The use of ultrasound and biochemical markers

in prediction of pregnancy outcome.

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By

Claire Janine Elson

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ProQuest LLC
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106-1346

Abstract

The use of ultrasound and biochemical markers in prediction of pregnancy outcome.
Claire Janine Elson

The aim of this thesis was to develop novel approaches to the management of early pregnancy complications.

The thesis is based on three studies of women in the first trimester of pregnancy. These studies examine the value of ultrasound and serum biochemistry in the prediction of outcome in women with an anembryonic gestational sac (Chapter 4), with ectopic pregnancies (Chapter 5), with failing pregnancies (Chapter 6). Statistical models comprising of logistic regression or decision tree analysis were developed for each study.

In women with anembryonic gestation sacs the probability of a viable pregnancy can be calculated using a logistic regression model, decision tree analysis or progesterone alone with equal accuracy. These models were validated prospectively.

Decision tree analysis was used to develop a model for the prediction of successful expectant management of ectopic pregnancy, based on both a single serum hCG measurement or using serial measurement. These models were also validated prospectively.

A novel approach to the prediction of the success of expectant management of miscarriages was developed using decision tree analysis and IGFBP-1 and inhibin A.

This thesis has developed algorithms that can be used in clinical practise to predict the success of expectant management in women with failing pregnancies. It has identified that novel biochemical markers may have a role to play in the prediction of successful expectant management of women with miscarriages.

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Chapter 1 Introduction

Early pregnancy failure is a large burden on health services because of its high incidence and complex clinical management, which often requires the use of multiple diagnostic tests and in-patient surgical treatment. Until recently there was little interest in the diagnosis and clinical management of early pregnancy complications. However in the last decade clinical management of early pregnancy failure has undergone a major transformation. Ultrasound is now used routinely to diagnose an early pregnancy and its complications. This in combination with biochemical markers enables a more complex approach to early pregnancy complications, which is similar to biophysical profile in late pregnancy. However, the value of this novel approach in the clinical management of early pregnancy failure is unknown.

One of the problems lies in a lack of well-defined criteria to differentiate between various types of early pregnancy failure, which causes continuous problems in clinical practice. In 1995 a number of cases where viable pregnancies had been misdiagnosed as miscarriages led to a government enquiry, which produced clinical guidelines in order to avoid similar problems occurring in the future (Hately et al 1995). Furthermore, the management of early pregnancy failure, which until recently often involved surgical evacuation of the uterus has become more complex and management options now also include medical and expectant treatment.

Early pregnancy units have become the accepted organisational structure within the health service, to look after women with early pregnancy problems (Walker & Shillito 1997). They offer easy and fast access to medical services with minimal social disruption. Centralising care in a specialised unit also means that there is continuity of

care, enabling an easily identifiable point of contact for women undergoing treatment and providing counselling and psychological support as necessary.

One of the major problems counterbalancing the benefits of early pregnancy units is a large number of non-diagnostic scans, which is about 20% in a typical unit (Cacciatore et al 1988, Hahlin et al 1995, Banerjee et al 1999). Although the majority of these pregnancies are failing, women whose scans fail to locate a pregnancy are usually managed as possible ectopic pregnancies, which causes considerable patients' anxiety and creates the need for extensive follow up. This significantly increases staff workload and the costs of running the service.

The detection of ectopic pregnancies is increasing due to liberal use of ultrasound and rapid access to human chorionic gonadotrophin (hCG) immunoassays. Thus a large number of tubal ectopic pregnancies, which would remain undetected in the past and undergo spontaneous resolution, are now actively treated. A challenge in modern clinical practice is therefore not only to detect an ectopic pregnancy, but also to develop management algorithms, which would facilitate expectant management of those ectopics, which are destined to resolve without causing any significant maternal morbidity.

The aim of this thesis was to develop novel approaches to the management of early pregnancy complications. In particular I tried to identify parameters, which can reliably predict the success of expectant management in women with failing pregnancies. The ability to do so would not only reduce the need for follow up but also decrease the need for surgery for both diagnostic and therapeutic indications. An accurate prediction of the outcome of expectant management is of great importance to women. Expectant

management often takes weeks to complete and success rates are variable depending on the type of early pregnancy abnormality, which is being treated. Failure of expectant management after prolonged follow-up is particularly disappointing for women and reduces overall benefits of the management strategy. The ability to estimate the likelihood of final outcome would almost certainly increase the uptake of expectant management by both clinicians and patients and would contribute to the overall care of women with early pregnancy problems.

Chapter 2: Literature Review

2.1 Normal Early Pregnancy

2.1.1 Physiology of early pregnancy development

Early pregnancy development and placentation

Fertilisation of the ovum by spermatozoa following ovulation occurs either in the peritoneal cavity or within the fimbrial end of the Fallopian tube. During the next 48 hour the conceptus travels along the Fallopian tube and into the uterine cavity. Three days after fertilisation the conceptus consists of 60 cells of which 10% will eventually form the embryo and 90% the trophoblast. The conceptus attaches to the endometrium by day 5 post ovulation but it is not until day 12 that the blastocyst is completely embedded into the endometrium. There is initial local decidualisation at the implantation site, which eventually extends to the whole endometrium The trophoblastic cells form the extra-embryonic tissue. These separate from the population of cells destined to be the embryo during days 4-5 of the embryogenic phase. Following blastocyst adhesion there is rapid trophoblast proliferation and cell fusion to form a multinucleated synctiotrophoblast which invades the uterine stroma. The trophobalst differentiates in two ways –the villous and extravillous trophoblast. The villous trophoblast remains attached to the villous membrane and the cells use to become the synctiotrophoblast reponsilbe for absorption, exchange and hormone secretion. The cells of the extravillous trophoblast migrate through the maternal tissue towards decidual arterial walls (interstitial invasion) (Lyall 2002) or infiltrate the lumens and walls of the arteries to cause endovascular invasion (Kaufmann et al 2003). Intra-arterial plugs consisting of endovascular trophoblasts prevent maternal blood from entering the intervillous space until the 12th week of gestation thus protecting the conceptus from high ocygen levels at this critical stage of development (Burton et al 1999). The

definitive structure of the placenta is apparent as early as day 21 post ovulation however the uteroplacental circulation is not fully functional until the end of the first trimester (Hustin & Jauniaux 2000).

During organgogeneis there is a limited circulation of blood through the vitelline duct between the fetus and the yolk sac. This takes place before trophoblast circulation becomes functional at 7-8 weeks. Cardiac activity starts at 5 weeks and 2 days when the fetal pole is around 1.5-3mm.

2.1.2 Biochemistry in early pregnancy

It is the syncytiotrophopblast of the developing placenta that plays a major part in hormone, protein and growth factor production and control in early pregnancy. Almost immediately after implantation the synctiotrophoblast secretes human chorionic gonadotropin (hCG). This, in turn, maintains the function of the corpus luteum which itself secretes hormones and growth factors. The corpus luteum activity decreases after the seventh week of pregnancy at which time the trophoblast and decidua become the main hormone producing unit.

Placental Proteins

Human chorionic gonadotropin (hCG) is a glycoprotein with a molecular weight of 36 kD secreted by the syncytiotrophoblast (Midgley & Pierce 1962). It is composed of two non-covalently linked α and β subunits. The α unit is shared by FSH and LH but the β subunit is specific to hCG. In normal pregnancies it is the corpus luteum that secretes steroid hormones in response to the hCG secreted from the developing trophoblast. By eight weeks this role is taken over by the trophoblast. hCG first enters the maternal

bloodstream on the day of implantation. It has been shown that its level in maternal serum doubles over 1.4-1.6 days from the time of first detection to the thirty-fifth day of pregnancy and then over 2.0-2.7 days from the thirty-fifth to the forty second day (Pittaway et al 1985). The half life of hCG is 32 to 37 hours. Levels of hCG are approximately 1000 IU/L at around 4 weeks of pregnancy, the time of initial visualisation on ultrasound scan. hCG secretion increases with advancing gestational age, reaching a maximal level of 50,000 – 100,000 IU/l at 10 weeks of gestation. hCG levels then decrease to around 10,000-20,000 IU/l by 20 weeks (Figure 2.1.1) and this plateau is maintained for the rest of pregnancy. Many different assay kits exist which are calibrated against different reference preparations of hCG. Thus when comparing results between different investigators this needs to be taken into account and appropriate values need to be developed for individual medical centres. βhCG may exist in the blood as part of the intact hCG molecule (α and β subunits) or as a free βhCG. Assay kits measure either intact hCG, βhCG or total hCG (intact plus βhCG).

In primate studies it is the exponential rise of hCG produced by the implanting embryo and synctiotophoblast that appears to extend the lifespan of the corpus luteum (Zeleznik 1998). hCG maintains the steroidogenesis of the corpus luteum until the ninth to tenth week of pregnancy, by which time placental steroidogenesis is established. It is thought that hCG produced by the placenta is involved with autoregulation of placental steroidogenesis (Menon & Jaffe 1973). As well as maintaitning the corpus luteum hCG regulates trophoblast differentiation by enhancing the spontaneous diffentiation of cytotrophoblast into syncytiotrophobast (Yang et al 2003). There is also evidence that hCG modulates tropholast invasion by interfering with endometrial matrix metalloproteinases (MMPS) and their tissue inhibitors (Licht et al 2001).

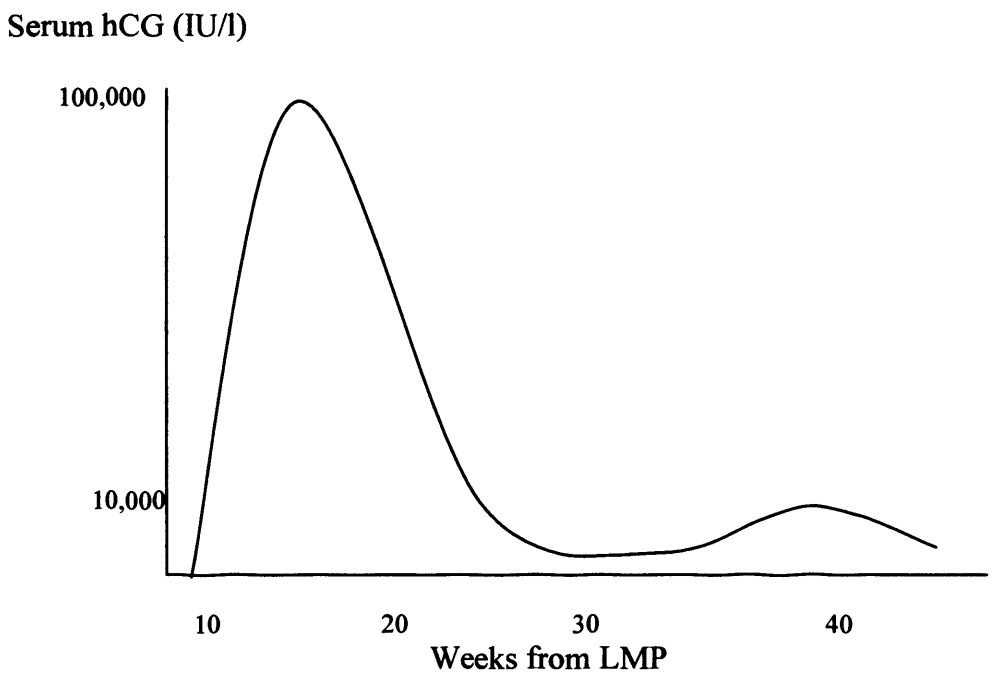


Figure 2.1.1 Schematic representation of concentration of human chorionic gonadotrophin (hCG) throughout gestation.

Peptides

Inhibin A is a heterodimeric glycoprotein, with a molecular weight of 32 KD composed of an inhibin α and $ß_A$ subunit linked by disulphide bridges. It is produced by the corpus luteum during the luteal phase of the menstrual cycle (Muttukrishna et al 1994). In early pregnancy inhibin A is also produced by the synctiotrophoblast. However it remains unclear whether synctiotrophoblast or the corpus luteum is the dominant source of inhibin production during early pregnancy. Santoro et al (1992) in a study looking at inhibin levels in women with premature ovarian failure and donor IVF pregnancies i.e. aluteal women showed that there was no early rise in inhibin as seen in normal pregnancies although the levels did reach normal values towards the end of the first trimester. Lockwood et al (1997) examined this further by comparing spontaneous pregnancies with those from frozen embryo transfer i.e. aluteal women. They observed that there was no difference in luteal and aluteal pregnancies and therefore they concluded that the fetoplacental unit must be the major source of inhibin A production. Inhibin A reaches a level on day 12 after ovulation of around 104.5pg/ml and then climbs from day 21 after ovulation to peak at 8 weeks of gestation (Illingworth et al 1996). The clearance of inhibin A is fast with a short half life of around 45minutes (Muttukrishna et al 1997). Inhibin is thought to be involved in regulating placental hCG production by inducing changes in GnRH secretion. It is also thought to play a part in the differentiation of cytotrophoblast into syncytiotrophoblast and therefore possibly trophoblast invasion (Debieve et al 2000). Animal studies have also suggested a role of inhibin A in maintaining luteal progesterone output (Webley et al 1994). However it's function in humans remains unclear.

Inhibin pro-αC circulates as a functionally inactive monomer and as part of high molecular weight functional dimers. Inhibin pro-αC-related immunoreactivity (pro-αC-RI) has been found to peak at 4 weeks and then fall until 11 weeks of gestation. Lockwood et al (1997) in their study of aluteal and luteal pregnancies compared serial levels of pro-αC-RI in early pregnancy in these two groups. They found that pro αC RI was significantly higher in those pregnancies with multiple corporea lutea compared to those with single coprorea lutuea and significantly lower in those women with conceptions from frozen embryos i.e. aluteal than those with fresh embryos i.e. luteal . They thus concluded that the corpus luteum must be the major source in early pregnancy. Inhibin pro αC RI is also thought to play a role as a paracrine and endocrine regulator of placental function.

Steroids

Progesterone is a steroid hormone derived from cholesterol. It is one of the primary products of the corpus luteum. Although, progesterone originates almost entirely from the corpus luteum before 6 weeks' gestational age, its production shifts more to the trophoblast after the 7th week. Beyond 12 weeks, the placenta is the dominant source of progesterone. Progesterone concentrations are less than 2 nmol/L during the follicular phase of the normal menstrual cycle (Abraham et al 1972; Lindbert, Johansson & Nilsson 1974). However, in the luteal phase of conception cycles, progesterone concentrations rise from about 2-4 nmol/L on the day of the LH surge to a plateau of approximately 20-70 nmol/L over the subsequent 7 days. Concentrations remain within this luteal-phase range until the 10th week from the last menstrual period, and then

show a continuous sustained rise until term (Figure 2.2.2). At term, progesterone concentrations can range from 200 to 600 nmol/L (Tulchinsky et al 1972).

Recent data from primate studies suggest that there are gonadotrophin-stimulated progesterone dependent processes that promote luteotrophic and suppress luteolytic patways in the corpus luteum and that this continues following pregnancy initiation (Duffy & Stouffer 1997). Luteal cells are progesterone receptor positive and would appear to be regulated in an autocrine manner by progesterone (Hild-Petito et al 1988) , possibly by the up-regualtion of progesterone receptors (Chaffin et al 1999).The ability of antiprogesterone agents to induce abortion confirms its crucial role in the maintenance of pregnancy. It is thought that it may act by inhibiting T lymphocytic cell-mediated responses involved in tissue rejection. Progesterone is known to be a potent inhibitor of leukaemia inhibitory factor (LIF) which itself plays an important role in trophobaslt invasion (Sunder & Lenton 2000). Synthetic progestagens have been shown to upregulate nitric oide synthase in the endometrium. Nitric Oxide appears to play a role in maintainin uterine quiescence (Cameron & Campbell 1998).

Serum Progesterone (nmol/L)

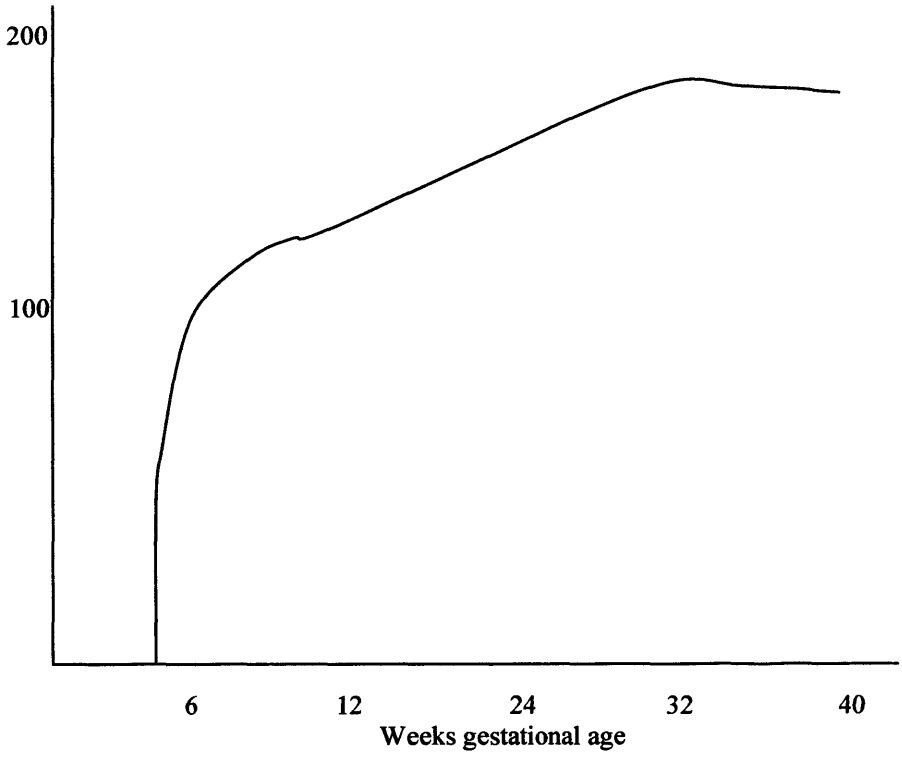


Figure 2.2.2

Circulating concentrations of progesterone during the course of human pregnancy.

17 α-OH progesterone (17OHP) is a steroid secreted in parallel to progesterone from the corpus luteum. 17 OH progesterone may be a better marker of corpus luteum function in early pregnancy than progesterone as there is limited placental hydroxylation at this stage. The plasma concentration of 17 OHP rises from 2.6ng/ml in the third week of pregnancy to 5.8 ng/ml at the fifth week and then declines to reach a nadir in the thirteenth week (Tulchinksy & Hobel 1973).

Placental Growth Factors

Insulin-like growth factor binding protein 1(IGFBP-1) is one of the six proteins that specifically binds insulin like growth factors in body fluids and tissues (Shimasaki & Ling 1992). IGFBP-1 contains 234 amino acids, with a molecular mass of 25 kD. The human IGFBP-1 gene is located on chromosome 7. Insulin growth factor binding protein (placental protein 12) is synthesised in large amounts in the decidua of early pregnancy (Rutanen 1992). It is also the predominant IGFBP in amniotic fluid, a major insulin-like growth factor (IGF) binding species in fetal plasma (Drop et al 1984) and it's concentrations increases in the maternal circulation during pregnancy. There is a rapid rise in the first trimester reaching a peak at 12-16 weeks. Levels then decrease after 33 weeks. In the fetus it is produced in the liver and pancreas. It has been suggested that IGFBP-1 acts as a barrier to trophoblast migration (Irwin & Giudice 1998). IGFBP-1 is a local modulator of IGF action in fetal growth. It is also able to independently interact with cytotrophoblast cells.

2.1.3 Ultrasound in early pregnancy

High resolution transvaginal ultrasound is now the method of choice when assessing early pregnancy and has been shown to be superior to transabdominal ultrasound in diagnostic accuracy (Cacciatore et al 1989).

The gestational sac is the first pregnancy structure that can be detected by ultrasound. It is usually visualised from 4^{+3} weeks gestation onwards when it measures 2-3mm in diameter (Yeh et al 1986). The gestational sac includes the chorionic cavity, a rim of invading chorionic villi and the underlying decidual reaction. It is usually located eccentrically in the upper part of the uterine cavity and grows approximately 1mm in diameter per day changing its shape from being round (up to the size of 1cm) to more elliptical thereafter. The early gestational sac contains two separate fluid filled compartments: the amniotic and exocoelomic (chorionic) cavity. In very early pregnancy the exocoelomic cavity predominates. From 8 weeks the amniotic cavity expands rapidly and it soon occupies most of the gestational sac. By the end of the first trimester the amniotic and chorionic membranes become fused resulting in the complete obliteration of the exocoelomic cavity. Normal ranges for gestational and amniotic sac size in early pregnancy have recently been established (Figure 2.1.3).

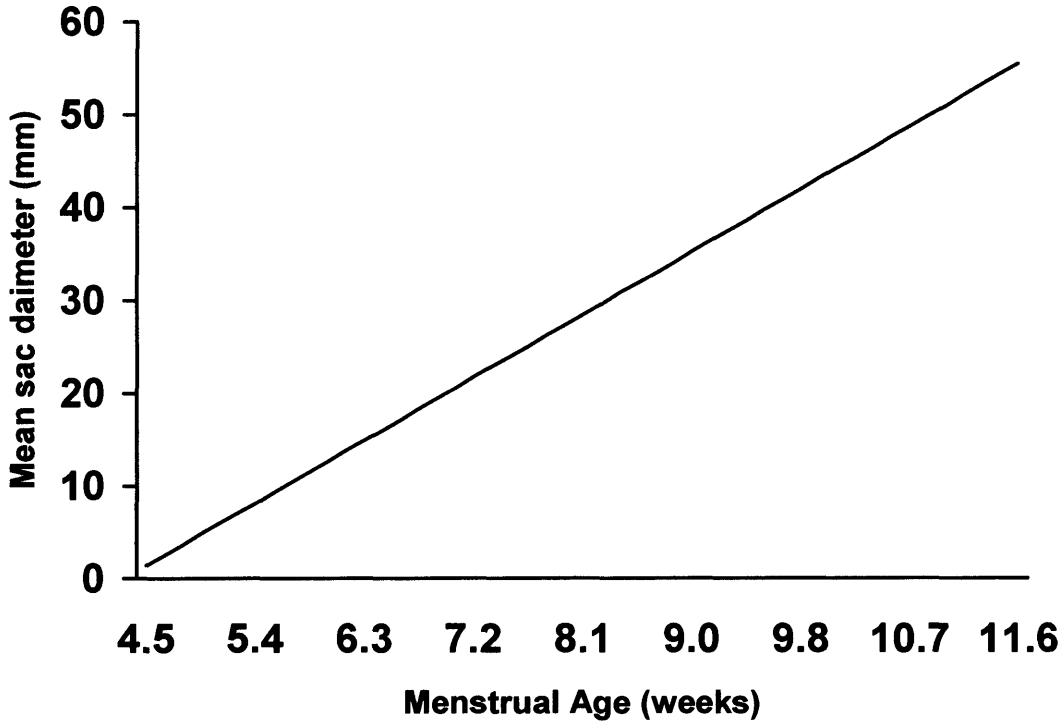


Figure 2.1.3

Gestational sac size correlated with menstrual age during the first 12 weeks (Nyberg et al 1987).

The yolk sac is first seen during the 5^{th} week as a circular, well defined, echo-free area within the gestational sac measuring 3-4mm in diameter. The yolk sac grows slowly until it reaches a maximum diameter of 6 mm at 10 weeks (Jauniaux et al 1991). It is the yolk sac that plays a major role in the nutrition of the early embryo. The yolk sac floats within the excocoelomic cavity and has been shown to have absorptive epithilia. It is thought that nutrients of maternal orign absorbed by the trophoblast pass into the coelomic fluid and are absorbed by the yolk sac (Burton et al 2001).

At the beginning of the sixth week the embryo measures around 2mm and can be demonstrated on transvaginal ultrasound. It is first seen as a straight echogenic line, adjacent to the yolk sac and close to the connecting stalk. The cardiac activity begins at approximately day 37 (5^{+2} weeks) menstrual age (Bree et al 1989). This corresponds to the crown rump length of 1.5-3mm. When the embryo reaches 5mm in length it can be consistently seen separate from the yolk sac and all embryos of that size should have a visible cardiac activity. That equals to a gestational age of 6^{+3} weeks and the sac diameter should measure 15-20mm. The embryo grows around 1 mm per day. The average crown-rump length measures around 12mm at 7^{+3} weeks and 20mm at 9 weeks.

2.2 Miscarriage

2.2.1 Epidemiology and aetiology

A miscarriage is an intrauterine pregnancy that ends spontaneously before the fetus has reached a viable gestational age. This is currently defined by the World Health Organisation as the spontaneous expulsion from its mother of a fetus weighing less than 500grams or before 24 weeks of gestation. A missed miscarriage is defined as an anembryonic pregnancy or where there is early fetal demise but the gestational sac is retained. An incomplete miscarriage is defined as one where part but not all of the products of conception have been passed from the uterus. A complete miscarriage is one where all the products of conception have been passed and the uterus is now empty.

Miscarriage is the most common complication of early pregnancy. It has been estimated that the overall miscarriage rate is around 40% (Wang et al 2003). Even though the majority of these losses occur before the missed menstrual period, bleeding complicates 21% of clinically detected pregnancies and 12-15% are lost (Kline et al 1988, Nybo Andersen et al 2000).

Fetal chromosomal abnormalities are the most common cause of miscarriage (Goddijn & Leschot 2000). Most of these abnormalities are numerical (86%) and a low percentage is caused by structural abnormalities (6%) or other genetic mechanisms, including chromosome mosaicism (8%). There is also extensive data confirming that there is a rising risk of chromosomal abnormalities with maternal age. The risk of spontaneous miscarriage can be seen to rise from 8.7% at the age of 22 years to over 84% at the age of 48 (Nybo Anderson et al 2000).

Maternal health also affects the risk of miscarriage, with diabetes mellitus, primary infections in the first trimester such as listeria, and rubella, and immune diseases such as antiphospholipid syndrome accounting for some miscarriages.

A small proportion of miscarriages may be related to uterine anomalies (Woelfer et al 2001).

2.2.2 Placentation

Spontaneous miscarriage is often characterised by inadequate placentation. Khong et al (1987) and Hustin et al (1990) both showed that by examining the histological specimens of spontaneous miscarriages, defective transformation of the spiral arteries, and a reduced trophoblastic infiltration of the decidua could be seen. Khong et al (1987), however, found that in missed miscarriages where the gestational sac was still growing the uteroplacental circulation was normally developed. Several studies have tried to assess the value of using transvaginal colour Doppler of the trophoblast, uterine and umbilical arteries to predict anembryonic or missed miscarriages (Alfirevic & Kurjak, 1990; Jaffe & Warsof,1992). They have however failed to find a significant difference between normal and anembryonic pregnancies. This is probably because Doppler measurements are an insensitive test to reflect abnormal transformation of the uteroplacental circulation. Jauniaux et al (1994) found that in missed miscarriages the trophoblastic shell was thinner and discontinous, and that the intervillous space and endometrium were massively infiltrated with blood. They suggest that this is due to reduction or absence of vascular plugs allowing free access of blood to the intervillous space with subsequent arrest of embryoplacental flow, or retroplacental haemorrhage and abruption. The mechanical cuase of most miscarriages is thought to be this

premature entry of maternal blood into the intervillous space. The low oxygen state of the early placenta would appear to be necessary for the differentiation of the trophoblast, angiogenesis and protein syntheis and increasing the oxygen flow to the intervillous space would appear to be a factor in early pregnancy failure (Jauniaux et al 2000).

2.2.3 Biochemical Markers

Several studies have examined the dynamics of placental hormones and biochemical markers and miscarriage. Lower levels of βhCG in pregnancies that are destined to fail have been well documented (Lower & Yovich, 1992). As hCG production is directly relate to the amount of trophoblast present it has been suggested that sub-optimal serial changes in βhCG may be a more accurate marker of trophoblast viability (Zegers-Hochschild et al 1994). However at the early stage of a missed miscarriage there may be a temporary rise in hCG and consequently progesterone in a response to stabilize the trophoblast in the presence of intervillous flow (Greenwold et al 2003). Progesterone production in early pregnancy reflects the dynamics of the corpus luteal-trophoblast axis and the status of the trophoblastic tissue. As progesterone has a shorter half life than hCG then the progesterone level will reflect any change in the dynamics of the pregnancy earlier. Progesterone is currently often used to try to reflect pregnancy location rather than viability (Stovall et al 1992, McCord et al 1996), although a number of small studies have also shown lower levels in nonviable pregnancies (Hahlin et al 1991, Cunningham et al 1993, Aksoy et al 1996). Progesterone receptors have been shown to be present in reduced numbers in the trophoblast of women with spontaneous miscarriages as opposed to normal pregnancies although it isn't clear whether this is a primary or secondary event (Hickman et al 2002).

Inhibin A is another marker that is known to reflect both trophoblast amount and the dynamics of the corpus luteum. It has a shorter half-life than either hCG or progesterone and therefore may be more sensitive at reflecting changes in the trophoblast. Lower levels have already been demonstrated in women with biochemical pregnancies and missed miscarriages (Glennon Phipps et al 2000). In women with medical termination of pregnancy inhibin A levels have been shown to fall following administration of misoprostol, which interrupts trophoblastic blood flow and leads to expulsion of the pregnancy (Lahiri et al 2003).

17 OHP is a marker of the corpus luteal –placental axis, and has been shown in one study to be lower in nonviable pregnancies (Check et al 1990). Inhibin pro α C is a product of the corpus luteum and had also been demonstrated to be lower in missed miscarriages. Interruption of the hormonal activity of the corpus luteum by administration of mifepristone in women undergoing medical termination of pregnancy leads to a drop in pro αC levels (Lahiri et al 2003).

IGFBP-1 is known to be associated with the placental-decidual interface and high levels are thought to protect the endometrium from invasion (Irwin & Giudice, 1998). Therefore any disruption in this interface may theoretically be reflected in the levels of IGFBP-1 although this has not previously been examined.

2.2.4 Ultrasound diagnosis of miscarriages

Ultrasound classification of miscarriages has superseded clinical classification, which was based on the amount of bleeding and the state of the internal cervical os on examination (Figure 2.2.1). A miscarriage is classified as one of the following on ultrasound scan; threatened, missed, incomplete and complete. A threatened miscarriage is usually diagnosed in women with a history of vaginal bleeding in whom a live fetus can be visualised on scan. Missed miscarriage is defined as the retention of a gestational sac within the uterus following embryonic or early fetal death. The diagnosis is usually based on the absence of cardiac activity within the fetal pole. When the fetal pole is very small or non detectable it is difficult to differentiate between early normal pregnancy and missed miscarriage. Nobody has prospectively evaluated these criteria.

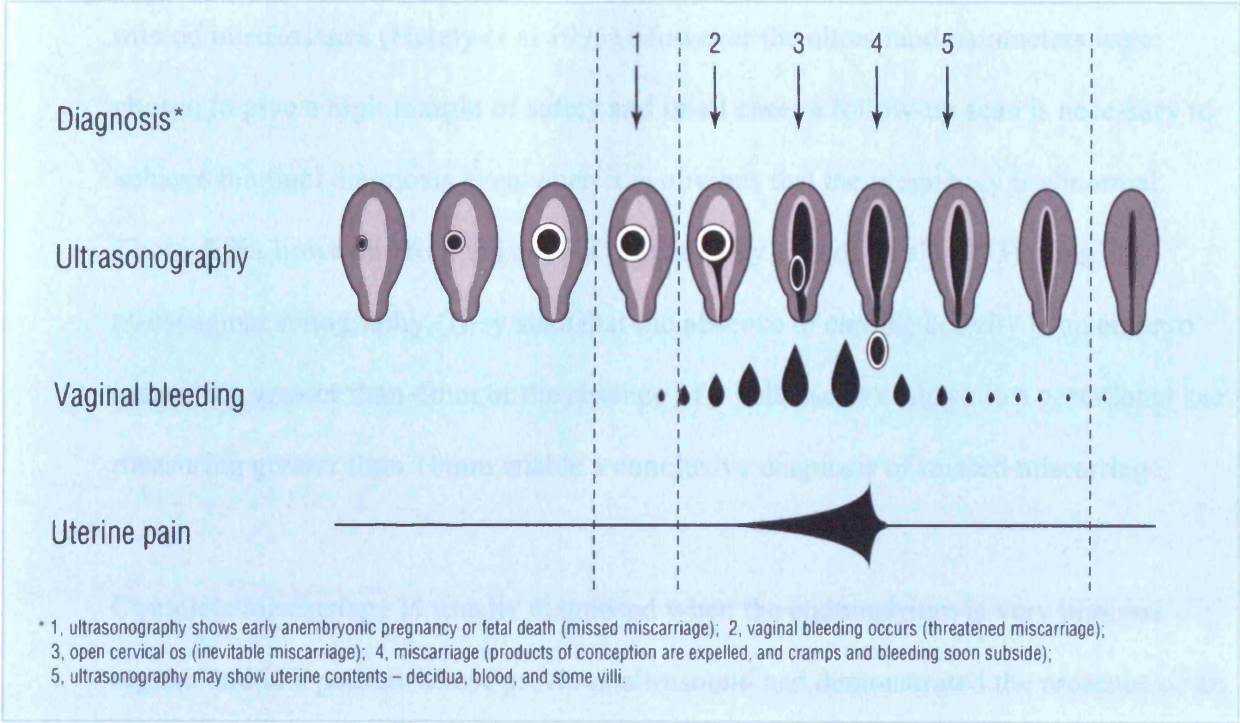


Figure 2.2.1
Natural course of miscarriage, with opportunities for intervention as illustrated by

Ankum et al 2001.

There are widely used guidelines (Table 2.2.1) that have been established to diagnose missed miscarriages (Hately et al 1995). However the ultrasound parameters were chosen to give a high margin of safety and in all cases a follow-up scan is necessary to achieve the final diagnosis even when it is obvious that the pregnancy is abnormal. These differ however from the criteria proposed by Ferazzi et al (1993) using transvaginal sonography. They state that the absence of cardiac activity in an embryo measuring greater than 4mm or the absence of a yolk sac or embryo in a gestational sac measuring greater than 10mm enable a conclusive diagnosis of missed miscarriage.

Complete miscarriage is usually diagnosed when the endometrium is very thin and regular and in a patient whose previous ultrasound had demonstrated the presence of an intrauterine pregnancy. The diagnosis of an incomplete miscarriage is more controversial and diagnostic criteria vary between different studies. The anteroposterior (AP) diameter of the endometrial cavity is the most frequently used criterion but there is no agreement regarding the cut-off level which differentiates an incomplete and complete miscarriage. Henshaw et al (1993) defined an incomplete miscarriage as one were the AP diameter on transvaginal ultrasound scan was greater than 10mm. The two studies from Gothenburg (Nielsen & Hahlin, 1995, Nielsen et al 1996) used a definition of 15-50mm AP diameter. Chipchase and James (1997) used a definition of retained products of conception measuring less than 50mm. However two studies have compared endometrial thickness (measured as the AP diameter of the endometrial cavity) with histological findings and both have found a high percentage of chorionic villi present even if the endometrial thickness were less than 5 mm (Kurtz et al 1991,

Table 2.2.1

Guidelines for establishing the death of an embryo by ultrasound (Hately et al 1995)

Ultrasound findings on initial scan	Action required
Gestational sac >20 mm with no embryo or yolk sac	Repeat scan in one week
Crown-rump length >10 mm with no heart action	Repeat scan in one week
Gestational sac <15mm or crown-rump length <10mm	Repeat scan in two weeks

Rulin et al 1993). Subjective assessment of the endometrium is therefore the preferred way to diagnose an incomplete miscarriage. Retained products of conception are usually seen as well-defined areas of hyperechoic tissue within the uterine cavity as opposed to blood clots which are more irregular.

2.2.5 Management of miscarriages

There are currently three options for the management of miscarriages

Surgical Management

Fifty years ago surgical evacuation of retained products was the universally accepted method of choice for the management of miscarriage. The rationale for the use of this approach was a perceived risk of sepsis and haemorrhage associated with spontaneous abortion. It is likely that a number of complicated miscarriages at that time represented retained products following illegal abortions, which contributed to the severity of clinical presentation. Women's general health has improved considerably since and most infections can nowadays be treated effectively using antibiotics. Legalisation of abortion in the UK has eliminated problems caused by criminal abortion although these remain in many developed countries.

In the last few years there has been a growing concern about the unconditional and non-selective use of surgery for the treatment of miscarriages. Apart from positive trends in the general health of women it has been argued that the number of interventions is increasing due to easier access to health care and improved diagnosis of miscarriages based on the use of transvaginal ultrasound and sensitive pregnancy tests. There is also concern about morbidity caused by surgical and anaesthetic complications.

Medical Management of Miscarriages

A few studies have examined the efficacy of medical treatment for non-surgical management of miscarriages. Medical management of miscarriage is the preferred choice of women over surgical management (Demetroulis et al 2001), and there are cost savings. However no statistical difference in efficacy has been shown between surgical and medical management in incomplete miscarriage (Muffley et al 2002). In women with incomplete miscarriages medical treatment has not been shown to offer any benefits over expectant management (Nielsen et al. 1999). A combination of the antiprogestin mifepristone and prostaglandins is used to treat cases of missed abortion. A group from Aberdeen achieved complete uterine evacuation in 85% -92% percent of cases (El-Refaey et al 1992, Kim & Hinshaw 1997). However, more recent studies reported success rates between 60% and 74% (Muffley et al 2002, Gronlund et al 2002).

Expectant management of miscarriages

Expectant management of miscarriages follows the natural history of the condition, avoids iatrogenic problems associated with both medical and surgical treatment and as such it is likely to be cost-effective. In their recent study Nielsen and Hahlin (1995) showed that 79% of women with clinical and ultrasound diagnosis of incomplete miscarriages had no evidence of retained products when re-examined three days later. These women had intrauterine tissue on ultrasound examination of 15-50mm. In this study women managed expectantly did not suffer an excess morbidity from infection and haemorrhage compared to those randomised to surgical intervention. Luise et al (2002) using a subjective diagnosis of retained products of conception made on

ultrasound scan, showed a 91% success rate of expectant management. Jurkovic et al (1998) studied the outcome of expectant management in 85 women with ultrasound diagnosis of missed miscarriages. Only 25% of women did not require any intervention and had complete miscarriages when followed until up to 6 weeks. The success rate of expectant management within the first week of follow up was only 13%.

Attempts have been made to determine the parameters with which to predict the success of expectant management. Schwarzler et al (1999) examined 108 patients with missed miscarriages, of which 78 underwent expectant management. They used colour Doppler to examine the intervillous blood flow and found that those with intervillous blood flow were more likely to undergo spontaneous miscarriage than those without. By examining the patients characteristics with logistic regression analysis, they showed that both hCG and progesterone had were able to predict spontaneous miscarriages. However only progesterone was statistically significant. Nielsen et al (1996) designed a logistic regression model to identify women with first trimester spontaneous miscarriages suitable for expectant management. They used data from 103 women undergoing expectant management of incomplete miscarriages of which 81 completed spontaneously and 22 went on to have surgical management. Those patients who miscarried completely were more likely to have lower serum progesterone, 17 hydroxyprogesterone, and hCG levels. They also had a significantly smaller intrauterine diameter of retained products of conception. They created a logistic regression model involving progesterone, hCG and intrauterine diameter measured by ultrasound scan. This model had a 98% positive predictive value (PPV) and 44% negative predictive value (NPV) for the probability that no more than 2% of women would undergo surgical management, and a 90% PPV and 67% NPV that 80% of the women with the

highest probability of complete miscarriage were managed expectantly. The time limit allowed for expectant management was only three days.

2.3 Ectopic Pregnancy

2.3.1 Epidemiology and Aetiology

An ectopic pregnancy is defined as implantation of the fertilised ovum outside the uterine cavity; 93% of them are tubal. The incidence of ectopic pregnancies increased from 4.9/1000 in 1970 to 11.1/1000 in 1999 in the UK. There were 13 maternal deaths resulting from ectopic pregnancy in the UK during the period 1997–9 (Confidential Enquiry into Maternal Death, 2001). Nearly 32 000 ectopic pregnancies are diagnosed in the UK annually (Confidential Enquiry into Maternal Death, 2001).

The incidence of ectopic pregnancy is known to rise with maternal age from 1.4% of all pregnancies at the age of 21 years to 6.9% of pregnancies by the age of 44 years (Nybo Anderson et al 2000). Ethnicity is also associated with an increased risk, this being higher in the black population than caucasians (Goldner et al 1993). Meta-analysis of twenty three case controls and one cohort study has shown an increased risk of ectopic pregnancy following pelvic inflammatory disease, especially *chlamydia trachomatis* infection (Ankum et al 1996). Current intrauterine contraceptive device use and sterilisation also carry an increase risk of an ectopic pregnancy.

2.3.2 Placentation

Normal implantation and placentation involves an interaction between a hormonally primed endometrium and the trophoblast. However, the fallopian tube undergoes little cyclical variation and therefore doesn't show any of the changes thought to be necessary for receptivity to the conceptus. Randall et al (1987) examined 105 tubal pregnancies to look at the processes of implantation and placentation in the tube. In the majority of tubal pregnancies there was no histological evidence of any decidual reaction. Normal placentation occurred only in mural or plicomural pregnancies. In the early tubal gestations the development of the placenta was seen as in intrauterine gestations. However in the later ectopic pregnancies the cytotrophoblast infiltrated and colonised the adjacent tubal tissues. Intravascular invasion of the extravillous trophoblast was also seen. However this was notably absent in 16 cases all of which were intratubal abortions. They found that tubal abortion was more likely to be the case in fimbrial and plical implantations where there was inadequate maternal surface for placentation. They also found that abruption was more common due to the fact that trophoblastic invasion occurs in maternal vessels that lack the haemostatic mechanisms within the uterus. Kemp et al (1999) also suggested that the placentation could be the key factor in the difference between viable and nonviable tubal pregnancies. They compared the histology in these two groups and found that the viable ectopic pregnancies were more likely to be implanted on the mesosalpingial side of the tube, that they had deeper trophoblastic invasion and that they had increased villous vascularisation.

2.3.3 Biochemical Markers

Biochemical markers have been used both to diagnose ectopic pregnancy and in its management.

Abnormal implantation leads to reduced levels of βhCG being seen in ectopic pregnancies. Traditionally an ectopic pregnancy is diagnosed if the hCG does not double in 2-3 days as seen in normal intrauterine pregnancies. However, this may also be the case in a failing intrauterine pregnancy. An abnormal doubling time is neither sensitive nor specific method to diagnose ectopic pregnancy (Shepherd et al 1990). Another approach used to diagnose ectopic pregnancy is as serum cut-off level above 1500 IU/l of βhCG at which point an intrauterine pregnancy should be seen by transvaginal sonography. However this fails to take into account the time to return to normal of serum βhCG levels following miscarriage or the diagnostic accuracy of ultrasound in the presence of uterine anomalies such as fibroids (Barnhart et al 1999). The serum level of hCG would appear to be higher in those women with deeper trophoblastic invasion into the tubal wall than in those where the ectopic trophoblast is limited to the lumen or tubal mucosa (Natale et al 2003).

Several studies have demonstrated reduced progesterone and 17OHP levels in ectopic pregnancies (Hahlin et al 1991,Choe et al 1992, Stewart et al 1995). This is thought to be due to abnormal implantation thus affecting the corporea luteal-placental axis (Sauer et al 1988). Progesterone and βhCG may therefore be used together with ultrasound for the diagnosis and management of pregnancies of unknown location as these pregnancies are likely to resolve spontaneously regardless of location (Banerjee et al 1999). Further evidence for the possible influence of lower levels of progesterone in ectopic

pregnancies is shown by Sadan et al (2003) who have demonstrated lower levels of progesterone receptors in the tubes of women with ectopic pregnancies versus non pregnant women. They suggest that this reduction in progesterone receptors leads to impaired transport of the fertilized egg to the uterus.

Only one small study has assessed the value of more novel biochemical markers in the diagnosis of ectopic pregnancy. Illingworth et al (1996) examined eight women with ectopic pregnancies and compared their inhibin A and pro α C RI levels with levels of eight women with ongoing intrauterine pregnancies. There was no significant difference between the two groups.

2.3.4 Ultrasound

Traditionally the findings of a positive pregnancy test and an empty uterus seen at the time of ultrasound scan have been synonymous with the presence of an ectopic pregnancy. However with the use of transvaginal ultrasound approximately 85% of ectopic pregnancies can be visualised directly (Ofili-Yebovi et al 2003). This is commonly in the form of an adnexal mass separate to the ovary.

Visualisation of the ectopic pregnancy is essential not only to facilitate diagnosis, but also to decide upon the best management option. Morphology varies and the relative frequency of different morphological features will depend on accessibility of ultrasound service, quality of equipment and experience of sonographers. The morphology of ectopic pregnancies ranges from an extrauterine gestational sac with a live embryo to an homogenous solid mass (Elson et al 2000). Studies published more than 10 years ago reported that an ectopic gestational sac was the most common appearance of an ectopic pregnancy on ultrasound scan, being present in 66-69% of cases, whilst solid ectopic

pregnancies were found only in 10-21% of cases (Cacciatore et al 1990, Nyberg et al 1991). Recent studies using high-resolution sonography have now shown the most common to be a solid ectopic pregnancy (Figure 2.3.1).

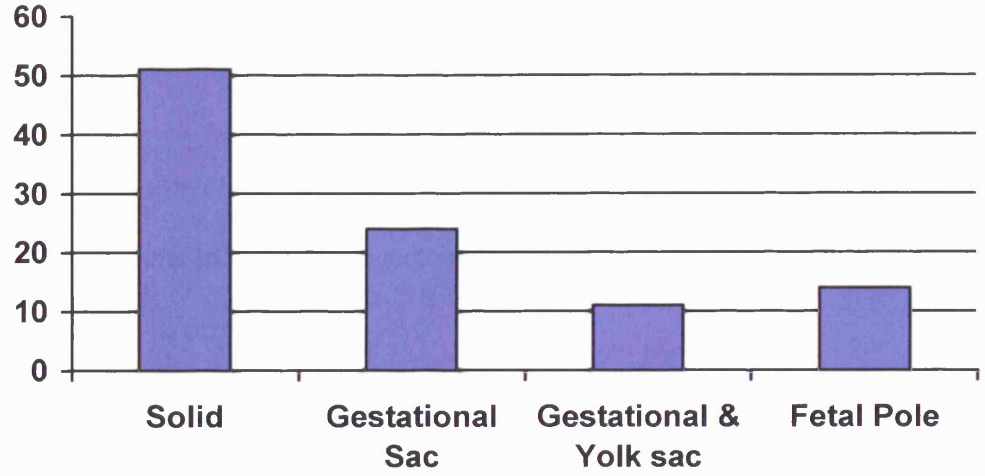


Figure 2.3.1

Frequency of morphological appearance of ectopic pregnancies on scan (Elson et al 2000)

2.3.5 Management of Ectopic Pregnancies

There are three main methods of currently managing an ectopic pregnancy; surgical, medical and expectant.

Surgical Treatment

Surgical options include salpingectomy or salpingostomy, done either as an open procedure or laparoscopically.

Three randomised trials have shown that laparoscopy is superior to laparotomy in haemodynamically stable patients (Vermesh et al 1989, Lundroff et al 1991, Koninckx et al 1991). In the laparoscopic approach there is less blood loss, less analgesic requirement and shorter hospital stays. There is also an economic benefit. Persistent ectopics are thought to be higher in the laparoscopic group than at laparotomy although the numbers are few (Yao & Tulandi 1997). Postoperative follow-up with weekly serum βhCG will enable persistent trophoblast to be detected. If the βhCG levels remain stable or start to rise then further medical treatment or radical surgery needs to be considered. There appears to be no difference in reproductive outcome following salpingostomy whatever the route. The reproductive outcomes of salpingostomy versus salpingectomy would appear to be comparable although the recurrent ectopic pregnancy rate is slightly higher after salpingotomy (Job-Spira et al 1996).

Medical Treatment

Methotrexate is used in the treatment of ectopic pregnancy – both tubal and non-tubal to minimise the need for surgical intervention. Methotrexate is a folic acid antagonist. It inhibits the enzyme dihydrofolate reductase, which reduces dihydrofolate to

tetrahydrofolate, the precursor of coenzymes essential for the formation of purines and pyrimidines, the nitrogen bases of DNA. This leads to interference with DNA synthesis and cell multiplication in the conceptus. Methotrexate may be given either intramuscularly or by direct injection into the ectopic pregnancy. This is either laparoscopically or under ultrasound guidance. The intramuscular dosage is $50mg/m^2$ and the direct injection dosage varies from 25-100mg. Pre-treatment bloods must be taken for liver and renal function tests and platelet count and these need to be repeated 7 and 14 days post treatment. Side effects include gastritis, stomatitis, alopecia, headaches, nausea and vomiting. Disturbances in hepatic and renal function and leukopenia or thrombocytopenia may occur. Success rate for treatment of ectopic pregnancy with methotrexate vary between 64-94% (Stovall & Ling 1993, Stika et al 1996). Serum hCG levels need to be monitored following methotrexate treatment and may actually increase over the first few days, and should then start decreasing until undetectable. There is a lag time of 6-10 days in the reduction of hCG levels in the response to methotrexate by the ectopic trophoblast which accounts for the initial rise seen at the traditional day 4 blood test followed by a decrease on day 7 (Schafer et al 1992). Methotrexate treatment is more likely to be successful if the initial hCG level is low. Treatment failure is also more likely the larger the initial ectopic pregnancy, although there is no absolute agreement about treatment thresholds. There is also evidence to support the use of methotrexate in women with evidence suggestive of persistent trophoblast after unsuccessful salpingotomy (Hajenius et al 1997). Fertility rates following methotrexate therapy are compatible with those following surgical treatment (Bouyer et al 2000).

Expectant Management

It is now well recognised that not all ectopic pregnancies require treatment as some will resolve spontaneously. Expectant management is becoming increasingly important as the ability to detect small ectopic pregnancies and tubal miscarriages increases. It is important that the ectopic pregnancy is actually visualised to avoid mistakenly managing expectantly live or large ectopic pregnancies where the risk of failure is high.

Studies show that around a quarter of ectopic pregnancies will be suitable for expectant management (Ylostalo et al 1992, Elson et al 2004). The selection criteria for expectant management varies but those ectopic pregnancies with a viable fetus or the presence of haemoperitoneum would be considered unsuitable for all but surgical management. In expectant management once the ectopic pregnancy is diagnosed management varies but consists of follow-up with a combination of serial ultrasound scans, hCG and progesterone measurements. The hCG levels are monitored until they drop below 20 IU/l indicating spontaneous resorption of the pregnancy. An increase in the size of the ectopic pregnancy or a rise in the serum hCG levels would be an indication to consider surgery.

Success rates for expectant management vary between 50-100% (Lund 1955, Sauer et al 1987). Fertility rates after expectant management have been examined and patients treated in this way have good long-term fertility outcomes with spontaneous pregnancy rates of around 80% (Carp et al 1986). The risk of repeat ectopic pregnancies is low, around 4%.

Several attempts have been made to examine the clinical, ultrasound and biochemical parameters that can predict the success of expectant management. Fernandes et al

(1988) looked at 14 patients with ectopic pregnancies confirmed by laparoscopy. 64% of these resolved spontaneously. This study found that a serum hCG below 1,000 IU/l appeared to be the best marker for successful expectant management. Garcia et al (1987) reported on 13 women with ectopic pregnancies of less than 4cm in size diagnosed at laparoscopy. Only one case required surgical intervention. They found that serum βhCG, progesterone and estradiol levels were all below the ranges expected for normal pregnancies but did not describe any threshold levels. Shalev et al (1995) examined 60 women with laparoscopically diagnosed ectopic pregnancies. They found that the presenting level of hCG, the rate of fall of hCG and the size of the ectopic pregnancy at laparoscopy were significant factors in predicting successful expectant management. They suggested that using a presenting level of hCG of <2,000 IU/L allowed a 60% success rate.

Ylostalo et al (1992) examined 83 patients and found that around 69% of these or 18% of all ectopic pregnancies resolved expectantly. They also used 4cm as a cut-off for the size of the ectopic pregnancy and included those ectopic pregnancies with a fetal pole and no fetal heartbeat. Whilst they also found that the hCG levels were significantly higher in the group with failed expectant management than those who finally resolved spontaneously, there were cases with high initial values with successful expectant management. No attempt to define whether the morphology of the ectopic pregnancies contributed to the final outcome was examined.

Cacciatore et al (1995) examined the sonographic findings and hCG levels in expectantly managed ectopic pregnancies. They found that 69% of 71 patients had spontaneously resolving ectopic pregnancies. They concluded that whilst initial hCG and size of the ectopic pregnancy did not differ between the two groups, a decrease in

the size of the ectopic pregnancy by day 7 was a significant predictor. Sauer et al (1987) compared the biochemical profiles of spontaneously resolving ectopics, viable ectopics and normal intrauterine pregnancies. They found that hCG, progesterone, 17-hydroxyprogesterone, and estradiol were all significantly lower in ectopic pregnancies. By using a low threshold for progesterone of 4 nmol/l, they found that the ectopic pregnancies with progesterone below this level had a shorter time to resolution. There was a high degree of correlation between 17-hydroxyprogesterone and progesterone. The fall in progesterone and 17-hydroxyprogesterone preceded the fall in hCG levels by 7-29 days.

Of these six studies (Garcia et al 1985, Shalev et al 1987, Sauer et al 1987, Ylostalo et al 1992, Cacciatore et al 1995, Fernandes et al 1998), examining expectant management of ectopic pregnancy, four looked at ectopic pregnancies diagnosed by laparoscopy with declining hCG levels (Garcia et al 1985, Shalev et al 1987, Sauer et al 1987, Fernandes et al 1998). Although two studies (Ylostalo et al 1992, Cacciatore et al 1995) using ultrasound excluded viable ectopic pregnancies, in the others the distinction between viable and nonviable ectopics was made on the finings of declining hCG levels, lack of haemoperitoneum and the size of the ectopic pregnancy. Therefore no further information on the morphology of these pregnancies is available. Only one study (Ylostalo et al 1992) examined the effect of size of the tubal pregnancy on the success of expectant management, the others using a variety of cut-off levels above which surgery was carried out e.g 2, 3, 4 or 5 cm.

Chapter 3: Materials and Methods

3.1 Patient Recruitment

Patients for all these studies were recruited from the Early Pregnancy Assessment Unit of Kings College Hospital during the period February 2000 and November 2001. The Unit serves a racially mixed inner city population with a high level of socio-economic deprivation. This is a tertiary referral unit seeing approximately 2,500 women per year. The unit has an open access policy and additionally sees women referred by their general practioner or hospital consultants. All women are triaged by a nurse and if appropriate undergo a urine pregnancy test (Clearview HCG II^{TM}, Unipath, Bedford, UK). This test is a monoclonal antibody test which according to the manufacturers specifications has a sensitivity of 99% at a urine β-hCG level greater than 25 IU/L. Those women with a positive test then undergo clinical examination, transvaginal ultrasound and biochemical testing as appropriate. Informed written consent was taken from all women prior to inclusion in the studies.

3.2 Ethics Committee approval

Approval for recruitment of women into all studies was granted by Kings College Hospital ethics committee (REC – 00-047 15/2/00 & REC 00-159 27/7/00)

3.3 Ultrasound

3.3.1 *Ultrasound Equipment*

The equipment used was an Aloka ultrasound system with a 5MHz transvaginal probe. (Aloka SSD-5000, Aloka Co. Ltd, Tokyo, Japan). The mechanical index (MI) was continuously displayed during examination and it was always kept <1.

3.3.2 *Ultrasound Method*

All women were scanned in the lithotomy position with a transvaginal probe. The bladder was emptied prior to scanning. The probe was introduced gently into the vagina and the cervix and uterus demonstrated in the sagittal plane. The probe was then rotated through 90° and the uterus examined in the coronal plane from fundus to the cervical region. Whilst in the coronal plane the tip of the probe was tilted to the patients right and the right ovary and adnexa examined in the coronal and sagittal planes. The tip of the probe was then tilted to the other side and the left ovary and adnexa then examined in the same way. Finally the pouch of Douglas was inspected for the presence of free fluid.

3.3.3 *Ultrasound Measurements*

All measurements were done on a frozen ultrasound image with callipers.

The endometrial thickness was measured from a longitudinal image through the thickest area of the endometrium, from the outermost border of the endometrium on one side to that on the other side (Figure 3.3.1).

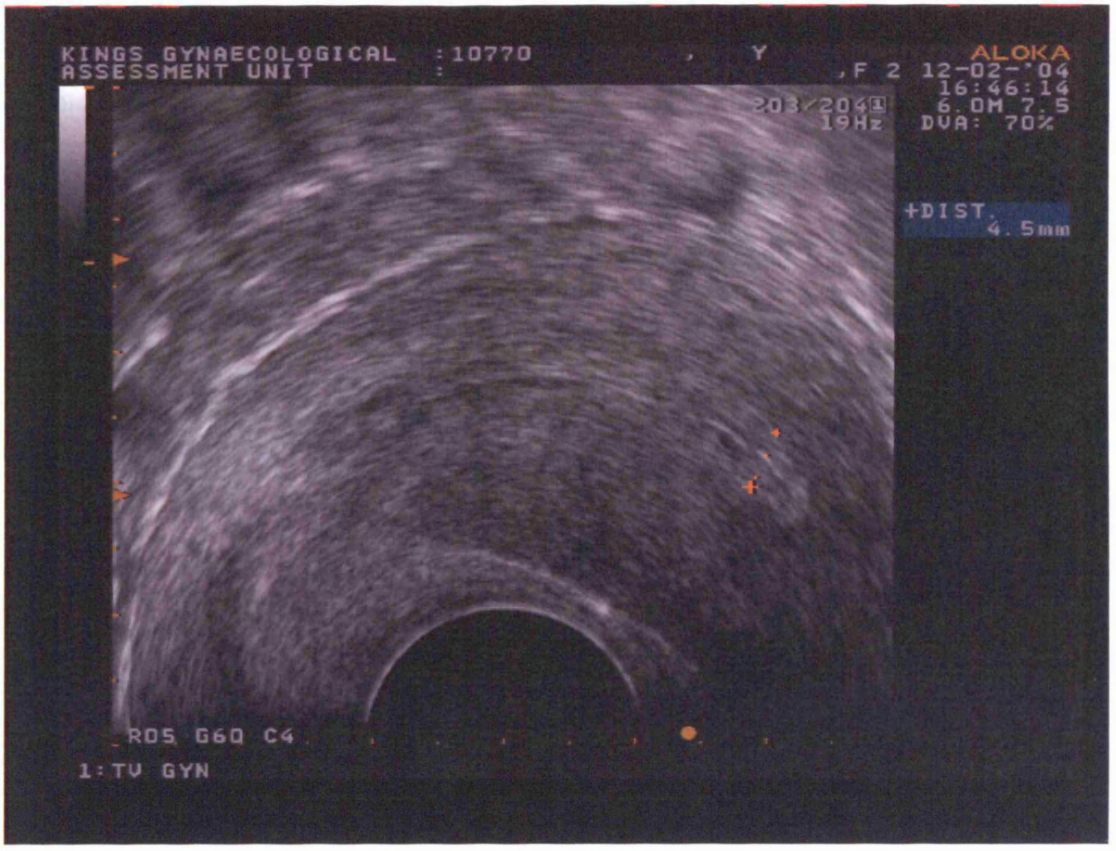


Figure 3.3.1

Longitudinal ultrasound image of the uterus, with the measurement of endometrial thickness from outermost border of the endometrium on one side to that on the other

In the case of an incomplete miscarriage, the intrauterine diameter of the retained products of conception was determined by taking two further measurements in the coronal plane at the thickest area and calculating the diameter as endometrial thickness x diameter 2 x diameter 3 divided by 3 (Figures 3.3.2 & 3.3.3). The diagnosis of incomplete miscarriage for the purpose of this study was made only in those women in whom a previous ultrasound scan had demonstrated an intrauterine pregnancy.

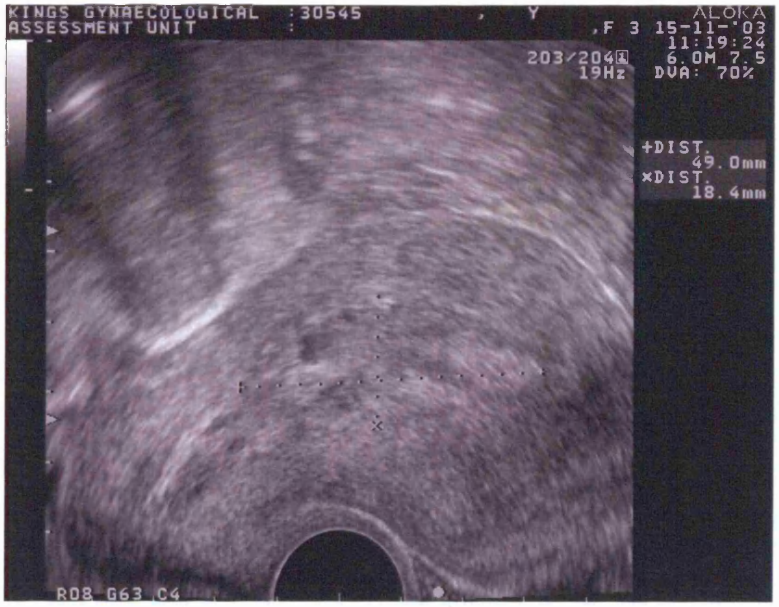


Figure 3.3.2

Ultrasound measurement of retained products of conception – longitudinal section

(Endometrial thickness 18.4mm, diameter 2 49.0mm)

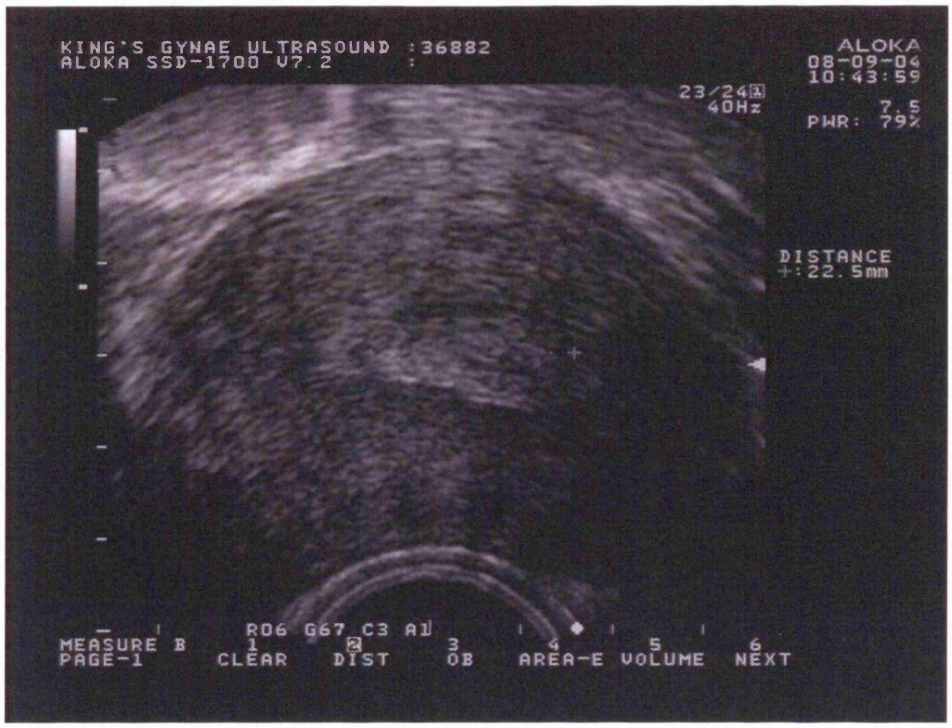


Figure 3.3.3

Ultrasound measurement of retained products of conception –transverse section

(Diameter 3 22.5mm)

Tubal ectopic pregnancies were diagnosed only when there was an adnexal mass with morphological characteristics of an ectopic separate to the ovary and corpus luteum. For ectopic pregnancies the average diameter of the ectopic pregnancy was calculated by measuring the ectopic pregnancy in three dimensions.

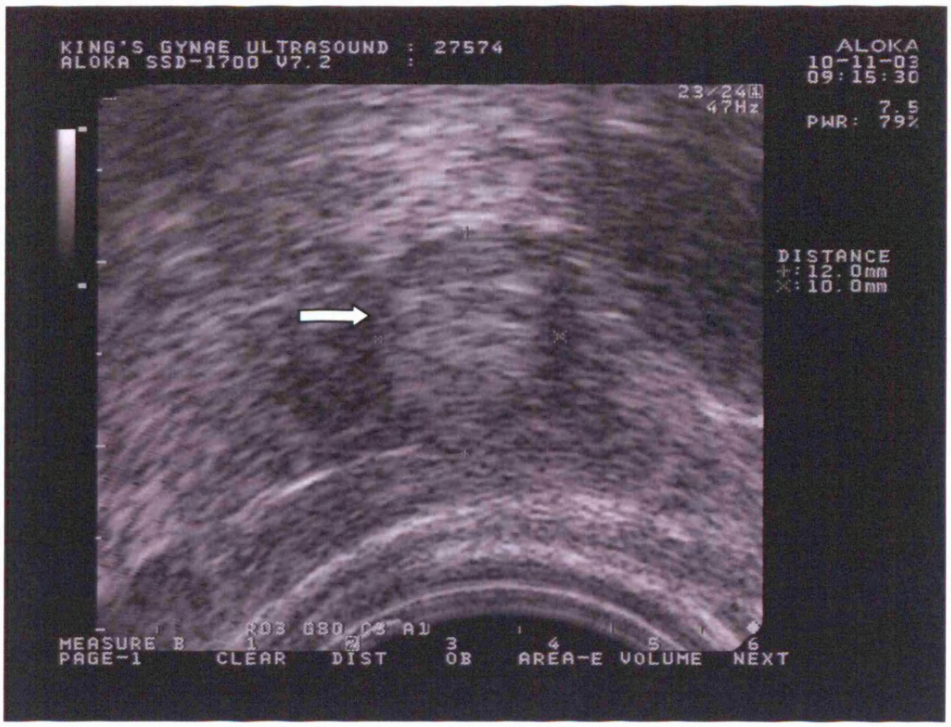
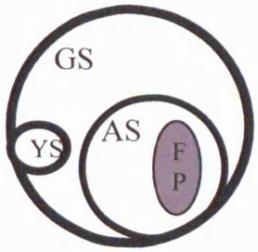


Figure 3.3.4

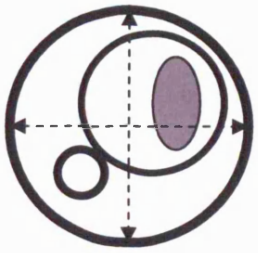
Ultrasound measurements of an ectopic pregnancy showing a solid tubal mass

The morphology of the ectopic pregnancy was classified into four categories: gestational sac with an embryo, gestational sac with a yolk sac, gestational sac with no detectable embryonic structures and homogenous or solid tubal mass.

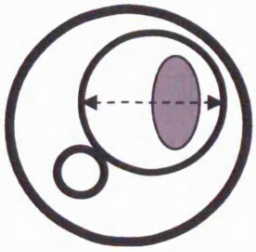
Measurements of intrauterine contents in missed miscarriages or normal pregnancies are demonstrated in Figure 3.3.5.



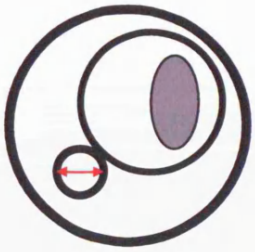
Gestational (chorionic) sac - Measurements should be performed from the inner edges of trophoblast in three planes. The diameters measured correspond to those of the chorionic cavity. The maximum and mean diameters should be recorded. The volume is calculated using formula for ellipsoid
V=AxBxCx 0.523.



Amniotic sac - The three perpendicular diameters should be measured and the mean diameter calculated. As the amnion is very thin the measurements should be taken from the centre of the membrane.



Yolk sac - Three diameters are measured from the outer wall of the yolk sac.



Crown- rump length - In early pregnancy this is the greatest length of the embryo as the crown and rump cannot be distinguished. From 7 weeks onwards the measurement should be taken in the sagittal section, with care taken not to include the yolk sac.

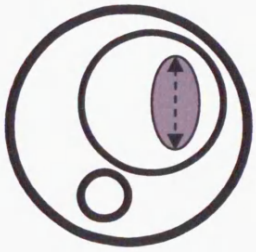


Figure 3.3.5 Schematic representation of measurement of ultrasound images of early pregnancy showing gestational sac (GS), yolk sac (YS), amniotic sac (AS) and fetal pole (FP)

3.4 **Biochemistry**

Blood samples were collected in plain tubes. All blood samples were centrifuged for 10 minutes at 1,000 RPM at room temperature and the serum extracted and frozen at -20 °C. The hCG and progesterone assays for those patients with either miscarriage and ectopic pregnancies were measured immediately. All others samples were frozen for later analysis.

3.4.1 *hCG Assay*

Serum hCG concentrations were quantified using an automated immunoassay technique and expressed in IU/L using the World Health Organisation Third International Reference 75/537. The immunoassay technique used was a heterogenous sandwich magnetic separation assay (MSA). The hCG antibody conjugate (R1) and the hCG antibody conjugate 2 (R2) are reacted with the patient sample and incubated on the Bayer Immuno 1 system at 37 ^{0}C. The monoclonal Immunomagnetic Particle (mIMP) reagent is added and a second incubation period occurs during which the antibody complex is bound. The mIMP/antibody complex is then washed and the para-nitrophenyl phosphate (pNPP) substrate is added. The alkaline phosphatase (ALP) in the antibody conjugate reacts with the pNPP to form para-nitrophenoxide and phosphate. Increasing absorbance due to formation of para-nitrophenoxide is monitored at 405 nm and 450 nm. The dose/response curve will be directly proportional to the hCG concentration in the sample. The inter and intra assay coefficients of variation are less than 10%.

3.4.2 *Progesterone Assay*

Progesterone levels were quantified using an automated immunoassay and expressed in nmol/L. The immunoassay method used is a heterogenous competitive immunoassay. Anti-progesterone antibody (R1) is reacted with the patient sample and incubated on the Bayer Immuno 1 system at 37 °C. Progesterone enzyme conjugate (R2), which competes with the progesterone in the sample for binding sites on the antibody is then added followed by the mIMP. A second incubation occurs during which the antibody/hapten complex is washed and the pNPP substrate is then added. The alkaline phosphatase in the antibody conjugate reacts with the pNPP to form para-nitrophenoxide and phosphate. Increasing absorbance due to formation of para-nitrophenoxide is monitored at 405 nm and 450 nm. The colour production in the reaction is inversely proportional to the progesterone concentration. The inter and intra assay coefficients of variation are less than 10%.

3.4.3 *17 – α –OH progesterone Assay*

This was quantified using an enzyme linked immunoassay (ELISA) (DRG Diagnostics, Germany) and expressed in ng/ml. This ELISA is based on the competition principle and microplate separation. The unknown amount of 17- α-OH progesterone (17OHP) present in the sample and a fixed amount of 17-OHP conjugated with horseradish peroxidase compete for the binding sites of a polyclonal 17OHP antiserum coated onto the wells. After one hour incubation the microtiter plate is washed to stop the competition reaction. Having added the substrate solution the concentration of 17 OHP is inversely proportional to the optical density measured. The intra-assay coefficients of variation were 8.1% at 0.99 ng/ml and 4.3% at 2.34 ng/ml, the inter-assay coefficients of variation were 9.5% at 0.95 ng/ml and 8.0% at 2.24 ng/ml.

Assay Procedure

1. Dispense 25µl of 17 OHP standards (1ml each of 0.15, 0.5, 1.5, 3,7.5, and 20ng/ml) into microtiter wells.

2. Dispense 25µl of sample ito selected wells.

3. Incubate plate for 5 minutes at room temperature.

4. Dipsense 200µl of enzyme conjugate into each well.

5. Mix the plate for 10 seconds.

6. Incubate the plate for 60 minutes at room temperature.

7. Shake out the contents of the wells

8. Rinse the wells with 400 µl each of diluted wash solution (30ml40x wash solution and 1170ml deionised water). Strike the wells nto absorbent paper to remove residual droplets.

9. 200µl of substrate solution to each well added at timed intervals

10. Incubate for 30 minutes at room temperature

11. Add 100µl of stop solution (0.5M H_2S0_4) to each well at the same timed intervals and determine the absorbacne of each well at 450+/- 10nm.

3.4.4 *Inhibin A Assay*

Inhibin A was quantified using a solid phase sandwich ELISA (Oxford Bio-innovation MCA 950 KZZ), and expressed as pg/ml. Before the ELISA, patient samples and standards are pre-treated with detergent 6% Sodium dodecyl-Sulphate (SDS) solution 060, heated to 100°C, and exposed to hydrogen peroxide. The samples and standards are then added to the wells of the microtiter plate. The wells of the microtiter plate are dry-coated with a monoclonal antibody specific for the beta-A subunit of inhibin. Samples are incubated in the wells overnight at room temperature so that the antigen binds to the

'capture' or immobilised' antibody via its beta-A subunit. Following washing of the plate a 'second' or detection antibody is added. This is a monoclonal antibody specific for the alpha subunit of inhibin coupled to alkaline phosphatase. Any unreacted material is then removed by washing before the detection of alkaline phosphatase using a sensitive amplified substrate reaction. This results in a red reaction product with a colour intensity which is directly proportional to the concentration of dimeric inhibin-A present in the original sample. The intra- and inter-assay coefficients of variation are less than 10%.

Assay Procedure

1. Into separate labelled 1.5ml microfuge tubes place 100 µl of each standard dilution (500, 250, 125, 62.5, 31.25, 15.6, 7.8, and 3.9 pg/ml) or patient sample. Into a further tube place 100µl of Foetal Calf Serum as a zero analyte sample.

2. To each tube (standards and samples) add 50 µl of Sodium Dodecyl Sulphate (SDS) 6% solution (SDS950). Mix.

3. Cap the tubes and place in water at 100°C for 3 minutes.

4. Allow to cool and then add 100µl of Dimeric Inhibin Assay Diluent to each tube and mix.

5. To each tube add 50µl of freshly prepared 6% hydrogen peroxide solution and mix briefly

6. Allow the tubes to stand at room temperature for 30 minutes. This pretreatment of the samples modifies, by oxidation, methionine residues in the epitope for the capture antibody, thereby improving the affinity of the reaction.

7. Sometimes the above procedure produces a slight gelatinous precipitate. Displace the material with a pipette tip before proceeding .

8. Add 80µl of each treated sample, in duplicate, to the wells of the plate.

9. Cover the plate and incubate overnight at Room Temperature.

10. To 1 vial of MCA950A, Alkaline Phosphatase conjugated Fab Mouse anti Human Inhibin Alpha Subunit, add 1ml of Dimeric Inhibin Assay Diluent .Replace the top and mix. Remove the contents and add to a further 5mls of assay diluent.

11. Wash the wells of the microtitre plate by filling to the top with diluted Inhibin-A washing Buffer (WB950) and then decanting or aspirating thoroughly. Repeat this procedure a further 3 times. Invert the plate to drain on absorbent paper.

12. Add 50 µl of MCA950A prepared in 10. to each well of the microtitre plate.

13. Cover the plate and incubate at room temperature for 1 hour.

14. Wash thoroughly as in step 11 but with 8 cycles and ending with the wells filled with buffer. Leave the plate to soak for 15 minutes at room temperature whilst preparing the substrate.

15. Prepare the substrate by adding the Substrate Diluent to the Lyophilised Substrate. Mix for 5 minutes.

16. Remove the buffer from the plate wells and further wash the plate for 2-3 cycles. Drain the plate dry by inversion on absorbent paper.

17. To each well of the plate add 50µ l of substrate solution.

18. Prepare the Amplifier by adding the Amplifier Diluent to the Lyophilised Amplifier Mix for 5 minutes.

20. To each well of the plate add 50 µl of amplifier solution. Agitate gently to mix.

21. Cover the plate and incubate at room temperature. Colour will appear quite rapidly. Read the absorbance values of each well at 490nm, preferably referencing at 620nm.

22. Stop the reaction by the addition of 50 µl of STOP solution to each well when the 500pg/ml standard has reached an absorbance of 2.0 (approximately 10-30 minutes depending on ambient temperature).

3.4.5 *Inhibin pro-αC Assay*

Inhibin pro-αC was quantified using a solid phase sandwich ELISA (Oxford Bio-Innovation MCA 1254KZZ) , and expressed as pg/ml. The wells of a microtitre plate come dry-coated with a monoclonal antibody specific for the pro region of the alpha subunit of inhibin. Samples are incubated in the wells so that the antigen binds to the 'capture' or immobilised antibody via its pro region of the alpha subunit. Following washing of the plate a 'second' or detection antibody is added. This is the Fab fragment of a monoclonal antibody specific to the alpha subunit of inhibin coupled to alkaline phosphatase. Any unreacted material is then removed by washing before the detection

of alkaline phosphatase using a sensitive amplified substrate reaction. This results in a red reaction product with a colour intensity which is directly proportional to the concentration of inhibin-pro-αC related materials present in the original sample. The assay has less than 0.1% cross reactivity with inhibin-A, inhibin –B, activin A, Activin B and follastatin.

Assay Procedure

1. Add 50µl of each sample and standard dilution in duplicate, to the wells of the microtitre plate (MCA1254K).

2. Add 50 µl of Assay Diluent in duplicate wells as a zero analyte sample. Cover the plate with a plate sealer and incubate overnight at 4°C.

3. To 1 vial of MCA1254A Alkaline Phosphatase conjugated Fab Mouse anti Human Inhibin Alpha Subunit, add 1ml of Pro-αC Assay Diluent .Replace the top and mix. Remove the contents and add to a further 5mls of assay diluent.

4. Wash the wells of the microtitre plate by filling each well to the top with Pro- αC Washing Buffer allowing to stand for about 15 seconds and then decanting or aspirating each well thoroughly. Repeat this step a further 3 times. Invert the plate to drain on absorbent paper.

5. Add 50 µl MCA1254A prepared in step 3. to each well of the microtitre plate.

6. Cover and seal the plate and incubate at room temperature for 1 hour.

7. Wash as in step 4. but with 8 cycles and ending with the wells filled with buffer. Leave the plate to soak for 15 minutes at room temperature whilst preparing the substrate.

8. Prepare the substrate by adding the Substrate Diluent to the Lyophilised Substrate . Mix for 5 minutes.

9. Remove the buffer from the plate wells and further wash the plate for 2-3 cycles. Drain the plate dry by inversion on absorbent paper.

10. To each well of the plate add 50 µl of substrate solution.

11. Cover and seal the plate and incubate at room temperature for 2 hours.

12. Prepare the Amplifier by adding the Amplifier Diluent to the Lyophilised Amplifier. Mix for 5 minutes.

13. To each well of the plate add 50l µl of Amplifier solution. Agitate gently to mix.

14. Cover the plate and incubate at room temperature. Colour will appear quite rapidly. Read the absorbance values, at 5 minute intervals, of each well at 490nm. Preferably referencing at 620nm.

15. Stop the reaction by the addition of 50 µl of STOP solution to each well when the 200pg/ml standard has reached an absorbance of 2.0 at 490nm (approximately 10-20 minutes depending on ambient temperature).

3.4.6 *IGFBP-1 Assay*

IGFBP-1 was quantified using an immunoenzymetric assay (IGFBP-1 IEMA test, Oxy Medix Biochemica Ab, Finland) and expressed as µg/L. A monclonal antibody specific to human IGFBP-1 is immobilised on microwell plates and another monoclonal antibody also specific to IGFBP-1 is conjugated with horseradish peroxidase (HRP). IGFBP-1 from the sample is bound to the plates. After a washing step, HRP conjugate is added. After a second washing step, enzyme substrate is added. The enzymatic reaction is proportional to the amount of IGFBP-1 in the sample. The reaction is terminated by adding stopping solution. Absorbance is measured on a plate reader at a wavelength of 405 nm. Inter- and intra-assay coefficients of variation were less than 10%.

Assay Procedure

1. Pipette 80µl of assay buffer into each well.
2. Pipette 20µl of standards (0,1,6,30 and 180µg/l), lysophilized control serum and serum samples into appropriate wells.
3. Cover the plate and incubate for 30 minutes at room temperature ona plate shaker
4. Aspirate and wash the wells 3 times with 300µl of washing solution.
5. Pipette 100 µl of enzyme conjugate (mouse monoclonal IGFBP-1 antibody conjugated with horseradish peroxidase) into the wells.
6. Cover the plate and incubate for 30 minutes at room temperature on a plate shaker.
7. Wash the wells 3 times with 300µl of washing solution.

8. At timed intervals add 100µl of horseradish peroxidase substrate solution into each well.
9. Cover the plate and incubate for 10 minutes at room temperature on a plate shaker.
10. Stop the reaction by adding 50µl of stopping solution into each well at timed intervals. Shake the plate to mix the solutions
11. Measure the absorbance at 414nm using a plate reader.

3.5 Statistical analysis

The Statistical Package for Social Sciences, version 11.0 (Statistical Analysis Systems, Chigaco, Illinois) and SPSS AnswerTree version 2.1 (SPSS Inc., Chicago, Illinois) were used for all calculations.

Chapter 4: Prediction of early pregnancy viability in the absence of a visible embryo

4.1 Introduction

Despite its common occurrence, there is no general consensus on the most reliable criteria to diagnose a missed miscarriage. In cases with visible embryonic pole, the absence of cardiac activity enables the conclusive diagnosis to be reached at the initial visit. However, when the embryo cannot be seen on scan, the differential diagnoses include a normal early intrauterine pregnancy of less than six weeks' gestation and early fetal demise, sometimes referred to as an anembryonic pregnancy or an empty gestational sac.

The aim of this study was to establish whether by combining clinical information, ultrasound findings and serum biochemistry, the differentiation between early viable pregnancies and early embryonic demise could be improved to allow a conclusive diagnosis to be reached at the initial visit.

4.2 Subjects & Method

This was a prospective observational study of women with an ultrasound finding of a mean gestational sac diameter <20mm. The validity of the statistical models created from the first study was then assessed using a further group of women with ultrasound finding of a gestational sac <20mm mean sac diameter.

Inclusion criteria included spontaneous conception, single intrauterine gestational sac when scanned, and no history of exogenous progesterone use in the current pregnancy. A full history was documented and a clinical examination carried out by the attending physician. All women had a transvaginal ultrasound scan, and peripheral blood samples to measure serum hCG and progesterone levels. All women had a subsequent follow up scan one to two weeks later. A diagnosis of miscarriage was made if the gestational sac

did not increase in size on follow up ultrasound scans or if the embryo failed to develop. In addition a miscarriage was also diagnosed in women with history of bleeding when the previously detected gestational sac was not visible on subsequent scan. A diagnosis of a viable intrauterine pregnancy was made only when there was an embryo with cardiac activity seen on subsequent scan.

Sample Size

Six variables were used in the regression analysis and therefore the sample size calculated allowed for 10 events per variable ie: at least 60 pregnancies (Altman 1999).

4.3 Statistical analysis

The database established for the aim of the study contained maternal age, date of last menstrual period, the presence or absence of vaginal bleeding (expressed as bleeding score 0 or 1 respectively), mean gestational sac diameter (calculated from measurements taken in three orthogonal planes) and the serum levels of progesterone and hCG.

All statistical analyses were carried out using SPSS version 10 (SPSS Inc., Chicago, Illinois). The outcomes were dichotomised into viable and non-viable pregnancy categories. Comparison of means of continuous variables was performed using Mann-Whitney or Student's-t tests depending on data distribution. Proportions were compared using the Yates corrected χ^2 test. A value of $P < 0.05$ was considered statistically significant.

The multivariate logistic regression analysis was performed with pregnancy viability as the dependent variable. Six independent variables were used for model construction and included maternal age, gestational age, pregnancy sac diameter, serum progesterone

level, serum hCG level, and presence or absence of bleeding (coded 1 and 0 respectively). All variables except the last were continuous.

The objective of the model building process was to obtain a "good fit" for the data, with the least number of independent variables.

The regression equation was derived by the forward stepwise selection of variables using the likelihood ratio test for determining which variables to include in the model.

Using these criteria, three variables were found to be independent with statistically significant coefficients. However, one of these variables (serum progesterone level) was found not to conform to a linear gradient after inspection of interactions using the Box-Tidwell transformation. Further analysis revealed that conformity to the linear gradient could be achieved by using the natural logarithm of the progesterone level in the model building process instead of the progesterone level itself. Interactions between each of the three independent variables were sought and found to be absent. The goodness of fit for the model was tested using the Hosmer and Lemeshow test. A non-significant p-value (0.978) suggested a favourable goodness of fit.

Substitution into the regression model with actual values for each case allowed the calculation of the probability of viability for each individual. Receiver operating characteristic (ROC) curves were then constructed to describe the relationship between the sensitivity and false-positive rate for different values of these probabilities and also for the raw value of serum progesterone. The ideal cut-off for predicting viability was derived from the ROC curve.

Validation of the logistic regression equation was carried out using Microsoft Excel 2000 and SPSS version 11.

The tree-based analysis was carried out using SPSS Answertree version 2.1 (SPSS Inc.Chicago, Illinois). To create the decision rules, the five outcomes were reclassified into three categories. These were viable pregnancy, miscarriage with spontaneous evacuation, and miscarriage requiring evacuation. Therefore, singleton and twin pregnancies were classed as viable pregnancies, complete miscarriages were classified as those with spontaneous evacuation, and incomplete and missed miscarriages as those requiring evacuation.

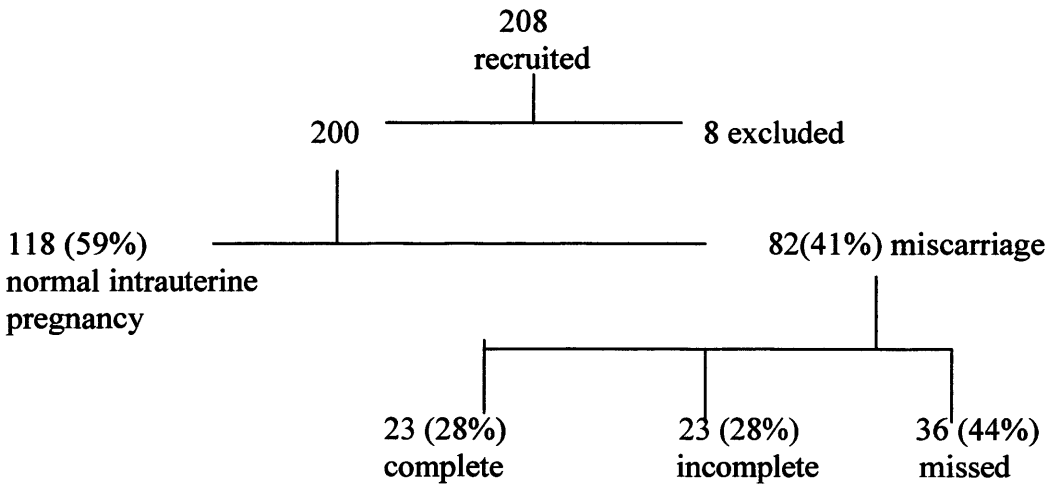
Six independent variables were used for construction of the decision tree and these included maternal age, gestational age, pregnancy sac diameter, serum progesterone level, serum hCG level, and presence or absence of bleeding. All except the last were entered as continuous variables.

Two separate tree-growing algorithms were employed for performing classifications. One tree was 'grown' using the Chi-Squared Automatic Interaction Detector (CHAID) and the other with Classification and Regression Trees (C&RT) method. For the CHAID-based tree, the stopping rules for the iterative process were that the tree should have a maximum of three levels, a minimum of 10 cases were to be present for a split to be calculated and any given split should not generate a group with less than 5 cases. With the C&RT-based tree, the stopping rules were similar except that a maximum of 5 levels were allowed. The accuracy of the models was judged by the risk estimate which gives the proportion of cases classified incorrectly.

Validation of the algorithms was carried out using Microsoft Excel version 2000. This group of women were classified according to the decision rules generated by the algorithms.

4.4 **Results**

208 women with an empty intrauterine gestational sac <20mm in size were identified on ultrasound scan. Data sets were incomplete in eight cases, which were excluded from further analysis.



Of the remaining 200 women 118 (59%) had a normal intrauterine pregnancy and 82 (41%) had a miscarriage on follow-up scans. The average length of follow up was 14 days (SD 7.28) until a final diagnosis was reached. In women with the final diagnosis of miscarriage at follow up visits 23 women (28%) had a spontaneous complete miscarriage, 23 (28%) had an incomplete miscarriage and 36 (44%) had a missed miscarriage. The indications for referral and the ethnic backgrounds of the women in the study are shown in Tables 4.4.1 and 4.4.2.

Table 4.4.1

Indications for referral

Indications for referral	Number of women (%)
Suspected early pregnancy complications	163 (78)
Past history of ectopic pregnancy	10 (5)
Dating scan	35 (17)
Total	208 (100)

Table 4.4.2

Ethnic backgrounds

Ethnicity	Number of women (%)
Caucasian	106 (51)
Black	77 (37)
Other	25 (12)
Total	208 (100)

Table 4.4.3 shows the different variables in women when compared according to outcome. There were significant differences in maternal age, gestational age, incidence of bleeding, gestational sac size and serum progesterone levels between women with viable and non-viable pregnancies at the initial visit.

Table 4.4.3

Comparison of measured variables in viable and non-viable pregnancies.

Variable	Viable pregnancies N= 118	Non-viable pregnancies N= 82	P
Maternal age (yrs)*	29.3 (6.2)	32.3 (7.4)	<0.01
Gestational age (days)*	42.8 (9.8)	59.8 (16.2)	<0.01
Vaginal bleeding (%)**	34.7	76.8	<0.01
Gestational sac diameter (mm)#	6.8 (4.2-8.3)	10.7 (6.0-15.8)	<0.01
hCG (IU/l)#	3974 (1661-8638)	3556 (1000-11083)	NS
Progesterone (nmol/l)#	84 (62 – 109)	31 (19 – 41)	<0.01

*Data distributed normally with values given as the mean and standard deviation; #data distributed non-parametrically with values given as the median (25th to the 75th interquartile range), **discrete data given as percentage of feature for each final outcome.

The regression equation was derived using forward stepwise selection of variables.

Maternal age, gestational sac diameter and serum progesterone were found to be independent with statistically significant coefficients, and were therefore included into a logistic regression model. Natural logarithm of serum progesterone levels was used to achieve conformity to linear gradient. The probability of pregnancy being viable was then calculated using formula:

$$\text{Probability of viability} = 1/(1+e^{-z})$$

where z = (6.091 x ln progesterone) – (0.159 x sac diameter) – (0.164 x maternal age) – 17.435

With this model, at a cut-off value of 10% probability of viability, the diagnosis of a viable pregnancy was made with sensitivity of 99.2% (95% CI 95.8 – 99.97) and specificity of 70.7% (95% CI 61.3 – 78.9). A comparison of ROC curves (Figure 4.4.1 and Table 4.4.4) showed that the logistic regression performs significantly better than all individual parameters except serum progesterone.

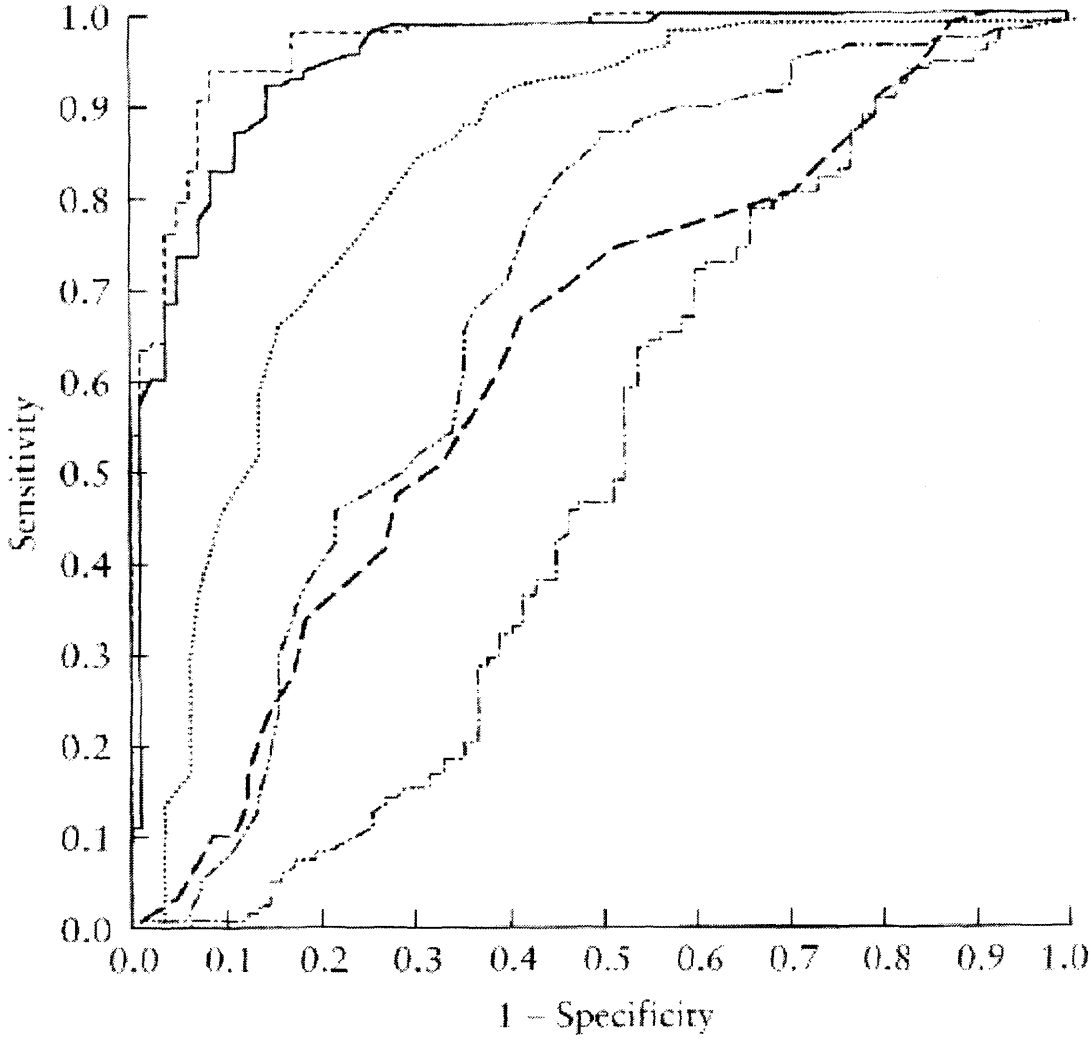


Figure 4.4.1

Receiver-operating characteristics (ROC) curves demonstrating the logistic regression model (----), serum progesterone (—), gestational age (.....), sac diameter (-••-••-), maternal age (----) and serum human chorionic gonadotropin (hCG) (-•-•-) in their ability to predict pregnancy viability.

Table 4.4.4

Comparisons of the diagnostic accuracy of the logistic regression model and individual diagnostic variables for the prediction of early pregnancy viability

Variable	Area under the curve	Standard Error	p
Logistic Model	0.9693	0.0109	NS
Progesterone	0.9493	0.0158	NS
Gestational Age	0.83	0.0316	<0.01
Gestational Sac Diameter	0.7032	0.04	<0.01
Maternal Age	0.6283	0.0408	<0.01
Human Chorionic Gonadotrophin	0.4906	0.0446	<0.01

In order to ensure that no cases of a viable pregnancy were wrongly classified as non-viable, the cut-off value of probability had to be decreased to 1%. At this level the sensitivity of 100% (95% CI 97.5 to 100) is reached, but the specificity decreases to 43.9% (95% CI 34.6 to 53.6). An almost identical result could be achieved by using serum progesterone at a cut-off level of 25 nmol/l. Viable pregnancies could be diagnosed with a sensitivity of 100% (95% CI 96.8 to 100) and a specificity of 40.2% (95% CI 31.1 to 50.0).

Decision tree analysis

Using the CHAID decision tree (Figure 4.4.2) progesterone was the best predictor of pregnancy viability. In women with serum progesterone of greater than 61nmol/l 89/95 (94%) had a viable pregnancy. When progesterone was between 39 and 61nmol/l 27/42 (64%) had a viable pregnancy. In women with a progesterone of between 39 and 61 nmol/l and under 33 years of age 26/32 (81%) had a viable pregnancy. In this subgroup, only 1/9 (11%) of women over 33 had a viable pregnancy. In those women with a progesterone of less than 39 only 2/63 (3%) had a viable pregnancy.

A gestational sac diameter of less than 8 mm meant that 2/26 (8%) had a viable pregnancy; the majority 16/26 (62%) going on to have a complete miscarriage. If the sac size was greater than 8 mm there were no women with viable pregnancies and the majority 33/37 (89%) had incomplete or delayed miscarriages.

The sensitivity of the CHAID tree was 97.5% (95% CI 94.6-100) and the specificity 85.4 (95% CI77.7-93). PPV 90.6 (95% CI 85.5-95.6) (Table 4.4.5)

Using the C&RT decision tree (Figure 4.4.3), progesterone was again the best predictor. In women with progesterone of greater than 38.5nmol/l 117/140 (84%) had a viable pregnancy. Only 1/60 (2%) had a viable pregnancy if the progesterone was under 38.5nmol/l. In this subgroup of women if the gestational sac diameter was less than 10.5mm 18/30 (60%) had a complete miscarriage but 1/30 (3%) went on to have a viable pregnancy. If the gestational sac diameter was greater than 10.5mmthere were no women with viable pregnancies and 28/30 (93%) had incomplete or delayed miscarriages. The sensitivity of the C&RT model was 99.2% (95% CI 97.5-100%) and specificity 72% (95% CI 62-81.7). The PPV was 83.6 (77.4-89.7) (Table 4.4.6)

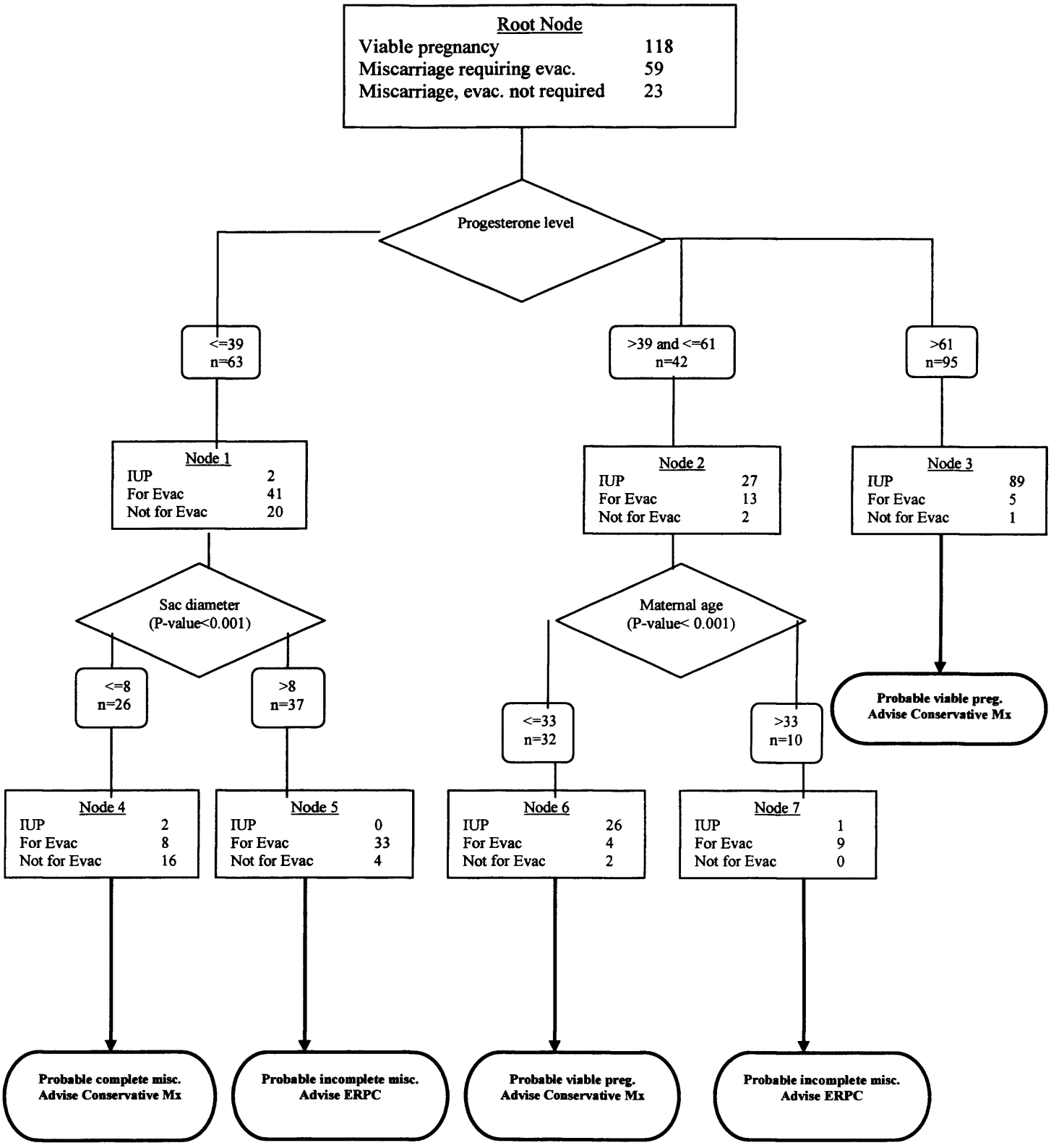


Figure 4.4.2

CHAID decision tree for prediction of pregnancy viability

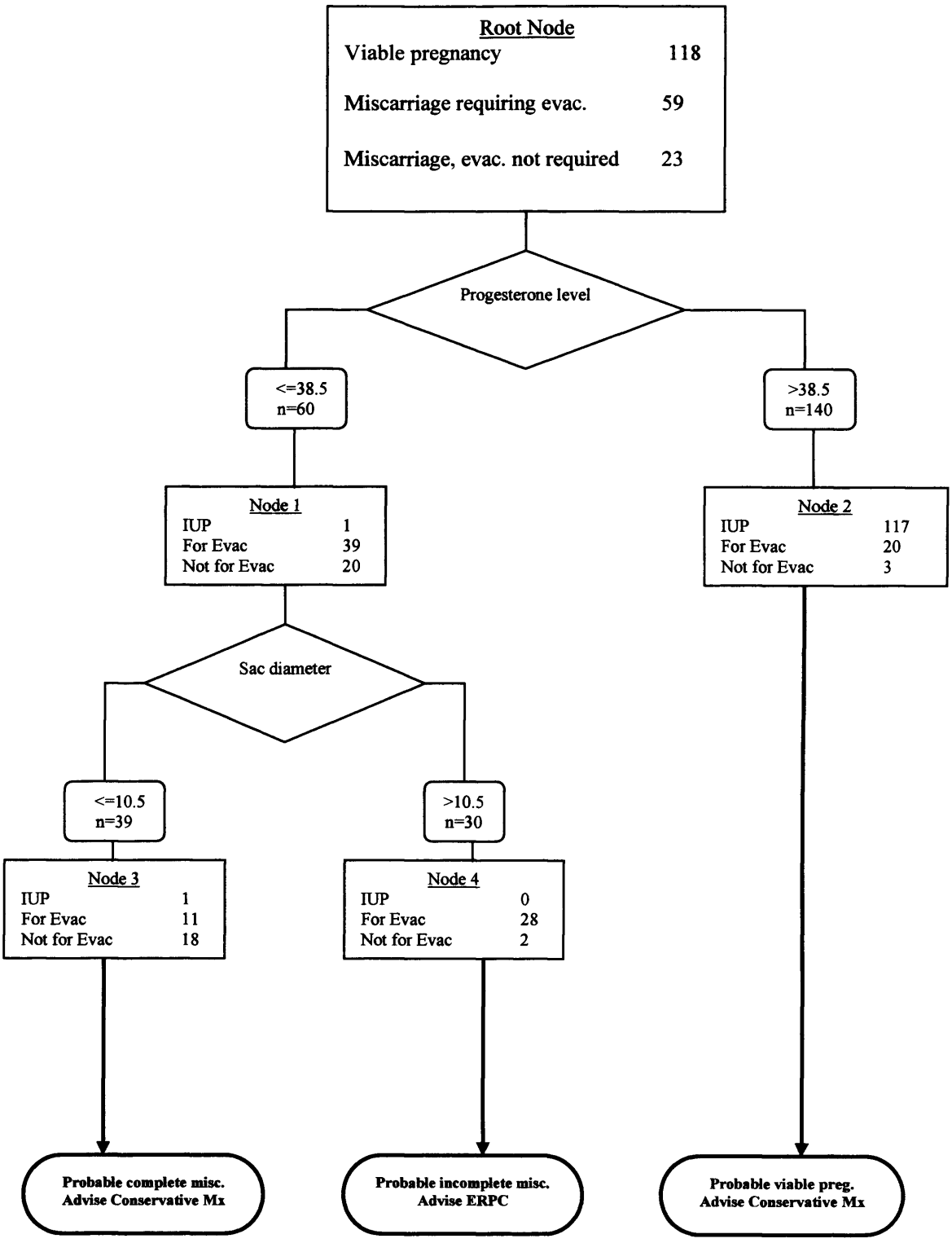


Figure 4.4.3

C&RT decision tree for pregnancy viability

Table 4.4.5

Classification table for the model built with CHAID algorithm.

		Actual Category			
		Miscarriage, ERPC not needed	Miscarriage, ERPC needed	Viable pregnancy	Total
Predicted Category	Miscarriage, ERPC not needed	16	8	2	26
	Miscarriage, ERPC needed	4	42	1	47
	Viable pregnancy	3	9	115	127
	Total	23	59	118	200

Table 4.4.6

Classification table for the model built with C&RT algorithm.

		Actual Category			
		Miscarriage, ERPC not needed	Miscarriage, ERPC needed	Viable pregnancy	Total
Predicted Category	Miscarriage, ERPC not needed	18	11	1	30
	Miscarriage, ERPC needed	2	28	0	30
	Viable pregnancy	3	20	117	140
	Total	23	59	118	200

Prospective testing

Both the logistic regression equation and the two trees were validated on a group of 100 women. Of these women 53 (53%) had a normal intrauterine pregnancy and 47 (47%) had a miscarriage on follow-up scans. The average length of follow up was 10 days (2-28 days) until the diagnosis was reached. In women with the final diagnosis of a miscarriage at follow up visits 17 women (36%) had a spontaneous complete miscarriage, 11 (24%) had an incomplete miscarriage and 19 (40%) had a missed miscarriage. When the logistic regression equation was applied at a cut-off value of 10% probability, the diagnosis of a viable pregnancy was made with a sensitivity of 98.1% (95% CI 94.45 – 100) and a specificity of 63.8% (95% CI 50.9 – 77.54). When the cut-off value of probability was decreased to 1% the sensitivity was 100% (95% CI 97.5 to 100) but the specificity decreases to 40.43% (95% CI 26.4-54.46). By using serum progesterone at a cut-off level of 25 nmol/l, viable pregnancies could be diagnosed with a sensitivity of 100% (95% CI 96.8 to 100) and a specificity of 38.30% (95% CI 24.4- 52.2). Comparing the prospective testing of the logistic regression model with the original model showed that the model performed equally as well on prospective testing (Table 4.4.7)

Table 4.4.7

Comparison of the diagnostic accuracy of the logistic model on prospective testing

Variable	Area under the curve	Standard Error	P
Original logistic model	0.9693	0.0109	NS
Prospective logistic model	0.965	0.018	<0.001

With the CHAID model (Table 4.4.8), 6 viable pregnancies would have been wrongly classified. However with node 4 these would have been in the conservative management group and would have therefore survived. The sensitivity was 88.7% (95% CI 80-97.2) and specificity was 78.7% (67-90.4). The PPV was 82.5 (72.6-92.3).

Table 4.4.8

Effects of the CHAID algorithm on the validation group

		Actual Category			
		Miscarriage, ERPC not needed	Miscarriage, ERPC needed	Viable pregnancy	Total
Predicted Category	Miscarriage, ERPC not needed	10	14	4	28
	Miscarriage, ERPC needed	5	8	2	15
	Viable pregnancy	2	8	47	57
	Total	17	30	53	100

With the C&RT model (Table 4.4.9), only 1 viable pregnancy would have been misclassified and this would have been in the conservative management group and thus survived. The sensitivity was 98.1% (95% CI 94-100), specificity 72% (95% CI 59.6-85.1). The PPV was 80 (70.3-89.7).

The ability of the decision trees to predict pregnancy viability on both the retrospective and prospective studies was then compared (Table 4.4.10)

Table 4.4.9

Effects of the C&RT algorithm on the validation group

		Actual Category			
		Miscarriage, ERPC not needed	Miscarriage, ERPC needed	Viable pregnancy	Total
Predicted Category	Miscarriage, ERPC not needed	13	16	1	30
	Miscarriage, ERPC needed	1	4	0	5
	Viable pregnancy	3	10	52	65
	Total	17	30	53	100

Table 4.4.10

Comparison of decision tree models

Decision Tree	PPV (95% CI)
CHAID Retrospective	90.6 (85.5-95.6)
CHAID Prospective	82.5 (72.6-92.3)
C&RT Retrospective	83.6 (77.4-89.7)
C&RT Prospective	80 (70.3-89.7)

4.5 Discussion

This study showed significant differences in demographic, clinical, biochemical and ultrasound parameters between early viable and non-viable pregnancies in women with an undetectable embryo on ultrasound scan.

Demographic & clinical features in the prediction of pregnancy viability

Significant differences in demographic and clinical features between early viable and non-viable pregnancies in women with an undetectable embryo on ultrasound scan have been shown. As expected, women who suffered miscarriage were older than those with normal pregnancies (Nybo Anderson 2000). In addition, vaginal bleeding was more common and gestational age (by menstrual dates) was longer in non-viable pregnancies. However the usefulness of these two parameters is limited as almost half of all pregnancies complicated by bleeding proceeded normally and the menstrual age is notoriously difficult to ascertain in women with early pregnancy complications (Falco et al 2003).

Biochemistry in the prediction of pregnancy viability

Most previous studies have concluded that total hCG measurements cannot be used to discriminate between viable and non-viable intrauterine pregnancies (Bateman et al 1990). In this study even when the patients were defined by the small size of the gestational sac the hCG levels were not significantly different between viable and non-viable pregnancies, yet this is the most common biochemical parameter in clinical use today.

No viable pregnancies with progesterone levels of less than 25nmol/L were seen in this study. The only pregnancy presenting with progesterone of less than 30nmol/l was found to be chromosomally abnormal and later presented for termination of pregnancy.

Previous studies examining the use of progesterone in the diagnosis of viability have differed significantly in their design, study populations and inclusion criteria. Most importantly a number of studies included patients who conceived following stimulation of ovulation or those receiving luteal support, both of which may affect serum progesterone levels. There are only a few studies, which have assessed the value of progesterone in very early gestations. Riss et al (1989) examined 71 women with a positive urine pregnancy test and an empty sac on ultrasound scan. The lowest recorded serum progesterone amongst the 23 women with normal pregnancies was 38.4 nmol/L (12 ng/ml). Using a threshold of > 48.0 nmol/L (15ng/ml) they could diagnose normal pregnancy with a sensitivity of 87% and specificity of 83%. Hahlin et al. (1995) examined a group of women with very early pregnancies, which could not be detected on ultrasound scan. In 73 pregnancies which progressed normally the lowest progesterone level was 28.8 nmol/L. Previous studies of a similar patient population found that in 57 normal early pregnancies the lowest serum progesterone was 28 nmol/L (Banerjee et al 1999, Banerjee et al 2001).

Ultrasound in the prediction of pregnancy viability

In women without the detection of a visible embryo on ultrasound the gestational sac diameter was found to be of diagnostic value. Although a large empty gestational sac is one of the main criteria for the diagnosis of a miscarriage, in this study, which was limited to a diameter of <20 mm, there was a considerable overlap between viable and non-viable pregnancies. It was therefore impossible to establish a particular cut-off to discriminate reliably between normal pregnancies and miscarriages. This study did not evaluate the validity of using the sac diameter of >20 mm to diagnose miscarriage (Hately et al 1995). This cut-off level has been widely used and it is believed that it includes a high safety margin. However, ultrasound is an operator dependent method and it is conceivable that an inexperienced operator may fail to detect an embryo in a relatively large sac due to a poor examination technique. Other previous studies have suggested that the mean diameter of >18 mm in a woman with an empty sac on the scan may be sufficient to diagnose a miscarriage (Rempen 1990). The results of this study, however, do not support this as three women with viable pregnancies presented initially with an empty sac measuring >18 mm.

Models for the prediction of pregnancy viability

Although serum progesterone is the single most powerful predictor of pregnancy viability, the gestational sac diameter and maternal age both contribute to the accuracy of the logistic model. As a result the logistic model discriminates between normal and abnormal pregnancies slightly better than does serum progesterone alone. In addition the model gives a numerical probability of the pregnancy being viable. When using decision tree analysis for predicting pregnancy viability both models select progesterone as the best predictor using a level of 39nmol/L for the CHAID model and 38.5 nmol/L

with the C&RT model. Below this level there were only 2 viable pregnancies. Both models then use gestational sac diameter in an attempt to refine the diagnosis, the CHAID model using a gestational sac diameter of 8.5 mm and the C&RT model using 10.5 mm. Although both values hold on prospective testing in view of the fact that ultrasound is an operator dependant tool then use of the C&RT model would offer higher safety margins in clinical practise. The CHAID model also uses a further subgroup of women with progesterone between 39 and 60nmol/l and attempts to refine prediction of outcome by using maternal age. However on both retrospective and prospective testing this leads to incorrect classification of viable pregnancies. By using an upper level of 39nmol/l as in the C&RT model 16-20% of women would have to wait longer before their miscarriage was diagnosed but no viable pregnancies would have been incorrectly classified. The C & RT table would appear to be the preferred method, as no inappropriate intervention would have been carried out with this method.

Chapter 5: Prediction of expectant management of ectopic pregnancy using hCG and progesterone

5.1 Introduction

Many of the ectopic pregnancies currently detected would not have been identified in the past and it is likely that many would have probably resolved without any treatment. Despite the increased sensitivity of screening tests for ectopic pregnancy and the detection of very mild forms of this condition all ectopic pregnancies are still perceived both by patients and clinicians as a potentially life threatening condition. As a result some form of therapeutic intervention is routinely used whenever the diagnosis of ectopic pregnancy is made regardless of clinical presentation.

Traditionally ectopic pregnancies have been managed surgically. With the advent of laparoscopic surgery patient morbidity has been reduced with improved long-term reproductive outcomes. In some centres medical treatment with methotrexate is being used in selected cases of ectopic pregnancy.

In recent years there have been few reports on the expectant management of ectopic pregnancy. However, this option is usually limited to a small proportion of ectopic pregnancies and reported success rates vary between different centres. The main difficulty with expectant management is the lack of selection criteria, which reliably predict the likelihood of successful spontaneous resolution of pregnancy.

The aim of this study was to identify clinical, demographic, ultrasound and biochemical factors that may be used to predict successful outcome of expectant management of tubal ectopic pregnancy.

5.2 Subjects & Methods

This was an observational study of women with ectopic pregnancies positively diagnosed on ultrasound scan. Inclusion criteria included spontaneous conception and no history of exogenous progesterone use in the current pregnancy. A full history was documented and a clinical examination carried out. All women underwent a transvaginal ultrasound scan. A diagnosis of tubal ectopic pregnancy was made when a mass with the ultrasound appearances of an ectopic pregnancy was seen in the adnexa, separate to the ovary and corpus luteum. The morphology of an ectopic pregnancy was classified into four categories as defined in section 2.3.4.Women who were either cardiovascularly unstable or who complained of moderate or severe pelvic pain were managed surgically. Other indications for surgery were the presence of a viable ectopic pregnancy and/or haemoperitoneum on ultrasound scan. All other women were offered expectant management. Those who consented to this management option had a blood sample taken for serum hCG and progesterone levels. The results of these investigations were available within two to six hours. Women were then asked to attend for another appointment 48-72 hours later for further measurement of serum hCG and progesterone.

All women were managed on an outpatient basis, but were advised not to travel, to avoid sexual intercourse and to return immediately if they experienced a significant increase in abdominal pain. Follow up continued every 48 hours until serum hCG levels decreased to <20 IU/l. Expectant management was discontinued and surgery undertaken in women who complained of increasing abdominal pain or the serum hCG did not decline on successive measurements.

Sample Size

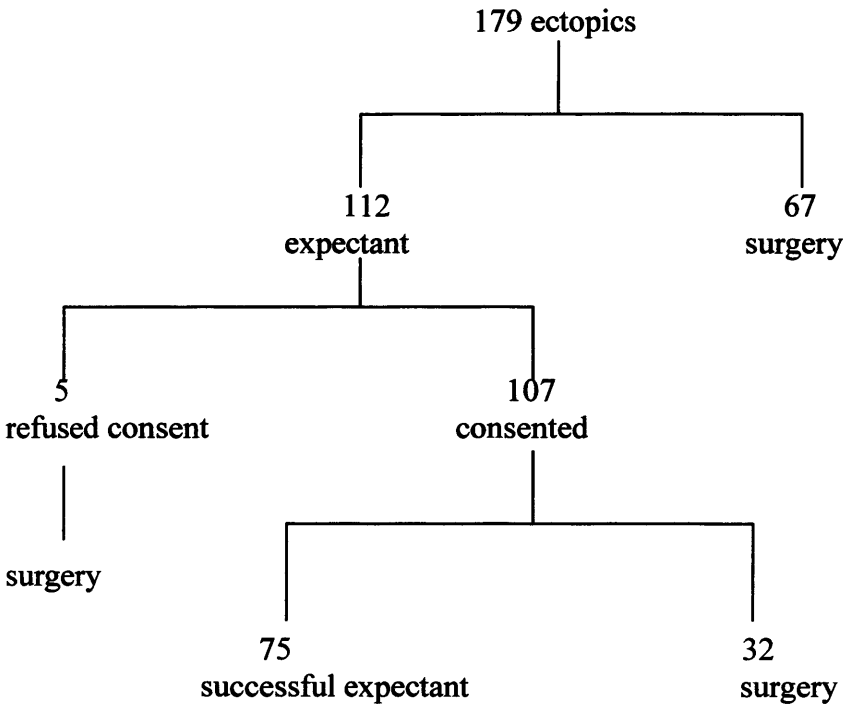
Nine variables were used in the regression analysis and therefore the sample size calculated allowed for 10 events per variable i.e.: at least 90 pregnancies (Altman 1999).

5.3 Statistical analysis

A database was established for the women's age, date of last menstrual period, clinical symptoms (pain and bleeding), size and morphology of ectopic pregnancy, serum hCG and progesterone measurements. The outcomes were dichotomised into expectant and surgical final management categories. Comparison of means of continuous variables was performed using Mann-Whitney or Student t-tests depending on data distribution. Proportions were compared using Yates corrected χ^2 test. A value of $p<0.05$ was considered statistically significant.

A decision tree was developed using the C&RT method. The stopping rules for the iterative process were that the tree should have a maximum of five levels, a minimum of five cases were to be present for a split to be calculated and any given split should not generate a group with less than two cases. These allow sequential analysis of variables to predict whether the final management would be expectant or surgical.

5.4 Results



A tubal ectopic pregnancy was diagnosed in 179 (3.9%) women but only 112 (63%) of these were considered suitable for expectant management; 107 of them consented to take part in the study. The management was successful in 75 (70%) cases, which represented 42% of all ectopics detected during the study period. There were no cases of tubal rupture in women with failed expectant management and none of these patients required blood transfusion. Ultrasound diagnosis of tubal ectopic pregnancy was confirmed in all 104 women who underwent surgery. The ethnic background of these women consisted of 48% Caucasian, 51% Black (Afro-Caribbean, African) and 1% from other ethnic backgrounds.

The pregnancy was removed laparoscopically in 30/32 (94%) women with failed expectant management. The remaining two women had laparotomies. This was similar to the laparoscopic surgery rate of 65/72 (90%) in women who were treated primarily by surgery (P>0.05). Salpingectomy rates in failed expectant and primary surgical group were also similar (24/32 (75%) vs. 53/72 (74%) (p>0.05).

Table 5.4.1 shows different variables in the women when compared according to outcome. There were significant differences in maternal age, initial hCG and progesterone levels in women with ectopic pregnancies that resolved spontaneously compared to those with unsuccessful expectant management

Table 5.4.1

Comparison of measured variables in ectopics managed expectantly and those requiring surgery

Variable	Expectant N=75	Surgical N=32	P value
Maternal Age (yrs) *	30.0 (5.5)	32.5 (5.2)	NS
Gestational age (days)*	44.2 (14.9)	47.8 (19.6)	NS
hCG (IU/l)#	246 (99-536)	628 (254-1402)	<0.01
Progesterone (nmol/l)#	10(6-22)	20(12-31)	<0.01
Largest diameter(mm)#	18(13-21)	16(12-21)	NS
Average diameter (mm)#	15.8 (11.3-17.9)	13 (10.5-16.3)	NS
Pain (%)**	70.7	75.0	NS
Bleeding (%)**	89.3	84.4	NS
Inhomogeneous (%)**	73.3	71.9	NS

* Data distributed normally with values given as mean and standard deviation; # data distributed non-parametrically with values given as the median (25^{th} to the 75^{th} interquartile range), ** discrete data given as percentage of feature for each final outcome

The mean length of follow up in women with failed expectant management was 9 days (SD 5.8), whilst those in whom expectant management was successful were followed for a mean of 15 days (SD 12).

Decision Tree analysis

Initial serum hCG level was the best predictor of the outcome of expectant management (Figure 5.4.1).

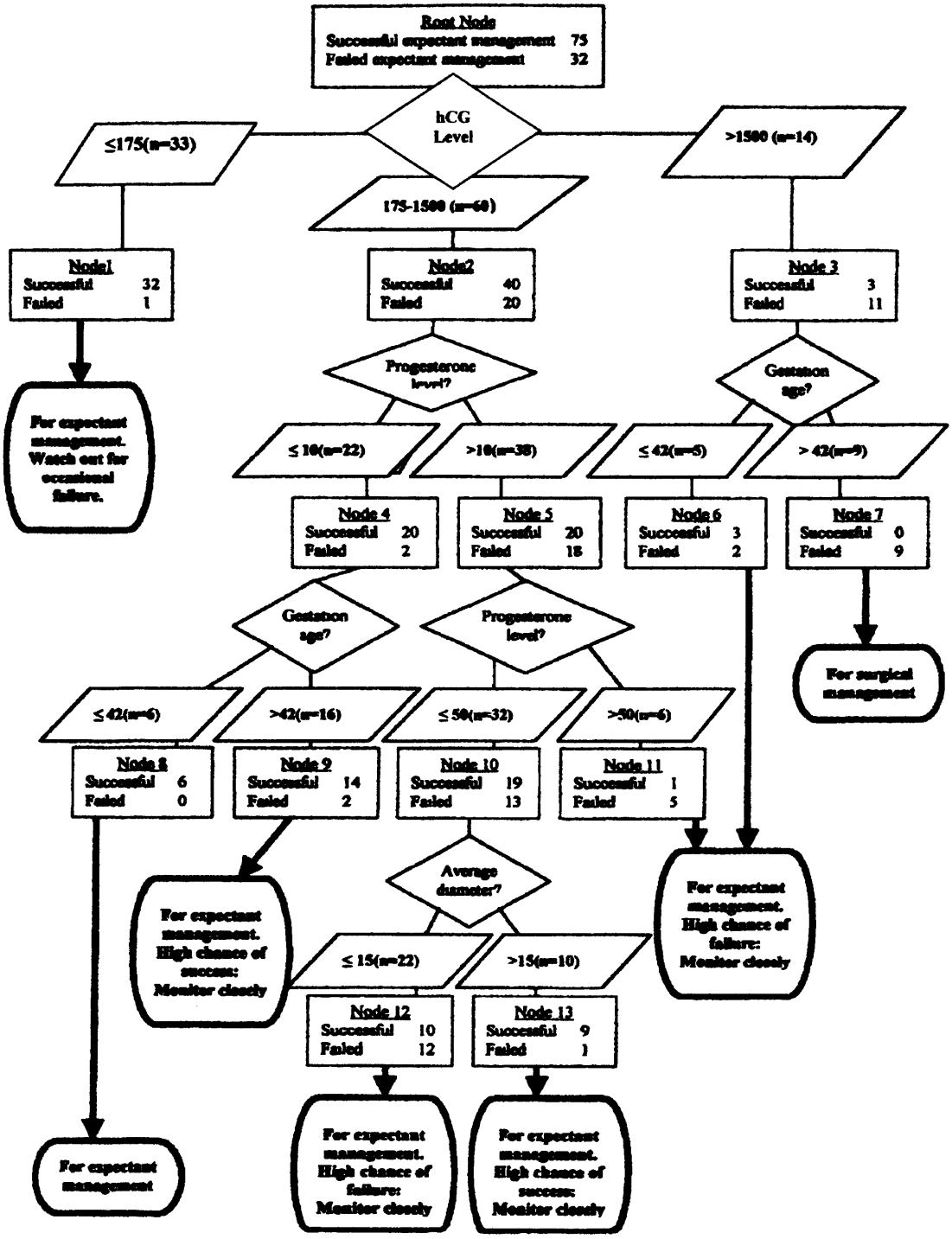


Figure 5.4.1

Decision tree analysis for expectant management of ectopic pregnancy

In women with serum hCG < 175 iu/l the expectant management was successful in 32/33 (96%) of cases. When hCG was between 175 and 1500 iu/l the success of expectant management was 40/60 (66%). In this subgroup of women, expectant management was more likely to be successful if progesterone levels were low (<10 nmol/l), gestational age was less than 42 days and the pregnancy measured >15mm in diameter. In the subgroup of women with initial hCG > 1,500 iu/L the success rate was only 3/14 (27%). Expectant management failed in all women in this subgroup who were more than 42 days pregnant (sensitivity 100% and specificity 28.2% (12.5-43.7)).

The second blood sample was taken from 97 women of the 107 who consented for expectant management. Of those, who did not have a second blood test, 5 were from the group that completed expectant management and 5 from those who eventually had surgery (i.e. expectant management failed). A second assessment was carried out in 4 of those 5 women from the expectant group and they were found to have a negative urine pregnancy test. From the failed expectant and surgically managed group four of those five women without a 2nd blood test had returned within 2 days with pain and were managed surgically. The remaining woman from this group was noted to have a fetal pole 4 days after initial assessment and went straight for surgery.

The mean time from first hCG to the 2nd assessment was 4.18 days (SD 2.6) for the successful expectantly managed group and 3.4 days (SD 2.0) for the failed expectant and then surgically managed group (p >0.05). Of the 97 women whom had a second blood test 70 women had a successful expectant management and 27 required surgery.

Initial hCG was again the most significant indicator with only one woman requiring surgery in the group with an initial hCG of less than 175 iu/l (Figure 5.4.2). The second hCG level in this woman had not fallen. All women whose initial hCG was less than 175 iu/l and in whom the second hCG level had fallen had successful expectant management. Of the women with an initial hCG > 175 whose second hCG had not fallen the majority 18/20 (90%) required surgery. Successful expectant management was more likely in those women with a serum hCG > 175 if the second hCG had fallen, and the average diameter was > 15mm (sensitivity 97.1% (95% CI 93-100) specificity 66.7% (95% CI 48.9-84.4)).

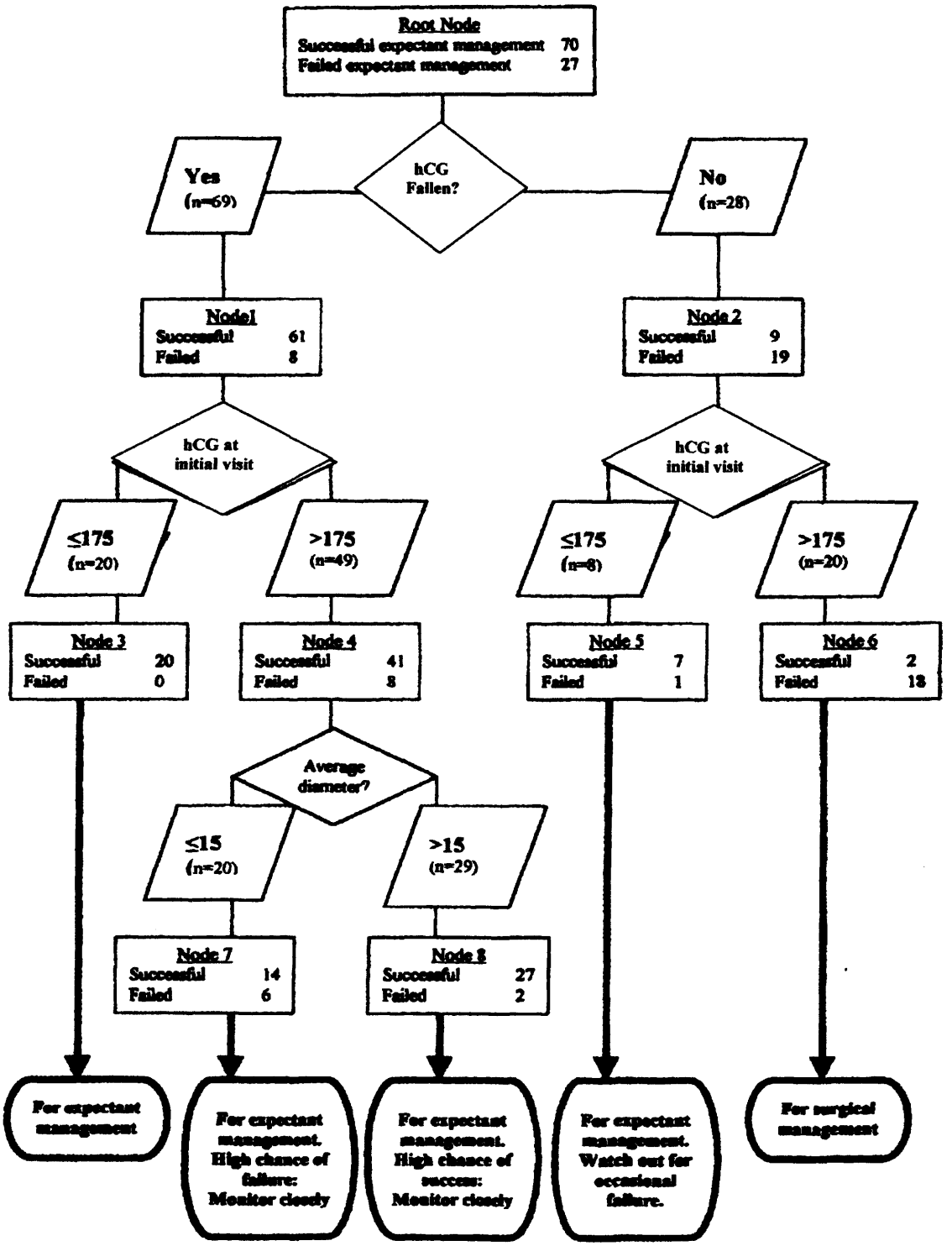


Figure 5.4.2

Decision tree analysis for successful expectant management with second hCG level

Prospective testing

The next 50 women presenting with an ectopic pregnancy suitable to be managed expectantly were used to prospectively test the decision tree analysis. Of those 50 women, 33 (66%) underwent successful expectant management. The decision tree analysis performed well (Table 5.4.2) with 22/28 (79%) of pregnancies identified as having a high chance of resolving spontaneously. Of those pregnancies identified as requiring surgery half were successful with expectant management. Of the pregnancies that were identified as appropriate for expectant management but with a high chance of failure, 10/23 (43%) required surgery (sensitivity 97.1% (95% CI 91-100) and specificity 5.9 (95% CI 0-17)).

Table 5.4.2

Effects of the C&RT algorithm on validation group

		Successful expectant management	Required surgery
Predicted		n=33	n=17
Expectant – High chance of success	Node 1	11	2
	Node 8	3	0
	Node 9	8	1
	Node 13	3	4
Expectant – Moderate chance of failure	Node 12	5	8
Expectant – High chance of failure	Node 11	2	1
Surgery	Node 7	1	1

All 50 women attended for a second appointment although only 39 had a second blood test. Of the 39 women, 23 (59%) had successful expectant management and 16 (41%) required surgery. The decision tree analysis performed well (Table 5.4.3) with 11/15 (73%) predicted as having a high chance of success with expectant management. Of the pregnancies predicted as requiring surgery 16/18 (89%) required surgery. Of the pregnancies that were identified as appropriate for expectant management but with a high chance of failure, 1/10 (10%) required surgery (sensitivity 91.3 (79-100) and specificity 68.8 (46-91.5)).

Table 5.4.3

Effects of the C&RT algorithm on validation group with 2nd hCG level

		Successful expectant Management n=23	Required surgery n=16
Expectant – High chance of success	Node 3	4	0
	Node 5	2	2
	Node 8	5	2
Expectant – High chance of failure	Node 7	10	1
Surgery	Node 6	2	11

The ability of the decision trees to predict successful expectant management from both the initial visit and the 2nd hCG levels on both retrospective and prospective data was compared. Both trees held well on prospective testing (Tables 5.4.4 & 5.4.5)

The positive predictive value of the initial model for the success of expectant management was 81.6% (95% CI 73.5-89.7). In women with a high chance of success, (61/65) the positive predictive value was 93.8% (95% CI 88-99.7) and in women (10/22) with a moderate chance of success it was 45.5% (95% CI 24.6-66.3). The overall positive predictive value in the prospective evaluation set was 69.6% (95% CI 56.3-82.9). In the 32 women with high chance of success the positive predictive value was 78.1% (95% CI 63.8-92.4) and in the 13 women with a moderate chance of success it was 41.7% (95% CI 13.8-69.6).

Table 5.4.4

Comparison of decision tree models for initial hCG level

Expectant management		Predicted success (retrospective data)	Actual success (prospective data)
High chance of success	Node 1	32/33	11/13
	Node 8	6/6	3/3
	Node 9	14/16	8/9
	Node 13	9/10	3/7
Moderate chance of failure	Node 12	10/22	5/13

Table 5.4.5

Comparison of decision tree models for 2nd hCG level

Expectant management		Predicted success (retrospective data)	Actual success (prospective data)
High chance of success	Node 3	20/20	4/4
	Node 8	27/29	5/7
	Node 5	7/8	2/4
Moderate chance of failure	Node 7	14/20	10/11

The positive predictive value of the 2nd hCG model for the success of expectant management was 88% (CI 81-95.5). In women with a high chance of success the positive predictive value was 94% (95% CI 88.9-100) and in women with a moderate chance of success it was 70% (95% CI 49.9-90). The overall positive predictive value in the prospective evaluation set was 80% (95% CI 65-95). In women with high chance of success the positive predictive value was 73% (95% CI 51-95.7) and in women with a moderate chance of success it was 90.9% (95% CI 73-100).

5.5 Discussion

Biochemistry in the management of ectopic pregnancies

The initial serum hCG was the best predictor of the final outcome of expectant management of ectopic pregnancies, with the probability of spontaneous resolution decreasing with increasing hCG levels. In women with low levels (<175 IU/L) the spontaneous resolution rate was 96%. This decreased to only 27% in women with a serum hCG >1,500 IU. Similar findings were reported by Korhonen et al (1994) who found that 88% of ectopics with initial hCG <200 IU/1 resolved spontaneously, compared to only 25% when hCG was >2000 IU/1.

However, the majority of ectopics in this study presented with hCG values between 175 and 1,500 IU/1. The success rate of expectant management in this subgroup was 66%, indicating the need for more refined selection criteria. The addition of serum progesterone <10 nmol/1 at the second level of the decision tree identified 30% of ectopics, which are very likely to resolve spontaneously. On the other hand a high serum progesterone >50 nmol/1 indicates that serum hCG levels are

rising, and consequently the chance of spontaneous resolution is slim. This is in agreement with a previous report by Ransom et al (1994) who also showed that ectopic pregnancies with serum progesterone <10 ng/l are more likely to be successfully treated by systemic methotrexate injection. Low serum progesterone is a reliable indicator of a failing pregnancy and tubal rupture is unlikely to occur in these cases (Banerjee et al 2001). Occasionally a tubal miscarriage may cause intra-abdominal bleeding requiring surgical treatment, which emphasises the need for follow up until serum hCG declines to non-pregnant levels. Sowter et al (2001) also showed that the initial serum βhCG level was the main determining factor of successful treatment with systemic methotrexate. In a group of women with βhCG >1,500 IU the success of methotrexate was 30%, which is only slightly better than the 21% success rate of expectant management in the same subgroup of patients in this study.

These results indicate that the improvement due to methotrexate to the successful treatment of ectopic pregnancies may be more limited than previously thought.

Medical treatments may be unnecessary in tubal miscarriages that present with very low hCG levels and it is probably not effective enough in ectopic pregnancies with the initial hCG levels >1,500 IU/1. However, the treatment may be helpful in women who present with medium hCG levels, which fail to decline on expectant management

Ultrasound in the management of ectopic pregnancy

In this study 40% of all tubal ectopic pregnancies detected during the study period resolved spontaneously on expectant management. This is significantly better than most previous studies, which reported overall success rates between 7 and 25% (Sauer et al 1987, Fernandez et al 1988, Makinen et al 1990, Ylostalo et al 1992). The difference may be as a result of more liberal selection criteria in this study, which did not include predefined cut-off levels for the size ectopic pregnancy or serum hCG measurements. As a result 63% of women with tubal ectopic pregnancies were considered suitable for expectant management compared to only 7-33% in earlier studies. However, these results were similar to those of Trio et al (1995) who also selected 63% of women with ectopic pregnancies for expectant management achieving 75% success rate. The overall spontaneous resolution rate was 44%. However, 39% of ectopic pregnancies in their study were not positively identified on ultrasound scan and therefore there is a possibility that some women with miscarriages of intrauterine pregnancies could have been treated as ectopic pregnancies contributing to the high resolution rate.

The high success of expectant management in this study may also be a result of the increased ability to detect early tubal miscarriages, which have a better chance of spontaneous resolution. Studies published more than 10 years ago reported that an ectopic sac was the most common appearance of an ectopic pregnancy on ultrasound scan, being present in 66-69% of cases, whilst solid ectopic pregnancies were found in only 10-21% of women (Cacciatore et al 1990, Nyberg et al 1991). In contrast, in this study the relative frequency of a tubal gestational sac was only 37%, whilst the solid

foci of ectopic trophoblast were the most common morphological presentation of ectopic pregnancy, accounting for 51% of cases. These pregnancies can only be detected by high-resolution transvaginal sonography, which has only recently become widely available.

It is likely that similar pregnancies were missed in the past and often managed as miscarriages of intra-uterine pregnancies. In all cases in this study, which required operative treatment, ultrasound diagnosis of ectopic pregnancy was confirmed at surgery. This shows that morphological features of ectopic pregnancy on ultrasound are specific enough to achieve a high level of diagnostic accuracy.

Models in the management of ectopic pregnancy

By combining different parameters, women with ectopic pregnancies, can be divided into four subgroups on the initial visit, which are all characterised by different probabilities of success or failure of expectant management. These subgroups consist of those women suitable for i) expectant management, ii) expectant management with high chance of success, iii) expectant management with high chance of failure, iv) surgical management. The prediction of outcome with a probability of >88% could be made in three out of four subgroups, which accounted for 69% of the study population. Furthermore in those women who attended for a second visit the prediction of outcome could be made with a probability of >88% for 3 out of the 4 groups accounting for 79% of the study population. This proved to be true on prospective testing.

Chapter 6: Prediction of expectant management of miscarriage and ectopic pregnancy using novel biochemical markers

6.1 Introduction

Evacuation of retained products of conception by operation has been the standard procedure for patients with an incomplete, or missed miscarriage over the last 60 years. However this is not without risks such as haemorrhage, and infection as well as the morbidity from general anaesthesia. More recently medical management of miscarriages has been described using antiprogestins and prostaglandins but these drugs are not without side effects. Expectant management of miscarriages has been shown to be effective and safe but with success rates varying from 25-90%. Traditionally ectopic pregnancies have also been managed surgically. With the advent of laparoscopic surgery patient morbidity has been reduced with improved long-term reproductive outcomes. In some centres, medical treatment with methotrexate has been used in selected cases of ectopic pregnancy.

In recent years there have been few reports on the expectant management of ectopic pregnancies. This option however, is usually limited to a small proportion of cases. The main difficulty with expectant management of both ectopic pregnancies and miscarriages is the lack of robust selection criteria, which reliably predict the likelihood of successful spontaneous resolution of the complications of pregnancy.

Over the last 5 years several new biochemical markers of the luteal-trophoblastic axis have been described. These markers may be useful in monitoring this axis in pregnancy.

The aim of this study was therefore to establish whether by combining clinical information, ultrasound findings and these newer markers could enable the successful outcome of expectant management of ectopic pregnancies and miscarriages to be predicted.

6.2 Study Design

This was an observational study of women with a diagnosis of miscarriage or ectopic pregnancy identified on ultrasound scan.

A diagnosis of a missed miscarriage was made based on one of the following criteria (i) the gestational sac was greater than 20mm in diameter with no intrauterine contents; (ii) the fetal crown rump length was greater than 5 mm with no fetal heart rate detected; (iii) the gestational sac had failed to develop from a previous scan more than 7 days ago.

An incomplete miscarriage was diagnosed in women with a history of bleeding in whom an intrauterine gestational sac had been previously seen on scan. The criteria to diagnose incomplete miscarriage were absence of an intact gestational sac and the presence of any amount of visible trophoblast in the uterine cavity. The sonographic features of visible trophoblast are irregular echoes in the midline of the uterine cavity.

An ectopic pregnancy was diagnosed when an adnexal mass separate to the ovary and corpus luteum was identified. Morphology was classified in four different categories (see section 6.3.3). Those women who were cardiovascularly unstable or who complained of significant pain or bleeding were managed surgically. Women with

ectopic pregnancies which were viable or who had haemoperitoneum were also managed surgically. All other women were offered expectant management.

All women had bloods taken for serum hCG and progesterone levels, and the new serum markers inhibin A, inhibin pro α-C, IGFBP-1, and 17 OHP.

Those women with miscarriage were asked to attend 7 days later for a urinary pregnancy test. If this was positive or if there was continued bleeding then a further transvaginal ultrasound was carried out. A complete miscarriage was diagnosed if the pregnancy test had become negative and the bleeding was settled. Those women whose miscarriage was not complete at the second visit were offered surgery or the chance to continue with expectant management. All women continuing with expectant management were followed up until the miscarriage was complete or they attended for surgery. Those women consenting to expectant management of ectopic pregnancy had a blood sample taken for baseline measurement of serum hCG and progesterone levels as well as serum 17 OHP, inhibin A, inhibin pro α-C and IGFBP-1. The results of the hCG and progesterone were available within six hours. Women were then asked to attend for another appointment 48-72 hours later for further measurement of serum hCG and progesterone. Follow up continued until serum hCG levels fell below 20IU/l. Expectant management was discontinued if the women complained of increasing abdominal pain or hCG did not decline on follow up measurements.

Sample Size

Ten variables were used in the decision tree analysis and therefore the sample size calculated allowed for 10 events per variable i.e.: at least 100 pregnancies (Altman 1999). However only 85 women were recruited within the study time period. This was reflected in the type of statistical analysis used.

6.3 Statistical analysis

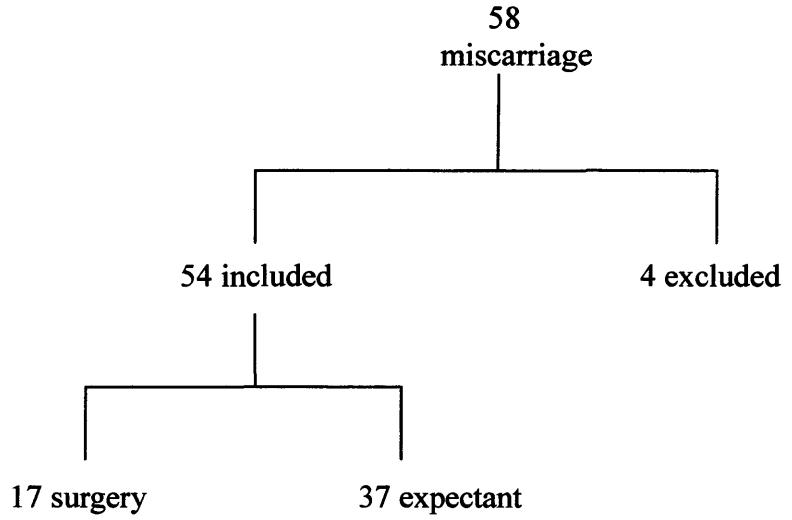
A database was established and the variables recorded included maternal age, date of last menstrual period, the presence or absence of vaginal bleeding (expressed as bleeding score 0 (absent) or 1 (present)), mean diameter of products of conception (calculated from measurements taken in three orthogonal planes) and the serum levels of progesterone, hCG, 17 OHP, inhibin A, pro αC inhibin, and IGFBP-1. All statistical analyses were carried out using SPSS version 10 (SPSS Inc., Chicago, Illinois). The outcomes were dichotomised into successful and failed expectant management categories. Comparison of means of continuous variables was performed using Mann-Whitney or Student's-t tests depending on data distribution. Proportions were compared using the Yates corrected χ^2 test. A value of $P < 0.05$ was considered statistically significant.

Data were analysed using decision tree analysis. This tree-based analysis was carried out using SPSS answerTree version 2.1 (SPSS Inc., Chicago, Illinois). Twelve independent variables were used for construction of the decision tree and these included maternal age, gestational age, diameter of products of conception, serum progesterone level, serum hCG level, serum inhibin A level, serum 17 OHP level, serum pro α C inhibin level, serum IGFBP-1 level, pregnancy type, presence or absence of pain and

presence or absence of bleeding. All except the last three were entered as continuous variables.

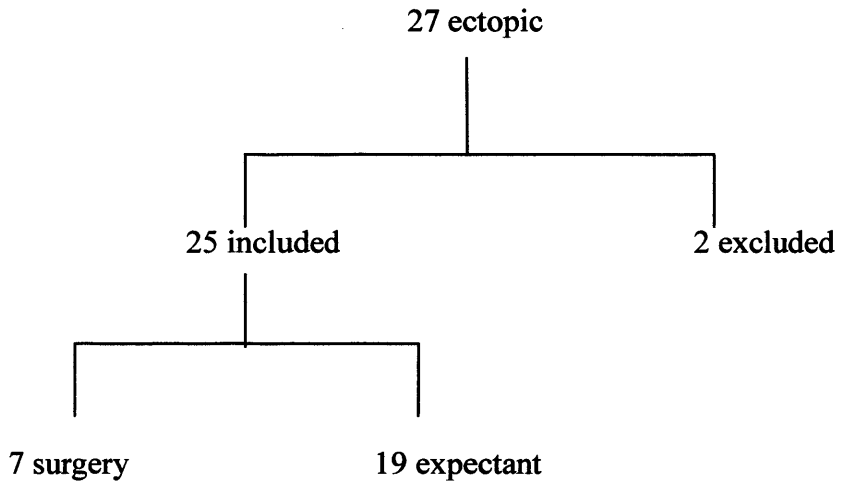
Two decision trees was developed using the Classification and Regression Trees (C&RT) method. The stopping rules for the iterative process were that the tree should have a maximum of five levels, a minimum of five cases were to be present for a split to be calculated and any given split should not generate a group with less than two cases. This allows sequential analysis of variables to predict whether the final management would be expectant or surgical. The first model regarded all pregnancies as one type of failing pregnancy. The second model differentiated in terms of type of pregnancy.

6.4 Results



Fifty eight women with a diagnosis of miscarriage were identified on ultrasound scan. Four women were lost to follow up and were therefore excluded from further analysis. The ethnic background of these women consisted of 54% Caucasians, 44% Blacks (Afro-Caribbean, African) and 1% from other ethnic backgrounds.

Of the remaining 54 women, 17 (31%) required surgery and in 37 (69%) had successful expectant management. The mean time to pregnancy resolution was 8.5 days (SD 4.5). An initial diagnosis of an incomplete miscarriage was made in 32 (60%) women and of a missed miscarriage in 22 (40%).



Twenty seven women had an ectopic pregnancy diagnosed on ultrasound scan. Of these, two were lost to follow up and were therefore excluded because of incomplete data sets. The ethnic background of these women consisted of 69% Blacks (Afro-Caribbean, African), 24% Caucasians and 4% from other ethnic groups. Seven (28%) required surgical management and 19 (72%) had successful expectant management. The mean time to resolution was 21 days (SD 13.94).

The indications for referral were: confirmation of pregnancy 5 (6%) and suspected early pregnancy complications 74 (94%)

Tables 6.4.1 and 6.4.2 show the different variables in the women when compared to outcome. There were significant differences in the size of retained products of conception, serum hCG, progesterone, inhibin A, and inhibin pro a C RI in miscarriages (Table 6.4.1). However for ectopic pregnancies the only significant variable was the serum hCG (Table 6.4.2).

When all failed pregnancies were regarded as one group, the initial serum hCG level was the best predictor of the outcome of expectant management (Figure 6.4.1)

In women with serum hCG <1800 iu/L the expectant management was successful in 89% of cases. When hCG was greater than 1800 IU/l the success of expectant management was 44%. In this subgroup of women, expectant management was more likely to be successful if IGFBP-1 levels were high (>15 μg/l), and pro α C inhibin levels were low (<400 pmol/l) (Figure 6.4.1). When distinguishing for pregnancy type then successful expectant management of miscarriages was more likely if inhibin A was unmeasurable (Figure 6.4.2). Where the inhibin A was unmeasurable 95.24% of miscarriages with resolved. Of those miscarriages with an inhibin A level over 3.9 pmol/l the likelihood of expectant management was 51.5%. In this subgroup of women successful expectant management was more likely if IGFBP-1 was high (>15 μg/l) and pro α C inhibin was low (<400 pmol/l) (Figure 6.4.2).

Table 6.4.1

Comparison of measured variables in miscarriages requiring surgery and those resolving spontaneously

Variable	Expectant N=37	Surgical N=17	p
Maternal age (yrs)*	32.3 (7.8)	32.2 (5.25)	NS
Gestational age (days)*	74 (13.6)	67.2 (26.2)	NS
Vaginal bleeding (%)**	95	76	NS
Diameter RPOC (mm)#	18.6 (16-44)	24.7 (22-35.5)	<0.05
hCG (iU/l)#	918 (254-2755)	5290 (2070-11742)	<0.001
Progesterone (nmol/l)#	7 (5-16)	18 (9-39)	<0.05
17 α OH Progesterone (ng/l)#	1.6 (0.9-2.1)	2.5 (1-2.9)	NS
IGFBP-1 (ng/l)#	30.9 (2.9-23.9)	29.2 (6.8-23.9)	NS
Inhibin A (pmol/l)#	24.6 (5.8-21.1)	74.8 (7.1-47.3)	<0.001
Inhibin pro α C RI (pmol/l)#	259 (139-192)	499 (168-419)	<0.05

*Data distributed normally with values given as the mean and standard deviation; #data distributed non-parametrically with values given as the median (25th to the 75th interquartile range), **discrete data given as percentage of feature for each final outcome.

Table 6.4.2

Comparison of measured variables in ectopics requiring surgery and those resolving spontaneously

Variable	Expectant N=18	Surgical N =7	p
Maternal age (yrs)*	28.38 (29)	30.57 (30)	NS
Gestational age (days)*	47.35 (46)	47 (49)	NS
Vaginal bleeding (%)**	83.3	100	NS
Diameter RPOC (mm)#	15.36 (12.41-17)	19.57 (15.33-19.67)	NS
hCG (iU/l)#	736.11 (102-288)	3030 (2085-3940)	<0.05
Progesterone (nmol/l)#	31.41 (11-34)	33 (17-48.5)	NS
17 OH progesterone (ng/l)#	3.4 (1.75-4.5)	3.17 (2.4-3.65)	NS
IGFBP-1 (μg/l)#	2.47 (0.93-2.5)	1.32 (1.75-1.85)	NS
Inhibin A (pmol/ml)#	17.40 (9.375-21.35)	13.54 (7.3-15.8)	NS
Inhibin pro α C RI (pmol/ml)#	909.61 (260-886.5)	766.42 (437-1005)	NS

*Data distributed normally with values given as the mean and standard deviation; #data distributed non-parametrically with values given as the median (25th to the 75th interquartile range), **discrete data given as percentage of feature for each final outcome.

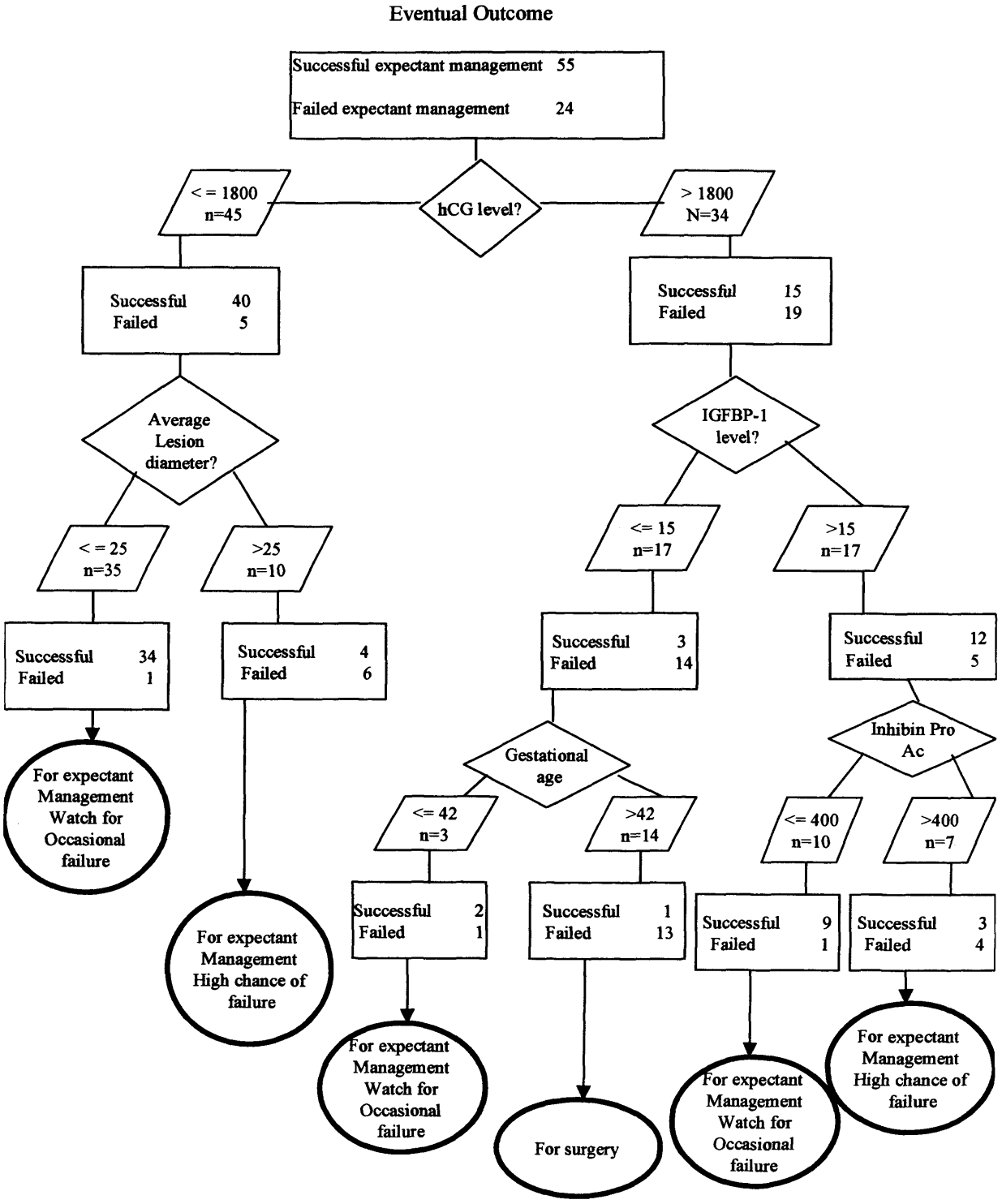
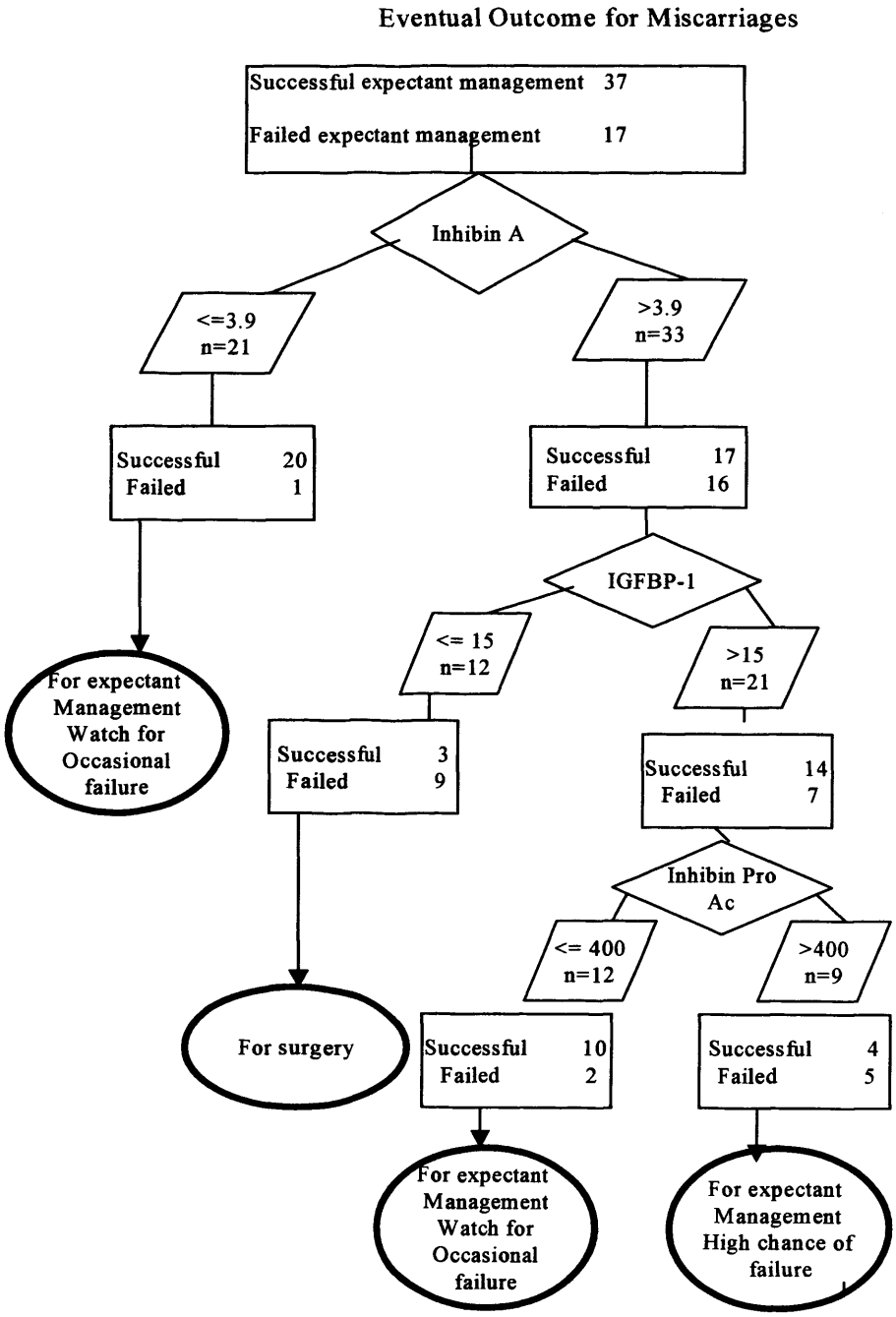


Figure 6.4.1

Decision tree for failed pregnancies

Figure 6.4.2
Decision tree for miscarriages alone



6.5 Discussion

Biochemistry in the management of miscarriages

hCG was the best discriminator for successful expectant management of a failed pregnancy. Those pregnancies with lower hCG and a small amount of trophoblast seen on ultrasound were more likely to resolve spontaneously. This agreed with the observations of Nielsen and Hahlin (1996) for miscarriages although they recorded much higher overall levels of hCG when compared to this study. In those women with higher levels of hCG, IGFBP-1 was the next best discriminator. Two hypotheses exist for the role of IGFBP-1in early placentation. One is that higher levels of IGFBP-1 are thought to inhibit binding of the trophoblast to the decidual cells. The second is that there is overproduction of IGFBP-1 by the decidua in response to defective implantation. In this study the presence of a raised IGFBP-1 levels was associated with an increased chance of successful expectant management. This is the first time that IGFBP-1 levels have been described in association with a miscarriage. It suggests that high levels reflect a defect in the attachment of the trophoblast to the decidua thus resulting in an increased chance of the retained products being spontaneously expelled. Low levels of IGFBP-1 were associated with a higher level of final surgical outcome.

In those women with high IGFBP-1 lower levels of inhibin pro αC RI were associated with an increased chance of successful expectant management. Inhibin pro αC RI is known to be a product of the corpus luteum and has been shown to drop after termination of pregnancy. It is not known whether the mechanism responsible for lower levels of inhibin pro α C RI in successful expectant management reflects a poorly functioning corpus luteum or whether inhibin pro α C RI levels are low in response to feedback from lower levels of hCG. Previous studies have suggested that pro α CRI

may be responsible for maintaining luteal progesterone output which may or may not be hCG mediated.

Once the diagnosis of a miscarriage had been established then inhibin A was the best predictor of expectant management of miscarriage. Unmeasurable inhibin A was strongly associated with successful expectant management reflecting either reduced amount of trophoblast or non-functioning trophoblast. Persistent functioning trophoblast is a probable mechanism for failed expectant management and it would seem that inhibin A reflects the trophoblast dynamics more accurately than hCG due to it's shorter half life. Of those pregnancies where inhibin was still measurable then again high levels of IGFBP-1 were more likely in those pregnancies, which were successfully expectantly managed. Low levels of pro α C RI in these women again improved the prediction of expectant outcome.

Ultrasound in the management of miscarriages

Once a miscarriage had been diagnosed, then ultrasound parameters were also seen to contribute to the prediction of the outcome of the miscarriage. This study showed a resolution rate of 69% for a combined group of missed and incomplete miscarriages. Whether the miscarriage was missed or incomplete was shown to be important with fewer pregnancies with the presence of a gestation sac resolving spontaneously. Other studies have shown success rates of 25% to 65% for missed miscarriage (Jurkovic et al 1998) and 79-100% when incomplete miscarriage alone (Nielsen & Hahlin, 1995; Chipchase & James, 1997). The amount of retained products of conception was also found to be significant in those pregnancies with less trophoblastic tissue that were more likely to resolve spontaneously. This agrees with studies by Nielsen and Hahlin

(1995), which examined expectant management of incomplete miscarriage, and Jurkovic and colleagues (1998) who examined expectant management of missed miscarriage.

However, there is a great deal of variation in previously published studies on the inclusion criteria. Studies evaluating the success of various methods of miscarriage management have defined completion of miscarriage using various criteria ranging from 15-25mm of products identified on ultrasound scan. This study included all women with a positive pregnancy test and ultrasound identification of products of conception regardless of ultrasound measurements. Previous authors have shown that chorionic villi may be present in 69-100% of women with an endometrial thickness of greater than 5mm (Rulin et al 1991; Kurtz et al 1993). Therefore the increased success rate of previous studies may be due to the fact that a proportion of women with incomplete miscarriages were not included in the analysis.

Models for the management of miscarriages

Using the decision tree analysis for the management of miscarriages alone, successful outcome could be predicted with a probability of >81% in 81% of the population. This compares favourably with the logistic regression model of Nielsen et al (1996). The use of colour Doppler as advocated by Schwarzler et al in 1999 predicts successful outcome with a probability of 80% but only in 54% of the population.

One of the limitations of clinical studies looking at expectant management of miscarriage is the difficulty in defining the end-point of expectant management. Some women in this study opted out of expectant management afer 7 days because of ongoing

bleeding or failure of the treatment whereas some women persisted for longer with the expectant management and achieved successful resolution of their pregnancy. This therefore may lead to therapeutic bias in interpretation of the results although is reflective of regular clinical practice.

Chapter 7: Discussion

The aim of this thesis was to see whether a combination of ultrasound findings with a variety of biochemical factors could help to overcome a number of problems that are faced in everyday practise. The combination of these factors in prediction of early pregnancy viability in the absence of a visible embryo and in the success of expectant management of failing pregnancies has been examined. This has led to the development of models that may be used in clinical practise to address these issues.

Why use models to predict pregnancy outcome?

For women in whom ultrasound assessment reveals an 'empty' gestational sac it is important to balance the goal of absolute certainty in the diagnosis of non viability with the practical considerations of sparing patients unnecessary follow-up examinations. This study has shown that the unique combination of progesterone levels with sonographic evidence, and maternal age can predict a failed pregnancy. This model allows for the fact that very early normal pregnancies may have low progesterone levels. Other suggested models for this clinical dilemma have used hCG measurements (Falco 2003) but have failed to correlate the hCG levels with the sac size (Nyberg & Filly 2003).

The main difficulty with expectant management of both ectopic pregnancies and miscarriages is the lack of criteria, which reliably predict the likelihood of successful spontaneous resolution of pregnancy without the need for surgical intervention. The

ability to predict successful expectant management of failing pregnancies would allow the number of surgical or medical procedures and their attendant risks to be reduced. Recently Wieringa-de Waard and colleagues (2002) demonstrated that using initial expectant management of miscarriage could reduce the number of surgical procedures required by 37%. The same group has also demonstrated improved mental health scores in women undergoing expectant management (Wieringa-de Waard 2002b).Women chose expectant management of miscarriage through a desire for a natural solution and a fear of operations. However those chosing this option can feel ill prepared and poorly informed (Ogden & Maker 2004). Being able to give a probability of success of up to 81% following one hospital visit would help these women to make an informed decision regarding their management. Whilst the model proposed by Nielsen et al (1996) relies on more commonly available biochemical markers it gives a probability of 80% in only 40% of the population. Bagratree et al (2004) in a randomised controlled trial comparing medical and expectant management of miscarriage had only a 44% success rate in an unselected population of women undergoing expectant management after 7 days. If women with only a high chance of success underwent expectant management as suggested by this thesis, then the success rate would be closer to 92%. However neither inhibin A nor IGFBP-1 are as yet in available in routine clinical practice. Luise et al (2002) reported a higher proportion of women achieving expectant management in women with incomplete miscarriages but with no restriction on length of treatment. However until the pregnancy has resolved women need ongoing psychological support (Ogden & Maker 2004) and this requires more dedicated early pregnancy units (Logan et al 2002).

Overall the model for the successful expectant management of ectopic pregnancy has a sensitivity of 100% and a specificity of 28%. This means that it is excellent at

predicting those women who will have a successful management but not very accurate for those whose expectant management will fail. If expectant management is recommended only to those women with a high chance of success then the sensitivity decreases to 81% and but the specificity increases to 87% meaning that it it becomes a better clinical tool. With methotrexate treatment there is evidence that women are prepared to accept the slightly lower success rate compared to surgery when offered as part of a nonsurgical approach to diagnosis and management of ectopic pregnancy (Nieuwerk et al 1998). This suggests that if expectant management can be offered with comparable success rates to methotrexate that women may prefer this option. Cost effectiveness studies of expectant management versus surgery or methotrexate have not been carried out. However methotrexate does appear to be cost effective versus surgery at low levels of hCG less than 1500iu/l (Mol et al 1999, Sowter et al 2001). As success rates between methotrxate treatment and expectant management appear similar it is likely that this too may prove a cost effective treatment versus surgery and probably methotrexate.

Logistic regression models examine multiple variables to reach a diagnosis. This reduces the risk of diagnostic errors, which are more likely to occur with the use of individual variables alone. In addition the model gives a numerical probability of the problem, which is likely to be helpful in clinical practice. This could improve counselling and decrease anxiety, which is often experienced by women when the results of diagnostic tests are inconclusive. However, when applying the models in clinical practice it is important that the population under observation is similar to that used in the index study. The second problem is that logistic models are more complex to use than traditional tests and necessitates the need for an electronic calculator or computer.

The use of decision tree analysis would appear to overcome this complexity and produces an easy to follow pathway for patient counselling. Decision tree analysis provides such information, which could be used both for patient selection and counselling. The analysis largely resembles the clinical decision making process, which may increase its appeal to the practising clinicians. The likelihood of the final outcome can also be easily conveyed to the patient, thus improving counselling and enabling women to make informed decisions about their management.

However to allow for the differences between ultrasound operators and biochemistry laboratories, any implementation of these models should be carefully audited. This would enable necessary adjustments to be made in order to define the optimal cut-offs for each individual unit and the most appropriate model for general use.

Chapter 8: Conclusions and Future Research Goals

This Thesis demonstrates that a combination of ultrasound and biochemical measurements can be used to assist in the management of women with missed miscarriages. The proposed logistic model was able to discriminate reliably between viable and non-viable pregnancies in both initial and prospective study. The proposed algorithm would be simple to implement into clinical practice and it would lead to a nearly 50% reduction in the need for follow up visits and additional biochemical assays. I have also shown that a large proportion of tubal ectopic pregnancies detected on the initial ultrasound scan can be safely managed without any intervention. The decision tree analysis described and prospectively tested in this study has a high positive predictive value for the diagnosis of a spontaneous pregnancy resolution. This algorithm could lead to up to a 40% reduction in operative interventions for tubal ectopic pregnancies, resulting in a significant reduction in morbidity associated with surgical treatment for ectopics and major cost saving for health care providers. This approach however requires a commitment from the patient to the possibility of a longer follow-up period that with surgical treatment.

The observation of increased serum IGFBP-1 and reduced serum inhibin A may form a basis for a novel approach to the expectant management of women with miscarriages. In recent years the expectant management of miscarriages has become a part of standard clinical practice and the measurement of these novel markers could improve counselling of women who wish to opt for non-interventional management of miscarriage. However, a larger prospective evaluation of this model is needed before its implementation into clinical practice could be recommended.

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