“FEDERICO II”

UNIVERSITY OF NAPLES

PhD Program

“Human Reproduction, Development and Growth”

Director

Prof. Claudio Pignata

PhD Thesis

“New Screening in Fetal Medicine: Prediction of preterm delivery by transvaginal cervical ultrasound in the first trimester of pregnancy”

Student:

Elena Greco, MD

Tutor:

Prof. Carmine Nappi

Academic year 2012-2013

Contents

CHAPTER 1

Preterm delivery: delineation of the problem

Preterm birth is defined as all births occurring before 37 completed weeks of gestation or fewer than 259 days since the first day of a woman's last menstrual period (WHO, 1977). In the absence of an explicit lower gestational age cut-off in official definitions to distinguish preterm birth from spontaneous abortion, the medical care given and whether or not birth and death registration occurs, classifications may vary between Countries and within Countries and depend on perceptions of viability (Goldenberg et al., 2008; Sanders et al., 1998).

Etiologically, preterm birth can be classified into two broad categories: (1) provider-initiated preterm birth, which accounts for 30% of cases and is defined as induction of labor or elective caesarean birth before 37 completed weeks of gestation for maternal or fetal indications; (2) spontaneous preterm birth, which account for about 70% of cases, occurring as a result of spontaneous onset of labor (45%) or following pre-labor premature rupture of membranes (pPROM) (25%); (Goldenberg et al., 2012) (Figure 1.1).

A further classification, based on gestational age at delivery, groups under "early preterm birth", all deliveries occurring before 34 weeks and "late preterm birth", those occurring between 34 and 37 completed weeks' gestation.

Preterm birth is the leading cause of perinatal morbidity and mortality worldwide and contributes to approximately 70% of neonatal mortality and approximately half of long-term neurodevelopmental disabilities (Liu et al., 2012, Mathews at al., 2004). Recent WHO data indicate that about 15 million (11.1%) of the 135 million live births worldwide in 2010, were born preterm, and of these, 1 million died (Blencowe et al., 2012). During the last 25 years, in spite of remarkable improvements in neonatal care leading to higher survival of very premature infants (Saigal 2008), the rate of preterm births has increased. Estimates assuming that the average annual increase in the rate of preterm birth observed between 2005 and 2010 is maintained, predict that over 2 millions babies will be born prematurely in 2025 (Blencowe et al., 2012) (Figure 1.2).

This increase in the rate of preterm birth has been attributed to a higher frequency of “indicated” preterm births in singleton gestations and preterm delivery in multiple gestations resulting, in part, from the use of assisted reproductive technologies (Ananth and Vintzileos, 2006; Chauhan et al., 2010; Pandian et al., 2004; Stone et al., 2008). However, the greatest portion of preterm births occur in pregnancies with no apparent risk factors, for reasons that remain mostly not understood and according to a path that is still in many respects, unpredictable.

Spontaneous preterm delivery is indeed considered to be one of the “great obstetrical syndromes” (Di Renzo, 2009; Romero, 1996), a term which emphasizes that obstetrical disorders with a similar phenotype are caused by multiple pathologic processes, have a long subclinical phase and may result from complex gene-environment interactions (Ma et al., 2011; Macones et al., 2004).

Innovative solutions to prevent preterm birth and hence reduce preterm birth rates are urgently needed. Indeed, a major impact on the associated mortality and morbidity will only be achieved through the development of a sensitive method to identify women at high-risk of preterm delivery and an effective strategy for prevention of this complication.

Prediction is the basis of prevention

Over the last decades, prediction and prevention of preterm birth has been the focus of intense research and ultrasound of the cervix during pregnancy has been established as the gold standard method for prediction of preterm delivery.

It has been known for decades that the process of parturition takes months of preparation and involves a number of histological and architectural changes in the lower part of the uterus – including the isthmus and the cervix- leading to the development of the lower uterine segment and the maturation of the cervix (Danforth, 1947; Wendell-Smith, 1954).

Prospective clinical investigations have established that a significant increased risk for preterm delivery is highly correlated with development of early effacement and maturation of the cervix and lower segment (Bouyer et al., 1986; Papiernik et al, 1986; Stubbs and Van Dorsten, 1988). However, these longitudinal observations have shown that the mechanism of cervical effacement in preterm delivery begins earlier but it is similar to the one observed at term in that it involves several months of clinically detectable cervical shortening and gradual cervical effacement.

The availability of high-resolution, non invasive obstetric ultrasound, has made it possible for these alterations in normal cervical anatomy to be measured accurately with the objectivity, safety and reproducibility that clinical digital examination cannot provide. Indeed ample evidence in the literature has supported the reliability of ultrasound evaluation of cervical shortening in the assessment of preterm cervical effacement (Celik et al., 2008; Heath et al., 1998; Iams et al., 1996; To et al., 2006). Early detection of such changes is paramount in allowing interventions the chance to work before the pathology is so far in its pathways as to thwart prevention.

Evaluation of the uterine cervix

Throughout the years, evaluation of the uterine cervix has evolved from digital examination to transabdominal, translabial and transvaginal ultrasound.

Physical examination

In the past, serial digital and speculum examinations were the traditional protocol to follow-up women at risk of preterm delivery. The physical changes appreciable on examination, determining the likelihood of preterm delivery included bulging membranes, pink discharge and softening of the cervix and of the lower uterine segment. However, at present, such practice has been nearly completely abandoned.

The relative lack of success of digital examination in predicting preterm birth is due to the fact that it is subjective, not accurate for evaluating the internal orifice and nonspecific. Studies have indeed demonstrated that digital examination is far less reproducible than ultrasound measurement of the cervical length, with inter-observer variability being 52% (Phelps et al., 1995). Digital examination cannot evaluate the internal orifice that is where initial physical changes leading to cervical effacement occur (Michaels et al., 1986). Moreover, manual estimations of CL have been shown to be, on average, shorter by 11 mm than sonographic measurements (Berghella et al, 1997b). The technique is non specific in that about 15% of primiparous and 17-35% of multiparous women who are delivered at term have cervices that are 1-2 cm dilated throughout the late second trimester (Floyd, 1961) and conversely, 74% of women with cervical funnelling at the internal orifice have a long cervix, with a closed external orifice (Berghella et al, 1997a).

Transabdominal ultrasound

Initial attempts at evaluating the cervix by ultrasound used the trans-abdominal approach (Figure 1.3). However, this technique has a number of substantial limitations for which it is currently used only when other approaches are not readily available. Such shortcomings include: (1) the need for the bladder to be sufficiently filled for a reliable image to be produced, leading to artificial lengthening of the cervix and potential masking of any funnelling at the internal os; (2) the inability to visualise the cervix in women with CL<20 mm, which represent the very group at increased risk of preterm delivery; (3) the difficulties to visualise the cervix when the distance between the probe and the cervix is increased, as in obese women, or whether fetal parts are interposed, which is more likely for examinations performed after 20 weeks of gestation (Berghella and Bega, 2008; Berghella et al, 2003a; Hassan et al, 2000; To et al., 2000).

Hassan (Hassan et al 2000) reports that the sensitivity of trasbdominal ultrasound in predicting PTB is only 8%.

All of these factors limit the use of transabdominal ultrasound in the evaluation of the uterine cervix as a screening tool for preterm birth.

Transperineal Ultrasound

Transperineal (also known as translabial) ultrasound was originally used in France in the 1980s and proved to be superior to the transabdominal approach. This technique involves having the patient lie on an examination table with the knees and hips in a flexed position, and placing a gloved transducer on the perineum between the labia majora, ensuring to keep the transducer in a sagittal orientation. A cushion may be placed underneath the patient’s back in order to lift up the hips and enhance visualisation of the cervix (Figure 1.4).

Compared with trans-abdominal approach, this technique offers significant improvements in that the image is not obstructed by fetal parts, the bladder does not have to be filled, and the transducer is closer to the cervix, thus allowing a clearer visualization of the whole cervical length in nearly 100% of cases. Other advantages offered by this technique include the fact that it does not require an additional transducer, the probe is closer to the cervix but does not enter the vagina (so no pressure can be exerted on the cervix) and is well accepted by most women (Berghella et al., 2003a). The main downfalls to translabial ultrasound include the possibility that gas in the rectum may interfere with the visualisation of the internal orifice and the technique is more challenging to master than other ultrasonographic methods (Owen et al., 1999).

Transvaginal Ultrasound

The first studies on the use of transvaginal ultrasound as an alternative approach to assess the pregnant uterine cervix also date back to the 1980s. This technique shares the advantages of translabial ultrasound with an improved visualisation of the cervix and without the interference of bowel gas (Bergella and Bega, 2008). It has therefore become the preferred, gold standard method of evaluating the cervix for prediction of preterm birth (Iams et al., 1996).

Measurement of the cervical length by transvaginal ultrasound: technical aspects

Several cervical parameters have been evaluated as predictors of preterm delivery. Among those, the cervical length, measured as the linear distance between the internal and external orifices along the endocervical canal, has emerged as the most reproducible and reliable indicator (Figure 1.5).

In order to obtain an accurate measurement of the cervical length, adherence to the appropriate technique is required (Iams et al., 1996). Table 1.1 summarises the main aspects of this technique.

Some authors have suggested that in case of a curved cervical canal, the cervical length should either be manually traced, or measured as the sum of 2 straight lines that follow the curve of the canal (Owen et al., 2001). However, a short cervix is always straight, while the presence of a curvilinear appearance of the canal is a reassuring finding as it almost invariably signifies a long cervix (>25 mm). Therefore such a distinction is irrelevant from either a diagnostic or a prognostic point of view.

In about 10% of low-risk women (Berghella et al., 2003b) and 25-35% of the high risk women (Berghella et al., 1997; Owen et al., 2001) the internal orifice presents some degree of “funnelling”, defined as a protrusion of the amniotic membranes 3 mm or more into the internal orifice as measured along the lateral border of the funnel (Iams et al., 1996). Funnelling of cervix typically occurs along a continuum: from the “T” appearance of the normally closed cervix, through the “Y” sign of the initial funnelling at the internal os, up to the ‘V” and “U” signs of much deeper openings (Figure 1.6). Studies evaluating the role of funnelling as predictor of preterm birth have found that funnelling is typically associated with a cervical length < 25 mm (Berghella et al., 2003b). Compared with a cervical length of <25 mm alone, the association with a funnelling increases the sensitivity of predicting preterm birth from 61% to 74% without changing specificity and positive and negative predictive value (Berghella et al., 1999). Conversely, in the presence of a normal functional cervical length (>25 mm), the finding of a funnelling does not increase the risk of preterm birth (Berghella and Roman, 2005). Therefore, it is far more important to report the cervical length rather than the presence of a funnelling, because it does not affect clinical management.

A number of other cervical parameters have been studied in transvaginal cervical imaging as potential prognosticators of preterm delivery. These include the length and the width of funnelling, the dilatation of the cervical canal, anterior and posterior cervical width, cervical position (horizontal versus vertical), lower uterine segment thickness, cervical angle, cervical vascularity, and presence of intra-amniotic sludge. However, none of those have been proven to be more reliable or predictive of preterm birth than cervical length (Berghella and Bega, 2008; Berghella et al., 1997a; Guzman et al., 2000).

Potential pitfalls in measuring the cervical length by transvaginal ultrasound

Although ultrasound assessment of the cervix by transvaginal ultrasound is usually straightforward, some anatomic or technical difficulties may be encountered in about one fourth of patients (Yost et al., 1999). A reliable measurement of the cervical length may be hampered by excessive pressure exerted on the probe or created by a partially empty bladder, as this may artificially elongate the cervix or mask funnelling. Conversely, uterine contractions may mimic the appearance of cervical funnelling of the internal os. In such instances the cervical canal may assume an “S” shape, and the walls of the uterine isthmus, either anteriorly, or posteriorly, or both, appear thickened and asymmetric (Yost et al., 1999). Unexplained, spontaneous, minute-to-minute fluctuations in the internal os, which may significantly alter the appearance of the length of the cervix, have also been described (Hertzberg et al., 1995). Finally, in some cases, and especially when attempting at measuring the cervix prior to 14 weeks' gestation, it may be challenging to identify the internal orifice thus the cervix may appear longer for inadvertent inclusion of part of the isthmus. In the first trimester of pregnancy these two structures indeed form a continuum ("cervico-isthmic complex") (Figure 1.7), as the gestational sac has not reached a sufficient size to completely expand the lower part of the uterus (Danforth, 1947; Danforth, 1980; Wendell-Smith, 1954). Therefore some authors have advocated that the cervix should not be measured before 14 weeks' gestation (Berghella et al., 2003b).

Cervical length as a screening for preterm delivery

Measurement of the cervical length by transvaginal ultrasound meets the criteria of an effective screening test (Table 1.2) (Grimes, 2000).

It is indeed used to *screen for an important condition*, as preterm birth is the leading cause of neonatal mortality and morbidity worldwide (Liu et al., 2012);

It is *safe*, as it has not been associated with complications, even in women with pPROM, who are at higher risk of ascending infection or inoculation of bacteria (Krebs-Jimenez and Neubert, 2002);

It has been shown to be *acceptable*, with over 90% of women reporting no or minimal discomfort and embarrassment (Heath et al., 1998);

It recognizes an *early, asymptomatic phase* of the process leading to preterm birth. Indeed, regardless of the underlying cause, the most likely pathophysiological sequence leading to spontaneous preterm delivery involves cervical effacement and dilation, both of which first occur at the level of the internal orifice (Romero et al., 2006);

It involves a *standardised, easy technique*. A study investigating the learning curve for measurement of cervical length by transvaginal ultrasound has indeed demonstrated that about 20 supervised scans are necessary for an operator with no experience in transvaginal ultrasound to master the technique, and substantially fewer (about 5) for an operator already familiar with this approach for other indications (Iams et al., 1996; Vayssière et al., 2002);

It is *highly reproducible*, as demonstrated by a study showing low (<10%) inter- and intra-observer variability. In 95% of cases studied, the difference in cervical length between 2 measurements obtained by the same observer and by two observers was indeed ≤ 3.5 and ≤ 4.2 mm, respectively (Heath at al., 1998);

It is *accurate*: measurement of the cervical length has been indeed shown to be predictive of preterm birth in all populations studied (Andersen et al., 1990; Conde-Agudelo et al., 2010; Crane et al., 2008; Hassan et al., 2000; Iams et al., 1996; To et al., 2001; Vaisbuch et al, 2010);

It involves the *early detection of a condition for which "early treatment" is effective*: the results of recent randomised controlled trials have indeed demonstrated that in asymptomatic women with a short cervix in the second trimester of pregnancy administration of progesterone reduces the risk of early preterm delivery by about 40% (Romero et al., 2012); similarly, ultrasound indicated cervical cerclage, when performed in women with a previous history of second trimester loss, has shown to reduce the risk of preterm delivery of about 40% (Berghella et al, 2011a; Berghella et al, 2011b).

Measurement of cervical length in the prediction of preterm birth

Measurement of the cervical length by transvaginal ultrasound has been shown to be predictive of preterm delivery in all populations examined (Andersen et al., 1990; Conde-Agudelo et al., 2010; Crane et al., 2008; Hassan et al., 2000; Iams et al., 1996; Kagan et al., 2006; To et al., 2001; Vaisbuch et al, 2010) (Table 1.3). Most studies found a cut-off of 25 mm to have the best predictive accuracy and used spontaneous preterm birth before 35 weeks' gestation as primary outcome.

However, the sensitivity of this method has been shown to vary according to a number of factors, including: number of fetuses, length of cervix, previous obstetric history, gestational age at detection of a short cervix and gestational age at screening.

Transvaginal screening for preterm delivery by cervical length is more effective in singleton than in multiple gestations. Indeed, most women with twins or triplets pregnancies who will eventually deliver preterm do not manifest a short cervix in the second trimester and therefore the sensitivity of this test is <50% (Goldenberg et al., 1996; Guzman et al., 2000; Owen et al., 2001).

The risk of preterm delivery is inversely related to the length of the cervix, with shorter cervices being associated with greater risks of preterm delivery. A cervix of 25 mm represents the tenth and twenty-fifth centile for low risk and high risk (for prior preterm delivery) populations of singleton gestations, respectively, and has been chosen as the cut-off at and above which a cervix can be called “normal”, and below which it can be called “short” (Andersen et al.,1990; Hassan et al., 2000; Heath et al.,1998; Iams et al.,1996; To et al., 2001; To et al., 2004; To et al., 2006). A cervix ≤ 25 mm at or before 28 weeks’ gestation is always abnormal and associated with increased risk of preterm delivery (Berghella et al., 2007).

The performance of the screening for preterm delivery by cervical length is higher in singleton pregnancies with previous history of preterm delivery and/or mid-trimester loss. From this group, over two-thirds of women (69%) destined to deliver preterm will be detected while asymptomatic by screening by cervical length (Owen et al, 2001). Sensitivity remains relatively high also in singleton gestations with other risk factors for preterm delivery such as previous cone biopsy, Mullerian anomaly, or previous multiple dilation & curettage (D&C) procedures (Airoldi et al., 2005; Berghella et al., 2007; Visintine et al., 2008). In low-risk women with singleton pregnancies, the sensitivity of this test is about 37% (Iams et al., 1996) (Table 1.3).

A study by the Fetal Medicine Foundation group has shown that it is possible to increase the sensitivity of the transvaginal cervical screening by combining cervical length to maternal characteristics and obstetric history in a model for individualised risk assessment. Such a model, for a 10% false positive rate, has a sensitivity of 80.6%, 58.5%, 53% and 28.6% for extreme, early, moderate and mild spontaneous preterm birth (Celik et al., 2008).

Gestational age at detection of a short cervix clearly impacts the probability of preterm birth. The earlier in gestation the short cervical length is detected, the higher is the risk of preterm delivery. As in most women who will have preterm delivery, cervical shortening reportedly occurs between 18 and 22 weeks (Berghella et al., 1997b; Owen et al., 2001), and this is also the time where interventions to predict preterm birth in low risk patients have been studied, the mid-trimester scan has been chosen as the best timing to perform transvaginal measurement of the cervical length as a screening for preterm delivery (Iams et al., 1996). After 28 weeks a cervix <25 mm may be physiological as the cervix starts to prepare for delivery many weeks before the process of labour becomes symptomatic and clinically recognisable (Berghella et al, 1997b).

Very few studies have investigated the role of cervical length in the prediction of preterm delivery prior to 14 weeks (Table 1.4) with most of them reporting the cervix to be longer than in the second trimester and not associated with the risk of spontaneous preterm birth (Carvalho et al., 2003; Conoscenti et al., 2003; Hasegawa et al., 1996; Ozdemir et al., 2007; Zorzoli et al., 1994). A limitation of very early screening, which may account for the reported low sensitivity of the method at this stage, is that measuring the cervix by transvaginal ultrasound before 14 weeks' gestation may be technically more difficult and prone to pitfalls than in the second trimester as the internal uterine orifice is poorly defined and the anterior and the posterior portions of the lower uterine segment lie in juxtaposition, mimicking the endocervical canal (Shalev 2003; Sonek and Shellhaas, 1998; Yost et al., 1999).

Effectiveness of interventions to prevent preterm birth based on a short cervical length

Certainly, the most important criterion to estimate the value of any screening test is to assess the availability of a treatment that effectively prevents the outcome predicted. Knowledge of the increased risk of preterm birth associated with shortening of the cervix had largely been academic, until findings from randomized controlled trials showed a benefit for interventions including vaginal progesterone and cervical cerclage in reducing the risk of preterm birth and improving neonatal outcomes.

Cervical cerclage

Cervical cerclage is a controversial procedure designed to prevent preterm birth by positioning a suture around the neck of the womb in order to give mechanical support to the cervix. Randomized clinical trials have been inconclusive regarding the value of cerclage in the prevention of preterm birth. However, such trials are particularly difficult to be performed, especially if the procedure is believed to be beneficial by many patients and their doctors; and to be compared, as they are extremely heterogeneous as regard to type of cerclage used, technique, suture material, height of the stitch, additional use of tocolytics, progesterone or antibiotics.

Overall, the effectiveness of cervical cerclage appears to be dependent upon the population studied with women with a previous history of preterm delivery representing the only population in which such intervention has been demonstrated to produce a significant reduction in the risk of preterm delivery (Berghella et al., 2011a). Such a benefit has not been confirmed in singleton pregnancies with no risk factors and a short cervical length in the mid-trimester. Randomised trials in twins and a short cervical length have shown cerclage to be associated with a much higher (75% versus 36%) incidence of preterm delivery <35 weeks, and a greater number of neonatal deaths in the treatment group compared with controls (Berghella et al., 2005). Therefore cerclage is not recommended in asymptomatic singleton pregnancies with a short cervix at mid-trimester, and should not be used in twin pregnancies with a cervical length <25 mm.

In a meta-analysis of five controlled trials that randomized women with a short cervix to either cerclage or no cerclage, there was a significantly reduced rate of preterm birth associated with cerclage in women with a prior spontaneous preterm birth, singleton gestation, and cervical length less than 25 mm by transvaginal ultrasound (28% for cerclage vs 41% for no cerclage; RR, 0.70; 95% CI, 0.55-0.89) (Berghella et al., 2011a). The combined data also showed a reduction in composite perinatal mortality and morbidity. These data provide support for recently published management guidelines (ACOG, 2012; Berghella, 2012) regarding the use of cerclage in women who have a history of preterm birth and who develop short cervix before 24 weeks' gestation (Figure 1.8). A separate analysis examined the results of four trials that randomized women with prior spontaneous preterm birth to cerclage based solely on obstetric history or cerclage based on results of transvaginal ultrasound screening for cervical length. There were no differences between the groups in the incidence of preterm birth or perinatal outcomes; however, 58% of women screened by transvaginal ultrasound did not have a short cervix and therefore were able to avoid the surgical procedure (Berghella et al., 2011b).

The optimal use of cerclage continues to be debated, but results of these analyses indicate that cervical length measurements may assist in identifying appropriate candidates for this procedure.

Progesterone

Progesterone is considered a key hormone for pregnancy maintenance, and a decline of progesterone action is implicated in the onset of parturition (Csapo 1969; Csapo 1977; Kerenyi, 2010). If such decline occurs in the midtrimester, the clinical, biochemical and morphologic changes associated with cervical ripening may occur, and this would predispose to preterm delivery. Therefore, an untimely decline in progesterone action has been proposed as an etiological precursor in the "preterm parturition syndrome" (Romero et al., 2006).

The mechanism by which progesterone maintains pregnancy is thought to involve the modulation of antibody production and a reduction in pro-inflammatory cytokine production. Current research suggests that the process of cervical ripening occurs after a decrease in progesterone concentration that leads to the release of pro-inflammatory cytokines. Infection as well as increases in inflammatory processes has been indeed associated to premature cervical ripening (Lee et al., 2008; Timmons et al., 2010).

Administration of progestogen therapy (including natural progesterone and synthetic progestins) has been studied extensively in the prevention of preterm (Romero et al., 2012). Despite early trials that showed contradictory results, particularly with synthetic progestins (Goldstein et al., 1989), data from the last 10 years have shown that the use of supplemental natural progesterone in mid-trimester is beneficial in the prevention of preterm birth, and that the decision to treat women with vaginal progesterone on the basis of cervical length have a greater effect than a decision made on the basis of obstetric history alone (Cetigoz et al., 2011; Fonseca et al., 2007; Hassan et al., 2011; O'Brien et al., 2007; Rode et al., 2011). Mixed populations of women, as well as use of different progestogen products, may explain the lack of consistent results in previous studies.

Results of a large meta-analysis of individual data from 775 women and 827 infants enrolled in 5 studies that evaluated the use of vaginal progesterone in women with sonographic short cervix demonstrated that treatment initiated in the mid-trimester for women with cervical length of 10 to 20 mm reduced the rate of preterm birth before 33 weeks' gestation by 42% (Table 1.5) (Romero et al., 2012). The number needed to treat for these significant reductions in preterm birth ranged from 11 to 18. In addition, administration of vaginal progesterone to women with a short cervix reduced neonatal morbidity and mortality as measured by admission to the NICU, respiratory distress syndrome, the need for mechanical ventilation, and a composite score of complications including intracranial haemorrhage, bowel problems, respiratory difficulties, infection, and death. The effect of vaginal progesterone was observed in women with short cervix regardless of prior history of preterm birth (Romero et al., 2012). Such report provides high-quality evidence in support of the efficacy and safety of vaginal progesterone to prevent preterm birth.

As a result, both the American College of Obstetricians and Gynecologists and the Society for Maternal-Fetal Medicine have recently published updated recommendations to reduce preterm birth in women with short cervix identified in the midtrimester (ACOG, 2012; Berghella 2012). These recommendations support the use of vaginal progesterone in asymptomatic women with short cervix (≤20 mm) at 24 weeks' gestation or earlier (Figure 1.8).

Although universal screening for short cervix continues to be debated, cost analyses based on these data indicate that routine cervical length screening by transvaginal ultrasound and subsequent treatment with vaginal progesterone in women with cervical length less than or equal to 15 mm are cost-effective (Cahill et al., 2010; Werner et al., 2011). The savings associated with preventing preterm birth result from improved infant outcomes and a decrease in long-term deficits.

Future studies will continue to refine the screening method and treatment approach of identifying at-risk women with cervical length measurement and targeted progesterone therapy. Some of the questions to be considered include: the timing of progesterone therapy, that is, whether earlier initiation of progesterone therapy would further reduce preterm births; whether to offer any intervention to women with cervical length less than 10 mm who have a higher risk of intra-amniotic infection and inflammation and have not been routinely included in clinical trials so far (Hassan et al., 2011); lastly, the effect of vaginal progesterone in multiple gestations is limited and results are varied based on small populations, different formulations of progesterone, and a lack of cervical length measurements (Romero et al., 2012).

CHAPTER 2

Prediction of preterm delivery by transvaginal ultrasound of the cervix in the first trimester of pregnancy

The disadvantages of measuring cervical length at 20-24 weeks are firstly, inevitable failure to identify cervical incompetence leading to miscarriage before this gestation and secondly, the effectiveness of prophylactic administration of progesterone or cervical cerclage may be inversely related to the gestation at which treatment is initiated (Romero et al., 2012).

Certainly in women who had a previous preterm birth or second-trimester loss cervical cerclage is either carried out electively in the first-trimester or it is reserved for those where serial scans, starting from the first-trimester, demonstrate cervical shortening (Althuisius *et al*., 2000).

In women with a short cervix administration of progesterone reduces the risk of spontaneous early preterm delivery by about 42% (Romero et al., 2012). However, progesterone is not as effective in women with cervical length below 11 mm as in those with a length of 10-20 mm (Fonseca at al., 2007; Hassan et al., 2011;). Consequently, it may be preferable to measure cervical length in earlier pregnancy before the critical length of 10 mm is reached.

Several studies reported that measurement of cervical length in the first-trimester is not predictive of preterm delivery (Carvalho et al., 2003, Conoscenti et al., 2003; Hasegawa et al., 1996; Ozdemir et al., 2007; Zorzoli et al., 1994). The mean or median cervical length in these studies (40-44 mm) was considerably longer than in the second trimester (Table 1.4). It is possible that cervical shortening in cases of preterm delivery is not apparent in the first trimester because it occurs after 16 weeks (Berghella et al., 2003). Alternatively, preterm delivery is associated with short cervix in the first trimester but this has not been recognized because in the measurement of cervical length sonographers inadvertently include the uterine isthmus.

With the present research programme, we aimed at:

1ST RESEARCH BRANCH

- Defining and standardising the technique for measuring the cervical length by transvaginal ultrasound at the time of the combined first trimester screening for aneuploidies (11-13^{+6} weeks' gestation);
- Investigating the potential value of this measurement in the prediction of spontaneous early preterm birth in a population of a 1500 unselected singleton pregnancies (pilot study).

2ND RESEARCH BRANCH

- Confirming the value of this technique as a prognosticator of spontaneous preterm birth before 34 weeks in 10.000 pregnancies and evaluating the performance of a model for individualised risk assessment for preterm delivery based on the combination of cervical length, maternal characteristics and obstetric history.

3RD RESEARCH BRANCH

- Demonstrating the feasibility of the technique, by investigating inter- and intra-observer variations and patients' acceptability;

- Investigating the time required for an operator with no or minimal experience in transvaginal ultrasound to master the technique.

1^{ST} RESEARCH BRANCH – DESCRIPTION OF THE METHOD

Prediction of spontaneous preterm delivery from endocervical length at 11 to 13 weeks: a pilot study

The aim of this study was to define and standardize the technique for measurement of cervical length at 11-13 weeks and determine the potential value of this measurement in the prediction of spontaneous early preterm birth.

Methods

The data for this study were derived from prospective screening for fetal abnormalities and pregnancy complications in women attending for their routine first hospital visit in pregnancy at University College Hospital, London, UK. In this visit, which is held at 11^{+0}-13^{+6} weeks of gestation, we record maternal characteristics and medical history and perform transabdominal and transvaginal sonography to firstly, determine gestational age from the measurement of the fetal crown-rump length (CRL), secondly, diagnose any major fetal abnormalities and thirdly, measure fetal nuchal translucency (NT) thickness as part of screening for chromosomal abnormalities (Robinson and Fleming 1975, Snijders et al., 1998). The transvaginal scan includes measurement of cervical length and if this is less than 15 mm the women are referred to a specialist high-risk for preterm delivery clinic. In our hospital all women at 20-24 weeks of gestation are offered another ultrasound scan for the diagnosis of fetal abnormalities, assessment of fetal growth and transvaginal measurement of cervical length.

The inclusion criteria for this study were singleton pregnancies with measurement of cervical length at 11-13 weeks and delivery at or after 24 weeks' gestation. We excluded pregnancies ending in termination, miscarriage or fetal death before 24 weeks and those with iatrogenic delivery before 34 weeks.

Measurement of endocervical and isthmic length

We have observed that in the majority of women undergoing cervical assessment before the development of the lower uterine segment, there is a persistent myometrial thickening (isthmus) between the endocervix and the gestational sac (Figure 1.7). This thickening is likely to represent the isthmus, rather than a contraction and it is much more consistent in the first than in the second trimester of pregnancy (Figure 2.1). Consequently, in the measurement of cervical length, which for the purpose of clarity we define as endocervical length, we undertake the following steps. First, the women are asked to empty their bladder and are placed in the dorsal lithotomy position. Second, the vaginal transducer (2.7-9.3 MHz) is introduced in the anterior fornix of the vagina and adjusted to obtain a sagittal view of the entire length of the cervical canal which may be either translucent or echodense. The canal is bordered by the endocervical mucosa, which is usually of decreased but occasionally of increased echogenicity compared to the surrounding tissues. Third, the probe is withdrawn until the image is blurred and then advanced gently until the image is restored without exerting undue pressure on the cervix. Fourth, the settings of the ultrasound machine are altered to obtain the widest viewing angle and the magnification is increased so that most of the screen is occupied by the tissues between the external cervical os at one end of the picture and gestational sac at the other end. Fifth, calipers are used to measure in sequence the linear distance between the two ends of the glandular area around the endocervical canal and the shortest distance between the glandular area and gestational sac (isthmus). The appropriate technique for measuring the cervical length by transvaginal ultrasound in the first trimester is summarised in Table 2.1.

All the operators performing the scans had received extensive training and had all passed a practical examination administered by an expert to demonstrate their competence in the technique. In addition all ultrasound images for every study subject were reviewed by a single investigator, who was unaware of the outcome of pregnancy, to ensure that measurements were made appropriately and consistently.

Diagnosis of spontaneous early preterm delivery

Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women and were also recorded in our database. The obstetric records of all patients delivering before 34 weeks were examined to determine if the preterm delivery was medically indicated or spontaneous. The latter included those with spontaneous onset of labor and those with preterm pre-labor rupture of membranes.

Statistical analysis

Comparison between the outcome groups was by Mann-Whitney U-test for continuous variables and $\chi 2$ test or Fisher's exact test for categorical variables. The significance of difference in endocervical length and cervico-isthmic complex length in the spontaneous early delivery and unaffected groups was determined. Regression analysis was used to examine the association in the measurements between 11-13 weeks and those at 20-24 weeks for the length of both the endocervix and the cervico-isthmic complex.

The statistical software package SPSS 16.0 (SPSS Inc.,Chicago, IL) was used for data analyses.

Results

During the study period (July 2009 to February 2010) we measured the cervical length at 11-13 weeks in 1,548 singleton pregnancies. We excluded 40 cases because the pregnancies resulted in miscarriage or termination (n=34) or there was iatrogenic delivery at 24-33 weeks (n=6), for preeclampsia in 4, maternal cerebral hemorrhage in 1 and bleeding from a placenta previa in 1. In the 1,508 cases included in the study 16 (1.1%) had spontaneous delivery before 34 weeks and 1,492 delivered after 34 weeks. The cervical length was also measured at 20-24 weeks in 1,320 of the cases, including 12 of those with subsequent spontaneous delivery before 34 weeks.

The maternal characteristics and obstetric history in the screened population are summarized in Table 2.2. In the group with spontaneous early delivery there was a higher prevalence of women of African racial origin and women with a previous miscarriage at 16-23 weeks or spontaneous delivery at 24-33 weeks.

Findings at 11-13 weeks

The maternal and pregnancy characteristics are presented in Table 2.2.

The median endocervical length at 11-13 weeks was 32.4 mm (5^{th} centile 25.6 mm, 95^{th} centile 40.2 mm) and median length of the cervico-isthmic complex was 45.3 mm (5^{th} centile 30.9 mm, 95^{th} centile 65.3 mm) (Figures 2.2 and 2.3). The median length of the isthmus was 13.8 (range 0-49.4) mm and it was above 5 mm in 1,287 (85.3%) of the 1,508 cases.

In the spontaneous early delivery group, compared to unaffected pregnancies, the median endocervical length was significantly shorter, but there was no significant difference in the length of the cervico-isthmic complex (Figure 2.3, Table 2.3).

The endocervical length was below the median in all 16 cases with spontaneous early delivery. The endocervical length was below 25 mm in 6 (37.5%) of the early preterm delivery group and 42 (2.8%) of the unaffected pregnancies. The respective values for endocervical length 25-29.9 mm and ≥30 mm were 9 (56.3%) vs 367 (24.6%) and 1 (6.3%) vs 1,083 (72.6%). Therefore, the rate of spontaneous early delivery decreased with endocervical length from 12.5% (6 of 48) for length below 25 mm to 2.4% (9 of 376) for length of 25-29.9 mm and 0.1% (1 of 1,084) for length of ≥30 mm ($\chi 2$ test, $p<0.0001$).

Relation of findings at 11-13 weeks and 20-24 weeks

In the 1,320 cases examined at 20-24 weeks the median endocervical length was 32.2 mm (5th centile 24.6 mm, 95th centile 40.2 mm) and median length of the cervico-isthmic complex was 40.4 mm (5th centile 26.1 mm, 95th centile 60.6 mm). The median length of the isthmus was 7.8 (range 0-51.0) mm and it was above 5 mm in 862 (65.3%) cases.

There was a significant association in the measurements between 11-13 weeks and those at 20-24 weeks for the length of the endocervix ($r=0.548$, $p<0.0001$; Figure 2.4), isthmus ($r=0.089$, $p=0.001$) and the cervico-isthmic complex ($r=0.194$, $p<0.0001$).

In the spontaneous early delivery group, compared to unaffected pregnancies, the length of both the endocervix and the cervico-isthmic complex was significantly shorter (Table 2.3).

Discussion

This study has demonstrated that firstly, in the measurement of cervical length it is important to distinguish between the endocervix and isthmus and secondly, the endocervical length at 11-13 weeks is shorter in pregnancies resulting in spontaneous delivery before 34 weeks than in those delivering after 34 weeks.

In the measurement of cervical length we adhered to the criteria suggested by Sonek and Shellhaas (1998). These authors highlighted the importance of including in the measurement only the portion of the cervix where the canal is bordered by the endocervical mucosa. Anatomical studies have demonstrated that during the early stages of pregnancy there is marked hypertrophy of the isthmic muscle (Danforth, 1947). In the second and third trimesters the isthmus undergoes a gradual unfolding, thinning and ‘taking up’ into the body of the uterus but this process leading to the formation of the lower uterine segment is completed only during labor (Wendell-Smith, 1954). Ultrasound studies in the 1990’s investigating changes in cervical length throughout gestation reported that the median or mean length in the first trimester was 42-52 mm and this remained stable throughout the second trimester with shortening only after 30 weeks (Ayers et al.,1998; Tongsong et al., 1997; Zorzoli et al.,1994). It is likely that in these studies the cervico-isthmic complex rather than the cervix was measured and the reported shortening in the third trimester was the consequence of formation of the lower segment with eventual obliteration of the isthmus.

At 11-13 weeks the median length of the endocervix and of the cervico-isthmic complex was 32 mm and 45 mm, respectively. The median endocervical length at 20-24 weeks was the same as at 11-13 weeks and there was a significant association in the measurements between the first and second trimesters. In contrast, between 11-13 weeks and 20-24 weeks there was shortening of the isthmus resulting in a decrease in the median length of the cervico-isthmic complex.

Previous studies investigating the potential value of first-trimester cervical length in the prediction of preterm delivery did not distinguish between the endocervix and isthmus and reported that the mean or median cervical length was 40-44 mm (Carvalho et al., 2003; Conoscenti et al., 2003; Hasegawa et al., 1996; Ozdemir et al., 2007; Zorzoli et al., 1994). It is therefore likely that in these studies the measurement was not confined to the endocervix but included the whole cervico-isthmic complex.

The endocervical length was below the median in all 16 cases with spontaneous early delivery. The endocervical length was below 25 mm in 6 (37.5%) of the early preterm delivery group and 42 (2.8%) of the unaffected pregnancies. The respective values for endocervical length 25-29.9 mm and $\geq$30 mm were 9 (56.3%) vs 367 (24.6%) and 1 (6.3%) vs 1,083 (72.6%). Therefore, the rate of spontaneous early delivery decreased with endocervical length from 12.5% (6 of 48) for length below 25 mm to 2.4% (9 of 376) for length of 25-29.9 mm and 0.1% (1 of 1,084) for length of $\geq$30 mm (χ^2 test, $p<0.0001$).

At 11-13 weeks the endocervical length in pregnancies complicated by subsequent spontaneous delivery before 34 weeks was shorter than in those delivering after 34 weeks and the risk for early delivery was inversely related to cervical length. This apparent contradiction between our results and those of previous first-trimester studies may be explained by the possibility that in the previous studies the cervico-isthmic complex rather than the endocervix was measured (Carvalho et al., 2003; Conoscenti et al., 2003; Hasegawa et al., 1996; Ozdemir et al., 2007; Zorzoli et al., 1994). We also found no significant differences in the length of the cervico-isthmic complex between the two outcome groups.

Larger studies involving several thousands of pregnancies will define the exact relation between cervical length at 11-13 weeks and spontaneous early delivery and the performance of such early screening either by cervical length alone or in combination with maternal characteristics and serum biomarkers (Beta *et al*., 2010).

PRENATAL DIAGNOSIS
Prenat Diagn 2011; **31**: 84–89.
Published online 28 December 2010 in Wiley Online Library
(wileyonlinelibrary.com) **DOI:** 10.1002/pd.2640

Prediction of spontaneous preterm delivery from endocervical length at 11 to 13 weeks

Elena Greco[1,2], Annabel Lange[1,2], Fred Ushakov[1], Jesus Rodriguez Calvo[1,2] and Kypros H. Nicolaides[1,2]*

[1]*Department of Fetal Medicine, University College Hospital, London, UK*
[2]*Harris Birthright Research Centre of Fetal Medicine, King's College Hospital, London, UK*

Objective To define the potential value of endocervical length at 11 to 13 weeks' gestation in the prediction of spontaneous early delivery.

Method The lengths of the endocervix and cervico–isthmic complex were measured by transvaginal ultrasound at 11 to 13 weeks in singleton pregnancies, including 1492 that subsequently delivered after 34 weeks and 16 (1.1%) who had spontaneous delivery before 34 weeks. In 1320 of the cases, the measurements were repeated at 20 to 24 weeks.

Results There were significant associations in the length of the endocervix and cervico-isthmic complex between 11 to 13 and 20 to 24 weeks ($r = 0.548$, $p < 0.0001$ and $r = 0.194$, $p < 0.0001$), and the respective median lengths were 32.4 and 32.2 mm for the endocervix and 45.3 and 40.4 mm for the cervico–isthmic complex. At 11 to 13 weeks in the early delivery group, compared to unaffected pregnancies, the median endocervical length was shorter (27.5 vs 32.5 mm, $p < 0.0001$), but there was no significant difference in the length of the cervico–isthmic complex (41.4 vs 45.4 mm, $p = 0.054$).

Conclusion In the measurement of cervical length, the endocervix should be distinguished from the isthmus. The endocervical length at 11 to 13 weeks is shorter in pregnancies resulting in spontaneous delivery before 34 weeks than in those delivering after 34 weeks.

KEY WORDS: first-trimester screening; preterm delivery; cervical length; endocervix; isthmus; transvaginal ultrasound

INTRODUCTION

Preterm birth is responsible for 75% of all neonatal deaths and over half the neurological handicap in children [McCormick, 1985; Centre for Maternal and Child Enquiries (CMACE), 2010]. Although all births before 37 weeks' gestation are defined as preterm, the vast majority of mortality and morbidity relates to early delivery before 34 weeks. Although improvements in neonatal care have led to higher survival of very premature infants, a major impact on the associated mortality and morbidity will only be achieved through the development of a sensitive method to identify women at high risk of preterm delivery and an effective strategy for the prevention of this complication.

The risk of spontaneous preterm birth is inversely related to cervical length measured by transvaginal sonography at 20 to 24 weeks' gestation (Iams *et al.*, 1996; Heath *et al.*, 1998; Kagan *et al.*, 2006; To *et al.*, 2006; Celik *et al.*, 2008). In women with a short cervix, administration of progesterone reduces the risk of spontaneous early preterm delivery by about 40% (Fonseca *et al.*, 2007). However, progesterone is not as effective in women with cervical length below 12 mm as in those with a length of 12 to 15 mm. An alternative treatment for women with a short cervix is cervical cerclage. This reduces the risk of spontaneous early preterm delivery by about 40% in women who had a previous preterm birth or second-trimester loss but not in those without such history (To *et al.*, 2004; Berghella *et al.*, 2005).

The disadvantages of measuring cervical length at 20 to 24 weeks are first, inevitable failure to identify cervical incompetence leading to miscarriage before this gestation and second, the effectiveness of prophylactic administration of progesterone or cervical cerclage may be inversely related to the gestation at which treatment is initiated. Certainly in women who had a previous preterm birth or second-trimester loss, cervical cerclage is either carried out electively in the first trimester or it is reserved for those where serial scans, starting from the first-trimester, demonstrate cervical shortening (Althuisius *et al.*, 2000).

Several studies reported that the measurement of cervical length in the first trimester is not predictive of preterm delivery (Zorzoli *et al.*, 1994; Hasegawa *et al.*, 1996; Carvalho *et al.*, 2003; Conoscenti *et al.*, 2003; Ozdemir *et al.*, 2007). The mean or median cervical length in these studies (40–44 mm) was considerably longer than in the second trimester. It is possible that cervical shortening in cases of preterm delivery is not apparent in the first trimester because it occurs after 16 weeks (Berghella *et al.*, 2003). Alternatively, preterm delivery is associated with short cervix in the first trimester but this has not been recognised because

*Correspondence to: Kypros H. Nicolaides, Harris Birthright Research Centre for Fetal Medicine, King's College Hospital, London SE5 9RS, UK. E-mail: kypros@fetalmedicine.com

Received: 10 August 2010
Revised: 3 September 2010
Accepted: 5 September 2010
Published online: 28 December 2010

in the measurement of cervical length sonographers inadvertently include the uterine isthmus.

The aim of this study was to define and standardise the technique for the measurement of cervical length at 11 to 13 weeks and to determine the potential value of this measurement in the prediction of spontaneous early preterm birth.

METHODS

The data for this study were derived from prospective screening for fetal abnormalities and pregnancy complications in women attending for their routine first hospital visit in pregnancy at University College Hospital, London, UK. In this visit, which is held at 11^{+0} to 13^{+6} weeks of gestation, we record maternal characteristics and medical history and perform transabdominal and transvaginal sonography to (1) determine gestational age from the measurement of the fetal crown-rump length, (2) diagnose any major fetal abnormalities and (3) measure fetal nuchal translucency thickness as part of screening for chromosomal abnormalities (Robinson and Fleming, 1975; Snijders *et al.*, 1998). The transvaginal scan includes the measurement of cervical length and if this is less than 15 mm the women who are at high risk for preterm delivery are referred to a specialist clinic. However, in this study, none of the patients had such a short cervix. In our hospital, all women at 20 to 24 weeks of gestation are offered another ultrasound scan for the diagnosis of fetal abnormalities, assessment of fetal growth and transvaginal measurement of cervical length.

The inclusion criteria for this study were singleton pregnancies with the measurement of cervical length at 11 to 13 weeks and delivery at or after 24 weeks' gestation. We excluded pregnancies ending in termination, miscarriage or fetal death before 24 weeks and those with iatrogenic delivery before 34 weeks.

Measurement of endocervical and isthmic length

We have observed that in the majority of women undergoing cervical assessment before the development of the lower uterine segment, there is a persistent myometrial thickening between the endocervix and the gestational sac (Figure 1). This thickening is likely to represent the isthmus rather than a contraction. Consequently, in the measurement of cervical length, which for the purpose of clarity we define as endocervical length, we undertake the following steps. First, the women are asked to empty their bladder and are placed in the dorsal lithotomy position. Second, the vaginal transducer (2.7–9.3 MHz) is introduced in the anterior fornix of the vagina and adjusted to obtain a sagittal view of the entire length of the cervical canal, which may be either translucent or echodense. The canal is bordered by the endocervical mucosa, which is usually of decreased but occasionally of increased echogenicity compared to the surrounding tissues. Third, the probe is withdrawn until the image is blurred and then advanced gently until the image is restored without exerting undue pressure on the cervix. Fourth, the settings of the ultrasound machine are altered to obtain the widest viewing angle and the magnification is increased so that most of the screen is occupied by the tissues between the external cervical os at one end of the picture and gestational sac at the other end. Fifth, callipers are used to measure in sequence the linear distance between the two ends of the glandular area around the endocervical canal and the shortest distance between the glandular area and the gestational sac (isthmus).

All the operators performing the scans had received extensive training and had all passed a practical examination administered by an expert to demonstrate their competence in the technique. In addition, all ultrasound images for every study subject were reviewed by a single investigator who was unaware of the outcome of pregnancy, to ensure that measurements were made appropriately and consistently.

Diagnosis of spontaneous early preterm delivery

Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women and were also recorded in our database. The obstetric records of all patients delivering before 34 weeks were examined to determine whether the preterm delivery was medically indicated or spontaneous. The latter included those with spontaneous onset of labour and those with preterm pre-labour rupture of membranes.

Statistical analysis

Comparison between the outcome groups was by Mann–Whitney U-test for continuous variables and χ^2-test or Fisher's exact test for categorical variables. The significance of difference in endocervical length and cervico–isthmic complex length (endocervical plus isthmic length) in the spontaneous early delivery and unaffected groups was determined. Regression analysis was used to examine the association in the measurements between 11 to 13 weeks and those at 20 to 24 weeks for the length of both the endocervix and the cervico–isthmic complex.

The statistical software package SPSS 16.0 (SPSS Inc., Chicago, IL, USA) was used for data analyses.

RESULTS

During the study period (July 2009 to February 2010), we measured the cervical length at 11 to 13 weeks in 1548 singleton pregnancies. We excluded 40 cases because the pregnancies resulted in miscarriage or termination ($n = 34$) or there was iatrogenic delivery at 24 to 33 weeks ($n = 6$), for preeclampsia in 4, maternal cerebral haemorrhage in 1 and bleeding from a placenta previa in 1. In the 1508 cases included in the study, 16 (1.1%) had spontaneous delivery before 34 weeks and

Prenat Diagn 2011; 31: 84–89.
DOI: 10.1002/pd

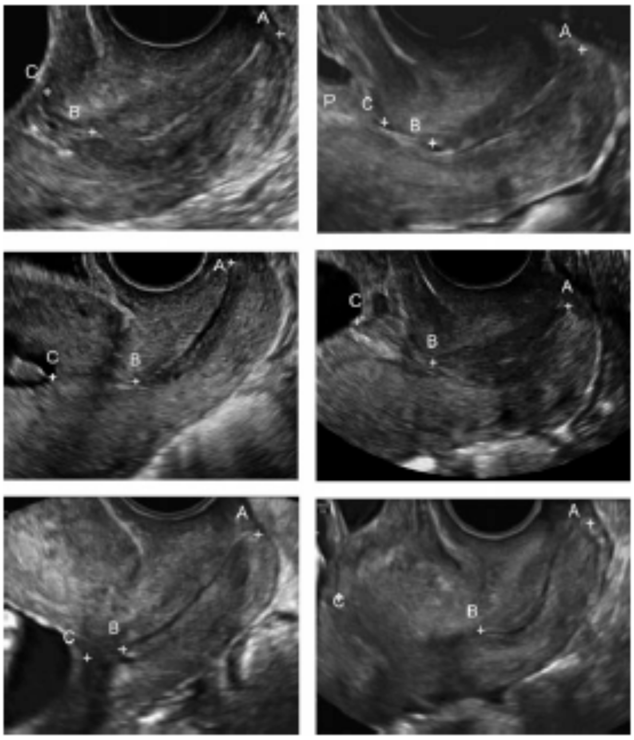


Figure 1—Ultrasound pictures illustrating the measurement of the length of the endocervix (A to B) and the isthmus (B to C). In one picture, the placenta (P) overlies the opening of the isthmus into the uterine cavity

1492 delivered after 34 weeks. The cervical length was also measured at 20 to 24 weeks in 1320 of the cases, including 12 of those with subsequent spontaneous delivery before 34 weeks.

The maternal characteristics and obstetric history in the screened population are summarised in Table 1. In the group with spontaneous early delivery, there was a higher prevalence of women of African racial origin and women with a previous miscarriage at 16 to 23 weeks or spontaneous delivery at 24 to 33 weeks.

Findings at 11 to 13 weeks

The maternal and pregnancy characteristics are presented in Table 1.

The median endocervical length at 11 to 13 weeks was 32.4 mm (5th centile 25.6 mm, 95th centile 40.2 mm) and median length of the cervico−isthmic complex was 45.3 mm (5th centile 30.9 mm, 95th centile 65.3 mm) (Figures 2 and 3). The median length of the isthmus was 13.8 (range 0−49.4) mm and it was above 5 mm in 1287 (85.3%) of the 1508 cases.

In the spontaneous early delivery group, compared to unaffected pregnancies, the median endocervical length was significantly shorter, but there was no significant difference in the length of the cervico−isthmic complex (Figure 3 and Table 2).

The endocervical length was below the median in all 16 cases with spontaneous early delivery. The endocervical length was below 25 mm in 6 (37.5%) of the early preterm delivery group and 42 (2.8%) of the unaffected pregnancies. The respective values for endocervical length 25 to 29.9 mm and ≥30 mm were 9 (56.3%) versus 367 (24.6%) and 1 (6.3%) versus 1083 (72.6%). Therefore, the rate of spontaneous early delivery decreased with endocervical length from 12.5% (6 of 48) for length below 25 mm to 2.4% (9 of 376) for length of 25 to 29.9 mm and 0.1% (1 of 1084) for length of ≥30 mm (χ^2-test, $p < 0.0001$).

Relation of findings at 11 to 13 weeks and 20 to 24 weeks

In the 1320 cases examined at 20 to 24 weeks, the median endocervical length was 32.2 mm (5th centile 24.6 mm, 95th centile 40.2 mm) and median length of the cervico−isthmic complex was 40.4 mm (5th centile 26.1 mm, 95th centile 60.6 mm). The median length

Table 1—Maternal characteristics and obstetric history in the screened population

Characteristics	Delivery ≥34 weeks (n = 1492)	Early preterm (n = 16)
Maternal age in years, median (IQR)	32.5 (29.3–35.6)	33.2 (29.7–35.9)
Maternal weight, median (IQR)	63.0 (57.2–70.7)	61.8 (55.5–81.0)
Maternal height in cm, median (IQR)	165 (160–170)	163 (156–167)
Racial origin		
Caucasian, n (%)	1096 (73.5)	10 (62.5)
African, n (%)	126 (8.4)	4 (25.0)*
South Asian, n (%)	149 (10.0)	1 6.3)
East Asian, n (%)	91 (6.1)	0
Mixed, n (%)	30 (2.0)	1 (6.3)
Cigarette smoker, n (%)	32 (2.1)	0
Conception		
Spontaneous, n (%)	1,402 (94.0)	16.0 (100)
Ovulation drugs, n (%)	90 (6.0)	0
Obstetric history		
Nulliparous—no pregnancy at >16 weeks, n (%)	937 (62.8)	9 (56.3)
Nulliparous—miscarriage at 16 to 23 weeks, n (%)	22 (15)	2 (12.5)*
Parous—preterm delivery 24 to 33 weeks, n (%)	14 (0.9)	2 (12.5)*
Parous—delivery >34 weeks, n (%)	519 (34.8)	3 (18.8)

Comparisons between groups (χ^2-test and Fisher's exact test for categorical variables and Mann–Whitney U-test for continuous variables).
*$p < 0.05$.

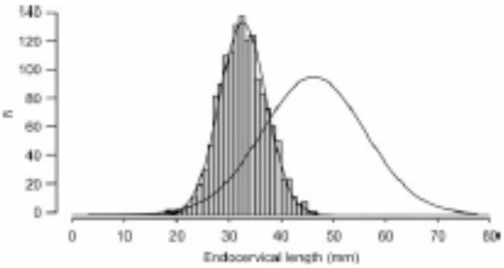


Figure 2—Distribution of endocervical length at 11 to 13 weeks (histograms and overlying curve). The curve on the right is the distribution of length of the cervico–isthmic complex

of the isthmus was 7.8 (range 0–51.0) mm and it was above 5 mm in 862 (65.3%) cases.

There was a significant association in the measurements between 11 and 13 weeks and those at 20 to 24 weeks for the length of the endocervix ($r = 0.548$, $p < 0.0001$; Figure 4), isthmus ($r = 0.089$, $p = 0.001$) and the cervico–isthmic complex ($r = 0.194$, $p < 0.0001$).

In the spontaneous early delivery group, compared to unaffected pregnancies, the length of both the endocervix and the cervico–isthmic complex was significantly shorter (Table 2).

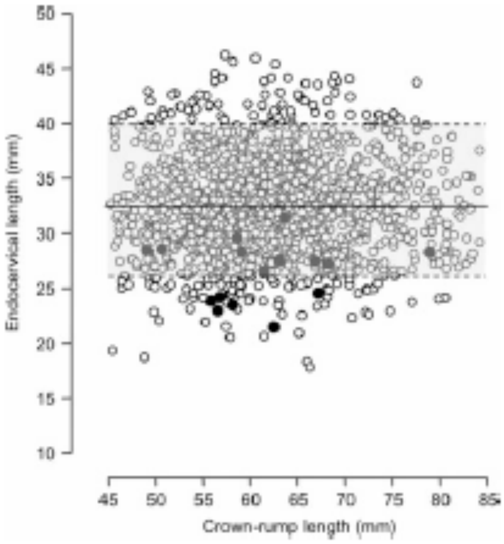


Figure 3—Reference range (50th, 5th and 95th centiles) and individual measurements in women with subsequent spontaneous delivery before 34 weeks (closed circles) and those delivering at or after 34 weeks (open circles)

DISCUSSION

This study has demonstrated that (1) in the measurement of cervical length it is important to distinguish between the endocervix and the isthmus and (2) the endocervical length at 11 to 13 weeks is shorter in pregnancies resulting in spontaneous delivery before 34 weeks than in those delivering after 34 weeks.

In the measurement of cervical length, we adhered to the criteria suggested by Sonek and Shellhaas (1998). These authors highlighted the importance of including in the measurement only the portion of the cervix where the canal is bordered by the endocervical mucosa. Anatomical studies have demonstrated that during the early stages of pregnancy there is marked hypertrophy of the isthmic muscle (Danforth, 1947). In the second and third trimesters, the isthmus undergoes a gradual

DOI: 10.1002/pd

Table 2—Comparison of the length of the endocervix and cervico–isthmic complex at 11 to 13 weeks and 20 to 24 weeks in women with spontaneous delivery before 34 weeks' gestation and those delivering at or after 34 weeks

Variables	Delivery ≥34 weeks	Delivery <34 weeks	p
11 to 13 weeks			
Endocervical length, median (IQR)	32.5 (29.5–35.6)	27.5 (24.0–28.6)	<0.0001
Cervico–isthmic length, median (IQR)	45.4 (39.0–53.1)	41.4 (34.8–45.8)	0.054
20 to 24 weeks			
Endocervical length, median (IQR)	32.2 (29.3–35.3)	20.6 (17.0–27.7)	<0.0001
Cervico–isthmic length, median (IQR)	40.7 (33.9–47.7)	27.0 (17.0–33.8)	<0.0001

IQR, interquartile range.
Comparisons between the outcome groups were by Mann–Whitney U-test.

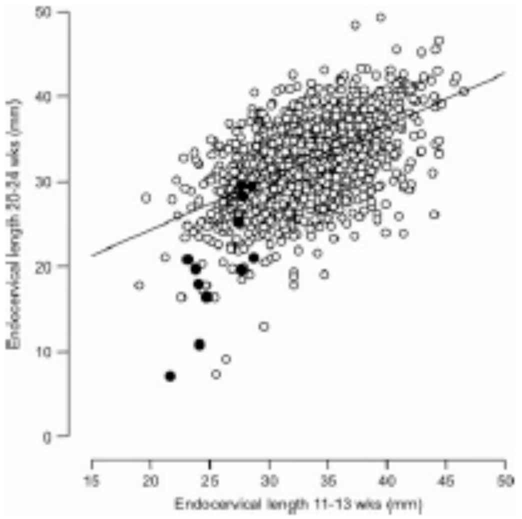


Figure 4—Relationship of endocervical length at 11 to 13 weeks with 20 to 24 weeks in women with subsequent spontaneous delivery before 34 weeks (closed circles) and those delivering at or after 34 weeks (open circles)

unfolding, thinning and 'taking up' into the body of the uterus but this process leading to the formation of the lower uterine segment is completed only during labour (Wendell-Smith, 1954). Ultrasound studies in the 1990s investigating changes in cervical length throughout gestation reported that the median or mean length in the first trimester was 42 to 52 mm and this remained stable throughout the second trimester with shortening only after 30 weeks (Zorzoli *et al.*, 1994; Tongsong *et al.*, 1997; Ayers *et al.*, 1998). It is likely that in these studies the cervico–isthmic complex rather than the cervix was measured and the reported shortening in the third trimester was the consequence of formation of the lower segment with eventual obliteration of the isthmus.

At 11 to 13 weeks, the median length of the endocervix and of the cervico–isthmic complex was 32 and 45 mm, respectively. The median endocervical length at 20 to 24 weeks was the same as at 11 to 13 weeks, and there was a significant association in the measurements between the first and the second trimesters. In contrast, between 11 to 13 weeks and 20 to 24 weeks there was shortening of the isthmus resulting in a decrease in the median length of the cervico–isthmic complex.

Previous studies investigating the potential value of first-trimester cervical length in the prediction of preterm delivery did not distinguish between the endocervix and isthmus and reported that the mean or median cervical length was 40 to 44 mm (Zorzoli *et al.*, 1994; Hasegawa *et al.*, 1996; Carvalho *et al.*, 2003; Conoscenti *et al.*, 2003; Ozdemir *et al.*, 2007). It is therefore likely that in these studies the measurement was not confined to the endocervix but included the whole cervico–isthmic complex.

The endocervical length was below the median in all 16 cases with spontaneous early delivery. The endocervical length was below 25 mm in 6 (37.5%) of the early preterm delivery group and 42 (2.8%) of the unaffected pregnancies. The respective values for endocervical length 25 to 29.9 mm and ≥30 mm were 9 (56.3%) versus 367 (24.6%) and 1 (6.3%) versus 1083 (72.6%). Therefore, the rate of spontaneous early delivery decreased with endocervical length from 12.5% (6 of 48) for length below 25 mm to 2.4% (9 of 376) for length of 25 to 29.9 mm and 0.1% (1 of 1084) for length of ≥30 mm (χ^2-test, $p < 0.0001$).

At 11 to 13 weeks, the endocervical length in pregnancies complicated by subsequent spontaneous delivery before 34 weeks was shorter than in those delivering after 34 weeks and the risk of early delivery was inversely related to cervical length. This apparent contradiction between our results and those of previous first-trimester studies may be explained by the possibility that in the previous studies the cervico–isthmic complex rather than the endocervix was measured (Zorzoli *et al.*, 1994; Hasegawa *et al.*, 1996; Carvalho *et al.*, 2003; Conoscenti *et al.*, 2003; Ozdemir *et al.*, 2007). We also found no significant differences in the length of the cervico–isthmic complex between the two outcome groups.

Larger studies involving several thousands of pregnancies will define the exact relation between cervical length at 11 to 13 weeks and spontaneous early delivery and the performance of such early screening either by cervical length alone or in combination with maternal characteristics and serum biomarkers (Beta *et al.*, 2010). Similarly, the extent to which early identification of the group at high risk of subsequent early delivery would improve pregnancy outcome through earlier intervention with such measures as prophylactic use of progesterone or cervical cerclage remains to be determined.

ACKNOWLEDGEMENT

This study was supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116).

REFERENCES

Althuisius SM, Dekker GA, van Geijn HP, Bekedam DJ, Hummel P. 2000. Cervical incompetence prevention randomized cerclage trial (CIPRACT): study design and preliminary results. *Am J Obstet Gynecol* **183**: 823–829.

Ayers JW, DeGrood RM, Compton AA, Barclay M, Ansbacher R. 1998. Sonographic evaluation of cervical length in pregnancy: diagnosis and management of preterm cervical effacement in patients at risk for premature delivery. *Obstet Gynecol* **71**: 934–944.

Berghella V, Talucci M, Desai A. 2003. Does transvaginal sonographic measurement of cervical length before 14 weeks predict preterm delivery in high-risk pregnancies?. *Ultrasound Obstet Gynecol* **21**: 140–144.

Berghella V, Odibo AO, To MS, Rust OA, Althuisius SM. 2005. Cerclage for short cervix on ultrasonography: meta-analysis of trials using individual patient-level data. *Obstet Gynecol* **106**: 181–189.

Beta J, Akolekar R, Ventura W, Syngelaki A, Nicolaides KH. 2010. Prediction of spontaneous preterm delivery from maternal factors and placental perfusion and function at 11–13 weeks. *Pren Diagn* xx: xx–xx.

Carvalho MH, Bittar RE, Brizot ML, Maganha PP, Borges da Fonseca ES, Zugaib M. 2003. Cervical length at 11–14 weeks' and 22–24 weeks' gestation evaluated by transvaginal sonography, and gestational age at delivery. *Ultrasound Obstet Gynecol* **21**: 135–139.

Celik E, To M, Gajewska K, Smith GC, Nicolaides KH; Fetal Medicine Foundation Second Trimester Screening Group. 2008. Cervical length and obstetric history predict spontaneous preterm birth: development and validation of a model to provide individualized risk assessment. *Ultrasound Obstet Gynecol* **31**: 549–554.

Centre for Maternal and Child Enquiries (CMACE). 2010. *Perinatal Mortality 2008: United Kingdom*. CMACE: London.

Conoscenti G, Meir YJ, D'Ottavio G, *et al.* 2003. Does cervical length at 13–15 weeks' gestation predict preterm delivery in an unselected population? *Ultrasound Obstet Gynecol* **21**: 128–134.

Danforth DN. 1947. The fibrous nature of the human cervix, and its relation to the isthmic segment in gravid and nongravid uteri. *Am J Obstet Gynecol* **53**: 541–560.

Fonseca EB, Celik E, Parra M, Singh M, Nicolaides KH. 2007. Progesterone and the risk of preterm birth among women with a short cervix. *N Engl J Med* **357**: 462–469.

Hasegawa I, Tanaka K, Takahashi K, *et al.* 1996. A prospective longitudinal study for the prediction of preterm delivery in a low-risk population. *J Matern Fetal Invest* **6**: 148–151.

Heath VC, Southall TR, Souka AP, Elisseou A, Nicolaides KH. 1998. Cervical length at 23 weeks of gestation: prediction of spontaneous preterm delivery. *Ultrasound Obstet Gynecol* **12**: 312–317.

Iams JD, Goldenberg RL, Meis PJ, *et al.* 1996. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. *N Engl J Med* **334**: 567–572.

Kagan K, To M, Tsoi E, Nicolaides K. 2006. Preterm birth: the value of sonographic measurement of cervical length. *BJOG* **113**: 52–56.

McCormick MC. 1985. The contribution of low birth weight to infant mortality and childhood morbidity. *N Engl J Med* **312**: 82–90.

Ozdemir I, Demirci F, Yucel O, Erkorkmaz U. 2007. Ultrasonographic cervical length measurement at 10–14 and 20–24 weeks gestation and the risk of preterm delivery. *Eur J Obstet Gynecol Reprod Biol* **130**: 176–179.

Robinson HP, Fleming JE. 1975. A critical evaluation of sonar "crown-rump length" measurements. *BJOG* **82**: 702–710.

Sonek J, Shellhaas C. 1998. Cervical sonography: a review. *Ultrasound Obstet Gynecol* **11**: 71–78.

Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH; Fetal Medicine Foundation First Trimester Screening Group. 1998. UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. *Lancet* **352**: 343–346.

To MS, Alfirevic Z, Heath VC, *et al.* 2004. Cervical cerclage for prevention of preterm delivery in women with short cervix: randomised controlled trial. *Lancet* **363**: 1849–1853.

To MS, Skentou CA, Royston P, Yu CK, Nicolaides KH. 2006. Prediction of patient-specific risk of early preterm delivery using maternal history and sonographic measurement of cervical length: a population-based prospective study. *Ultrasound Obstet Gynecol* **27**: 362–367.

Tongsong T, Kamprapanth P, Pitaksakorn J. 1997. Cervical length in normal pregnancy as measured by transvaginal sonography. *Int J Gynecol Obstet* **58**: 313–315.

Wendell-Smith CP. 1954. The lower uterine segment. *BJOG* **61**: 87–93.

Zorzoli A, Soliani A, Perra M, Caravelli E, Galimberti A, Nicolini U. 1994. Cervical changes throughout pregnancy as assessed by transvaginal sonography. *Obstet Gynecol* **84**: 960–964.

DOI: 10.1002/pd

2ND RESEARCH BRANCH – VALIDATION OF THE METHOD IN A SCREENING SETTING

First trimester screening for spontaneous preterm delivery by maternal characteristics and cervical length

The aim of this screening study is to examine the potential value of cervical length at 11-13 weeks' gestation in the prediction of spontaneous preterm delivery.

Methods

This was a prospective screening study for spontaneous preterm delivery in women attending for their routine first hospital visit in pregnancy at King's College Hospital, University College London Hospital and Medway Maritime Hospital Gillingham. In this visit, which is held at 11^{+0}-13^{+6} weeks' gestation, we recorded maternal characteristics and medical history and performed transabdominal and transvaginal sonography to firstly, determine gestational age from the measurement of the fetal crown-rump length (CRL), secondly, diagnose any major fetal abnormalities, thirdly, measure fetal nuchal translucency thickness as part of screening for aneuploidies and fourthly, measure endocervical length (Greco et al., 2011; Nicolaides, 2011a ; Snijders et al., 1998; Syngelaki et al., 2011; Robinson and Fleming, 1975). The policy was to refer women with cervical length of less than 15 mm to a specialist high-risk for preterm delivery clinic. However, in this study none of the patients had such a short cervix. All women were offered another scan at 20-24 weeks' gestation for the diagnosis of fetal abnormalities, assessment of fetal growth and transvaginal measurement of cervical length and if the length was less than 15 mm the women were treated by cervical cerclage or vaginal progesterone.

Measurement of endocervical length

The measurement of endocervical length by transvaginal sonography was performed as previously described (Greco et al., 2011; Figure 2.1). First, the women were asked to empty their bladder and were placed in the dorsal lithotomy position. Second, the vaginal transducer (2.7–9.3 MHz) was introduced in the anterior fornix of the vagina and adjusted to obtain a sagittal view of the entire length of the cervical canal, which may be either translucent or echodense. The canal is bordered by the endocervical mucosa, which is usually of decreased but occasionally of increased echogenicity compared to the surrounding tissues. Third, the probe was withdrawn until the image was blurred and then advanced gently until the image was restored without exerting undue pressure on the cervix. Fourth, the settings of the ultrasound machine were altered to obtain the widest viewing angle and the magnification was increased so that most of the screen was occupied by the tissues between the external cervical os at one end of the picture and gestational sac at the other end. Fifth, calipers were used to measure in sequence the linear distance between the two ends of the glandular area around the endocervical canal (Figure 2.5). All the operators performing the scans had received extensive training and had all passed a practical examination administered by an expert to demonstrate their competence in the technique.

Outcome measures

The outcome measures were spontaneous preterm delivery before 34 weeks (early preterm) and at 34^{+0}-36^{+6} weeks (late preterm). Data on pregnancy outcome were obtained from the maternity computerised records or the general medical practitioners of the women and were also recorded in our database. The obstetric records of all patients delivering before 37 weeks (< 259 days) were examined to determine whether the preterm delivery was medically indicated or spontaneous. The latter included those with spontaneous onset of labour and those with preterm pre-labour rupture of membranes.

Statistical analysis

Descriptive data were presented in median and interquartile range (IQR) for continuous variables and in numbers and percentages for categorical variables. Comparison between the outcome groups was by Chi-square or Fisher exact test for categorical variables and Mann-Whitney-U test for continuous variables.

The distribution of cervical length was logarithmically transformed to obtain a symmetric distribution of residuals with approximately constant standard deviation. This was assessed by inspecting histograms and probability plots. Multivariate regression analysis was used to determine which of the factors among the maternal characteristics; obstetric history and gestation were significant predictors of $\log_{10}$ cervical length in the term delivery group. The distribution of $\log_{10}$ cervical length, expressed as multiple of the median (MoM) of the term delivery group, was determined in the early and late spontaneous preterm delivery groups. Comparison of cervical length MoM between outcome groups was by Mann-Whitney U test, with post-hoc Bonferroni correction. The a priori risk for early spontaneous preterm delivery based on maternal characteristics and obstetric history was determined as previously described (Beta et al., 2011). Likelihood ratios were computed from the fitted distributions of $\log_{10}$ MoM values in the term delivery group and in each of the two preterm delivery groups. The a posteriori risks for early and late spontaneous preterm delivery were derived by multiplying the a priori risk with the likelihood ratio. The performance of screening was determined by the area under the receiver operating characteristic curve (AUROC) (Zweig and Campbell, 1993).

The statistical software package SPSS 18.0 (SPSS Inc.,Chicago, IL) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for all data analyses.

Results

We prospectively examined 10,870 singleton pregnancies between July 2009 and March 2011. We excluded 896 (8.2%) because they had missing outcome data (n=423), the pregnancies resulted in miscarriage before 24 weeks' gestation (n=91) or termination (n=105), there was iatrogenic delivery at 24 to 33 weeks (n=192) or the women had either cervical cerclage (n=37) or vaginal progesterone (n=48). In the remaining 9,974 pregnancies included in the study spontaneous preterm delivery before 34 weeks occurred in 104 (1.0%) cases and between 34 and 36 completed weeks in 213 (2.1%) cases. The maternal characteristics of each of the outcome groups are compared in Table 2.4.

Multivariate regression analysis in the term delivery group demonstrated that for the $\log_{10}$ cervical length significant independent contributions were provided by fetal CRL, maternal height, age, racial origin and parity (R^2=0.018; Table 2.5). The median cervical length MoM was significantly lower in both the early (p<0.0001) and late (p=0.009) spontaneous preterm delivery groups than in the term delivery group (Table 2.6).

In the term delivery group, African women had shorter cervical length than Caucasian women (Table 2.5) but cervical length MoM was not significantly different between the two racial groups (p=0.628; Figure 2.6). Similarly, in the early and late preterm delivery groups, the median cervical length MoM was not significantly different between African and Caucasian women (p=0.264; p=0.966; Figure 2.6).

The frequency distributions of cervical length MoM in the term delivery group and early spontaneous preterm delivery group are presented in Figure 2.7. The overlapping Gaussian distributions of $\log_{10}$ cervical length MoM in the term delivery group and each of the spontaneous preterm delivery groups were used to calculate the likelihood ratios for preterm delivery (Figure 2.8, Table 2.7).

The a priori risk for early spontaneous preterm delivery based on maternal characteristics and obstetric history was determined as previously described (Beta et al., 2011) and using this model, the expected number of early spontaneous preterm delivery was 101 (95% prediction interval 83-123), which is similar to the observed number of 104. The a posteriori risks for spontaneous preterm delivery were derived by multiplying the a priori risk by the likelihood ratio for cervical length.

The AUROC and the detection rates of early and late spontaneous preterm delivery for false positive rates of 5% and 10% in screening by maternal characteristics, cervical length and their combination are given in Figure 2.9 and Table 2.8. In the prediction of early spontaneous preterm delivery, the AUROC was significantly improved to 0.840 (95% CI 0.833-0.847) when maternal characteristics (0.714, 95% CI 0.705-0.723) was combined with cervical length (0.782, 95% CI 0.774-0.790; $p<0.0001$). In the prediction of late spontaneous preterm delivery, the AUROC was significantly improved to 0.583 (95% CI 0.573-0.593) when maternal characteristics (0.563, 95% CI 0.553-

0.573) were combined with cervical length (0.551, 95% CI 0.541-0.561; p=0.042).

Discussion

The findings of this screening study demonstrate that in singleton pregnancies resulting in spontaneous preterm delivery cervical length at 11-13 weeks' gestation is shorter than in women delivering at term. An algorithm combining maternal characteristics and cervical length can identify about 55% of pregnancies resulting in delivery before 34 weeks at a false positive rate of 10%. The detection rate for delivery at 34-36 weeks was only 20%.

The study confirmed that spontaneous preterm delivery is associated with certain maternal characteristics and the observed number of preterm deliveries in this study was similar to that predicted from a multivariable regression model published previously (Beta et al., 2011). The risk for spontaneous early preterm delivery increases with maternal age and decreases with height, it is higher in women of African and South Asian racial origin than in Caucasians, in cigarette smokers and in those conceiving after the use of ovulation induction drugs. The risk of preterm delivery is also influenced by the outcome of previous pregnancies. The risk is inversely related to the gestation at previous spontaneous delivery decreasing from about 7% if the gestation was 16-24 weeks to 3% if 31-33 weeks and 0.6% if all deliveries were at term. Additionally, the risk is affected by the number of previous spontaneous deliveries at 16-30 weeks, increasing from about 6% to 19% if there are two rather than one such delivery. In women with previous preterm deliveries there is a protective effect against recurrence if they also had a delivery at term and for women with one or two deliveries at 16-30 weeks the risk of recurrence decreases from about 6% to 1.5% and from 19% to 10%, respectively. Screening at 11-13 weeks by an algorithm combining maternal characteristics and obstetric history (Beta et al., 2011) identified about 38% of our pregnancies resulting in spontaneous delivery before 34 weeks and 20% of those delivering at 34-36 weeks, at a false positive rate of 10%.

In the measurement of cervical length care was taken to include only the portion of the cervix where the canal is bordered by the endocervical mucosa (Greco et al., 2011; Sonek and Shellhaas, 1998). At 11-13 weeks' gestation the median cervical length in women delivering at term was 32 mm. Multivariate regression analysis in the normal outcome group demonstrated that cervical length increases with fetal CRL, maternal age and height and is lower in women of African and South Asian racial origin than in Caucasians. After adjustment for these maternal characteristics the median cervical length MoM was significantly reduced in the early and to a lesser extent in the late spontaneous preterm delivery groups. The patient-specific risk for spontaneous preterm delivery was inversely related to the cervical length MoM and the individual risk can be derived by multiplying the a priori risk, obtained from maternal characteristics and obstetric history, by the likelihood ratio for cervical length.

In women of African racial origin the cervical length in both the term and early spontaneous preterm delivery groups was shorter than in Caucasians. In the calculation of MoMs, after correction for maternal characteristics including racial origin, the cervical length in those with spontaneous delivery before 34 weeks was shorter in women of African than Caucasian origin but this difference was not significant. Similarly, the performance of screening for preterm delivery in women of African origin was higher, but not significantly so, than in Caucasians. The extent to which larger studies will demonstrate significant differences between racial groups in the short cervical length related preterm delivery rate and therefore in the mechanisms leading to preterm delivery remains to be determined.

The study has provided evidence that spontaneous preterm delivery can be added to the list of pregnancy complications that can now be identified by screening at 11-13 weeks' gestation (Nicolaides, 2011b). Further studies will prove that such technique is easy to be mastered and has the characteristics of reproducibility and patients' acceptability required in a screening setting.

Finally, randomized studies, based on first-trimester screening to identify the high-risk group for subsequent early delivery, will investigate the extent to which pregnancy outcome would improve through early intervention with such measures as prophylactic use of progesterone.

Original Paper

Fetal Diagnosis and Therapy

Fetal Diagn Ther 2012;31:154–161
DOI: 10.1159/000335686

Received: November 14, 2011
Accepted after revision: December 11, 2011
Published online: March 6, 2012

First-Trimester Screening for Spontaneous Preterm Delivery with Maternal Characteristics and Cervical Length

Elena Greco[a, b] Rachna Gupta[a, b] Argyro Syngelaki[a] Leona C.Y. Poon[a]
Kypros H. Nicolaides[a–c]

[a]Harris Birthright Research Centre of Fetal Medicine, King's College Hospital, and [b]Department of Fetal Medicine, University College Hospital, London, and [c]Department of Fetal Medicine, Medway Maritime Hospital, Gillingham, UK

Key Words

First-trimester screening · Preterm delivery · Cervical length · Pyramid of pregnancy care

Abstract

Objective: It was the aim of this study to examine the potential value of cervical length at 11–13 weeks' gestation in the prediction of spontaneous preterm delivery. ***Methods:*** This was a screening study for spontaneous preterm delivery in singleton pregnancies from cervical length measured by transvaginal ultrasound at 11–13 weeks' gestation. The performance of screening for preterm delivery by cervical length alone and with maternal characteristics was estimated. ***Results:*** In the 9,974 pregnancies included in the study, spontaneous delivery before 34 weeks occurred in 104 (1.0%) cases. Multivariate regression analysis in the term delivery group demonstrated that for the $\log_{10}$ cervical length, significant independent contributions were provided by fetal crown-rump length, maternal height, age, racial origin and parity. The median cervical length multiple of the median (MoM), corrected for maternal characteristics, was significantly lower in the preterm (0.892 MoM, 95% CI 0.829–0.945) than in the term delivery group (0.994 MoM, 95% CI 0.919–1.082; $p < 0.0001$). In screening by a combination of maternal characteristics and cervical length, the estimated detection rate of preterm delivery was 54.8% (95% CI 44.7–64.6), at a false-positive rate of 10%. ***Conclusions:*** Effective first-trimester screening for spontaneous early preterm delivery can be provided by a combination of maternal characteristics and cervical length.

Introduction

Preterm birth is the main cause of neonatal death and neurological handicap in children [1–3]. Consequently, prediction and prevention of this complication is a major challenge in pregnancy care. Whilst all births before 37 weeks' gestation are defined as preterm, the vast majority of mortality and morbidity relates to early delivery before 34 weeks.

The risk of spontaneous preterm birth is inversely related to the cervical length measured by transvaginal sonography at 20–24 weeks' gestation [4–7]. In women with a short cervix, administration of progesterone reduces the risk of spontaneous early preterm delivery by about 45% [8, 9]. However, progesterone is not as effective in women with cervical length <10 mm as in those with a length of 10–20 mm. Consequently, it may be preferable to measure cervical length in earlier pregnancy before the critical length of 10 mm is reached. Several studies reported that measurement of cervical length in the first

KARGER

Fax +41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1015–3837/12/0313–0154$38.00/0

Accessible online at:
www.karger.com/fdt

Prof. K.H. Nicolaides
Harris Birthright Research Centre for Fetal Medicine, King's College Hospital
Denmark Hill
London SE5 9RS (UK)
Tel. +44 20 3299 8256, E-Mail kypros@fetalmedicine.com

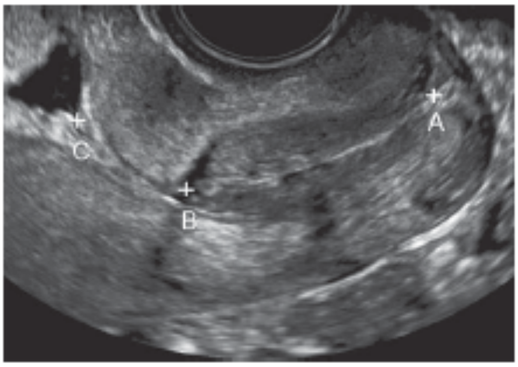


Fig. 1. Ultrasound picture illustrating the measurement of the length of the cervix (A to B) and the isthmus (B to C).

trimester is not predictive of preterm delivery [10–14]. However, in these studies, the mean or median cervical length was 40–44 mm, which is considerably longer than in the second trimester suggesting that in the measurement of cervical length, sonographers may have inadvertently included the uterine isthmus. In a study of 1,508 singleton pregnancies, we reported that the median lengths of the cervix and the cervico-isthmic complex at 11–13 weeks were 32 and 45 mm, respectively [15]. In the 16 (1.1%) cases that subsequently delivered spontaneously before 34 weeks, the median cervical length (28 mm) was shorter [15].

The aim of this screening study is to examine the potential value of cervical length at 11–13 weeks' gestation in the prediction of spontaneous preterm delivery.

Methods

This was a prospective screening study for spontaneous preterm delivery in pregnant women attending their routine first hospital visit at King's College Hospital, University College London Hospital and Medway Maritime Hospital Gillingham. At this visit, which is held at 11^{+0}–13^{+6} weeks' gestation, we recorded maternal characteristics and medical history and performed transabdominal and transvaginal sonography to firstly determine gestational age from the measurement of the fetal crown-rump length (CRL), secondly, diagnose any major fetal abnormalities, thirdly, measure fetal nuchal translucency thickness as part of screening for aneuploidies, and fourthly, measure endocervical length [15–19]. The policy was to refer women with a cervical length <15 mm to a specialist high-risk for preterm delivery clinic. However, in this study, none of the patients had such a short cervix. All women were offered another scan at 20–24 weeks' gestation for the diagnosis of fetal abnormalities, assessment of fetal growth and transvaginal measurement of cervical length, and if the length was <15 mm, the women were treated by cervical cerclage or vaginal progesterone.

Measurement of Endocervical Length

The measurement of endocervical length by transvaginal sonography was performed as previously described [15]. First, the women were asked to empty their bladder and were placed in the dorsal lithotomy position. Second, the vaginal transducer (2.7–9.3 MHz) was introduced in the anterior fornix of the vagina and adjusted to obtain a sagittal view of the entire length of the cervical canal, which may be either translucent or echodense. The canal is bordered by the endocervical mucosa, which is usually of decreased but occasionally of increased echogenicity compared to the surrounding tissues. Third, the probe was withdrawn until the image was blurred and then advanced gently until the image was restored without exerting undue pressure on the cervix. Fourth, the settings of the ultrasound machine were altered to obtain the widest viewing angle and the magnification was increased so that most of the screen was occupied by the tissues between the external cervical os at one end of the picture and the gestational sac at the other end. Fifth, callipers were used to measure in sequence the linear distance between the two ends of the glandular area around the endocervical canal (fig. 1). All the operators performing the scans had received extensive training and had all passed a practical examination administered by an expert to demonstrate their competence in the technique.

Outcome Measures

The outcome measures were spontaneous preterm delivery before 34 weeks (early preterm) and at 34^{+0}–36^{+6} weeks (late preterm). Data on pregnancy outcome were obtained from the maternity computerized records or the general medical practitioners of the women and were also recorded in our database. The obstetric records of all patients delivering before 37 weeks (<259 days) were examined to determine whether the preterm delivery was medically indicated or spontaneous. The latter included those with spontaneous onset of labour and those with preterm prelabour rupture of membranes.

Statistical Analysis

Descriptive data were presented as medians and interquartile ranges for continuous variables and as numbers and percentages for categorical variables. Comparison between the outcome groups was done by χ^2 or Fisher's exact test for categorical variables and by the Mann-Whitney U test for continuous variables.

The distribution of cervical length was logarithmically transformed to obtain a symmetric distribution of residuals with approximately constant standard deviation. This was assessed by inspecting histograms and probability plots. Multivariate regression analysis was used to determine which of the factors among the maternal characteristics, obstetric history and gestation were significant predictors of $\log_{10}$ cervical length in the term delivery group. The distribution of $\log_{10}$ cervical length, expressed as multiple of the median (MoM) of the term delivery group, was determined in the early and late spontaneous preterm delivery groups. Comparison of cervical length MoM between outcome groups was done by the Mann-Whitney U test, with post-hoc Bonferroni correction. The a priori risk for early spontaneous preterm deliv-

Table 1. Maternal characteristics in the three outcome groups

Maternal variables	Delivery >37 weeks (n = 9,657)	Spontaneous delivery	
		<34 weeks (n = 104)	34–36 weeks (n = 213)
Maternal age, years	31.9 [28.0–35.4]	31.4 [27.4–35.5]	30.6 [26.6–34.2]*
Maternal weight, kg	64.6 [58.0–74.1]	65.3 [57.3–78.1]	62.7 [56.5–72.7]
Maternal height, cm	164 [160–169]	163 [158–167]	164 [159–168]
Racial origin			
Caucasian	7,214 (74.7)	58 (55.8)*	138 (64.8)*
African	1,179 (12.2)	30 (28.8)*	30 (14.1)
South Asian	639 (6.6)	8 (7.7)	22 (10.3)
East Asian	392 (4.1)	4 (3.8)	11 (5.2)
Mixed	233 (2.4)	4 (3.8)	12 (5.6)*
Cigarette smoker	597 (6.2)	8 (7.7)	20 (9.4)
Assisted conception	437 (4.5)	8 (7.7)	9 (4.2)
Obstetric history			
No delivery at or beyond 16 weeks	5,313 (55.0)	51 (49.0)	123 (57.7)
Delivery at 16–30 weeks (1 event)	95 (1.0)	6 (5.8)*	4 (1.9)
Delivery at 16–30 weeks (2 events)	0	4 (3.8)*	0
Delivery at 16–30 weeks (1 event) plus ≥37 weeks	7 (0.1)	3 (2.9)*	0
Delivery at 31–36 weeks	118 (1.2)	14 (13.5)*	14 (6.6)*
Delivery at 31–36 weeks plus ≥37 weeks	17 (0.2)	3 (2.9)*	1 (0.5)
Delivery at ≥37 weeks	4,107 (42.5)	23 (22.1)*	71 (33.3)*

Data are medians with interquartile ranges in brackets, or number of patients with percentages in parentheses. Comparison between the outcome groups was done by χ^2 or Fisher's exact test for categorical variables and by Mann-Whitney U test for continuous variables. * $p < 0.0167$, post-hoc Bonferroni correction.

ery based on maternal characteristics and obstetric history was determined as previously described [20]. Likelihood ratios were computed from the fitted distributions of $\log_{10}$ MoM values in the term delivery group and in each of the two preterm delivery groups. The a posteriori risks for early and late spontaneous preterm delivery were derived by multiplying the a priori risk with the likelihood ratio. The performance of screening was determined by the area under the receiver operating characteristic curve (AUROC) [21].

The statistical software package SPSS 18.0 (SPSS Inc., Chicago, Ill., USA) and Medcalc (Medcalc Software, Mariakerke, Belgium) were used for all data analyses.

Results

We prospectively examined 10,870 singleton pregnancies between July 2009 and March 2011. We excluded 896 (8.2%) because they had missing outcome data (n = 423), the pregnancies resulted in miscarriage before 24 weeks' gestation (n = 91) or termination (n = 105), because there was iatrogenic delivery at 24–33 weeks (n = 192) or the women had either cervical cerclage (n = 37) or vaginal progesterone (n = 48). In the remaining 9,974 pregnancies included in the study, spontaneous preterm delivery before 34 weeks occurred in 104 (1.0%) cases and delivery between 34 and 36 completed weeks in 213 (2.1%) cases. The maternal characteristics of each of the outcome groups are compared in table 1.

Multivariate regression analysis in the term delivery group demonstrated that for the $\log_{10}$ cervical length significant independent contributions were provided by fetal CRL, maternal height, age, racial origin and parity ($R^2 = 0.018$; table 2). The median cervical length MoM was significantly lower in both the early ($p < 0.0001$) and late ($p = 0.009$) spontaneous preterm delivery groups than in the term delivery group (table 3).

In the term delivery group, African women had shorter cervical length than Caucasian women (table 3) but cervical length MoM was not significantly different between the two racial groups ($p = 0.628$; fig. 2). Similarly, in the early and late preterm delivery groups, the median cervical length MoM was not significantly different between African and Caucasian women ($p = 0.264$ and 0.966, respectively; fig. 2).

Table 2. Multivariate regression analysis for the prediction of $\log_{10}$ cervical length in the term delivery group

Independent variable	Coefficient	Standard error	p
Intercept	1.360143	0.017832	<0.0001*
Fetal CRL	0.000222	0.000066	0.0008*
Maternal height	0.000480	0.000078	<0.0001*
Maternal age	0.002926	0.000800	0.0003*
(Maternal age)^2	–0.000036	0.000013	0.0047*
Racial origin			
Caucasian	0		
African	–0.004102	0.001591	0.0099*
South Asian	–0.008513	0.002117	0.0001*
East Asian	–0.004224	0.002651	0.1112
Mixed	–0.001738	0.003349	0.6038
Cigarette smoking	0.002660	0.002195	0.2256
Assisted conception	0.000432	0.002902	0.8816
Obstetric history			
No delivery at or beyond 16 weeks	0		
Delivery at 16–30 weeks (1 event)	–0.005124	0.005220	0.3263
Delivery at 16–30 weeks (1 event) plus ≥37 weeks	–0.036067	0.018994	0.0576
Delivery at 31–36 weeks	0.002786	0.004678	0.5515
Delivery at 31–36 weeks plus ≥37 weeks	–0.021189	0.012219	0.0829
Delivery at ≥37 weeks	0.003595	0.001059	0.0007*

* $p < 0.05$.

Table 3. The median and interquartile range of cervical length in the three outcome groups

	Number	Term delivery	Spontaneous delivery			
			n	<34 weeks	n	34–36 weeks
Cervical length, mm						
Total	9,657	32 (30–35)	104	29 (26–30)*	213	31 (29–34)*
Caucasian	7,214	32 (30–35)	58	29 (27–31)*	139	31 (29–34)*
African	1,179	31 (29–35)**	30	27 (25–30)*	30	31 (28–34)
Cervical length, MoM						
Total	9,657	0.994 (0.919–1.082)	104	0.892 (0.829–0.945)*	213	0.977 (0.892–1.059)*
Caucasian	7,214	0.995 (0.921–1.081)	58	0.894 (0.832–0.984)*	139	0.967 (0.890–1.057)
African	1,179	0.992 (0.911–1.087)	30	0.863 (0.769–0.944)*	30	0.962 (0.884–1.066)

Comparison between the outcome groups was done by the Mann-Whitney U test. * $p < 0.0167$, post-hoc Bonferroni correction. Comparison between Caucasian and African racial groups in each outcome group was done by the Mann-Whitney U test. ** $p < 0.05$.

The frequency distributions of cervical length MoM in the term delivery group and early spontaneous preterm delivery group are presented in figure 3. The overlapping Gaussian distributions of $\log_{10}$ cervical length MoM in the term delivery group and each of the spontaneous preterm delivery groups were used to calculate the likelihood ratios for preterm delivery (fig. 4, table 4).

The a priori risk for early spontaneous preterm delivery based on maternal characteristics and obstetric history was determined as previously described [20], and using this model, the expected number of early spontaneous preterm delivery was 101 cases (95% prediction interval 83–123), which is similar to the observed number of 104 cases. The a posteriori risks for spontaneous pre-

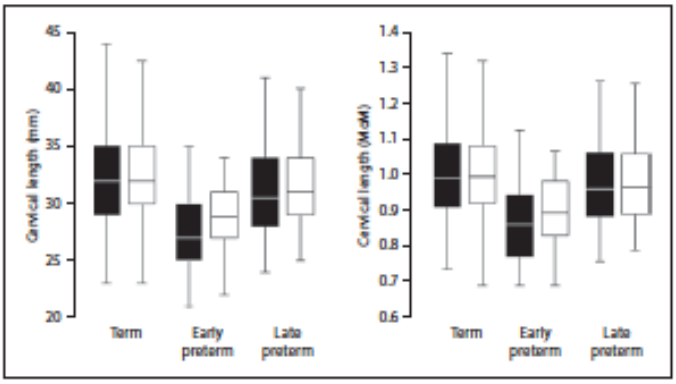


Fig. 2. Box-whisker plot of cervical length and its MoM values of Caucasian (□) and African (■) women in the three outcome groups.

term delivery were derived by multiplying the a priori risk by the likelihood ratio for cervical length.

The AUROC and the detection rates of early and late spontaneous preterm delivery for false-positive rates of 5 and 10% in screening by maternal characteristics, cervical length and their combination are given in figure 5 and table 5. In the prediction of early spontaneous preterm delivery, the AUROC was significantly improved to 0.840 (95% CI 0.833–0.847) when maternal characteristics (0.714, 95% CI 0.705–0.723) were combined with cervical length (0.782, 95% CI 0.774–0.790; $p < 0.0001$). In the prediction of late spontaneous preterm delivery, the AUROC was significantly improved to 0.583 (95% CI 0.573–0.593) when maternal characteristics (0.563, 95% CI 0.553–0.573) were combined with cervical length (0.551, 95% CI 0.541–0.561; $p = 0.042$).

In the 85 patients with cervical length <15 mm at 20–24 weeks' gestation who were treated with either cervical cerclage or vaginal progesterone, there were 3 with spontaneous delivery before 34 weeks, 2 with delivery between 34 and 36 completed weeks and 80 with delivery at or after 37 weeks. In the 85 cases, the cervical length at 11–13 weeks was <32 mm, which is the median of the normal range, and <25 mm, which is the 1st centile, in 65 (76.5%) and 18 (21.2%) women, respectively.

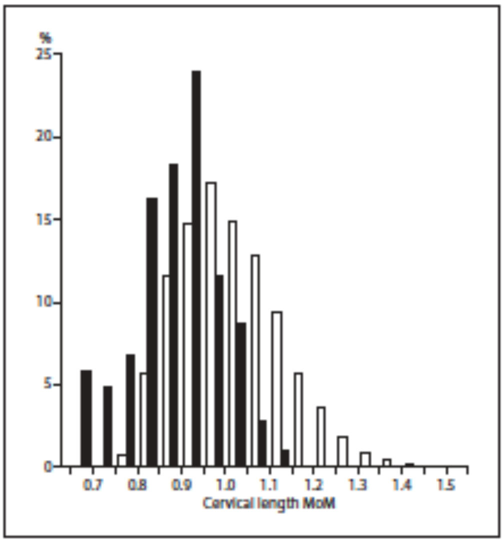


Fig. 3. Frequency distributions of cervical length MoM in the term delivery group (□) and early spontaneous preterm delivery group (■).

Discussion

The findings of this screening study demonstrate that in singleton pregnancies resulting in spontaneous preterm delivery, cervical length at 11–13 weeks' gestation is shorter than that in women delivering at term. An algorithm combining maternal characteristics and cervical length can identify about 55% of pregnancies resulting in delivery before 34 weeks, at a false-positive rate of 10%. The detection rate for delivery at 34–36 weeks was only

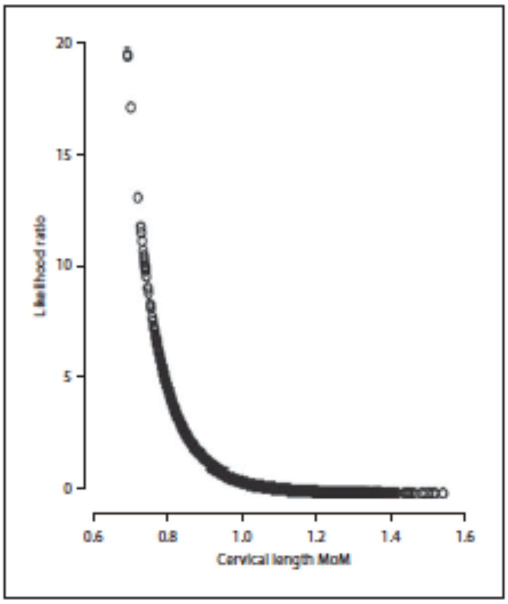


Fig. 4. Likelihood ratios for early spontaneous preterm delivery from cervical length MoM.

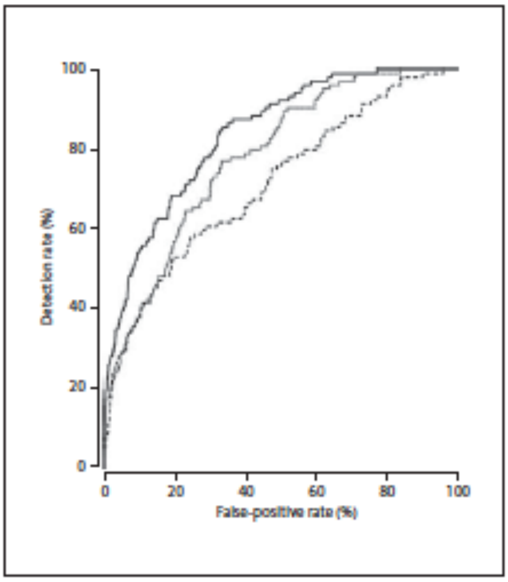


Fig. 5. Receiver operating characteristics curves of maternal characteristics (------), cervical length (- - - - -) and their combination (-------) in the prediction of early spontaneous preterm delivery.

20%. It is likely that the performance of screening would have been higher if some of the women with a short cervix were not excluded from the study because they were treated with cervical cerclage or progesterone.

The study confirmed that spontaneous preterm delivery is associated with certain maternal characteristics, and the observed number of preterm deliveries in this study was similar to that predicted from a multivariable regression model published previously [20]. The risk for spontaneous early preterm delivery increases with maternal age and decreases with height; it is higher in women of African and South Asian racial origin than in Caucasians, in cigarette smokers and in those conceiving after the use of ovulation induction drugs. The risk of preterm delivery is also influenced by the outcome of previous pregnancies. The risk is inversely related to gestation at previous spontaneous delivery decreasing from about 7% if the gestation was 16–24 weeks to 3% if 31–33 weeks and to 0.6% if all deliveries were at term. Additionally, the risk is affected by the number of previous spontaneous deliveries at 16–30 weeks, increasing from about 6–19% if there are two rather than

Table 4. Likelihood ratios for early and late spontaneous preterm delivery from $\log_{10}$ cervical length MoM

$\log_{10}$ cervical length MoM	Likelihood ratio	
	early spontaneous preterm delivery	late spontaneous preterm delivery
–0.16	19.76 (18.80–20.72)	2.04 (2.02–2.06)
–0.14	12.17 (10.52–13.82)	1.82 (1.76–1.88)
–0.12	7.82 (6.74–8.90)	1.64 (1.59–1.69)
–0.10	5.00 (4.36–5.64)	1.49 (1.45–1.53)
–0.08	3.22 (2.81–3.63)	1.35 (1.31–1.39)
–0.06	2.04 (1.78–2.30)	1.24 (1.21–1.27)
–0.04	1.29 (1.12–1.46)	1.13 (1.10–1.16)
–0.02	0.82 (0.72–0.92)	1.04 (1.02–1.06)
0	0.52 (0.45–0.59)	0.97 (0.95–0.99)
0.02	0.33 (0.29–0.37)	0.90 (0.88–0.92)
0.04	0.21 (0.18–0.24)	0.84 (0.82–0.86)

Figures in parentheses are 95% CIs.

Table 5. Comparison of the performance of screening for early and late spontaneous preterm delivery by maternal characteristics, cervical length and their combination

Screening test	AUROC	
	early spontaneous preterm delivery	late spontaneous preterm delivery
Maternal history		
Total	0.714 (0.705–0.723)	0.563 (0.553–0.573)
Caucasian	0.692 (0.682–0.703)	0.533 (0.522–0.545)
African	0.729 (0.703–0.754)	0.645 (0.617–0.672)
Cervical length		
Total	0.782 (0.774–0.790)	0.551 (0.541–0.561)
Caucasian	0.772 (0.762–0.781)	0.563 (0.552–0.575)
African	0.797 (0.773–0.820)	0.553 (0.525–0.581)
Combined test		
Total	0.840 (0.833–0.847)	0.583 (0.573–0.593)
Caucasian	0.836 (0.828–0.845)	0.565 (0.553–0.576)
African	0.829 (0.806–0.850)	0.662 (0.635–0.689)

	Detection rate for fixed false-positive rate			
	5%	10%	5%	10%
Maternal history				
Total	28.9 (20.4–38.6)	37.5 (28.2–47.5)	9.9 (6.2–14.7)	19.7 (14.6–25.7)
Caucasian	25.9 (15.3–39.0)	34.5 (22.5–48.1)	8.0 (4.1–13.8)	12.3 (7.3–19.0)
African	26.7 (12.3–45.9)	26.7 (12.3–45.9)	16.7 (5.7–34.7)	20.0 (7.8–38.6)
Cervical length				
Total	28.9 (20.4–38.6)	38.5 (29.1–48.5)	9.4 (5.8–14.1)	14.6 (10.1–20.0)
Caucasian	25.9 (15.3–39.0)	39.7 (27.1–53.4)	8.7 (4.6–14.7)	15.2 (9.7–22.3)
African	40.0 (22.7–59.4)	50.0 (31.3–68.7)	10.0 (2.2–26.6)	13.3 (3.8–30.7)
Combined test				
Total	39.4 (30.0–49.5)	54.8 (44.7–64.6)	12.2 (8.1–17.4)	20.2 (15.0–26.2)
Caucasian	41.4 (28.6–55.1)	53.5 (39.9–66.7)	10.1 (5.7–16.4)	13.0 (7.9–19.8)
African	43.3 (25.5–62.6)	60.0 (40.6–77.3)	13.3 (3.8–30.7)	26.7 (12.3–45.9)

Figures in parentheses are 95% CIs.

one such delivery. In women with previous preterm deliveries, there is a protective effect against recurrence if they also had a delivery at term, and for women with one or two deliveries at 16–30 weeks, the risk of recurrence decreases from about 6 to 1.5% and from 19 to 10%, respectively. Screening at 11–13 weeks by an algorithm combining maternal characteristics and obstetric history [20] identified about 38% of our pregnancies resulting in spontaneous delivery before 34 weeks and 20% of those delivering at 34–36 weeks, at a false-positive rate of 10%.

In the measurement of cervical length, care was taken to include only the portion of the cervix where the canal is bordered by the endocervical mucosa [15, 22]. At 11–13 weeks' gestation, the median cervical length in women delivering at term was 32 mm. Multivariate regression analysis in the normal outcome group demonstrated that cervical length increases with fetal CRL, maternal age and height and is lower in women of African and South Asian racial origin than in Caucasians. After adjustment for these maternal characteristics, the median cervical length MoM was significantly reduced in the early and to a lesser extent in the late spontaneous preterm delivery groups. The patient-specific risk for spontaneous preterm delivery was inversely related to the cervical length MoM, and the individual risk can be derived by multiplying the a priori risk, obtained from maternal characteristics and obstetric history, by the likelihood ratio for cervical length.

In women of African racial origin, the cervical length in both the term and early spontaneous preterm delivery groups was shorter than in Caucasians. In the calculation of MoMs, after correction for maternal characteristics including racial origin, the cervical length in those with spontaneous delivery before 34 weeks was shorter in women of African than of Caucasian origin, but this difference was not significant. Similarly, the performance of screening for preterm delivery in women of African origin was higher, but not significantly so, than in Caucasians. The extent to which larger studies will demonstrate significant differences between racial groups in the short cervical length related preterm delivery rate and therefore in the mechanisms leading to preterm delivery remains to be determined.

The study has provided evidence that spontaneous preterm delivery can be added to the list of pregnancy complications that can now be identified by screening at 11–13 weeks' gestation [23]. Randomized studies, based on first-trimester screening to identify the high-risk group for subsequent early delivery, will investigate the extent to which pregnancy outcome would improve through early intervention with such measures as prophylactic use of progesterone.

Acknowledgment

The study was supported by a grant from The Fetal Medicine Foundation (UK Charity No. 1037116).

References

1 Centre for Maternal and Child Enquiries (CMACE): Perinatal Mortality 2008: United Kingdom. London, CMACE, 2010.

2 Goldenberg RL, Culhane JF, Iams JD, Romero R: Epidemiology and causes of preterm birth. Lancet 2008;371:75–84.

3 McCormick MC: The contribution of low birth weight to infant mortality and childhood morbidity. N Engl J Med 1985;312:82–90.

4 Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, Thom E, McNellis D, Copper RL, Johnson F, Roberts JM: The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med 1996;334:567–572.

5 Heath VC, Southall TR, Souka AP, Elisseou A, Nicolaides KH: Cervical length at 23 weeks of gestation: prediction of spontaneous preterm delivery. Ultrasound Obstet Gynecol 1998;12:312–317.

6 To MS, Skentou CA, Royston P, Yu CK, Nicolaides KH: Prediction of patient-specific risk of early preterm delivery using maternal history and sonographic measurement of cervical length: a population-based prospective study. Ultrasound Obstet Gynecol 2006;27:362–367.

7 Celik E, To M, Gajewska K, Smith GC, Nicolaides KH, Fetal Medicine Foundation Second Trimester Screening Group: Cervical length and obstetric history predict spontaneous preterm birth: development and validation of a model to provide individualized risk assessment. Ultrasound Obstet Gynecol 2008;31:549–554.

8 Fonseca RB, Celik E, Parra M, Singh M, Nicolaides KH: Progesterone and the risk of preterm birth among women with a short cervix. N Engl J Med 2007;357:462–469.

9 Hassan SS, Romero R, Vidyadhari D, Fusey S, Baxter JK, Khandelwal M, Vijayaraghavan J, Trivedi Y, Soma-Pillay P, Sambarey P, Dayal A, Potapov V, O'Brien J, Astakhov V, Yuzko O, Kinzler W, Dattel B, Sehdev H, Mazheika L, Manchulenko D, Gervasi MT, Sullivan L, Conde-Agudelo A, Phillips JA, Creasy GW, PREGNANT Trial: Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol 2011;38:18–31.

10 Zorzoli A, Soltani A, Perra M, Caravelli E, Galimberti A, Nicolini U: Cervical changes throughout pregnancy as assessed by transvaginal sonography. Obstet Gynecol 1994;84:960–964.

11 Hasegawa I, Tanaka K, Takahashi K, Tanaka T, Aoki K, Torii Y, Okai T, Saji F, Takahashi T, Sato K, Fujimura M, Ogawa Y: A prospective longitudinal study for the prediction of preterm delivery in a low-risk population. J Matern Fetal Invest 1996;6:148–151.

12 Carvalho MH, Bittar RE, Brizot ML, Maganha PP, Borges da Fonseca ES, Zugaib M: Cervical length at 11–14 weeks' and 22–24 weeks' gestation evaluated by transvaginal sonography, and gestational age at delivery. Ultrasound Obstet Gynecol 2003;21:135–139.

13 Conoscenti G, Meir YJ, D'Ottavio G, Rustico MA, Pinzano R, Fischer-Tamaro L, Stampalija T, Natale R, Maso G, Mandruzzato G: Does cervical length at 13–15 weeks' gestation predict preterm delivery in an unselected population? Ultrasound Obstet Gynecol 2003;21:128–134.

14 Ozdemir I, Demirci F, Yucel O, Erkorkmaz U: Ultrasonographic cervical length measurement at 10–14 and 20–24 weeks gestation and the risk of preterm delivery. Eur J Obstet Gynecol Reprod Biol 2007;130:176–179.

15 Greco E, Lange A, Ushakov F, Calvo JR, Nicolaides KH: Prediction of spontaneous preterm delivery from endocervical length at 11 to 13 weeks. Prenat Diagn 2011;31:84–89.

16 Robinson HP, Fleming JE: A critical evaluation of sonar 'crown-rump length' measurements. Br J Obstet Gynaecol 1975;82:702–710.

17 Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH: Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11–13 weeks. Prenat Diagn 2011;31:90–102.

18 Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH, Fetal Medicine Foundation First Trimester Screening Group: UK multicentre project on assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10–14 weeks of gestation. Lancet 1998;352:343–346.

19 Nicolaides KH: Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn 2011;31:7–15.

20 Beta J, Akolekar R, Ventura W, Syngelaki A, Nicolaides KH: Prediction of spontaneous preterm delivery from maternal factors, obstetric history and placental perfusion and function at 11–13 weeks. Prenat Diagn 2011;31:75–83.

21 Zweig MH, Campbell G: Receiver-operating characteristic (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem 1993;39:561–577.

22 Sonek J, Shellhaas C: Cervical sonography: a review. Ultrasound Obstet Gynecol 1998;11:71–78.

23 Nicolaides KH: Turning the pyramid of prenatal care. Fetal Diagn Ther 2011;29:183–196.

3RD RESEARCH BRANCH – FEASIBILITY OF THE METHOD IN A SCREENING SETTING

Measurement of the endocervical length by transvaginal ultrasound in the first trimester of pregnancy: a feasibility study

The aim of the present study was to investigate the feasibility of incorporating routine measurement of trans-vaginal cervical length to the first trimester ultrasound screening for aneuploidies.

Therefore, we aimed firstly, at assessing intra- and inter-operator reproducibility of the measurements; secondly, at evaluating patient acceptability to the procedure.

Methods

<u>*Repeatability of measurements*</u>

This study involved 100 healthy women with singleton pregnancies attending their first hospital visit at King's College Hospital, London and Medway Maritime Hospital Gillingham. In this visit, which is held at 11^{+0}-13^{+6} weeks' gestation, we recorded maternal characteristics and medical history and performed trans-abdominal and trans-vaginal sonography to firstly, determine gestational age from the measurement of the fetal crown-rump length (CRL), secondly, diagnose any major fetal abnormalities, thirdly, measure fetal nuchal translucency thickness as part of screening for aneuploidies and fourthly, measure endocervical length (Greco et al., 2011; Nicolaides, 2011; Robinson and Fleming, 1975; Snijders et al., 1998; Syngelaki et al., 2011).

The study was approved by the institutional review board of the local institution, and informed consent was obtained from all participants.

Cervical length was measured on each patient by two experienced examiners (Operator 1, E.G., and Operator 2, D.M.) who had previously carried out at least 150 trans-vaginal cervical assessments in the first trimester. Practical circumstances determined whether Operator 1 or Operator 2 performed the measurement first. Women were examined in the lithotomy position with an empty bladder, according to the standardised technique described elsewhere (Greco et al., 2011; Table 2.1.). All measurements were carried out using a Voluson GE 730 or E6 system (GE Voluson 730 or GE E6, General Electric Medical System, Germany) with a 2.7-9.3 MHz trans-vaginal transducer and digitally stored. To assess repeatability of different components of variability, six measurements of cervical length were made on each patient. The study protocol used is shown in Figure 2.10. The first operator generated the appropriate image and measured cervical length in the usual way and then generated a new image and repeated the measurement (intra-observer repeatability). The process was then repeated for the second operator. All images acquired were stored in the digital archive, with the second of each set being saved without calipers, so that by reviewing the picture *off line* each operator had to reset the calipers and generate a new measurement (caliper placement repeatability). Thus, six measurement were obtained: A1, A2, BP, B1, B2, and AP, were A and B represent the two operators, 1 and 2 indicate the two normal measurements, and P indicates the measurement acquired *offline* which involved only placement of the calipers. Numeric displays on the screen were covered with opaque tape so that the operators were blinded to the actual measurement. All measurements obtained *real time* were performed at a single examination, with the only break allowed being the actual time for operators to swap. *Offline* measurements were obtained by reviewing images without calipers randomly stored in a digital archive. The investigators were not present during each other's examinations, both during the *real time* and the *offline* components of the protocol, and were kept unaware of each other's results until after the completion of the study.

Repeatability of cervical length measurements and between-observer comparisons were assessed using the four normal measurements on each patient (A1, A2, B1 and B2 described above). Intra-observer variation was analysed by calculating the standard deviation (SD) of the differences between A1 and A2 and between B1 and B2 from the 200 pairs of measurements. Inter-observer variation was analysed by calculating the SD of differences between the means of pairs of measurements made by two observers on the same patients (100 observations of the mean of A1 and A2 compared to the mean of B1 and B2). Caliper placement repeatability was assessed from the SD of the 200 pairs of between observer differences (A2-BP or B2-AP).

The time required to perform an individual measurement was recorded throughout the study.

Statistical analysis was performed with SPSS for Windows 15.0 software package (Chicago, IL, USA). $P<0.05$ was considered to be statistically significant.

<u>*Patient acceptability*</u>

At the end of the ultrasound examination, 100 women who were examined consecutively were asked to complete a questionnaire aimed at assessing the acceptability of the procedure. They were ask to record the degree of discomfort, pain or embarrassment caused by the scan, and to compare the degree of discomfort to that caused by the speculum examination.

Results

Repeatability of measurements

Cervical length was successfully measured by both operators in all 100 cases, and a total of 600 measurements were made. The median cervical length in the 100 patients, using the mean of four measurements (A1, A2, B1, B2) was 32.7 mm. The standard deviation of differences between repeat readings by the same observer, pooled across the two observers, was 1.75 mm. Likewise, the between-observer standard deviation was 1.99 mm for the mean of two measurements. Consequently, on 95% of occasions, the difference between two measurements by the same observer (intra-observer) would not differ by more than 3.2 mm and the corresponding number for two observers (inter-observers) would be 4.1 mm. No significant relationship between mean cervical length and both within-observer difference and between-observer difference was observed (Figure 2.11). When we considered only measurements below the median (32.7 mm), no trend towards a reduction in both the intra-observer and inter-observer standard deviation was observed, with values being 1.7 mm and 2.0 mm, respectively.

The between-observer SD when the image was reviewed *offline* and calipers were placed was 2.02 mm (95% of the time the two readings would not differ by more than 4 mm). Therefore, the greatest contribution to the between observer variation is given by caliper placement.

Patient acceptability

Out of the 100 women questioned about the acceptability of the transvaginal scan, 90% reported that the procedure was associated with no or only minimal discomfort and 85% reported no or only mild embarrassment (Figure 2.12). The degree of pain experienced by the women was recorded on a linear scale measuring 10 cm with 0 representing no pain and 10 representing extremely severe pain. The median score was 1.5 (range 0-6.5). Compared to the speculum examination, the ultrasound scan was recorded as being less, equally or more uncomfortable by 85%, 6% or 4%, respectively (Figure 2.12).

Median time required to obtain an individual measurement was 3.5 minutes (range 2.5-5.0 minutes).

Discussion

This study has demonstrated the feasibility of trans-vaginal sonographic assessment of the cervix at the time of the routine ultrasound examination performed between 11-13^{+6} weeks for the prediction of spontaneous preterm delivery before 34 weeks. The examination takes less than 5 minutes to complete and is associated with a minimal degree of discomfort to the patients. In our study, 80% of the women agreed to have cervical assessment and about 90% of these reported that the procedure was associated with no or only minimal discomfort and embarrassment and found the ultrasound scan to be equally or less uncomfortable than a speculum examination.

Measurement of the cervical length was highly reproducible and, on 95% of occasions, the difference between two measurements by the same observer and by two observers was 3.2 mm and 4.1 mm or less, respectively; the reproducibility of the measurements was not affected by the length of the cervix as the standard deviations of both within-observer and between-observer variation remained stable also when considering mean cervical length values below the median.

A number of studies have shown the measurement of cervical length by transvaginal scan to be highly reproducible in the second trimester of pregnancy (Burger et al., 1997; Heath et al., 1998; Valentin and Bergelin 2002). This study represents the first attempt to demonstrate that measurement of the cervical length by transvaginal scan in the first trimester of pregnancy is as reproducible as in the second trimester, provided that strict adherence to a standardised technique and appropriate training of the operators are respected.

Data on intra-observer and within-observer variability are similar to those previously reported from our group for the measurement of cervical length in the second trimester of pregnancy (Heath et al., 1998). In the second trimester 95% of differences between the two measurements obtained by the same observer (intra-observer) and for two observers (inter-observer) were within 3.5 and 4.2 mm, respectively. By contrast, in the first trimester, repeatability of measurements seems to be less dependent on the length of the cervix than in the second trimester, where the variability is significantly reduced for cervical lengths below the median (Iams et al., 1996; Heath et al., 1998). This finding may reflect the multiple architectural changes that take place at the level of both cervical epithelium and the stroma throughout pregnancy resulting in a progressive softening of the cervix. It is indeed likely that reproducibility of measurements may be inversely related to stage of gestation rather than to the length of the cervix. At the beginning of pregnancy, the cervix is somewhat firmer, thus less prone to be distorted by excessive compression applied through the vaginal probe. With advancing gestational age, intense collagenolysis and water accumulation underlying cervical effacement in addition to initial funnelling at the internal os, lead to increased cervical dynamism. This in turn may produce a number of potential anatomic and technical pitfalls when examining the cervix by ultrasound (Burger et al., 1997; Yost et al., 1999). Yost *et al*. have compared the measurement of the cervical length in the second trimester of pregnancy to an attempt to take a snapshot of a moving target (Yost et al., 1999). Some of these dynamic features of the cervix include unexplained minute-to-minute fluctuations at the internal os, changes due to uterine contractions, and a greater compressibility under pressure exerted by the vaginal probe on the anterior lip.

In contrast with the results of the previous study published by our group on the feasibility of transvaginal cervical assessment in the second trimester of pregnancy (Heath et al., 1998), the between-observer SD is similar both for measurements obtained real-time and for those generated *off-line* by replacing calipers. This finding seems to suggest that in the second trimester of pregnancy, only a part of the between observer variation is due to placing of the calipers, with the remaining difference being due to the true dynamic changes of the cervix throughout the scan. Conversely, in the first trimester of pregnancy, as the cervix is less dynamic, the greatest contribution to the between-observer variation is given by calipers placement. The main issue in assessing the cervix in the first trimester is therefore the correct identification of the internal orifice, as the cervix is often a continuum with the uterine isthmus, which may be falsely "counted" in the cervical length (Greco et al., 2011; Greco et al., 2012; Sonek and Shellaas,1998).

We have previously demonstrated that in order to achieve an accurate measurement of the cervical length in the first trimester of pregnancy it is indeed essential to distinguish the endocervical length, bordered by the glandular area, by the isthmus (Greco et al., 2011; Greco et al., 2012; Souka et al., 2011) and that appropriate training is mandatory.

Learning curve for transvaginal sonographic measurement of cervical length at 11+0 to 13+6 weeks' gestation.

The aim of this study was to determine the number of scans necessary for training sonographers to measure accurately the cervical length by transvaginal ultrasound at 11^{+0} to 13^{+6} weeks' gestation.

Methods

Overall design

This prospective study involved 5 pairs of sonographers. Each pair included a sonographer accredited for first-trimester measurement of NT by The Fetal Medicine Foundation, but not for the assessment of the cervical length and with little or no experience in transvaginal ultrasound (trainee). Each of these trainees was coupled with an expert operator (trainer), who had performed at least 150 examinations for the measurement of the cervical length in the first trimester. Each trainee received a theoretical training on how to measure the cervical length by transvaginal ultrasound, prior to commencing a series of one-to-one practical training sessions, which consisted of 10 transvaginal ultrasound examinations performed under direct supervision of the trainer. After each session of training, measurements from the set were reviewed. This cycle of training-review continued until the trainee was judged to have obtained competence in the technique.

Ultrasound examination

In order for all five sonographers to complete their learning curve for the measurement of the cervical length, 220 ultrasound vaginal examinations were performed in 220 women with singleton pregnancies attending the first-trimester screening for aneuploidies at King's College Hospital, London and Medway Maritime University Hospital, Gillingham. All measurements were obtained transvaginally using a Voluson 730 or GE E6 ultrasound system (GE Medical System, Germany) equipped with a 2.7-9.3 MHz endocavitary probe. The study was approved by the institutional review board of the local institution, and informed consent was obtained from all participants.

Training and data analysis

All trainees, prior to commencement of the study, had been given theoretical training on how to measure the cervical length in the first trimester, according to published standards (Greco et al., 2011; Greco et al., 2012;) (Table 2.1.).

For each examination, the trainee generated the first measurement of the cervical length. Then the trainer obtained a second measurement of the cervical length (designated as the "true" measurement) and completed the examination. Measurements on the ultrasound screen were covered with opaque tape during the whole duration of the examination. All measurements were performed at a single examination with no break other than the actual time necessary for the two operators to swap. During the training session, the trainer was always present and restricted to assist the trainee (only throughout the first training session) if he had any difficulty in visualising the cervix but not in the actual process of measurement. Once the examination had been completed, the trainee was informed of the true cervical length measurement and explained any mistakes or errors made during the measurement. All images were saved as hard copies and stored in a digital archive. After completion of a training session, each set of 10 couples of measurements was then analysed and the delta value, defined as the difference between the measured cervical length and the true measurement, was calculated for each of them. A measurement was considered “successful” if the delta value was <10, and “failed” if the delta value was 10% or greater. Learning curve was considered completed when all measurements in a training session were successful (i.e. the delta value was <10% in all 10 cases). Figure 2.13 summarises the training strategy.

A total of 220 scans were performed. Data sets were analysed per group of 10 scans (training session) and in each the number of failed scans was calculated. Regression analysis was used to examine whether successful measurement of the cervical length was significantly related to stage of gestation (expressed by crown-rump length in mm), cervical length, uterine version (anteverted, retroverted), obstetric history (nulliparity, multiparity, previous one or more cesarean section), body mass index (BMI) in Kg/m^2, maternal ethnic origin (Caucasian, Afro-Caribbean, Asian, Oriental or mixed) and number of scans (in chronological order by trainee sonographer).

Results

All five trainees achieved competence in measuring the cervical length in the first trimester, by completing the training process. One sonographer required only three training sessions (30 scans) to pass the examination, two required four sessions (40 scans), one required five (50 scans) and one six sessions (60 scans). A total of 220 transvaginal ultrasound examinations were performed for the study.

The median number of cases required for a sonographer to become competent in measuring cervical length by transvaginal ultrasound in the first trimester was 40 (range 40-60). The median cervical length was 32 mm (range 24-39mm). Mean gestational age at the time of the examination was 12 weeks (range 11^{+0}-13^{+6}); the median CRL was 63.4mm (range 45-84mm). In 139 (63.1%) cases the uterus was anteverted, in the remaining 81 (36.9%) the uterus was retroverted.

Eighty-nine women (40.4%) involved in the study were primigravidae, 131 (59.6%) multiparous with 38 (17.2%) of them having had one or more previous caesarean sections. The ethnic origin of the women was Caucasian in 147 (66.8%), Afro-Caribbean in 39 (17.7%), Asian in 17 (7.7%), Oriental in 4 (1.9%) and mixed in 13 (5.9%). The median maternal body mass index was 24.5 (range 15.4-53.5) kg/m^2.

Table 2.9 and Figure 2.14 show the number of failed measurements by training session. The median number of failed cases (out of a total of 10) progressively reduced with increased training, from 5 at the first session to 0 at the sixth. Regression analysis showed that the ability to obtain an accurate measurement of the cervical length (which differs <10% from the true value) depends on the experience of the operator (defined as cumulative number of scans) but not cervical length, fetal CRL, BMI, obstetric history, uterine version and ethnic origin (Table 2.10).

Discussion

The findings of this study demonstrate that competence in the measurement of cervical length by transvaginal ultrasound at 11^{+0}-13^{+6} weeks is achieved only after appropriate supervised training. Sonographers with no or minimal experience in transvaginal ultrasound required on average 44 scans before the delta value of their measurements as compared to the true measurements (obtained by expert operators) was consistently <10% thus their learning curve was judged completed. Although one of the 5 pairs achieved this standard within 30 scans, 60 scans were needed for all the sonographers to become competent in the technique. The study has also shown that the learning curve for the measurement of cervical length in the first trimester is significantly related to the experience of the operator (expressed as cumulative number of scans, in chronological order) but not to any of the other factors, which may, theoretically, interfere with easy execution of the measurement. The latters included: uterine factors such as cervical length, uterine version; obstetric factors, such as gestational age; maternal characteristics such as ethnic origin, body mass index, obstetric history. In particular, it has been speculated that multiparity may determine changes in the connective and glandular elements of the cervix, thus impeding the visualization by ultrasound of the endocervical area or making it more challenging (Pires et al., 2006); similarly, scar tissue from previous caesarean section usually creates a shadow which may interfere with the correct identification of the internal uterine orifice.

A study assessing the learning curve for the measurement of the cervical length in the second trimester of pregnancy (Vayssiere et al., 2002) found that a sonographer with no experience in transvaginal ultrasound required 23 examinations under supervision to master the technique, whist an operator expert in gynaecological ultrasound required only 5 scans. This study involved only two trainee sonographers. In our study, one sonographer with no experience in gynaecological ultrasound, achieved competence in the technique within the first 30 scans, with only one measurement failed out of the set of 10 of the second training session. This suggests that mastering the technique for the measurement of cervical length in the first trimester could in fact be equally challenging than in the second trimester. However, understanding modifications of the cervico-isthmic complex throughout pregnancy (including progressive shortening of the isthmus and formation of the lower uterine segment in the third trimester) and adherence to a standardised technique is an essential prerequisite.

Previous studies conducted by our group on learning curve in the measurement of nuchal translucency (Braithwaite et al., 1996), assessment of the nasal bone (Cicero et al., 2003), Doppler assessment of ductus venosus flow (Maiz et al., 2008) and measurement of the fronto-maxillary angle (Yang et al., 2010) in the first-trimester scan found that NT-accredited trainees achieved competency after a minimum of 80 scans.

Achieving competency in the measurement of the cervical length in the first trimester appears to have a steeper learning curve than other techniques routinely offered for the first trimester ultrasound screening of aneuploidies.

Notably, none of the trainee sonographers selected for this study had achieved FMF competency in the measurement of the cervical length in the second trimester or had any meaningful experience in gynaecological transvaginal ultrasound techniques. In similar studies investigating the learning curve for various ultrasound techniques, the length of the training required was found to significantly correlate with the experience of the operator in the same ultrasound technique (Vayssiere et al., 2002; Yang et al., 2010). Therefore, it is highly likely that with more experienced sonographers time to train in order to achieve competence would be still further reduced.

In conclusion, we have previously demonstrated that in experienced hands, measurement of cervical length by transvaginal ultrasound at 11-14 weeks is highly reproducible. Like other sonographic examinations and measurements, such as nuchal translucency, these highly reproducible results could only have been achieved through the appropriate training. The results of this study demonstrate that the learning curve for the measurement of the cervical length in the first trimester is similar to that related to the same measurement in the second trimester and shorter than the learning curve for the measurement of other markers currently checked for the first trimester ultrasound screening of fetal aneuploidies.

Such techniques may have a significant impact on future studies for the prevention of preterm delivery (including cervical cerclage and progestogens) evaluating the effectiveness of different measures when commenced at an early stage of gestation. It is therefore advisable that an increasing number of sonographers acquire competency and confidence in this technique.

Conclusions

In conclusion, the present research programme has provided evidence that spontaneous preterm delivery can be added to the list of pregnancy complications that can now be identified by screening at 11-13 weeks' gestation (Nicolaides, 2011b).

1. In the first pilot study we have defined the correct technique for measurement of the cervical length in the first trimester, and demonstrated that in singleton pregnancies resulting in spontaneous preterm delivery, cervical length at 11-13 weeks' gestation is shorter than in women delivering at term.
2. In the second study, on a larger population of 10,000 women, we have confirmed the findings of our pilot study and demonstrated that an algorithm combining maternal characteristics and cervical length can identify about 55% of pregnancies resulting in delivery before 34 weeks at a false positive rate of 10%.
3. In the third study we have demonstrated the feasibility of the method by assessing patients' acceptability and reproducibility of the technique. Our data have shown that the examination takes less than 5 minutes to be carried out and it is well accepted by 90% of women reporting the procedure to be associated with no, or only minimal, discomfort and any feelings of embarrassment. Moreover, measurement of the cervical length has proven highly reproducible with the difference between two measurements by the same

observer and by two observers being, on 95% of occasions, 3.2 mm and 4.1 mm or less, respectively.

4. Finally, in the fourth study we have demonstrated that competence in the measurement of cervical length by transvaginal ultrasound at 11^{+0}-13^{+6} weeks can be achieved after appropriate supervised training. Sonographers with no recognised experience in transvaginal ultrasound required on average 44 scans before the *delta* value of their measurements was consistently within a variation of <10% of the true measurements (obtained by expert operators) - thus their learning curve was judged completed.

Further studies investigating the value of measuring the cervical length at the time of the first trimester screening in different high-risk populations, including multiple pregnancies, previous cervical surgery and mullerian abnormalities, are needed.

Randomised studies investigating the effectiveness of interventions including vaginal progesterone and/or cervical cerclage commenced as early as the first trimester of pregnancy for women with short cervix will define whether such early screening can provide any further decrease in spontaneous preterm birth rate and reduce neonatal morbidity.

ACKNOWLEDGEMENTS

These studies were supported by a grant from the Fetal Medicine Foundation (Charity No: 1037116).

REFERENCES

1. Airoldi J, Berghella V, Sehdev H, Ludmir J. Transvaginal ultrasonography of the cervix to predict preterm birth in women with uterine anomalies. Obstet Gynecol. 2005; 106:553-6.
2. Althuisius SM, Dekker GA, van Geijn HP, Bekedam DJ, Hummel P. Cervical incompetence prevention randomized cerclage trial (CIPRACT): study design and preliminary results. Am J Obstet Gynecol. 2000; 183:823-9.
3. American College of Obstetricians and Gynecologists. Incidentally detected short cervical length. Committee Opinion No. 522. Obstet Gynecol. 2012; 119:879-82.
4. Ananth CV, Vintzileos AM. Maternal-fetal conditions necessitating a medical intervention resulting in preterm birth. Am J Obstet Gynecol. 2006; 195:1557-63.
5. Andersen HF, Nugent CE, Wanty SD, Hayashi RH. Prediction of risk for preterm delivery by ultrasonographic measurement of cervical length. Am J Obstet Gynecol. 1990; 163:859-67.
6. Ayers JW, DeGrood RM, Compton AA, Barclay M, Ansbacher R. Sonographic evaluation of cervical length in pregnancy: diagnosis and management of preterm cervical effacement in patients at risk for premature delivery. Obstet Gynecol. 1998; 71: 934-44.
7. Beghella V and Roman A. Does funnelling increase the incidence of preterm birth in women with normal cervical length? Am J Obstet

Gynecol. 2005; 193:S147.

8. Berghella V, Bega G, Tolosa J, Berghella M. Ultrasound assessment of the cervix. Clin Obstet Gynecol. 2003a; 46:947-61.
9. Berghella V, Bega G. Ultrasound evaluation of the cervix. In Callen PW (ed): Ultrasonography in Obstetrics and Gynecology (ed 5). Philadelphia, PA, Saunders Elsevier, 2008, pp. 698-720.
10. Berghella V, Daly SF, Tolosa JE, DiVito MM, Chalmers R, Garg N, Bhullar A, Wapner RJ. Prediction of preterm delivery with transvaginal ultrasonography of the cervix in patients with high risk pregnancies: does cervical cerclage prevent prematurity? Am J Obstet Gynecol. 1999; 181:809-15.
11. Berghella V, Kuhlman K, Weiner S, Texeira L, Wapner RJ. Cervical funnelling: sonographic criteria predictive of preterm delivery. Ultrasound Obstet Gynecol. 1997a; 10:161-6.
12. Berghella V, Mackeen AD. Cervical length screening with ultrasound-indicated cerclage compared with history-indicated cerclage for prevention of preterm birth: a meta-analysis. Obstet Gynecol. 2011b; 118:148-55.
13. Berghella V, Odibo AO, To MS, Rust OA, Althuisius SM. Cerclage for short cervix on ultrasonography: meta-analysis of trials using individual patient-level data. Obstet Gynecol. 2005; 106:181-9.
14. Berghella V, Pereira L, Gariepy A, Simonazzi G. Prior cone biopsy: prediction of preterm birth by cervical ultrasound. Am J Obstet Gynecol. 2004; 191:1393-7.

15. Berghella V, Rafael TJ, Szychowski JM, Rust OA, Owen J. Cerclage for short cervix on ultrasonography in women with singleton gestations and previous preterm birth. Obstet Gynecol. 2011a; 117:663- 71.
16. Berghella V, Roman A, Daskalakis C, Ness A, Baxter JK. Gestational age at cervical length measurement and incidence of preterm birth. Obstet Gynecol. 2007; 110:311-7.
17. Berghella V, Talucci M, Desai A. Does transvaginal sonographic measurement of cervical length before 14 weeks predict preterm delivery in high-risk pregnancies? Ultrasound Obstet Gynecol. 2003b; 21;140-4.
18. Berghella V, Tolosa JE, Kuhlman KA et al. Cervical ultrasonography compared to manual examination as a predictor of preterm delivery. Am J Obstet Gynecol. 1997b; 177:723-30.
19. Berghella V, Tolosa JE, Kuhlman K, Weiner S, Bolognese RJ, Wapner RJ. Cervical ultrasonography compared with manual examination as a predictor of preterm delivery. Am J Obstet Gynecol. 1997; 177:723-30.
20. Berghella V. Progesterone and preterm birth prevention: translating clinical trials data into clinical practice. Society for Maternal-Fetal Medicine Publications Committee, with assistance of Vincenzo Berghella. Am J Obstet Gynecol. 2012; 206:376-86.
21. Beta J, Akolekar R, Ventura W, Syngelaki A, Nicolaides KH. Prediction of spontaneous preterm delivery from maternal factors, obstetric history, and placental perfusion and function at 11-13 weeks. Prenat Diagn. 2011; 31:75-83.

22. Blencowe H, Cousens S, Oestergaard MZ, Chou D, Moller AB, Narwal R, Adler A, Vera Garcia C, Rohde S, Say L, Lawn JE. National, regional, and worldwide estimates of preterm birth rates in the year 2010 with time trends since 1990 for selected countries: a systematic analysis and implications. Lancet. 2012 Jun 9; 379:2162-72.
23. Bouyer J, Papiernik E, Dreyfus J, Collin D, Winisdoerffer B, Gueguen S. Maturation signs of the cervix and prediction of preterm birth. Obstet Gynecol. 1986; 68:209-14.
24. Braithwaite JM, Kadir RA, Pepera TA, Morris RW, Thompson PJ, Economides DL. Nuchal translucency measurement: training of potential examiners. Ultrasound Obstet Gynecol. 1996; 8:192-5.
25. Burger M, Weber-Rossler T, Willmann M. Measurement of the pregnant cervix by transvaginal sonography: an interobserver study and new standards to improve the interobserver variability. Ultrasound Obstet Gynecol. 1997; 9:188-93.
26. Cahill AG, Odibo AO, Caughey AB, Stamilio DM, Hassan SS, Macones GA, Romero R. Universal cervical length screening and treatment with vaginal progesterone to prevent preterm birth: a decision and economic analysis. Am J Obstet Gynecol. 2010; 202:548.e1-8.
27. Carvalho MH, Bittar RE, Brizot ML, Maganha PP, Borges da Fonseca ES, Zugaib M. Cervical length at 11–14 weeks' and 22–24 weeks' gestation evaluated by transvaginal sonography, and gestational age at delivery. Ultrasound Obstet Gynecol. 2003; 21:135–9.

28. Celik E, To M, Gajewska K, Smith GC, Nicolaides KH. Cervical length and obstetric history predict spontaneous preterm birth: development and validation of a model to provide individualized risk assessment. Ultrasound Obstet Gynecol. 2008; 31:549–54.
29. Cetingoz E, Cam C, Sakalli M, Karateke A, Celik C, Sancak A. Progesterone effects on preterm birth in high-risk pregnancies: a randomized placebo-controlled trial. Arch Gynecol Obstet. 201; 283:423–9.
30. Chauhan SP, Scardo JA, Hayes E, Abuhamad AZ, Berghella V. Twins: prevalence, problems, and preterm births. Am J Obstet Gynecol. 2010; 203:305–15.
31. Cicero S, Dezerega V, Andrade E, Scheier M, Nicolaides KH. Learning curve for sonographic examination of the fetal nasal bone at 11-14 weeks. Ultrasound Obstet Gynecol. 2003; 22:135-7.
32. Conde-Agudelo A, Romero R, Hassan SS, Yeo L. Transvaginal sonographic cervical length for the prediction of spontaneous preterm birth in twin pregnancies: a systematic review and metaanalysis. Am J Obstet Gynecol. 2010; 203:128, e1–12.
33. Conoscenti G, meir YJ, D'Ottavio G, Rustico MA, Pinzano R, Fischer-Tamaro L, Stampalija T, Natale R, Maso G, Mandruzzato G. Does cervical length at 13-15 weeks' gestation predict preterm delivery in an unselected population? Ultrasound Obstet Gynecol. 2003; 21:128-34.
34. Crane JM, Hutchens D. Transvaginal sonographic measurement of cervical length to predict preterm birth in asymptomatic women at

increased risk: a systematic review. Ultrasound Obstet Gynecol. 2008; 31:579–87.

35. Csapo A. The luteo-placental shift, the guardian of pre-natal life. Postgrad Med J. 1969; 45:57–64.
36. Csapo AI. The ‘see-saw’ theory of parturition. Ciba Found Symp. 1977:159–210.
37. Danforth DN. 1947. The fibrous nature of the human cervix, and its relation to the isthmic segment in gravid and nongravid uteri. Am J Obstet Gynecol. 53: 541-60.
38. Danforth DN. Early study on the anatomy and physiology of the human cervix and implications for the future. In Naftolin F, Stubblefield PG, eds. Dilatation of the uterine cervix: Connective tissue biology and clinical management. New York: Raven, 1980:3-16.
39. Di Renzo GC. The great obstetrical syndromes. J Matern Fetal Neonatal Med. 2009; 22:633–5.
40. Floyd WS. Cervical dilatation in the midtrimester of pregnancy. Obstet Gynecol. 1961; 18:380-1.
41. Fonseca RB, Celik E, Parra M, Singh M, Nicolaides KH. Progesterone and the risk of preterm birth among women with a short cervix. N Engl J Med 2007; 357:462-9.
42. Goldenberg RL, Culhane JF, Iams JD, Romero R. Epidemiology and causes of preterm birth. Lancet 2008; 371:75-84.

43. Goldenberg RL, Iams JD, Miodovnik M, Van Dorsten JP, Thurnau G, Bottoms S, Mercer BM. The preterm prediction study: risk factors in twin gestations. Am J Obstet Gynecol. 1996; 175:1047-53.
44. Goldenberg RL, Gravett MG, Iams J, Papageorghiou AT, Waller SA, Kramer M, Culhane, J, Barros F, Conde-Agudelo A, Bhutta ZA, Knight HE, Villar J. The preterm birth syndrome: issues to consider in creating a classification system. Am J Obstet Gynecol. 2012; 206:113-8.
45. Goldstein P, Berrier J, Rosen S, Sacks HS, Chalmers TC. A meta-analysis of randomized control trials of progestational agents in pregnancy. Br J Obstet Gynaecol. 1989; 96:265-74.
46. Greco E, Gupta R, Syngelaki A, Poon LC, Nicolaides KH. First-trimester screening for spontaneous preterm delivery with maternal characteristics and cervical length. Fetal Diagn Ther. 2012; 31:154-61.
47. Greco E, Lange A, Ushakov F, Calvo JR, Nicolaides KH. Prediction of spontaneous preterm delivery from endocervical length at 11 to 13 weeks. Prenat Diagn. 2011; 31:84-9.
48. Grimes DA. Understanding screening tests: the key to avoiding pitfalls in interpretations. Contemp Obstet Gynecol. 2000; 6:466-8.
49. Guzman ER, Walters C, Ananth CV, O'Reilly-Green C, Benito CW, Palermo A, Vintzileos AM. A comparison of sonographic cervical parameters in predicting spontaneous preterm birth in high-risk singleton gestations. Ultrasound Obstet Gynecol. 2001;18:204-10.
50. Guzman ER, Walters C, O'reilly-Green C, Meirowitz NB, Gipson K, Nigam J, Vintzileos AM. Use of cervical ultrasonography in prediction of

spontaneous preterm birth in triplet gestations. Am J Obstet Gynecol. 2000; 183:1108-13.

51. Hasegawa I, Tanaka K, Takahashi K, Aoki K, Torii Y, Okai T, Saji F, Takahashi T, Sato K, Fujimura M, Ogawa Y. A prospective longitudinal study for the prediction of preterm delivery in a low-risk population. J Matern Fetal Invest. 1996; 6:148–51.

52. Hassan SS, Romero R, Berry SM, Dang K, Blackwell SC, Treadwell MC, Wolfe HM. Patients with ultrasonographic cervical length ≤ 15 mm have a 50% risk of early spontaneous preterm delivery. Ultrasound Obstet Gynecol. 2000; 182:1458-67.

53. Hassan SS, Romero R, Vidyadhari D, Fusey S, Baxter JK, Khandelwal M, Vijaraghavan J, Trivedi Y, Soma-Pillay P, Sambarey P, Dayal A, Potapov V, O'Brien J, Astakhov V, Yuzko O, Kinzler W, Dattel B, Sehdev H, Mazheika L, Manchulenko D, Gervasi MT, Sullivan L, Conde-Agudelo A, Phillips JA, Creasy GW: PREGNANT Trial: Vaginal progesterone reduces the rate of preterm birth in women with a sonographic short cervix: a multicenter, randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2011; 38:18-31.

54. Heath VC, Southall TR, Souka AP, Elisseou A, Nicolaides KH: Cervical length at 23 weeks of gestation: prediction of spontaneous preterm delivery. Ultrasound Obstet Gynecol. 1998; 12:312-17.

55. Hertzberg BS, Kliewer MA, Farrell TA, DeLong DM. Spontaneously changing gravid cervix: clinical implications and prognostic features. Radiology 1995; 196:721-4.

56. Iams JD, Goldenberg RL, Meis PJ, Mercer BM, Moawad A, Das A, Thom E, McNellis D, Copper RL, Johnson F, Roberts JM. The length of the cervix and the risk of spontaneous premature delivery. National Institute of Child Health and Human Development Maternal Fetal Medicine Unit Network. N Engl J Med. 1996; 334:567-72.
57. Kagan K, To M, Tsoi E, Nicolaides K. Preterm birth: the value of sonographic measurement of cervical length. BJOG 2006; 113: 52–6.
58. Kerenyi T. Forgotten “father of progesterone”. Am J Obstet Gynecol. 2010; 202:e10–1.
59. Krebs-Jimenez J, Neubert AG. The microbiological effects of endovaginal sonographic assessment of cervical length. J Ultrasound Med 2002; 21:727-9.
60. Lee SE, Romero R, Park CW, Jun JK, Yoon BH. The frequency and significance of intraamniotic inflammation in patients with cervical insufficiency. American Journal of Obstetrics and Gynecology 2008; 198:633 e631-638.
61. Liu L, Johnson HL, Cousens S, Perin J, Scott S, Lawn JE, Rudan I, Campbell H, Cibulskis R, Li M, Mathers C, Black RE; Child Health Epidemiology Reference Group of WHO and UNICEF. Global, regional, and national causes of child mortality: an updated systematic analysis for 2010 with time trends since 2000. Lancet 2012; 379:2151-61.
62. Ma S, Yang L, Romero R, Cui Y. Varying coefficient model for gene-environment interaction: a non-linear look. Bioinformatics. 2011; 27:2119–26.

63. Macones GA, Parry S, Elkousy M, Clothier B, Ural SH, Strauss JF 3rd. A polymorphism in the promoter region of TNF and bacterial vaginosis: preliminary evidence of gene-environment interaction in the etiology of spontaneous preterm birth. Am J Obstet Gynecol. 2004; 190:1504-8.

64. Maiz N, Kagan KO, Milavanovic Z, Celik E, Nicolaides KH. Learning curve for Doppler assessment of ductus venosus flow at 11+0 to 13+6 weeks' gestation. Ultrasound Obstet Gynecol. 2008; 31:503-6.

65. Mathews TJ, Menacker F, MacDorman MF. Infant mortality statistics from the 2002 period: linked birth/infant death data set. Natl Vital Stat Rep. 2004; 53:1–29.

66. Michaels WH, Montgomery C, Karo J, Temple J, Ager J, Olson J. Ultrasound differentiation of the competent from the incompetent cervix: prevention of preterm delivery. Am J Obstet Gynecol. 1986; 154:537-46.

67. Nicolaides KH. Screening for fetal aneuploidies at 11 to 13 weeks. Prenat Diagn. 2011a; 31:7-15.

68. Nicolaides KH. Turning the pyramid of prenatal care. Fetal Diagn Ther. 2011b; 29:183-96.

69. O'Brien JM, Adair CD, Lewis DF, Hall DR, Defranco EA, Fusey S, Soma-Pillay P, Porter K, How H, Schackis R, Eller D, Trivedi Y, Vanburen G, Khandelwal M, Trofatter K, Vidyadhari D, Vijayaraghavan J, Weeks J, Dattel B, Newton E, Chazotte C, Valenzuela G, Calda P, Bsharat M, Creasy GW. Progesterone vaginal gel for the reduction of recurrent preterm birth: primary results from a randomized, double-blind, placebo-controlled trial. Ultrasound Obstet Gynecol. 2007; 30:687–96.

70. Owen J, Neely C, Northern A. Transperineal versus endovaginal ultrasonographic examination of the cervix in the midtrimester: a blinded comparison. Am J Obstet Gynecol. 1999; 181:780-3.

71. Owen J, Yost N, Berghella V, Thom E, Swain M, Dildy GA 3rd, Miodovnik M, Langer O, Sibai B, McNellis D; National Institute of Child Health and Human Development, Maternal-Fetal Medicine Units Network. Mid-trimester endovaginal sonography in women at high risk for spontaneous preterm birth. JAMA 2001; 286:1340-8.

72. Ozdemir I, Demirci F, Yucel O, Erkorkmaz U. Ultrasonographic cervical length measurement at 10-14 and 20-24 weeks gestation and the risk of preterm delivery. Eur J Obstet Gynecol Reprod Biol. 2007; 130:176-9.

73. Pandian Z, Templeton A, Bhattacharya S. Modification of assisted reproduction techniques to prevent preterm birth. Clin Obstet Gynecol. 2004; 47:833–41.

74. Papiernik E, Bouyer J, Collin D, Winisdoerffer G, Dreyfus J. Precocious cervical ripening and preterm labor. Obstet Gynecol. 1986; 67:238-42.

75. Phelps JY, Higby K, Smyth MH, Ward JA, Arredondo F, Mayer AR. Accuracy and intraobserver variability of simulated cervical dilatation measurements. Am J Obstet Gynecol. 1995; 173:942-5.

76. Robinson HP and Fleming JE: A critical evaluation of sonar "crown-rump length" measurements. Br J Obstet Gynaecol 1975; 82:702-10.

77. Rode L, Klein K, Nicolaides K, Krampl-Bettelheim E, Tabor A. Prevention of preterm delivery in twin gestations (PREDICT): A multicentre

randomised placebo-controlled trial on the effect of vaginal micronised progesterone. Ultrasound Obstet Gynecol. 2011; 38:272-80.

78. Romero R, Espinoza J, Kusanovic JP, Gotsch F, Hassan S, Erez O, Chaiworapongsa T, Mazor M. The preterm parturition syndrome. BJOG. 2006; 113:17–42.

79. Romero R, Nicolaides K, Conde-Agudelo A, Tabor A, O'Brien JM, Cetingoz E, Da Fonseca E, Creasy GW, Klein K, Rode L, Soma-Pillay P, Fusey S, Cam C, Alfirevic Z, Hassan SS. Vaginal progesterone in women with an asymptomatic sonographic short cervix in the midtrimester decreases preterm delivery and neonatal morbidity: a systematic review and metaanalysis of individual patient data. Am J Obstet Gynecol. 2012 Feb; 206:124.e1-19.

80. Romero R. Prenatal medicine: the child is the father of the man. 1996. J Matern Fetal Neonatal Med. 2009; 22:636–9.

81. Saigal S, Doyle LW. An overview of mortality and sequelae of preterm birth from infancy to adulthood. The Lancet 2008; 371: 261-9.

82. Sanders MR, Donohue PK, Oberdorf MA, Rosenkrantz TS, Allen MC. Impact of the perception of viability on resource allocation in the neonatal intensive care unit. Journal of Perinatology, 1998; 18:347-51.

83. Shalev J, Mashiach R, Bar-Chava I, Kaplan B, Bar J, Orvieto R, Meizner I. The 'virtual' cervical internal os: diagnosis during the first trimester of pregnancy. Ultrasound Obstet Gynecol. 2003; 21:145-8.

84. Snijders RJ, Noble P, Sebire N, Souka A, Nicolaides KH; Fetal Medicine Foundation First Trimester Screening Group: UK multicentre project on

assessment of risk of trisomy 21 by maternal age and fetal nuchal-translucency thickness at 10-14 weeks of gestation. Lancet 1998; 352:343 -6.

85. Sonek J and Shellhaas C. Cervical sonography: a review. Ultrasound Obstet Gynecol 1998; 11:71-8.

86. Souka AP, Papastefanou I, Michalitsi V, Salambasis K, Chrelias C, Salamalekis G, Kassanos D. Cervical length changes from the first to second trimester of pregnancy, and prediction of preterm birth by first-trimester sonographic cervical measurement. J Ultrasound Med. 2011; 30:997-1002.

87. Stone J, Ferrara L, Kamrath J, Getrajdman J, Berkowitz R, Moshier E, Eddleman K. Contemporary outcomes with the latest 1000 cases of multifetal pregnancy reduction (MPR). Am J Obstet Gynecol. 2008; 199:406, e1–4.

88. Stubbs TM, Van Dorsten JP. Preterm cervical examination. Am J Obstet Gynecol. 1988;159:789-90.

89. Syngelaki A, Chelemen T, Dagklis T, Allan L, Nicolaides KH. Challenges in the diagnosis of fetal non-chromosomal abnormalities at 11-13 weeks. Prenat Diagn. 2011; 31:90-102.

90. Timmons B, Akins M, Mahendroo M. Cervical remodeling during pregnancy and parturition. Trends Endocrinol Metab. 2010; 21:353-61.

91. To MS, Alfirevic Z, Heath VC, Cicero S, Cacho AM, Williamson PR, Nicolaides KH; Fetal Medicine Foundation Second Trimester Screening Group. Cervical cerclage for prevention of preterm delivery in women

with short cervix: randomised controlled trial. Lancet. 2004; 363(9424): 1849-53.

92. To MS, Skentou C, Cicero S, Nicolaides KH. Cervical assessment at the routine 23 weeks' scan: problems with transabdominal sonography. Ultrasound Obstet Gynecol 2000; 15:292-6.

93. To MS, Skentou C, Liao AW, Cacho A, Nicolaides KH. Cervical length and funneling at 23 weeks of gestation in the prediction of spontaneous early preterm delivery. Ultrasound Obstet Gynecol. 2001; 18:200–3.

94. To MS, Skentou CA, Royston P, Yu CK, Nicolaides KH. Prediction of patient-specific risk of early preterm delivery using maternal history and sonographic measurement of cervical length: a population-based prospective study. Ultrasound Obstet Gynecol. 2006; 27:362-7.

95. Tongsong T, Kamprapanth P, Pitaksakorn J. Cervical length in normal pregnancy as measured by transvaginal sonography. Int J Gynecol Obstet. 1997; 58:313-5.

96. Vaisbuch E, Romero R, Erez O, Kusanovic JP, Mazaki-Tovi S, Gotsch F, Romero V, Ward C, Chaiworapongsa T, Mittal P, Sorokin Y, Hassan SS. Clinical significance of early (< 20 weeks) vs. late (20–24 weeks) detection of sonographic short cervix in asymptomatic women in the mid-trimester. Ultrasound Obstet Gynecol. 2010; 36:471–81.

97. Valentin L, Bergelin I. Intra- and interobserver reproducibility of ultrasound measurements of cervical length and width in the second and third trimesters of pregnancy. Ultrasound Obstet Gynecol. 2002; 20:256-62.

98. Vayssière C, Morinière C, Camus E, Le Strat Y, Poty L, Fermanian J, Ville Y. Measuring cervical length with ultrasound: evaluation of the procedures and duration of a learning method. Ultrasound Obstet Gynecol. 2002; 20:575-9.

99. Visintine J, Berghella V, Henning D, Baxter J. Cervical length for prediction of preterm birth in women with multiple prior induced abortions. Ultrasound Obstet Gynecol. 2008; 31:198-200.

100. Wendell-Smith CP. The lower uterine segment. J Obstet Gynaecol Br Emp.1954; 61:87-93.

101. Werner EF, Han CS, Pettker CM, Buhimschi CS, Copel JA, Funai EF, Thung SF. Universal cervical-length screening to prevent preterm birth: a cost-effectiveness analysis. Ultrasound Obstet Gynecol. 2011; 38:32-7.

102. WHO: recommended definitions, terminology and format for statistical tables related to the perinatal period and use of a new certificate for cause of perinatal deaths. Modifications recommended by FIGO as amended October 14, 1976. Acta Obstetricia et Gynecologica Scandinavica 1977; 56: 247-53.

103. Yang X, Chen M, Wang HF, Leung TY, Borenstein M, Nicolaides KH, Sahota DS, Lau TK. Learning curve in measurement of frontomaxillary facial angle at 11-13 weeks of gestation. Ultrasound Obstet Gynecol. 2010; 35:530-4.

104. Yost NP, Bloom SL, Twickler DM, Leveno KJ. Pitfalls in ultrasonic cervical length measurement for predicting preterm birth. Obstet Gynecol. 1999; 93:510-6.

105. Zorzoli A, Soliani A, Perra M, Caravelli E, Galimberti A, Nicolini U. Cervical changes throughout pregnancy as assessed by transvaginal sonography. Obstet Gynecol. 1994; 84:960-4.
106. Zweig MH, Campbell G. Receiver-operating characteristics (ROC) plots: a fundamental evaluation tool in clinical medicine. Clin Chem. 1993; 39:561-77.

APPENDIX: PICTURES & TABLES

CHAPTER 1

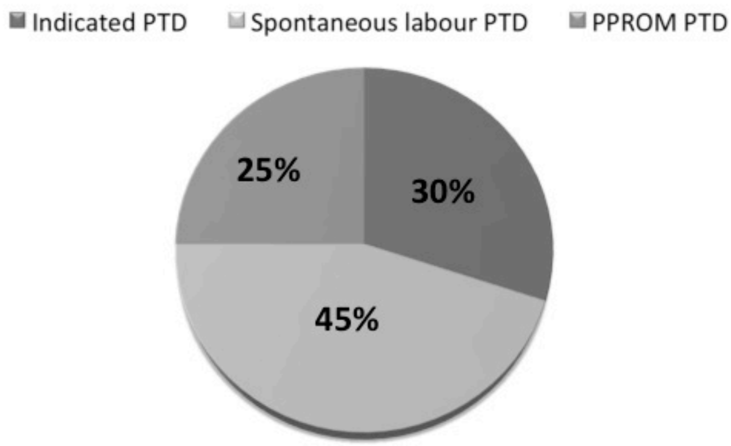


Figure 1.1. Obstetric precursors of preterm delivery
(from *Goldenberg, 2008*)
PTD: preterm delivery: pPROM: premature pre-labor rupture of membrane.

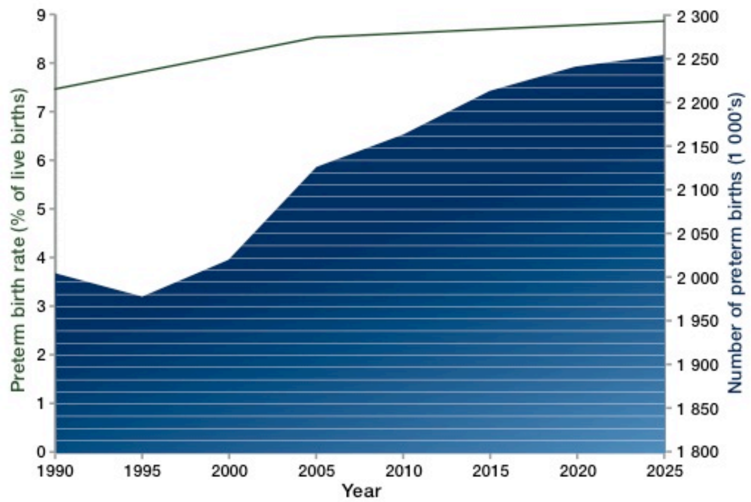


Figure 1.2. Time trends in preterm birth rate for Developed Countries, Latina America and Caribbean projecting to 2025. Projections are based on the assumption that the average annual rate of change observed from 2005 to 2010 is maintained.
(adapted *from Blencowe et al, 2012*)

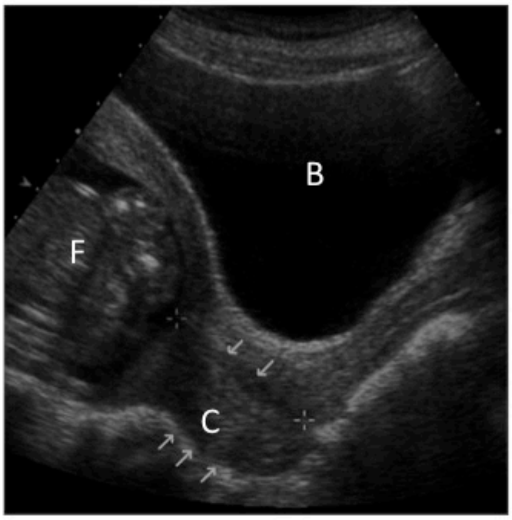


Figure 1.3. Measurement of the cervical length by trans-abdominal ultrasound. C, cervix; **B**, bladder; **F**, fetus. The white arrows indicate the cervical canal; the calipers indicate the external and internal orifices.

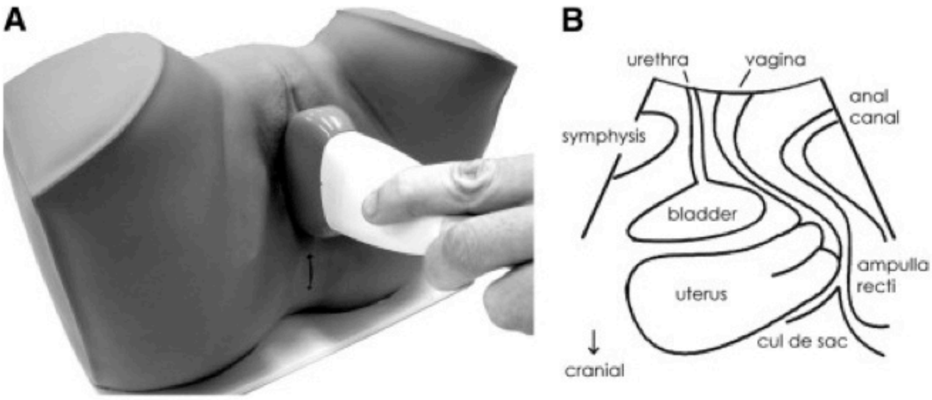


Figure 1.4. Technique for transperineal ultrasound. The patient lies on an examination table with the knees and hips in a flexed position; a gloved transducer is placed on the perineum between the labia majora, ensuring to keep the transducer in a sagittal orientation.

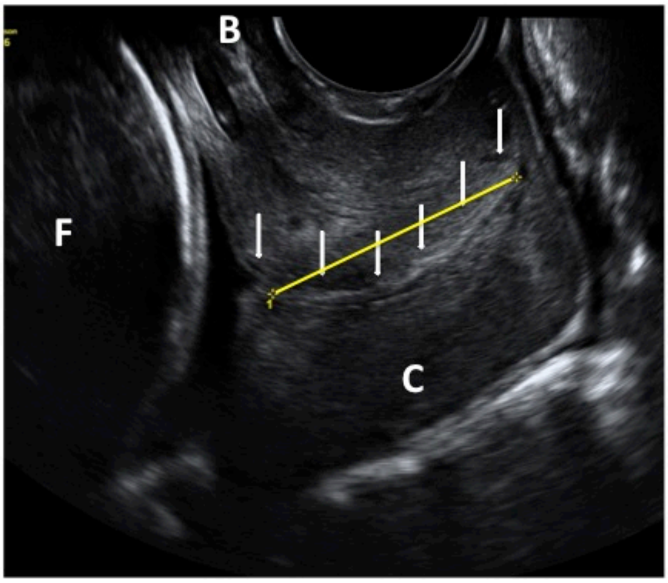


Figure 1.5. Measurement of the cervical length by trans-vaginal ultrasound. C, cervix; **B**, bladder; **F**, fetus. The white arrows indicate the cervical canal; measurement of the cervical length is indicated by the yellow line.

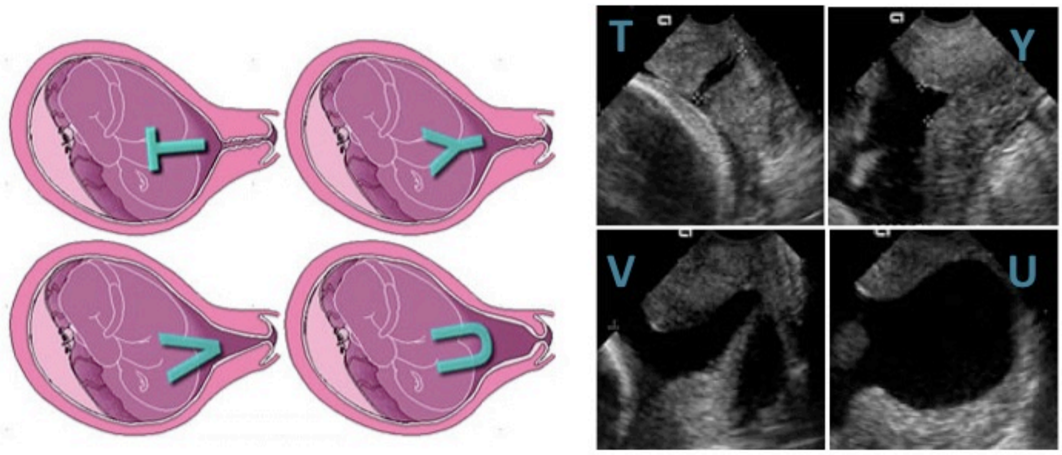


Figure 1.6. Funnelling. Funnelling of cervix typically occurs along a continuum: from the "T" appearance of the normally closed cervix, through the "Y" sign of the initial funnelling at the internal os, up to the 'V" and "U" signs of much deeper openings. Diagrams and corresponding transvaginal ultrasound images.

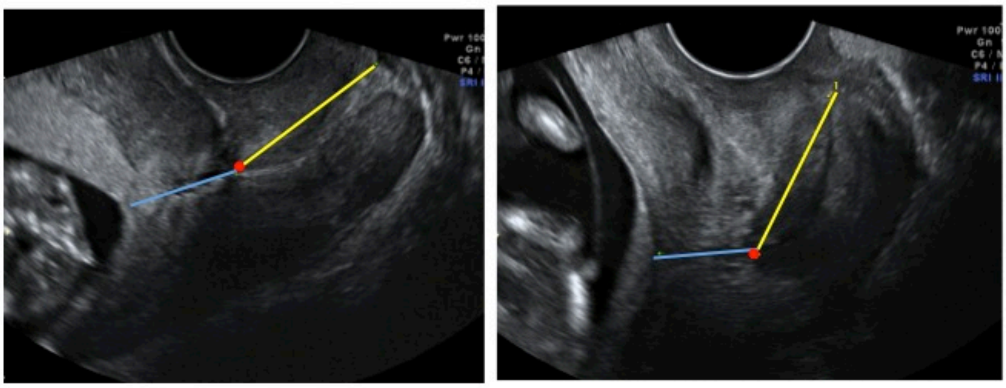


Figure 1.7. Ultrasound appearance of the cervico-isthmic complex in the first trimester. The endocervical length is measured by the yellow line; the isthmus is measured by the blue line; the internal orifice is represented by the red dot. Endocervical length + isthmus = cervico-isthmic complex.

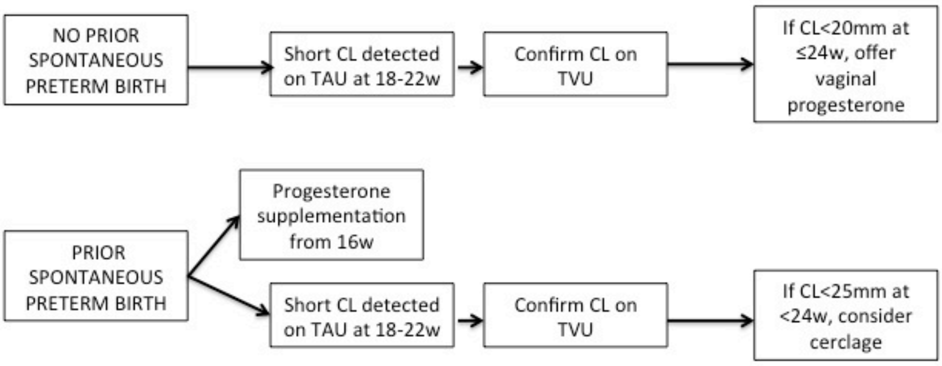


Figure 1.8. Recommended management of short cervical length in women with singleton gestations based on prior history of spontaneous preterm birth, as summarised from the American College of Obstetricians and Gynecologists and Society for Maternal-Fetal Medicine guidelines.

CL: cervical length; TVU: trans-vaginal ultrasound; TAU: trans-abdominal ultrasound: w: weeks' gestation.

Table 1.1. Technique for measurement of cervical length by transvaginal ultrasound as a screening for prediction of preterm birth

1. Have the woman empty her bladder just before ultrasound
2. Insert the gloved probe in the anterior fornix of the vagina
3. Obtain a sagittal, long-axis view of the entire endocervical canal, identified by the location of the triangular area of echodensity at the external os, a V-shaped notch at the internal os and a faint line of echodensity or echolucency between the two
4. Withdraw the probe until the image is blurred, and reapply just enough pressure to restore the image (to avoid undue pressure on the cervix that might artificially increase its length)
5. Enlarge the image so that the cervix occupies at least 2/3 of the screen, and both external and internal os are seen
6. Measure the cervical length from the internal os to the external os using as a guide to the true position of the internal os the echogenic endocervical mucosa, thereby avoiding confusion with the isthmus
7. Obtain at least three measurements, and record the shortest best measurement (in mm)
8. The entire examination should last at least 3 minutes, in order to allow time for dynamic changes of the cervix to take place

Table 1.2. Criteria for an effective screening test

1. Important condition
2. Safe and acceptable
3. Recognisable early, asymptomatic phase
4. Technique well described
5. Reproducible
6. Accurate
7. Early treatment is effective (prevention)

(adapted *from Grimes, 2000*)

Table 1.3. Prediction of preterm delivery by trans-vaginal ultrasound in different populations of asymptomatic pregnant women

Author	N	PTD (%)	Definition of PTD (w)	GA (w)	CL cut-off (mm)	Abn (%)	Sens	Spec	PPV	NPV	RR
Singleton: low-risk Iams et al.,1996	2915	4.3	<35	22-25	25	10	37	92	18	97	6.2
Singleton: prior PTD Owen et al., 2001	183	26	<35	16-24	25	_	69	80	55	88	4.5
Singleton: prior cone biopsy Berghella et al., 2004	109	13	<35	16-24	<25	28	64	78	30	94	4.7
Singleton: mullerian anomaly Airoldi et al., 2005	64	11	<35	14-24	<25	16	71	91	50	96	13.5
Singleton: prior D&C Visintine et al., 2008	131	30	<35	14-24	<25	51	53	75	48	78	2.2
Twins Goldenberg et al.,1996	147	32	<35	22-24	≤ 25	18	30	88	54	74	3.2
Triplets Guzman et al., 2000	47	34	<32	15-20	≤ 25	8.5	25	100	100	72	N/A

PTD: preterm delivery; w: weeks of gestation; CL: cervical length; Abn: abnormals; Sens: sensitivity; Spec: specificity; PPV: positive predictive value; NPV: negative predictive value; RR: relative risk; N/A: not available.

adapted from Mella and Berghella, 2009

Table 1.4. Prediction of preterm delivery by trans-vaginal ultrasound measurement of the cervical length prior to 14 weeks' gestation

Author	GA (w)	Population	Total N	CL mean/ median (mm)	PTD definition	N PTD (%)	P
Carvalho et al., 2003	11-14	Unselected	529	42.2	<37w; <33W	23/529 (4.3)	NS; 0.0007
Conoscenti et al., 2003	13-16	Unselected	2469	44.2	<34w	6/2469 (0.2)	NS
Hasegawa et al., 1996	8-12	Unselected	298	_	<37w	_	S
Ozdemir et al., 2007	10-14	Unselected	152	40.5	<35w	16/152 (10.5)	NS
Zorzoli et al., 1994	12	Unselected	154	43	<37w	3/154 (1.9)	NS

PTD: preterm delivery; GA: gestational age; w: weeks of gestation; CL: cervical length; N: number; N/S: non significant; S: significant for P<0.005.

Table 1.5. Meta-Analysis of 5 studies in women with short cervix evaluating the effect of vaginal progesterone on the risk of preterm delivery

Outcome	Number of trials	Number of events/Total number: Vaginal Progesterone	Number of events/Total number: Placebo	Pooled Relative Risk (95% CI)	Number needed to treat (95% CI)
PTD < 35w	5	79/388	118/387	0.69 (0.55-0.88)	11 (7-27)
PTD < 33w	5	39/388	71/387	0.57 (0.40-0.81)	13 (9-29)
PTD < 28w	5	21/388	43/387	0.50 (0.30-0.81)	18 (13-47)

PTD: preterm delivery; w: weeks of gestation; CI: confidence interval.

Source: Romero et al, 2012

APPENDIX: PICTURES & TABLES

CHAPTER 2

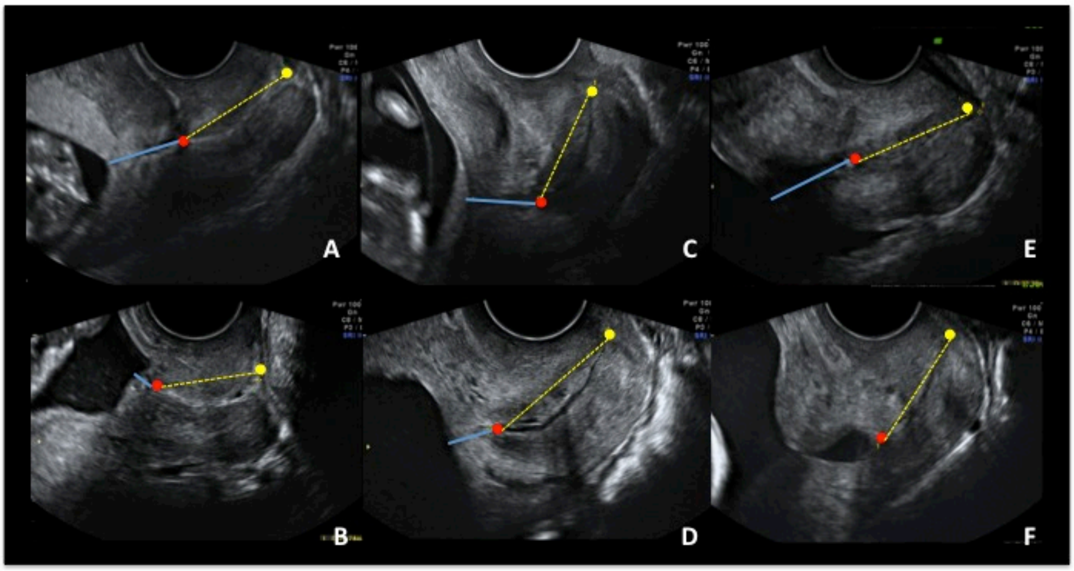
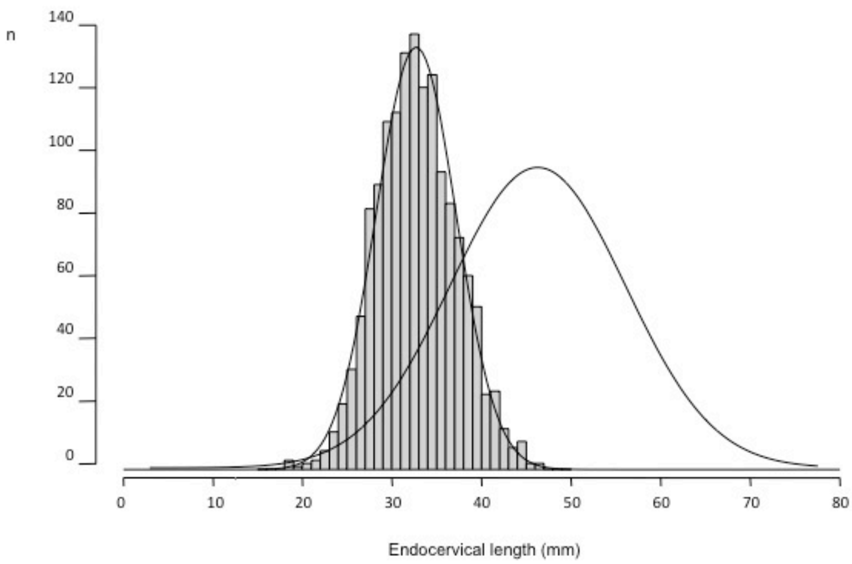
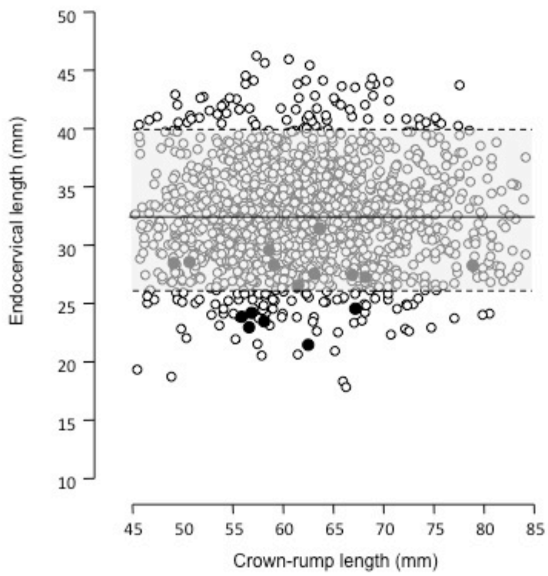


Figure 2.1 . Ultrasound appearance of cervix by transvaginal ultrasound in the first trimester (A,C,E) and corresponding images (B,D,F) in the second trimester. Cervical length is measured by the yellow dashed line, as the linear distance beween the external (yellow dot) and internal orifice (red dot); The myometrial thicknening (isthmus) is measured by the blue line, as the shortest linear distance between the internal orifice and the gestational sac. The isthmus is more consistent in the first than in the second trimester.



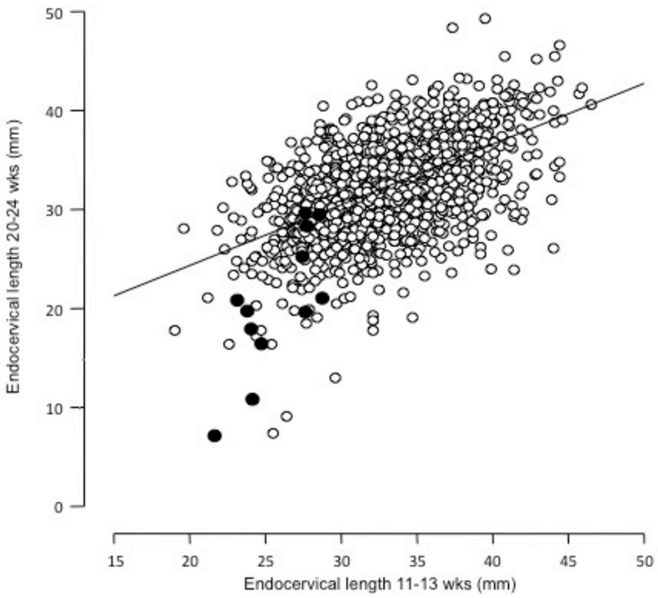
Source: Greco et al, 2011

Figure 2.2. Distribution of endocervical length at 11-13 weeks (histograms and overlying curve). The curve on the right is the distribution of lengthy of the cervico-isthmic complex.



Source: Greco et al, 2011

Figure 2.3. Reference range (50th, 5th and 95th centiles) and individual measurements in women with subsequent spontaneous delivery before 34 weeks (closed circles) and those delivering at or after 34 weeks (open circles).



Source: Greco et al, 2011

Figure 2.4. Relationship of endocervical length at 11-13 with 20-24 weeks in women with subsequent spontaneous delivery before 34 weeks (closed circles) and those delivering at or after 34 weeks (open circles).

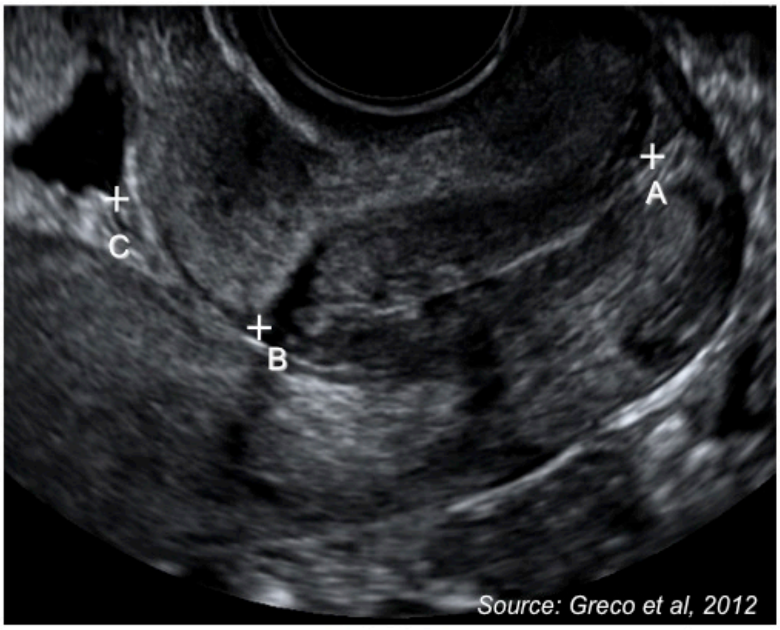
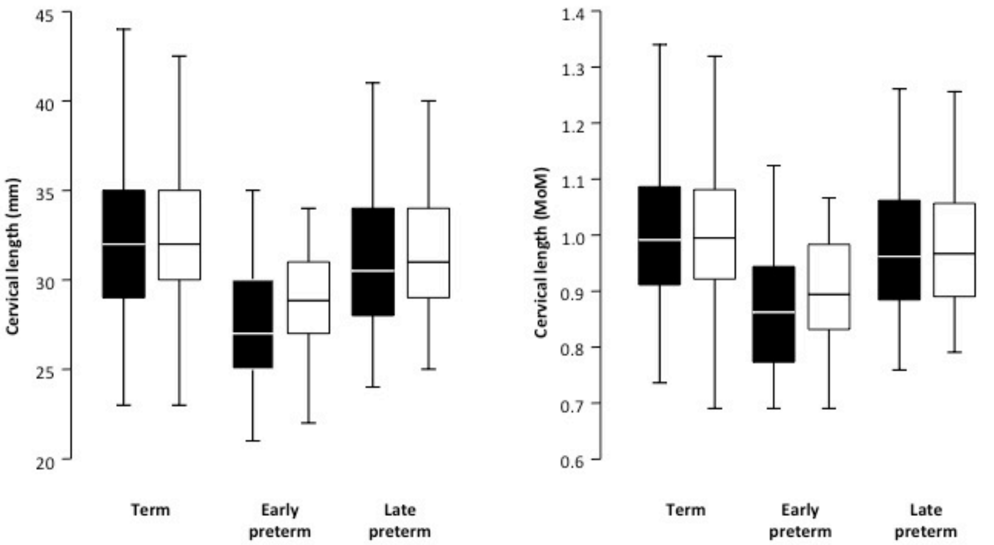


Figure 2.5. Measurement of endocervical length at 11-13 weeks by transvaginal ultrasound
Measurement of cervical length (A to B) and isthmus (B to C) are shown.



Source: Greco et al, 2012

Figure 2.6. Box-whisker plot of cervical length (left) and its multiple of the median (MoM) values (right) of Caucasian (white) and African (black) women in the three outcome groups.

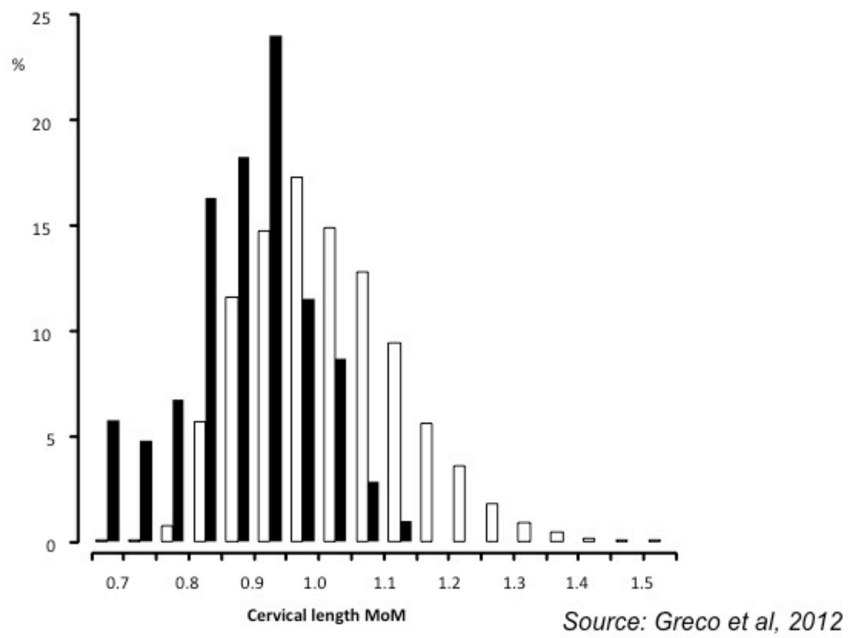


Figure 2.7. Frequency distributions of cervical length MoM in the term delivery group and early spontaneous preterm delivery group.

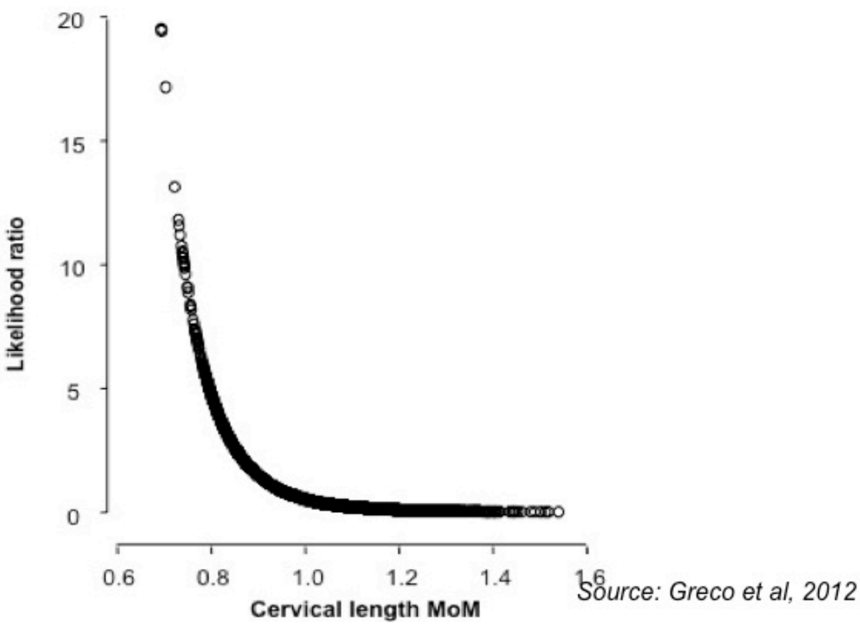
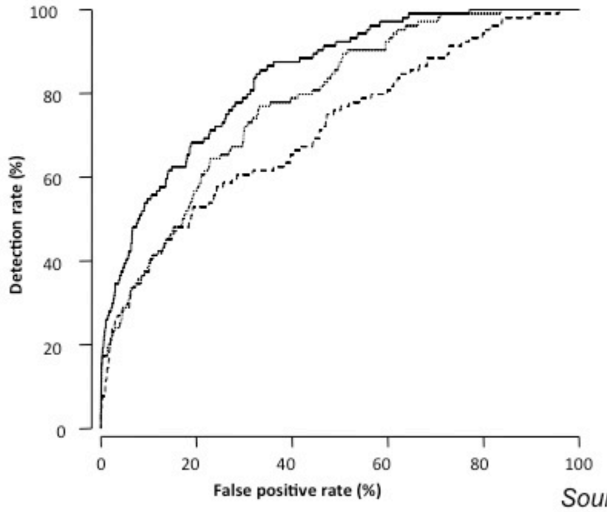


Figure 2.8. Likelihood ratios for early spontaneous preterm delivery from cervical length multiple of the median (MoM).



Source: Greco et al, 2012

Figure 2.9. Receiver operating characteristics curves of maternal characteristics, cervical length and their combination in the prediction of early spontaneous preterm delivery.

Maternal characteristics curve is shown as a large, dashed line (- - -); cervical length curve is shown as a tight, dashed line (......); the combination of the two is shown as a continuous line (____)

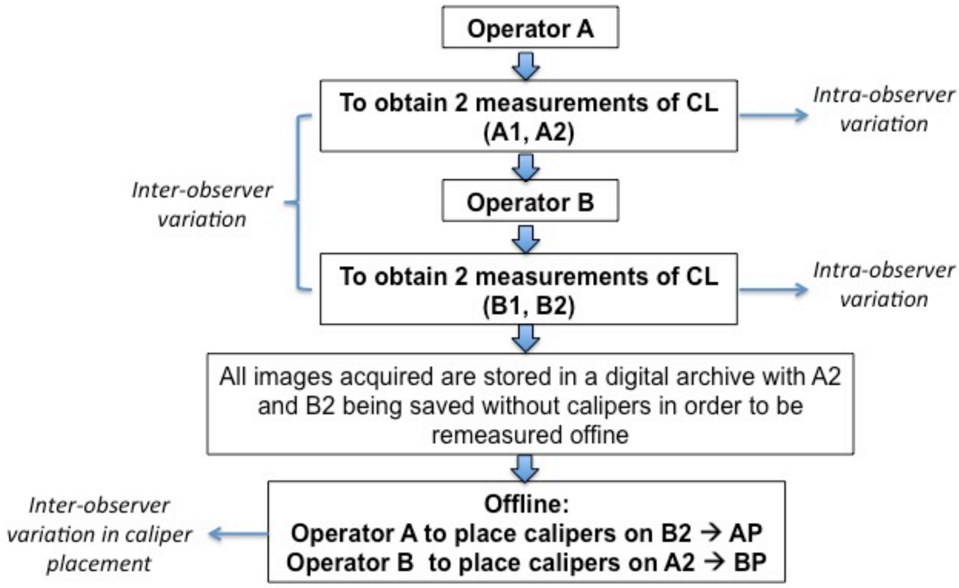


Figure 2.10. Study protocol for assessing intra- and inter-observer variation in the measurement of cervical length by transvaginal ultrasound at 11-13^{+6} weeks.

CL: cervical length.

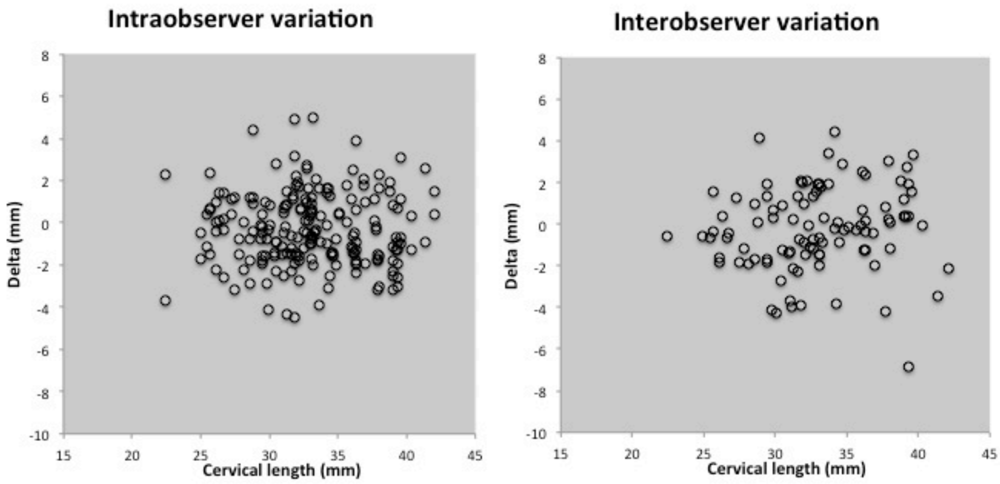


Figure 2.11. Relationship between mean cervical length and intra-observer difference (left) and inter-observer difference (right)

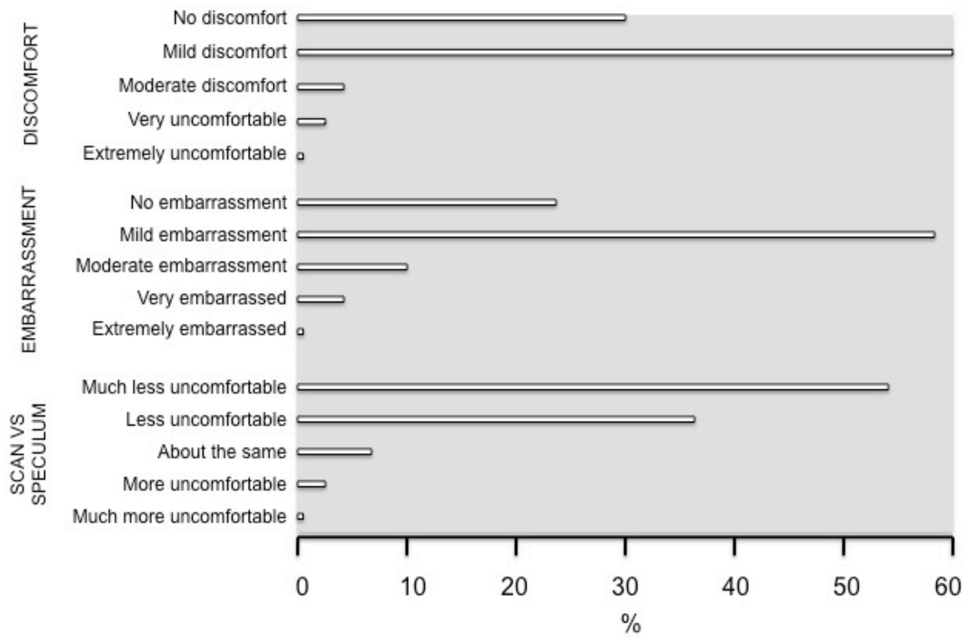


Figure 2.12. Acceptability of transvaginal sonographic assessment of cervical length. The answers to questions on degrees of discomfort and embarassment and comparison of the scan to the speculum examination are given as percentages.

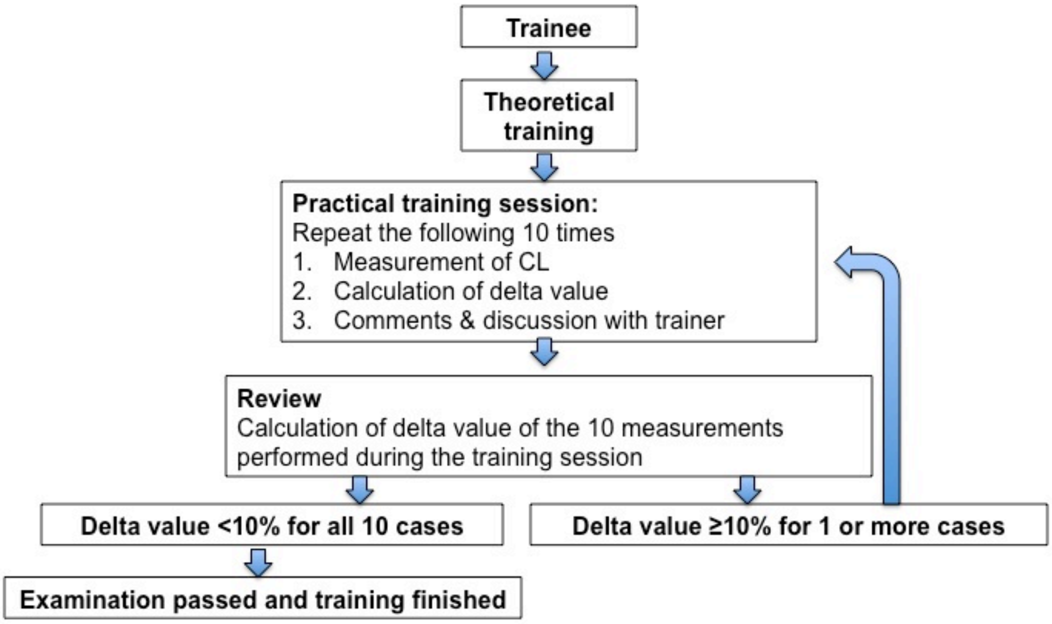


Figure 2.13. Training strategy for measurement of cervical length at 11^{+0}-13^{+6} weeks' gestation (FMF)

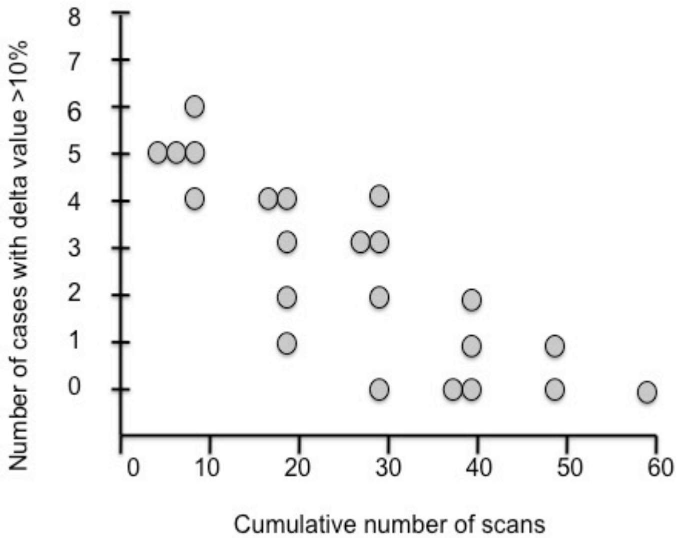


Figure 2.14. Performance of five sonographers in measuring cervical length in the first trimester of pregnancy after different stages of training

Number of cases, out of a total of 10, in which the difference between measured and true values of CL (delta value) was >10%. Each dot represents a trainee. All five trainees participated the first training session. Since those who passed the examination were not required to carry on with the study, the number of candidates in subsequent examinations reduced progressively.

Table 2.1. Technique for measurement of cervical length by transvaginal ultrasound in the first trimester

1. Have the woman empty her bladder just before ultrasound and lying in the dorsal lithotomy position
2. Insert the gloved transducer in the anterior fornix of the vagina
3. Obtain a sagittal, long-axis view of the entire endocervical canal, bordered by the endocervical mucosa which is usually of decreased, but occasionally of increased echogenicity compared to the surrounding tissues
4. Withdraw the probe until the image is blurred, and reapply just enough pressure to restore the image (to avoid undue pressure on the cervix)
5. Use the widest viewing angle and enlarge the image so that most of the screen is occupied by the tissues between the external os, at one end of the picture, and the gestational sac at the other end (cervico-isthmic complex)
6. Measure the cervical length as the linear distance between the two end of the glandular area around the endocervical canal
7. Measure the isthmus as the shortest distance between the glandular area and the gestational sac

Source: Greco et al, 2011

Table 2.2 Maternal characteristics and obstetric history in the screened population.

Maternal characteristics	Delivery > 34 wks (n=1,492)	Early preterm (n=16)
Maternal age in years, median (IQR)	32.5 (29.3-35.6)	33.2 (29.7-35.9)
Maternal weight, median (IQR)	63.0 (57.2-70.7)	61.8 (55.5-81.0)
Maternal height in cm, median (IQR)	165 (160-170)	163 (156-167)
Racial origin		
- Caucasian, n(%)	1,096 (73.5)	10 (62.5)
- Afro-caribbean, n(%)	126 (8.4)	4 (25.0)*
- South Asian, n (%)	149 (10.0)	1 (6.3)
- East Asian, n (%)	91 (6.1)	0
- Mixed, n (%)	30 (2.0)	1 (6.3)
Cigarette smoker, n (%)	32 (2.1)	0
Conception		
- Spontaneous, n (%)	1,402 (94.0)	16.0 (100)
- Ovulation drugs, n (%)	90 (6.0)	0
Obstetric history		
- Nulliparous – no pregnancy at >16w, n (%)	937 (62.8)	9 (56.3)
- Nulliparous – miscarriage at 16-23w, n (%)	22 (15)	2 (12.5)*
- Parous, preterm delivery 24-33w, n (%)	14 (0.9)	2 (12.5)*
- Parous, delivery >34w, n (%)	519 (34.8)	3 (18.8)

Comparisons between groups (χ^2-test and Fisher exact test for categorical variables and Mann Whitney U-test for continuous variables): * $p < 0.05$. IQR: interquartile range

Source: Greco et al, 2011

Table 2.3 Comparison of the length of the endocervix and cervico-isthmic complex at 11-13 weeks and 20-24 weeks in women with spontaneous delivery before 34 weeks' gestation and those delivering at or after 34 weeks

Variables	Delivery ≥ 34 weeks	Delivery ≤ 34 weeks	*P*
11-13 weeks			
- Endocervical length, median (IQR)	32.5 (29.5-35.6)	27.5 (24.0-28.6)	<0.0001
- Cervico-isthmic length, median (IR)	45.4 (39.0-53.1)	41.4 (34.8-45.8)	0.054
20-24 weeks			
- Endocervical length, median (IQR)	32.2 (29.3-35.3)	20.6 (17.0-27.7)	<0.0001
- Cervico-isthmic length, median (IQR)	40.7 (33.9-47.7)	27.0 (17.0-33.8)	<0.0001

Comparisons between the outcome groups were by Mann Whitney-U test.

Source: Greco et al, 2011

Table 2.4. Maternal characteristics and obstetric history in the three outcome groups.

Maternal characteristics	Delivery ≥ 37w (n=9,657)	Delivery < 34w (n=104)	Delivery between 34-36w (n=213)
Maternal age in years, median (IQR)	31.9 (28.0-35.4)	31.4 (27.4-35.5)	30.6 (26.6-34.2)*
Maternal weight in Kg, median (IQR)	64.4 (58.0-74.1)	65.3 (57.3-78.1)	62.7 (56.5-72.7)
Maternal height in cm, median (IQR)	164 (160-169)	163 (158-167)	164 (159-168)
Racial origin			
- Caucasian, n(%)	7,214 (74.7)	58 (55.8)*	138 (64.8)*
- Afro-caribbean, n(%)	1,179 (12.2)	30 (28.8)*	30 (14.1)
- South Asian, n (%)	639 (6.6)	8 (7.7)	22 (10.3)
- East Asian, n (%)	392 (4.1)	4 (3.8)	11 (5.2)
- Mixed, n (%)	233 (2.4)	4 (3.8)	12 (5.6)*
Cigarette smoker, n (%)	597 (6.2)	8 (7.7)	20 (9.4)
Assisted conception, n (%)	437 (4.5)	8 (7.7)	9 (4.2)
Obstetric history			
- Nulliparous – no pregnancy at >16w, n (%)	5,313 (55.0)	51 (49.0)	123 (57.7)
- Delivery at 16-30w – 1 event- , n (%)	95 (1.0)	6 (5.8)*	4 (1.9)
- Delivery at 16-30w - 2 events, n (%)	0	4 (3.8)*	0
- Delivery at 16-30w – 1 event + ≥37w, n (%)	7 (0.1)	3 (2.9)*	0
- Delivery at 31-36 w, n (%)	118 (1.2)	14 (13.5)*	14 (6.6)*
- Delivery at 31-36w + ≥37w, n (%)	17 (0.2)	3 (2.9)*	1 (0.5)
- Delivery at ≥37w, n (%)	4,107 (42.5)	23 (23.0)*	71 (33.3)*

Comparisons between the outcome groups was by χ^2-test or Fisher exact test for categorical variables and Mann Whitney U-test for continuous variables. * $p < 0.0167$. IQR: interquartile range

Source: Greco et al, 2012

Table 2.5. Multivariate regression analysis for the prediction of $\log_{10}$ cervical length in the term delivery group

Independent variable	Coefficient	Standard error	*P*
Intercept	1.360143	0.017832	<0.0001*
Fetal crown-rump length in mm	0.000222	0.000066	0.0008*
Maternal height in m	0.000480	0.000078	<0.0001*
Maternal age in years	0.002926	0.000800	0.0003*
(Maternal age in years)^2	-0.000036	0.000013	0.0047*
Racial origin			
- Caucasian, n(%)	0		
- Afro-caribbean, n(%)	-0.004102	0.001591	0.0099*
- South Asian, n (%)	-0.008513	0.002117	0.0001*
- East Asian, n (%)	-0.004224	0.002651	0.1112
- Mixed, n (%)	-0.001738	0.003349	0.6038
Cigarette smoker, n (%)	0.002660	0.002195	0.2256
Assisted conception, n (%)	0.000432	0.002902	0.8816
Obstetric history			
- Nulliparous – no pregnancy at >16w, n (%)	0		
- Delivery at 16-30w – 1 event- , n (%)	-0.005124	0.005220	0.3263
- Delivery at 16-30w – 1 event + ≥37w, n (%)	-0.036067	0.018994	0.0576
- Delivery at 31-36 w, n (%)	0.002786	0.004678	0.5515
- Delivery at 31-36w + ≥37w, n (%)	-0.021189	0.012219	0.0829
- Delivery at ≥37w, n (%)	0.003595	0.001059	0.0007*

*P<0.05

Source: Greco et al, 2012

Table 2.6. Median and interquartile range of cervical length in the three outcome groups.

	N	Term delivery	Spontaneous delivery			
			N	<34 wks	N	34-36 wks
Cervical length (mm)						
Total	9,657	32 (30-35)	104	29 (26-30)*	213	31 (29-34)*
Caucasian	7,214	32 (30-35)	58	29 (27-31)*	139	31 (29-34)*
Afro-caribbean	1,179	31 (29-35)+	30	27 (25-30)*	30	31 (28-34)*
Cervical length (MoM)						
Total	9,657	0.994 (0.919-1.082)	104	0.892 (0.829-0.945)*	213	0.977 (0.892-1.059)*
Caucasian	7,214	0.995 (0.921-1.081)	58	0.894 (0.832-0.984)*	139	0.967 (0.890-1.057)
Afro-caribbean	1,179	0.992 (0.911-1.087)	30	0.863 (0.769-0.944)*	30	0.962 (0.884-1.066)

Comparisons between the outcome groups was by Mann Whitney U-test for continuous variables. * $p < 0.0167$. Comparison between Caucasian and Afro-caribbean racial groups in each outcome group was by Mann-Whitney U test. +$p<0.05$: N: number; wks: weeks' gestation; MoM: multiple of median.

Source: Greco et al, 2012

Table 2.7. Likelihood ratios for early and late spontaneous preterm delivery from $\log_{10}$ cervical length multiple of median.

Log_{10} cervical length MoM	Likelihood ratio (95% CI)	
	Early spontaneous preterm delivery	Late spontaneous preterm delivery
-0.14	19.76 (18.80-20.72)	2.04 (2.02-2.06)
-0.14	12.17 (10.52-13.82)	1.82 (1.76-1.88)
-0.12	7.82 (6.74-8.90)	1.64 (1.59-1.69)
-0.10	5.00 (4.36-5.64)	1.49 (1.45-1.53)
-0.08	3.22 (2.81-3.63)	1.35 (1.31-1.39)
-0.06	2.04 (1.78-2.30)	1.24 (1.21-1.27)
-0.04	1.29 (1.12-1.46)	1.13 (1.10-1.16)
-0.02	0.82 (0.72-0.92)	1.04 (1.02-1.06)
0	0.52 (0.72-0.92)	0.97 (0.95-0.99)
0.02	0.33 (0.29-0.37)	0.90 (0.88-0.92)
0.04	0.21 (0.18-0.24)	0.84 (0.82-0.86)

MoM: multiple of median.

Source: Greco et al, 2012

Table 2.8. Comparison of the performance of screening for early and late spontaneous preterm delivery by maternal characteristics, cervical length and their combination.

Screening test	Area under receiver operating curve (95% CI)			
	Early spontaneous preterm delivery		Late spontaneous preterm delivery	
Maternal history				
Total	0.714 (0.705-0.723)		0.563 (0.553-0.573)	
Caucasian	0.692 (0.682-0.703)		0.533 (0.522-0.545)	
African	0.729 (0.703-0.754)		0.645 (0.617-0.672)	
Cervical length				
Total	0.782 (0.774-0.790)		0.551 (0.541-0.561)	
Caucasian	0.772 (0.762-0.781)		0.563 (0.552-0.575)	
African	0.797 (0.773-0.820)		0.553 (0.525-0.581)	
Combined test				
Total	0.840 (0.833-0.847)		0.583 (0.573-0.593)	
Caucasian	0.836 (0.828-0.845)		0.565 (0.553-0.576)	
African	0.829 (0.806-0.850)		0.662 (0.635-0.689)	
	Detection rate (%) for fixed false positive rate (95% CI)			
	5%	10%	5%	10%
Maternal history				
Total	28.9 (20.4-38.6)	37.5 (28.2-47.5)	9.9 (6.2-14.7)	19.7 (14.6-25.7)
Caucasian	25.9 (15.3-39.0)	34.5 (22.5-48.1)	8.0 (4.1-13.8)	12.3 (7.3-19.0)
African	26.7 (12.3-45.9)	26.7 (12.3-45.9)	16.7 (5.7-34.7)	20.0 (7.8-38.6)
Cervical length				
Total	28.9 (20.4-38.6)	38.5 (29.1-48.5)	9.4 (5.8-14.1)	14.6 (10.1-20.0)
Caucasian	25.9 (15.3-39.0)	39.7 (27.1-53.4)	8.7 (4.6-14.7)	15.2 (9.7-22.3)
African	40.0 (22.7-59.4)	50.0 (31.3-68.7)	10.0 (2.2-26.6)	13.3 (3.8-30.7)
Combined test				
Total	39.4 (30.0-49.5)	54.8 (44.7-64.6)	12.2 (8.1-17.4)	20.2 (15.0-26.2)
Caucasian	41.4 (28.6-55.1)	53.5 (39.9-66.7)	10.1 (5.7-16.4)	13.0 (7.9-19.8)
African	43.3 (25.5-62.6)	60.0 (40.6-77.3)	13.3 (3.8-30.7)	26.7 (12.3-45.9)

CI: confidence interval.

Source: Greco et al, 2012

		Examination results			
				Number of cases with delta value >10%	
Training session	Cumulative number of cases	Number of trainees	Number of trainees passing examination	Median	Range
1st	10	5	0	5	4-6
2nd	20	5	0	3	1-4
3rd	30	5	1	3	0-4
4th	40	4	2	0.5	0-2
5th	50	2	1	0.5	0-1
6th	60	1	1	0	0

Table 2.9. Performance of five sonographers in measuring cervical length in the first trimester of pregnancy after different stages of training

	Univariate analysis		*Multivariate analysis*	
Prediction	*Odds ratio (95% CI)*	*P*	*Odds ratio (95% CI)*	*P*
Crown-rump length (mm)	1.02 (1.003-1.041)	0.012	1.01 (0.996-1.043)	0.106
Cervical length (mm)	0.99 (0.977-1.016)	0.307	–	
Body mass index (Kg/m^2)	0.87 (0.850-1.014)	0.231	–	
Cumulative number of scans	1.05 (1.048-1.057)	<.0001	1.05 (1.048-1.057)	<.0001
Obstetric history			–	
- Nulliparity	1			
- Multiparity	1.46 (0.768-15.555)	0.264		
- Previous C/S	1.79 (0.345-3.547)	0.307		
Uterine version			–	
- anteverted	0.97 (0.560-2.762)	0.330		
- retroverted	0.87 (0.340-1.758)	0.763		
Ethnic origin			–	
-Caucasian	1			
- Afro-Caribbean	1.09 (0.714-1.656)	0.679		
- Asian	1.33 (0.430-4.725)	0.373		
- Oriental	2.70 (0.295-18.457)	0.702		
- Mixed	0.56	0.440		

Table 2.10. Regression analysis in the prediction of adequate measurement of cervical length