by gestational age

However, when stratifying the cohort by gestational age, a definite diagnosis was given by 7 days (Figure 4.13):

- In 89% of cases (17/19), at a gestation up to 5 weeks+6 days
- In 73.3% of cases (7/11), at gestation up to 6 weeks +6 days
- In 66.7% of cases(6/9), at gestation over 7 weeks

The observations for this cohort show that there is no clear advantage of stratifying the follow-up by gestational age. In fact, the results indicated that 89% of PUV patients who presented early, prior to 6 completed weeks (5+6), received a definite final diagnosis by 7days.

4.7.3 Follow-up stratification by MSD

A study by Preisler et al. (2015), whose recommendations were adopted by the ASUM (Mizia et al., 2018), recommended that if the MSD measured <12mm on initial scan, a 14-day follow-up should be performed as opposed to a 7-day review in the case of an MSD >12mm (Mizia et al., 2018; Preisler et al., 2015).

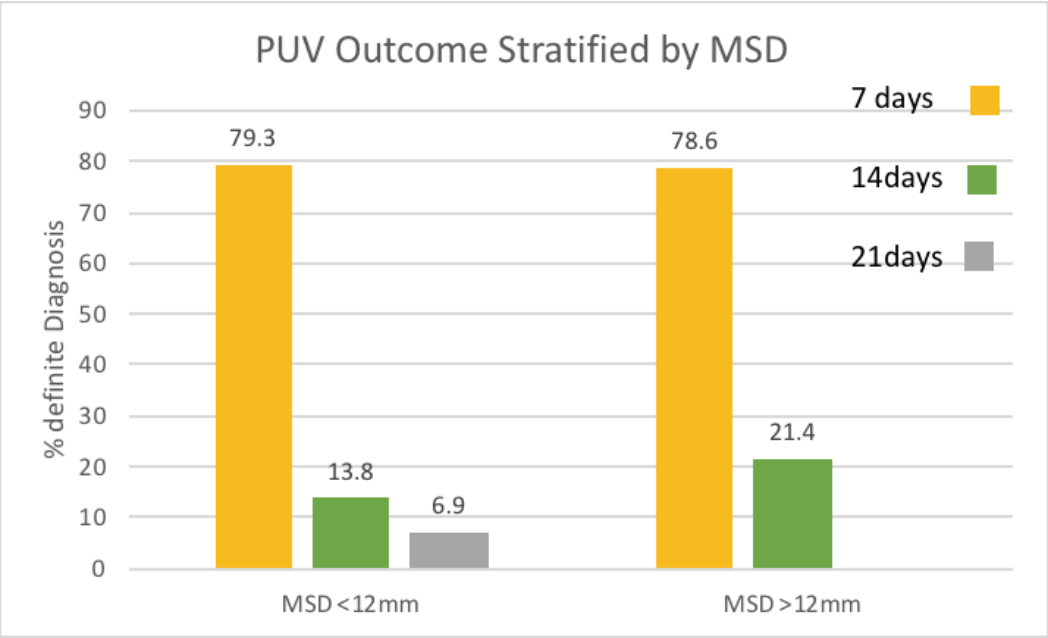

Figure 4.14 Definite Outcome Stratified by MSD

These recommendations aimed at reducing review ultrasound scans. However, when this PUV cohort was stratified using the 12mm cut-off point for MSD, as recommended by Preisler et al., (2015) (Figure 4.14):

- 79.3% of PUV with MSD <12mm had a definite diagnosis by 7 days
- 78.6% of PUV with MSD >12mm had a definite diagnosis by 7 days.

The results showed that the proportion of cases diagnosed by 7 days, irrespective of MSD, was similar to the 79% overall diagnosis for the unstratified cohort. Hence, it may be deduced that, in this PUV cohort, there was no clear advantage in stratifying the follow-up of patients according to MSD as recommended by the ASUM (Mizia et al.2018).

4.7.4 Considering stratification based on first ultrasound findings

The Collège National des Gynécologues Obstétriciens Français (CNGOF, French College of Gynaecologists and Obstetricians) (Huchon et al., 2016), although graded as Grade C (low level) evidence, recommended to stratify follow-up of patients according to what gestational structures were seen on the initial ultrasound. Therefore, in case of an empty gestation sac a 14-day review was recommended. In case of a gestation sac with a visible yolk sac, an 11-day review was suggested; whereas if a fetal pole was visible in the first scan, a 7-day interval was recommended. This was not adopted by ACOG (2018) or NICE (2019). Stratifying the PUV dataset in this manner, showed minimal difference in the obtaining definite results by 7 days as shown in Figure 4.15.

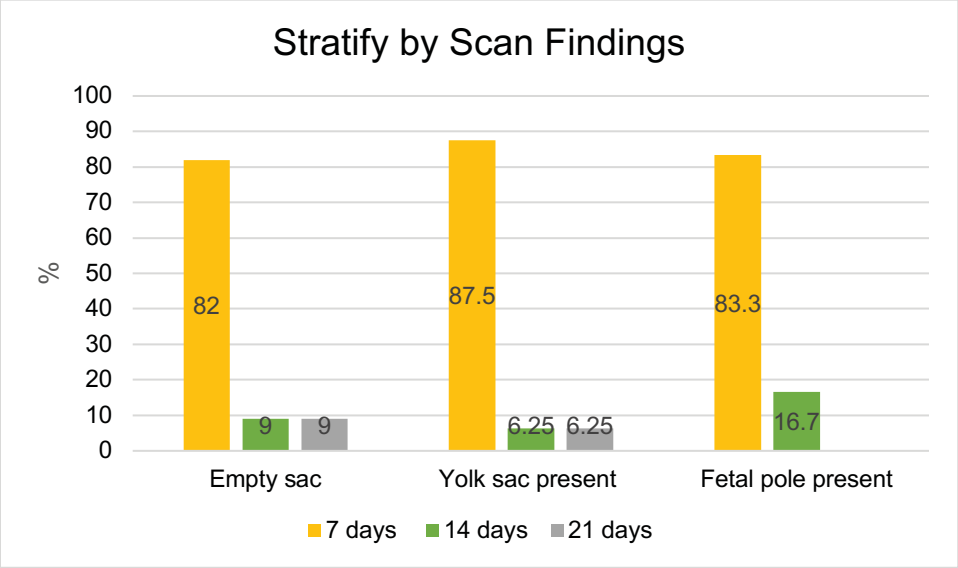

Figure 4.15 Outcomes stratified by scan findings

- 82% of PUV having an empty sac receive a definite diagnosis by 7 days.
- 87.5% of PUV having a visible yolk sac receive a definite diagnosis by 7 days.
- 83.8% of PUV having a visible fetal pole receive a definite diagnosis by 7 days.

Comparing these results to the overall 79% definite diagnosis, one may conclude that stratifying the review strategy by visible ultrasound features confers no advantage.

4.7.5 Considering stratification by prognostic factors

Some studies have recommended using their predictive models to stratify the follow-up care with the aim of reducing visits and cost. Bottomley et al. (2011) and Guha et al. (2013), devised predictive models and scoring systems for PUV outcome. Both studies suggested that those patients who were predicted to have a positive outcome could be seen at a longer time interval (dating scan 11-13 weeks). On the other hand, those women who were scored as having a high risk for pregnancy loss would be reviewed early in 7 to 14 days. However, as NICE (2019) pointed out in its research recommendations, the follow-up strategy was still vaguely described as 7 to 14 days with no evidence to back it. In the PUV study, considering the recommended model offers a 72.2% sensitivity and an 88% specificity, one may opt to suggest a similar stratification system. However, the 7-day review has been shown to provide a definite diagnosis in 79% of the current dataset, conferring little advantage in stratifying by using predictors.

4.8 Strengths And Weaknesses Of The PUV Study

4.8.1 Sample size

The small sample size of the PUV study constituted a weakness. As explained by Hulley et al., (2013), due to this, the results cannot be generalised to the whole target population, but are rather restricted to the cohort of participants. Attempts to reduce this problem included immediate commencement of recruitment of participants as soon as ethical approval was given.

4.8.2 Selection Bias

Another possible limitation of the study was selection bias due to convenience recruitment and the over-representation of non-Maltese nationals in the cohort could suggest this. The study could not explain the reasons for this (Section 4.3.1). From the researcher's experience it reflect the impact of private health care or later presentation. However, since PUV is an interim ultrasound diagnosis, the PUV population is a clinical one. Hence, research data is intended to be applicable only to other hospital/clinic attenders and not to pregnant women in the community (Hulley et al. 2013).

4.8.3 Confounding factors

Confounding factors are known to be a challenge in observational studies (Aronson et al. 2018). Due to constraints in the PUV study it was not possible to correct for smoking and body mass index and any other unknown confounders. This has been a recognized limitation even in a study conducted

by Puget et al. (2018). However, the viable PUVs may be considered as internal controls for this cohort study as suggested by Hulley et al. (2013).

4.9 Conclusion

This chapter focussed on a discussion of the study results drawing comparison to the literature review findings. The next chapter will draw the clinical conclusions derived from the results obtained, indicating how the set aims were reached. Recommendations for further research are also made based on the outcome of this study and on the literature reviewed.

CHAPTER 5:

CONCLUSIONS AND RECOMMENDATIONS

5.1 Introduction

The aim of this chapter is to present the conclusions that have been drawn from the PUV study, subdivided into the predictive (section 5.2) and strategic (section 5.3) aims as designed at the outset of the study and described in Section 1.5 The researcher makes recommendations for clinical practice in section 5.5, followed by suggestions for future research in section 5.6.

5.2 Conclusions From The Predictive Aim Of The PUV Study

The first aim of the study was to investigate the possibility of predicting PUV outcome using clinical, biochemical or ultrasonographic markers. Summarizing the predictive findings, the univariate analysis suggests that there are three strongly significant factors that are predictive of miscarriage in PUV. All three of them concur with the current literature available.

- Miscarriage rate was higher in the presence of bleeding. The rate increased from 16.7% to 66%, p=0.003;
- PUVs presenting at a later gestational age are more likely to miscarry, p= 0.000;
- Low progesterone level (<16mmol/l) is highly suggestive of miscarriage, p=0.000.

Other factors were indicative but did not reach statistical significance.

- A history of previous miscarriage increases the risk of miscarriage from 32% to 60%, but was not statistically significant, p=0.078;
- The association of increased MSD with increased risk of miscarriage, p=0.42;

- The MSD daily growth rate was greater in viable pregnancies (1.161mm/day) than in those that miscarried (0.769mm/day). This did not reach statistical significance, p=0.159;
- The association of abnormal yolk sac size with an increased risk of miscarriage, p= 0.403.

This PUV study also developed a predictive model using Bleeding, Progesterone and MSD. This showed that:

- Each 1mm increase in MSD increased the miscarriage risk by 13.1%;
- Each 1mmol/ml rise in Progesterone decreased the miscarriage risk by 13.3%;
- Bleeding caused an 8-fold increase in the risk of miscarriage.

The findings of the predictive aspect of the study have corroborated what the literature has previously shown, in terms of the prognostic factors for PUV (Section 2.9-2.13). No new discriminatory values could be put forward to predict miscarriage with certainty. Even when applying the predictive model to the data, one cannot give a definitive diagnosis, since there is a sensitivity rate of 72.2% and a specificity rate of 88%. This means that there will always be a false positive and a false negative component to the model prediction. The absolute need of certainty in the diagnosis of miscarriage supersedes any desire or need for an early diagnosis. The Hippocratic oath “first do not harm” is to be respected at all times (Bourne & Bottomley, 2012; Hately et al., 1995).

However, the prognostic findings, may be useful in guiding the clinician to exercise more caution or to be more reassuring in his/her approach when advising women diagnosed with a pregnancy of uncertain viability. This is the same conclusion that other similar predictive studies have drawn using their predictive models (Bottomley et al., 2011; Guha et al., 2012).

5.3 Conclusions Drawn From The Strategic Aim

The second aim of the study was to explore the best ultrasound timing strategy for PUV patients in Malta. This was done by comparing ultrasound findings at 7 and 14 days as recommended by the NICE research recommendations (NICE , 2019).

The timing stratification aspect of the PUV study can be summarized as follows:

- 79% of the whole cohort received a definitive diagnosis in 7 days;
- 95.3% of the whole PUV cohort received a definitive diagnosis by 14 days;
- 89% of the PUV cohort presenting before 6 completed weeks received a definitive diagnosis by 7 days;
- 79.3% of the PUV cohort with an MSD under 12mm received a definitive diagnosis by 7 days;
- 82% of PUVs with a visible empty sac received a definite diagnosis by 7days.

The study has fulfilled its aim in comparing the ultrasound findings at 7 and 14 days. The results showed that, by 7 days, nearly 80% of the women had a definitive diagnosis without the need to stratify them by gestational age, sac size, scan features or a predictive model.

5.3.1 Impact of a 7-day strategy

A 7-day review strategy imparted a definitive diagnosis to 79% of PUV cases. This means that 48.8% of the whole cohort (n=21) were reassured of a viable pregnancy and were referred straight to antenatal booking. A further 30.2% (n=13) were given a diagnosis of miscarriage and were directed into their management pathways. This 7-day timing strategy had the advantage of faster reassurance, and the avoidance of an unnecessary wait for 79% of the cohort. In addition, this approach had the potential of reducing emergency visits, although quantifying this was not the purpose of the study. Only 16.3% of the whole cohort had an inconclusive result on day 7 and were recalled a week later.

5.3.2 Impact of a 14-day Strategy

An alternative approach would be to omit the 7-day review altogether and recommend a 14-day strategy, to maximize the diagnostic potential to over 95%. It has the advantage of reducing the time and cost of a visit and an ultrasound scan in 16.3% of cases. This could be considered advantageous to the pragmatic health service provider.

However, based on the results of this study, a 14-day ultrasound strategy would result in an unnecessary and inconvenient extra 7-day wait for the 79% of patients who could have been diagnosed earlier (at 7 days), as per section 5.3.1. This unnecessary wait may result in a negative psychological impact on patients. As discussed in Section 2.6.2, the uncertainty of PUV has been shown to cause increased anxiety and stress scores as compared to a certain diagnosis, whether it is a positive or a negative one (Richardson et al.,

2015). Another concern in adopting a 14-day strategy is the potential for emergency admissions in the interim period. Whilst an expectant management of silent miscarriage is considered a safe option, it has been found to increase emergency visits and emergency surgical evacuations with potential morbidity to patients including blood transfusions (Trinder et al., 2006). The current PUV study results do not look into quantifying this latter scenario.

5.4 Overall Conclusions

The purpose of the PUV study was to optimize the care of patients diagnosed with pregnancy of uncertain viability. Both the predictive and the ultrasound timing aspects of the study were designed to ultimately attempt to recommend the ideal management strategy for PUV in Malta.

It is the researcher's opinion that any strategy should harmonise three factors.

- The first and non-negotiable one is an accurate diagnosis i.e. having a zero tolerance to false positive diagnosis of miscarriage (Bourne, 2016).
- The second reconciles the impact that a chosen strategy may have on patients, in terms of inconvenience, morbidity and the anxiety that couples in this situation are known to face (Richardson et al., 2017).
- The third considers the workload and financial impact on the health service (NICE 2019).

The study concludes that a 7-day review strategy offers an accurate diagnosis in a very high percentage of cases (79%), leading to a shorter wait for patients. A comparatively low percentage of patients, 16.3%, as discussed above,

require recall due to inconclusive results. Hence, it is the researcher's opinion that a 7-day review adequately fulfills the aforementioned three criteria for a good strategy. The role of prognostic factors does not improve on the 7-day strategy; however, the clinician may use this model to individualise the prognosis for PUV patients who require it.

5.5 Clinical Recommendations

Thus, the researcher recommends that in cases of PUV:

- A 7-day follow-up is offered, explaining that the chance of having a definite diagnosis is very high. A 14-day option is offered, if the patient so prefers, with an explanation of how and when to self-refer in case of increased bleeding.
- To offer the use of a prognostic tool that involves a blood test (progesterone) to be able to offer an individualized prognosis as indicated by the results.

The researcher suggests it is an opportune time to set up an Early Pregnancy Unit in the local state hospital to manage the various complications of early pregnancy.This could improve clinical services whilst providing ample opportunity for ongoing auditable research relating to ultrasound findings and management outcomes.

5.6 Recommendations For Future Research

Further research is recommended in the area of PUV and early pregnancy complications.

5.6.1 PUV study follow-up

The researcher recommends that the current PUV study could be replicated on a larger scale, over an 18-month period, to improve its generalisability and external validity. It would be useful to assess if the significant associations detected would be reproduced, and if the ones that did not reach statistical significance would show clearer results in a larger cohort. One could apply the prediction model prospectively to a new cohort of participants, and hence validate it. Moreover, one could investigate the acceptability of prediction testing to the local population.

5.6.2 Auditing the local emergency service users

An interesting aspect that emerged from the study was the proportion of non-Maltese nationals in the cohort. The researcher recommends that an audit be carried out to assess the local emergency service users and, whether their needs and outcomes vary depending on their nationality with a view to improve health care services.

5.6.3 Psychological impact of ultrasound results on patients

An area which has raised considerable interest in the last 5 years has been the psychological impact of early pregnancy complications on patients (Farren et al., 2018; Richardson et al. 2017). The researcher proposes to compare

mental health outcomes over time, following the different ultrasound diagnoses given to patients: viable pregnancy, PUV, PUL and miscarriage.

5.6.4 Ongoing early pregnancy research in a dedicated clinical unit

A research lacuna that exists in the field of early pregnancy is the knowledge of the local incidence of early pregnancy complications (including PUV), and their outcomes.An Early Pregnancy Assessment unit could provide such ongoing data in the main state general hospital that caters for a vast proportion of the population of Malta.

5.7 Conclusion

The PUV study has addressed the two main aims it set out to analyse as introduced in section 1.5. The study has demonstrated the significance of prognostic factors in the predicition of miscarriage: presentation at a later gestational age, the presence of bleeding and a low progesterone level. A prediction model has been devised that may help the clinician to offer patients an individual risk assessment. The second aim of the study was to explore an optimal ultrasound review strategy. Based on the study results, the researcher recommends a 7-day review strategy for the management of PUV in Malta.

The word count of this study is approximately 27000.

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APPENDIX A

Ethical Approval Forms

Dr. Helga Consiglio MD FRCOG
Resident Specialist in Obstetrics and Gynaecology
Department of Obstetrics and Gynaecology
Mater Dei Hospital
15th May 2018

Prof. Yves Muscat Baron MD FRCOG FRCPI PhD
Head of Department
Department of Obstetrics and Gynaecology

Dear Prof. Muscat Baron,

I am currently reading for a Masters degree in Radiography-Ultrasound Abdomen and Pelvis (Course Intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patients' standard management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way.
I have obtained consent from the Consultants in charge of the patients and other stakeholders including the Midwifery officer, the sonographers and intermediaries involved in order to carry out my study. Consent has also been sought from the CEO and Data Protection Officer to access the required patient files and isoft data.
In this regard, I kindly ask your permission to be able to conduct my study as indicated above.

Regards

Dr. Helga Consiglio MD FRCOG
Reg 2480
Resident Specialist
Msc Student
[redacted]

Mrs. Karen Borg Grima
MSc (Nuclear Medicine)
Assistant Lecturer- FHS
Supervisor
[redacted]

CONSULTANT OBSTETRICIANS AND GYNAECOLOGISTS

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

MATER DEI HOSPITAL

MALTA

I , the undersigned Consultant within the Department of Obstetrics and Gynaecology at Mater Dei Hospital, confirm that I am aware that Dr. Helga Consiglio, Obstetrician and Gynaecologist is currently reading for a Masters degree in radiography and is investigating Pregnancy of Uncertain Viability for her dissertation. I have no objection to patients who are admitted under my care being included in this project and that their data be collected in accordance to standard hospital data protection practices.

Yours truly

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Dr. Helga Consiglio MD FRCOG
Resident Specialist in Obstetrics and Gynaecology
Department of Obstetrics and Gynaecology
Mater Dei Hospital
15th May 2018

Ms Astrid Zarb
Midwifery Officer
Obstetric Ward 2
Department of Obstetrics and Gynaecology
Mater Dei Hospital

Dear Ms Zarb,
I am a qualified full time Resident Specialist in Obstetrics and Gynaecology, currently reading for a Masters degree in Radiography-Ultrasound Abdomen and Pelvis (Course Intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patients' usual management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way.
I have obtained consent from the Head Of Department, Consultants in charge of the patients and other stakeholders, the sonographers and intermediaries involved in order to carry out my study. Consent has also been sought from the CEO and Data Protection Officer in order to access the required patient files and isoft data.
I would appreciate if you would endorse this project that could be of benefit to the local Pregnancy services.

Regards

Dr. Helga Consiglio MD FRCOG
Reg 2480
Resident Specialist
Msc Student
[redacted]

Mrs. Karen Borg Grima
MSc (Nuclear Medicine)
Assistant Lecturer FHS
Supervisor
[redacted]

I endorse this project

Dr. Helga Consiglio MD FRCOG
Resident Specialist in Obstetrics and Gynaecology
Department of Obstetrics and Gynaecology
Mater Dei Hospital
15th May 2018

Dear Sonographer,

I am Dr. Helga Consiglio, a Resident Specialist in Obstetrics and Gynaecology, and I am currently reading for a Masters degree in Radiography- Ultrasound Abdomen and Pelvis (Course Intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patients' management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way.
I have obtained consent from the Head Of Department, Consultants in charge of the patients and other stakeholders and intermediaries involved in order to carry out my study. Consent has also been sought from the CEO and the Data Protection Officer to access the required patient files and isoft data.

In this regard, I would appreciate if you would participate in this project by offering your scanning expertise. The study could be of benefit to the local Early pregnancy service provision.

Regards

Dr. Helga Consiglio MD FRCOG
Reg 2480
Resident Specialist
Msc Student
[redacted]

Mrs. Karen Borg Grima
MSc (Nuclear Medicine)
Assistant Lecturer-FHS
Supervisor
[redacted]

I endorse the about

Marygrose Vella
Senior Allied Health Professional
(Radiography)

I accept

Karen Borg Grima
Allied Health Professional

Dr. Helga Consiglio MD FRCOG
Resident Specialist in Obstetrics and Gynaecology
Department of Obstetrics and Gynaecology
Mater Dei Hospital
15th May 2018

Dear Sir/Madam

I am Dr. Helga Consiglio, a Resident Specialist in Obstetrics and Gynaecology and I am currently reading for a Masters degree in Radiography-Ultrasound Abdomen and Pelvis (Course Intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patient's management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way.
I have obtained consent from the Head Of Department, Consultants in charge of the patients and other stakeholders involved. Consent has also been sought from the Data Protection Officer to access the required patient files and isoft data.

I would appreciate if you would endorse this project by acting as an intermediary to invite or consent from patients involved in this study that can be of benefit to the local our Pregnancy service provision.

Regards

Dr. Helga Consiglio MD FRCOG
Reg 2480
Resident Specialist
Msc Student
[redacted]

Mrs. Karen Borg Grima
MSc (Nuclear Medicine)
Assistant Lecturer- FHS
Supervisor
[redacted]

Dr. Helga Consiglio MD FRCOG
Resident Specialist in Obstetrics and Gynaecology
Department of Obstetrics and Gynaecology
Mater Dei Hospital
15th May 2018

Dr.Chris Barbara
Chairman of Department of Pathology
Mater Dei Hospital

Dear Dr. Barbara,
I am Helga Consiglio, a full time Resident Specialist Obstetrician and Gynaecologist, at Mater Dei Hospital. I am currently for reading a Masters degree in Radiography- Ultrasound Abdomen and Pelvis (Course intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patients' management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way.
I have obtained consent from the Consultants in charge of the patients, the Midwifery Officer, intermediaries as well as from the Head of the Department of Obstetrics and Gynaecology, Prof. Y. Muscat Baron, in order to carry out my study.
In this regard, I kindly request your permission to request a CBC, HCG and Progesterone at the first encounter. Consent has also been sought from the CEO and the Data Protection Officer to access the isoft system and case notes to complete the data collection.
Should you require further clarification regarding my study, kindly contact me.

Regards

Dr. Helga Consiglio MD FRCOG
Reg 2480
Resident Specialist
Msc Student
[redacted]

Mrs. Karen Borg Grima
MSc (Nuclear Medicine)
Assistant Lecturer- FHS
Supervisor
[redacted]

Dr. Helga Consiglio MD FRCOG
Resident Specialist in Obstetrics and Gynaecology
Department of Obstetrics and Gynaecology
Mater Dei Hospital
15th May 2018.

Dr Ethel Felice
Consultant Psychiatrist
Lead of Perinatal Mental Health Services
Mater Dei Hospital

Dear Dr. Felice,

I am Dr Helga Consiglio, a full time Resident Specialist in Obstetrics and Gynaecology and I am currently reading for a Masters degree in Radiography-Ultrasound Abdomen and Pelvis (Course Intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patients' usual management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way.

I have obtained permission from the Head of Department, Prof. Muscat Baron, the Obstetric Consultants to carry out the study together with other stakeholders including the Midwifery officer, the sonographers and intermediaries involved. Consent from the CEO and the Data Protection Officer to access the required patient files and isoft data has also been sought.

There are no foreseeable risks to patients being involved in this project. However, in the unlikely event that a subject may experience anxiety as a result of her participation, I would like to ask for permission to refer such a patient to your services should the need arise . Patients will be informed of this at the time of consent.

I would appreciate if you would endorse this study by offering your expertise should the need arise.

Regards

Dr. Ethel Felice
M.D., B.C. Psych (Lond.),
M.Phil. (Cantab.)
Sen.(Perinatal) Mental Health Glasgo
Consultant Psychiatrist
1735

Dr. Helga Consiglio MD FRCOG
Reg 2480
Resident Specialist
M.Sc. Student

Mrs. Karen Borg Grima
MSc (Nuclear Medicine)
Assistant Lecturer- FHS
Supervisor

From: **Helga Consiglio**
Subject: Fwd: Data Protection Permission to access patient's Soft data for a Master's Study
Date: 21 May 2018 at 21:59
To:

---------- Forwarded message ----------
From: **Data Protection at MDH** <datapro.mdh>
Date: 21 May 2018 at 11:19
Subject: RE: Data Protection Permission to access patient's Soft data for a Master's Study
To: "helga.consiglio"
Cc: Aquilina Graziella at Health-MDH Buhagiar Nadine at Health-MDH

Dear Dr Consiglio

On the basis of the documentation you submitted, from the MDH data protection point of view you have been cleared to proceed with your study provided that you obtain approval from MDH CEO and the University Ethics Committee.

Please contact Ms. Nadine Buhagiar on or Ms. Graziella Aquilina on to present a copy of your approvals and fill in the appropriate Data Protection Form.

Remember that in no way should you retain any personal details you obtain from your research and this should be destroyed at the end of your study.

All medical records are to be viewed at the Medical Records Department MDH.

You are requested to submit a copy of your findings to this office at the end of your study.

Regards

Sharon Young

Data Protection Officer

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Mater Dei Hospital, | Tel | www.mdh.gov.mt

Think before you print.

From: **Helga Consiglio**
Sent: 21 May 2018 10:38 AM
To: Data Protection at MDH
Subject: Data Protection Permission to access patient's Soft data for a Master's Study

Dear Ms Young,

I am Dr. Helga Consiglio, a Resident Specialist in Obstetrics and Gynaecology and I am currently reading for a Masters degree in Radiography-Ultrasound Abdomen and Pelvis (Course Intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patients' usual management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way. No vulnerable or underage groups will be involved in the study.

I have obtained consent from Prof. Y. Muscat Baron, Head of Department of Obstetrics and Gynaecology, the Consultants in charge of the patients and other stakeholders including the Midwifery officer, the sonographers and intermediaries involved, in order to carry out my study as indicated above. Consent has also been sought from Mr Ivan Falzon, Chief Executive Officer at Mater Dei Hospital in order to access the patients' clinical files.

In this regard, I would appreciate if you could give me your permission to access the patients' I soft data in order to be able to collect the information required for my research. Please see attached documents. Should you require any further clarifications regarding my research please do not hesitate to contact me. The supervisor of my research is Ms Karen Borg-Grima, Assistant Lecturer within the Faculty of Health Sciences, Radiography department.

From: **Falzon Ivan at Health-MDH**
Subject: RE: Request for permission to carry out Master's Research Project
Date: 22 May 2018 at 16:21
To: Helga Consiglio
Cc:

Proceed in line with established policies regulating such activities.

Ivan Falzon
Chief Executive Officer | TeaMDH

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Mater Dei Hospital, | Tel | www.careandcure.gov.mt

From: Helga Consiglio
Sent: 21 May 2018 22:23
To: Falzon Ivan at Health-MDH
Cc:
Subject: Request for permission to carry out Master's Research Project

Dear Mr. Falzon,

I am Helga Consiglio, a full time Resident Specialist Obstetrician and Gynaecologist, at Mater Dei Hospital. I am currently for reading a Masters degree in Radiography- Ultrasound Abdomen and Pelvis (Course intake 2016). As part of my Degree I am preparing my dissertation for which I will be investigating problems in early pregnancy. The proposed title of my dissertation is :

Pregnancy of Uncertain Viability: can the outcome be predicted?

This involves an observational study which will include collecting clinical, biochemical and ultrasonic data that are part of the patients' standard management and hence will not impact their care pathway in any way. Patients will be invited to participate through an intermediary person and consented should they choose to participate in the study. It will be clearly explained to the patients that all information collected will be anonymised during the data processing and data will be handled in a strictly confidential manner, available only to the researcher. Participants will be reassured in the information letter that they can leave the study at any point, and, that should they choose to do so it will not impact their care in any way.
I have obtained consent from the Consultants in charge of the patients, the Midwifery Officer, intermediaries as well as from the Head of the Department of Obstetrics and Gynaecology, Prof. Y. Muscat Baron, in order to carry out my study. (see attached pdf)

Consent has also been obtained from the Data Protection Officer to access the isoft data of the patients (see e-mail below)
In this regard, I kindly request your permission to access and collect the required information from the patient's medical files in order to be able to carry out my study as indicated above. Should you require further clarification regarding my study, kindly contact me or my supervisor Ms Karen Borg Grima, MSc. nuclear medicine, assistant lecturer FHS.

Regards

Helga Consiglio MD FRCOG Reg 2480

Fwd: Ethics Forms to be Collected Inbox ×

Helga Consiglio
to me

---------- Forwarded message ---------
From: **Christabel Vella**
Date: Tue, 13 Nov 2018 at 09:09
Subject: Ethics Forms to be Collected
To: Helga Consiglio

Dear Applicants,

Please call at my office to collect your approved Ethics Forms on:

Wednesday 14th or **Friday 16th November** between **08:00-12:00**.

If you cannot make it during the times mentioned, you can ask someone to collect your documents on your behalf.

Sincere Regards,
Christabel

Christabel Vella
Administration Specialist

University of Malta
Faculty of Health Sciences
Room 76, Block A, Level 1
Mater Dei Hospital
☎

APPENDIX B

Patient Information Sheet

and

Consent Form In English and Maltese

PARTICIPANT- INFORMATION SHEET

Dear Madam,

I am Dr. Helga Consiglio, an Obstetrician and Gynaecologist and, I am following a master's degree in Ultrasound at the University of Malta. As part of my final year I am carrying out a study entitled
Pregnancy of uncertain viability: can the outcome be predicted? under the supervision of Ms. Karen Borg-Grima, assistant lecturer at the Faculty of Health Sciences.

Bleeding and pain in early pregnancy are very common and not all women will miscarry if this happens. The first ultrasound taken when bleeding occurs may not always show us what is happening. This is called ***"***Pregnancy of Uncertain Viability"***.*** The study aims to find out if we can predict which of the pregnancies will actually miscarry. You have been invited to participate since you are currently in this situation.

Your participation will simplyinvolve allowing the staff to collect information that is relevant to the study from you or your medical records. These include: information about your bleeding, your first blood results and ultrasound results. The staff will do this every time you come in until your diagnosis is confirmed. Your care will be the same as when you come through the emergency service. You will not have extra visits. If your pregnancy is successful you will be followed till the end of the first 3 months. There are no risks nor benefits to you or your current pregnancy if you take part since we will be only observing what is naturally taking place However, it will certainly help us improve our early pregnancy services and the results may also benefit you and other women needing our services in future.

In order to save-guard your anonymity as much as possible, intermediary members of staff will be approaching you the first time and explaining the study to you whilst asking for your consent to participate. Your details will be kept confidential and the information will be processed using a code(pseudonym) which will be stored separately from your data. Collected data will remain under lock and key and handled by myself as the researcher and by the supervisor . The examiner may also have access to the data for verification purposes. Once the study is completed the data will be erased.
Participation is on a voluntary basis. Even if you choose to take part in the study, you are free to terminate your participation at any point without giving a reason and without any loss of benefit to which you are otherwise entitled. Should you decide to withdraw any data collected will be deleted or retained only in an anonymous form. As a participant, you have the right under the General Data Protection Regulation (GDPR) and national legislation to access, rectify, and where applicable ask for the data concerning you to be erased. A copy of this information letter and of the consent form will be given to you for your own records.

I would like to thank you in advance for your participation. Should you have any questions please contact me on the details below.

Regards

Dr. Helga Consiglio MD FRCOG
Resident Specialist Obs &Gynae
MSc Student

Ms. Karen Borg-Grima MSc
Assistant Lecturer FHS
Supervisor

CONSENT FORM

1. I confirm that I have read and understood the information sheet.

2. I understand that taking part in the study causes no risks/ benefits to me or my pregnancy.

3. I understand that my participation is voluntarily and that I am free to withdraw or discontinue participation at any time without any prejudice.

4. I understand that all data collected will be pseudonymised and kept confidential throughout the study. Data will be accessible to the researcher, the supervisor and to the examiner for verification purposes. They will be erased once the study is complete.

5. I accept that the study will involve collection of clinical information, ultrasound and blood results.

6. I accept that my case will be followed up until the diagnosis is confirmed . In case of a live pregnancy, this will be until the end of the first trimester. For this purpose, I accept to communicate with the researcher using a method of my choice (see below).

7. I have been made aware that this research has been reviewed and approved by the University Research Ethics Committee.

8. I voluntarily agree to participate in this study and have been given a copy of this consent form and the information sheet.

9. I understand that in the rare event that any anxiety or concern arises during the study, I may ask for help and can be referred to the perinatal mental Health services.

10. I understand that under the General Data Protection Regulation (GDPR) and national legislation, I have a right to access, rectify, and erase data concerning myself .

Participant's signature _____

Date_____

Contact method of participant's choice_____

Dr. Helga Consiglio MD FRCOG
Resident Specialist Obs &Gynae
MSc Student

Ms. Karen Borg-Grima MSc
Assistant Lecturer FHS
Supervisor

INFORMAZZJONI

Għażiża Sinjura,

Jiena Dr. Helga Consiglio, speċjalista tal- maternità. Qed insegwi kors ta' livell Masters fl-ultrasound fl- Università ta' Malta. F'din l-aħħar sena qed nagħmel studju jismu: "Tqala ta' futur inċert. Nistgħu nbassru x'se jiġri?"

Dan ser isir taħt is-superviżjoni ta' Karen Borg Grima, Assistent Lettur fil-Fakulta` tal-Health Sciences.

Xi demm u wġigħ fil-bidu tat-tqala huma komuni ħafna. Mhux kull min jiġrilu hekk ikorri. Xi drabi, l-ewwel ultrasound ma jurix biċ-ċert x'ser jiġri. Din tissejjaħ " Tqala Ta' Futur Inċert". L-istudju se jipprova jinduna liema tqala se tkompli u liema le. Inti ġejt mistiedna biex tieħu sehem għax jidher li qed tgħaddi minn din is-sitwazzjoni.
Il-parteċipazzjoni tiegħek tinvolvi biss li tagħtina l-permess li niġbru l-informazzjoni, li hija relevanti għall-istudju, minn għandek jew min-noti tiegħek. Dan jinvolvi: informazzjoni dwar kemm qed tara demm, l-ewwel test tad-demm tiegħek u r-riżultati tal-ultrasound. Dan nagħmluh kull darba li tiġi għal viżta sakemm ikollna dijanjosi ċerta. Il-kura tiegħek tkun l-istess daqs li kieku ġejt bħala emerġenza. Ma jkollokx viżti żejda. Pero` jekk it-tqala tkompli, nkomplu nsegwuk sa'ma tagħlaq tliet xhur. M'hemmx riskji jew benefiċċji għalik jew għat-tqala tiegħek jekk tieħu sehem għax inkunu qegħdin biss nosservaw dak li qed jiġri Pero' l-istudju żgur ser itejjeb is-servizz li nagħtu fit-tqala bikrijau r-riżultati għad ighinu lilek u lil nisa oħra li jeħtieġu s-servizzi tagħna fil-futur.

Biex nħarsu l-kunfidenzjalita` tiegħek, persuna tal-istaff, ser tkun intermedjarja biex tispjegalek dwar l-istudju u teħodlok il-kunsens. Id-dettalji tiegħek jibqgħu kunfidenzjali u nipproċessawhom permezz ta' numru ta' kodiċi (pswedonimu) li jinżamm separat mill-bqija tal-informazzjoni. It-tagħrif dwarek ikun imsakkar u immaniġjat minni bħala riċerkatriċi u mis-supervisor. L-eżaminatur ukoll jista' jkollu aċċess għall-informazzjoni għal skopifijiet ta' verifika tal-istudju. Meta jispiċċa l-istudju, l-informazzjoni tiġi mħassra.

Tagħżel li tieħu sehem b'mod volontarju. Tista' tagħżel li ma tieħux sehem jew li twaqqaf il-parteċipazzjoni tiegħek meta trid mingħar ma tagħti raġuni u mingħajr ma titlef benefiċċji li tgawdi minnhom bħalissa.. Jekk tagħżel li tieqaf, l-informazzjoni dwarek tiġi mħassra. Għandek tkun taf li taħt il-liġi tal-General Data Protection Regulation (GDPR) u l-liġijiet tal-pajjiż, il-parteċipanti għandhom id-dritt li jaċċessaw, ibidlu jew iħassru l-informazzjoni dwarhom. Kopja ta' din l-ittra u tal-kunsens ser tingħatalek. Nixtieq jirringrazzjak bil-quddiem għall-parteċipazzjoni tiegħek. Jekk ikollok xi domandi tista' tikkuntattjani fuq id-dettalji t' hawn isfel.

Dejjem tiegħek

Dr. Helga Consiglio MD FRCOG
Resident Specialist Obs &Gynae
Studenta tal-Masters

Ms. Karen Borg-Grima MSc
Assistant Lecturer FHS
Assistent Lettur, Supervisor

FORMOLA TA' KUNSENS

1. Nikkonferma li qrajt u fhimt din l-ittra ta'informazzjoni.

2. Nifhem li m'hemmx riskji/ benefiċċjigħalija u għat-tqala tiegħi f'dan l-istudju.

3. Nifhem li qed nipparteċipa b'mod volontarju u li nista' nwaqqaf il-parteċipazzjoni tiegħi fl-istudju meta nixtieq. Dan ma jaffettwax il-kura tiegħi issa u 'l quddiem.

4. Nifhem li l-informazzjoni dwari tkun permezz ta' kodici psewdonimu u li tinżamm b'mod kunfidenzjali matul l-istudju. L-informazzjoni hija aċċessibli għar-riċercatur, għas-supervisor u għall-eżaminatur għal skopijiet ta' verifika. Id-data tkun imħassra wara li jintemm l-istudju.

5. Naċċetta li f'dan l-istudju tingħabar informazzjoni dwar saħħti, riżultati tal-ultrasound u tad-demm.

6. Ser isegwu l-każ tiegħi sa ma jkunu jafu biċ-ċert x'se jiġri. Jekk tkompli t-tqala, jsegwuni sat-tmiem tal-ewwel tliet xhur. Għalhekk naċċetta li nikkomunika mar-
riċerkaturi b'mezz li nagħżel jien (ara isfel).

7. Jiena nifhem li ngħata permess għal dan l-istudju mill- University Research Ethics Committee.

8. Jiena nagħżel li nieħu sehem f'dan l-istudju b'mod volontarju. Jien ingħatajt kopja tal- fuljett ta' informazzjoni u kunsens.

9. Nifhem li jekk inħoss xi ansjeta` jew xi problemi oħra matul dan l-istudju nista' nitlob għall-għajnuna mit-tim tar-riċerka u huma jistgħu jirriferuni lill-Perinatal Mental Health Services jekk ikun hemm bżonn.

10. Bil-liġi tal- General Data Protection Regulation (GRPR), jien għandi d-dritt li nara, nibdel jew inħassar kull informazzjoni dwari nnifsi.

Firma tal-
parteċipanta_____Data_____

Mezz ta'kuntatt magħżul mill- parteċipanta _____

Dr. Helga Consiglio MD FRCOG
Resident Specialist Obs &Gynae
Studenta tal-Masters

Ms. Karen Borg-Grima MSc
Assistant Lecturer FHS
Assistent Lettur, Supervisor

APPENDIX C

PUV Study Operations Manual

PUV Study Ultrasound Operations Manual

PUV STUDY - OPERATIONS MANUAL (9/2018)

RESEARCHER CONTACT DETAILS – Helga Consiglio	
Pager	*** ****
Work Mobile	********

AIMS:

a) To investigate the possibility of predicting the outcome of PUV by using clinical biochemical and ultrasonic markers.

b) To explore the optimal ultrasound follow-up strategy for these patients.

INCLUSION CRITERIA:

- Age 18 to 50 ; <13 weeks seen at emergency obstetric service.
- Symptoms: bleeding, pain and/or loss of pregnancy symptoms.
- Patients clinically stable.
- Ability to communicate in Maltese or English for consent.
- Women residing in Malta for the duration of pregnancy.
- TV ultrasound performed by trained sonographer/obstetrician.
- Ultrasound performed using only the Equipment on the Emergency Obstetric Ward.
- **A single intrauterine PUV- MSD <25mm with no visible fetal pole or CRL <7mm**

EXCLUSION CRITERIA:

- Multiple pregnancy.
- Pregnancies with a definite diagnosis already
- Women already on Progesterone supplementation (oral or vaginal route).
- Women who decline transvaginal ultrasound.

PARTICIPANT PATHWAY:

- Check eligibilty criteria
- Intermediary to invite to participate
- Explain PUV study, Give information sheet and consent form
- Give copy of information sheet and consent to the participant to keep
- Book HCG and Progesterone- chase result after case is closed.
- Organise the 7 and 14 day follow up at Emergency obstetric service.
- If subsequent scan confirms viability refer for antenatal booking
- if subsequent scan confirms miscarriage offer expectant, medical or surgical management as per usual protocol.

DATA COLLECTION SHEET:

- Fill in clinical data- code, basic history as indicated.
- **PBAC** – check participant's panty liner and compare to PBAC chart **Score as nil/ light/moderate/fills a pad/ clots or flooding**
- Fill in **Ultrasound data** – MSD, Mean Yolk sac size , CRL , presence of haematomas

PUV STUDY- ULTRASOUND OPERATIONS MANUAL

CONSIDER EVERY PATIENT WITH PUV AS A POSSIBLE PARTICIPANT

Health and Safety guidelines:

1. Use hand and probe hygiene
2. Use Probe cover
3. ALARA principle

Patient preparation	Equipment preparation
• Verbal Consent for Trans vaginal ultrasound • Ensure empty bladder • Offer adequate cover	• Use transvaginal probe • Endovaginal preset

Ultrasound measurements – document on ultrasound proforma

1. Confirm intra-uterine pregnancy

2. Gestational Sac:
    a. Measure sac (Inner to inner) in 3 orthogonal measurements and calculate mean – MSD.
    b. Observe and document apperance, postion of gestation sac

3. Yolk Sac:
    a. If yolk sac is present, measure outer to outer in 3 orthogonal measurements and calculate mean.
    b. Observe and document appearance of yolk sac – absent/normal/echogenic/calcified/irregular

4. Fetal Pole:
    a. If fetal pole is present, measure Crown Rump Length *
    b. Check and document fetal heart pulsations.

    **In case of viable pregnancy – no need to measure yolk sac and MSD.*

5. Check and document haematomas and measure in 3 planes

6. Check and document for any other uterine abnormalities eg fibroids

7. Check adnexae, document corpus luteum and any abnormalities.

APPENDIX D

Data Collection Sheet

SUPERVISORS: Ms Karen Borg Grima MSc Radiography
Mr Mark Formosa MD FRCOG M.Phil

PATIENT CODE _____ CONSENT OBTAINED □ INFORMATION □

CLINICAL HISTORY
AGE _____

GRAVIDA_____ PARA_____ SPONTANEOUS/ ASSISTED

LMP_____ CYCLE _____ REGULAR/IRREGULAR

PMH_____

FIRST VISIT **Date_____**

PBAC SCORE: No Bleeding / Light / Moderate/ Soaks towel/ Clotting or flooding

MSD _____mm SAC APPEARANCE _____

CRL _____mm/ Absent **FETAL HEART - NO**

YOLK SAC MEAN DIAMETER_____mm

YOLK SAC APPEARANCE: _____

HCG _____ PROGESTERONE _____

DAY 7 VISIT **Date**_____

PBAC SCORE : No Bleeding / Light/ Moderate/ Soaks towel/ clots or flooding

MSD _____mm SAC APPEARANCE_____

CRL _____mm/ Absent

YOLK SAC MEAN DIAMETER _____mm

YOLK SAC APPEARANCE_____

FETAL HEART - YES-------Confirmed Live Pregnancy---------Referred

NO -------Confirmed Miscarriage ----------Referred

NO--------PUV---------- Book 14 day review

DAY 14 VISIT **Date**_____

PBAC SCORE : No Bleeding / Light/ Moderate/ Soaks towel/ clots or flooding

MSD _____mm SAC APPEARANCE_____

CRL _____mm/ Absent

YOLK SAC MEAN DIAMETER _____mm

YOLK SAC APPEARANCE_____

FETAL HEART - YES-------Confirmed Live Pregnancy---------Referred

NO -------Confirmed Miscarriage ----------Referred

NO--------PUV---------- Book 14 day review

APPENDIX E

Raw Data

CODE	AGE	NATIONALITY	PARITY	GEST	PBAC/0	MSD/0	CRL/0	YS/0	YS/0	HCG	PROG	MSD/7	CRL/7	YS/7	DIAGNOSIS /7	DIAG/ 14	DIAG/21	OUTCOME	Days to outcome
O1	30	1	3	5.57	2	9.6		2	2.4	6548	41.7	10			2	2	2	2	7
N1	31	1	2	6.147	1	10		2	2.9	2503	17.8	15.9	4.8	4.3	1	1	1	1	7
N2	30	1	1	5.29	1	10.5		1		11919	38.2	18.2	4.1	2.13	1	1	1	1	7
N3	36	2	1	5.71	1	4.9		1		2667	66.5	13	2.4	3.23	3	1	1	1	14
N4	31	2	1	6	2	10.5		1		31366	47	30			2	2	2	2	5
O2	18	1	4	6.29	1	12.8		2	1.73	16884	44.8		5		1	1	1	1	7
N5	30	1	1	9.71	2	14.3	4.6	3	3.5	3209	15				2	2	2	2	1
D2	40	2	3	6.28	1	6.3		1		1683	28.4	14.7	2	4	3	1	1	1	14
D3	29	1	1	5.71	1	11.4		2	2.6	8533	38.5	21	5.8	4.8	1	1	1	1	7
D4	25	2	2	5.57	1	15.9	2.6	2	3.7	26319	38.8	27.7	8	5	1	1	1	1	7
D5	34	1	1	5.14	1	5.9		2	1.8	2472	42.9	11.7	4.3	2.53	1	1	1	1	7
N7	24	2	1	7.28	1	12.7	2.7	2	4.7	36233	24.2	16.2	6.9		3	2	2	2	14

D6	38	2	3	9.43	2	15		1		8683	25.5				2	2	2	2	2
D7	36	2	2	5.85	2	10.5	2.9	2	3.3	9081	39.4	20.2	8.3	5.2	1	1	1	1	7
D8	36	1	3	5.57	2	10.5		1		107	0.97				2	2	2	2	7
D9	31	1	4	6	2	15.6		2	4.5	21040	39.8	24.6	10.8	5.13	1	1	1	1	7
D10	38	1	3	5.147	1	16.3	2.9	2	4.23	17848	37	23.4	7	4.7	1	1	1	1	7
J1	26	1	3	7.147	4	17	3.9	1		1581	18.3				2	2	2	2	2
J2	29	1	1	9.57	2	20.1		1		19456	17	19.5			2	2	2	2	7
J3	40	1	2	6.57	2	13.3		2	7.1	11945	32.4		14.5		1	1	1	1	7
D12	35	1	3	6.71	2	7.13		1		3122	12.1	8.4	2	2.6	3	2	2	2	14
J4	28	2	3	8.85	3	15.96	2.7	2	1.6	2994	11.7				2	2	2	2	4
J5	19	2	1	6.71	2	7.7	2.3	2	2.8	3000	13.7	12.4	2.3		2	2	2	2	7
J6	42	1	3	6	1	6		2	2.6	2049	22.1	11.9	2.9	5.9	3	3	2	2	21
J7	33	2	3	5	1	8.1		2	3.7	16290	37.5	20.4	5.1	4.8	1	1	1	1	7

J8	33	1	2	6.147	2	7.3		2	3.6	1548	15.6				2	2	2	2	3
J9	35	2	2	5.28	1	7.5		2	2.9	19447	23	17.8	4.5	4.8	1	1	1	1	7
J10	19	1	1	5.57	1	5		2	1.7	1818	22		2.3		1	1	1	1	7
J11	20	1	1	5.28	2	8.5		2	3.4	6176	37.5		4.8		1	1	1	1	7
J12	27	2	2	5	1	6		1		2980	38.5	12	3.4	4.7	1	1	1	1	7
J13	30	1	2	5	1	6.13		2	1.1	3106	42.6	9	2.9	4	1	1	1	1	7
F1	32	1	1	6.85	2	11.9	1.7	2	3.7	8248	26.6	12.7	5	4.5	1	1	1	1	7
J14	32	2	2	5	1	19.3		1		22977	29.2	29.3	7.7	4.8	1	1	1	1	7
J15	40	1	2	8.29	1	5.3		1		4808	26.7	16	2.8	4.5	3	1	1	1	14
F2	31	1	3	6.43	2	4.9		2	1.7	944	2.75				2	2	2	2	4
J16	35	2	2	5.29	1	5.7		1		19447	23	17.8	4.5	4.8	1	1	1	1	7
F3	28	2	1	5.57	2	2		1		622	35	4.5			3	3	1	1	21
F5	23	1	1	6	2	8	2.3	2	3.1	9711	23.9		9	5.73	1	1	1	1	7
f6	29	2	2	7.147	2	7		1		7103	12.1				2	2	2	2	7

m1	38	2	3	5.57	1	7.57		1		10075	26.6	19.5	5.7	5.2	1	1	1	1	7
f7	40	2	4	6.29	3	4.3		2	1.6	1445	4.71				2	2	2	2	2
m2	40	1	2	6.29	1	14	1.8	2	6.1	14912	19.9	16.4	2.6	7.6	3	2	2	2	14
m3	30	2	2	7.29	2	14		2	5.9	23524	26.2	13	4.3	6	3	2	2	2	14

APPENDIX F

Predictive Model based on Raw Data

MSD	Progesterone	Bleed	Miscarriage	Viable	Predicted	Actual
9.6	41.7	Bleeding	0.165	0.835	Viable	Miscarriage
10	17.8	No bleeding	0.432	0.568	Viable	Viable
10.5	38.2	No bleeding	0.042	0.958	Viable	Viable
4.9	66.5	No bleeding	0	1	Viable	Viable
10.5	47	Bleeding	0.093	0.907	Viable	Miscarriage
12.8	44.8	No bleeding	0.022	0.978	Viable	Viable
14.3	15	Bleeding	0.941	0.059	Miscarriage	Miscarriage
6.3	28.4	No bleeding	0.096	0.904	Viable	Viable
11.4	38.5	No bleeding	0.045	0.955	Viable	Viable
15.9	38.8	No bleeding	0.072	0.928	Viable	Viable
5.9	42.9	No bleeding	0.012	0.988	Viable	Viable
12.7	24.2	No bleeding	0.298	0.702	Viable	Miscarriage
15	25.5	Bleeding	0.796	0.204	Miscarriage	Miscarriage
10.5	39.4	Bleeding	0.234	0.766	Viable	Viable
10.5	1	Bleeding	0.987	0.013	Miscarriage	Miscarriage
15.6	39.8	Bleeding	0.351	0.649	Viable	Viable
16.3	37	No bleeding	0.096	0.904	Viable	Viable
17	18.3	Bleeding	0.933	0.067	Miscarriage	Miscarriage
20.1	17	Bleeding	0.961	0.039	Miscarriage	Miscarriage
13.3	32.4	Bleeding	0.541	0.459	Miscarriage	Viable
7.1	12.1	Bleeding	0.91	0.09	Miscarriage	Miscarriage
16	11.7	Bleeding	0.969	0.031	Miscarriage	Miscarriage
7.7	13.7	Bleeding	0.896	0.104	Miscarriage	Miscarriage
6	22.1	No bleeding	0.201	0.799	Viable	Miscarriage
8.1	37.5	No bleeding	0.035	0.965	Viable	Viable
7.3	15.6	Bleeding	0.862	0.138	Miscarriage	Miscarriage

7.5	23	No bleeding	0.21	0.79	Viable	Viable
5	22	No bleeding	0.184	0.816	Viable	Viable
8.5	37.5	Bleeding	0.239	0.761	Viable	Viable
6	38.5	No bleeding	0.023	0.977	Viable	Viable
6.1	42.6	No bleeding	0.013	0.987	Viable	Viable
11.9	26.6	Bleeding	0.694	0.306	Miscarriage	Viable
19.3	29.2	No bleeding	0.318	0.682	Viable	Viable
5.3	26.7	No bleeding	0.107	0.893	Viable	Viable
4.9	2.8	Bleeding	0.967	0.033	Miscarriage	Miscarriage
5.7	23	No bleeding	0.176	0.824	Viable	Viable
2	35	Bleeding	0.168	0.832	Viable	Viable
8	23.9	Bleeding	0.674	0.326	Miscarriage	Viable
7	12.1	Bleeding	0.908	0.092	Miscarriage	Miscarriage
7.6	26.6	No bleeding	0.138	0.862	Viable	Viable
4.3	4.7	Bleeding	0.953	0.047	Miscarriage	Miscarriage
14	19.9	No bleeding	0.48	0.52	Viable	Miscarriage
14	26.2	Bleeding	0.757	0.243	Viable	Miscarriage

DATA LEGEND

PARITY • Nullipara 1 • Parous 2 • Previous Miscarriage And Also Live Child 3 • Previous Miscarriage, No Live Child 4	PBAC. • Nil 1 • Light 2 • Moderate 3 • Soaks Towel 4 • Clotting Or Flooding 5	FETAL HEART • Yes 1 • No 2
WEEKS AMENORRHOEA in weeks	MSD in mm	DIAGNOSIS • Viable. 1 • Miscarriage 2 • PUV 3
GESTATION SAC: • Normal/Spherical 1 • Abnormal/ Irregular /Flattened 2 • Absent3 • POC /Complete 4	YOLK SAC • Absent 1 • Normal 2 • Calcified 3 • Echogenic 4 • Abnormal Shape 5	NATIONALITY • Maltese 1 • Non Maltese 2
FETAL POLE • Present 1 • Absent 2	CRL in mm PROGESTERONE in mmol/l HCG in miU/l	YOLK SAC DIAMETER in mm