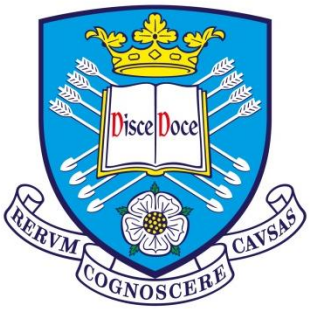

The University Of Sheffield.

ELECTRICAL IMPEDANCE SPECTROSCOPY OF THE CERVIX: A USEFUL SCREENING TEST FOR PRETERM BIRTH?

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Abstract

Reducing the rate of preterm birth (PTB) is a cornerstone of global efforts to address child mortality. However, without accurate techniques to identify those at risk, success will be limited. Existing tests offer imperfect prediction, particularly for universal screening.

Cervical electrical impedance spectroscopy (EIS) is a novel technique to quantify the ripening changes which precede labour. For the first time, this thesis provides a comprehensive assessment of its use in PTB screening by: (1) confirming measurement reliability; (2) evaluating predictive accuracy in pregnant women with no symptoms of preterm labour; (3) evaluating predictive accuracy in women presenting with symptoms of preterm labour and (4) assessing test acceptability. Cervical length (CL) and fetal fibronectin (FFN) measurements were employed alongside EIS to allow assessment of its performance in isolation and in conjunction with these conventional tests.

Significantly lower mid-trimester cervical impedance was observed in untreated asymptomatic women destined to deliver preterm. EIS-based prediction compared favourably with CL and FFN in unselected, high risk and low risk groups. Incorporating obstetric history further improved predictive accuracy. Moreover, a trend towards superior prediction via multimodal testing was observed.

Lower impedance was also observed in symptomatic women delivering shortly after assessment. In this cohort, EIS had good ability to discriminate those at risk of imminent delivery, with comparable and superior performance to FFN and CL respectively. Again, a trend towards optimal prediction via multimodal testing was noted.

Mixed-methods analysis of test acceptability suggests EIS is well tolerated and acceptable to high and low risk women, with reduced anxiety noted following screening.

Overall, cervical EIS may offer a useful, acceptable test to predict PTB. Further large studies are required to determine the value of EIS in specific groups, the effect of prophylactic treatment on measurements and the potential for incorporation into existing risk algorithms and treatment pathways.

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Awards Publications and Presentations

Awards

Best Oral Presentation (Pregnancy Outcome), Conference of the British Maternal and Fetal Medicine Society, 2018.

Finalist, Max Perutz Science Writing Award (for MRC funded researchers), 2015.

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Submitted articles:

Stern V; Jones GL; Senbeto S; Anumba DOC (2021) The acceptability of cervical electrical impedance spectroscopy: A mixed methods study examining women's experiences of a novel test to predict preterm birth. *(Submitted for consideration of publication to BMC Pregnancy & Childbirth)*

Conference presentations

Oral Presentations:

Oral presentation at BMFMS 2018 – "Cervical electrical impedance spectroscopy predicts preterm delivery in asymptomatic women – the ECCLIPPx TM studies" (Prize awarded – best presentation: Pregnancy Ouctome).

Oral presentation at RCOG Annual Academic Meeting 2016 – "Prediction of Preterm Birth by Electrical Impedance Spectroscopy of the Cervix - A Useful Addition to Current Tests?"

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Work also presented at Society for Reproductive Medicine International Conference in San Diego 2018 by Prof Anumba.

Poster Presentations: (first author posters only)

Poster presentation at Society for Reproductive Medicine Annual Meeting, San Diego 2018 – "Women's Experiences of Incorporating Cervical Electrical Impedance Spectroscopy into Conventional Clinical Assessment of Risk of Preterm Birth"

Poster presentation at Society for Reproductive Medicine Annual Meeting, Montreal 2016 – "Predicting Preterm Birth –The Performance Of Ultrasound Cervical Length, Quantitative Fetal Fibronectin and Vaginal Fluid pH Differs By Risk Of Preterm Birth and Symptoms Of Preterm Labour"

Poster presentation at Society for Reproductive Medicine Annual Meeting, Montreal 2016 – "The Association Of Cervicovaginal Fluid Metabolites At 20-22 Weeks Gestation With Quantitative Fetal Fibronectin, Ultrasound Cervical Length and Gestational Age At Delivery In Asymptomatic Pregnant Women"

Poster presentation at BMFMS, Birmingham 2016 – "Screening for preterm birth with serial cervical length measurement and fetal fibronectin quantification - does rate of change predict early delivery?"

Poster presentation at BMFMS, London 2015 - "Diversity of the vaginal microbiome and cervico-vaginal fetal fibronectin levels: Is there an association?"

Poster presentation at Society for Reproductive Medicine Annual Meeting, San Francisco 2015 - "The ECCLIPPx study: Electrical Impedance Prediction of Preterm Birth: preliminary results"

Poster presentation at BMFMS, Harrogate 2014 - "The ECCLIPPx study: Electrical Impedance Prediction of Preterm Birth"

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List of Abbreviations

AC	Alternating current
ACA	Anterior cervical angle
16S-rRNA	16S ribosomal ribonucleic acid
17-OHP	17α-hydroxyprogesterone caproate
AF	Amniotic fluid
AHR	Asymptomatic high risk
ALR	Asymptomatic low risk
AMP	Anti-microbial peptide
ASYMP	Asymptomatic
AUC	Area under the curve
BMI	Body mass index
BV	Bacterial vaginosis
CAI	Chorioamnionitis
CAP	Contraction associated protein
CCI	Cervical Consistency Index
CGA	Cervical Gland Area
CI	Confidence interval
CL	Cervical length
COX-2	Cyclo-oxygenase 2
CR	Cervical resistivity
CRF	Clinical research fellow
CRH	Corticotropin releasing hormone
CROWN	Core Outcomes in Women's and Newborn health
CST	Community state type
CVF	Cervico-vaginal fluid
ECM	Extracellular matrix
EGF	Epidermal growth factor
EIS	Electrical Impedance Spectroscopy
FDR	False Discovery Rate
FFN	Fetal fibronectin
FIRS	Fetal Inflammatory Response Syndrome
FPR	False Positive Rate
FSCN	Fascin-1
FWER	Family wise error rate
GAG	Glycosaminoglycan
GBS	Group B Streptococcus
HA	Hyaluronic acid
HIV	Human Immunodeficiency Virus
HRW	High risk women
HTA	Health Technology Assessment
ICC	Intraclass correlation coefficient
ICER	Incremental Cost Effectiveness Ratios
IL	Interleukin
IMD	Index of Multiple Deprivation

IUD	Intra-uterine death
IUT	In-utero transfer
IV	Intravenous
JAM-A	Junctional adhesion molecule A
KHz	Kilohertz
LED	Light emitting diode
LLETZ	Large loop excision of transformation zone
LLOA	Lower limit of agreement
LOA	Limit of agreement
LR-	Negative likelihood ratio
LR+	Positive likelihood ratio
LRW	Low risk women
LSCS	Lower segment caesarean section
MIAC	Microbial invasion of the amniotic cavity
miRNA	Micro Ribonucleic Acid
MMP	Matrix metalloproteinase
MRI	Magnetic resonance imaging
MTL	Mid-trimester loss
mTORC	mammalian target of rapamycin signalling
NF-κB	Nuclear factor kappa B
NHS	National Health Service
NI	Non-inferiority
NICE	National Institute for Health and Care Excellence
NICU	Neonatal Intensive Care Unit
NIHR	National Institute for Health Research
NLR	Nod-like receptor
NND	Neonatal death
NNT	Number needed to treat
NPV	Negative predicitve value
NR	Not reported
OR	Odds ratio
PAMG	Placental alpha macroglobulin-1
PCM	Pericellular matrix
PG	Prostaglandin
phILGFBP-1	Phosphorylated insulin-like growth factor binding protein 1
PI	Principal Investigator
PIS	Patient information sheet
PNM&M	Perinatal morbidity and mortality
PPI	Present Pain Intensity
PPROM	Preterm pre-labour rupture of membranes
PPV	Positive predictive value
PREBIC	Preterm Birth International Collaborative
PRI	Pain Rating Index
PROM	Pre-labour rupture of membranes
PTB	Preterm birth
PTL	Preterm labour

QALY	Quality adjusted life year
QUAL	Qualitative
QUANT	Quantitative
QUiPP app	Quantitative Instrument for the Prediction of Preterm Birth application
RANTES	Regulated on Activation Normal T Expressed and Secreted
RCT	Randomised controlled trial
REC	Research Ethics Committee
RM	Research Midwife
RNA	Ribonucleic acid
ROC	Receiver Operator Curve
RR	Relative risk
SASP	senescence-associated secretory phenotype
SBLv2	Saving Babies' Lives Version 2
SD	Standard deviation
SE	Standard error
SL	Senior Lecturer
spPTB	Spontaneous preterm birth
SR	Systematic review
SRM	Spontaneous rupture of membranes
SSI	Semi-structured interview
STAI-6	Six question, short form of the Spielberger State-Trait Anxiety Inventory
SWSE	shear wave speed elastography
SYM	Symptomatic
TA	Transabdominal
TAC	Transabdominal cerclage
TLR	Toll-like receptor
TNF	Tumour necrosis factor
TVUSS	Transvaginal ultrasound scan
UK	United Kingdom
ULOA	Upper limit of agreement
VAS	Visual analogue scale

Chapter 1 – General Introduction and Outline of Thesis

1.1 Introduction

1.1.1 Definition, epidemiology and impact of preterm birth

Obstetric care has advanced rapidly over the last century and throughout the world, maternal[1] and perinatal mortality[2] rates have generally declined. Despite such progress, preterm labour (PTL) still presents a major clinical challenge. Preterm birth (PTB) is defined as delivery before 37 completed weeks' gestation. Globally around 11% of pregnancies deliver preterm and over 1 million children die from complications of prematurity every year[3, 4]. In England and Wales approximately 8% of all births are preterm[5]. The majority occur after 28 weeks, but a significant minority (~5%) occur before this[6]. The earlier the gestation at delivery and the lower the birth weight, the higher the chance of significant perinatal morbidity and mortality[7, 8]. The sequelae of PTB are numerous and include problems with respiratory function, feeding, neurodevelopment and vulnerability to sepsis. As survival rates improve, the long term effects of PTB are becoming clearer. Even moderate to late preterm births (32 to <37 weeks)[9] are associated with an increased risk of developmental and behavioural problems in later life[10, 11] and higher rates of metabolic and cardiovascular disease[12, 13]. Whilst one in four early deliveries are iatrogenic, due to severe maternal or fetal disease[6], the majority follow spontaneous PTL.

The economic burden of PTB should not be underestimated. In the UK, the average cost of a day of neonatal intensive care is £1081[14], and PTB has been estimated to cost the National Health Service (NHS) £2.9 billion per year[15]. Analysis has suggested that delaying every premature delivery in the NHS by just one week could realise savings of up to £260 million annually[15]. However, in order to develop and optimise methods of preventing PTL, we first need to be able to predict exactly which women will deliver early. Otherwise we risk exposing low risk women to unnecessary intervention and we may underestimate the efficacy of preventative treatment if we cannot reliably target it at truly high risk patients.

1.1.2 Preterm birth screening and Electrical Impedance Spectroscopy

Historically, the provision of PTB screening in UK hospitals has been variable[16, 17]. Recent guidance aims to address this[18], but challenges persist. Surveillance of cervical length (CL) by serial trans-vaginal ultrasound scans (TVUSS) for women with PTB risk factors is well

established[19], but prediction is imperfect and less reliable for other groups (particularly low risk[20] and nulliparous women[21]). The evidence for screening tests will be reviewed later, but overall there is scope for improvement.

Electrical impedance spectroscopy (EIS) was first used to assess the pregnant cervix in 1996[22]. The technique applies a small electrical current at a range of frequencies to a biological tissue in order to evaluate its structure. It was pioneered in Sheffield and studies have demonstrated its ability to quantify cervical epithelial change during colposcopy[23]. Its use as a practical clinical tool in pregnant women bears further investigation. The existing data shows promise: EIS can predict the outcome of induced labour as efficiently as the Bishop score[24] (the digital assessment of cervical favourability used routinely in clinical practice); Furthermore, a recent pilot study demonstrated good correlation between cervical resistivity (CR) readings and delivery gestation, with comparable performance to CL scans[25]. If EIS can accurately predict PTB, it may have broad applications. PTB is a global problem and geographical differences in outcome are stark: in low-income countries, ~50% of babies born ≤32 weeks survive, whereas equivalent rates are seen at 24 weeks in high-income settings[9]. Explanations for such differences are multifactorial but limitations in neonatal care contribute significantly. Thus, a preventative approach to PTB has significant potential to improve morbidity and mortality. EIS may be particularly suited to PTB screening in such settings: the equipment is significantly cheaper than an ultrasound machine and CR measurements can be obtained with less training than a CL scan, enabling easier detection of women who may benefit from prophylactic treatment.

In order to understand the background to this thesis, it is helpful to consider current knowledge regarding the aetiology of preterm labour and the structure and function of the pregnant cervix. The utility of current screening tests for preterm labour will then be evaluated. An overview of the theory underlying electrical impedance spectroscopy, and the literature regarding its use in pregnancy will be provided. Finally, current therapeutic options for PTB prevention will be summarised.

1.2 Mechanisms of Parturition

Approaches to PTB have historically been simplistic, with spontaneous PTL treated as a homogenous condition[26]. Recently, clinicians and academics have begun to utilise "phenotypic classifications" of PTB[27], prompting careful consideration of its full range of

pathogenic triggers. One suggested prototype incorporates (i) assessment of the presence of significant maternal, fetal and/or placental conditions which might contribute to PTB, (ii) evidence of initiation of parturition (e.g. cervical shortening, uterine activity) and (iii) the observed pathway to delivery (i.e. iatrogenic or spontaneous)[27]. This paradigm shift has increased understanding of the complex, diverse pathways leading to PTB, although the precise molecular mechanisms are the focus of intense ongoing investigation. It is unsurprising that knowledge regarding the pathological processes of preterm labour is incomplete, given that even our understanding of term labour is imperfect. A brief overview of current theories of term labour is provided below, as the degree of overlap, or indeed divergence, between preterm and term parturition will inform predictive and therapeutic approaches.

1.2.1 Mechanisms of term labour

There are multiple triggers for term labour including: uterine stretch[28, 29]; rising maternal corticotrophin releasing hormone levels; increased oestrogen levels[28, 29]; functional progesterone withdrawal[30-32]; fetal hypothalamo-pituitary axis activation[33] and increased levels of fetal surfactant, surfactant associated proteins and epidermal growth factor (EGF) in amniotic fluid[34-39]. No trigger has clear primacy over the rest and the multiplicity of pathways promoting parturition is plausibly an evolutionary safety net to safeguard against postmaturity[38]. Overall, the switch from progesterone to oestrogen dominance and balance of maternal-fetal hypothalamo-pituitary axis activity acts to convert the uterus from a quiescent to highly contractile organ. There is increased secretion of, and sensitivity to, uterotonins such as oxytocin and prostaglandins (PGs) which affects both uterus and membranes[33]. Complementary changes in the cervix take place alongside the shift in uterine behaviour to form a common "effector pathway"[34] (the triad of contractions, membrane activation/rupture and cervical dilatation) which ultimately leads to birth. Mechanisms of cervical remodelling will be reviewed in detail in section 1.3.2.

1.2.2 Mechanisms of preterm labour

Romero *et al.* prompted the aforementioned shift in attitudes to PTB when they described the "preterm parturition syndrome" in 2006[40]. Their theory acknowledges that all labours share common routes to birth, but term labour follows physiological activation of these pathways, whereas preterm labour follows a spectrum of pathological triggers. Examples

are summarised in Figure 1-1. Multiple PTB precipitants may be present and there may be interaction between them. For example, the mechanism by which cervical weakness precipitates preterm labour seems to be partly explained by compromised barrier function and increased vulnerability to ascending infection, but also by mechanical weakness[41-43].

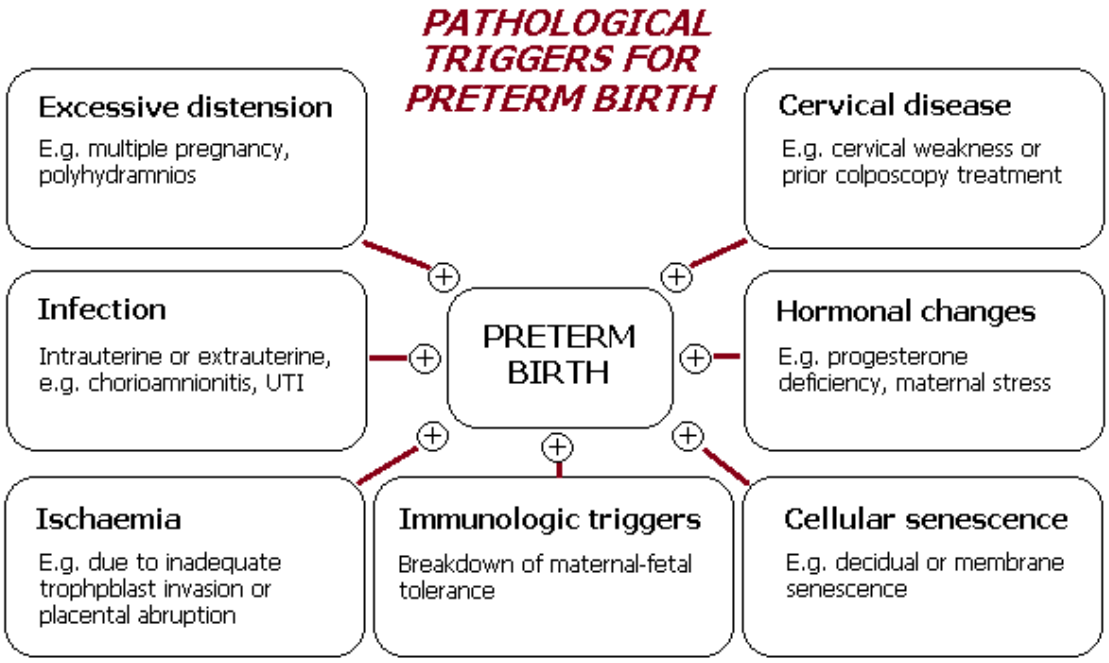


Figure 1-1 Causes of Preterm Labour
(Adapted from[26, 40]). Abbreviations: UTI – urinary tract infection

Characterisation of PTL as a syndrome is helpful – it provides a comprehensive framework for further research, ensuring all investigative and therapeutic avenues are explored. It also highlights the difficulty in devising effective PTB screening programmes: targeting specific triggers of PTB could allow prophylactic intervention at an early stage, but risks missing patients with PTL of different aetiology. This problem will be considered further in section 1.2.3, below.

The mechanisms by which the common pathological triggers might precipitate early labour will now be considered in turn.

1.2.2.1 Infection and preterm birth

Infection is the commonest trigger of PTL, implicated in up to 40% of early births[44]. Intra-uterine infection most often arises from ascending vaginal pathogens, but also via trans-placental haematogenous spread; from the peritoneal cavity via the fallopian tubes; or iatrogenically via invasive procedures[40]. The mechansims underlying infection-associated

PTB are incompletely elucidated, although numerous studies have confirmed causation in both animal and human models[45-47]. Many reproductive tissues express pattern recognition receptors, capable of triggering an inflammatory response to pathogenic infection, e.g. toll-like receptors (TLRs) and Nod-like receptors (NLRs) within the membranes and decidua[48, 49]. Receptor binding typically triggers the release of pro-inflammatory cytokines and chemokines (such as interleukins(ILs) 1β, 6 and 8 and tumour necrosis factor (TNF))[48, 50] promoting tissue infiltration by leucocytes and increased local production of PGs and matrix metalloproteases (MMPs). Thus, the common pathway of parturition can be triggered.

Studies also suggest a 'heterogeneity of response' to infection[47]. This may be due to inherent genetic suscepitibility: for example, women with polymorphisms for genes encoding various inflammatory mediators are at higher risk of PTB[51]. However, other variables such as the gestation at which infection occurs, its location and duration, the nature and abundance of the causative pathogen and other 'inflammatory modifiers' (e.g. co-exisiting viral infection) may significantly influence host repsonse to infection and thus the individual risk of infection-associated preterm birth. Techniques such as whole exome sequencing could provide further detail of specific polygenic changes which confer increased susceptibility to PTB. Preliminary studies suggest an association between mutations in genes which negatively regulate the innate immune response and which encode anti-microbial peptides and PPROM[52]. Interestingly, similar genetic mutations have been associated with peri-odontal disease and inflammatory bowel disease (conditions which appear to confer a higher risk of PTB) potentially suggesting a "shared genetic substrate" [52]. It remains to be seen whether these mutations might be used as biomarkers of PTB risk, or if their discovery could further contribute to our understanding of the mechanisms leading to infection-associated PTB.

Of late, the precise detail of the vaginal environment has been brought into focus by a new sphere of investigation, which will be considered in the following section.

The vaginal microbiome

16s rRNA (ribosomal ribonucleic acid) gene sequencing technology has facilitated intensive research into the vaginal microbiome. In pregnancy, various *Lactobacillus* species predominate (*L. crispatus, L gasseri, L. iners* and *L. jensenii*) and greater microbiome stability is seen than in the non-pregnant state[53]. Such changes plausibly confer resistance to pathogens and form a "finely tuned mutualistic relationship" with elements of the host

immune defence[54]. Higher oestrogen levels increase vaginal glycogen, which acts as a substrate for lactobacilli, promoting and supporting their growth. Lactobacilli produce lactic acid (which maintains a low vaginal pH) and anti-microbial compounds (e.g. hydrogen peroxide and bacteriocins[55]) rendering the environment more hostile to potential pathogens.

Prospective observational studies assessing the vaginal microbiota and PTB risk report inconsistent results. Some observe increased PTB and PPROM (preterm pre-labour rupture of membranes) risk in women with higher microbial richness and diversity[56-58], yet the findings of other studies are diametrically opposed and associate lower diversity with PTB[59, 60]. Conclusions regarding the relative abundance of different *Lactobacillus* species and other bacteria are similarly varied; some datasets associate *L.iners* dominance with PTB and deem *L. crispatus* protective[61-63], others show no variation in delivery gestation[56, 64]. Such differences may result from methodological variation: some studies were longitudinal, with serial swabs obtained during pregnancy whereas others utilised one-off samples obtained in early pregnancy. Additionally, study populations varied in nationality, ethnic mix and PTB risk status; variable definitions of PTB were used; and sample sizes were generally small. This makes overall synthesis of knowledge challenging. However, it is possible that these variable results suggest the presence of multiple microbiome-mediated pathways to preterm birth, with varied pathogenesis between high and low risk women and early and late preterm birth. Potential mechanisms by which the cervico-vaginal microbiota influence host defences and might lead to PTB are discussed further in section 1.3.3.2.

1.2.2.2 Stretch as a trigger of preterm birth

Excessive distension due to multiple pregnancy[65], uterine anomalies[66] and polyhydramnios[67] has long been associated with increased PTB risk. Stretch plays an important role in term labour and such conditions probably mean that term-equivalent levels of distension are reached early. Mechanistically, the effect of stretch appears two-fold: it stimulates myometrial contractility and initiates changes within the membranes which predispose to rupture[40]. Within the myometrium, stretch increases the expression of CAPs (contraction-associated proteins, e.g. gap junctions[68]), increases PG levels[69, 70] and upregulates and activates receptors (including the oxytocin receptor[71]). Stretch-induced changes observed within fetal membranes *ex vivo* include higher levels of collagenase and IL-8[72]; furthermore

these, and other mediators, may not only weaken the membranes, but also increase cervical ripening[40].

1.2.2.3 Cellular senescence and preterm birth

Cellular senescence is an irreversible arrest of cell proliferation, without cell death. This terminal differentiation provokes an inflammatory response within the local tissue environment which has been termed the "senescence-associated secretory phenotype" (SASP)[73]. It is typically observed during tissue ageing, but physiological stressors may promote earlier senescence (e.g. genotoxic stress, including telomere loss[74], and oxidative stress[75]). Both decidual and membrane senescence have been implicated in the timing of birth[38]. Many of the inflammatory mediators released as part of the SASP overlap with those implicated in the pathways of both term and PT parturition (e.g. cytokines, chemokines and MMPs[76]). Decidual senescence may promote weakening of the "decidual anchor" between membranes and the uterine wall, promoting separation and withdrawal of decidual support for the pregnancy[73]. In mice, decidual senescence has been linked with PTB due to increased mammalian target of rapamycin signalling (mTORC)[77], which might be amenable to treatment with mTORC1 inhibitors[77] and metformin[78]. Senescence of the membranes themselves could promote tissue weakening and membrane rupture, although these changes have predominantly been investigated with respect to term labour[38]. This remains an active area of investigation, with the potential for novel therapeutic insights in future.

1.2.2.4 Placental vascular disease and preterm birth

A broad range of placental vascular abnormalities have been associated with spontaneous PTB. Retroplacental abruption and decidual haemorrhage confer an increased risk of early labour, likely due to the stimulatory effect of thrombin on the myometrium[26]. Ischaemic placental lesions, particularly those associated with maternal vascular under-perfusion and failed transformation of the spiral arteries (as commonly seen in pre-eclampsia/fetal growth restriction) are also associated with a higher chance of PTL[79, 80]. Meta-analysis shows that women at risk of pre-eclampsia who take aspirin prophylaxis antenatally have lower rates of spontaneous PTL compared to those on no or placebo treatment, perhaps reflecting a reduction in uteroplacental iscahemia[81]. Given the shared etiology of placental ischaemia in pre-eclampsia, fetal growth restriction and a subset of spPTB and the proven preventive effect of aspirin on preeclampsia/growth restriction, a randomised trial of aspirin for PTB prophylaxis in women with prior PTB is ongoing[82].

1.2.2.5 Immunological triggers of preterm birth

These stimuli represent a relatively less studied area of PTL aetiology. It has been suggested that rejection of the fetal 'allograft' due to a disruption of maternal-fetal tolerance might be responsible for a subset of spontaneous PTB[83]. The reasons for such rejection are unclear, but it appears to result in placental abnormalities including chronic chorioamnionitis and villitis of unknown origin[84]; however, such lesions are also observed in patients delivering at term, albeit at a lower rate[85].

1.2.2.6 Cervical triggers of preterm birth

The structure, function and remodelling of the pregnant cervix are discussed in detail below. At this point, however it is worth noting that the cervical conditions which predispose to early delivery appear heterogeneous. Disruption of cervical anatomy, with resultant mechanical weakness and reduced barrier function, likely explains the increased risk of PTB in women following surgical therapy (e.g. colposcopy treatment/trachelectomy)[86] and with congenital or drug induced anomalies[87]. Recurrent mid trimester loss (MTL) and clinically 'silent' dilation of the cervix are often explained by 'incompetence' of the cervix despite apparently normal anatomy – this may result from premature ripening with or without local infection[40].

1.2.3 Screening for preterm birth in the face of diverse precipitants

It should now be clear that the pathway to preterm delivery is complex and may be activated at a variety of points. This makes screening difficult – how can one test and detect the plethora of physiological and biochemical changes described above? As understanding of the molecular basis of preterm labour has increased, clinical studies are increasingly assessing diagnostic and screening tests in combination to try to address this problem[88-90]. It also makes sense to evaluate elements of the common pathway of parturition during screening: regardless of the cause of PTB, ultimately delivery must involve a change in the cervix, or birth will not occur. This is part of the appeal of cervical EIS as a putative investigation – it should enable detection of women with early cervical change resulting from a range of problems, from infection and excessive uterine distension to apparently isolated cervical incompetence.

1.3 Structure and Function of the Pregnant Cervix

The function of the pregnant cervix is delicately balanced. It must provide an effective barrier to retain the fetus in utero until term, then, change rapidly and dynamically to a compliant structure which can dilate before vaginal delivery. After birth it resumes its previous form rapidly, aiding haemostasis and limiting the access of pathogens. Knowledge of the molecular pathways underlying cervical remodelling has advanced significantly in recent years. Studies utilising techniques typically rooted in engineering and mathematical modelling, in addition to more conventional biologic and genomic approaches, have provided new explanations for the cervical behaviour we observe clinically. As the focus of this thesis is prediction of PTB via cervical EIS, this section will summarise knowledge regarding macro- and microscopic anatomy of the cervix and review theories of cervical change prior to term and preterm birth to elucidate the processes which EIS might quantify.

1.3.1 Cervical anatomy and histology

1.3.1.1 Macroscopic anatomy

The cervix sits beneath the muscular uterine corpus and provides a tubular communication with the lower genital tract, measuring around 3-5cm in length and 2cm in diameter[91]. For the majority of pregnancy, it retains this closed conformation, then prior to labour it softens and becomes thinner and shorter during cervical effacement and opens gradually as cervical dilatation commences.

1.3.1.2 Microscopic anatomy

Cervical tissue can predominantly be divided into two main subtypes: an epithelium, which covers the luminal and endocervical surfaces of the cervix, providing an important protective barrier, and the underlying stroma, which provides both strength and compliance[91]. The epithelial cells are themselves surrounded by a pericellular matrix(PCM)[92] and produce mucous which organises to form a plug within the cervical canal[93].

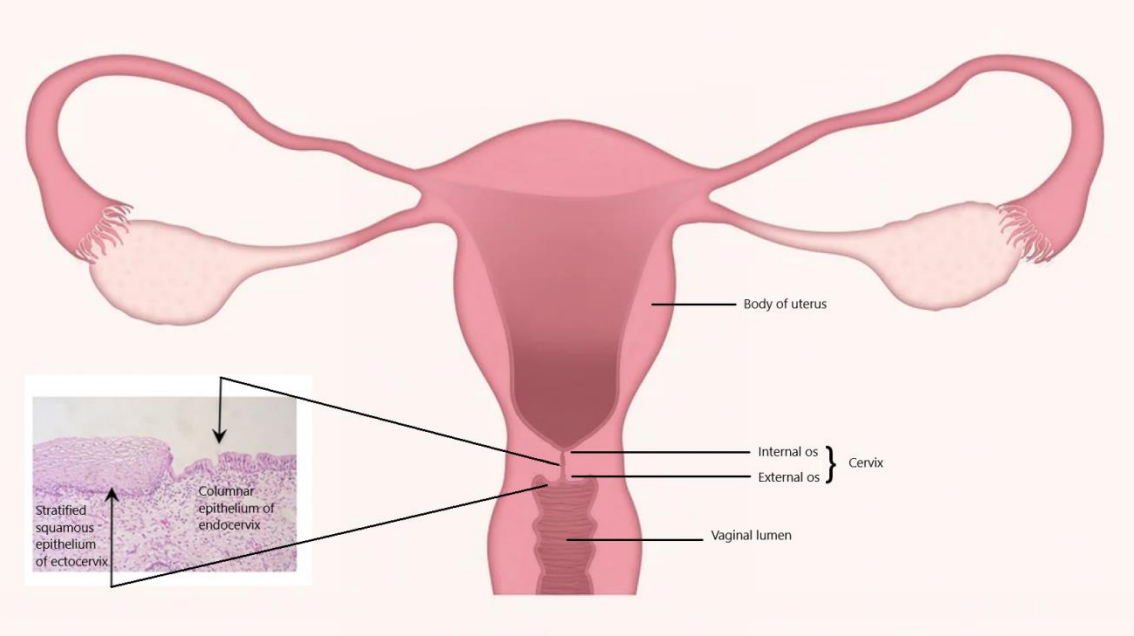


Figure 1-2 Cervical Anatomy

The cervix functions as a barrier between the uterine cavity and the vaginal environment. The endocervical canal is lined by secretory columnar epithelium, whereas the ectocervical epithelium comprises stratified squamous cells.

Cervical epithelium:

The cervical epithelium is comprised of varied cell types. The intra-vaginal ectocervix is covered with stratified squamous epithelium. The endocervical canal is lined with columnar epithelium and at the boundary between the two - the squamo-columnar junction - squamous metaplasia occurs. The predominant role of the cervical epithelium is preservation of tissue integrity and defence against infection. It is immunologically active, with the ability to detect a variety of pathogens via TLRs[94] and expresses multiple components of the downstream innate immune response including cytokines and chemokines[95, 96].

Pericellular matrix:

The cervical epithelium produces its own extracellular matrix which Nallasamy *et al.* term the pericellular matrix[92]. It is rich in hyaluronic acid (HA), with increasing concentrations at advancing gestation, and appears to act synergistically with the epithelial cells to maintain barrier function.

Cervical stroma:

Beneath the epithelium lies the cervical stroma. The cells here are relatively sparse, consisting predominantly of fibroblasts, with a smaller number of smooth muscle cells

(~85% vs 15%[97]). Its main structure lies within the extracellular matrix (ECM), where a network of collagen fibres is supported by the glycoprotein rich ground substance. Elastin fibres and vascular capillaries are also present along with a large amount of water (which comprises ~85% of human cervical tissue in the third trimester[98]). It is likely that the mechanical properties of the cervix during pregnancy are predominantly determined by alterations in the collagen network and supporting ECM[99], however the precise mechanisms by which changes occur require ongoing investigation. Certainly there appears to be considerable tissue heterogeneity and differences between the internal and external cervical os[100-105] and inner subglandular *vs.* outer stromal zones[106] are increasingly recognised.

Collagen network:

Collagen represents between 54-77% of the dry weight of the cervix[107] and provides it with its tensile strength and ability to resist the load placed upon it by pregnancy. Both type I and type III collagen fibres are present[108] and defects in their synthesis can significantly affect the function of the cervix in pregnancy[91, 109]. Early histological studies suggested collagen levels might decline during pregnancy[110]. However, more recent work[105, 111] contradicts this: when normalised to dry weight, the amount of cervical collagen during pregnancy does not appear to change in rodents or humans. Instead, collagen fibres seem to be processed and assembled differently as gestation advances. This is mediated by changes in collagen cross-linking (with a falling ratio of mature:immature cross-links, correlated with decreased tissue strength[111]) and altered expression of matricellular proteins which modify collagen fibril assembly (e.g. thrombospondin and tenascin)[92]. Imaging studies suggest collagen fibrils increase in diameter and spacing with advancing gestation, and fibres become less linear[112]. The overall structure of the collagen matrix was originally thought to be arranged in discrete zones, with outer and inner layers of longitudinally orientated fibres providing secure attachment to the uterine lower segment, and a central layer of circumferentially orientated fibres providing strength to keep the cervix closed[104]. However recent work has suggested a more complex ultrastructure, with heterogeneous 'interweaving zones' of collagen scaffolding which differ in fibre orientation[102] and cross-linking[105] between the internal and external cervical os. It is unclear precisely what role this differential alignment plays in maintaining cervical function but nevertheless, a progressive disorganisation of the collagen matrix is a notable feature of cervical softening[107].

Ground substance:

The ground substance is an abundant ECM, containing proteoglycans, glycosaminoglycans and matricellular proteins, which supports and modifies the collagen network. Many of its constituent molecules are known to bind and regulate assembly of collagen fibres[113] and early observational data suggested they may play a role in remodelling. For example, dermatan sulphate may decrease during ripening[114] (it normally stabilises and binds both fibronectin and collagen fibres, therefore loss of this support affects tissue strength[91]) and versican and biglycan levels may increase during ripening (they associate with hydrophilic HA and plausibly increase the gaps within the collagen network[115]). However, more recent work demonstrated that global levels of sulfated GAG do not change with gestation – rodent studies show an increase in the ratio of HA:sulfated GAG but this predominantly appears to be mediated by an increase in HA[116]. Nevertheless, HA deficient mice are still able to demonstrate cervical ripening[117]. It is possible that more subtle changes in proteoglycan composition, such as altered GAG chain length, underlie remodelling, thus further work is required to illuminate the precise contribution of ECM constituents to cervical change.

Smooth muscle:

In comparison to the uterus, the distribution of smooth muscle cells within the cervix has always been considered relatively sparse. Tissue strength and function has been attributed to the collagen and ECM structure described above. However, in recent years, multi-disciplinary research focusing on tissue mechanics, has shifted focus towards the internal cervical os[99, 102, 118, 119]. Human studies have demonstrated significant heterogeneity of muscle distribution within the cervix, with ~50% of tissue at the internal os consisting of smooth muscle arranged in a circumferential, sphincter-like, configuration[100]. A gradient of distribution is evident, with lower concentrations noted caudally.

Cervical mucous:

In pregnancy, cervical glands secrete tenacious mucous which is classically retained within the canal until shortly before labour[93]. This provides reinforcement to the physical barrier properties of the cervix and innate immunological protection from pathogens, as partly evidenced by the presence of antimicrobial peptides (AMPs) within the mucous (including elafin, secretory leukoprotease inhibitor and defensins)[92]. Interestingly, recent observational studies[43, 120] have detected differences in the properties of cervical mucous in women destined to deliver preterm, specifically increased permeability and extensibility,

similar to the spinnbarkeit changes observed physiologically in non-pregnant women at ovulation, when cervical mucous becomes more permeable to sperm.

1.3.2 Cervical change during pregnancy

It is unsurprisingly difficult to obtain serial samples from the pregnant human cervix for tissue analysis. Ethical and logistical barriers limit the number and size of studies providing information on the histological structure of the human cervix antenatally. Obtaining biopsies from women at high risk or symptomatic of preterm birth is even harder. Consequently, much research utilizes animal models, especially mice, to generate and test hypotheses. Murine research should be cautiously extrapolated to human parturition: mice have markedly different reproductive anatomy (with two uterine horns); routinely carry multiple pregnancies, and, as bioengineering researchers have emphasized, are subject to very different mechanical forces as quadrupeds[101]. However, there are similarities in the biochemical triggers of labour between mice and humans (predominantly a decline in progesterone function) and there is some evidence to suggest that common processes of remodelling are shared between species[112, 121]. In contrast, limitations of existing human studies include: small sample sizes; variable timing and techniques of cervical biopsy; frequent use of post-partum biopsies (which may reflect changes associated with dilatation and post-natal repair, rather than remodelling) and frequent lack of gestational age matched controls[104]. Nevertheless, they represent the best available information on the true *in-vivo* changes occurring under a range of conditions.

1.3.2.1 Phases of cervical change

A degree of cervical softening occurs early in pregnancy (detectable clinically even at 4 weeks – the 'Hegar' sign'[122]). This is followed by cervical ripening - an accelerated phase of change in late pregnancy when marked biomechanical modification occurs and the strong, competent cervix becomes pliable, shortened and effaced[123]. It generally overlaps with the dilation phase, depending on when contractions commence. Deficiencies in cervical ripening are associated with poorer obstetric outcomes, emphasising the vital role remodelling plays in parturition – contractions alone are not enough for an uncomplicated birth[124, 125]. The three phases of remodelling – softening, ripening and dilation – have been extensively studied in rodents, with additional insights provided by human studies[111, 112, 116, 126-132].

Cervical softening:

Cervical softening appears to be predominantly mediated by changes in the collagen matrix of the stroma. The observed changes in cross-linking described above (with a shift from mature, strongly cross-linked collagen to newly synthesized, immature, poorly cross-linked fibres) correlate with the decline in tissue stiffness seen between ~day 10 to 15 of rodent pregnancy[92]. Expression of matricellular proteins such as thrombospondins and tenascins also decline at this stage - with potential effects on fibril assembly[133]. However, cross-linking changes are only observed up to day 15, whereas tissue stiffness continues to fall prior to ripening at day 18[92, 111] with unclear mechanisms responsible for the late increase in compliance.

Cervical ripening:

Ripening is associated with further changes in the collagen network. In mice, day 18 stromal collagen fibres are thicker, wavier and more widely spaced[92] (illustrated in Figure 1-3). This dispersion is likely mediated by ECM changes, although the interaction between various stromal components is incompletely understood. Certainly, there is an increase in HA synthesis in both mice and humans during cervical ripening[112, 134, 135] which plausibly increases tissue hydration, enhances collagen spacing and may increase compliance. In addition, an increase in hyaluronidase activity at term has been described (with resultant shift from high to low molecular weight HA)[112], and HA binding to molecules such as versican within the ECM[136] and even to the toll-like receptors of nearby vaginal epithelium may facilitate ripening further[133, 137].

Furthermore, the collagen scaffold and ECM appear to be disrupted by an influx of immune cells (particularly macrophages) which provoke a sterile inflammatory response[132, 138, 139] (characterised by tissue oedema, cellular hypertrophy and reduced cell nuclei density[121]). This is also associated with local release of collagenase and matrix metalloproteinase (MMP) enzymes which also contribute to structural degradation.

Recent studies employing non-invasive techniques to assess the 3rd trimester cervix in pregnant women support these murine models of cervical change: longitudinal cervical Raman spectroscopy measurements show a linear decline in peaks associated with ECM proteins with advancing gestation[140] and third trimester diffusion tensor MRI confirms increased hydration and increasing collagen fibre disorganisation in the subglandular cervical stroma at 36-38 weeks[106].

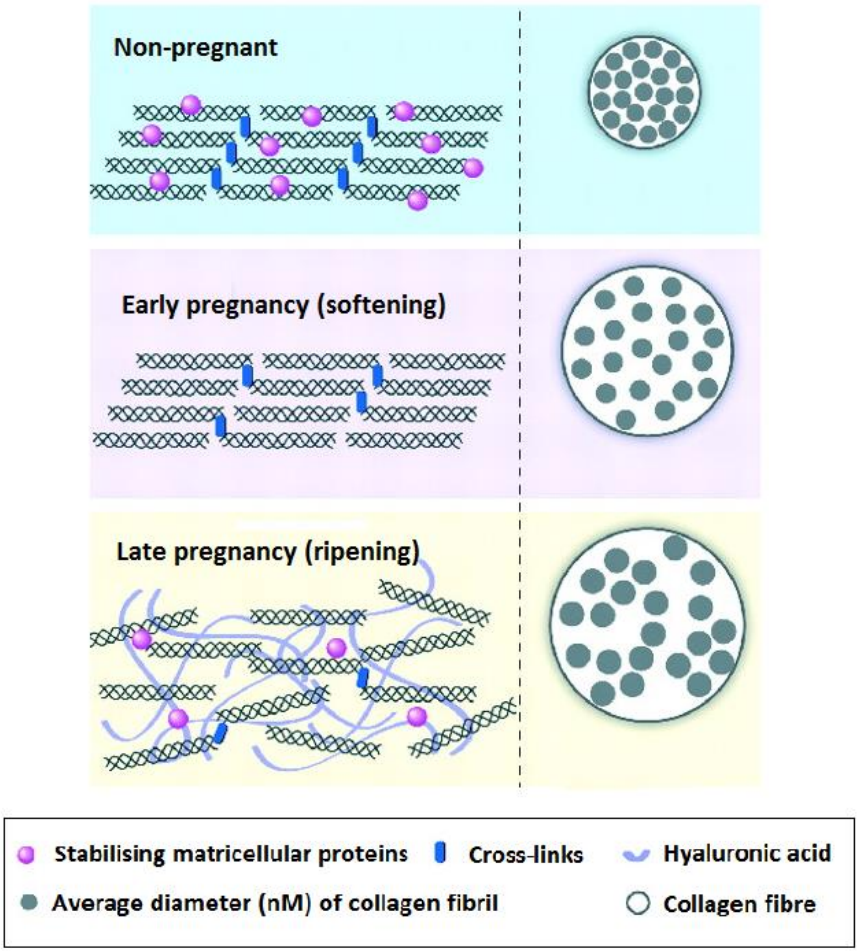


Figure 1-3 Structural Changes in Cervical Stroma during Pregnancy

(Adapted from [112], informed by murine research). In early pregnancy expression of stabilising matricellular proteins (e.g. tenascin) declines and collagen crosslinking changes mediate increased matrix turnover. Thicker, wider spaced collagen fibres are evident. In late pregnancy the accelerated ripening phase is associated with disorganisation of collagen fibres, an increase in hydrophilic hyaluronic acid and maximal diameter/spacing of immature, poorly crosslinked collagen fibres.

Cervical dilatation and postpartum repair:

Progressive cervical dilation typically results from the mechanical effect of increasingly strong and regular uterine contractions on the now compliant cervix. The processes underlying post-partum repair have been less studied than those preceding birth. They are likely to have less relevance to preterm birth screening, and as such, will not be considered further.

1.3.3 Cervical dysfunction during pregnancy

1.3.3.1 Cervical weakness

Sphincter failure at the internal os:

Previously cited work focusing on muscle cell distribution within the cervix has led to the hypothesis that 'sphincter failure' at the internal os might be a significant contributor to PTB. Evidence for its behaviour as a specialised sphincter includes the finding of a higher density of smooth muscle fibres, circumferentially orientated around the canal[141]; moreover biopsy specimens at this level are more contractile when stimulated in *in vitro* than those from the external os[100]. Clinically, funnelling may be a precursor to cervical shortening and/or PTB[142] (suggesting an association with cervical weakness) and postnatally, the internal os closes more rapidly than the external os. Observations that PTB risk is increased following caesarean sections in advanced labour may reflect underlying sphincter damage. Collagen fibre arrangement[119, 143, 144] and cross-linking[105] also varies at internal *vs.* external os, again suggesting level-dependent, differential mechanical function

A "new paradigm" of cervical function has been suggested, in which muscular contractility at the internal os is critical to maintaining cervical competence[104]. This raises new avenues for ongoing mechanistic research; however the findings of clinical studies do not entirely support the hypothesis. A recent secondary analysis of cohort data from nulliparous women undergoing cervical length screening demonstrated no significant association between the presence of funnelling and PTB before 34 weeks[145]. Moreover, an interesting recent study comparing the cervical tissue of pregnant women with cervical weakness to gestation-matched controls suggests that no inherent defect in smooth muscle contractility is present; instead, smooth muscle contractility was influenced by the softness of the adjacent ECM[141]. Overall, it is unclear how internal os dysfunction might fit in with other regulators of cervical function, and whether it might develop early or late in the process of pathological cervical change.

Tissue weakness:

Inherent defects in cervical stroma

Various groups have attempted to identify inherent deficiencies in the constituents of the cervical stroma amongst women with a history of "cervical incompetence"[146-153]. However, there is marked methodological heterogeneity and inconsistent results between studies[103]. Biopsy site/technique varied, many utilised non-pregnant index and/or control cases and

variable definitions of cervical incompetence were employed. Suggestions that women with cervical incompetence exhibit lower stromal collagen concentrations than controls[141, 146] have not been consistently replicated[148, 149, 151]. Given the paucity of evidence in this area, future studies, employing strict case/control definitions and utilising novel non-invasive methods of assessing the collagen network and other constituents (e.g. Raman[140, 154] or fluorescence spectroscopy[155-157]) may yield useful insights. Novel genomic approaches also support a role for stromal deficiency in cervical weakness. A recent study of 21 women with cervical insufficiency identified variants in 12 genes linked to cervical dysfunction, including 10 associated with non-syndromic cervical weakness due to clear functional changes in collagen and/or ECM synthesis[158].

Premature ripening

The process of premature ripening has received considerable research attention. It seems clear that in some PTBs, aberrant, early ripening is triggered as opposed to there being an inherent, pre-existing tissue weakness. Furthermore, there appear to be different mechanisms by which this premature ripening is mediated: ripening which occurs during infection-associated PTB[130, 131], and that which follows a "sterile inflammatory process" similar to that occurring at term[132, 139, 159]. Evidence from animal models suggests this latter progress may be triggered by functional progesterone withdrawal and the action of tissue macrophages[132, 138, 159] although the precise role and nature of immune cells implicated in term and preterm ripening is somewhat controversial[92]. Certainly, many groups have described stromal infiltration by leucocytes (including monocytes[160], macrophages[139] and neutrophils[161]) even if they have not reached consistent conclusions about their effects. Evidence from murine studies suggests infection-mediated remodelling may be dependent on the action of local prostaglandins, whereas mifepristone-induced remodelling is not associated with elevated PG levels. Overall, it is clear that further work is required to fully characterise the varied processes which may underlie premature cervical ripening. In the meantime, it is reasonable to suppose that the molecular mechanisms underlying premature ripening may be as heterogeneous and complex as upstream pathways involved in labour initiation.

1.3.3.2 Deficiency of the infection barrier

Cervical function is likely to play a significant role in infection-mediated preterm birth. A shortened and/or funnelled cervix plausibly provides a less effective barrier to the ascent of

vaginal pathogens. Clinical observation supports this – higher rates of MIAC are noted in association with reduced CL[42]. There is also increasing evidence that cervical epithelium may act as a critical gatekeeper of infection-mediated preterm birth and dysfunction has been associated with higher rates of early delivery[92, 117, 162-164]. Whether cervical EIS is able to detect the changes in epithelial structure and permeability that modulate this barrier's immunological function, and whether such changes occur in a temporal fashion that can be exploited to predict PTB remains to be determined.

Several human studies have investigated the barrier function of cervical epithelium following insights from microRNA (miRNA) studies. MicroRNAs are short, non-coding sections of single-stranded RNA which regulate gene expression. In one study, asymptomatic high-risk women exhibited significantly higher expression of two miRNAs targeting molecules associated with epithelial barrier function (including the junctional adhesion molecule A (JAM-A)). Accordingly, women experiencing PTB had lower JAM-A levels, particularly those with short CL[164]. These findings offer a hypothetical mechanism for infection-mediated PTB, summarised in Figure 1-4.

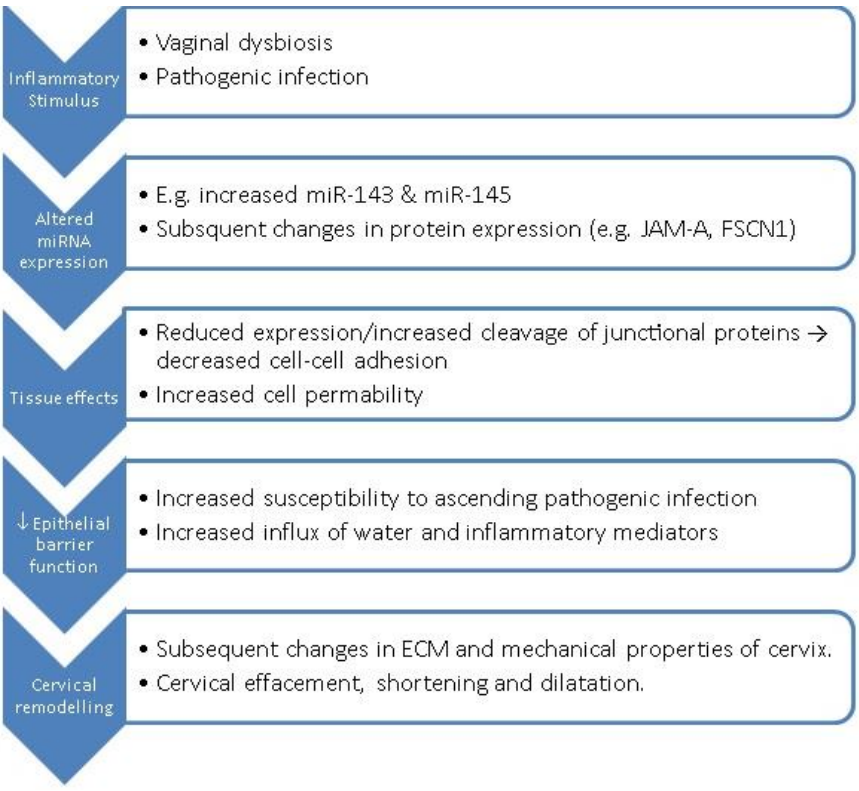


Figure 1-4 Cervical Epithelial Dysfunction and Infection-Mediated Preterm Birth
Potential mechanism by which vaginal dysbiosis or ascending infection might trigger premature cervical remodelling. Abbreviations: miR microRNA; JAM-A Junctional Adhesion Molecule A; FSCN Fascin-1; ECM extracellular matrix.

This hypothesis is further supported by *in vitro* work utilising human cervical cell cultures[162, 163, 165]. This has not only confirmed epithelial dysfunction following upregulation of specific miRNAs but has also illustrated how dysbiotic commensal bacteria (specifically *L. iners* and *Gardnerella vaginalis*) might initiate this pathway[162, 163]. Interestingly, *L. cripatus* may protect the epithelium, ameliorating miRNA upregulation and increases in permeability[163], which raises exciting therapeutic possibilities. Thus far, antibiotic treatment of vaginal dysbiosis has had limited effect on PTB rates[166]. If the protective effects of additive *L. crispatus* can be realised *in vivo* then this may advance clinical care significantly.

An interesting study from the Mahendroo group has provided new insights into the role HA plays in cervical remodelling, with particular reference to the role of the PCM and epithelium. Whilst HA was not a pre-requisite for stromal ripening and mechanical cervical change, marked differences in the epithelia of HA deficient mice were observed. Cells appeared disorganised, with reduced mucus secretion and loss of ordered differentiation from basal to terminally differentiated epithelial cells. Epithelial cell permeability was increased and reduced staining for the tight junction protein occludin was noted. This conferred an increased susceptibility to infection-mediated PTB via vaginal inoculation of live bacteria, suggesting a causal link between impaired epithelial barrier function and PTB due to ascending infection[117]. The interplay between the PCM and epithelial cells in maintaining protection from potential pathogens requires ongoing investigation.

Overall it is clear that epithelial and stromal components have a role to play in cervical remodelling and thus in preterm birth. Understanding the structural changes which take place will facilitate accurate interpretation of the cervical impedance spectra obtained in our clinical studies of pregnant women. It is also clear that mechanisms of cervical change ahead of term and preterm birth are complex, incompletely understood and likely multifactorial. The evidence presented in this section suggests that the determinants of cervical 'competence' are more elaborate than simple clinical definitions would suggest. Much like preterm parturition, cervical function comprises multiple elements, and is subject to numerous influences which may trigger (or themselves be triggered by) cervical remodelling. Figure 1-5 summarises the factors currently thought to contribute to cervical function and potential disruptive influences.

Screening approaches which appreciate this complexity and attempt to address it via multimodal assessment are likely to be more successful in identifying a higher proportion of women destined to deliver preterm.

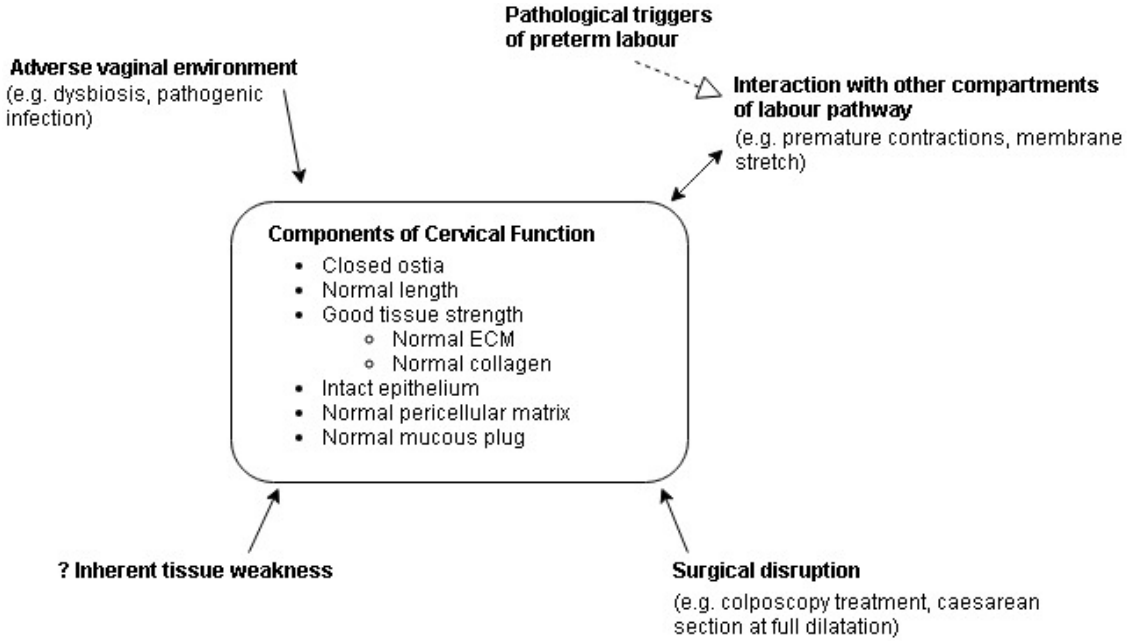


Figure 1-5 Cervical Function – Determinants and Influences
ECM - extracellular matrix

1.4 Prediction of Preterm Birth in Singleton Pregnancies

Numerous tests have been investigated in the hunt for a reliable predictor of PTB. Combining studies for the purposes of systematic review/meta-analysis, or for simple comparison of tests is difficult: study populations vary (high risk vs. low risk, symptomatic vs. asymptomatic, singleton vs. multiple pregnancy); outcome measures differ (e.g. prediction of PTB at varying gestations); and definitions of 'abnormal' test results vary (e.g. threshold for a 'short' cervix on TVUSS)[167]. There are increasing calls to standardise data collection and outcome measures in PTB research to reduce the heterogeneity of evidence available to guide clinical practice[168, 169]. This section will focus on tests predicting PTB in singleton pregnancies with intact membranes (i.e. the group recruited to our EIS studies). Evidence for tests in current widespread use will be reviewed, with asymptomatic low risk

(ALR), asymptomatic high risk (AHR) and symptomatic women considered separately. In addition to conventional tests, proposed novel screening methods will be briefly reviewed, with particular attention paid to techniques which assess cervical structure and function.

1.4.1 Risk factor based 'triage'

Risk factor based screening is generally used to identify women at risk of preterm birth who require additional antenatal surveillance[19, 170]. A previous history of preterm birth (PTB) is the most predictive risk factor[171], with a positive likelihood ratio (LR+) of 4.62 (95% CI 3.28–6.52) and a negative likelihood ratio (LR–) of 0.68 (95% CI 0.56–0.82)[142]. Screening for other factors does not confer additional predictive benefit in women with a known CL at 20 weeks[172]. Risk factor screening may increase the predictive ability of 'multi-marker' screening packages combining history with, for example, serum biomarkers and CL[173-175].

1.4.2 Techniques for screening asymptomatic women

1.4.2.1 Table summarising the evidence base for conventional predictors of preterm birth in asymptomatic singleton pregnancies

Important studies providing evidence for the main conventional predictive tests in each group are summarised in Table 1-1. Where available, systematic reviews and meta-analyses of diagnostic studies are summarised, but large or notable single studies are also included.

Table 1-1 Evidence base for Conventional Tests for Predicting Preterm Birth in Asymptomatic Singleton Pregnancies

Screening test	Patient population	Study	Methodology	Sn	Sp	PPV	NPV	LR+	LR-	Comments
Cervical length	High risk	Crane and Hutchens (2008)[176]	• Systematic review and meta-analysis of 14 studies. • "Increased risk" defined as prior spPTB, uterine anomaly or previous excisional cervical treatment. Subgroup analysis of women with prior PTB only. • Figures here for prediction of SpPTB <35 weeks. • Range of outcomes and test thresholds reported.	NR	NR	NR	NR	4.31	0.68	Asymptomatic women "at increased risk"; CL<25mm <20 weeks (4 studies, 742 women).
				65.4	75.5	33.0	92.0	2.78	0.55	Asymptomatic women "at increased risk"; CL<25mm 20-24 weeks (4 studies, 830 women).
				60.3	78.5	41.4	88.7	2.85	NR	AHR women with prior PTB; CL<25mm <24 weeks (5 studies, 651 women)
				NR	NR	NR	NR	11.30	NR	AHR women with prior PTB; CL<25mm <20 weeks (2 studies, 236 women)
	Unselected or low risk	Iams *et al.* (1996) [177]	• Prospective cohort study of 2915 unselected asymptomatic women (10 centres). • CL measured at 24 + 28 weeks • Outcome: SpPTB <35 weeks • Range of CL thresholds reported.	37.3	92.2	17.8	97.0	NR	NR	Unselected asymptomatic women with CL <25mm at 24 weeks.
				49.4	86.8	11.3	98.0	NR	NR	Unselected asymptomatic women with CL <25mm at 28 weeks.
		Heath *et al.* (1998)[178]	• Prospective cohort study of 2567 unselected asymptomatic women (single centre). • CL measured at 23 weeks • Range of outcomes and test thresholds reported. • Figures here for prediction of spPTB ≤32 weeks. • Incidence of PTB <32 weeks ~2%.	~60	NR	NR	NR	2.7	NR	Unselected asymptomatic women with CL ≤15mm at 23 weeks. 43 patients had CL ≤15mm of whom 22 underwent cerclage.
		Taipale *et al.* (1998)[179]	• Prospective cohort study of 3694 unselected asymptomatic women (single centre).	7.0	100.0	15.0	99.3	NR	NR	Unselected asymptomatic women with CL ≤25mm at 18-22 weeks.

			• CL measured at 18-22 weeks. • Range of outcomes and test thresholds reported. • Figures here for prediction of spPTB <35 weeks. • Incidence of PTB low (2.4% <37 and 0.8% <35 weeks).							
		Hassan *et al.* (2000)[180]	• Retrospective cohort study of 6877 unselected asymptomatic women (single centre). • CL scan performed 14-24 weeks. • TA measurement initially with TVUSS only if CL <30mm. • Range of outcomes and test thresholds reported. • Figures here for prediction of spPTB ≤32 weeks. • PTB rate: 10% <37 & 3.6% <32 weeks.	8.2	99.7	47.6	96.7	NR	NR	Unselected asymptomatic women with CL ≤15mm at 24 weeks.
				10.6	99.4	40.6	96.8	NR	NR	Unselected asymptomatic women with CL ≤20mm at 24 weeks.
				14.7	98.8	31.6	96.9	NR	NR	Unselected asymptomatic women with CL ≤25mm at 24 weeks.
		To *et al. (*2006)[181]	• Prospective cohort study of 39,284 unselected asymptomatic women (7 centres). • CL scan performed at 22-24+6. • Outcome: SpPTB <32 weeks • Test threshold: CL<15mm	Specificity, PPV and NPV not reported. For fixed false positive rates of 5 and 10 % sensitivity was 48 and 55%.						Incorporating maternal risk factors into predictive model improved sensitivity. 368 patients had CL <15mm of whom 129 underwent cerclage.
		Van der Ven *et al.* (2015)[182]	• Prospective cohort study of 11943 low risk asymptomatic women (>200 centres, Dutch registry study); 5710 nulliparous, 6233 multiparous.	9.1	98.7	NR	NR	NR	NR	ALR nulliparous women with CL <30mm at 16-21+6 weeks
				10.8	98.0	NR	NR	NR	NR	ALR multiparous women with CL <30mm at 16-21+6 weeks.

			• Exclusions: prior PTB<34 weeks; symptoms PTL/PPROM; cerclage in situ; fetal anomaly. • CL scan performed 16-21+6 weeks. • Figures here for prediction of spPTB <34 weeks. • Incidence of PTB: 5.3% <37 and 0.7% <32 weeks for nulliparous and : 2.6% <37 and 0.2% <32 weeks for multiparous women. • Incidence of CL<30mm low, 1.8%	NR	NR	NR	NR	40.0	NR	ALR nulliparous women with CL <20mm at 16-21+6 weeks.
				NR	NR	NR	NR	124.0	NR	ALR multiparous women with CL <20mm at 16-21+6 weeks.
		Esplin *et al.* (2017)[21]	• Prospective cohort study of 9410 low risk nulliparous women (8 centres). • CL scans performed at 16-22+6 and 22-30+6 weeks. • Range of outcomes and test thresholds reported. • Figures here for prediction of spPTB ≤32 weeks. • Incidence of spPTB: 5.0% <37 and 0.8% <32 weeks	23.9	97.7	7.4	99.4	10.39	0.78	ALR nulliparous women with CL ≤25mm at 16-22+6 weeks.
				14.9	98.8	8.6	99.3	12.26	0.86	ALR nulliparous women with CL ≤20mm at 16-22+6 weeks.
				52.0	93.0	2.1	99.9	7.39	0.52	ALR nulliparous women with CL ≤25mm at 22-30+6 weeks.
				52.0	96.3	3.9	99.9	13.98	0.50	ALR nulliparous women with CL ≤20mm at 22-30+6 weeks.
		Honest *et al.* (2003)[183]	• Systematic review and meta-analysis of 18 studies. • Pooled studies of AHR and ALR women. • Range of outcomes and test thresholds reported. • Figures here for prediction of spPTB <34 weeks.	NR	NR	NR	NR	6.29	0.79	Asymptomatic women (both ALR and AHR) with CL <25mm <20 weeks (5 studies, 4263 women)
				NR	NR	NR	NR	4.40	0.67	Asymptomatic women (both ALR and AHR) with CL <25mm at 20-24 weeks (3 studies, 3330 women).
Fetal fibronectin	High risk	Abbott *et al.* (2015)[184]	• Prospective masked observational study.	46.5	88.7	23.7	95.6	4.10	0.60	AHR women with FFN ≥50ng/ml at 22-28 weeks.

			• 1448 AHR women (defined by ≥1 previous spPTB, PPROM or late miscarriage; previous cervical surgery or CL<25mm) • FFN measured at 22-28 weeks. • Range of outcomes and test thresholds reported. Figures here for prediction of spPTB <34 weeks.							
				28.7	96.4	37.7	94.7	7.97	0.74	AHR women with FFN ≥200ng/ml at 22-28 weeks.
		Faron *et al.* (2018)[185]	• Systematic review and meta-analysis of 193 studies with 53 subgroup analyses (also included symptomatic women and multiple pregnancies). • Criteria for inclusion in AHR subgroup not clearly defined. • FFN swabs performed at variable gestations. • Range of outcomes reported. • Test threshold: FFN ≥50ng/ml	NR	NR	NR	NR	2.5	0.8	AHR women with FFN ≥50ng/ml, at varied gestations, for spPTB <37 weeks (12 studies, 2469 women). Average prevalence 20.3%
				NR	NR	NR	NR	3.3	0.7	AHR women with FFN ≥50ng/ml, at varied gestations, for spPTB <34 weeks (11 studies, 2409 women). Average prevalence 9.6%
				NR	NR	NR	NR	6.3	0.3	AHR women with FFN ≥50ng/ml, at varied gestations, for spPTB <30 weeks (9 studies, 2841 women). Average prevalence 3.5%
	Low risk	Esplin *et al.* (2017)[21]	• Summary as above (CL section) • Self-obtained vaginal FFN swabs performed at 6-14+6, 16-22+6 and 22-30+6 weeks. • Range of outcomes and test thresholds reported. • Figures here for prediction of spPTB ≤32 weeks. • Incidence of spPTB: 5.0% <37 and 0.8% <32 weeks	15.6	96.0	2.9	99.3	3.87	0.88	ALR nulliparous women with FFN ≥50ng/ml at 16-22+6 weeks
				7.8	98.3	3.4	99.3	4.59	0.94	ALR nulliparous women with FFN ≥200ng/ml at 16-22+6 weeks
				32.1	96.7	3.1	99.8	9.70	0.70	ALR nulliparous women with FFN ≥50ng/ml at 22-30+6 weeks
				21.4	98.8	5.6	99.7	17.92	0.80	ALR nulliparous women with FFN ≥200ng/ml at 22-30+6 weeks

		Faron *et al.* (2018)[185]	• Summary as above • Criteria for inclusion in AHR subgroup not clearly defined. • FFN measured at variable gestations. • Outcome spPTB <27 weeks. • Test threshold: FFN ≥50ng/ml	NR	NR	NR	NR	3.3	0.6	ALR women with FFN ≥50ng/ml, at varied gestations, for spPTB <37 weeks (6 studies, 2806 women). Average prevalence NR.
Combined testing	High risk	Kuhrt *et al.* (2016)[186]	• Unblinded prospective cohort study (5 centres). • 1249 AHR women (defined by ≥1 previous spPTB, PPROM or late miscarriage; previous cervical surgery or CL<25mm • Exclusions: multiple pregnancy, fetal anomaly, blood stained swab or sexual intercourse within preceding 24 hours. • Treated women (cerclage/ progesterone) included. • Serial CL and quantitative FFN swabs performed 2-4 weekly at 22 -30 weeks. • Survival analysis used to generate a predictive model incorporating CL, FFN level and history of prior spPTB/PPROM. • Range of outcomes reported.	54.5	90.4	17.1	98.2	5.7	0.5	Prediction of PTB <30 weeks (prevalence 3.5%, figures generated from validation set)
				71.2	77.7	24.5	96.8	3.6	0.4	Prediction of PTB <34 weeks (prevalence 8.3%, figures generated from validation set)
				74.5	63.5	26.5	93.4	2.0	0.4	Prediction of PTB <37 weeks (prevalence 15.0%, figures generated from validation set)
		Tran *et al.* (2019)[187]	• Prospective cohort study (single centre) of 109 AHR women (defined by defined by ≥1 previous spPTB <37 weeks) • Exclusions: multiple pregnancy, PPROM, vaginal bleeding, recent intercourse or vaginal exam.	63.6	82.1	29.2	95.1	3.6	0.4	Prediction of spPTB<35 weeks by FFN level ≥50ng/ml OR CL<25mm.

			Treated women (cerclage/ progesterone) included. • FFN swab and CL scan performed at 18-24 weeks. Clinicians blinded to FFN results but not CL. • Range of outcomes and test thresholds reported. Figures here for prediction of spPTB <35 weeks (prevalence 10.5%).							
	Low risk	Jwala *et al.* (2016)[188]	• Prospective cohort study (single centre) of 528 ALR women. • Exclusions: multiple pregnancy, PPROM, vaginal bleeding, recent sexual intercourse or vaginal examination. Treated women (progesterone) included. • FFN swab and CL scan performed at 18-24 weeks. Clinicians blinded to FFN results but not CL. • Range of outcomes and test thresholds reported. Figures here for prediction of spPTB <37 weeks (prevalence 6.8%).	61.1	55.1	9.1	95.1	NR	NR	Prediction of spPTB<37 weeks by FFN level ≥5ng/ml OR CL<20mm. This threshold of FFN was identified as optimising sensitivity and specificity for prediction of spPTB<37. However, 45% of women had a FFN level ≥5ng/ml and overall, predictive accuracy compromised by combining tests.
		Esplin *et al.* (2017)[21]	• Summary as above • Self-obtained vaginal FFN swab and CL scan performed at 6-14+6, 16-22+6 & 22-30+6 weeks. • Range of outcomes and test thresholds reported. • Figures here for prediction of spPTB <37 weeks (prevalence 5.0%).	Sensitivity, specificity, NPV, PPV and LRs not reported for combined testing. ROC AUC for CL + FFN was 0.67, which was identical to the AUC for CL scanning alone.						No benefit of combined testing in this cohort.

1.4.2.2 Cervical length

An inverse association between CL and PTB risk has long been recognised[177, 179, 189, 190]. Thresholds used to define a short cervix vary and different cut-offs may be appropriate for different populations (e.g. when considering prophylaxis for low *vs*. high risk women). Large studies have established the distribution of mid-trimester CL measurements during uncomplicated pregnancy[191, 192]. Centiles generated from such work guide interpretation of CL scans at different gestations. A degree of physiological shortening (~1mm per week) is normal as pregnancy progresses, but rapid change, or measurements <25mm before 24 weeks (approximately the 10th centile in mid gestation) are associated with higher rates of PTB[193].

There is also an increasing acknowledgment that cervical shortening represents a "continuum of risk"[194]. Focusing exclusively on whether a measurement is more or less than 25mm, thus utilising CL as a binary variable is likely to limit predictive performance. More sophisticated predictive modelling should allow better estimation of individual risk, especially if the exact CL is combined with other biomarkers[186]. In addition, TVUSS can assess more than just CL: the presence of funnelling at the internal os [142]; intra-amniotic sludge[195]; utero-cervical angle[196]; and the rate of CL change over serial scans[197, 198] may all provide additional prognostic information (see Figure 1-6).

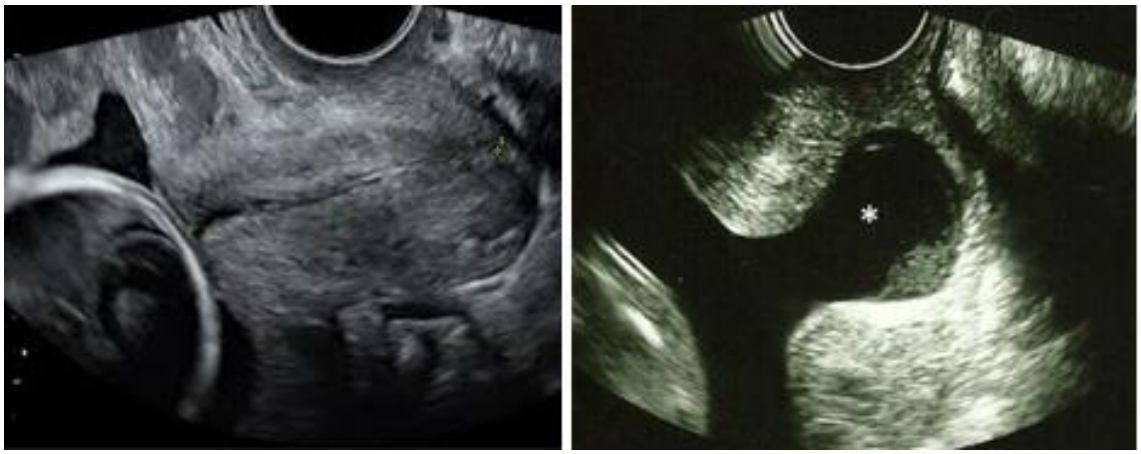


Figure 1-6 Trans-vaginal Ultrasound Images of Cervical Length
The left hand image demonstrates a closed cervix; the right hand image depicts funnelling, cervical shortening and the presence of intra-amniotic sludge.

Asymptomatic high-risk women

There is widespread acceptance that AHR women should be offered serial CL screening, unless history-indicated cerclage is planned[199]. This should identify women with signs of

cervical weakness during the latent period prior to cervical dilatation, so that ultrasound-indicated cerclage can be offered in a timely fashion. Meta-analysis suggests the positive likelihood ratio (LR+) for CL <25mm is 2.9 (95% CI 2.1-3.0) between 20-24 weeks and 11.3 (95% CI 3.6-35.6) before 20 weeks, when predicting spPTB before 35 weeks[176]. Although the combination of short CL and prior PTB seems to identify women at particularly high risk of spPTB, other research suggests the number and gestational age of previous PTBs does not further modify the predictive performance of CL<25mm[200]. However, whilst CL has a well-established role in managing patients with prior PTB, there is room for improvement: the sensitivity of measurements <25mm generated by the aforementioned systematic review was only 60.3%, with a corresponding positive predictive value (PPV) of 41.4%[176].

Unselected, low risk and nulliparous asymptomatic women

Another systematic review[183] has evaluated the performance of CL screening in asymptomatic women in general. Meta-analysis was hampered by methodological heterogeneity amongst the included studies; however, the largest pooled sub-group yielded a LR+ of 6.29 (95% CI 3.3-12.0) and LR- of 0.79 (95% CI 0.65-0.95) for prediction of spPTB before 34 weeks by CL<25mm before 20 weeks. As this systematic review incorporated several studies of exclusively high-risk populations, it is likely that these LRs over-estimate the performance of CL in a truly unselected obstetric cohort.

A more representative evaluation of CL performance in the general antenatal population is provided by large observational studies[177, 179-181, 189, 190]. The largest[181], evaluated prediction of early PTB <32 weeks in 39,284 unselected women attending for anomaly scans. The study was not blinded, and around one third of women with CL<15mm underwent cervical cerclage. However, accepting this potential source of bias, the sensitivity of CL measurement for PTB <32 weeks was only 48% for a fixed false positive rate (FPR) of 5%. Incorporating maternal factors (obstetric history, smoking status, ethnicity, age, BMI and prior cervical surgery) increased detection rates to 57%. Other studies yielded limited sensitivities ranging from 7-60%[177, 179, 180, 190] although variable CL thresholds and outcome measures were used.

Given the increasing prevalence of dedicated PTB clinics, two recent studies are of interest[21, 182]. If HRW are seen separately outwith general screening, then the performance of CL scanning in *exclusively* low risk women should be carefully evaluated. Van der Ven *et al*.[182] measured CL in 11,943 LR women - a measurement ≤30mm identified just 6% of spPTB. Esplin *et al.*[21] screened 9410 LR nulliparous women; in their cohort, CL <25mm at 16-22

weeks had a sensitivity of only 8% for delivery <37 weeks. The explanation for the limited sensitivity of CL scanning in these studies is not clear. The low prevalence of PTB is a contributor – this was ~5% in both studies, which is not unexpected for a low risk population. It is possible that the PTBs within these cohorts were provoked by factors not amenable to detection via CL screening. Alternatively, rapid change in CL prior to PTB may not have been picked up by timing of screening tests. Finally, it is likely that CL measurement most sensitively detects extreme PTBs and, given the low risk nature of the study participants, most early deliveries occurred at moderate to late preterm gestations. Another vital consideration is the incidence of short CL within these populations. Whilst the LR+s yielded by very short CLs were sometimes large, the frequency with which these observations were made was very low, for example a CL <20mm was observed in just 0.3% of LR nulliparous and 0.096% of LR multiparous patients within the Dutch cohort[182]. This renders the numbers needed to screen to prevent one preterm birth impractically large. If found incidentally, a very short CL should not be ignored in this population, but there seems insufficient evidence to mandate routine screening of LR women.

1.4.2.3 Fetal fibronectin estimation

The first use of fetal fibronectin (FFN) as a PTL biomarker occurred in the 1990s[201]. It is widely employed in the clinical assessment of symptomatic women and also for risk assessment in the AHR group[194]. The FFN glycoprotein is usually localised to the chorio-decidual interface, consequently mid-gestation (~18-34 weeks), FFN levels in cervico-vaginal secretions are normally low. Premature membrane activation/separation is associated with a release of the FFN adhesion molecule from the tissue boundary due to mechanical or inflammatory disruption; thus, the detection of FFN at a higher than expected level in vaginal discharge is associated with a higher risk of subsequent PTB[201]. FFN was initially used as a qualitative test (with levels ≥50ng/ml denoting a positive test). More recent work suggests the use of quantitative assays may enhance test performance, particularly with respect to its PPV[184].

Asymptomatic high-risk women

AHR women are the group most likely to benefit from PTB prophylaxis. As such, they have been the subject of particular interest when considering the optimal application of FFN screening. A recent meta-analysis yielded a limited LR+ and LR- for delivery <37 weeks of 2.5 and 0.8[185]. However, these studies utilised one-off qualitative FFN tests, so are less representative of current clinical usage in this patient population (as serial quantitative

measurements are commonly employed in PTB clinics). When prediction of earlier PTB was considered, LR+s improved, reflecting the particular utility of FFN as a short-term predictor of PTB. Although LR+s remain modest (2.8 - 6.3), they are comparable to those of many other tests in current clinical usage, in particular CL scanning. A large observational study[184] (not included within the aforementioned meta-analysis) has specifically examined the diagnostic accuracy of quantitative FFN in asymptomatic HRW. Higher FFN thresholds were associated with higher LR+s. The LR- of a FFN level ≤10ng/ml was 0.37, with a corresponding negative predictive value of 97.3% for delivery <34 weeks. When quantitative and qualitative tests were directly compared via ROC curve analysis, the use of quantification improved predictive accuracy, generating an AUC of 0.78 for delivery <34 weeks, *vs.* an AUC of 0.68 for qualitative testing ($p<0.001$). The authors highlight the ability of negative FFN results to normalise HRW, facilitating a targeted approach to preventative treatment.

Asymptomatic low risk women

FFN does not perform well as a screening test for LRW. The meta-analysis by Faron *et al.*[185] generated a LR+ of 3.3 and LR- of 0.6, suggesting only a slight effect on post-test probability of disease. In addition, 5 of the 6 included studies analysed swabs taken regularly from 24 to 34-36 weeks gestation – a strategy which is unlikely to prove economically viable to screen LRW. The inclusion of swabs taken as late as 36 weeks may also have exaggerated test performance, whilst providing little clinical utility: given the good outcomes of deliveries at 36 weeks, prediction of very late PTB confers limited benefit. Notably, all studies in this review utilised a threshold of FFN ≥50ng/ml for a positive test. However, prospective work has also investigated the use of quantitative FFN testing in LRW[21]. In isolation, FFN measurement at 16-22 weeks was of limited benefit in predicting PTB <37 and <32 weeks, with LR+s/LR-s of 1.85/0.97 and 2.27/0.82 respectively. Employing a higher positive test threshold did not significantly improve performance: ROC curve analysis generated AUCs of 0.52 (<37 weeks) and 0.58 (<32 weeks) at FFN ≥50ng/ml, compared to AUCs of 0.51 and 0.56 at ≥200ng/ml. However, the results should be interpreted in light of the methodology employed – patients provided self-obtained vaginal swabs for fibronectin estimation, whereas samples of CVF taken from the posterior fornix during speculum examination seem to offer superior prediction[185].

1.4.2.4 Combination approaches

The concept of combining predictive tests to maximise screening accuracy in asymptomatic women is not new[174] and HR women attending dedicated PTB clinics often undergo both CL scans and FFN quantification antenatally to aid risk assessment and guide management decisions. However, evidence of the superior performance of this approach has been lacking until recently. The Shennan group have developed an application for use in HR asymptomatic women which incorporates CL, quantitative FFN estimation and obstetric history[186, 202]. They originally reported LR+s of 2.0 - 5.7 and LR-s of 0.4 - 0.5 for prediction of delivery before 30, 34 and 37 weeks, with higher LR+s for delivery within 2 and 4 weeks of testing (33.3 and 15.0)[186]. Validation of a recent application update demonstrated ROC AUCs between 0.75 and 0.90 for the same outcome measures using combined testing[202]. These results compare favourably with single test characteristics reported elsewhere. It is notable that this cohort of women received both prophylactic and preparatory interventions for preterm birth (e.g. cerclage, progesterone, admission), thus the model might perform less well for clinical populations receiving different management regimens. Tran et al.[187] have also evaluated CL and FFN screening in HRW. In their cohort, the addition of FFN improved sensitivity and NPV, at the expense of a slight reduction in specificity and PPV. High NPV is useful in the normalisation of high risk women with reassuring screening results[203], therefore their findings suggest the addition of FFN may be clinically useful. Further work incorporating standardised treatment pathways (dependent on combined test results) is required to prove the benefit of this approach.

Combination screening in low risk women has been the subject of even less research. The largest two studies of CL measurement plus FFN estimation in low risk women do not support the approach in this population[21, 188].

1.4.3 Techniques for screening symptomatic women

Symptomatic women have particular need of accurate predictive tests to guide their treatment. The PPV of a clinical diagnosis of threatened PTL is notoriously poor[204]: in a large RCT of antibiotics for spontaneous PTL 85% of women in threatened PTL were still pregnant 7 days after presentation[205] (regardless of treatment). If predictive testing is not available (or indeed provides a false positive result) significant costs arise, including hospitalisation, tocolytic medication and in utero transfer, not to mention personal costs to the woman and her family. Therefore, it is unsurprising that this group have been the focus of much

research. This section aims to summarise those tests currently accepted for widespread use and some novel approaches which might be useful in future.

1.4.3.1 Table summarising the evidence base for conventional predictors of preterm birth in symptomatic singleton pregnancies

As for Table 1-1, the data synthesis which follows in Table 1-2 aims to summarise the results of available systematic reviews and meta-analyses, but also includes notable stand-alone studies investigating less researched topics (e.g. the application of quantitative FFN thresholds or combination testing in symptomatic women).

Table 1-2 Evidence base for Conventional Tests for Predicting Preterm Birth in Symptomatic Singleton Pregnancies

Screening test	Patient population	• Study	Methodology	Sn	Sp	PPV	NPV	LR+	LR-
Cervical length	Honest *et al.* (2003)[183]	• Systematic review and meta-analysis of 9 studies of symptomatic women with singleton pregnancies. • Range of outcomes and test thresholds reported.	NR	NR	NR	NR	2.15	0.32	Pooled LRs for spPTB <34 weeks in symptomatic women with CL <30mm >20 weeks (2 studies)
			NR	NR	NR	NR	1.98	0.28	Pooled LRs for spPTB <37 weeks in symptomatic women with CL <30mm >20 weeks (3 studies)
	Honest *et al.* (2009) [142, 171]	• HTA systematic review and meta-analysis examining 319 studies of 22 different tests: 19 studies (2849 women) assessing CL. • Range of outcomes and test thresholds reported (birth within 48 hours, 7 days, before 34 and 37 weeks)	NR	NR	NR	NR	1.86	0.30	Pooled LRs for spPTB <34 weeks in symptomatic women with CL <30mm (4 studies)
			NR	NR	NR	NR	2.29	0.29	Pooled LRs for spPTB <37 weeks in symptomatic women with CL <30mm (3 studies)
			NR	NR	NR	NR	8.61	0.03	Pooled LRs for spPTB within 7 days in symptomatic women with CL <15mm (6 studies)
	Sotiriadis *et al.* (2010) [206]	• Systematic review and meta-analysis of 28 studies. • Range of outcomes (summarised) and test thresholds reported.	71.1	86.6	28.9	97.5	5.92	0.35	Pooled statistics for delivery within 48 hours if CL<15mm (3 studies, 1266 women)
			59.9	90.5	44.0	94.8	5.71	0.51	Pooled statistics for delivery within 7 days if CL<15mm (6 studies, 1781 women). Marked heterogeneity. Increased sensitivity at expense of specificity at higher CLs.
			46.2	93.7	62.0	88.7	4.31	0.63	Pooled statistics for delivery before 34 weeks if CL<15mm (4 studies, 429 women)

	Boots *et al.* (2014)[173]	• Systematic review and meta-analysis of CL, FFN and fetal breathing movements to predict PTB in symptomatic women. • 24 studies of CL (5112 women) included. • Focused on short term prediction (delivery within 48 hours and 7 days). • Variety of test thresholds considered (most data for 15mm cut off)	77.0	88.0	NR	NR	6.4	0.26	Pooled statistics for delivery within 48 hours if CL<15mm (9 studies)
			74.0	89.0	NR	NR	6.8	0.29	Pooled statistics for delivery within 7 days if CL<15mm (24 studies)
	Berghella *et al.* (2017)[207]	• Systematic review and meta-analysis investigating whether clinician knowledge of CL prevents PTB in symptomatic women. • 3 RCTs of 287 singleton pregnancies included.	n/a	n/a	n/a	n/a	n/a	n/a	Clinician knowledge of CL was associated with lower risk of PTB <37 weeks: RR 0.64 (95% CI, 0.44–0.94).
	Berghella and Saccone (2019)[208]	• Cochrane systematic review and meta-analysis investigating whether clinician knowledge of CL prevents PTB in a range of patient populations. • 7 RCTs of 923 women of which 4 studied symptomatic women with singleton pregnancies.	n/a	n/a	n/a	n/a	n/a	n/a	Clinician knowledge of CL was associated with lower risk of PTB <37 weeks in symptomatic singletons: RR 0.59, 95% CI 0.26 to 1.32; (2 studies, 242 women) with birth delayed by ~4 days. Evidence quality noted to be very low.
Fetal fibronectin	Honest *et al.* (2002)[209]	• Systematic review and meta-analysis examining qualitative FFN use in symptomatic and asymptomatic women.	NR	NR	NR	NR	3.64	0.32	Pooled statistics for spPTB<34 weeks if FFN positive (8 studies)
			NR	NR	NR	NR	3.27	0.48	Pooled statistics for spPTB <37 weeks if FFN positive (33 studies)

		• 40 studies of symptomatic women included. • SpPTB within 7-10 days of testing and before 34 weeks assessed. • Qualitative FFN test used (positive/negative with 50ng/ml threshold).	NR	NR	NR	NR	5.42	0.25	Pooled statistics for delivery within 7-10 days if FFN positive (17 studies)
	Honest *et al.* (2009) [142, 171]	• HTA systematic review and meta-analysis examining 319 studies of 22 different tests: 40 studies (4209 women) assessing qualitative FFN in symptomatic women. • Range of outcomes reported.	NR	NR	NR	NR	3.53	0.24	Pooled statistics for delivery within 7 days if FFN positive (18 studies)
			NR	NR	NR	NR	3.98	0.33	Pooled statistics for delivery before 34 weeks if FFN positive (8 studies)
			NR	NR	NR	NR	7.97	0.13	Pooled statistics for delivery before 37 weeks if FFN positive (31 studies)
	Deshpande *et al.* (2013)[210]	• Updated HTA systematic review and meta-analysis examining FFN specifically. • 54 studies using qualitative FFN assessment of symptomatic women included. • Reported pooled sensitivities and specificities rather than LRs.	75.8	81.1	NR	NR	NR	NR	Pooled statistics for delivery within 7-10 days if FFN positive (12 studies of singletons)
			76.4	82.4	NR	NR	NR	NR	Pooled statistics for delivery before 34 weeks if FFN positive (9 studies of singletons)
			66.4	85.6	NR	NR	NR	NR	Pooled statistics for delivery before 37 weeks if FFN positive (16 studies of singletons)
	Boots *et al.* (2014)[173]	• Systematic review and meta-analysis of CL, FFN and fetal breathing movements to predict PTB in symptomatic women. • 38 studies of FFN (6383 women) included. • Qualitative (positive/negative) FFN employed.	62.0	81.0	NR	NR	3.3	0.47	Pooled statistics for delivery within 48 hours if FFN positive (4 studies)
			75.0	79.0	NR	NR	3.6	0.31	Pooled statistics for delivery within 7 days if FFN positive (37 studies)

		• Focused on short term prediction (delivery within 48 hours and 7 days).							
	Faron *et al.* (2018)[185]	• Systematic review and meta-analysis of 193 studies of FFN prediction in varied populations. • Women with and without symptoms, and with singleton and multiple pregnancies studied. • Qualitative and quantitative FFN used in different studies, with varied regimens (single vs serial testing). • Wide variety of outcome measures considered (delivery before 37, 35, 34 and 32 weeks and within 48 hours and 7, 10, 14, 21 and 28 days of testing)	NR	NR	NR	NR	3.9	0.5	Pooled statistics for delivery before 34 weeks if FFN positive (32 studies, 4848 women)
			NR	NR	NR	NR	3.6	0.6	Pooled statistics for delivery before 37 weeks if FFN positive (68 studies, 9139 women)
			NR	NR	NR	NR	4.8	0.5	Pooled statistics for delivery within 48 hours if FFN positive (7 studies, 1064 women)
			NR	NR	NR	NR	3.8	0.4	Pooled statistics for delivery within 7 days if FFN positive (54 studies, 11255 women)
	Melchor *et al.* (2018)[211]	• Systematic review and meta-analysis comparing prediction of spPTB in symptomatic women by FFN, PAMG-1 and phILGFBP-1. • 40 studies of 7431 women included for qualitative FFN. • Meta-analysis focused on prediction of spPTB within 7 days.	58.0	84.0	34.0	93.0	3.63	0.50	Pooled statistics for delivery within 7 days if FFN positive
	Abbott et al. (2013)[212]	• Prospective blinded cohort study investigating use of quantitative	82.4 90.0	59.3 64.0	98.2 98.5	10.9 19.4	2.02 2.52	0.30 0.16	FFN ≥10 ng/ml to predict delivery within 14 days. FFN ≥10 ng/ml to predict delivery before 34 weeks.

		FFN to predict spPTB in symptomatic women (n=300). • Different FFN cut-offs compared.	76.5 70.0	81.1 85.7	98.3 96.8	19.7 31.8	4.04 4.90	0.29 0.35	FFN ≥50 ng/ml to predict delivery within 14 days. FFN ≥50 ng/ml to predict delivery before 34 weeks.
			58.8 55.0	93.9 96.7	97.4 95.8	37.0 61.1	9.69 16.5	0.44 0.47	FFN ≥200 ng/ml to predict delivery within 14 days. FFN ≥200 ng/ml to predict delivery before 34 weeks.
			35.3 45.0	97.5 98.6	96.1 95.0	46.2 75.0	14.12 31.5	0.66 0.56	FFN ≥500 ng/m FFN threshold 10 ng/ml to predict delivery within 14 days. FFN ≥500 ng/ml to predict delivery before 34 weeks.
	Radford et al. (2018)[213]	• Prospective observational cohort study investigating use of quantitative FFN to predict spPTB in symptomatic women (n=120). • Three different FFN cut-offs compared. • Delivery within 14 days and before 37 weeks assessed.	100.0 50.0	69.0 68.0	13.89 22.22	100.0 88.41	3.22 1.59	NR NR	FFN ≥10 ng/ml to predict delivery within 14 days. FFN ≥10 ng/ml to predict delivery before 37 weeks.
			60.0 25.0	92.0 92.0	27.27 36.36	97.87 87.23	7.50 3.18	NR NR	FFN ≥50 ng/ml to predict delivery within 14 days. FFN ≥50 ng/ml to predict delivery before 37 weeks.
			60.0 18.75	97.0 96.63	50.0 50.0	97.98 86.87	20.0 5.56	NR NR	FFN ≥200 ng/ml to predict delivery within 14 days. FFN ≥200 ng/ml to predict delivery before 37 weeks.
	Nguyen et al. (2019)[214]	• Retrospective observational cohort study investigating use of quantitative FFN to predict spPTB in symptomatic women (n=380). • Three different FFN cut-offs compared.	87.5 81.8 80.0	68.8 68.8 68.5	5.7 7.4 9.8	99.6 99.2 98.8	2.8 2.62 2.54	0.18 0.26 0.29	FFN ≥10 ng/ml to predict delivery within 48 hours. FFN ≥10 ng/ml to predict delivery within 14 days. FFN ≥10 ng/ml to predict delivery before 34 weeks.
		Delivery within 48 hours, 14 days and before 34 weeks assessed.	62.5	89.0	10.9	99.1	5.70	0.42	FFN ≥50 ng/ml to predict delivery within 48 hours.

			63.6 66.7	88.7 89.2	14.6 20.8	98.8 98.4	5.62 6.18	0.41 0.37	FFN ≥50 ng/ml to predict delivery within 14 days. FFN ≥50 ng/ml to predict delivery before 34 weeks.
			50.0 54.6 46.7	97.0 97.8 97.7	26.7 42.9 46.7	99.2 98.6 97.7	16.9 24.7 20.53	0.52 0.47 0.55	FFN ≥200 ng/ml to predict delivery within 48 hours. FFN ≥200 ng/ml to predict delivery within 14 days. FFN ≥200 ng/ml to predict delivery before 34 weeks.
	Varley-Campbell *et al.* (2019)	• HTA systematic review and meta-analysis comparing prediction of spPTB by qualitative FFN with quantitative FFN (i.e. threholds other than 50ng/ml), ILGFBP-1 and PAMG-1. • 20 studies included. • Limited evidence detected to allow direct comparison of biomarker accuracy (2 studies)	93.8 to 95.7	32.3 to 42.3	NR	NR	NR	NR	Only 2 eligible studies of quantitative FFN identified therefore meta-analysis not performed (ranges of sensitivity and specificity provided). Prediction of PTB within 7 days using FFN threshold 10ng/ml
			70.8 to 71.0	78.6 to 83.6	NR	NR	NR	NR	Prediction of PTB within 7 days using FFN threshold 200ng/ml
			29.2 to 42.0	94.3 to 95.7	NR	NR	NR	NR	Prediction of PTB within 7 days using FFN threshold 500ng/ml
	Berghella *et al.* (2016)[215]	• Systematic review and meta-analysis investigating whether clinician knowledge of FFN prevents PTB in symptomatic women. • 6 RCTs of 546 singleton pregnancies included.	n/a	n/a	n/a	n/a	n/a	n/a	Clinician knowledge of FFN level was not associated with lower incidence of PTB at any gestation threshold but was associated with higher healthcare costs.
	Berghella and Saccone (2019)[216]	• Cochrane systematic review and meta-analysis investigating whether clinician knowledge of	n/a	n/a	n/a	n/a	n/a	n/a	Management based on knowledge of FFN results was associated with a lower rate of PTB<37 weeks (20.7% vs 29.2%

		FFN prevents PTB in symptomatic women. • 6 RCTs of 546 singleton pregnancies included.							with RR 0.72 but 95% CI 0.52 to 1.01). No evidence of lower rates of earlier PTB/other neonatal outcomes. Evidence quality low.
ILGFBP-1	Conde-Agudelo and Romero (2016)[217]	• Systematic review and meta-analysis of 43 studies (10293 women) of ILGFBP-1 prediction in varied populations. • Women with and without symptoms, and with singleton and multiple pregnancies studied – statistics summarised here for symptomatic women with singleton pregnancies. • Varied outcome measures reported (PTB <34 and 37 weeks and within 48 hours, 7 and 14 days of testing). • Significant heterogeneity noted.	85.0	67.0	NR	NR	2.6	0.2	Pooled statistics for delivery within 48 hours if ILGFBP positive (3 studies, 406 women).
			68.0	78.0	NR	NR	3.1	0.4	Pooled statistics for delivery within 7 days if ILGFBP positive (14 studies, 1668 women).
			68.0	81.0	NR	NR	3.5	0.4	Pooled statistics for delivery within 14 days if ILGFBP positive (5 studies, 521 women).
			62.0	78.0	NR	NR	2.9	0.5	Pooled statistics for delivery<34 weeks if ILGFBP positive (6 studies, 911 women).
			65.0	79.0	NR	NR	3.1	0.4	Pooled statistics for delivery<37 weeks if ILGFBP positive (12 studies, 1010 women).
	Melchor *et al.* (2018)[211]	• Systematic review and meta-analysis comparing prediction of spPTB in symptomatic women by FFN, PAMG-1 and phILGFBP-1. • 22 studies of 3192 women included for phILGFBP-1. • Meta-analysis focused on prediction of spPTB within 7 days.	93.0	76.0	35.0	99.0	3.8	0.09	Pooled statistics for delivery within 7 days if ILGFBP positive.
	Varley-Campbell *et al.* (2019) [218]	• HTA systematic review and meta-analysis comparing prediction of	77.0	81.0	NR	NR	NR	NR	Pooled statistics for delivery within 7 days if ILGFBP positive (16 studies).

			spPTB by qualitative FFN with quantitative FFN (i.e. threholds other than 50ng/ml), ILGFBP-1 and PAMG-1. • 20 studies included. • Limited evidence detected to allow direct comparison of biomarker accuracy (2 studies)	87.0	73.0	NR	NR	NR	NR	Pooled statistics for delivery within 48 hours if ILGFBP positive (6 studies).
PAMG-1		Melchor *et al.* (2018)[211]	• Systematic review and meta-analysis comparing prediction of spPTB in symptomatic women by FFN, PAMG-1 and phILGFBP-1. • 14 studies of 2278 women included for PAMG-1. • Meta-analysis focused on prediction of spPTB within 7 days.	76.0	97.0	76.0	97.0	22.51	0.24	Pooled statistics for delivery within 7 days if ILGFBP positive.
		Varley-Campbell *et al.* (2019)[218]	• HTA systematic review and meta-analysis comparing prediction of spPTB by qualitative FFN with quantitative FFN (i.e. threholds other than 50ng/ml), ILGFBP-1 and PAMG-1. • 20 studies included. • Limited evidence detected to allow direct comparison of biomarker accuracy (2 studies)	83.0	95.0	NR	NR	NR	NR	Pooled statistics for delivery within 7 days if PAMG-1 positive (4 studies).
Combined testing		DeFranco *et al.* (2013)[90]	• Systematic review and meta-analysis assessing prediction of spPTB in symptomatic women by combined CL and FFN testing	71.4	96.8	45.4	98.9	22.0	0.3	Pooled statistics for delivery within 7 days (2 studies, 192 women).
				33.3	86.0	10.3	96.4	2.4	0.8	Pooled statistics for delivery within 14 days (2 studies, 203 women).

			• 9 studies of 1194 women included. Mixture of singleton/twin pregnancies included although low numbers of twins noted. Varied CL cut offs used; all studies used FFN≥50ng/ml to define positive test.	53.8	84.3	36.8	91.5	3.4	0.5	Pooled statistics for delivery<34 weeks (3 studies, 270 women).
				36.8	83.0	49.4	74.4	2.2	0.8	Pooled statistics for delivery<37 weeks (4 studies, 346 women).
		Bruijn *et al.* (2016) [219]	• Post hoc analysis of frozen cervico-vaginal fluid samples from a multicentre cohort study of 714 women with threatened PTL, although only samples from those with CL<30mm processed (n=350). • Qualitative FFN, quantitative FFN, CL and combined approaches compared. • Focused on prediction of spPTB within 7 days.	NR	NR	NR	NR	NR	NR	Complex statistical analysis focused on stratification into lower and higher risk of delivery within 7 days. Predictive accuracy only compared by means of ROC AUCs: Quantitative FFN AUC=0.85; CL + qualitative FFN AUC =0.89; CL + quantitative FFN AUC=0.91.
		Kumari *et al.* (2017) [220]	• Prospective observational cohort study investigating use of CL plus ILGFBP-1 to predict spPTB in symptomatic women (n=98). • CL <25mm defined short cervix. • Varied outcome measures assessed. • Improved AUCs for all outcomes by employing combined rather than individual tests.	72.0	74.0	29.0	94.0	2.74	0.38	Prediction of spPTB within 48 hours.
				70.0	79.61	33.35	94.63	3.34	0.53	Prediction of spPTB within 7 days.
				64.0	81.4	33.9	93.78	3.43	0.44	Prediction of spPTB within 14 days.
				80.0	79.7	37.06	96.38	3.94	0.25	Prediction of spPTB before 34 weeks.
				61.0	81.0	32.0	93.0	3.11	0.49	Prediction of spPTB before 37 weeks.
		Fuchs *et al.* (2017) [221]	• Prospective observational cohort study investigating use of CL plus ILGFBP-1 to predict spPTB in	92.9	51.8	14.0	98.9	1.9	0.2	Prediction of spPTB within 7 days.
				89.5	52.8	18.3	97.7	1.9	0.2	Prediction of spPTB within 14 days.

			symptomatic women with CL <25mm (n=180). Clinicians and women blinded to ILGFBP-1 result. • Short CL in this cohort (with already shortened CL) defined as <15mm. • Varied outcome measures assessed.	87.0	53.8	21.7	96.6	1.9	0.3	Prediction of spPTB before 34 weeks.
				76.5	58.7	42.9	86.0	1.9	0.4	Prediction of spPTB before 37 weeks. Overall limited predictive performance of ILGFBP-1 and LRs of combined testing not superior to CL alone.
		Levine *et al.* (2019) [222]	• Prospective blinded observational cohort study investigating use of CL plus FFN to predict PTB in symptomatic singleton pregnancies (n=439). • Short CL defined as <20mm in nulliparous women and <25 in multiparous women. • Outcome: spPTB before 37 weeks or PPROM resulting in PTB before 37 weeks.	72.7	77.6	21.1	97.2	NR	NR	Positive screen = positive FFN ≥20ng/ml OR CL<20mm (145 nulliparous women) Predictive accuracy similar to CL and FFN alone.
				36.4	NR	44.4	NR	NR	NR	Positive screen = positive FFN ≥20ng/ml AND CL<20mm. Higher PPV but this combination of test results rarely occurred and difference in PPV was not significant to that for CL and FFN alone.

1.4.3.2 Cervical length

In the UK, the National Institute for Health and Care Excellence (NICE) recommend that women with PTL symptoms ≥30 weeks undergo CL scanning (when available) in preference to other predictive tests. Their economic analysis found that screening at lower gestational ages was not cost effective. However, no economic model can account for the full complexity/heterogeneity of clinical presentations, particularly when factors such as neonatal intensive care unit (NICU) bed capacity may be an issue. Certainly, the literature suggests predictive benefit of CL measurement at a range of gestations. NICE did not perform formal meta-analysis but noted that 8 studies of 1614 women produced LR+s ranging from 4.3 to 20.0 and LR-s from 0.03 to 0.77 for the prediction of delivery within 7 days of a CL ≤15mm. CLs ≥30mm (3 studies, 712 women) yielded LR-s from 0.15 – 0.23. The LR ranges are rather wide (although most suggested at least moderate positive predictive benefit with LR+>5), a fact also noted by three systematic reviews and meta-analyses conducted over the last 15 years[173, 183, 206]. The source of variation in predictive test performance between the individual studies was not easily explained by methodological variation or threshold effects, and in view of this, the meta-analysis results should be viewed with some caution. Nevertheless, the three studies produced summary LR+s ranging from 5.7 to 12.8 for delivery <7 days with Boots *et al.* and Sotiriadis *et al.* using a threshold of 15mm and Honest *et al.* using 20mm.

Further research has considered the impact of CL scanning on outcome in symptomatic women. Following a 2013 Cochrane review which suggested a non-significant trend towards lower PTB rates when clinicians knew the CL result[167], an individual patient data meta-analysis of trials randomising to knowledge/ concealment of CL result was conducted in 2017[207]. Despite small numbers, the control group experienced more PTB than those randomised to screening with knowledge of results. RCTs incorporating standardised management protocols dependent on CL result suggest TVUSS may also reduce unnecessary treatment of PTL[223, 224].

1.4.3.3 Fetal fibronectin

Qualitative FFN testing has a modest ability to rule out PTB in women presenting with symptoms of threatened PTL: meta-analyses suggest pooled sensitivity and specificity of 77 and 83% for delivery within 7-10 days[210] and summary LR+ of 5.42 and LR- of 0.25. The Deshpande *et al.* HTA economic analysis[210] suggests implementation of FFN screening in

symptomatic women is associated with a small reduction in costs, but other data contradicts this[215, 225]. It is likely that a multitude of factors influence clinician's interpretation and response to predictive test results including gestational age of the patient, and availability of tertiary neonatal support. Such behaviour is difficult to account for by modelling and may partly explain the conflicting cost effectiveness data.

Quantitative FFN estimation has also been used to assess symptomatic women and may allow more nuanced assessment of risk, given the continuous nature of FFN levels. Use of varying thresholds can be tailored to optimise NPV or PPV depending on the clinical situation and pre-test probability of PTL[194]. The original report in this cohort noted an NPV of 98.2% if FFN levels were ≤10ng/ml and a PPV of 75% when levels reached ≥500ng/ml (for delivery <34 weeks)[212]. Whilst subsequent cohort studies have again reported enhanced discrimination of LR and HR patients by the quantitative test[213, 214], it is notable that it is the most expensive of the currently available biomarker tests for PTL[226] and cost effectiveness is far from certain. A recent NIHR Health Technology Assessment[218] suggests possible cost savings by use of higher test thresholds, but at the expense of a slight reduction in infant/maternal Quality Adjusted Life Years (QALYs) (£25,209 and £17,025 per QALY loss for 200 and 500 ng/ml cut offs respectively compared to 50ng/ml). This compared to Incremental Cost Effectiveness Ratios (ICERs) of £56,030 per QALY loss for Phosphorylated insulin-like growth factor binding protein 1 (phILGFBP-1) and £81,922 per QALY loss for Placental alpha macroglobulin-1 (PAMG-1), although the authors noted a high degree of uncertainty regarding test accuracy, particularly for the alternative biomarkers.

1.4.3.4 Phosphorylated Insulin-like Growth Factor Binding Protein 1

Phosphorylated insulin-like growth factor binding protein 1 (phILGFBP-1), is secreted by decidual cells[201, 227]. Similar to FFN, it is normally absent from cervicovaginal secretions in the middle portion of pregnancy, but disturbance of the maternal-fetal interface during activation of parturition pathways leads to premature protein release. It was first utilised as a biomarker for PTB in 2001[228, 229] and was proposed to have several advantages over FFN: it is unaffected by the presence of semen, urine or lubricating jelly in the vagina[227]; testing costs are lower (£7-10 vs. £50); and processing times are quicker[230]. However, recent meta-analysis showed limited predictive performance in symptomatic women[217]. PhILGFBP-1 performed best when identifying those unlikely to deliver within 48 hours (LR- 0.2, 95% CI 0.1 – 0.5) but values for other outcomes (delivery within 7 and 14 days of testing and before

34 and 37 weeks) were imperfect at 0.3 to 0.5. LR +s were also consistently modest with values ranging from 2.6 to 3.1.

1.4.3.5 Placental alpha macroglobulin 1

Placental alpha macroglobulin-1 (PAMG-1) is another glycoprotein molecule originating from the decidua and was originally investigated as a marker of premature membrane rupture due to its high level in amniotic fluid[231]. Subsequent studies have employed it as a biomarker for PTB in women presenting with threatened PTL[232-238]. A recent comparative systematic review and meta-analysis demonstrated significantly improved positive prediction of delivery within in 7 days by PAMG-1 when compared to FFN and ILGFBP-1[211]. However, it is notable that 54% of studies included within this meta-analysis fulfilled ≤2 quality criteria. Moreover, recent NICE diagnostics guidance concluded that there was insufficient evidence to recommend routine adoption of FFN, phILGFBP-1 or PAMG-1 detection for PTL diagnosis, predominantly due to wide variation in accuracy estimates between studies; limited detail regarding delivery outcomes; heterogeneity of study populations; and incomplete information regarding PTL management[226]. The HTA review mentioned above[218] reached similar conclusions, and the results of ongoing large observational studies are awaited.

1.4.3.6 Combined approaches

As in asymptomatic cohorts, combining predictive tests may offer enhanced accuracy in the assessment of symptomatic women. Several studies have described improved predictive performance when CL and FFN are used in combination[90, 219, 222, 239], whereas others show limited or no additional benefit[222, 240]. It is plausible that the most cost effective approach may employ contingent testing, where FFN estimation is reserved for women with an 'equivocal' CL of 15-30mm[241]; indeed, some authors have suggested this might reduce unnecessary admissions/treatment by ~10%[219]. A multicentre UK study is currently assessing the impact of multi-modality testing on subsequent rates of inappropriate management – this protocol incorporates the use of a validated decision-making aid combining clinical history, symptoms, CL and quantitative FFN results[242].

Other combinations of predictive tests have been relatively less studied in symptomatic women. With respect to the combination of CL and phILGFBP-1, two prospective cohort studies report opposing results; Kumari *et al.* describe improved prediction with an AUC of 0.83 for combined testing vs. 0.75 and 0.79 for phILGFBP-1 and CL alone[220]; conversely,

Fuchs *et al.* show no benefit of combined testing[221]. Further research is necessary to clarify this situation, although the qualitative nature of current phILGFBP-1 assays may well result in inferior performance when compared to quantitative FFN.

1.4.4 Other biomarkers

A vast number of biomarkers have been evaluated as PTB predictors. A recent umbrella systematic review (SR)[243] identified 21,614 references, including 542 individual SRs! Outwith FFN, other biomarkers with fair predictive performance included maternal serum IL-6, alpha fetoprotein and C-reactive protein. Two earlier SRs had failed to identify any effective single maternal biomarkers for PTB despite similarly numerous references[39, 244]. Furthermore, a review in 2017 conducted by the Preterm Birth International Collaborative (PREBIC) looked specifically at biomarkers identified in studies employing multiplex assays, in an attempt to establish whether multiple markers improve predictive accuracy[245]. Even within this relatively smaller analysis of ten studies there was considerable heterogeneity due to variable study populations, sample type (amniotic fluid, CVF, maternal serum etc.) assay and analytic techniques, therefore meta-analysis was not performed. The two PTB related proteins identified in ≥3 studies were RANTES (Regulated on Activation Normal T Expressed and Secreted) and IL-10. Further evaluation of the markers identified by these reviews is required to validate their utility.

1.4.5 Other cervical techniques

Assessment of cervical structure and function during pregnancy is not limited to cervical length measurement. In addition to EIS, a variety of novel methods have been used to interrogate the cervix, with varying success. None have yet been adopted in routine clinical practice, but the evidence supporting those which have been evaluated via human studies will be briefly reviewed here. Many tests have been evaluated in mixed populations of symptomatic and asymptomatic women – to avoid repetition an overall synthesis will be provided.

1.4.5.1 Ultrasonic techniques

These include semi-quantitative (strain) elastography[246-252]; shear wave speed elastography[253-255] (SWSE); measurement of the cervical consistency index[256-258] (CCI); acoustic attenuation[259, 260]; assessment of cervical gland area[261-267] (CGA); and measurement of the anterior cervical angle (ACA) [196, 268-271]. Both strain elastography[272] and acoustic

attenuation[272, 273] use analytic techniques which assume tissue homogeneity (which is not true of the cervix[274]) which is likely to limit their predictive potential and broader application. SWSE can be used in combination with CL scanning to identify cervices which are both short and soft but it requires a specialised TV probe and sensitivity of the technique to predict PTB was poor (19-33%) in the largest available study[254].

The techniques which use conventional TV probes (CCI, CGA and ACA) are intuitively appealing as they could most easily be incorporated into current screening programmes without the need for specialist software. CCI measurement involves taking antero-posterior measurements from a longitudinal section of the cervix during minimal and maximal compression by a TV probe. The ratio between measurements is the CCI which shows a linear association with gestational age. Three studies including 1915 women have noted significantly lower CCI in women destined to deliver preterm[256-258]. Whilst this technique appears promising, validation of predictive performance in larger groups and repeatability between centres is required. Interestingly CCI performed less well in a high risk population, although results may well have been confounded by the inclusion of patients with prior colposcopic treatment and cerclage[258].

CGA assessment requires visualisation of the area around the cervical canal during a conventional CL scan. If hyper or hypoechogencity is present, the CGA is visible. Absence of the CGA is associated with PTB[261, 262, 264-267] but sensitivity estimates are currently too low to merit use for asymptomatic screening (with estimates of 2.3-39% across 3 studies of 3974 women[261, 266, 267]).

ACA measurement is also obtained from a longitudinal section of the cervix. Linear calipers are placed along the anterior lower uterine segment up to the internal os, and along the cervical canal - the anterior angle between the two represents the ACA. Multiple studies have noted higher ACA in women who subsequently experience PTB[196, 268-271] however there is considerable heterogeneity, with variation in study population, outcome measures reported, and thresholds used to define 'higher' ACA. In a recent systematic review, Dasakalakis *et al.*[275] were unable to perform meta-analysis, despite identifying 11 studies, including 6 evaluating test performance in unselected asymptomatic women attending for universal CL screening. The largest individual study[196], demonstrated reasonable sensitivity using ACA>95^{o} for PTB <37 weeks and ACA>105^{o} for PTB <34 weeks (80 and 81%). It is notable that average ACA in both term and preterm groups varied significantly between

studies – this may reflect differing gestations at measurement and ACA appears to increase towards term. Ultimately further prospective studies are required to define the normal distribution of ACA measurements by gestational age and the patterns observed in women who deliver preterm.

1.4.5.2 Magnetic Resonance Imaging

Magnetic resonance imaging (MRI) has been used to evaluate normal three-dimensional anatomy of the cervix with advancing gestation (to inform mechanical modelling research)[276] but has also been investigated as a predictive tool for PTB. Two studies have been published using MRI to assess symptomatic[277] and HR asymptomatic[278] women respectively. Both noted stromal differences in women destined to deliver preterm, however predictive performance was modest in the symptomatic study, and methodological concerns (recruitment of women with antepartum haemorrhage/fetal anomaly only) hamper the generalisability of the asymptomatic study. Overall, there has been no proven benefit of MRI in PTB screening and given the lower cost and clinical utility of ultrasound, this seems unlikely to change unless modified techniques such as diffusion-tensor imaging[106, 119, 279] are shown to confer predictive benefit.

1.4.5.3 Spectroscopic techniques

A variety of spectroscopic techniques have been proposed for cervical investigation in pregnancy. Of these, only EIS has currently been used in a clinical study of PTB prediction[25] (see section 1.5.3). However, this is a highly active research area, and two other putative spectroscopic tools for PTB screening, Raman Spectroscopy[140, 154] and Light Induced Fluorescence[156, 157, 273 155, 280] may prove useful in future.

Other techniques used to assess the biomechanical properties of the cervix, include back scattered power loss measurement[281]; external mechanical compression of the cervix; use of endocervical balloons and other instruments[282] to assess canal compliance; and aspiration of ectocervical tissue using a specialised suction device[283]. These methods have either not been assessed directly in PTB prediction or have limited utility (especially for use in HR women) due to concerns about the invasiveness of the technique. Several comprehensive reviews summarise other putative methods for biomechanical assessment of the cervix and include further information on techniques used *ex vivo*, and in term and non-pregnant cervices which may in future become relevant to PTB screening[273, 284, 285].

1.5 Electrical Impedance Spectroscopy

The processes underlying preterm birth (particularly those occurring within the cervix) and existing predictive technologies have now been reviewed. The rationale for developing improved tests targeting the latent period of cervical change which precedes preterm birth should thus be clear. In this section the underlying principles and development of impedance spectroscopy techniques will be summarised. The literature concerning the use of EIS to assess the pregnant cervix will be reviewed in order to explain the evidence in favour of continued investigation and development of this technique as a tool for clinical practice.

1.5.1 Background and Principles of Bioimpedance

Bioimpedance techniques utilise materials' ability to conduct and resist electrical current. When current flows through tissue, a potential difference is generated across it. The size of this voltage can be measured and used to calculate the resistance of the material[286, 287]. Current flows through tissue via the movement of free and bound carriers of charge. These can be free ions, such as those in solution within the intracellular and extracellular fluid, or bound charges, which may be attached to membranes or complex proteins. The mobile ions produce a conduction current when they move through the tissue – opposition to this movement is the tissue resistance[288].

The bound charges within a tissue cannot move enough to produce a flow of electrical current but they can polarise when an electrical field is applied, creating a displacement current. These areas of tissue with bound charges act as capacitors - they can store and release energy when an alternating current is applied[289]. Direct current does not flow through a capacitor to any significant extent. When an alternating current (AC) is applied, the direction of polarisation is constantly shifting, and the displacement current flows across the capacitor[289]. At high frequencies, alternating electrical current flows easily, whilst at low frequencies the charges pass backwards and forwards less rapidly. The term reactance is used to describe how difficult it is for current to pass through a capacitor, and it is inversely proportional to current frequency[286]. The complex impedance of a tissue is made up of both tissue resistance and tissue reactance, hence alternating current is used in bioimpedance techniques.

A simple circuit model of the cell, as proposed by Fricke and Morse[290], describes how individual cell components behave in response to electricity. They class extracellular and intracellular fluid as resistors and the cell membrane as a capacitor. At low frequencies, current passes through the resistive extracellular fluid rather than through the capacitive cell membrane. As it is passing through a smaller amount of the tissue, impedance is relatively high. At high frequencies current can navigate the cell membrane and pass though the intracellular fluid too - tissue impedance is therefore lower[289]. This is demonstrated in Figure 1-7.

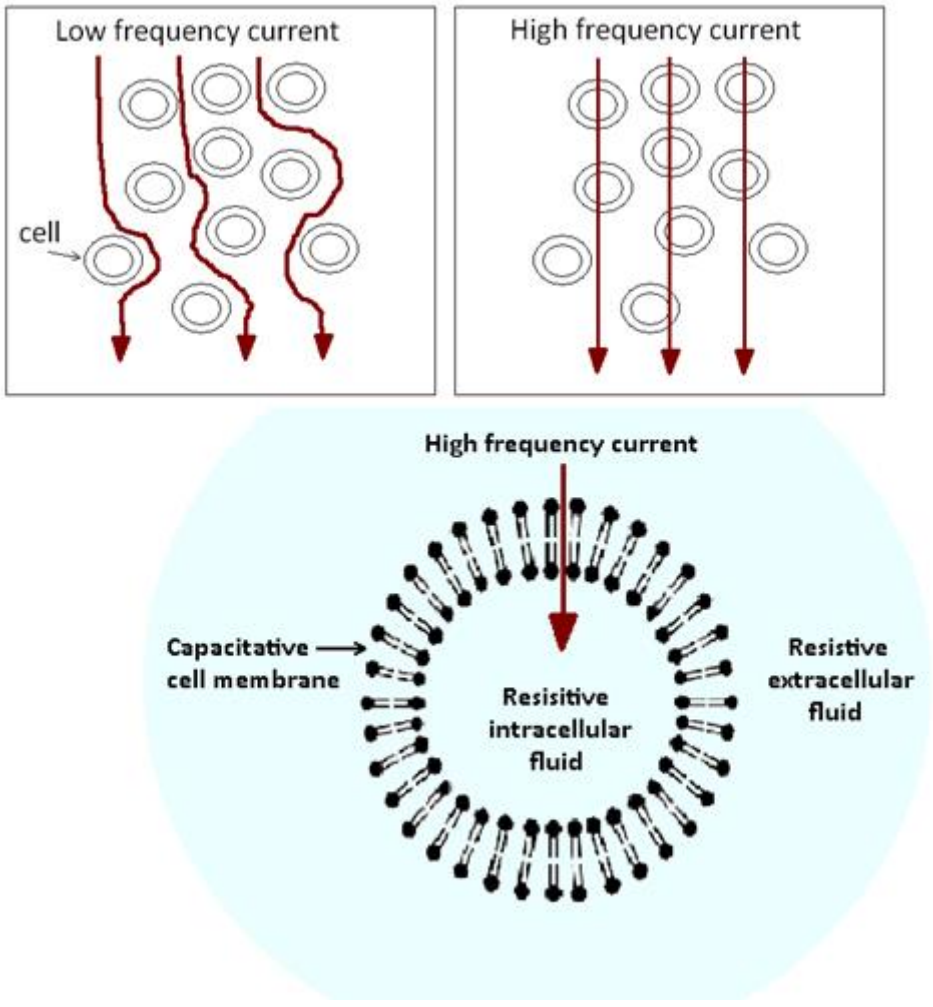


Figure 1-7 Movement of current through tissues at different frequencies
(Adapted from[286]). At low frequencies, current flows predominantly via circuitous extracellular pathways, with high resistance to flow. At high frequencies, current can cross the capacitative cell and nuclear membranes and flow directly through a higher proportion of tissue, thus resistance falls.

1.5.2 Assessment of the pregnant cervix

Investigation of the electrical properties of the pregnant cervix has proceeded alongside colposcopy-based studies assessing abnormal cervical epithelium[23, 291-297]. The efficacy of EIS

in detecting cervical intra-epithelial neoplasia has been established to the extent that commercially available probes can be used in routine clinical practice[298]. Obstetric studies have aimed to assess not only whether EIS can assess tissue changes at the level of the cervical epithelium, but also whether it could interrogate deeper, stromal tissues and thus detect evidence of ripening/remodelling[22, 24, 299-302]. Conducting research with novel diagnostic methods in an obstetric population is inevitably challenging. Whilst the colposcopy studies have been able to directly compare cervical impedance spectra measured *in vivo* with contemporaneously-obtained histological samples, such methodology is not easily replicated in pregnant women due to ethical/ safety constraints. This, coupled with incomplete understanding of the process of cervical remodelling during pregnancy, has made interpretation of the results of obstetric EIS studies more difficult.

Early work used *in vitro* techniques to measure the impedance spectra of cervical biopsies obtained during third trimester caesarean sections[22]. The gestational range studied was fairly narrow (35 - 42 weeks) and the sample size small (n=6), but this pilot showed that the R/S ratio of cervical tissue (a ratio of the resistance of extracellular vs. intracellular space) decreased with advancing gestation. This provided preliminary evidence that EIS could quantify cervical remodelling – most plausibly that it was detecting increased tissue hydration (i.e. more fluid in the extracellular space reduced extracellular resistance and thus tissue impedance). This provided the foundation for a subsequent *in vivo* study: in 2000, O'Connell *et al.* compared the impedance of the pregnant and non-pregnant cervix[303]. They obtained EIS readings using a 5.5mm pencil probe at a single frequency of 4.8 kHz and showed the cervical resistivities of 78 pregnant patients to be around 50% lower ($p < 0.001$) than those of 195 non-pregnant patients. Again, this was felt to reflect increased tissue hydration but possible alternative explanations included changes in the inherent electrical activity of the cervix/muscle cell connections, in connective tissue structure or in cell orientation.

Further research aimed to characterize the impedance of the pregnant cervix prior to labour[302]. Impedance measurements were taken from 86 women before induction of labour, using an 8mm pencil probe and a single frequency of 4.8 kHz. Concurrent vaginal examination enabled conventional assessment of cervical ripening. Women with a favourable cervix (Bishop score ≥5) had lower tissue resistivity compared to the unfavourable group (Bishop score ≤4) (5.34 Ωm vs. 7.03 Ωm, $p = 0.016$). Cervical resistivity

and the Bishop score correlated similarly with interval to delivery as (r= 0.42 for resistivity and -0.43 for Bishop score). ROC curve analysis demonstrated that resistivity better discriminated between women achieving normal delivery and those requiring caesarean section for failed induction or delay in the 2nd stage, with an area under the curve of 0.66 vs. 0.38 respectively (no p value provided).

A 2006 study also compared the electrical impedance of pregnant and non-pregnant cervices, and described mean values during the first, second and third trimesters of pregnancy[300]. Interestingly the group's findings differed from that of O'Connell *et al.* [303] They noted higher average cervical impedance in the pregnant versus non-pregnant patients, and in the third vs. the first and second trimesters, in conflict with earlier work[22]. The authors hypothesized that their observations might be due to increased tissue cellularity following the influx of inflammatory cells prior to labour. However, it is notable that their measurements were obtained from women undergoing pre-labour LSCS from 34 weeks gestation with low Bishops scores, *vs.* the earlier study's term induction cohort[303]. It is possible therefore that their measurements preceded the accelerated ripening changes and matrix degradation of the late third trimester, or that their subjects underlying reasons for caesarean delivery (e.g. previous LSCS for failed induction/labour dystocia with a non-compliant cervix) may have confounded their observations. In addition, differences in the EIS technology utilised hampers direct comparison: O'Connell *et al.* assessed impedance using 5.5mm and 8mm probes and a single frequency of alternating current[302, 303] whilst Gandhi *et al.* assessed the tissue at multiple frequencies (from 2-1625 kHz) with a 9mm probe[300]. The differing frequencies of the studies and the differences in the relative contribution of cervical and stromal tissue elements to derived resistivity values attributable to the probe sizes may have further confounded observations.

Subsequent work sought to explore the optimal design and reliability of EIS probe equipment[299, 304]. Probe size has been shown to affect impedance readings with one study assessing how inter-electrode distance affects EIS measurements[299]. Employing two probes of differing diameters (5mm and 9mm) they demonstrated that the use of the 5mm gave approximately two-fold higher resistivity values compared to the 9mm probe. This corresponds with values obtained with different sized probes across earlier studies[302, 303]. The effect was particularly marked at lower frequencies, with a significant difference noted for frequencies from 4 - 819 kHz. Finite element modelling studies suggested that current

applied with the larger probe achieved better stromal penetration than the small probe, even at high frequencies. Stromal tissue has a lower resistivity than epithelial tissue and therefore the researchers suggested that their results were predominantly explained by this variable depth of penetration. They did acknowledge that other factors, such as inconsistent probe application pressure, may have contributed to the observed differences. However, the intra-observer variation noted across their data was small, suggesting good repeatability, regardless of any pressure variation. Overall, they concluded that, whilst small probes might be optimal for assessing cervical epithelium, the ideal probe for assessing remodelling in pregnancy could be larger to allow both epithelial and stromal assessment.

A more detailed assessment of the reliability and reproducibility of EIS and the effect of variable probe application pressure was published by Jokhi *et al.* in 2009[304]. Cervical impedance measurements were obtained from 11 women prior to term elective caesarean section. Repeated readings were obtained using 2 probes (3mm and 12mm), at frequencies from 0.076 – 625 kHz, by two observers using firm and soft pressures. Pressure variation did not appear to cause a significant difference in impedance readings, especially at high current frequencies. Intra-observer repeatability was good across both pressures and probe sizes. Inter-observer agreement was less strong, but best when the larger probe was applied with firm pressure (intra-class correlation coefficient 0.528-0.638). A concurrent study by the same group, assessing the outcome of induced labour[24], measured cervical resistivity in 200 pregnant women. Four probe sizes were evaluated (3, 6, 9 and 12mm) at a frequency range identical to the earlier variability study[304]. Only resistivity values obtained with the 12mm probe were shown to correlate with labour outcomes – patients with higher resistivity readings were more likely to have a labour duration >24 hours and to require syntocinon augmentation. Those with lower resistivity values were more likely to achieve vaginal delivery (OR 3.9). However, CR readings were not predictive of time to onset of labour, or induction delivery interval <24 hours, whilst the Bishop score was. The greater depth of penetration of current with the 12 mm probe may have contributed to the observations since remodelling predominantly takes place at a stromal, rather than epithelial, level. Although the demonstrated predictive value of EIS in this series was modest, it remains to be determined whether device improvements may enable some clinical utility in labour management.

Overall, the literature reported thus far suggests a pattern of falling cervical resistivity in the late third trimester as ripening occurs (which mirrors the rising Bishop's score). Whilst EIS measurements at term have not been shown to be a reliable predictor of labour outcome, observed correlations between low CR and higher vaginal delivery rates and high CR and prolonged labour[24]/LSCS[302] suggest it does have ability to objectively assess cervical ripening. Moreover, cervical compliance is clearly not the only factor affecting successful term labour – fetal position, adequacy of uterine activity and the presence of any cephalo-pelvic disproportion could all adversely influence labour progress, even if a low CR has been detected. Thus, there remains a potential role for EIS in detecting premature cervical ripening as a precursor to PTB.

1.5.3 Use in Preterm Birth screening

Indeed, a recent pilot study has provided preliminary evidence regarding the utility of EIS in PTB screening[25]. Fortnightly measurements of CR between 14 and 28 weeks were obtained from 40 women at high risk of preterm birth, alongside serial CL scans. There was a strong positive correlation between CR at 20-28 weeks and delivery gestation, and EIS measurements at 39kHz predicted PTB<37 weeks with an AUC of 0.88 and <34 weeks with an AUC of 0.96. The equivalent AUCs for a CL <24mm were 0.97 and 0.98.

Two other groups have described preliminary use of EIS in PTB screening, however both devices are at a considerably earlier stage of development than the Sheffield EIS probe. One combines the use of impedance spectroscopy with cervical fluorescence[305], for which results from just one patient are available (this device employs a cup like probe tip with bipolar EIS electrode configuration in contrast to the Sheffield probe's tetrapolar pencil tip). The team acknowledge that it was challenging to maintain good electrode contact with the cervix, even in the absence of cervical shortening, which could plausibly limit device utility moving forwards. The other group's device exclusively uses EIS (using a similar pencil tip to the Sheffield probe, but with a linear tetrapolar arrangement), however it has only been tested in vitro (with lab-based work confirming higher impedance with increasing collagen concentration in pre-prepared gel samples)[306]. Moreover, measurement repeatability using biological tissue has not been confirmed.

Collectively, the obstetric EIS studies to date illustrate the potential merit of EIS as a screening test for PTB. They therefore provide the foundation for the studies presented

within this thesis. However, PTB screening can only be justified if effective treatments are available for those women identified as high risk. The next section will briefly review prophylactic and preparatory therapies designed to reduce the risk of PTB and its downstream complications.

1.6 Prediction-based therapies for Preterm Birth

1.6.1 Progesterone

Supplemental progesterone is widely used as PTB prophylaxis. Vaginal progesterone is most commonly prescribed in the UK and national guidance recommends its use in women with a CL <25mm[19], with or without a history of PTB. However, there is some debate regarding its effectiveness. Multiple meta-analyses demonstrate reductions in PTB rates with progesterone (particularly in women with prior PTB and/or a short cervix, with RRs ranging from 0.50-0.79 depending on the group and outcome studied[307-310]). However, the largest and highest quality randomised studies have not yielded positive results[311, 312]. Although the OPPTIMUM study[311] failed to demonstrate a reduction in the primary outcomes of PTB before 34 weeks or composite adverse neonatal outcome with progesterone, rates of neonatal death and brain injury were significantly lower in the group who received active treatment. Moreover, no long term harm after treatment was noted at 2 year follow up. A further, independent, individual patient data meta-analysis is in progress which aims to address some of the concerns levelled at earlier data syntheses[313] and clarify treatment effects. Critics of progesterone use cite the absence of an 'identifiable deficiency syndrome' as a key concern underlying their scepticism[314]. However, it is increasingly clear that local tissue-level fluctuations in progesterone concentration and function (rather than global, systemic reductions) are likely to contribute to parturition signalling. Progesterone appears to have local effects on the cervical ECM[315], fibroblast-ECM adhesion[316] and anti-microbial[317] and anti-inflammatory[318] effects within both uterine and cervical compartments. Greater understanding of the interaction between endogenous and supplemental progestogens and the molecular mechanisms by which they take effect may guide more precise therapeutic use in future.

1.6.2 Cervical Cerclage

Cervical cerclage involves placing an encircling purse-string suture around the cervix to prevent premature effacement/dilatation. It can be inserted trans-vaginally (with or without

prior bladder reflection - Shirodkar and Macdonald techniques) or trans-abdominally, via an open or laparoscopic approach. Trans-abdominal cerclage (TAC) is typically reserved for patients with previous failed trans-vaginal cerclage attempts[319], or those with a history of trachelectomy for cervical malignancy.

Meta-analysis of 10 studies of 2927 women, suggests a trend towards improved outcomes with cerclage use: RR of perinatal death in treated women was 0.82, although the 95% CI crossed 1 (0.65 to 1.04)[320]. The risk of PTB before 28, 34 and 37 weeks was also reduced. However, there is ongoing debate regarding the population most likely to benefit from treatment. A systematic review of international guidelines suggests consensus on three issues: women with three prior PTB/mid-trimester losses should be offered history-indicated cerclage; women prior PTB and CL <25mm before 24 weeks should be offered ultrasound-indicated cerclage; and low risk women with a short cervix should not be routinely offered cerclage[199]. Ongoing research is required regarding the relative benefits of different prophylactic treatments in different patient groups; the use of cerclage in patients with prior colposcopy treatment; the use of rescue cerclage; and the risk/benefit balance of combined therapies for PTB prophylaxis (for which evidence is limited thus far[321]). Given the complexity of pathways leading to preterm birth, it is unsurprising that cerclage is no universal panacea. Nevertheless, it is highly plausible that it helps women with cervical weakness, thus, the development of tests which can identify this group more readily should allow prompt, targeted treatment to be administered.

1.6.3 Cervical Pessary

Pessaries are a non-invasive method of providing external mechanical support to the cervix. Early research suggested possible reduction in PTB rates when used in singleton pregnancies[322]. However meta-analyses have shown less clear evidence of benefit[323] and other therapies, notably progesterone, may be more effective[324].

1.6.4 Tocolysis

Despite intense research, tocolytic medication, designed to induce uterine relaxation, has had limited success in preventing PTB. Multiple drug classes have been studied, most commonly calcium channel blockers (e.g. nifedipine), oxytocin receptor antagonists (e.g. atosiban) and progestogens[19]. Current UK guidance advises use of nifedipine initially, with oxytocin receptor antagonists reserved for those with contraindications. Calcium channel

blockers reduce delivery within 48 hours of presentation (RR 0.30, 95% CI 0.21 to 0.43), although evidence regarding effect on PTB rates is inconsistent[325]. This facilitates preparatory treatments for those in PTL, aimed at reducing morbidity (see below). Network meta-analysis suggests calcium channel blockers have the highest likelihood of beneficial effects on neonatal morbidity (e.g. respiratory distress, intraventricular haemorrhage and sepsis) with minimal fetal risks[19, 326]. Although widely used, oxytocin receptor antagonists have not been shown to be superior to placebo or other tocolytic drugs in prolonging pregnancy or improving outcomes[327]. However, many of the studies included in the aforementioned SRs are relatively small, and quality assessment suggests a significant risk of bias within the primary evidence base[328]. One included RCT in particular[329] did not stratify recruits by gestational age, and an excess of women at extremely preterm gestations were recruited to the atosiban arm, which likely biased neonatal outcomes. Furthermore, randomised comparisons of nifedipine *vs.* atosiban have failed to demonstrate superiority of either drug[330] and atosiban is associated with a better maternal side effect profile than other tocolytic classes[327]. Certainly, the use of tocolysis should be carefully considered and targeted to those populations most likely to benefit –prolonging fetal exposure to an adverse intrauterine environment (e.g. if chorioamnionitis is present) may also cause harm. Incorporating established predictive tests into future randomised studies of tocolysis may provide stronger evidence of benefits and risks: A large, multicentre, double blind, placebo controlled RCT of atosiban tocolysis is ongoing, and aims to recruit 1514 women in PTL with short CL and/or a positive FFN or premature rupture of mebranes, powered to detect a 4% reduction in adverse neonatal outcome[331].

1.6.5 Preparation for PTB

1.6.5.1 Antenatal corticosteroids

Antenatal corticosteroid therapy prior to PTB is associated with a significant reduction in neonatal morbidity and mortality. Meta-analysis of 30 studies showed lower rates of perinatal death, respiratory distress, intraventricular haemorrhage, necrotising enterocolitis and systemic infections in those born to treated mothers[332]. Duration of benefit is debated and further research is necessary to clarify optimal care for women at high risk of PTB who remain pregnant following administration of an initial course of steroids. Single repeat 'rescue' courses may be beneficial but are associated with a reduction in birthweight[333].

Accurate prediction of PTB may therefore facilitate optimal timing of steroid therapy to maximise benefit and minimise risks.

1.6.5.2 Magnesium Sulphate

Administration of intravenous magnesium sulphate (MgSO4) to women at risk of PTB reduces the risk of cerebral palsy in their offspring (RR 0.68 95% CI 0.54-0.87) with a NNT to benefit of 63[334]. In the UK, NICE advise that MgSO4 is offered to women in preterm labour between 24+0 and 29+6 weeks and considered between 30+0 and 33+6 weeks[19]. Evidence regarding the optimal dose and administration regime[335, 336] is limited, but it typically necessitates close monitoring on labour ward for a prolonged period, thus is best targeted to patients at highest risk of PTB.

1.6.5.3 In-Utero Transfer

In-utero transfer (IUT) is typically arranged for women presenting before 28 weeks with threatened PTL to obstetric units without a co-located NICU; it may also be required when cot availability is an issue. In the UK, delivery in a hospital with a busy level 3 NICU is associated with improved survival at extremely preterm gestations[7]. However, limitations in current predictive tests mean that many transfers ultimately prove unnecessary[337], with financial and personal costs to health services and patients[338].

1.7 Justification for Project

Well established criteria for assessing screening tests exist[339] (Table 1-3). The literature reviewed thus far has established the clinical importance of PTB, current understanding of its natural history and the existence (in many cases) of a latent period amenable to treatment. The range of therapeutic options has also been summarised.

Table 1-3 Wilson and Jungner Screening Criteria (reproduced from [339])

1. The condition sought should be an important health problem.
2. There should be an accepted treatment for patients with recognized disease.
3. Facilities for diagnosis and treatment should be available.
4. There should be a recognizable latent or early symptomatic stage.
5. There should be a suitable test or examination.
6. The test should be acceptable to the population.
7. The natural history of the condition, including development from latent to declared disease, should be adequately understood.
8. There should be an agreed policy on whom to treat as patients.
9. The cost of case-finding (including diagnosis and treatment of patients diagnosed) should be economically balanced in relation to possible expenditure on medical care as a whole.
10. Case-finding should be a continuing process and not a "once and for all" project

Current screening tests are limited, in particular by modest positive predictive values. Electrical impedance spectroscopy can provide useful, objective information about the resistivity (and thus structure) of cervical tissue. It should therefore confer additional predictive ability to the range of tests presently available. It targets the common pathway of parturition, maximising its ability to predict PTBs with varied aetiology; it carries negligible risks; it is easy to use; and the equipment is relatively inexpensive. To justify wider use this thesis will aim to: confirm patterns of CR change in women ahead of PTB; confirm the utility of EIS as a predictive test; determine test accuracy in symptomatic and asymptomatic groups; and evaluate test acceptability. Analysis will also establish whether it can be used as a stand-alone test or as part of multi-modal screening.

1.8 Hypotheses

1. The Mark V version of the Sheffield EIS probe allows repeatable and reproducible measurements of cervical resistivity following the addition of an application pressure sensor and blinding of the operator to individual EIS spectra (see Chapter 3).
2. Cervical resistivity is lower in asymptomatic women destined to deliver preterm due to the epithelial and stromal changes which accompany pre-labour softening and ripening (see Chapter 4).
3. Cervical EIS measurements obtained from asymptomatic women in the mid-trimester of pregnancy constitute a useful predictive test both in isolation and combination with existing screening techniques (see Chapter 4).
4. Similar measurable changes in cervical resistivity are expected to occur in women with symptoms of preterm labour. EIS may therefore be a useful bedside test in assessing the risk of early delivery in this group (see Chapter 5).

5. Although the effects of prior colposcopy treatment on subsequent cervical resistivity are unknown, in the absence of ongoing dyskaryosis, EIS may have potential to identify pre-labour cervical remodelling in women with prior LLETZ destined to deliver preterm (see Chapter 4).
6. EIS represents an acceptable screening test for preterm birth in asymptomatic women at both high and low risk of preterm birth (see Chapter 6).

1.9 Aims

1. Confirm EIS test variability with the updated Mark V probe.
2. Confirm the changes in cervical impedance seen ahead of preterm birth.
3. Evaluate the accuracy of cervical spectroscopy in predicting preterm birth in asymptomatic women (incorporating both high and low risk groups).
4. Perform a preliminary assessment of whether EIS detects similar changes prior to PTB in women with previous colposcopy treatment.
5. Evaluate the accuracy of cervical spectroscopy in predicting preterm birth in a pilot study of symptomatic women.
6. Assess the acceptability of EIS screening to asymptomatic women at low and high risk of preterm birth.

Chapter 2 - Materials and Methods for Prospective Cohort Studies

2.1 Overview of studies and target populations:

The scale and impact of preterm birth is clear. Unless clinicians have access to improved predictive technologies, the benefit of therapeutic measures to reduce the risk of preterm birth will always be limited.

Our group has previously presented pilot data demonstrating the ability of cervical EIS to predict PTB in high risk asymptomatic women[25]. Those destined to deliver preterm had significantly lower cervical resistivity at 20-28 weeks than their term counterparts. We therefore hypothesized that mid-trimester cervical impedance measurements with the Sheffield Mark V device would accurately predict PTB. In order to test this hypothesis further, two prospective cohort studies were designed.

The primary cohort consisted of asymptomatic women with and without risk factors for PTB. Women with a prior history of PTB and/or late miscarriage have the highest incidence of premature delivery and therefore, if the predictive ability of EIS is confirmed, will stand to benefit most from prophylactic interventions. For the purposes of this study, women were defined as high risk if they had history of delivery before 37 weeks' gestation, mid-trimester loss after 14 weeks gestation or a cervical length <25mm before 24 weeks gestation. Asymptomatic women at low risk of PTB were also recruited - the ability of EIS to predict PTB has not been evaluated before in this group and both nulliparous and multiparous women were included. This subgroup were anticipated to have a low rate of PTB, however, they represent an important group to study: approximately 85% of preterm births occur in women with a low risk obstetric history[340] and current tests demonstrate poor predictive ability in such patients[21, 182]. If effective screening tests for these women can be found, then significant reductions in the rate of PTB may be realised.

The second cohort included women presenting with symptoms of threatened preterm labour. Access to effective screening tests in this group should allow both preventative and preparatory treatments to be delivered at the optimal time-point to minimise the morbidity and mortality associated with PTB. Interventions such as antenatal

corticosteroid administration and neuroprotection with magnesium sulphate are best timed shortly before birth and therapeutic benefit may be lost if they are administered too soon[341]. Additionally, if the modest positive predictive values of current tests can be improved upon, unnecessary hospital admissions and in utero transfers may be reduced, with significant cost savings.

Results from these two cohorts will be presented in Chapters 4 and 5. Within this Chapter, as there is significant methodological overlap, the overall structure of the studies will be discussed; the process of data acquisition explained; and intended outcome measures and analyses described. Prior to commencing the main cohort studies, formal assessment of the repeatability and reproducibility of cervical EIS measurements with an updated EIS probe was performed. The conduct and results of this variability study are described in Chapter 3.

An assessment of the acceptability of EIS has also been performed; however methodological considerations for this part of the project are reported separately in Chapter 6.

2.2 Ethical Approval

This research was reviewed and approved by the Yorkshire & Humber (Sheffield) Committee of the UK National Research Ethics Service (REC Number 13/YH/0167) (see Appendix A).

2.3 Sample size calculation

On the basis of data from our group's earlier pilot study, a sample size calculation for the subgroup of high risk asymptomatic women was performed employing the methods of Buderer[342] which incorporate estimates of disease (PTB) prevalence and hypothesized values of sensitivity and specificity to ensure the test performs with clinically acceptable precision. Given that EIS had been shown to predict delivery <37 weeks with a sensitivity and specificity of 85%[25], if a 95% confidence interval for sensitivity of 75-95% is used, 49 high risk women needed to deliver prematurely in our study population to enable us to confidently confirm the sensitivity of EIS prediction in this group. The prevalence of PTB in the high risk pilot group was ~25%, consistent with existing literature[343]. Therefore approximately 200 high risk women needed to be recruited to the asymptomatic cohort.

There is no published data regarding prediction of PTB by EIS in low risk asymptomatic women. The prevalence in this subgroup was likely to be around 5% or less and they were also expected to be harder to recruit as they would be no clinical reason for them to routinely undergo speculum examination, transvaginal ultrasound or attend for additional appointments. The combined effect of lower PTB prevalence and anticipated lower acceptance rates would likely have required the study team to have approached an unrealistically large number of women. Although 7000 women book for antenatal care in the Jessop Wing each year, not all would be eligible for participation (either due to exclusion criteria or higher risk status), and funding and project team size meant the timescales for conducting the study were finite, thus realistically not every patient attending for booking could be approached or contacted. HTA approval at this stage of the project allowed a single rather than multicentre study (as CE marking would have been required for extension to sites outwith Sheffield). We therefore set a pragmatic recruitment target of 250 low risk asymptomatic women. If EIS performs similarly for those without prior PTB, future evaluation within a larger cohort will likely be necessary. Participants in the symptomatic cohort were recruited opportunistically at the time of presentation – this data constitutes pilot work and a sample size of 50 was planned.

2.4 Inclusion/exclusion

Entry criteria to the two cohorts were as follows:

- Group 1 participants were **asymptomatic** women either judged to be:
    - at **high risk** of PTB on account of their past obstetric history (≥ one previous spPTB < 37 weeks gestation or ≥ 1 miscarriage >14/40) or cervical length <25mm (**AHR group).**
    - or at **low risk** of PTB (no prior PTB/late miscarriage/short cervix) (**ALR group**).
- Group 2 participants were women admitted to the triage area or the labour and delivery suite with intact membranes and **symptoms** of premature labour but not in established labour (cervical dilatation < 3cm).

Exclusion criteria were:

- A history of recent abnormal cervical smear (if a normal smear or colposcopic examination had subsequently excluded the presence of CIN then recruitment was permitted).
- Women with current clinical cervical infection or vaginal bleeding.
- Multiple pregnancy.
- Known fetal anomaly.

The rationale for excluding women with a current abnormal smear arose from knowledge regarding EIS use in colposcopy settings – the presence of CIN materially affects cervical resistivity[294] and could confound any observations occurring due to premature cervical remodelling. The effect of prior colposcopic treatment on cervical resistivity is unknown. However, such history may confer a significant risk of preterm birth in both parous and nulliparous women[344]. Therefore, as long as they had a subsequent normal smear test, women with a history of excisional procedures were recruited and an additional subgroup analysis was planned to investigate potential treatment effects.

Women with cervical infections or vaginal bleeding were excluded due to the potential for such states to affect fetal fibronectin test results. The risk of PTL in multiple pregnancy is complex and multi-factorial and pathways to PTB may differ somewhat from those preceding singleton PTB. In addition, the evidence base for preventative therapy differs significantly from that in singletons[345]. Thus, women carrying multiple pregnancies were excluded. Fetal anomalies again may provoke PTB via different mechanisms to those affecting uncomplicated pregnancies and would affect rates of perinatal morbidity and mortality within the study cohort, therefore patients carrying an affected fetus were not recruited.

2.5 Process of recruitment

Asymptomatic women were screened at an early stage in pregnancy and given information about the study after their dating scans. If they agreed to participate, the first visit was timed to coincide with the routine anomaly scan at approximately 20 weeks.

AHR women attended for two visits – the first at 20-22 weeks and the second at 26-28 weeks. The timing of visit 1 and 2 reflects the most predictive gestations demonstrated by the earlier pilot study.

ALR women attended for a one-off assessment at 20-22 weeks. As a subgroup, they were less likely to agree to attend multiple study visits, as they would not otherwise need assessment in hospital. In addition, if EIS is adopted for use in the routine assessment of low risk pregnant women, it would be most feasible to perform the test at this stage, so

it was the most practical starting point for our study. Figure 2.1 summarises the study pathway for asymptomatic women.

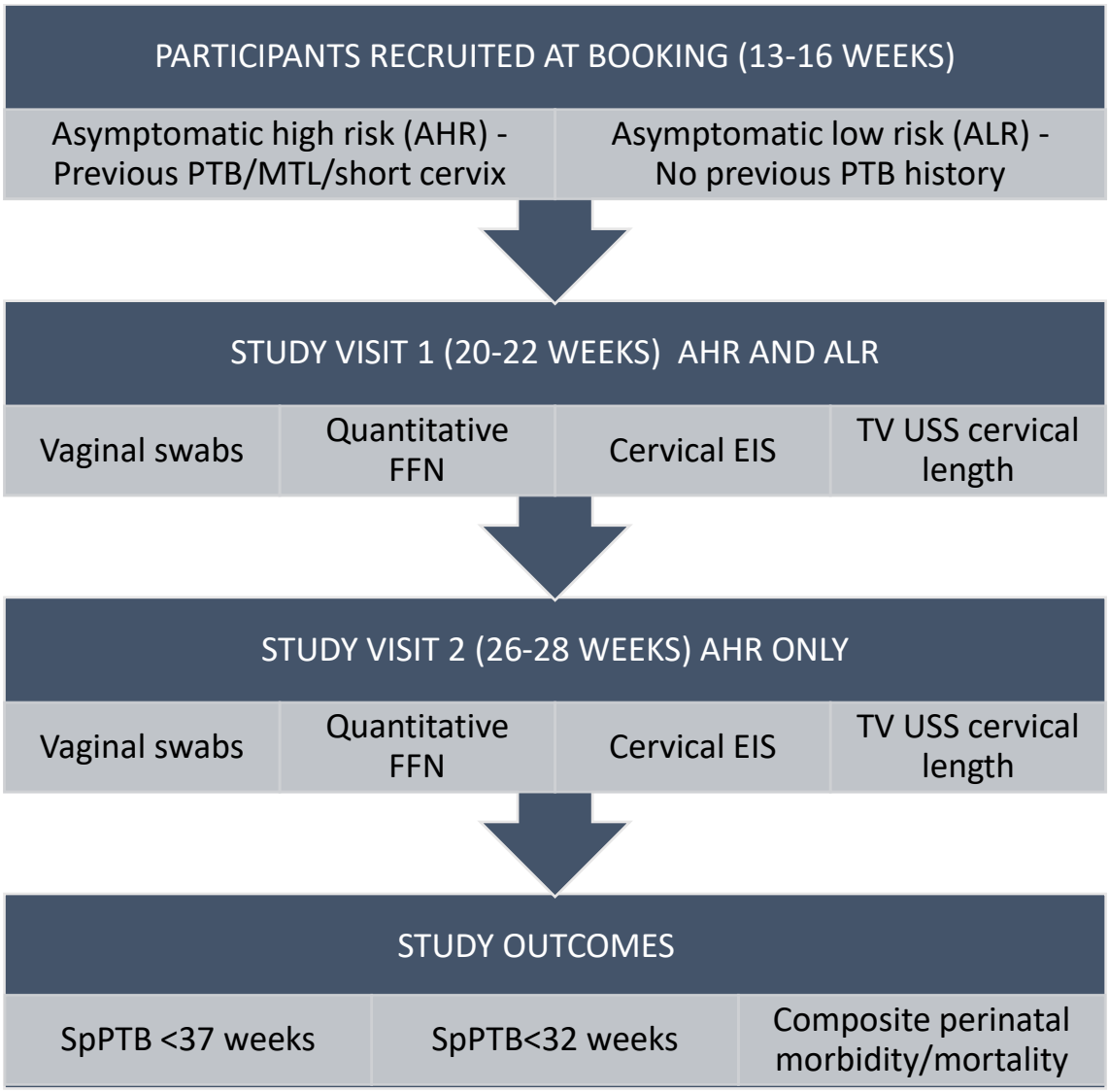


Figure 2-1 Flow Diagram of Studies in Asymptomatic Women

Abbreviations: PTB preterm birth; MTL mid-trimester loss; FFN fetal fibronectin; EIS electrical impedance spectroscopy; TV USS transvaginal ultrasound scan; SpPTB spontaneous PTB.

Participants in the symptomatic cohort (SYM) were recruited at the time of presentation to hospital, or within 24 hours of admission if clinical symptoms remained present. The flow diagram in Figure 2-2 summarises study conduct for this group.

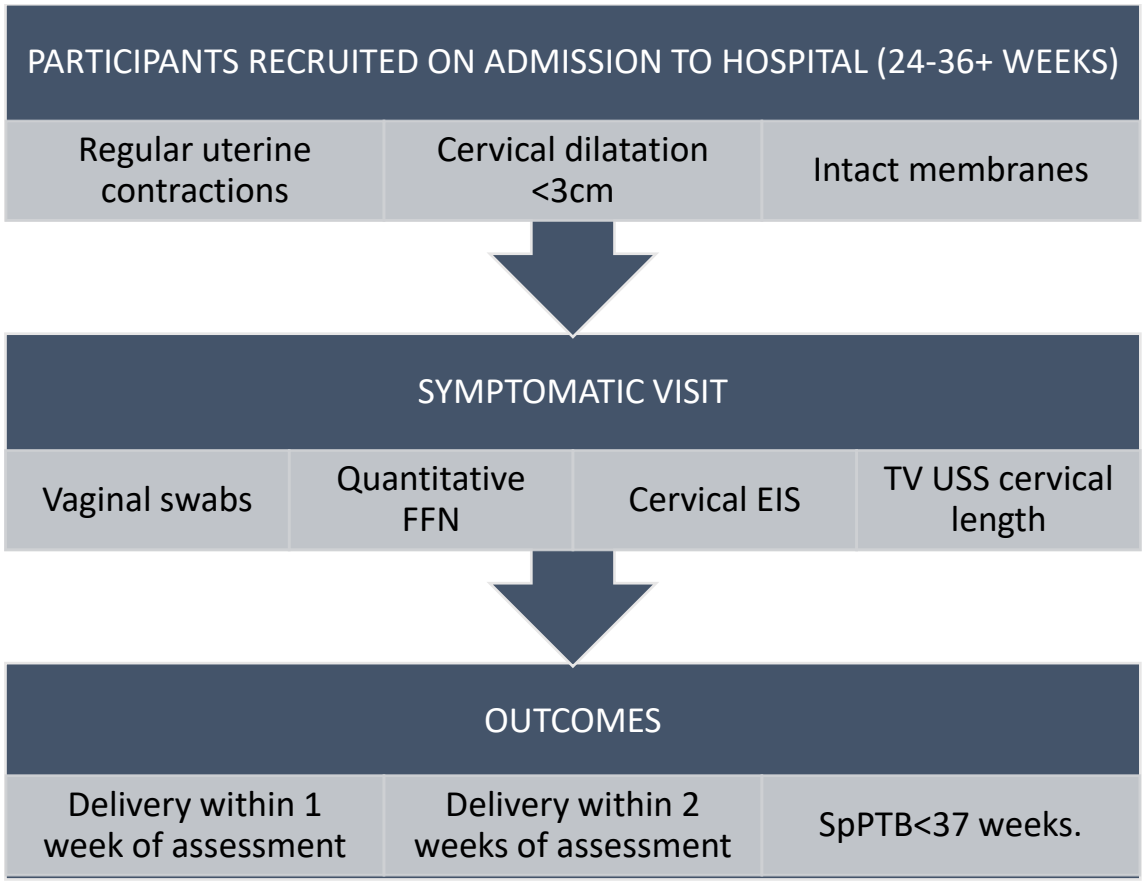


Figure 2-2 Flow Diagram of Study in Symptomatic Women

Abbreviations: FFN fetal fibronectin; EIS electrical impedance spectroscopy; TV USS transvaginal ultrasound scan; SpPTB spontaneous PTB.

All women received a written patient information sheet (PIS) and were given time to reflect on whether they wished to participate before informed consent was obtained. Copies of the PIS and consent form for each study group are included in Appendix B.

2.6 Conduct of main study visits

For each participant (AHR, ALR or SYM) study visits were conducted in the same way: after obtaining written informed consent, a full history was taken and all demographic and clinically relevant information was recorded contemporaneously in a purpose designed database. The woman was asked to empty her bladder and a speculum examination was then performed to visualise the cervix. Only water was used to facilitate this procedure as lubricating gel and obstetric cream may affect fetal fibronectin (FFN) measurements. The appearance of the cervix was noted, and then swabs taken from the posterior vaginal fornix and lateral walls for FFN quantification, conventional

microbiological culture, pH measurement, microbiome and metabolome analysis). The fibronectin sample was analysed using the Rapid FFN 10Q System®, as per manufacturer's instructions. Vaginal pH was estimated using narrow range indicator paper (pH 3.6 – 6.1). The microbiome and metabolome analysis did not form part of this thesis but were the basis of nested research published elsewhere[346-348].

Any excessive discharge or cervical mucus was gently removed from the cervix then the tip of the Mark V EIS probe was positioned in the 12 'o'clock position on the anterior lip and applied with a pressure of 2N. A series of EIS readings were obtained at 1-minute intervals – optimally three, but sometimes one or two depending on comfort of the participant and ease of measurement.

A transvaginal ultrasound was then performed and cervical length measured as follows: The covered transvaginal probe was gently introduced, whilst observing the image on screen and taking care not to compress the cervix excessively. A longitudinal section of the uterus and cervix was obtained, noting the presence of any funnelling or "sludge" at the level of the internal os. This image was magnified to occupy two-thirds of the screen. Once a suitable image demonstrating the full length of the cervix had been obtained, the transducer was withdrawn slightly, enough to minimise application pressure to the cervix but maintain image quality. The calipers were then placed on the internal and external os and the cervical length measured. This was repeated 3 times and the shortest obtained cervical length recorded.

Participants were informed of the results of their cervical length scan and fetal fibronectin result at the time of the study visit. Positive microbiological swabs (e.g. for candida or Group B streptococcus) were managed according to local clinical protocols (see Appendix C). Participants and study investigators were blinded to the results of the EIS measurements.

Participants with an abnormal cervical scan result or positive fibronectin swab received appropriate management and follow up according to local clinical protocols (see Appendices D and E).

2.7 Probe design

The Medical Engineering department in Sheffield have pioneered the development of clinical impedance spectroscopy devices and probe technology has evolved over the course of extensive laboratory and clinical experimentation [22-24, 291-297, 299-304]. The most recent EIS probe is the Mark V device, which differs from its predecessors by virtue of a pressure sensor. This custom designed EIS system was used for the studies reported herein, and consists of a handheld EIS probe, linked wirelessly via Bluetooth with a database housed on the hospital intranet. The probe was designed and manufactured by the Medical Engineering department at Sheffield Teaching Hospitals. The Scientific Computing department produced a custom database (in collaboration with me) which linked with the EIS probe and enabled recording of relevant clinical data captured during study visit alongside the EIS spectra.

Previous EIS devices manufactured in Sheffield have utilised a tetrapolar electrode system to obtain impedance measurements. This avoids measurement error due to resistance of the electrodes themselves, as one pair of electrodes function as the 'injecting' pair through which current flows, and the other function as the 'measuring' pair by which the potential difference is measured. The system used in our studies in fact utilised eight electrodes, with a small inner ring (4 electrodes spaced 3mm apart) and a larger outer ring (4 electrodes spaced 9mm apart), conferring the ability to obtain measurements with both sizes. Electrode spacing is known to influence the magnitude of impedance measurements and finite element modelling has demonstrated increased depth of tissue penetration by electrical current when larger EIS tips are used[293, 299]. However, the pilot data evaluating preterm birth prediction[25] showed superior sensitivity of the 3mm *vs.* 9mm tip for discriminating cervical tissue characteristics, hence measurements from the smaller ring were used throughout these studies.

The incorporation of a pressure sensor in the Mark V probe means that during clinical measurements, the EIS tip is applied to the tissue of interest at a standardised, predetermined application pressure (Figures 2-3 to 2-6 illustrate the current probe design). This is important as previous work has identified a relationship between application pressure and the magnitude of resistivity readings (in vitro work has suggested an increase in tissue resistivity at higher application pressures[349], in vivo work demonstrated improved repeatability and reproducibility at higher pressures[304]). It was

hypothesized that the addition of the pressure sensor would improve intra-observer repeatability and inter-observer reproducibility. The variability study reported in Chapter 3 tests this hypothesis.

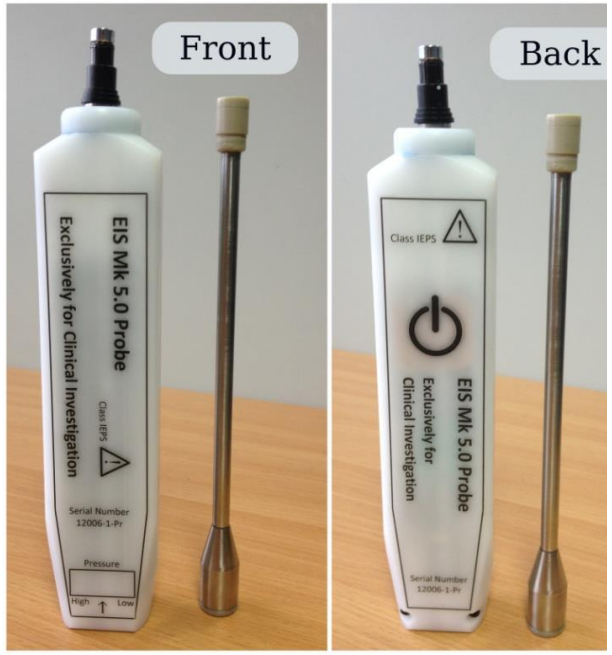


Figure 2-3 Mark V Electrical impedance spectroscopy probe with tip detatched.
LED pressure gauge visible at bottom of front view of probe. LED – light emitting diode

Figure 2-4: Mark V probe tip.
3mm and 9mm electrode rings are present.

Figure 2-5: LED pressure gauge.
Difficult to obtain clear photo of colour of lights therefore see also diagram below.

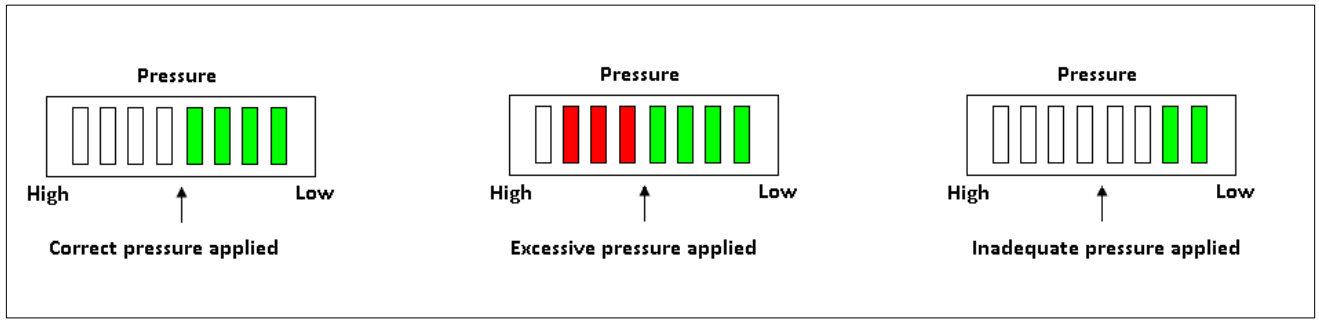


Figure 2-6: LED pressure gauge in detail.

As pressure is applied to probe tip, green LEDs sequentially light up – once level with marker arrow, the predetermined pressure has been achieved. Red LEDs light up if the desired pressure has been exceeded.

2.8 Infection control

Medical devices can be classified as non-critical, semi-critical and critical, depending on the nature of their use and the associated infection risk[350]. The EIS tip is a semi-critical instrument as it comes into contact with intact mucous membranes, similar to trans-vaginal, trans-oesophageal or trans-rectal ultrasound probes[351]. However, the nature of its design means that it cannot be subjected to normal heat-based sterilisation procedures due to electronic components within the tip. At present the probe does not work with covers (such as those used for trans-vaginal ultrasound probes) although further engineering work may remedy this for future device iterations. In the meantime the spectroscopy tips required high level disinfection between each use. Appendix F details the process of high level disinfection used to clean the Mark V probe and tips.

Chapter 3 - Variability Study

3.1 Introduction

The Mark V EIS probe utilised for this research incorporated new features compared to previous versions. Firstly it has a pressure sensor, designed to standardise the tip application force prior to measurement. Secondly, the measurement recording software now blinds operators to the impedance readings to minimise bias. Prior to commencing the substantive cohort studies described in chapter 2, a variability study was conducted to assess the effect of these modifications on measurement reliability.

In order to usefully measure differences in tissue composition, the ideal instrument should have good repeatability (close agreement of repeat readings by the same operator) and good reproducibility (close agreement of repeat readings by different operators)[352]. It was hypothesized that the incorporation of the pressure sensor in particular should improve intra- and inter-observer reliability. The effect of blinding was unknown. A previous EIS variability study utilised the unblinded Mark IV probe and showed high reproducibility and repeatability[304], therefore it was anticipated that the net effect of both probe modifications would be to improve or maintain measurement reliability.

3.2 Methods

3.2.1 Subjects

A pragmatic sample size of 13 patients scheduled for elective Caesarean section were recruited. They were booked for Caesarean section for a variety of indications, but all were at term gestation (mean 39+1 weeks, range 38+3 to 40+3) and gave full informed consent. The PIS and consent form for this study are included in Appendix B. The majority (77%) were multiparous with a mean age of 31.2 years. Patient characteristics are summarised in table 3-1. Exclusion criteria included a recent abnormal smear or history of colposcopy treatment, multiple pregnancy, fetal anomaly and current cervical infection or vaginal bleeding.

Table 3-1 Characteristics of Variability Study Participants

Mean gestation	39+1 weeks (38+3 to 40+3)
Parity	10 multiparous, 3 primiparous
Mean age	31.2 years (26.0 to 36.5)

3.2.2 Cervical EIS Studies

Under spinal anaesthesia, a speculum examination was performed with the patient in a supine position. A cotton swab was used to remove any cervical mucus or copious discharge as excessive amounts can interfere with impedance measurements[295]. Impedance readings were then obtained: the probe tip was positioned in the 12 o' clock position on the anterior lip of the cervix and two readings taken, approximately a minute apart, by the first observer at the first of two designated pressures. Two further readings were then taken by the same observer at the second application pressure. Finally, a repeat reading at both pressures was obtained by the second observer – overall a total of 6 EIS measurements were obtained. Each measurement recorded tissue impedance at 14 electrical current frequencies ranging from 76 Hz to 625 KHz. Once the study measurements were completed, the patient was repositioned and the surgical team proceeded with caesarean delivery as planned.

The above method is similar to that described in previous studies[304]. Initial measurements were obtained using application pressures of 1 and 2N – this was felt to provide a satisfactory balance between adequate pressure to ensure optimal repeatability whilst minimising patient discomfort (likely to be more of an issue in later studies without anaesthesia). However, after obtaining data on 3 patients, it became clear that a 1N application pressure was in fact unrealistically light – operators noted that it was difficult to achieve as the gauge recorded excessive pressure as soon as the tip was in contact with cervical tissue. A pragmatic decision to change the application pressures to 2N and 3N from patient 4 onwards was therefore made. This also enabled data analysis to investigate the effect of the three different pressures on cervical resistivity.

3.2.3 Statistical Analysis

Normality testing demonstrated that the data were not normally distributed ($p<0.05$, Shapiro-Wilk), therefore logarithmic transformation of the entire set was performed to facilitate subsequent analyses[353]. Intra-observer repeatability and inter-observer reproducibility were assessed through calculation of intra-class correlation coefficients (ICCs) and their 95% confidence intervals. For within observer comparisons a two-way, mixed effects model (absolute agreement) was utilised; for between observer comparisons a two-way, random effects (absolute agreement) model was chosen. Reliability was regarded as poor if ICC values were <0.5, moderate if values were between 0.5 and 0.75, good if values were between 0.75 and 0.9 and excellent if values exceeded 0.9[354] In addition, limits of agreement (LOA) were calculated in order to produce Bland-Altman plots[353]. This graphical method of examining measurement variability enables detection of systematic bias (e.g. one operator consistently obtaining different measurements to another), assessment of any relationship between observed differences and measurement magnitude and identification of outliers. In order to perform this analysis, the differences between readings and means of the two repeats were calculated within and between observers. Normality testing suggested that, similar to the measurements themselves, the between-reading differences were generally not normally distributed: using the Shapiro-Wilk test to assess intra-observer differences, $p<0.05$ for 10 out of 14 frequencies at 2N and 8 out of 14 frequencies at 3N. Similarly, non-parametric distribution of differences was noted at multiple frequencies for the inter-observer dataset. Log transformation was therefore employed for these analyses as well as the ICC calculations.

Statistical analysis was performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp). For both repeatability and reproducibility analyses, test performance at each of the 14 frequencies and two application pressures was evaluated.

3.3 Results

3.3.1 Intra-observer repeatability

At a 2N application pressure, intra-observer repeatability was excellent at 11 out of 14 current frequencies (from 76.3 to 78125 Hz), with ICCs >0.9. For the remaining 3

frequencies, repeatability was good at 156250 and 625000 Hz (with ICCs of 0.86 and 0.82 respectively. The only frequency at which repeatability values yielded a more moderate ICC was 312500 Hz, with an ICC of 0.629. These ICCs evaluated the reliability of single measurements (as only two repeat readings by observer one were obtained in this study) – if the average measure ICCs are considered, then reliability would increase, with all generated ICCs >0.75. The results of these analyses are summarised in Table 3-2, with 95% confidence intervals (CIs) provided. The majority of CIs were narrow, again consistent with good to excellent measurement reliability at the 2N application pressure.

Table 3-2 Repeatability of cervical resistivity measurements taken at a 2N application pressure (n=12)

Current frequency (Hz)	REPEATABILITY Single measures			REPEATABILITY Average measures		
	ICC (3,1)	95% CI		ICC (3,1)	95% CI	
76.3	**.960**	.868	.988	**.980**	.930	.994
152.6	**.948**	.832	.985	**.974**	.909	.992
305.2	**.948**	.835	.985	**.973**	.910	.992
610.4	**.940**	.809	.982	**.969**	.895	.991
1220.7	**.945**	.824	.984	**.971**	.904	.992
2441.4	**.934**	.789	.980	**.966**	.882	.990
4882.8	**.926**	.762	.978	**.961**	.865	.989
9765.6	**.945**	.820	.984	**.972**	.901	.992
19531.3	**.961**	.875	.989	**.980**	.934	.994
39062.5	**.945**	.824	.984	**.972**	.903	.992
78125	**.934**	.796	.980	**.966**	.886	.990
156250	**.859**	.598	.957	**.924**	.749	.978
312500	**.629**	.130	.876	**.773**	.231	.934
625000	**.823**	.495	.946	**.903**	.662	.972

When the EIS tip was applied with 3N of pressure, intra-observer repeatability was best at mid to high current frequencies (9765.6 to 312500 Hz) with ICCs between 0.82 and 0.99 (good to excellent). Moderate reliability was noted for impedance readings at 152.5 to 4882.8 Hz (ICCs 0.60 to 0.72), with good reliability noted at 76.3 and 312500 Hz (ICCs 0.77 and 0.82 respectively). The ICC confidence intervals were noticeably wider at the lower current frequencies, and also at the highest current frequency. Again, when average measure ICCs were considered, values improved, such that all but one frequency yielded ICCs>0.75 (good to excellent). The results of the 3N ICC analyses are provided below in Table 3-3.

Table 3-3 Repeatability of cervical resistivity measurements taken at a 3N application pressure (n=10)

Current frequency (Hz)	REPEATABILITY Single measures			REPEATABILITY Average measures		
	ICC (3,1)	95% CI		ICC (3,1)	95% CI	
76.3	**.771**	.316	.938	**.871**	.481	.968
152.6	**.724**	.208	.924	**.840**	.344	.961
305.2	**.675**	.155	.907	**.806**	.269	.951
610.4	**.646**	.120	.896	**.785**	.214	.945
1220.7	**.604**	.061	.881	**.753**	.116	.937
2441.4	**.620**	.083	.887	**.765**	.153	.940
4882.8	**.715**	.202	.921	**.834**	.336	.959
9765.6	**.915**	.635	.979	**.956**	.777	.990
19531.3	**.974**	.902	.994	**.987**	.948	.997
39062.5	**.991**	.965	.998	**.995**	.982	.999
78125	**.987**	.944	.997	**.993**	.971	.998
156250	**.974**	.904	.993	**.987**	.949	.997
312500	**.815**	.418	.950	**.898**	.590	.975
625000	**.568**	.013	.868	**.724**	.026	.929

Table 3-4 summarises the mean differences between reading 1 and 2 for observer 1 at 2 and 3N. The non-transformed data is provided to permit clinical evaluation of the magnitude of mean differences. Mean differences were highest at low current frequencies and smallest at the penultimate frequency of 312500 Hz. From 76 to 19531 Hz, mean differences were smaller at a 2 vs 3N application pressure. Between 39062 and 312500 Hz, differences were smaller at the higher pressure.

Table 3-4 Summary Table of Average Repeat Measures (Non transformed data) Within Observer Repeatability at 2N (n=12) and 3N (n=10)

Current Freq (Hz)	2N Repeatability			3N Repeatability		
	Mean R1 (Ohm)	Mean R2 (Ohm)	Mean Difference (R1 – R2)	Mean R1 (Ohm)	Mean R2 (Ohm)	Mean Difference (R1 – R2)
76.3	101.331	97.232	**4.099**	79.698	96.998	**-17.300**
152.6	121.178	114.323	**6.856**	95.108	117.615	**-22.507**
305.2	124.186	116.623	**7.563**	99.264	120.761	**-21.497**
610.4	120.715	112.940	**7.775**	98.866	116.978	**-18.112**
1220.7	106.901	100.876	**6.025**	88.286	104.476	**-16.190**
2441.4	90.842	86.788	**4.053**	77.323	88.193	**-10.870**
4882.8	77.867	75.173	**2.693**	67.776	75.136	**-7.360**
9765.6	67.794	67.443	**0.352**	61.401	64.946	**-3.545**
19531.3	59.940	60.827	**-0.887**	56.984	58.297	**-1.313**

39062.5	53.543	55.561	**-2.018**	52.827	52.691	**0.136**
78125	46.478	47.904	**-1.426**	47.265	46.578	**0.687**
156250	34.258	35.871	**-1.613**	35.736	35.287	**0.449**
312500	13.804	14.604	**-0.800**	15.526	15.261	**0.265**
625000	-6.958	-6.696	**-0.263**	-6.559	-7.788	**1.229**

Tables 3-5 and 3-6 summarise the results of the Bland-Altman analyses for repeat readings by Observer 1 at 2N and 3N respectively. These analyses utilised log transformed data (in order to fulfil the assumption of the Bland-Altman model[353] that differences between paired measurements are normally distributed) therefore the mean differences differ from those detailed in Table 3-4. The parameters calculated were then used to produce the Bland Altman plots in Figures 3-1 to 3-6.

A plot is provided for each current frequency at both pressures, to allow graphical inspection of the data variability. At both pressures, measurement variability reduces with rising current frequency and limits of agreement narrow accordingly on the sequential Bland Altman plots (with the exception of the LOA for 625000Hz at 3N). In general, the data are homoscedastic, i.e. variability does not seem to be affected by the magnitude of the resistivity reading, and a random scatter of differences is noted at both pressures and for each current frequency.

There is limited bias at either application pressure, especially at the higher current frequencies, as the mean difference (central dotted line) lies close to zero (closer for 2 than 3N). Limits of agreement were somewhat narrower at higher current frequencies for the 3 vs 2N application pressure (figure 3-2 vs figure 3-5). However, the LOA at 2N are also narrow enough to yield clinically useful results (i.e. where between-subject variance in the presence/absence of premature cervical ripening would exceed inherent measurement variability).

Table 3-5 Mean Differences and Limits of Agreement on log transformed 2N data (Within Observer Repeatability) (n=12)

Current frequency (Hz)	Mean Difference (R1 – R2)	SD	SE	Lower 95% CI of mean	Upper 95% CI of mean	SE for Limits of Agreement	Lower Limit of Agreement	LLOA lower 95% CI	LLOA upper 95% CI	Upper Limit of Agreement	ULOA lower 95% CI	ULOA upper 95% CI
76.3	**.00474**	.07489	.02162	-.04284	.05232	0.0374	**-0.1420**	-0.2154	-0.0687	**0.1515**	0.0781	0.2249
152.6	**.00800**	.08359	.02413	-.04511	.06111	0.0418	**-0.1558**	-0.2378	-0.0739	**0.1718**	0.0899	0.2538
305.2	**.01260**	.07525	.02172	-.03522	.06041	0.0376	**-0.1349**	-0.2086	-0.0611	**0.1601**	0.0863	0.2338
610.4	**.01381**	.07187	.02075	-.03185	.05948	0.0359	**-0.1271**	-0.1975	-0.0566	**0.1547**	0.0842	0.2251
1220.7	**.01276**	.06500	.01876	-.02855	.05406	0.0325	**-0.1146**	-0.1783	-0.0509	**0.1402**	0.0765	0.2039
2441.4	**.00747**	.06453	.01863	-.03353	.04847	0.0323	**-0.1190**	-0.1822	-0.0558	**0.1339**	0.0707	0.1972
4882.8	**.00290**	.06061	.01750	-.03561	.04141	0.0303	**-0.1159**	-0.1753	-0.0565	**0.1217**	0.0623	0.1811
9765.6	**-.00258**	.04524	.01306	-.03132	.02617	0.0226	**-0.0913**	-0.1356	-0.0469	**0.0861**	0.0418	0.1304
19531.3	**-.00691**	.03073	.00887	-.02643	.01261	0.0154	**-0.0671**	-0.0973	-0.0370	**0.0533**	0.0232	0.0834
39062.5	**-.01152**	.02830	.00817	-.02949	.00646	0.0142	**-0.0670**	-0.0947	-0.0393	**0.0439**	0.0162	0.0717
78125	**-.00870**	.02570	.00742	-.02503	.00763	0.0129	**-0.0591**	-0.0843	-0.0339	**0.0417**	0.0165	0.0669
156250	**-.01171**	.03747	.01082	-.03552	.01210	0.0187	**-0.0852**	-0.1219	-0.0484	**0.0617**	0.0250	0.0985
312500	**-.01028**	.04070	.01175	-.03614	.01558	0.0204	**-0.0901**	-0.1299	-0.0502	**0.0695**	0.0296	0.1094
625000	**-.00539**	.04516	.01304	-.03408	.02330	0.0226	**-0.0939**	-0.1382	-0.0496	**0.0831**	0.0389	0.1274

Table 3-6 Mean Differences and Limits of Agreement on log transformed 3N data (Within Observer Repeatability) (n=10)

Current frequency (Hz)	Mean Difference (R1 – R2)	SD	SE	Lower 95% CI of mean	Upper 95% CI of mean	SE for Limits of Agreement	Lower Limit of Agreement	LLOA lower 95% CI	LLOA upper 95% CI	Upper Limit of Agreement	ULOA lower 95% CI	ULOA upper 95% CI
76.3	**-.05589**	.09444	.02987	-.12345	.01167	0.0517	**-0.2410**	-0.3424	-0.1396	**0.1292**	0.0278	0.2306
152.6	**-.06386**	.09621	.03042	-.13268	.00496	0.0527	**-0.2524**	-0.3557	-0.1491	**0.1247**	0.0214	0.2280
305.2	**-.05889**	.09944	.03145	-.13002	.01224	0.0545	**-0.2538**	-0.3605	-0.1470	**0.1360**	0.0293	0.2428
610.4	**-.05182**	.09326	.02949	-.11854	.01489	0.0511	**-0.2346**	-0.3347	-0.1345	**0.1310**	0.0309	0.2311
1220.7	**-.05177**	.08995	.02844	-.11611	.01258	0.0493	**-0.2281**	-0.3246	-0.1315	**0.1245**	0.0280	0.2211
2441.4	**-.04195**	.07366	.02329	-.09465	.01074	0.0403	**-0.1863**	-0.2654	-0.1072	**0.1024**	0.0233	0.1815
4882.8	**-.03406**	.05318	.01682	-.07210	.00398	0.0291	**-0.1383**	-0.1954	-0.0812	**0.0702**	0.0131	0.1273
9765.6	**-.01826**	.02667	.00843	-.03734	.00081	0.0146	**-0.0705**	-0.0992	-0.0419	**0.0340**	0.0054	0.0626
19531.3	**-.00770**	.01629	.00515	-.01935	.00396	0.0089	**-0.0396**	-0.0571	-0.0221	**0.0242**	0.0067	0.0417
39062.5	**.00103**	.01018	.00322	-.00626	.00831	0.0056	**-0.0189**	-0.0299	-0.0080	**0.0210**	0.0101	0.0319
78125	**.00529**	.00962	.00304	-.00159	.01218	0.0053	**-0.0136**	-0.0239	-0.0032	**0.0241**	0.0138	0.0345
156250	**.00445**	.01530	.00484	-.00650	.01539	0.0084	**-0.0255**	-0.0420	-0.0091	**0.0344**	0.0180	0.0509
312500	**.00348**	.02202	.00696	-.01227	.01923	0.0121	**-0.0397**	-0.0633	-0.0160	**0.0466**	0.0230	0.0703
625000	**.05503**	.11659	.03687	-.02837	.13844	0.0639	**-0.1735**	-0.2986	-0.0483	**0.2835**	0.1584	0.4087

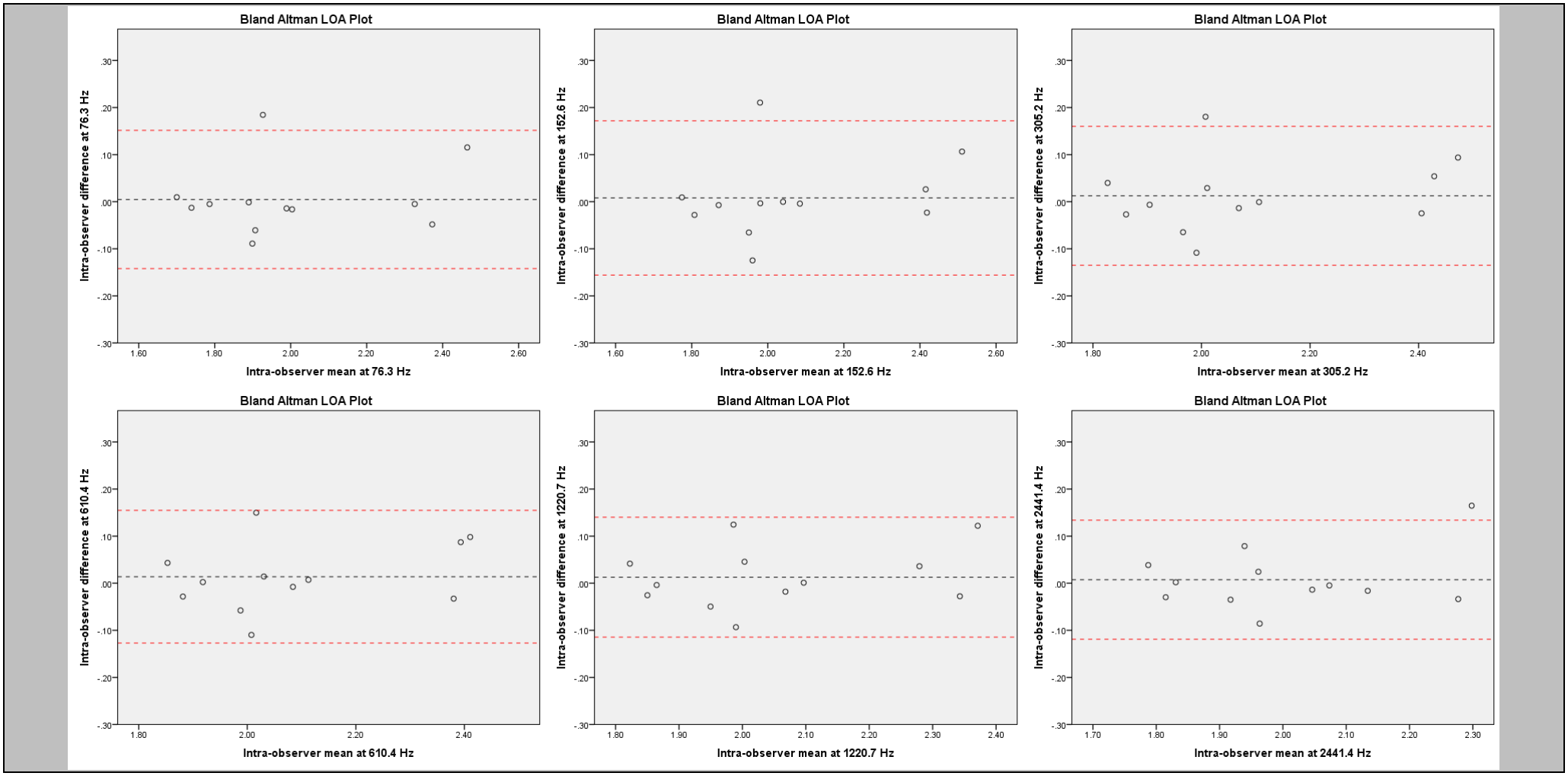


Figure 3-1 Bland Altman plots of log transformed 2N data at frequencies 1 to 6 (76.3 to 2441.4 Hz) (Within Observer Repeatability, n=12)

LOA - limits of agreement

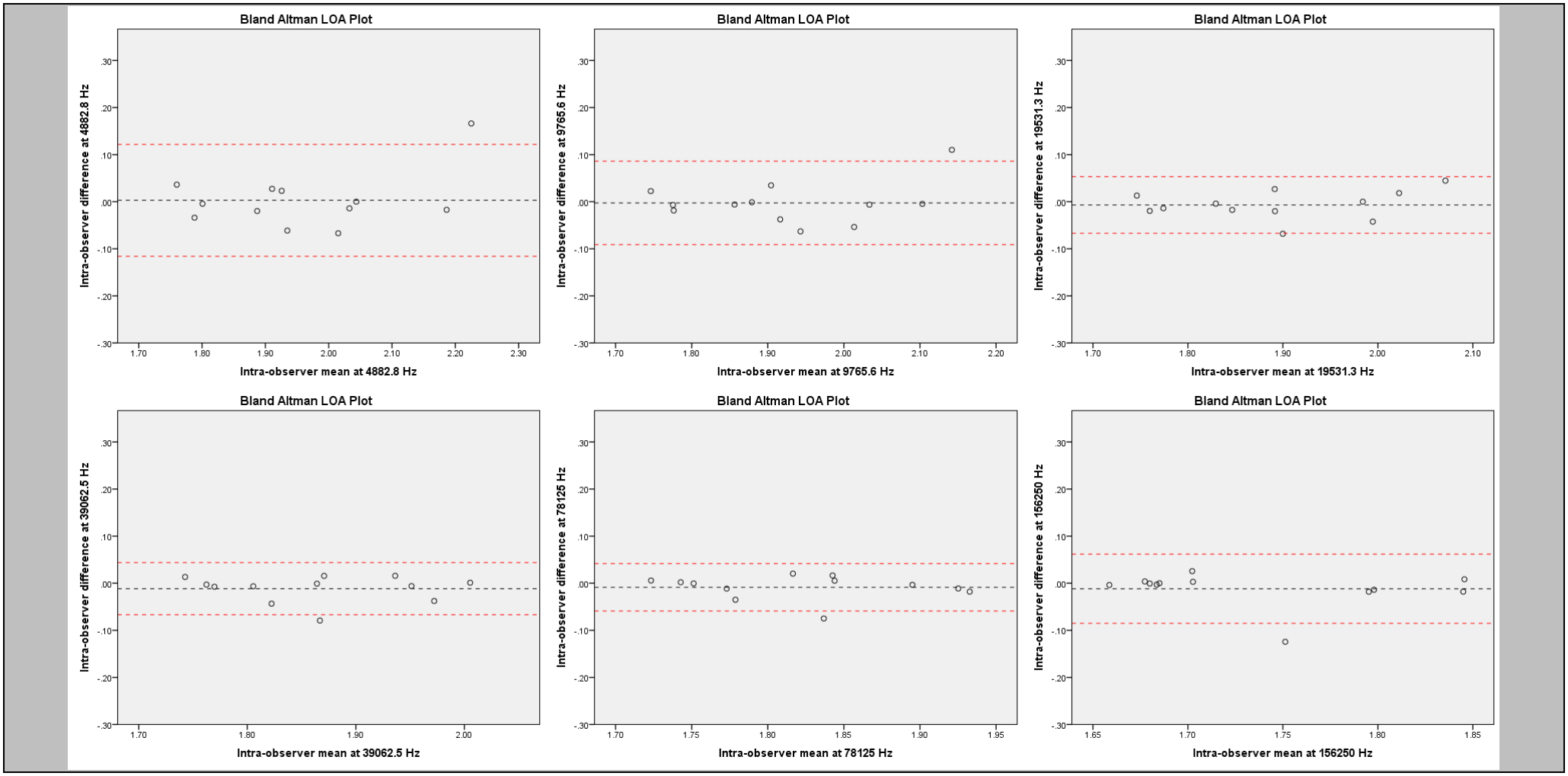


Figure 3-2 Bland Altman plots of log transformed 2N data at frequencies 7 to 12 (4882.8 to 156250 Hz) (Within Observer Repeatability, n=12)

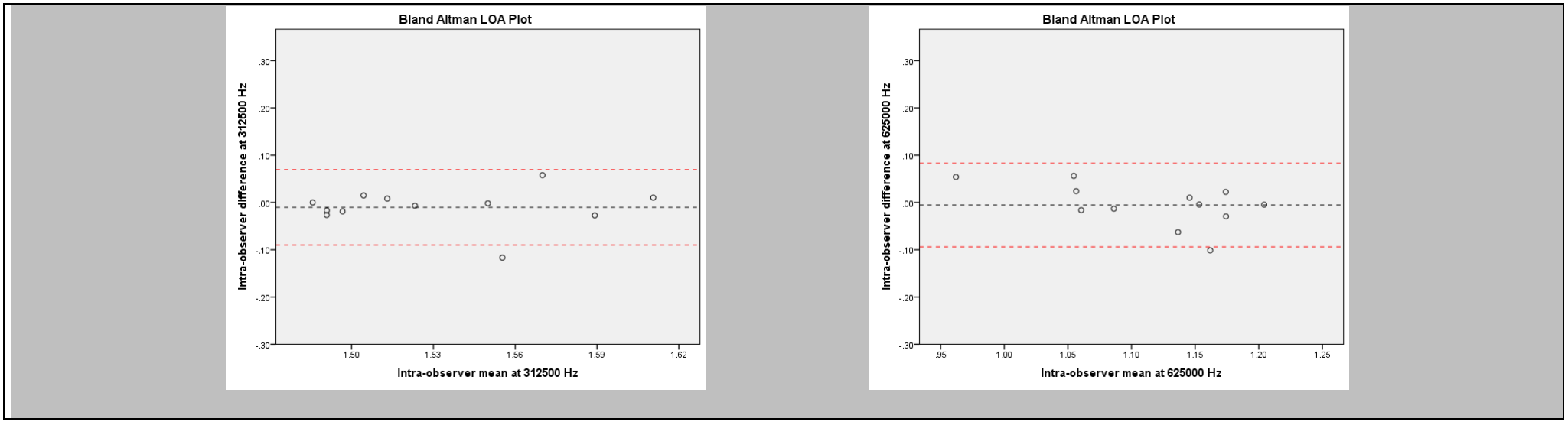


Figure 3-3 Bland Altman plots of log transformed 2N data at frequencies 13 & 14 (312500 and 625000 Hz) (Within Observer Repeatability, n=12)

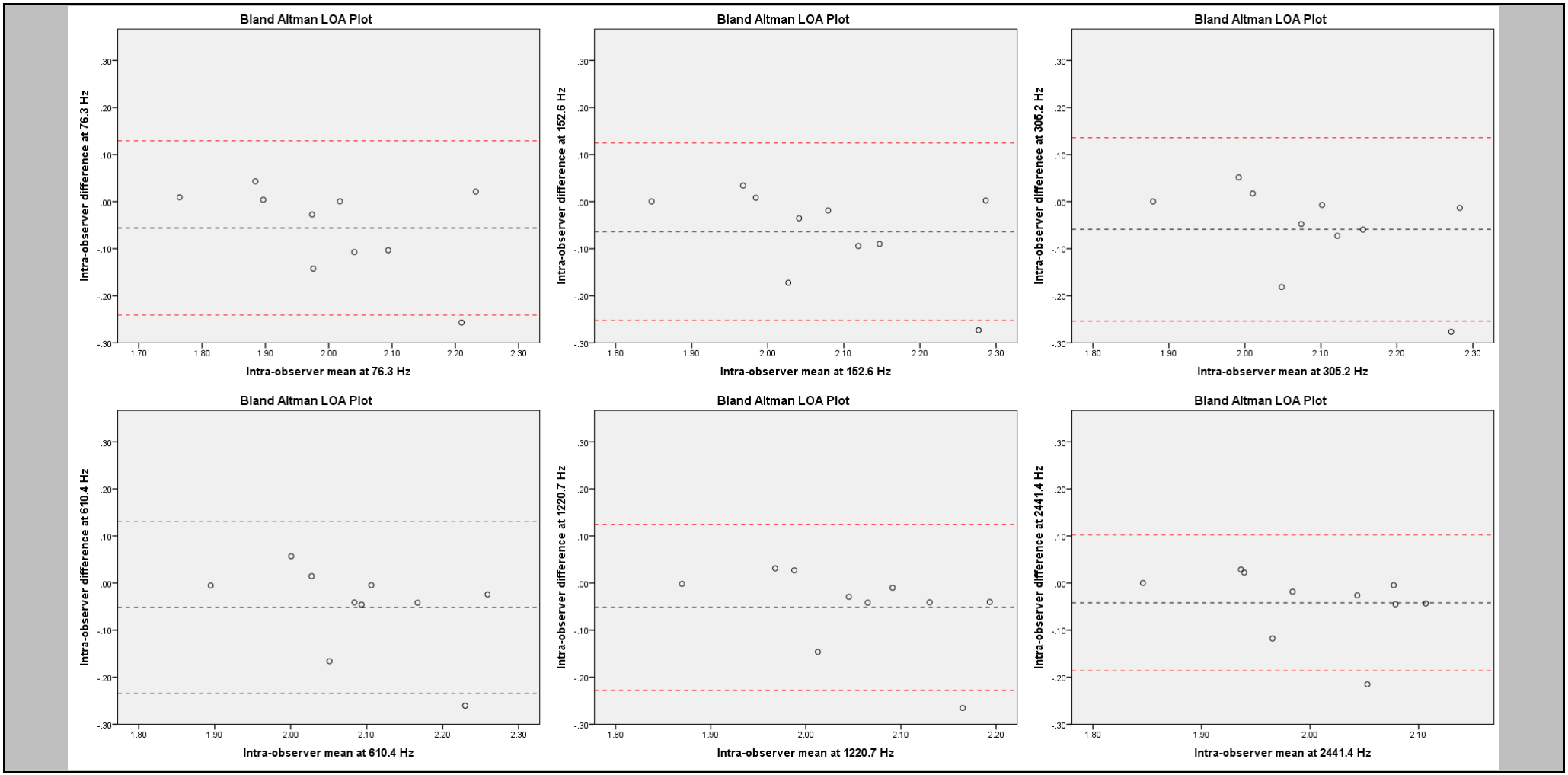


Figure 3-4 Bland Altman plots of log transformed 3N data at frequencies 1 to 6 (76.3 to 2441.4 Hz) (Within Observer Repeatability, n=12)

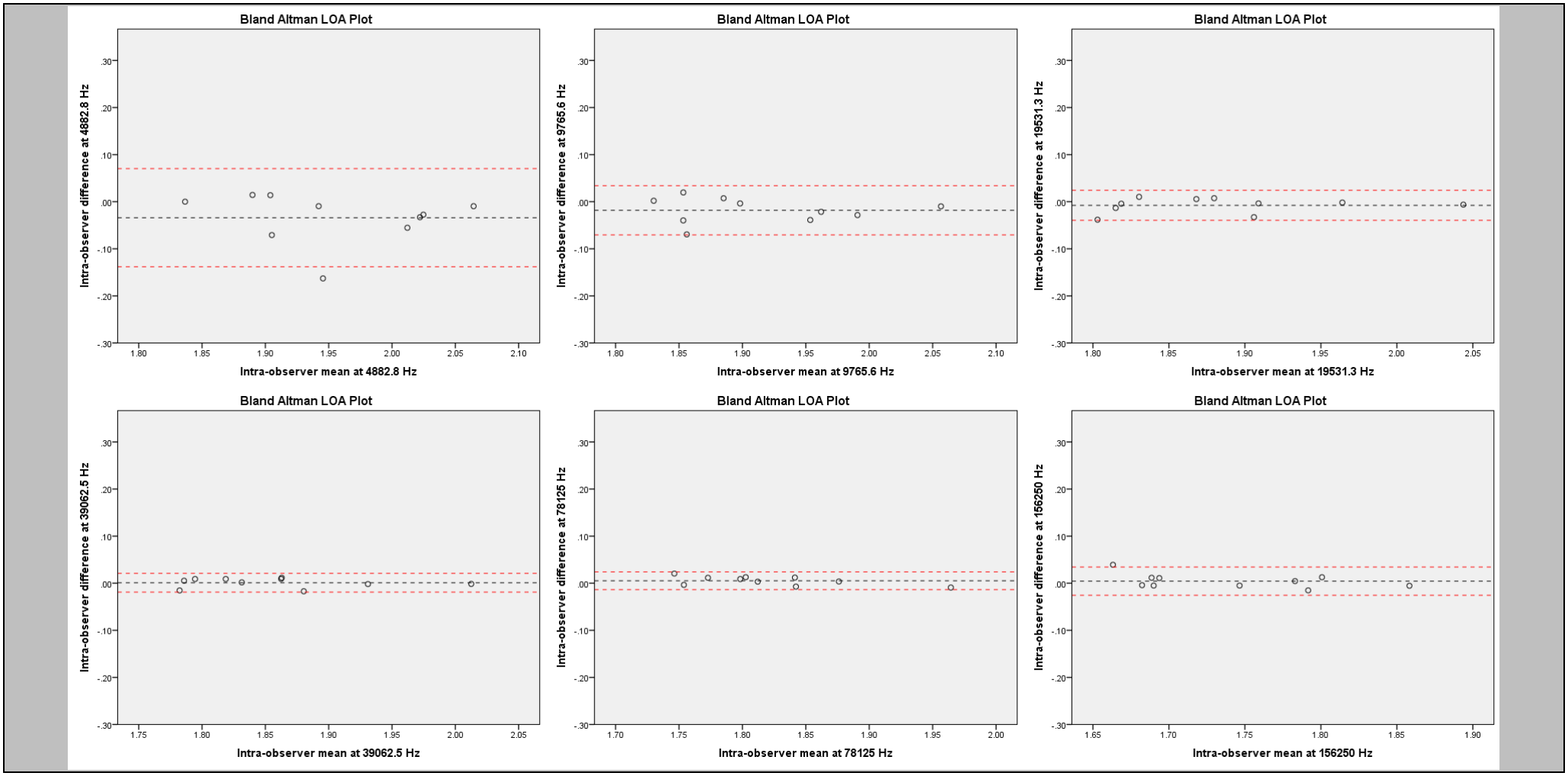


Figure 3-5 Bland Altman plots of log transformed 3N data at frequencies 7 to 12 (4882.8 to 156250 Hz) (Within Observer Repeatability, n=12)

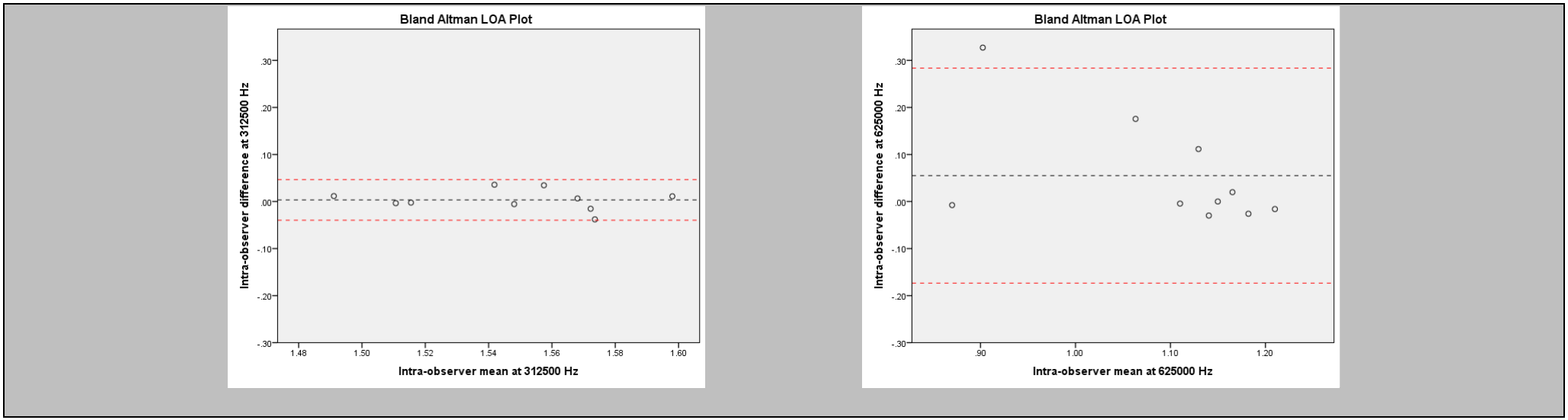


Figure 3-6 Bland Altman plots of log transformed 3N data at frequencies 13 & 14 (312500 and 625000 Hz) (Within Observer Repeatability, n=12)

3.3.2 Inter-observer reproducibility

To evaluate measurement reproducibility between observers, the first EIS readings by each clinician were compared. The ICC selected for these reproducibility analyses was calculated using a two-way, random effects model (absolute agreement) to allow generalisation of the results to other future raters[354]. When considering the 2N dataset (Table 3-7) reproducibility was poor at the lowest current frequencies (ICCs <0.5 for frequencies less than 4882 Hz, however, readings within this range were not expected to be of predictive benefit on the basis of the pilot study data[25]. Moderate reproducibility was noted in in the middle of the frequency range (4882 – 19531 Hz when considering average measures, 19531 and 39062 Hz for single measures) and good to excellent reproducibility was achieved at frequencies of 78125 and 156250 Hz (single measures) and 39062 – 312500 Hz (average measures). However, except for the ICCs at 156250 Hz, generated confidence intervals are wide, thus these ICC estimates must be viewed with some caution.

Table 3-7 Reproducibility of cervical resistivity measurements taken at a 2N application pressure (n=12)

Current frequency (Hz)	REPRODUCIBILITY Single measures			REPRODUCIBILITY Average measures		
	ICC (2,2)	95% CI		ICC (2,2)	95% CI	
76.3	**.041**	-.500	.575	**.079**	-2.001	.730
152.6	**.092**	-.467	.610	**.169**	-1.754	.757
305.2	**.123**	-.451	.630	**.219**	-1.645	.773
610.4	**.181**	-.408	.666	**.307**	-1.379	.800
1220.7	**.285**	-.310	.723	**.444**	-.899	.839
2441.4	**-.258**	.753	2.024	**-.695**	.859	2.024
4882.8	**.399**	-.211	.781	**.570**	-.536	.877
9765.6	**.453**	-.159	.807	**.624**	-.377	.893
19531.3	**.549**	-.031	.848	**.709**	-.064	.917
39062.5	**.650**	.127	.886	**.788**	.226	.940
78125	**.762**	.347	.926	**.865**	.516	.961
156250	**.920**	.747	.976	**.959**	.855	.988
312500	**.649**	.130	.886	**.787**	.230	.939
625000	**.288**	-.199	.705	**.447**	-.497	.827

At a 3N application pressure, the calculated ICCs suggested good reproducibility at a higher proportion of applied current frequencies (39062 – 312500 Hz for single measures and 9765 – 625000 Hz for average measures). However the confidence intervals were

wider than those generated for the 2N dataset, with a lower limit of <0.5 in the majority of cases.

Table 3-8 Reproducibility of cervical resistivity measurements taken at a 3N application pressure (n=10)

Current frequency (Hz)	REPRODUCIBILITY Single measures			REPRODUCIBILITY Average measures		
	ICC (2,2)	95% CI		ICC (2,2)	95% CI	
76.3	**.209**	-.479	.725	**.346**	-1.836	.840
152.6	**.159**	-.534	.702	**.274**	-2.291	.825
305.2	**.229**	-.456	.734	**.373**	-1.675	.846
610.4	**.291**	-.379	.760	**.451**	-1.222	.864
1220.7	**.355**	-.314	.789	**.524**	-.916	.882
2441.4	**.470**	-.166	.834	**.640**	-.398	.910
4882.8	**.563**	-.013	.867	**.720**	-.026	.929
9765.6	**.613**	.074	.884	**.760**	.137	.939
19531.3	**.712**	.235	.918	**.832**	.380	.957
39062.5	**.756**	.317	.932	**.861**	.481	.965
78125	**.790**	.385	.942	**.882**	.556	.970
156250	**.761**	.302	.935	**.865**	.464	.966
312500	**.764**	.278	.936	**.866**	.435	.967
625000	**.733**	.249	.926	**.846**	.399	.961

Bland Altman analysis was performed as detailed in section 3.2.3, this time evaluating agreement between observers. Table 3-9 summarises the mean differences observed, prior to log transformation. Again, difference magnitude fell with increasing current frequency. Measurements by Observer 2 were, on average, higher than those by Observer 1, except at 625000 Hz. Greater mean differences are seen between rather than within observers (Table 3-9 vs Table 3-4). This is consistent with the ICC analysis, suggesting the repeatability of CR readings is somewhat more consistent than the reproducibility.

Table 3-9 Summary Table of Average Repeat Measures (Non transformed data) Between Observer Reproducibility at 2N (n=12) and 3N (n=10)

Current Freq (Hz)	2N Reproducibility			3N Reproducibility		
	Mean R1 Observer 1 (Ohm)	Mean R1 Observer 2 (Ohm)	Mean Difference (R1 – R2)	Mean R1 Observer 1 (Ohm)	Mean R1 Observer 2 (Ohm)	Mean Difference (R1 – R2)
76.3	101.33	145.86	**-44.53**	79.70	97.48	**-17.79**
152.6	121.18	169.98	**-48.80**	95.11	113.62	**-18.51**
305.2	124.19	169.75	**-45.56**	99.26	117.30	**-18.03**
610.4	120.72	159.32	**-38.60**	98.87	116.59	**-17.72**

1220.7	106.90	138.49	**-31.59**	88.29	105.33	**-17.04**
2441.4	90.84	114.14	**-23.30**	77.32	92.20	**-14.88**
4882.8	77.87	92.11	**-14.24**	67.78	80.96	**-13.18**
9765.6	67.79	76.25	**-8.45**	61.40	72.72	**-11.31**
19531.3	59.94	64.07	**-4.13**	56.98	64.55	**-7.57**
39062.5	53.54	55.13	**-1.58**	52.83	57.44	**-4.61**
78125	46.48	46.99	**-0.52**	47.27	50.17	**-2.90**
156250	34.26	34.46	**-0.20**	35.74	37.39	**-1.66**
312500	13.80	13.91	**-0.10**	15.53	15.63	**-0.11**
625000	-6.96	-8.56	**1.60**	-6.56	-7.68	**1.12**

Tables 3-10 and 3-11 summarise the calculated limits of agreement at 2 and 3N for the reproducibility dataset following log transformation. These were then utilised to produce the series of Bland Altman plots in figures 3-7 to 3-12. Examination of these graphs again confirms data homoscedasticity, with a random scatter of differences noted, regardless of measurement magnitude. The slight bias between observers, with Observer 2 producing greater measurements than Observer 1 is evident as the plotted mean difference (dotted central line) is <0 on almost every plot.

The limits of agreement are wider than those generated for the repeatability dataset (indeed the scale differs slightly for the reproducibility plots (Figures 3-7 to 3-12) compared to the repeatability plots (Figures 3-1 to 3-6)) suggesting less agreement for readings between rather than within observers. Again, the limits of agreement narrow with increasing current frequency, and the LOAs at 156250 and 312500 Hz are similar for within and between observer comparisons, suggesting improved measurement reliability at higher frequencies.

When comparing the 2N to 3N reproducibility plots (Figures 3-7 to 3-9 vs Figures 3-10 to 3-12), the narrowing of LOAs with increasing frequency is more rapid for the 3N dataset, but the narrowest LOAs are in fact achieved at a 2N application pressure with current frequencies of 156250 and 312500 Hz (see figures 3-8, bottom right plot and 3-9, left hand plot), which is consistent with the ICC analyses. Given this, neither application pressure provides clearly superior reproducibility.

Table 3-10 Mean Differences and Limits of Agreement on log transformed 2N data (Between Observer Reproducibility) (n=10)

Current frequency (Hz)	Mean Difference (R1 – R2)	SD	SE	Lower 95% CI of mean	Upper 95% CI of mean	SE for Limits of Agreement	Lower Limit of Agreement	LLOA lower 95% CI	LLOA upper 95% CI	Upper Limit of Agreement	ULOA lower 95% CI	ULOA upper 95% CI
76.3	**-0.128**	0.388	0.112	-0.375	0.118	0.194	**-0.889**	-1.269	-0.509	**0.632**	0.252	1.013
152.6	**-0.119**	0.377	0.109	-0.359	0.121	0.189	**-0.859**	-1.228	-0.489	**0.621**	0.251	0.991
305.2	**-0.102**	0.345	0.100	-0.321	0.117	0.172	**-0.778**	-1.116	-0.440	**0.574**	0.236	0.912
610.4	**-0.085**	0.304	0.088	-0.278	0.109	0.152	**-0.681**	-0.979	-0.383	**0.512**	0.214	0.810
1220.7	**-0.074**	0.269	0.078	-0.245	0.097	0.135	**-0.602**	-0.866	-0.338	**0.454**	0.190	0.718
2441.4	**-0.059**	0.239	0.069	-0.211	0.092	0.119	**-0.527**	-0.761	-0.293	**0.409**	0.175	0.643
4882.8	**-0.040**	0.204	0.059	-0.170	0.090	0.102	**-0.440**	-0.641	-0.240	**0.360**	0.160	0.561
9765.6	**-0.024**	0.168	0.049	-0.131	0.084	0.084	**-0.354**	-0.519	-0.189	**0.307**	0.142	0.472
19531.3	**-0.012**	0.125	0.036	-0.091	0.068	0.063	**-0.257**	-0.380	-0.134	**0.233**	0.111	0.356
39062.5	**-0.003**	0.088	0.025	-0.059	0.053	0.044	**-0.175**	-0.261	-0.089	**0.169**	0.083	0.255
78125	**0.000**	0.056	0.016	-0.036	0.035	0.028	**-0.110**	-0.165	-0.055	**0.109**	0.054	0.164
156250	**-0.001**	0.029	0.008	-0.019	0.017	0.014	**-0.057**	-0.085	-0.029	**0.055**	0.027	0.083
312500	**-0.003**	0.035	0.010	-0.025	0.019	0.017	**-0.071**	-0.105	-0.037	**0.066**	0.031	0.100
625000	**0.076**	0.136	0.039	-0.011	0.162	0.068	**-0.191**	-0.325	-0.058	**0.343**	0.209	0.476

Table 3-11 Mean Differences and Limits of Agreement on log transformed 3N data (Between Observer Reproducibility) (n=10)

Current frequency (Hz)	Mean Difference (R1 – R2)	SD	SE	Lower 95% CI of mean	Upper 95% CI of mean	SE for Limits of Agreement	Lower Limit of Agreement	LLOA lower 95% CI	LLOA upper 95% CI	Upper Limit of Agreement	ULOA lower 95% CI	ULOA upper 95% CI
76.3	**-0.049**	0.211	0.067	-0.200	0.102	0.116	**-0.463**	-0.689	-0.236	**0.364**	0.138	0.591
152.6	**-0.044**	0.207	0.065	-0.192	0.104	0.113	**-0.449**	-0.671	-0.227	**0.361**	0.139	0.583
305.2	**-0.043**	0.179	0.057	-0.171	0.085	0.098	**-0.394**	-0.586	-0.202	**0.307**	0.115	0.499
610.4	**-0.043**	0.158	0.050	-0.157	0.070	0.087	**-0.354**	-0.524	-0.184	**0.267**	0.097	0.437
1220.7	**-0.041**	0.153	0.048	-0.151	0.068	0.084	**-0.341**	-0.506	-0.177	**0.259**	0.094	0.423
2441.4	**-0.039**	0.134	0.042	-0.135	0.056	0.073	**-0.302**	-0.445	-0.158	**0.223**	0.079	0.367
4882.8	**-0.042**	0.112	0.035	-0.122	0.038	0.061	**-0.261**	-0.381	-0.141	**0.177**	0.057	0.297
9765.6	**-0.042**	0.095	0.030	-0.110	0.026	0.052	**-0.229**	-0.331	-0.127	**0.144**	0.042	0.246
19531.3	**-0.032**	0.075	0.024	-0.085	0.021	0.041	**-0.178**	-0.259	-0.098	**0.114**	0.034	0.194
39062.5	**-0.022**	0.060	0.019	-0.065	0.021	0.033	**-0.140**	-0.204	-0.075	**0.096**	0.031	0.160
78125	**-0.015**	0.047	0.015	-0.049	0.019	0.026	**-0.108**	-0.159	-0.057	**0.078**	0.027	0.129
156250	**-0.011**	0.050	0.016	-0.046	0.025	0.027	**-0.108**	-0.161	-0.055	**0.087**	0.034	0.140
312500	**0.000**	0.029	0.009	-0.021	0.021	0.016	**-0.057**	-0.089	-0.026	**0.057**	0.026	0.089
625000	**0.040**	0.069	0.022	-0.009	0.090	0.038	**-0.095**	-0.169	-0.021	**0.176**	0.102	0.250

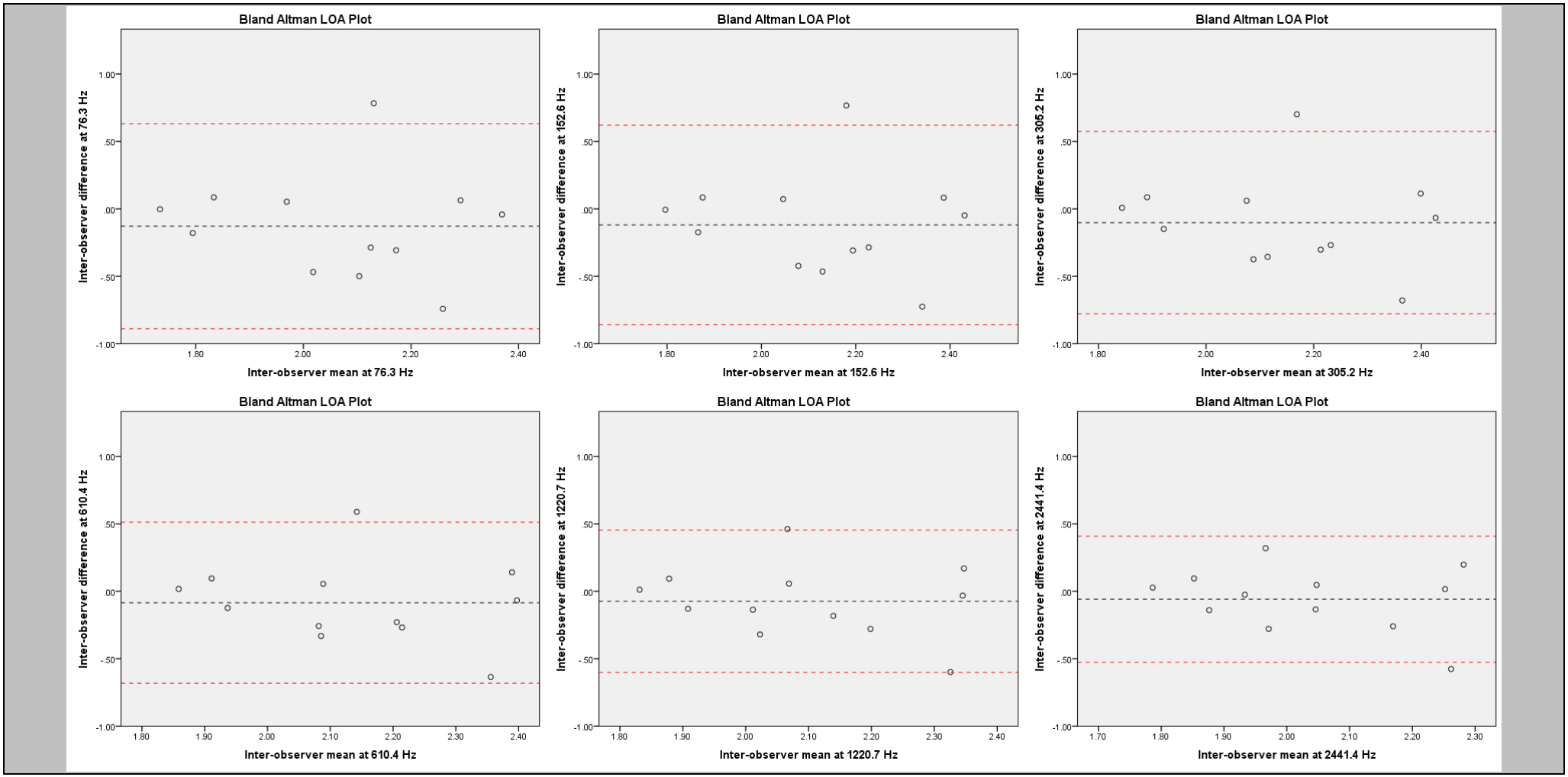


Figure 3-7 Bland Altman plots of log transformed 2N data at frequencies 1 to 6 (76.3 to 2441.4 Hz) (Between Observer Reproducibility, n=10)

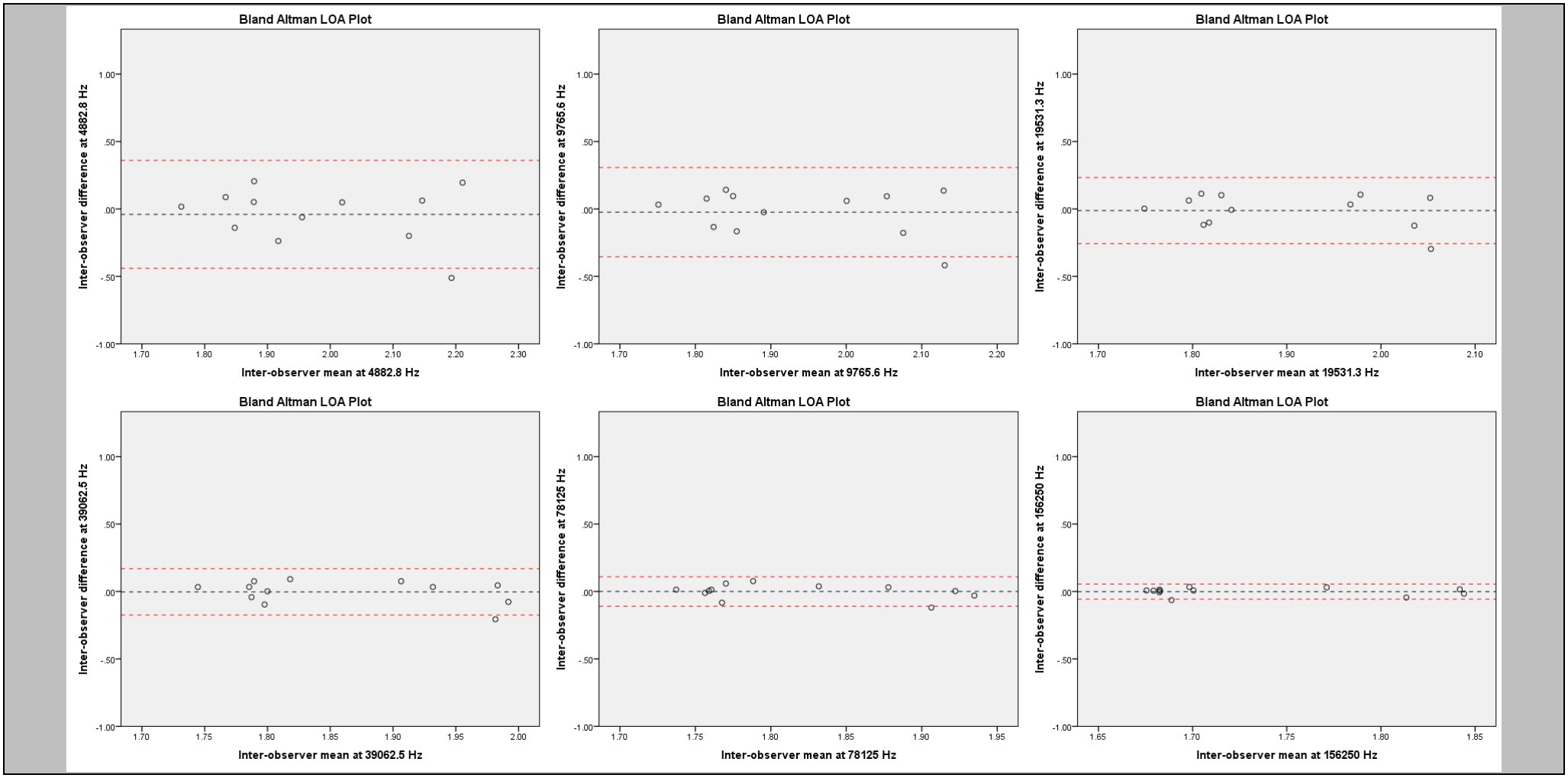


Figure 3-8 Bland Altman plots of log transformed 2N data at frequencies 7 to 12 (4882.8 to 156250 Hz) (Between Observer Reproducibility, n=10)

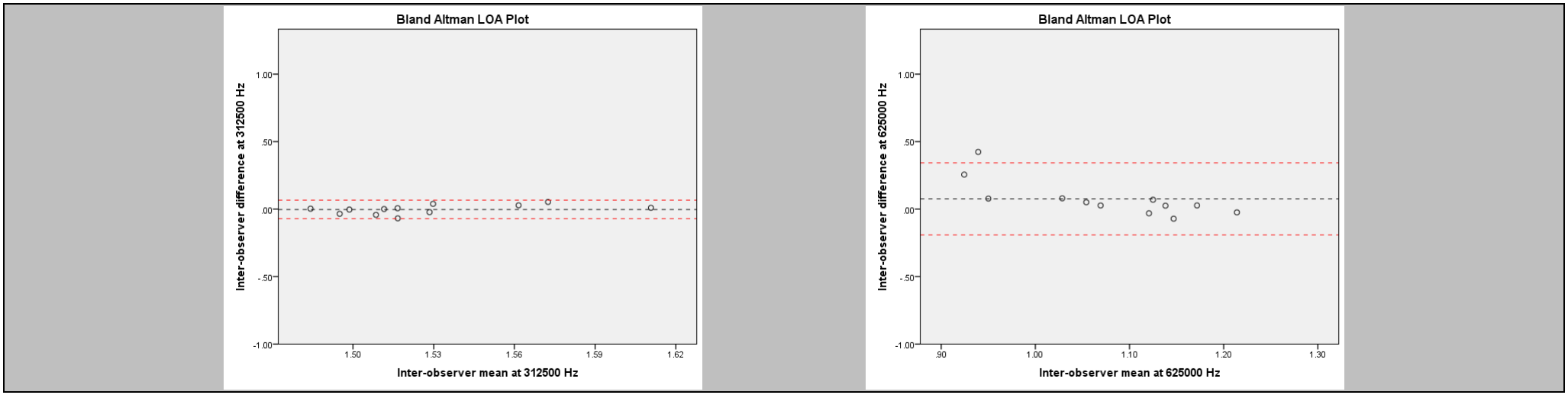


Figure 3-9 Bland Altman plots of log transformed 2N data at frequencies 13 & 14 (312500 and 625000 Hz) (Between Observer Reproducibility, n=10)

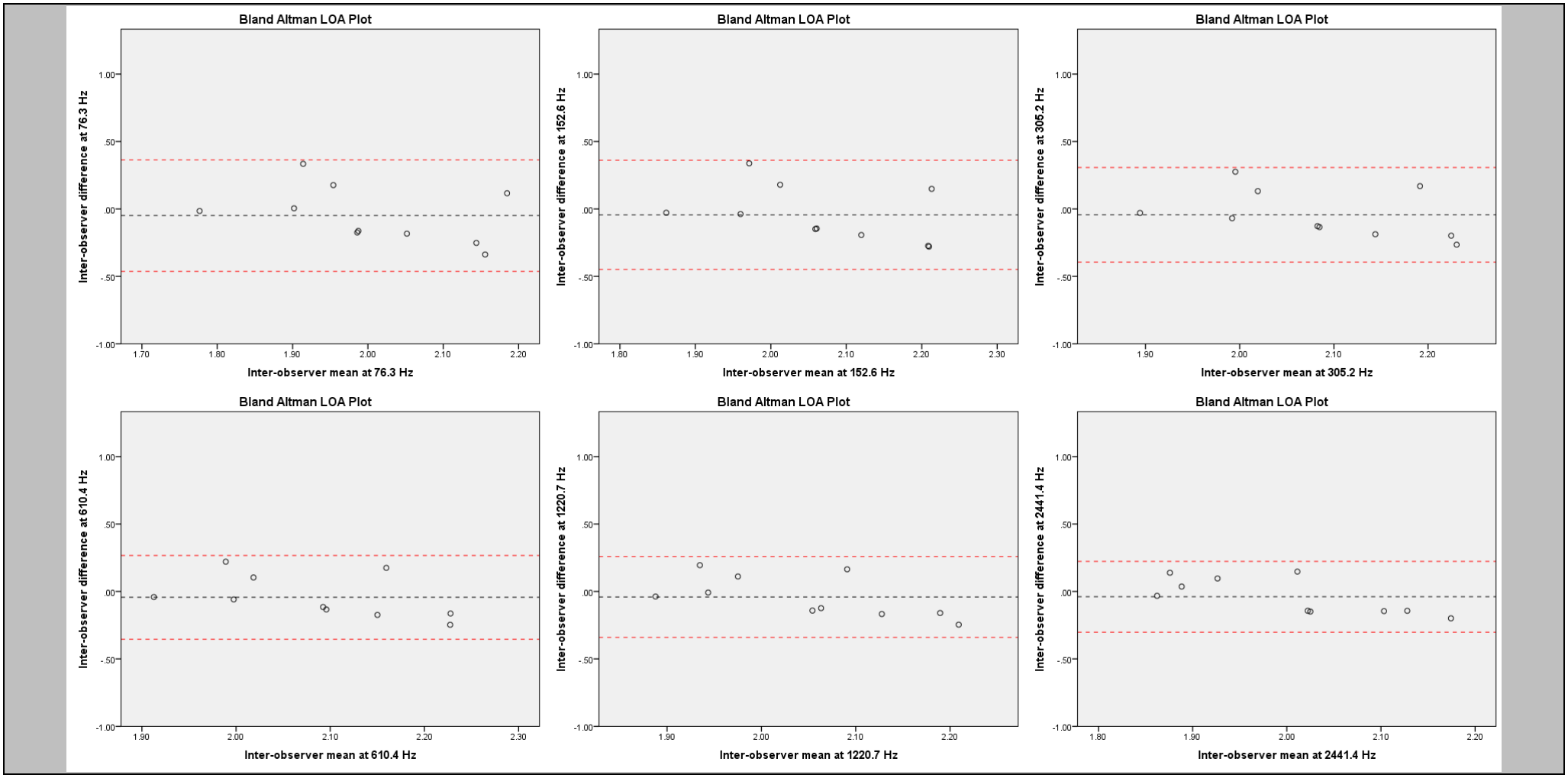


Figure 3-10 Bland Altman plots of log transformed 3N data at frequencies 1 to 6 (76.3 to 2441.4 Hz) (Between Observer Reproducibility, n=10)

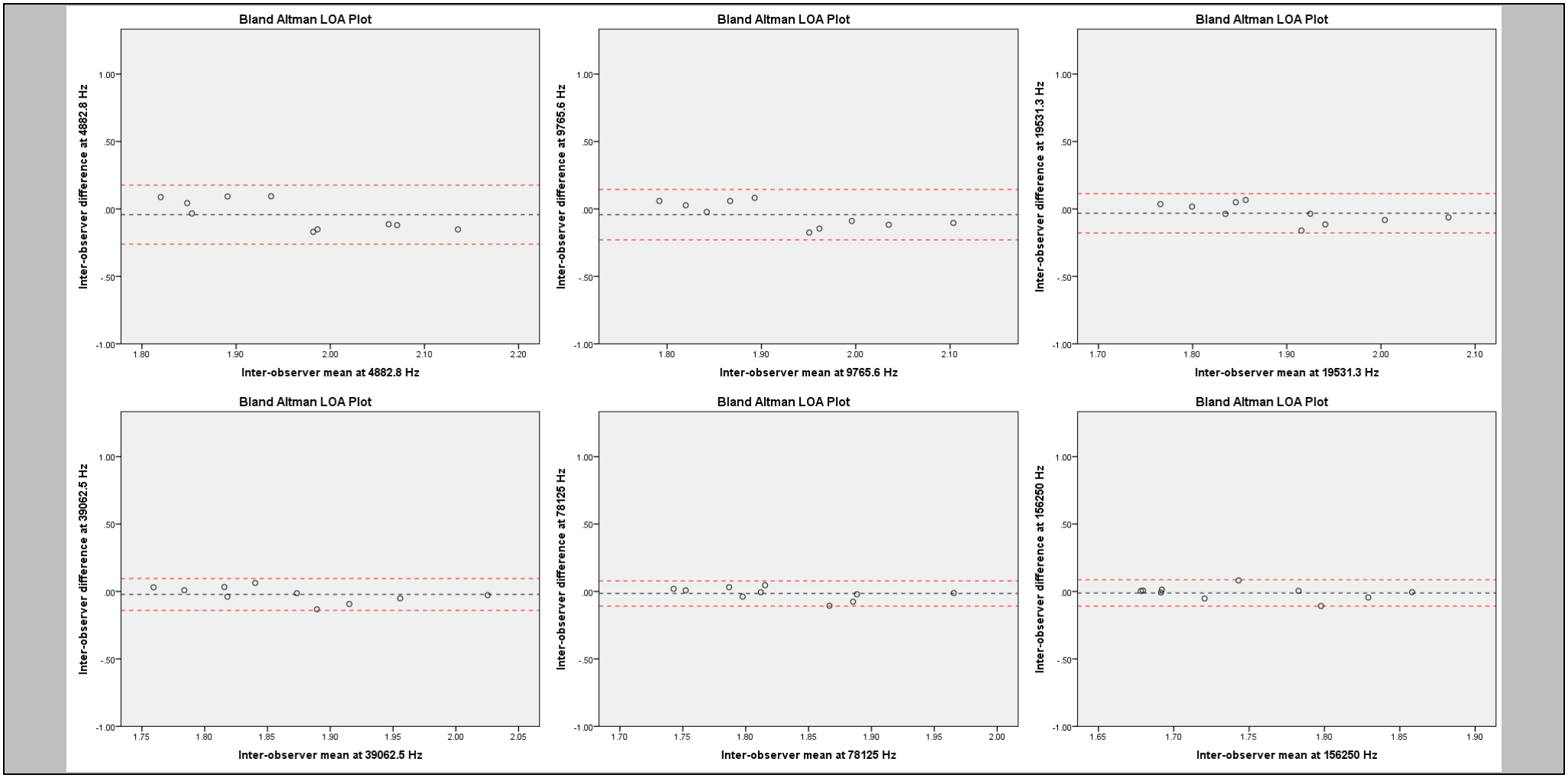


Figure 3-11 Bland Altman plots of log transformed 3N data at frequencies 7 to 12 (4882.8 to 156250 Hz) (Between Observer Reproducibility, n=10)

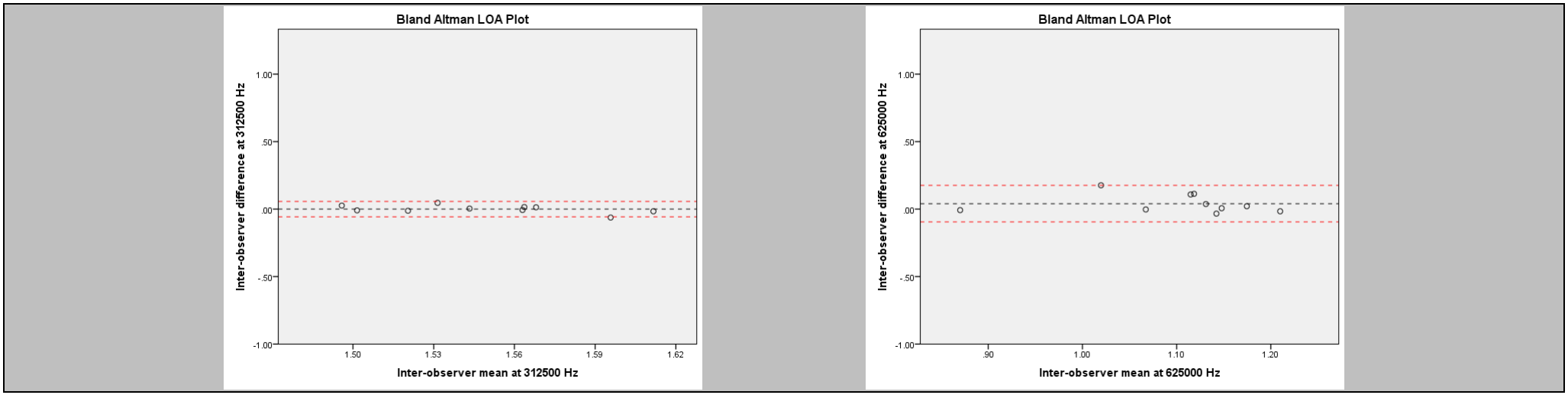


Figure 3-12 Bland Altman plots of log transformed 3N data at frequencies 13 & 14 (312500 and 625000 Hz) (Between Observer Reproducibility, n=10)

3.3.3 Variation in Cervical Resistivity with Tip Pressure

Previous research has investigated the effect of probe application pressure on tissue resistivity readings[304, 349]. In this study the variation in cervical impedance with three different tip pressures is demonstrated. A soft application pressure of 1N produces lower mean resistivity readings than those obtained at 2 and 3N, although the sample size of readings at 1N only included three women, thus hypothesis testing was not possible. The difference between spectra obtained at the two higher pressures was less marked, particularly at higher current frequencies, and non-parametric testing of the non-transformed data revealed no significant differences at any frequency between 2 and 3N readings (all p values>0.05, Wilcoxon signed ranks test). Figure 3-13 demonstrates the variation in mean cervical impedance at each application pressure for Observer 1.

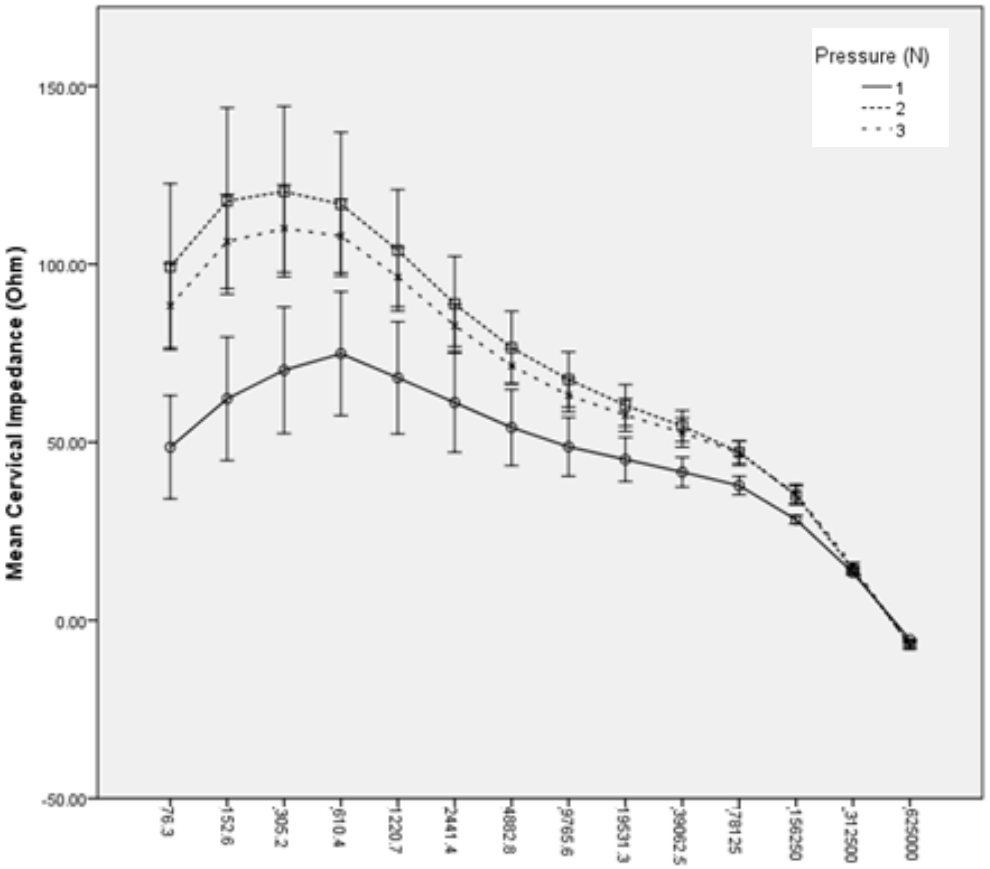


Figure 3-13 Variability of Cervical Impedance Spectra by Application Pressure
(Mean reading obtained by observer 1; n=3 for 1N, n=12 for 2N and n=10 for 3N).

3.4 Discussion

This study aimed to assess how the addition of a pressure sensor and observer blinding affected the reliability of CR measurements obtained with the Mark V EIS probe. Establishing

acceptable repeatability and reproducibility was a pre-requisite prior to employing the probe for use in larger cohort studies.

The data presented above demonstrate good within-observer repeatability of CR readings, with high ICCs at both application pressures. In the pilot study of PTB prediction by cervical EIS, readings obtained at mid to high current frequencies proved most predictive of early delivery, with optimal test performance at 39 kHz[25]. Therefore, the good repeatability obtained at similar frequencies (indicated by high ICCs and narrow limits of agreement from 9.7 to 312 kHz) during this variability study is encouraging. In fact, this could in part explain the observations of the pilot study – it is possible that EIS more reliably distinguishes ripe from unripe cervices in the higher frequency range, whereas at lower frequencies its discriminatory ability is confounded by inherent measurement variability. When comparing repeatability at 2 and 3N, high ICCs with narrow confidence intervals were obtained at a wider range of frequencies at 2N, however limits of agreement were slightly narrower at 3N. Overall this suggests similar repeatability at both pressures, with no clear improvement yielded with firmer tip application.

Previous work with an earlier iteration of the EIS probe demonstrated improved repeatability with increasing application pressure[304], however that study compared approximate application pressures of 1 and 2N (with no real-time force measurement) and did not evaluate the effect of forces above 2N. This work therefore builds upon those findings, suggesting that the improvement in repeatability with firmer pressures plateaus beyond 2N. It may be that, at 1N, application pressures are insufficient to provide reliable contact between the EIS electrodes and cervical epithelial cells thus measurements may be affected by variable amounts of cervical mucous, or the pericellular matrix of the epithelial cells. It is plausible that higher pressures facilitate direct contact between electrodes and epithelium, but that once this contact has been achieved, limited difference is then observed. This is supported by the observations presented in section 3.3.3, showing a greater separation between CR readings at 1N vs the higher application pressures, but no significant difference between CR magnitude at 2 and 3N.

The repeatability ICCs obtained by this study are fractionally lower than those yielded by earlier research (ICCs at 2N 0.94 to 0.99 with 3mm tip and 0.91 – 0.99 with a 12mm tip in the study by Jokhi *et al.*[304]), although this is only true at 3 of the 14 current frequencies. Overall, test performance still suggests good to excellent repeatability, therefore the

addition of operator blinding (with concealment of impedance curves) has not unduly affected repeatability. Use of the blinded probe in the larger cohort studies will therefore allow a potential source of bias to be removed, without compromising data reliability.

CR measurements obtained by different observers were more variable than within-observer repeats (i.e. the repeatability of Mark V probe measurements exceeds reproducibility). However, at mid to high current frequencies (≥39 kHz), calculated ICCs suggest acceptable reproducibility, especially if average measures are used (with ICCs of 0.79-0.95 at 2N and 0.86-o.85 at 3N). Readings with the Mark V probe were most reproducible when a current frequency of 156 kHz was applied with a tip pressure of 2N (ICC 0.92, 95% CI 0.75 – 0.98 single measures; ICC 0.96, 95% CI 0.86 – 0.99 average measures) – at other current frequencies, the 95% CIs are broader, rendering the ICC estimates less precise. The measurement reproducibility obtained with this probe is superior to that of earlier devices. In the study by Jokhi *et al.,* the highest ICCs were generated by firm application of a 12mm probe tip (ICC 0.64) with only poor reproducibility noted for the 3mm tip, regardless of application pressure (highest ICC 0.16)[304]. Therefore in this dataset, although significant variation in reproducibility was noted with applied current frequency, and reliability appears poorer at low frequencies, the addition of the pressure sensor has demonstrably improved test performance, with less inter-observer variation seen at the frequencies of greatest predictive interest.

As for intra-observer repeatability, inter-observer reproducibility was similar at 2 and 3N pressures in this study. The likeliest explanation is again that both pressures are sufficient to achieve epithelial-electrode contact – beyond this, the pressure effect appears minimal.

Although measurement reproducibility appears acceptable for clinical use, possible reasons for the difference in repeatability and reproducibility must be considered. Cervical tissue is known to be somewhat heterogeneous[101]. Measurements in this study were obtained at a "12 o' clock" position on the anterior lip of the cervix, avoiding visible ectopy when present. Inevitably, choice of this position involves a degree of subjectivity. Whilst one observer may be able to accurately place the EIS tip repeatedly in their selected location, it is credible that a second observer might select a slightly different location. Thus, reproducibility may be lower than repeatability due to small variations in tip position and subtle differences in the underlying tissue composition at adjacent measurement points. However, given that both repeatability and reproducibility improve at higher frequencies, and that tissue penetration

is deeper in this frequency range[295], it may be that the deeper epithelial and stromal layers evaluated by this range are less heterogeneous than the lumen-facing, superficial epithelial cells (which are more exposed to chemical, mechanical and microfloral challenges from the vaginal environment).

It is also plausible that EIS readings exhibit a 'training effect' where more experienced users can obtain more consistent measurements than operators who are new to the technique. The procedure for obtaining readings is not complex, but it can be challenging if certain patient characteristics are present (e.g. obesity, significant vaginal laxity, a highly mobile cervix). Whilst both observers in this study had prior experience of EIS measurement, observer 1 had more measurement experience than observer 2. Therefore the high repeatability exhibited by observer 1 may in part reflect their expertise with the Mark V probe.

Observer 2 tended to obtain slightly higher CR measurements than Observer 1 (the negative bias illustrated on figures 3-7 to 3-12. Interestingly, similar trends were seen in earlier variability work[304], and also in *in vitro* studies examining the effects of variable tip application pressures[349] where impedance tended to increase during repeated measurements. González-Correa *et al.*[349] attributed this to progressive tissue deformation due to the compressive force of the EIS tip causing reduction of the extracellular space and squeezing of intracellular fluid. However they used much higher application pressures (1-50 kPa) than those employed for clinical research and actually observed indentation of their tissue samples following measurement. Whilst subtle deformation of superficial tissue may have occurred during this study, another explanation is that, by the time of the fifth and sixth repeat (observer 2's readings), practical measurement difficulties became more likely. These included cervical mobility (necessitating repositioning of the speculum) and bleeding. Patients with frank blood loss were excluded, so this is unlikely to explain the observed differences. Overall, abrasion from prolonged examination or repeated measurements may act to increase CR via compressive/sloughing effects on cervical mucous and/or the epithelial pericellular matrix. The presence of a thicker mucous layer overlying surface epithelium is associated with lower impedance values, as a greater proportion of current flows through the mucous, bypassing the tissue itself[295]. Mechanical interruption of this layer could plausibly cause an increase in impedance measurements.

This study has some limitations. In particular, practical problems with the EIS probe affected the choice of methodology. The engineering required to incorporate the additional features of the Mark V probe (in particular the addition of the accelerometer which forms the basis of the pressure sensor) was highly complex. The medical physics team wrote a bespoke piece of software in order to allow selection of the multiple application pressures required to perform the study. Unfortunately this software was a source of some problems during the conduct of the study. 'Arming' the probe at each change of pressure took longer than anticipated, resulting in a delay between repeat readings. This was thought to be due to interference in the Bluetooth connectivity between probe and laptop in the operating theatre environment and unanticipated software bugs, which the engineering team worked to resolve over the course of the study. In practice this meant that, although additional repeat readings by both observers would have been methodologically ideal, a pragmatic approach was necessary. Thus, the focus was on obtaining an adequate number of repeats to perform the planned analyses, without compromising patient care and unduly delaying the performance of participants' caesarean sections.

3.5 Conclusions

Measurements of cervical resistivity with the Mark V EIS Probe are highly repeatable at both 2 and 3N, particularly at high current frequencies. Measurement reproducibility is limited at low frequencies, but improves significantly in the frequency range which is most likely to be useful for PTB prediction. The addition of blinding does not appear to have compromised test reliability, and the incorporation of the pressure sensor is likely to be responsible for the improved reproducibility exhibited here compared to earlier EIS probes[304]. Therefore the probe modifications represent an important advance in EIS technology, and have enhanced the instrument prior to use in further clinical studies.

Both repeatability and reproducibility were similar at 2 and 3N application pressures, and CR magnitude did not differ significantly using the higher force. These findings informed the design of the substantive cohort studies: a 2N application pressure was chosen as it was felt to provide the best balance of test reliability, patient comfort and speed and ease of measurement. Repeat measures were also planned, to improve reliability and enhance the strength of the cohort study datasets.

Chapter 4 - Study of Asymptomatic Women: Results and Discussion

4.1 Introduction

The most successful screening programmes target disease processes at an early stage and institute effective preventative or curative treatments[340]. Although there is still a degree of uncertainty regarding the precise pathogenesis of PTB, a proportion of patients (e.g. those with cervical weakness) have an asymptomatic prodrome before PTL, which might be amenable to treatment with prophylactic cerclage or progesterone. Consequently, a primary aim of this thesis is to assess the predictive performance of cervical impedance measurements in asymptomatic women during the mid-trimester of pregnancy. Pilot data has demonstrated significantly lower cervical impedance in high-risk women destined to deliver preterm[25]. This study aimed to evaluate the discriminatory ability of EIS in a larger asymptomatic cohort to assess its utility as a standalone test and as an addition to conventional PTB screening.

It is widely acknowledged that a prior history of preterm birth is one of the strongest risk factors for recurrent early delivery[355]. Women with previous PTB therefore represent a population who are particularly likely to benefit from accurate screening. By the time of presentation with symptoms of threatened preterm labour, therapeutic options are limited. Therefore, pre-specified subgroup analyses were planned to investigate test performance in asymptomatic women at both high and low risk of PTB.

4.2 Study design and population

The sample size calculation for this study and details of the conduct of study visits are described in Chapter 2. In summary, a minimum sample size of 200 AHR women was required in order to demonstrate the discriminatory ability of EIS with sufficient power. A formal sample size calculation for the ALR subgroup was not possible due to a lack of data regarding predictive performance in this group – a pragmatic target of 250 ALR women was therefore set.

In total 211 AHR women were recruited to allow some leeway to accommodate loss to follow up. The majority (n = 187, 88.6%) were recruited at booking and attended two study visits at 20-22 and 26-28 weeks. 19 participants (9%) attended Visit 1 only: 5 of

these women delivered before visit 2, the remainder were either unable to attend a second study visit, were lost to follow up or attended a visit but EIS measurements were not obtained due to technical issues. 5 participants (2.4%) attended Visit 2 only, either due to late presentation/transfer of care to the Jessop Wing, or due to technical issues with the EIS probe at visit 1 (see Figure 4.2, below). All recruits had experienced at least one prior preterm birth or mid-trimester miscarriage, or had a CL <25mm before 24 weeks.

250 ALR women were recruited although two crossed to the HR subgroup due to having a CL<25mm at visit 1, one was lost to follow up and in five, usable EIS data was not obtained due to participant intolerance of speculum examination (n=2) or technical issues with the EIS probe early in the study (n=3). In total, CR measurements were obtained from 242 ALR women for whom delivery outcomes were available (see Figure 4-1, below).

The exclusion criteria for the study are also detailed in Chapter 2 (page 84). Patients with a prior history of colposcopy treatment and/or who had received prophylactic treatment for preterm birth (i.e. cerclage or vaginal progesterone) were not excluded, but treatment information was recorded to permit appropriate subgroup analysis.

At each study visit women had swabs taken for FFN quantification and microbiological screening, followed by EIS measurements and finally a transvaginal ultrasound of cervical length. Both investigators and participants were blinded to the EIS results, but not to CL and FFN measurements. Short CL (<25mm before 24 weeks) and positive FFN (>50ng/ml) were managed according to standard protocols (see Appendix D). Similarly, positive microbiological swabs were managed according to local protocols, with antibiotics/antifungals as appropriate (see Appendix C). Consequently, a proportion of women were commenced on prophylactic treatment between study visits – further information regarding women receiving PTB prophylaxis is provided in the results section below.

Delivery information and other clinical outcomes were obtained from maternity and neonatal databases and case notes where electronic information was incomplete. Overall two patients were lost to follow up due to moving out of area. Statistical analysis was performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows,

Version 22.0. Armonk, NY: IBM Corp). Additional ROC curve analyses were performed using MedCalc (MedCalc Software bvba. Released 2018. MedCalc Statistical Software, Version 18.2.1. Ostend, Belgium)

4.3 Results

4.3.1 Participant demographics

Table 4-1 summarises demographic and basic clinical information for the entire asymptomatic cohort, with comparison by delivery outcome. As discussed above, participants had varied obstetric histories. In total, 37% of women were nulliparous. When split by outcome, nulliparous women made up a significantly lower proportion of the spontaneous preterm birth group compared to those women delivering at term ($p=0.015$, χ^2 test). This is mainly because the majority of high-risk recruits were parous (i.e. with a history of prior PTB rather than exclusive mid-trimester miscarriages).

Table 4-1 Demographic and Clinical Details of Whole Asymptomatic Cohort (n=452)

	Spontaneous PTB (n=43)	Iatrogenic PTB (n=23)	Term birth (n=386)
Age (Mean (SD))	31.3 yrs (4.8)	29.9 yrs (5.0)	29.9 yrs (5.0)
BMI (Mean (SD))	28.2 (6.6)	27.4 (6.6)	26.3 (5.4)
BMI >30	16 (37.2%)	6 (26.1%)	82 (21.2%)*
Parity			
• **Nulliparous (either prev MTL or CL<25)**	9 (20.9%)	5 (21.7%)	154 (39.9%)*
• **Multiparous**	34 (79.1%)	18 (78.3%)	232 (60.1%)*
○ **Previous Term deliveries only (plus either prev MTL or CL<25)**	4 (9.3%)	1 (4.3%)	106 (27.5%)
○ **Previous Preterm deliveries only**	16 (37.2%)	7 (30.4%)	74 (19.2%)
○ **Term & preterm deliveries**	14 (32.6%)	10 (43.5%)	52 (13.5%)
Ethnicity			
• **Caucasian**	36 (83.7%)	20 (87.0%)	362 (93.8%)
• **Asian**	1 (2.3%)	1 (4.3%)	9 (2.3%)
• **African**	5 (11.6%)	2 (8.6%)	7 (1.9%)
• **Mixed race**	1 (2.3%)	0 (0%)	4 (1.0%)
• **Afro-Caribbean**	0 (0%)	0 (0%)	3 (0.8%)

• **Arabic**	0 (0%)	0 (0%)	1 (0.3%)
Non-white ethnicity	7 (16.3%)	3 (13%)	24 (6.6%)*
Smoking			
• **Yes**	14 (32.6%)	6 (26.1%)	50 (13.0%)**
Previous colposcopy treatment			
• **Yes**	8 (18.6%)	4 (17.4%)	44 (11.4%)
Antenatal progesterone therapy (at any point)			
• **Yes**	6 (14.0%)	5 (21.7%)	20 (5.2%)*
Antenatal cervical cerclage (at any point)			
• **Yes**	5 (11.6%)	6 (26.1%)	14 (3.6%)*

* Significant differences noted between term and spontaneous PTB groups with $p<0.05$ (χ^2 test)
** $p<0.005$

When demographic aspects are considered, maternal age was similar across all three outcome groups. Women experiencing a spontaneous preterm birth were significantly more likely to have a BMI>30, be of non-white ethnicity and to smoke than their term-delivering counterparts (p=0.018, 0.020 and 0.001 respectively, χ^2 test). There was a trend towards higher colposcopy rates in the spontaneous PTB group, but this did not achieve significance (p=0.172). Women delivering preterm were significantly more likely to have received a cervical cerclage or progesterone supplementation (p=0.016 and 0.022, χ^2 test).

4.3.2 Delivery outcomes

Within the entire asymptomatic cohort there were 66 preterm births (a rate of 14.6%), of which 43 (65.1%) were spontaneous and 23 (34.8%) were iatrogenic. 386 women delivered at term (85.4%). Figures 4-1 and 4-2 summarise delivery outcomes for ALR and AHR women, with additional information regarding the proportion of women with a history of treatment (previous colposcopy or PTB prophylaxis). The untreated subgroup of women (summarised on the left side of both figures) will be the focus of the majority of the analyses which follow, although section 4.3.6 will consider prediction of PTB in those women with a history of prior LLETZ only. As Table 4-1 shows, the numbers of women receiving antenatal cerclage and progesterone overall were small. Furthermore, women often had a history of more than one treatment (e.g. a prior LLETZ plus PTB prophylaxis or treatment with both progesterone and cerclage) rendering subgroup analysis according to prophylactic treatment impractical.

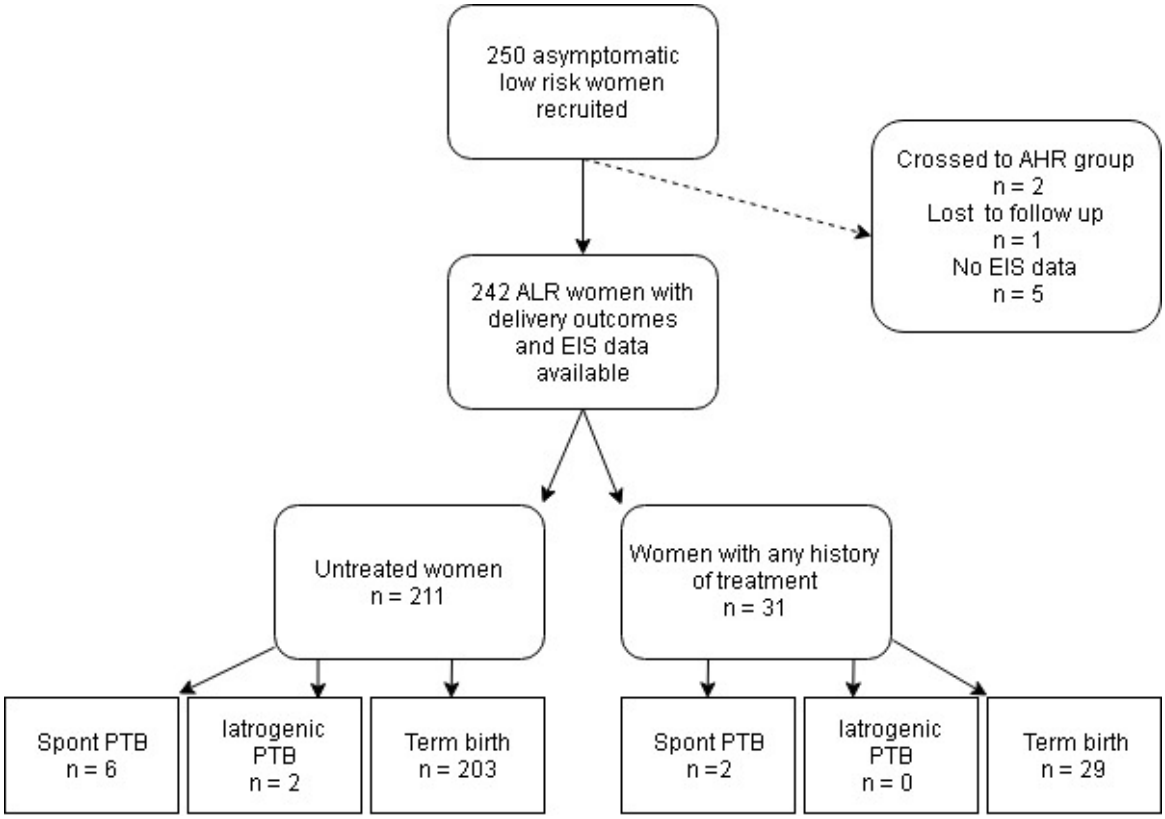


Figure 4-1 Delivery Outcomes for Asymptomatic Low Risk Women

Abbreviations: ALR = asymptomatic low risk; AHR = asymptomatic high risk; EIS = electrical impedance spectroscopy; PTB = preterm birth.

Within the untreated asymptomatic subgroup there were 28 spontaneous preterm births (6 to ALR women and 22 to AHR women of whom one only attended visit 2), 12 iatrogenic preterm births and 317 term births (203 to ALR women and 114 to AHR women of whom two only attended visit 2). Thus, the preterm birth rate amongst untreated women was 11.2% (70% spontaneous PTB and 30% iatrogenic PTB). As expected, when subdivided by risk grouping, untreated AHR women had a higher PTB rate than untreated ALR women (21.7 vs 3.8%). Of the 12 iatrogenic preterm births, 2 patients were induced for preterm premature rupture of membranes. The remainder were delivered early for other maternal or fetal indications.

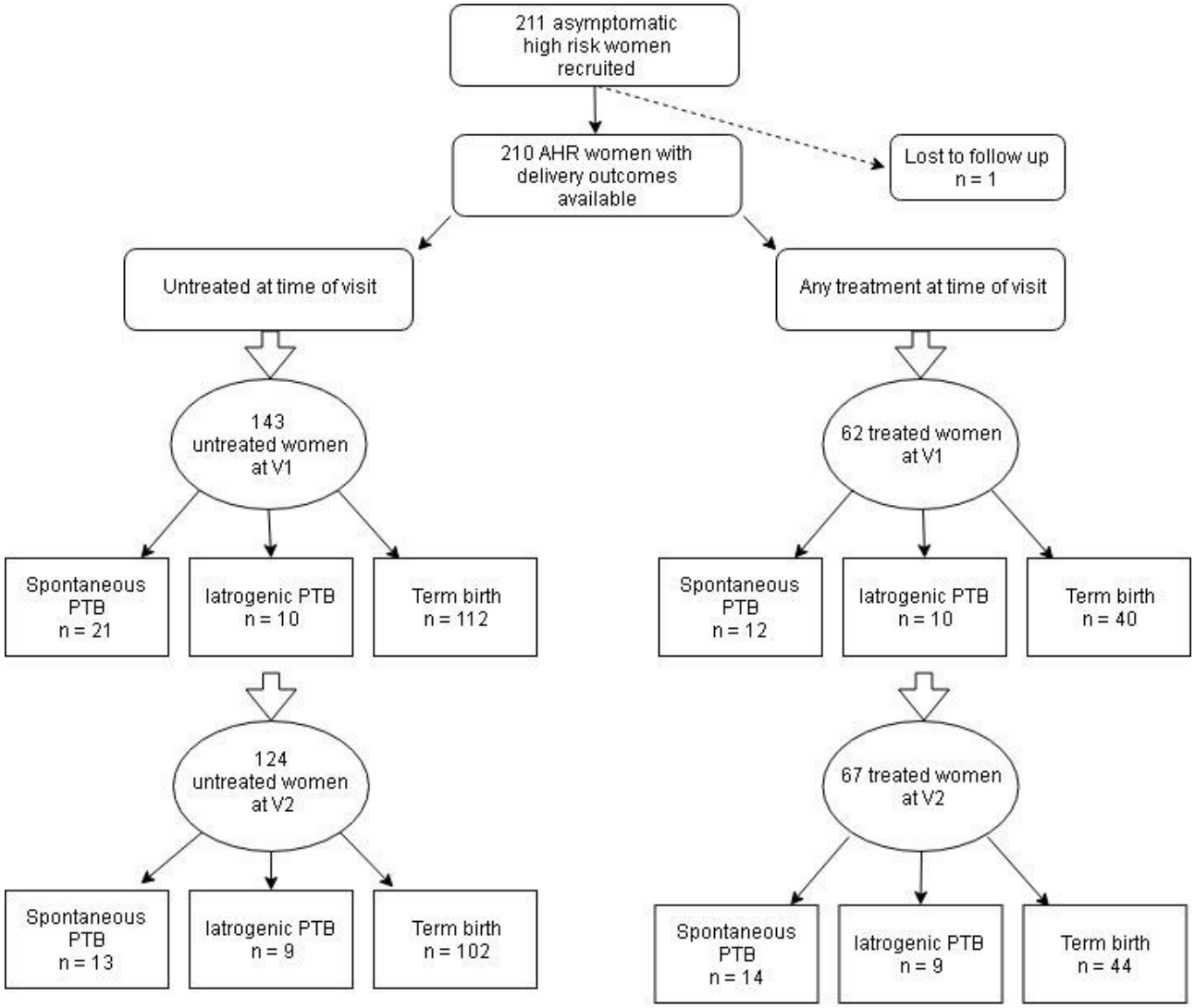


Figure 4-2 Delivery Outcomes for Asymptomatic High-Risk Women

4.3.3 Additional clinical outcomes

Table 4-2 summarises the rates of primary and secondary clinical outcomes across the various asymptomatic subgroups, namely spontaneous PTB and late miscarriage, mean delivery gestation and birthweight, rates of perinatal morbidity, mortality and hospital/NICU admission duration.

Table 4-2 Summary of Primary and Secondary Clinical Outcomes by Treatment Group

Subgroup	Whole ASYMP cohort n =452	Untreated ASYMP cohort n = 359	Untreated AHR women n =147	Untreated ALR women n=211	ASYMP women with prior LLETZ only n = 50
spPTB <37 weeks	43 (9.5%)	28 (7.8%)	22 (15.0%)	6 (2.8%)	5 (10.0%)
spPTB <32 weeks	14 (3.1%)	8 (2.2%)	7 (4.8%)	1 (0.5%)	1 (2.0%)
SpPTB or PPROM <37 weeks	50 (11.0%)	30 (8.4%)	24 (16.3%)	6 (2.8%)	7 (14.0%)
Midtrimester losses (14-23 weeks)	1 (0.2%)	0	0	0	0
Stillbirths	2 (0.4%)	1 (0.3%)	0	1 (0.5%)	0
Mean gestation at delivery (weeks) and range	38.9 (20.9 to 42.3)	39.2 (23.3 to 42.3)	38.0 (23.3 to 41.6)	40.0 (28.3 to 42.3)	38.6 (27.0 to 42.1)
Mean birthweight (g) and range	3255.4 (320 to 5120)	3313.8 (675 to 5120)	3080.4 (675 to 5090)	3477.7 (990 to 5120)	3247.5 (1048 to 4990)
Birthweight <2500g	52 (11.5%)	34 (9.5%)	26 (17.7%)	8 (3.8%)	7 (14.0%)
NICU admission	18 (4.0%)	15 (4.2%)	20 (13.6%)	8 (3.8%)	6 (12.0%)
Duration of NICU admission (days)	35.6 (1 to 132)	33.1 (3 to 91)	35.9 (3 to 91)	27.0 (6 to 75)	37.3 (14 to 86)
Duration of maternal admission (days)	2.9 (0 to 18)	2.7 (0 to 14)	3.1 (0 to 14)	2.5 (0 to 13)	3.2 (1 to 13)
Neonatal death	1 (0.2%)	1 (0.3%)	1 (0.7%)	0	0
Respiratory distress syndrome	27 (5.9%)	15 (4.2%)	11 (7.5%)	4 (1.9%)	3 (6.0%)

Intra-ventricular haemorrhage	7 (1.5%)	2 (0.6%)	2 (1.4%)	0	2 (4.0%)
Necrotising enterocolitis	1 (0.2%)	1 (0.3%)	1 (0.7%)	0	0
Sepsis	20 (4.4%)	10 (2.8%)	7 (4.8%)	3 (1.4%)	4 (8.0%)
Perinatal mortality (stillbirth + early NND)	3 (0.7%)	2 (0.6%)	1 (0.7%)	1 (0.5%)	0 (0.0%)
Composite of perinatal mortality and morbidity	37 (8.1%)	20 (5.6%)	14 (9.5%)	6 (2.8%)	5 (10.0%)

Rates of spPTB <37 weeks in the whole asymptomatic cohort are broadly consistent with national UK figures[5] at 9.5%. Unsurprisingly, rates of spPTB, low birthweight, NICU admission and perinatal morbidity and mortality were highest amongst AHR women. The spPTB rate in this subgroup is perhaps lower than one might expect at 15%, but as these are untreated AHR women, the highest risk women (e.g. those with abnormal screening/histories mandating prophylaxis) have been excluded.

The perinatal mortality rate was low, partly due to the low rate of extreme preterm birth: only two patients delivered before 28 weeks (one at 23+2 with subsequent neonatal death at 20 days of age, one at 26+5 who survived). Of the two cases of stillbirth, one occurred in an ALR participant and was unexplained, the other occurred in an AHR participant with history-indicated cerclage followed by PPROM at 35/40 and sudden onset of fulminant chorioamnionitis.

4.3.4 Patterns of cervical resistivity by birth outcome

Untreated asymptomatic women destined to experience spPTB exhibited lower mean cervical impedance across the majority of spectral frequencies; at visit 1 lower CR was noted from frequency 5 (1.2 kHz), at visit 2 impedance was lower in the SpPTB group across all 14 frequencies. These differences were statistically significant in the 39.1 to 312.5 kHz range at visit 1 (a mixed group of AHR and ALR women) and in the 39.1 to 625 kHz range at visit 2 (AHR only), as illustrated by Figures 4-3 and 4-4.

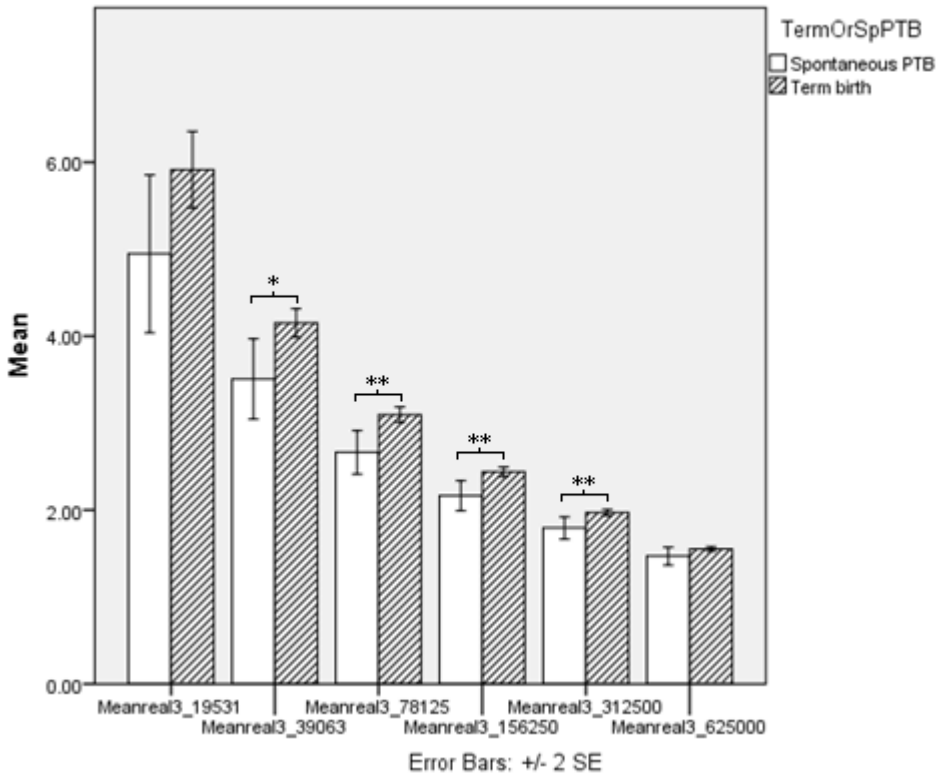


Figure 4-3 Differences in Mean Cervical Resistivity (Ohm.m) at Visit 1, at Frequencies 9 to 14 (19.5 to 625 kHz) (Untreated Asymptomatic women, n=342)

**p<0.005; *p<0.05

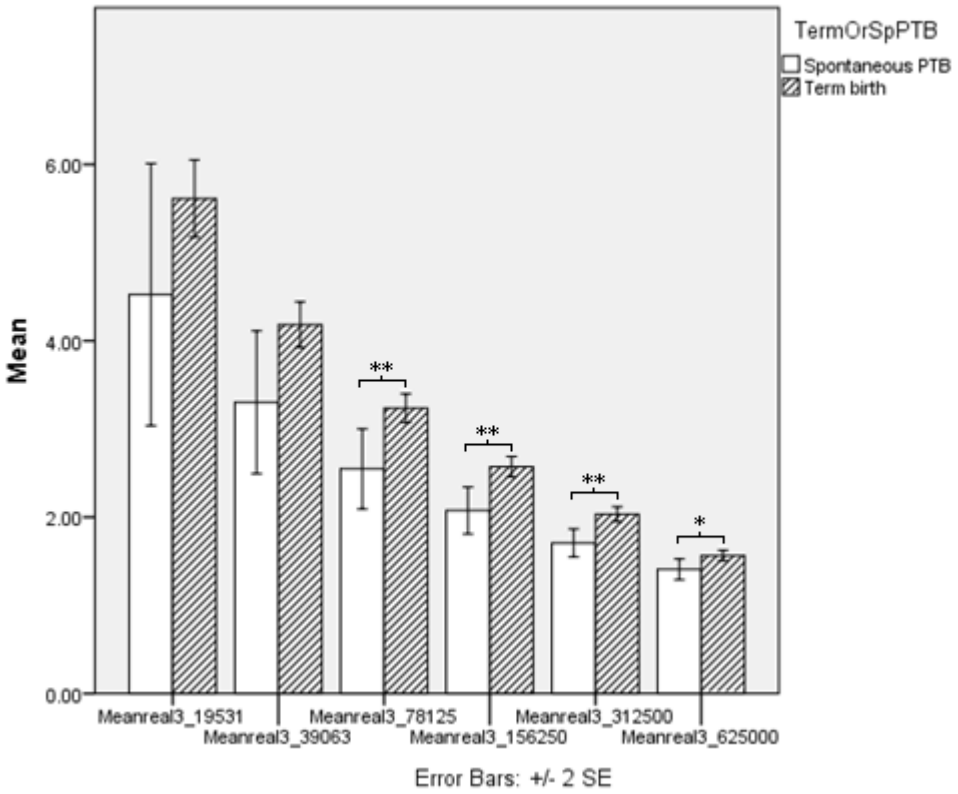


Figure 4-4 Differences in Mean Cervical Resistivity (Ohm.m) at Visit 2, at Frequencies 9 to 14 (19.5 to 625 kHz) (Untreated Asymptomatic women n=114)

**p<0.005; *p<0.05

The violin plots in figures 4-5 and 4-6 provide further illustration of the differences in cervical resistivity observed between term and spPTB groups for the 5 highest current frequencies (i.e. those where significant differences were noted). At Visit 1 (Figure 4-5) the density curves are fairly similar in shape between outcome groups, suggesting a comparable distribution of resistivity readings. Much like the comparisons of mean resistivity presented in Figures 4-3 and 4-4, the box plots within each violin demonstrate lower resistivity in women destined to experience spPTB, although there is significant overlap between the respective distributions. Some outliers with low cervical resistivity are noted within the term birth group at frequencies of 156 – 625kHz.

At Visit 2 (Figure 4-6), the density curves for the spPTB group suggest a possible multi-modal distribution of resistivity readings at the top four current frequencies. However, given the small numbers in this group (only 14 women who attended visit 2 went on to experience spPTB) this observation should be viewed with caution.

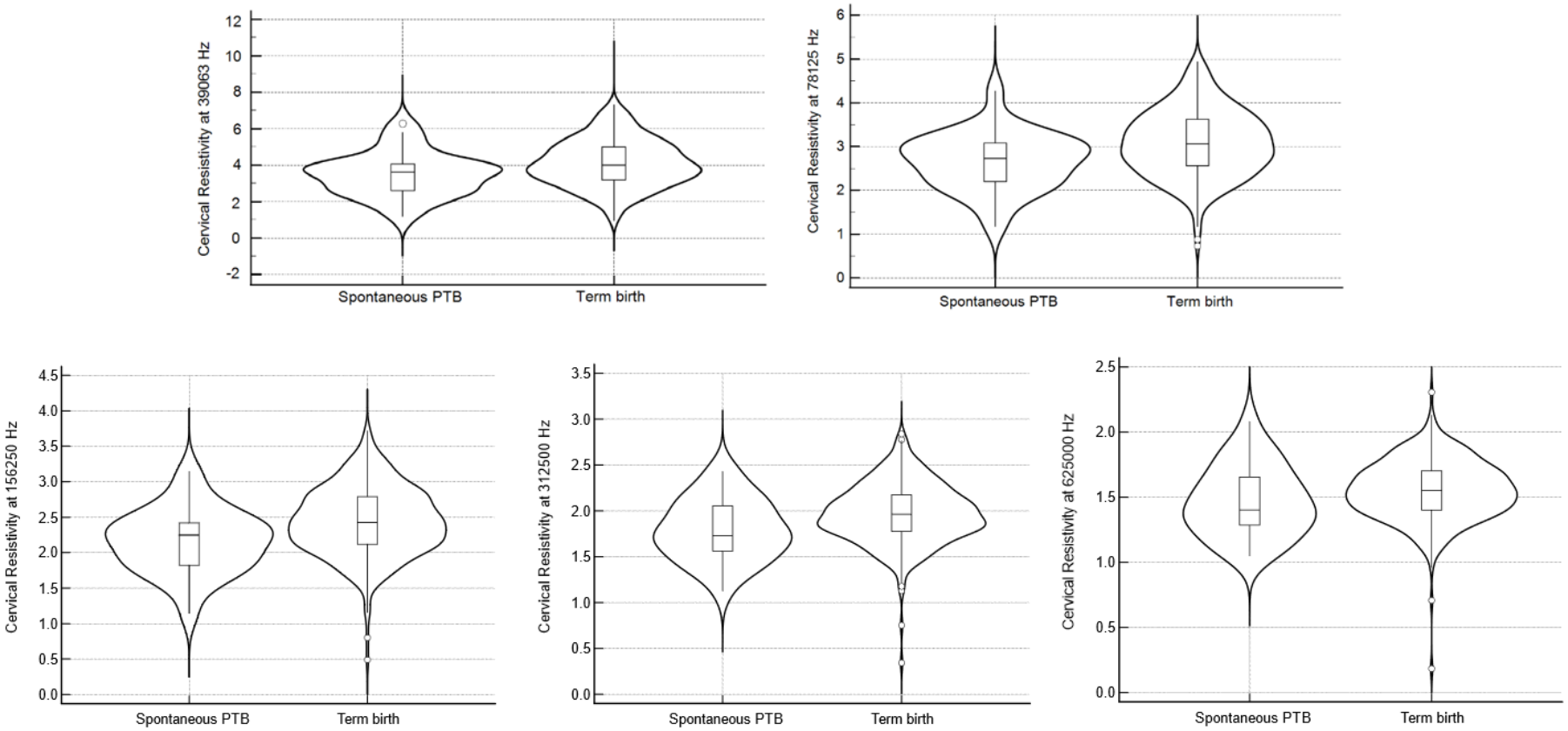


Figure 4-5 Violin Plots Illustrating Differences in Cervical Resistivity (Ohm.m) at Visit 1, at Frequencies 10 to 14 (39 to 625 kHz) (Untreated Asymptomatic women, n=342, 27 spPTB *vs.* 315 term births)

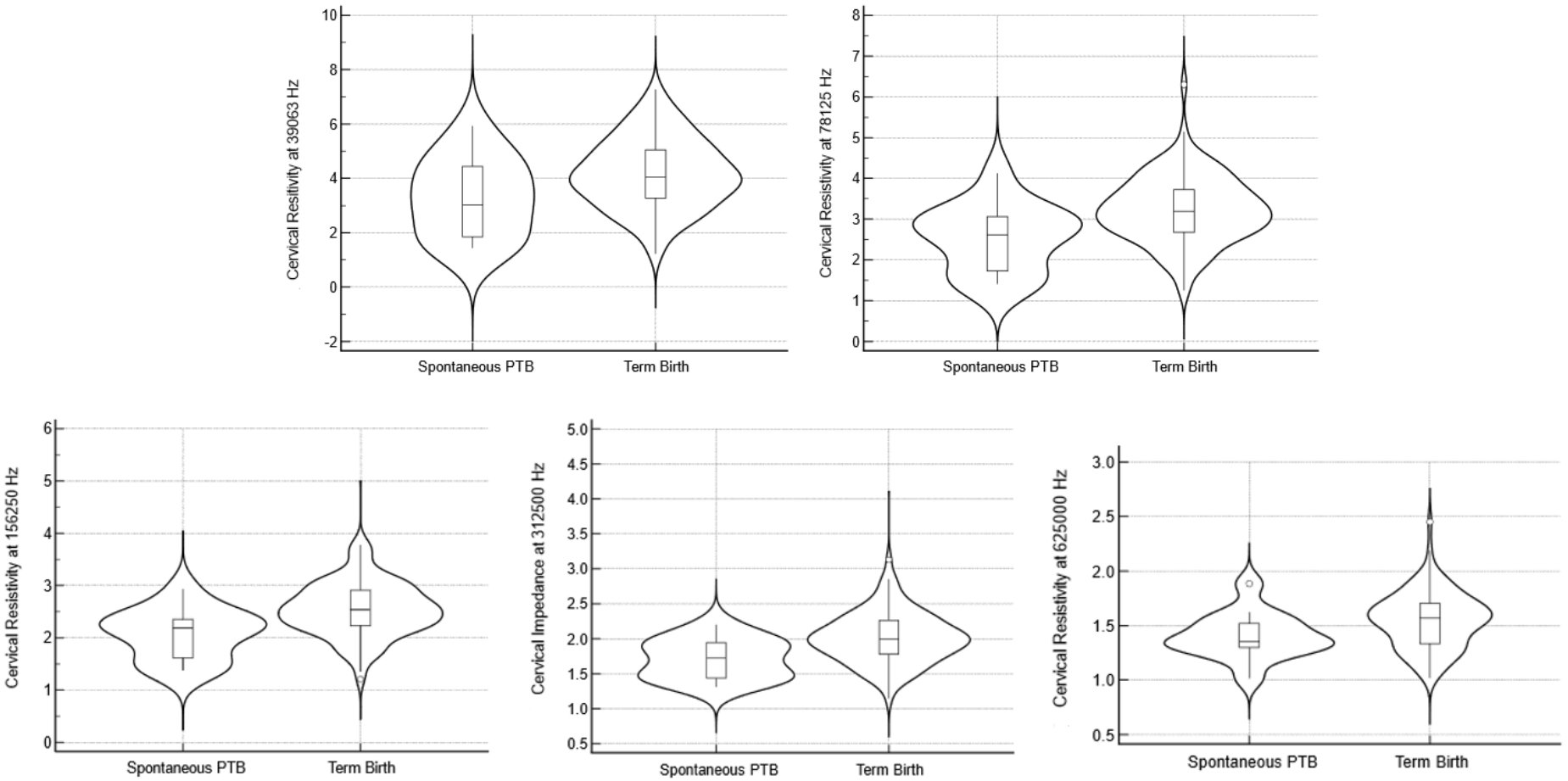


Figure 4-6 Violin Plots Illustrating Differences in Cervical Resistivity (Ohm.m) at Visit 2, at Frequencies 10 to 14 (19.5 to 625 kHz) (Untreated Asymptomatic women n=114, 13 spPTB *vs.* 101 term births)

Tables 4-3 and 4-4 summarise the mean cervical impedance at each frequency for SpPTB and term groups at both visits, and the results of statistical comparison of these means.

Table 4-3 Comparison of Mean Cervical Resistivity (Ohm.m) in Term and Preterm Delivering Women at Visit 1 (Untreated Asymptomatic Women, n=342)

Current frequency (Hz)	SpPTB <37/40 N=27		Term Birth N=315		P value for comparison of means (Mann Whitney U)
	Mean CR (Ohm.m)	95% CI	Mean CR (Ohm.m)	95% CI	
76.3	30.41	19.14 to 41.68	29.72	27.19 to 32.26	0.877
152.6	28.78	18.16 to 39.40	29.08	25.73 to 30.43	0.870
305.2	26.68	17.05 to 36.31	26.10	23.97 to 28.23	0.838
610.4	23.60	15.50 to 31.69	23.29	21.45 to 25.13	0.796
1220.7	19.42	13.34 to 25.51	19.67	18.19 to 21.15	0.748
2441.4	14.81	10.71 to 18.90	15.49	14.38 to 16.61	0.668
4882.8	10.54	7.91 to 13.16	11.59	10.77 to 12.41	0.461
9765.6	7.24	5.59 to 8.96	8.36	7.75 to 8.96	0.201
19531.3	4.95	4.01 to 5.88	5.91	5.48 to 6.35	0.070
39062.5	3.51	3.03 to 3.98	4.15	3.99 to 4.31	**0.023***
78125	2.66	2.40 to 2.92	3.09	3.01 to 3.18	**0.005****
156250	2.16	1.99 to 2.34	2.44	2.39 to 2.49	**0.004****
312500	1.79	1.66 to 1.93	1.97	1.93 to 2.01	**0.008****
625000	1.47	1.36 to 1.58	1.55	1.53 to 1.58	0.055

*p<0.05, **p<0.005

Table 4-4 Comparison of Mean Cervical Resistivity (Ohm.m) in Term and Preterm Delivering Women at Visit 2 (Untreated Asymptomatic Women, n=114)

Current frequency (Hz)	SpPTB <37/40 N=13		Term Birth N=101		P value for comparison of means (Mann Whitney U)
	Mean CR (Ohm.m)	95% CI	Mean CR (Ohm.m)	95% CI	
76.3	19.37	8.41 to 30.32	28.46	24.52 to 32.41	0.070
152.6	18.45	8.01 to 28.88	26.82	23.14 to 30.51	0.074
305.2	17.31	7.54 to 27.10	24.75	21.42 to 28.08	0.081
610.4	15.70	6.90 to 24.51	21.78	18.95 to 24.61	0.096
1220.7	13.51	6.10 to 20.93	18.06	15.83 to 20.29	0.122
2441.4	11.03	5.17 to 16.90	14.02	12.40 to 15.65	0.150
4882.8	8.54	4.29 to 12.79	10.48	9.36 to 11.59	0.163
9765.6	6.30	3.53 to 9.07	7.68	6.96 to 8.40	0.180
19531.3	4.52	2.91 to 6.14	5.61	5.18 to 6.05	0.138
39062.5	3.30	2.42 to 4.18	4.18	3.93 to 4.44	0.054
78125	2.55	2.05 to 3.04	3.23	3.07 to 3.40	**0.008****
156250	2.08	1.79 to 2.37	2.57	2.46 to 2.69	**0.003****
312500	1.71	1.54 to 1.88	2.03	1.95 to 2.12	**0.004****
625000	1.41	1.28 to 1.54	1.56	1.51 to 1.62	**0.031***

*p<0.05, **p<0.005

During these analyses, consideration was given to the effect of multiple hypothesis testing. Corrections which aim to control the Family Wise Error Rate (FWER) such as the Bonferroni procedure[356] are highly conservative[357] and whilst very effective at minimising Type 1 errors, they risk inflating the risk of Type 2 errors (i.e. missing a true difference). This is particularly true for related datasets[356], including spectral measurements, and even modified approaches such as the Holm method may lack power in this situation[358]. The main purpose of these analyses was exploratory – to identify the frequency range which might optimally be used to predict preterm birth. Thus an overly stringent approach was less appropriate[358, 359]; we preferred to accept the potential consequences of including a non-discriminatory frequency in our summary measure of CR, than risk excluding one at the borderline of statistical significance, which might in fact prove useful in determining outcome. Therefore, rather than controlling FWER, the two-stage linear step-up procedure of Benjamini, Krieger and Yekutieli[360] procedure, which controls the false discovery rate (FDR), was employed. These calculations were performed using GraphPad Prism (Version 9.0.0. for Windows, GraphPad Software, San Diego, California USA). A Q value of 0.1 was selected (meaning the potential FDR would not exceed 10%). When procedure was applied to the p values for the 28 individual hypothesis tests, a new significance threshold of $p<0.033$ was generated suggesting the previously highlighted significant differences were not false discoveries.

4.3.5 Results of conventional predictive tests

Amongst the whole untreated, asymptomatic cohort, mean cervical length was significantly lower in women who went on to have a spontaneous PTB at both visit 1 and visit 2 ($p=0.001$ and $p=0.03$, T test). Higher levels of fetal fibronectin were noted in the cervico-vaginal secretions of the spPTB group, but these differences did not achieve statistical significance ($p=0.55$ and 0.74 at visits 1 and 2 respectively, Mann Whitney U). The comparative distributions of cervical length and fetal fibronectin concentrations in term and spPTB groups are depicted by the box plots in Figure 4-7, below.

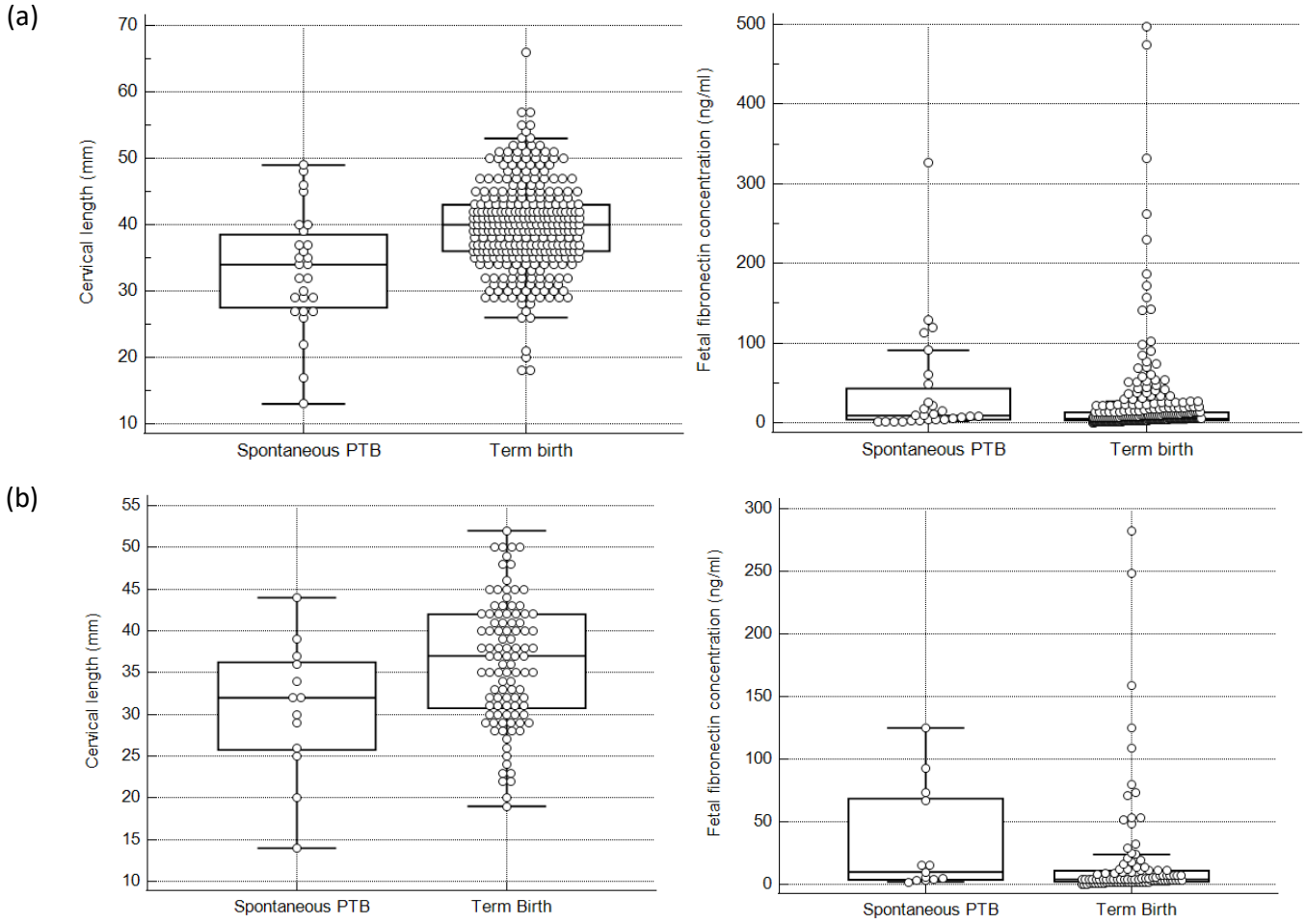


Figure 4-7 Differences in Cervical Length and Fetal Fibronectin Concentration at (a) Visit 1 and (b) Visit 2 (Untreated Asymptomatic women, n=342 and 114 respectively)

4.3.6 Effect of treatment on EIS measurements

As acknowledged previously, prior colposcopy treatment can increase the risk of preterm birth, with higher risk conferred by multiple or deep excisions. It was therefore important to consider whether CR varied similarly with delivery gestation amongst the subgroup of women with a history of previous LLETZ prior to considering the application of EIS as a screening tool. 56 women within the asymptomatic cohort had a history of previous LLETZ, of whom 6 also received PTB prophylaxis (cerclage or progesterone). For clarity, those receiving prophylaxis were excluded from further analysis, given the uncertain effect that such treatment might have on cervical impedance. Of the 50 remaining women, 5 (10%) experienced a spontaneous preterm birth, 3 (6%) an iatrogenic PTB and 42 (84%) a term birth. All 50 women attended a first study visit, a further 21 attended a second visit (with 3 spPTB and 16 term births). 45 women had a history of one previous LLETZ, 4 had two previous LLETZ and 1 had undergone a solitary cone biopsy.

As Figure 4-8 demonstrates, in contrast to the differences observed in untreated women, women with prior LLETZ experiencing spPTB on average had generally higher CR than their term counterparts. The majority of these differences did not reach significance, although mean impedance was significantly higher in the spPTB group at visit 2 for frequencies of 78.1 and 156.2 kHz ($p=0.023$ and 0.047, Mann Whitney U).

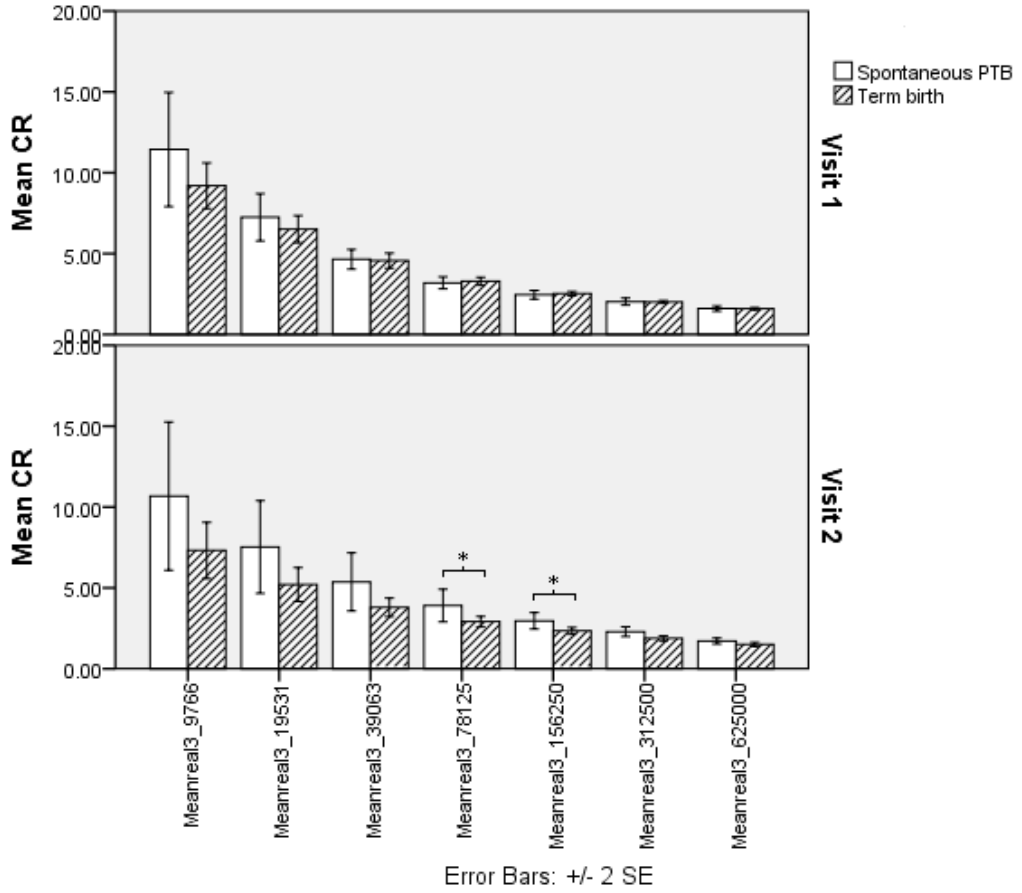


Figure 4-8 Differences in Mean Cervical Resistivity between Term and Preterm Delivering Women with a History of Previous Colposcopy Treatment only (n=50)

(All p>0.05)

Figures 4-9 and 4-10 emphasise the differential pattern of higher resistivity in women with prior LLETZ destined to deliver preterm. Although a wider distribution of CR readings was noted amongst term-delivering treated women at visit 1 (as illustrated by the elongated density curve), both the combined box plots and density curves depict a consistent pattern of higher CR in preterm delivering women at both timepoints. The separation between outcome groups appears greater at 26-28 weeks, but only 3 women with prior LLETZ went on to experience spPTB after attending visit 2.

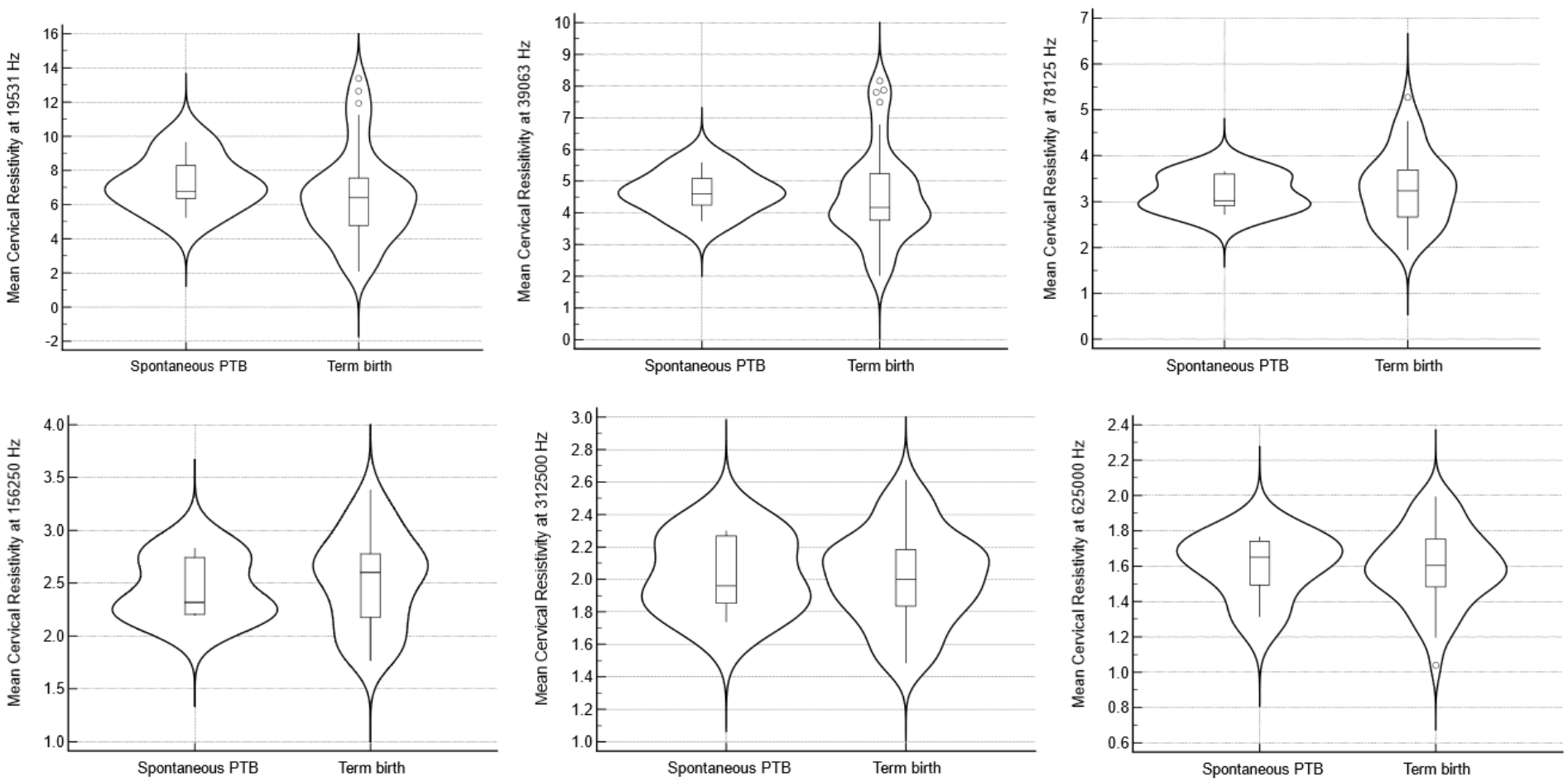


Figure 4-9 Differences in Cervical Resistivity between Term and Preterm Delivering Women with a History of Previous Colposcopy Treatment only at Visit 1 (n=47, 5 spPTB *vs.* 42 term)

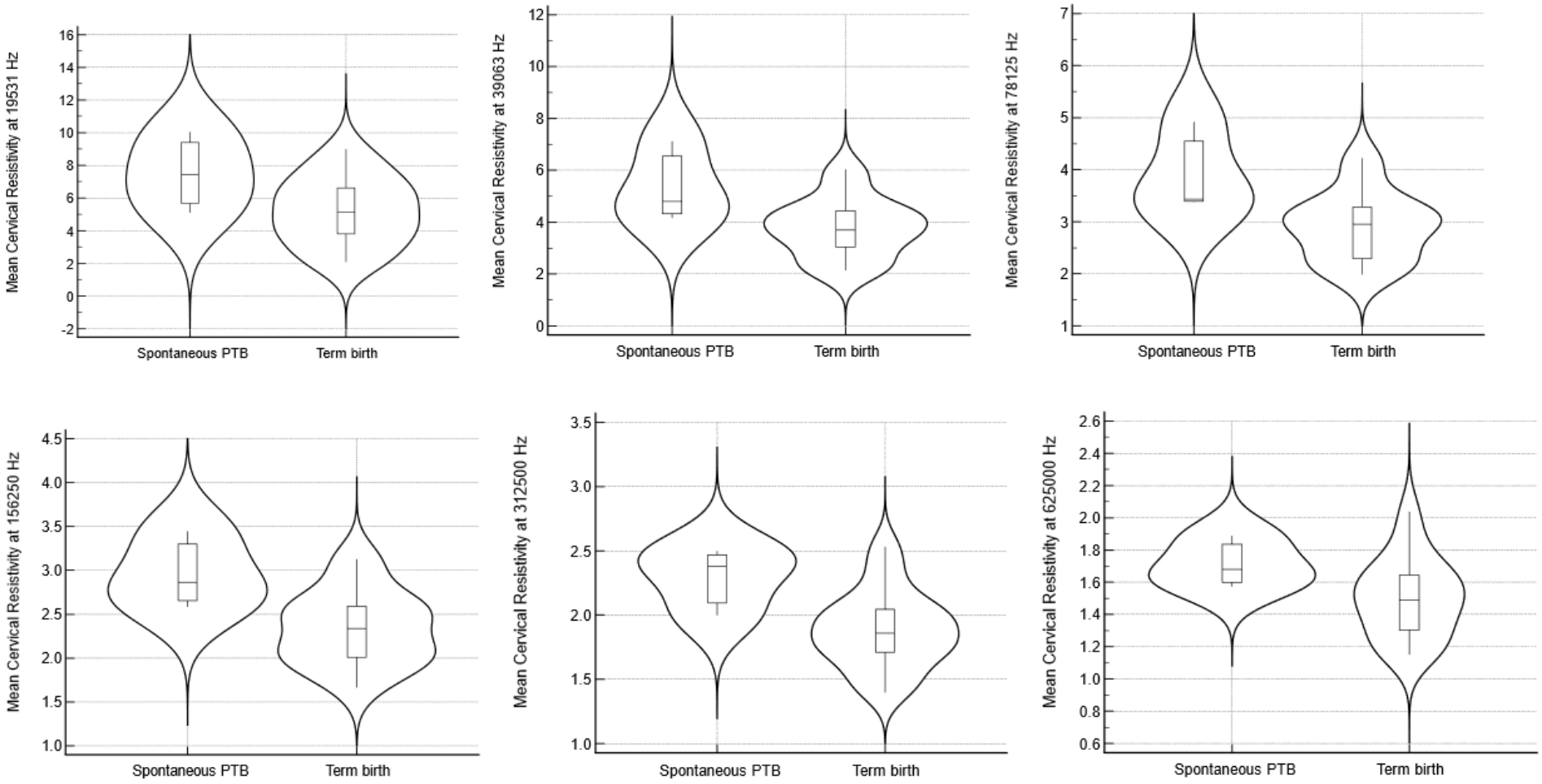


Figure 4-10 Differences in Cervical Resistivity between Term and Preterm Delivering Women with a History of Previous Colposcopy Treatment only at Visit 2 (n=19, 3 spPTB *vs.* 16 term)

4.3.7 Results of infection screening

The results of conventional microscopy and culture results from the high vaginal swabs taken at each study visit are summarised in Figure 4-11. The majority of women received a normal result (68% at visit 1 and 69% at visit 2), with *Candida albicans* (21% at visit 1 and visit 2), *Group B Streptococcus* (10% at visit 1 and 6% at visit 2) and bacterial vaginosis (4% at both visits) representing the most common positive results. Higher rates of bacterial vaginosis were noted in the SpPTB *vs.* term group at visit 1 (7.4 *vs.* 4.1%) and visit 2 (7.7 *vs.*4%) but these differences did not reach significance (p= 0.34 and 0.46, Fishers exact test).

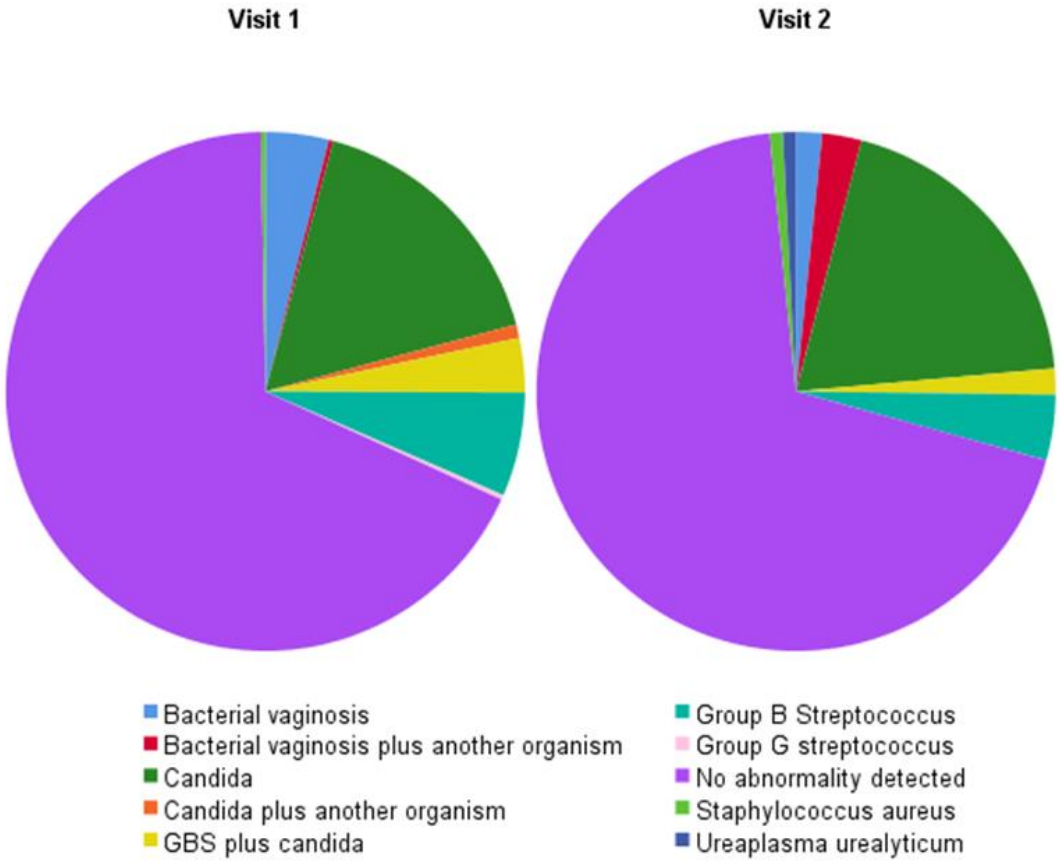


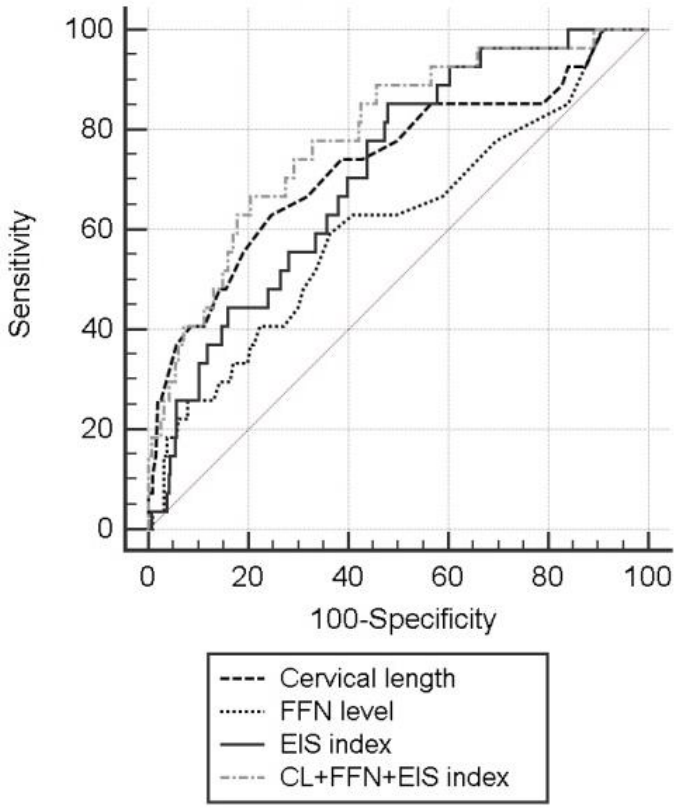
Figure 4-11 High Vaginal Swab Results for the Entire Untreated Asymptomatic Cohort
(n=355 at visit 1 and n=123 at visit 2)

Consideration was given to assessing the BV positive group, to see if their impedance spectra varied significantly from those with negative results, however, small numbers precluded meaningful analysis.

4.3.8 Predictive performance of EIS and conventional screening tests

When evaluating the predictive performance of EIS, the frequencies for which significant differences were observed between term and preterm groups were combined to create a summary measure of CR. In determining significance, the p value threshold of 0.033, (discussed in section 4.3.4) was used, to maximise the inclusion of potentially discriminatory frequencies. Hormonally mediated changes of the cervical epithelium such as ectropion formation, squamous metaplasia[361], deciduosis[362] and Arias-Stella reaction[363] are more common in pregnancy. It was therefore also relevant to consider the nature of epithelial cells covering the cervices from which our spectral data was obtained. Previous studies employing EIS to detect pre-malignant changes in the cervix[23] and other tissues[364] have demonstrated enhanced accuracy when a process of template matching is incorporated into predictive models. In short, this technique systematically compares all obtained spectral data to templates for normal squamous and columnar epithelia[295] and matches them via a least squares minimisation technique to derive a probability that the readings have been obtained from either subtype[23]. These probabilities were subsequently combined with CR readings (in the range 39.1-625 kHz) via multivariate regression to produce a final EIS index. The performance of EIS was then compared to CL and FFN by means of ROC curve analysis (with areas under the ROC curve (AUCs) of 0.9-1 categorised as demonstrating excellent prediction, 0.8-0.9 as good, 0.7-0.8 as fair and 0.6-0.7 as poor prediction). Standard binomial logistic regression was employed to incorporate all three tests into a multi-modal predictive model for all outcomes of interest. For the various regression analyses standard procedures were followed (including checking assumptions of linearity using the Box Tidwell procedure and checking for outliers using casewise diagnostics to identify cases with a standardized residual of >2.5). Where significant outliers were identified, inspection of the individual spectral measurements was performed as an additional quality control. When cases were identified in which one or more measurements had not produced a plausible impedance spectra (with loss of the smooth S shaped curve that is necessarily produced by multi-frequency measurement), the individual erroneous spectral measurements were removed and analyses repeated. Goodness of fit was assessed by means of the Hosmer and Lemeshow test (all p values>0.05, suggesting well-fitting models).

Figures 4-12 and 4-13 summarise the performance of EIS, CL and FFN in isolation and combination for predicting the primary outcome of spontaneous PTB before 37 weeks in the entire untreated asymptomatic cohort.



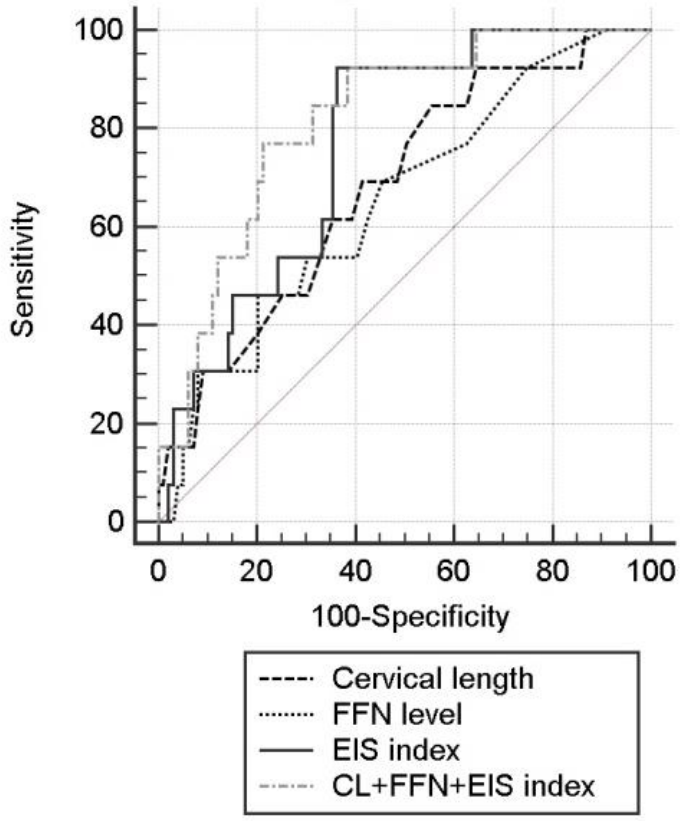
Untreated Asymptomatic Women at Visit 1 (n=342, 27 spPTB *vs.* 315 term)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤35mm	0.73	0.68 to 0.78	**<0.001**
FFN	>7ng/ml	0.61	0.56 to 0.66	0.071
EIS index	≤0.89	0.72	0.67 to 0.76	**<0.001**
CL+FFN+EIS	≤0.89	0.79	0.74 to 0.83	**<0.001**

Figure 4-12 ROC curves for Individual and Combined Predictive Tests (Prediction of SpPTB <37 weeks in Untreated Asymptomatic Women at Visit 1)

At Visit 1, cervical length and EIS yielded significant areas under the curve (AUCs). They performed similarly (p=0.835), with AUCs of 0.73 and 0.72 suggesting fair prediction of preterm birth. Sensitivity, specificity, and positive and negative likelihood ratios (LR+ and LR-) were 63.0%, 75.2%, 2.54 and 0.49 for cervical length and 85.2%, 52.4%, 1.79 and 0.28 for EIS. FFN levels at 20-22 weeks were a relatively poor predictor of PTB in this unselected asymptomatic group with a non-significant AUC of 0.61. Table 4-5 summarises the AUC, sensitivity, specificity, LR+ and LR- for the three tests in isolation and combination. Overall, the generated likelihood ratios suggest slight shifts in the pre-test probability of disease

with positive and negative test results. Combining EIS, CL and FFN generated the highest AUC of 0.79 (Sn 66.7%, Sp 79.6%, LR+ 3.26, LR- 0.42) but this was only significantly higher than the AUC for FFN in isolation (p=0.004, *vs.* 0.118 and 0.102 for CL and EIS alone).



Untreated Asymptomatic Women at Visit 2 (n=114, 13 spPTB *vs.* 101 term)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤37mm	0.69	0.59 to 0.77	**0.016**
FFN	>14ng/ml	0.65	0.56 to 0.74	0.060
EIS index	≤0.89	0.77	0.68 to 0.84	**<0.001**
CL+FFN+EIS	≤0.87	0.82	0.73 to 0.88	**<0.001**

Figure 4-13 ROC curves for Individual and Combined Predictive Tests (Prediction of SpPTB <37 weeks in Untreated Asymptomatic Women at Visit 2)

At Visit 2, EIS continued to demonstrate good prediction of preterm birth with an AUC of 0.77 (Sn 92.3%, Sp 64.4%, LR+ 2.59, LR- 0.12). The performance of CL was poorer at this later gestation with an AUC of 0.69 (Sn 84.6%, Sp 45.4%, LR+ 1.55, LR- 0.34). FFN was not a significant predictor of PTB at this timepoint (p value for ROC AUC 0.06). However, in combination, all three tests generated an AUC of 0.82 suggesting good multi-modal prediction. It is unclear if there is a definite additive effect from combining predictors – the

AUC for EIS+CL+FFN was significantly higher than that of CL alone (p=0.01) but not of EIS alone (p=0.10).

When prediction of earlier spontaneous PTB, prior to 32 weeks, is considered, predictive performance improves for all three tests. ROC curve analysis was only possible for data gathered at visit 1, as only one woman attending visit 2 went on to have a spPTB <32 weeks. Table 4-6 summarises predictive test performance for this outcome. Good prediction by EIS was noted with AUC 0.82 (Sn 75.0%, Sp 90.12%, LR+ 7.59, LR- 0.28) and by CL with an AUC of 0.81 (Sn 62.5%, Sp 89.8%, LR+ 6.14, LR- 0.42). The optimal threshold for a positive test for CL was ≤30mm at this timepoint. Prediction of earlier PTB by FFN level was reasonable with an AUC of 0.79 (Sn 75.0%, Sp 79.5%, LR+ 3.66, LR- 0.31).

Again a trend towards improved prediction with multi-modal screening was noted with the highest AUC generated by the combination of EIS+CL+FFN at 0.87 (CI 0.83 to 0.90 suggesting good to excellent prediction of spPTB <32 weeks) although this difference in AUC did not achieve statistical significance (p=0.082, 0.278 and 0.558 compared with CL, FFN and EIS alone). Sensitivity and specificity are relatively high at 75.0% and 92.8% with a LR+ of 10.37 suggesting a large shift in the pre-test probability of disease following a positive multi-modal screen.

Table 4-5 Prediction of Spontaneous PTB <37 weeks in Untreated Asymptomatic Women

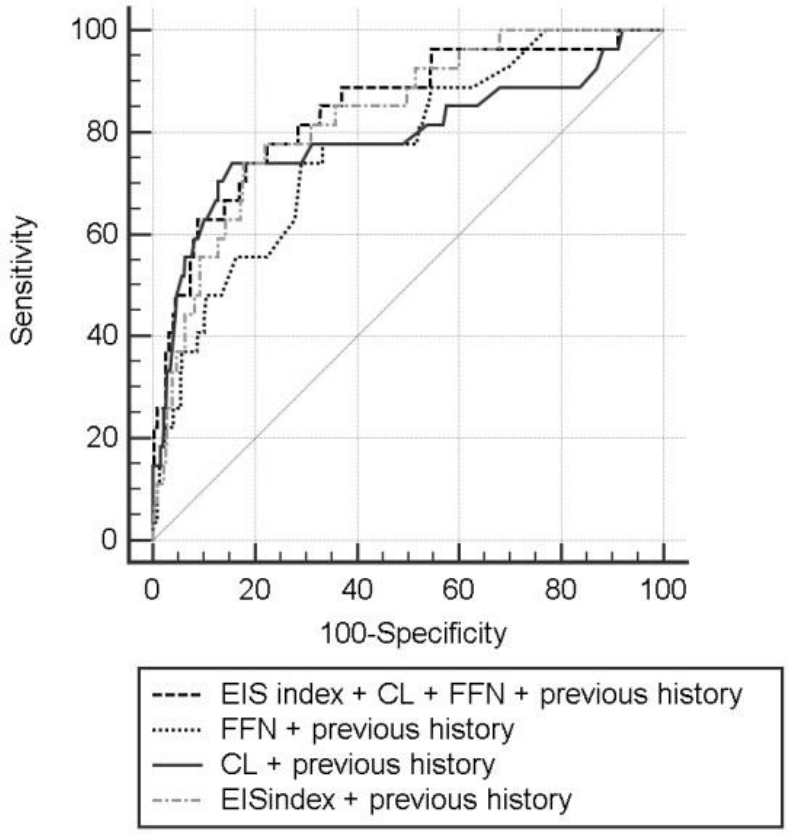
Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value	Sensitivity	Specificity	LR +	LR -
Visit 1 (n=342, 27 spPTB vs 315 term)								
EIS index	≤0.89	0.72	0.67 to 0.76	**<0.001**	85.19	52.38	1.79	0.28
Cervical length	≤35 mm	0.73	0.68 to 0.78	**<0.001**	62.96	75.24	2.54	0.49
FFN	>7ng/ml	0.61	0.56 to 0.66	0.071	59.26	63.58	1.63	0.64
EIS+CL+FFN	≤0.89	0.79	0.74 to 0.83	**<0.001**	66.67	79.55	3.26	0.42
Visit 2 (n=114, 13 spPTB vs 101 term)								
EIS index	≤0.89	0.77	0.68 to 0.84	**<0.001**	92.31	64.36	2.59	0.12
Cervical length	≤37 mm	0.69	0.59 to 0.77	**0.016**	84.62	45.54	1.55	0.34
FFN	>14ng/ml	0.65	0.56 to 0.74	0.060	46.15	79.80	2.28	0.67
EIS+CL+FFN	≤0.87	0.82	0.73 to 0.88	**<0.001**	76.92	78.79	3.63	0.29

Table 4-6 Prediction of Spontaneous PTB <32 weeks Untreated Asymptomatic Women at Visit 1 (insufficient numbers for analysis at visit 2)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value	Sensitivity	Specificity	LR +	LR -
Visit 1 (n=342, 8 spPTB vs 334 term)								
EIS index	≤0.94	0.82	0.77 to 0.86	**<0.001**	75.00	90.12	7.59	0.28
Cervical length	≤30mm	0.81	0.77 to 0.85	**<0.001**	62.50	89.82	6.14	0.42
FFN	>17ng/ml	0.79	0.74 to 0.83	**0.006**	75.00	79.52	3.66	0.31
EIS+CL+FFN	≤0.97	0.87	0.83 to 0.90	**<0.001**	75.00	92.77	10.37	0.27

4.3.9 Effect of incorporating obstetric history

When considering universal PTB screening for asymptomatic women, it is relevant to consider how previous obstetric history might combine with predictive tests to refine risk estimation. As discussed in Chapter 1 (section 1.4.1), a history of prior PTB confers a higher risk of recurrence in subsequent pregnancies[171.] Additional binary logistic regression analysis was therefore performed to incorporate a previous history of a least one PTB/mid-trimester miscarriage with CL, FFN estimation and EIS individually and in combination. Figures 4-14, 4-15 and Table 4-7 summarise the results of these analyses.



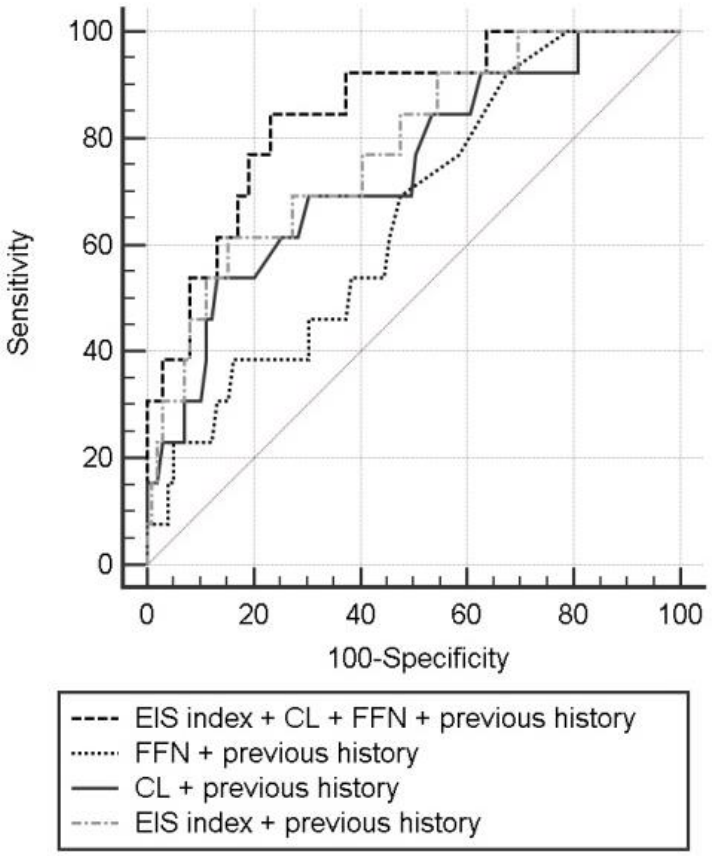
Untreated Asymptomatic Women at Visit 1 (n=342, 27 spPTB *vs.* 315 term)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
CL + history	≤0.90	0.79	0.74 to 0.83	**<0.001**
FFN + history	≤0.89	0.76	0.71 to 0.80	**<0.001**
EIS index + history	≤0.86	0.82	0.78 to 0.86	**<0.001**
CL+FFN+EIS + history	≤0.91	0.84	0.80 to 0.88	**<0.001**

Figure 4-14 ROC Curves Evaluating the Effect of Incorporating Obstetric History with Screening Tests to Predict SpPTB <37 weeks in Untreated Asymptomatic Women (Visit 1)

Adding an assessment of women's previous obstetric history had the effect of improving AUCs for both individual and multi-modal testing with the highest values produced by a combination of EIS + history (AUC 0.82, Sn 66.7%, Sp 82.5%, LR+ .82, LR- 0.40) and EIS + CL + FFN + history (AUC 0.84, Sn 74.1%, Sp 81.8%, LR+ 4.07, LR- 0.32) at Visit 1. These AUCs were significantly higher than those for that of FFN + history (p=0.0.025 compared to EIS + history and 0.004 for EIS + CL + FFN + history) but not for CL + history (p=0.525 and 0.074). Figure 4-14 shows the comparative ROC curves at visit 1 with confidence intervals and p values.

Similarly at Visit 2, the best performing combinations were EIS + history (AUC 0.79, Sn 61.5%, Sp 87.1%, LR+ 4.78, LR- 0.44) and EIS + CL + FFN + history (AUC 0.85, Sn 84.6%, Sp 76.8%, LR+ 3.64, LR- 0.20). The predictive performance of CL falls somewhat as gestation advances (this is true for both CL alone and CL + history in this cohort) whereas prediction of PTB by EIS later in pregnancy is better maintained. Multi-modal screening by EIS + CL + FFN + history was more accurate than CL + history (p=0.028) and FFN + history (p=0.005) but not significantly different to EIS + history alone (0.326). Figure 4-15 summarises the comparative ROC curves and relevant statistics at Visit 2.



Untreated Asymptomatic Women at Visit 2 (n=114, 13 spPTB *vs.* 101 term)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
CL+ history	≤0.74	0.74	0.65 to 0.82	**0.001**
FFN + history	≤0.89	0.65	0.56 to 0.74	**0.042**
EIS index + history	≤0.78	0.79	0.71 to 0.86	**<0.001**
CL+FFN+EIS + history	≤0.85	0.85	0.77 to 0.91	**<0.001**

Figure 4-15 ROC Curves Evaluating the Effect of Incorporating Obstetric History with Screening Tests to Predict SpPTB <37 weeks in Untreated Asymptomatic Women (Visit 2)

Table 4-7 Effect of Incorporating Obstetric History of Previous PTB or Late Miscarriage into the Predictive Model (Untreated Asymptomatic Women)

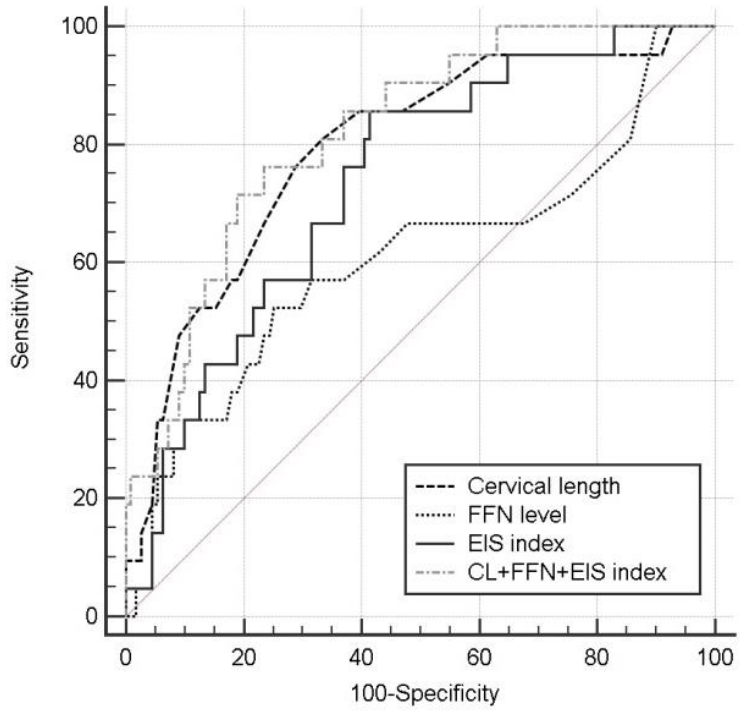
Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value	Sensitivity	Specificity	LR +	LR -
Visit 1 (n=342, 27 spPTB vs 315 term). SpPTB < 37 weeks.								
EIS index + history	≤0.86	0.82	0.78 to 0.86	**<0.001**	66.67	82.54	3.82	0.40
CL + history	≤0.90	0.79	0.74 to 0.83	**<0.001**	74.07	83.81	4.58	0.31
FFN + history	≤0.89	0.76	0.71 to 0.80	**<0.001**	77.78	66.77	2.34	0.33
EIS+CL+FFN+ history	≤0.91	0.84	0.80 to 0.88	**<0.001**	74.07	81.79	4.07	0.32
Visit 2 (n=114, 13 spPTB vs 101 term). SpPTB < 37 weeks.								
EIS index + history	≤0.78	0.79	0.71 to 0.86	**<0.001**	61.54	87.13	4.78	0.44
CL + history	≤0.74	0.74	0.65 to 0.82	**0.001**	53.85	87.13	4.18	0.53
FFN + history	≤0.89	0.65	0.56 to 0.74	**0.042**	92.31	32.32	1.36	0.24
EIS+CL+FFN+ history	≤0.85	0.85	0.77 to 0.91	**<0.001**	84.62	76.77	3.64	0.20
Visit 1 (n=342, 8 spPTB vs 334 term). SpPTB < 32 weeks.								
EIS index + history	≤0.95	0.85	0.81 to 0.89	**<0.001**	75.00	89.82	7.37	0.28
CL + history	≤0.99	0.86	0.82 to 0.89	**<0.001**	87.50	76.95	3.80	0.16
FFN + history	≤0.98	0.87	0.83 to 0.91	**<0.001**	87.50	84.94	5.81	0.15
EIS+CL+FFN+ history	≤0.99	0.89	0.85 to 0.92	**<0.001**	87.50	85.24	5.93	0.15

4.3.10 Performance of predictive tests within different risk subgroups

An alternative perspective on the use of EIS as a screening tool may be gained by assessing its performance in different risk groups.

4.3.10.1 AHR women only

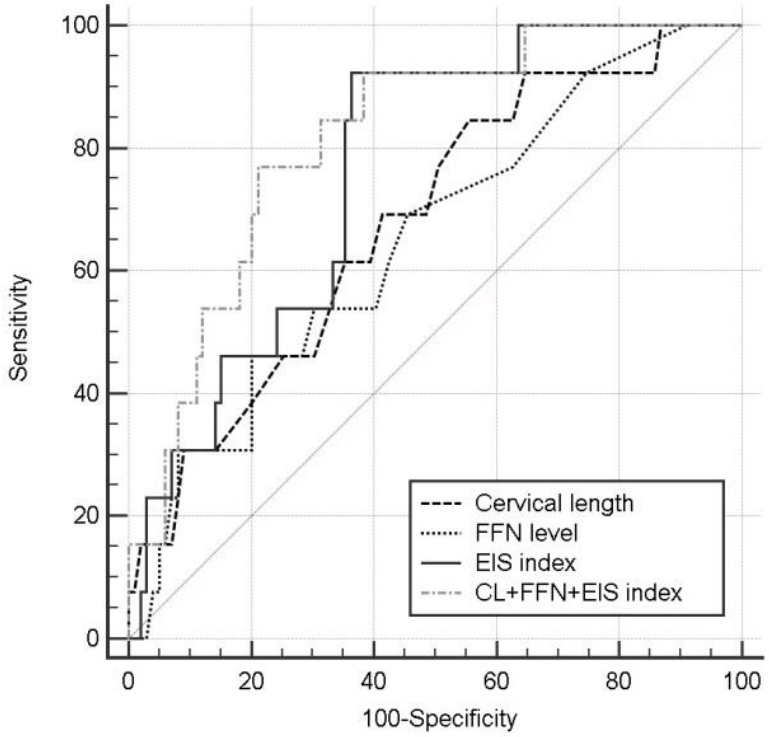
In AHR women, both EIS and CL measurement were fair predictors of spPTB at visit 1 with significant AUCs of 0.74 and 0.79 respectively (both p<0.001). The AUCs for FFN suggest poor discrimination (0.61 visit 1, 0.65 visit 2) and did not achieve significance at either timepoint (p=0.167 and 0.06 respectively). At Visit 2 EIS outperformed CL scanning, with an AUC of 0.76 vs 0.68 (p<0.001 and 0.015). At both timepoints, there was a trend towards improved prediction by combining all three predictive methods (AUC=0.82 at both visits suggesting good prediction, p<0.001) although the curves did not differ significantly from that of EIS alone. These results are summarised in Figures 4-16 and 4-17.



Untreated AHR Women at Visit 1 (n=132, 21 spPTB *vs.* 111 term)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤36mm	0.793	0.714 to 0.859	**<0.001**
FFN	>14ng/ml	0.607	0.519 to 0.691	0.167
EIS index	≤0.86	0.737	0.653 to 0.809	**<0.001**
CL+FFN+EIS	≤0.89	0.821	0.744 to 0.882	**<0.001**

Figure 4-16 Prediction of SpPTB <37 weeks at Visit 1 in Asymptomatic High-Risk Women: Comparison of ROC Curves



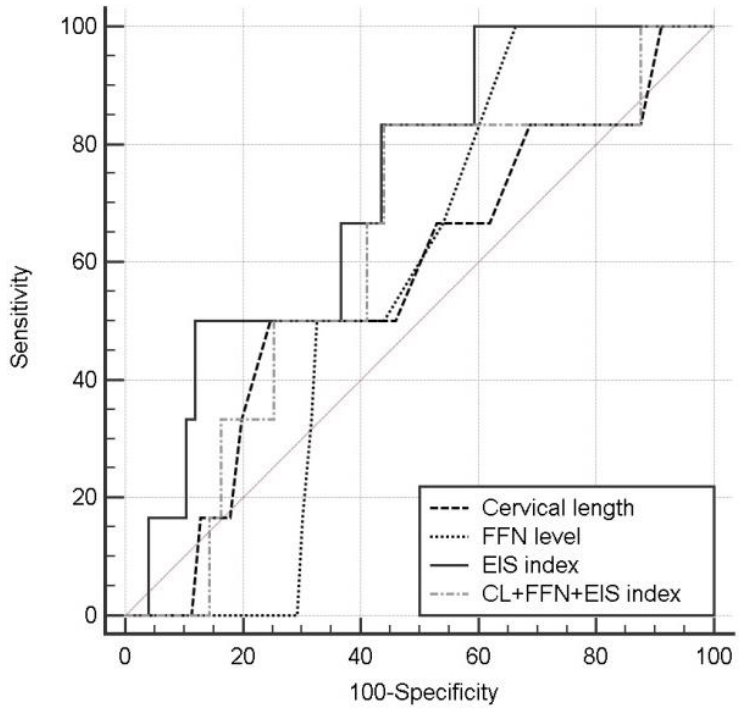
Untreated AHR Women at Visit 2 (n=112, 13 spPTB *vs.* 99 term)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤37mm	0.680	0.585 to 0.765	**0.015**
FFN	>14 ng/ml	0.652	0.556 to 0.739	0.060
EIS index	≤0.89	0.763	0.673 to 0.838	**<0.001**
CL+FFN+EIS	≤0.87	0.817	0.733 to 0.884	**<0.001**

Figure 4-17 Prediction of SpPTB <37 weeks at Visit 2 in Asymptomatic High-Risk Women: Comparison of ROC Curves

4.3.10.2 ALR women only

As a screening test in ALR women attending at 20-22 weeks, EIS performed better than either CL or FFN measurement and generated the only significant AUC with ROC curve analysis (AUC 0.72, p=0.015 for EIS, 0.57, p=0.58 for CL and 0.56, p=0.34 for FFN). The numbers of spPTBs in this subgroup are small (as would be expected from their low risk status), thus these results must be considered with a degree of caution. Combining all three tests did not improve prediction in this subgroup (AUC 0.62, p=0.30).



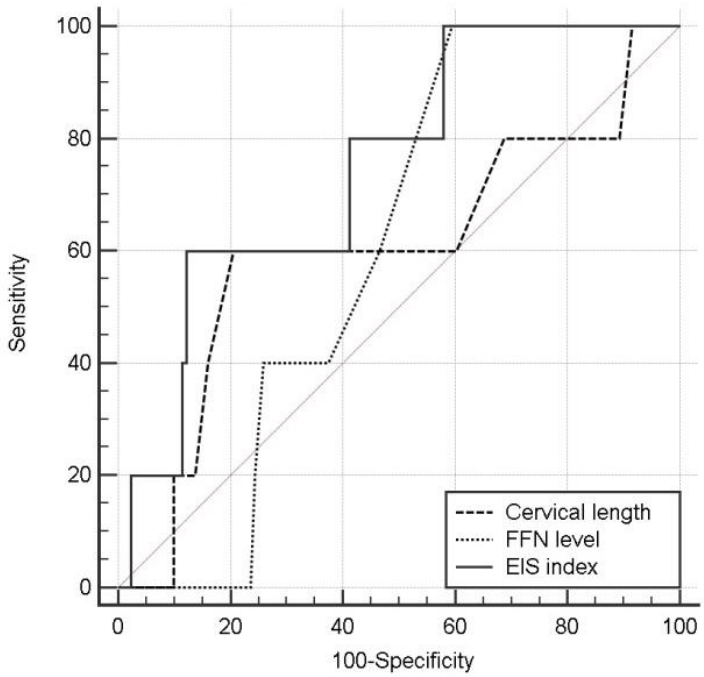
Untreated ALR Women at Visit 1 (n=208, 6 spPTB *vs.* 202 term)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤44	0.571	0.501 to 0.639	0.577
FFN	>3ng/ml	0.563	0.493 to 0.632	0.340
EIS index	≤90	0.724	0.658 to 0.783	**0.015**
CL+FFN+EIS	≤0.94	0.619	0.549 to 0.685	0.295

Figure 4-18 Prediction of SpPTB <37 weeks at Visit 1 in Asymptomatic Low-Risk Women: Comparison of ROC Curves

4.3.10.3 Nulliparous ALR only

Nulliparous women represent a particularly important group when considering PTB screening. With no obstetric history to guide risk assessment, selecting those at risk of early birth has proved challenging[21]. When considering this group alone within our cohort, EIS again compares favourably to conventional screening techniques with an AUC of 0.75 (p=0.008), whereas the AUCs for CL (0.60) and FFN (0.61) suggest poor discrimination with both p values >0.05. Multi-modal prediction was similarly limited (AUC 0.62 p=0.40). Validation within a larger cohort is necessary to confirm whether the promise of EIS can be replicated.



Untreated Nulliparous Women at Visit 1 (n=137, 5 spPTB *vs.* 132 term)

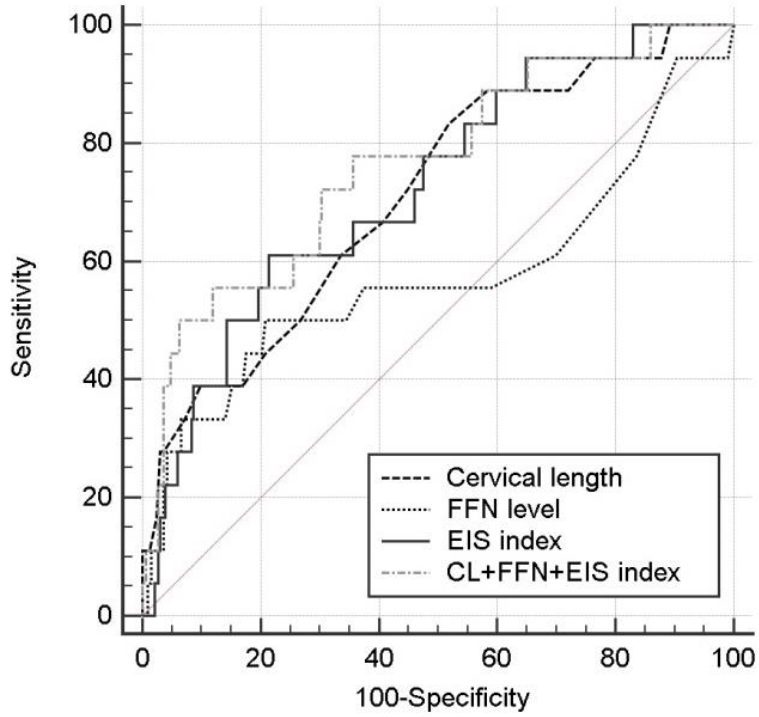
Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤44mm	0.604	0.516 to 0.687	0.523
FFN	>3ng/ml	0.605	0.518 to 0.688	0.150
EIS index	≤0.82	0.750	0.668 to 0.820	**0.008**
CL+FFN+EIS	≤0.94	0.618	0.531 to 0.700	0.399

Figure 4-19 Prediction of SpPTB <37 weeks at Visit 1 in Asymptomatic Nulliparous Women: Comparison of ROC Curves

4.3.11 Prediction of other secondary outcome measures

When considering prediction of PTB, it is important to consider not just gestational age at birth, but also the sequelae which result from early delivery. The ideal test would be able to predict not just premature birth, but particularly those with adverse consequences. Data on a range of secondary outcome measures was collected however, as Table 4-2 has already shown, the number of neonates experiencing the individual complications (neonatal death/perinatal mortality, respiratory distress syndrome, intraventricular haemorrhage, necrotising enterocolitis and sepsis) was thankfully relatively small. In view of this, a composite measure of perinatal morbidity and mortality (PNM&M) forms the basis of the analyses which follow.

Prediction of PNM&M by EIS amongst the entire untreated cohort was fair with an AUC of 0.73 at visit 1 (p<0.001). This was similar to the AUC generated by CL measurement (AUC=0.71, p=0.001, p value for comparison 0.89). FFN concentration was a poor predictor of PNM&M (AUC 0.58, p=0.40). A higher AUC was generated by multi-modal prediction using all three tests (AUC=0.76, p<0.001) but this was only significantly superior to prediction by FFN alone (p=0.01, whereas p>0.05 for other comparisons). These results are summarised in Figure 4-20.

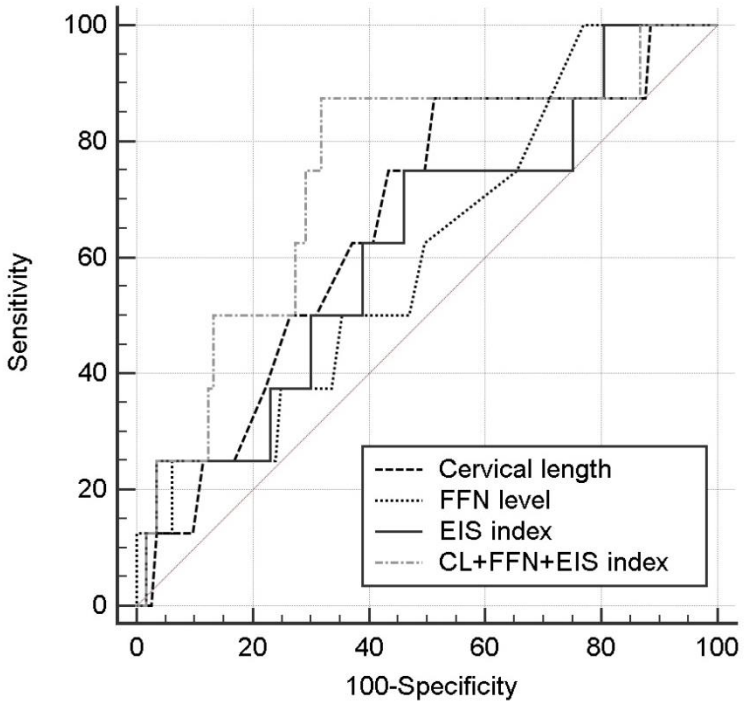


Untreated Asymptomatic Women at Visit 1 (n=354, 18 with perinatal morbidity or mortality *vs*. 336 without)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤39mm	0.712	0.662 to 0.758	**0.001**
FFN	>17ng/ml	0.575	0.522 to 0.628	0.401
EIS index	>0.10	0.725	0.675 to 0.770	**<0.001**
CL+FFN+EIS	>0.11	0.764	0.716 to 0.807	**<0.001**

Figure 4-20 Prediction of Composite Perinatal Morbidity and Mortality in Untreated Asymptomatic Women at Visit 1

At Visit 2, only multi-modal prediction generated a significant ROC AUC of 0.74 with p=0.015 suggesting fair predictive ability (compared to AUC 0.66, p=0.09 for CL alone; AUC 0.61, p=0.29 for FFN alone and AUC 0.63 p=0.24 for EIS alone).



Untreated Asymptomatic Women at Visit 2 (n=121, 8 with perinatal morbidity or mortality *vs*. 113 without)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤36mm	0.660	0.568 to 0.743	0.094
FFN	>2ng/ml	0.608	0.516 to 0.696	0.286
EIS index	>0.09	0.626	0.534 to 0.712	0.243
CL+FFN+EIS	>0.08	0.742	0.655 to 0.817	**0.015**

Figure 4-21 Prediction of Composite Perinatal Morbidity and Mortality in Untreated Asymptomatic Women at Visit 2

4.4 Discussion

This is the first study to definitively assess the use of cervical EIS to predict PTB in a general population of asymptomatic pregnant women. The cohort was broadly reflective of the antenatal population in Sheffield although non-Caucasian ethnic groups were slightly less well represented. It is unclear whether this suggests variation in cultural attitudes to research in general or to the study investigations specifically. The qualitative work which follows later in this thesis aims to explore differences in test acceptability between study participants and offers some insight here.

The observation that the SpPTB group were more likely to be obese, smoke and be of non-white ethnicity is consistent with existing literature - these factors are recognised to increase the risk of PTB[365-368]. The trend towards higher colposcopy rates in the SpPTB group is also unsurprising given the longstanding recognition of an association between SpPTB and colposcopy treatment[369]. However the difference did not reach statistical significance, which may reflect both a limited sample size in the 'previous colposcopy' subgroup and the fact that one small prior LLETZ procedure is not consistently associated with a significant increase in subsequent PTB rates[370]. The higher rate of prophylactic treatment (progesterone and cerclage) seen in those women destined to deliver preterm is unlikely to represent a causal association, but rather indicates that women receiving prophylactic therapy were significantly higher risk than those who did not require treatment. Indeed, whilst a proportion of women received history-indicated prophylaxis, a significant number commenced treatment due to abnormal predictive test results. Unfortunately the numbers of participants receiving prophylaxis was too small to permit meaningful subgroup analysis, but it would be interesting to assess the effect of cerclage and progesterone on cervical impedance in future work.

The pattern of lower cervical impedance in women destined to deliver preterm demonstrated here confirms the results of earlier pilot work in AHR women[25]. It is not unexpected that differences between term and preterm CR only reach significance in the mid to high frequency range. At low frequencies, the proportion of applied current which can cross the capacitative barrier of the cell membrane to reach sub-epithelial structures is limited[299]. Low frequency readings are more influenced by variation in tissue surface conditions (e.g. amount and composition of cervical mucous)[289] and therefore are likely to reflect pathogenic differences in tissue structure less reliably. Moreover, the frequency range at which significant differences in CR were identified is consistent with the pilot study which demonstrated optimal predictive performance of EIS at 39.1 kHz[25]. The observed differences reported in section 4.3.4 underpinned the decision to incorporate CR at 39 to 625 kHz in the EIS index ultimately used for PTB prediction.

The findings from the participants with a history of 'LLETZ only' treatment, reported in section 4.3.6, provide interesting insight regarding the effect of colposcopy treatment on cervical resistivity. The numbers within this subgroup are small, and the majority of differences seen did not achieve statistical significance, but the trend towards higher CR in

preterm delivering women may suggest that greater scarring following colposcopy treatment has the effect of increasing both cervical impedance and susceptibility to PTB. Whether more scarring is directly associated with a reduction in mechanical strength or barrier function (with resultant susceptibility to ascending infection) is unclear - if it is, then there could be a putative role for EIS in PTB prediction in this group. Moreover, these findings justify the exclusion of treated women from the main asymptomatic analyses as their results would act to confound the discriminatory ability of EIS.

The variation seen in CL and FFN levels between term and preterm groups is again consistent with existing research. The use of universal CL scanning has often been proposed as a useful screening test for PTB[177, 190, 340, 371] and the finding of significantly shorter cervical length in women who went on to experience spPTB is in broadly in accord with such work. However, it is notable that at both study visits, mean CL in the spPTB group was >25mm, in keeping with what would conventionally be considered a normal cervical length[19]. The differences in FFN level did not achieve statistical significance, although the finding of higher mean FFN concentrations in the discharge of preterm delivering women was seen at both visits. Nevertheless, mean FFN level in the spPTB was again in keeping with what would be considered a normal result clinically (i.e. <50ng/ml – a negative result)[203].

The primary aim of this study was to evaluate the performance of cervical EIS as a predictive test for PTB and the results reported in sections 4.3.8 to 4.3.10 validate the promising results of Anumba *et al*.[25] ROC curve analysis demonstrates that the predictive performance of EIS is superior to FFN and comparable to CL at 20-22 weeks gestation. Moreover, predictive ability is maintained with advancing gestation: the accuracy of CL scanning declines at 26-28 weeks whereas the accuracy of EIS actually improves (with AUCs of 0.73 and 0.69 for CL and 0.72 and 0.77 for EIS at visits 1 and 2 respectively). These results are in keeping with previous observational studies which have demonstrated that a significant proportion of women with short cervices in the early third trimester do not proceed to PTB[240, 372, 373]. It is also striking that the optimal thresholds for CL and FFN were ≤35 mm and >7ng/ml at visit 1 and ≤37 mm and >14ng/ml at visit 2 (perhaps unsurprising given the mean values described above). In practice, adoption of such thresholds would likely result in a significant proportion of women screening positive which limits their clinical utility – the potential for false positive screening is increased, with resultant effects on both patient anxiety and increased risk of complications from unnecessary prophylactic therapy.

The relatively poor performance of FFN in this asymptomatic cohort is consistent with other research which demonstrated limited utility in asymptomatic populations without prior PTB[21, 188]. Whilst the ROC AUCs and other predictive parameters improved slightly at visit 2 compared to visit 1, the AUC remained non-significant. However, FFN did have better ability to predict earlier PTB: as Table 4-6 summarises, all three tests yielded comparable AUCs of 0.79 to 0.82 (all p values for comparison >0.05) for prediction of SpPTB<32 weeks. Given the particular utility FFN has in predicting PTB in symptomatic women[212], and the fact that FFN release into cervicovaginal secretions appears to be a predictor of more imminent PTB[203], the improvement in performance with greater proximity to delivery is predictable.

Incorporating a history of at least one prior PTB/late miscarriage into risk assessments in combination with predictive technology is a promising strategy in this cohort. The higher AUCs and sensitivities, specificities and likelihood ratios generated by this approach (as demonstrated in figures 4-14 and 4-15 and Table 4-7) suggest it may have merit when employed for screening an unselected asymptomatic antenatal population. EIS as a standalone test performs comparably well when paired with obstetric history to the combination of EIS+CL+FFN+previous history (AUC 0.82 vs 0.84 p=0.42 at visit 1, AUC 0.79 vs 0.85, p=0.39 at visit 2) suggesting it could play a particular role in screening when access to ultrasound is difficult (e.g. due to resource or training issues).

When participants are considered according to their *a priori* risk grouping, EIS again performs well, with maintained predictive ability at both study visits (ROC AUCs of 0.74 and 0.76 at visits 1 and 2 respectively) compared with a decline in the accuracy of CL scanning (ROC AUCs of 0.79 and 0.68) when used in AHR women (Figures 4-16 and 4-17). Interestingly, despite previous research suggesting a role for FFN in AHR screening[184, 374], it had less utility in our cohort (generating no significant AUCs for prediction of SpPTB<37/40). This may in part be explained by the use of different outcome measures (e.g. prediction of earlier PTB *vs.* spontaneous delivery <37 weeks) as its particular strength lies in the short term exclusion of PTB. Amongst the ALR and nulliparous groups of women, prediction of PTB by EIS was superior to either CL or FFN measurement (Figures 4-18 and 4-19). The small numbers of spPTBs amongst these subgroups mean the data must be interpreted cautiously, but further evaluation should be considered. Accordingly, a power calculation was conducted to estimate the necessary sample size for a substantive evaluation of EIS prediction in such women. The prevalence of preterm birth in the ALR and nulliparous ALR

subgroups was particularly low at 2.9 and 3.6% respectively. Larger studies of low risk and nulliparous women suggest a prevalence of 5% is more representative of similar obstetric populations in high resource settings[21, 188]. A possible explanation for the low prevalence observed in our cohort may lie in the demographic make-up of these subgroups. The Jessop Wing is a direct neighbour of the University of Sheffield, the Royal Hallamshire, Weston Park and Dental Hospitals and a significant number of women who agreed to take part were either University or NHS staff. Whilst information on occupation was not routinely recorded during data collection, it is known that socio-economic deprivation[375, 376] and lower educational level[377-379] are associated with higher PTB rates. It may be that such women were relatively under-represented in the low risk sub-groups with a resultant effect on PTB rate. Therefore, for the sample size calculation which follows, a pragmatic decision was made to use an estimated PTB prevalence of 5%. Using the method described by Buderer[342], given a sensitivity estimate of 60.0% and a specificity of 87.9% (from previous ROC curve analyses) 1844 nulliparous low risk participants would need to be recruited to reliably assess test performance with a 95% confidence interval width of 10% and a type 1 error rate of 0.05. For a more precise confidence interval width of 5%, ~7400 women would need to be recruited. Use of predictive parameters for ALR women more generally yields very similar sample sizes.

The final results section considers the prediction of fetal/neonatal outcome rather than delivery gestation. It could be argued that prediction of birth before 37 weeks alone is immaterial – what matters is whether harm ensues as a result of that PTB. However, a potential problem with using PNM&M as an outcome measure in this context is that morbidity and mortality clearly occur for reasons unrelated to prematurity. The selected predictive tests are unlikely to be useful in identifying patients at risk (for example) of late onset fetal growth restriction, placental abruption or unexplained stillbirth, all of which were causes of morbidity/mortality in this cohort. Thus, sensitivity and specificity will inevitably be somewhat lower when composite PNM&M is selected as the outcome of interest. Nevertheless, when Figures 4-20 and 4-21 are considered, it is evident that some predictive ability is maintained (particularly at visit 1) with significant AUCs of 0.71, 0.73 and 0.76 generated by CL, EIS and multimodal screening respectively. Numbers were smaller at visit 2 and only multimodal testing generated a significant AUC of 0.74 at this time-point. This is explained by the small numbers of women who proceeded to earlier spPTB after attending visit 2 – only 1 delivered before 32 weeks. Therefore, a higher proportion of the

cases experiencing PNM&M after visit 2 had complications due to reasons other than prematurity. Ultimately, a full and comprehensive assessment of any screening programme would need to assess the effect of both predictive technology and prophylactic/preparatory treatment in tandem. Use of core outcome sets, such as those proposed by the CROWN initiative[168] will ensure that the effect on outcomes important to both clinicians and families is thoroughly evaluated.

4.5 Conclusions

This prospective cohort study sought to determine the ability of cervical EIS to predict spontaneous PTB in asymptomatic women. This novel technique has only previously been utilised for PTB screening in a single pilot study of high risk women[25] and one case report[305]. This is the first investigation of its application in a general antenatal population. An additional aim of the research was to compare the performance of EIS to conventional techniques such as CL measurement and FFN estimation and consider its implementation as both a stand-alone test and as part of multi-modal screening. The study findings indicate that EIS offers an effective alternative to CL screening when employed in the mid and early third trimester. It performs well as a solitary test (particularly when combined with a history of previous PTB) and offers several advantages to transvaginal ultrasound: the equipment is cheaper, obtaining measurements is easier (thus clinicians able to perform a speculum examination could easily be taught to measure cervical impedance) and it does not appear to exhibit the same loss of predictive accuracy as CL at later gestational ages. Taken together, these observations support a particular role for EIS screening in obstetric facilities where access to cervical length scanning is challenging. This naturally raises the possibility of application to low-resource settings; however, even within the NHS, the increasing pressure on ultrasound services means that measures to reduce the number of scans performed may be welcomed by commissioning teams. Alternatively, where CL screening is already in routine use, the addition of EIS measurement may offer further enhance the predictive accuracy of screening.

The research presented here is not without limitations. It became clear early in recruitment that subgroup analysis of participants receiving prophylactic treatment or with a history of LLETZ excision would be challenging given the frequency with which multiple treatments (e.g. LLETZ plus stitch, progesterone then cerclage) occurred. Concern regarding the potential confounding effect of such treatments on CR readings led to the decision to

exclude such women from the main study analyses. Small numbers within the 'stitch only' and 'progesterone only' subgroups also precluded appraisal of prophylactic treatment effects. Exclusion of treated women strengthens confidence in the finding of lower CR in women destined to deliver preterm. However, it also means that some of the highest risk patients in the cohort were not included within the main analyses and 23 preterm births (14 spPTB) were excluded with a resultant reduction in power. Thus, these findings cannot easily be generalised to women who have received any kind of treatment, which might limit application in a PTB clinic setting. Similarly, the observation that impedance patterns may differ in women with prior LLETZ treatment destined to deliver preterm means screening this group requires further investigation before wider implementation. This limits the potential coverage of universal screening to some extent.

There is considerable scope for further research in this area. Firstly, larger prospective studies are required to refine the estimates of predictive accuracy reported here. The sample size calculations in section 4.4 provide a basis for studies of ALR/nulliparous women. Given a sample target of over 1800, a multi-centre design would facilitate recruitment in the timeliest fashion. Similarly, larger studies screening unselected and AHR women could strengthen the case for wider use of EIS. Measurements are easy to perform and staff would require limited additional training. Therefore, EIS could potentially be used for screening during routine antenatal appointments. The UK has a recognised shortage of ultrasonographers[380] and consequently many units have finite scan capacity, which hampers wider implementation of CL screening. However, the pool of staff able to perform EIS screening would be larger – anyone able to perform a speculum examination, could be trained to measure CR. Screening acceptance rates in studies of unselected women would also provide further insight into patient acceptability and build upon the findings of the qualitative work presented in Chapter 6

Secondly, to determine whether the introduction of wider EIS screening would result in a reduction in the spPTB rate, future work could incorporate standardised treatment protocols for women found to be at increased risk as a result of either CL or EIS measurements. The optimal design could incorporate randomisation to one of three study arms: EIS screening alone, CL screening alone or a combination of EIS and CL measurement, enabling comparison of both clinical and economic benefits from a variety of screening approaches. This design would be ideally suited to recruitment of a general asymptomatic

population, similar to that studied here. However, given the low prevalence of PTB in the general obstetric population, and the modest effect size of progesterone prophylaxis, this would require a substantial sample size (e.g. To *et al.* measured CL in over 47,000 women to recruit 253 women to their RCT of cervical cerclage *vs.* expectant management[381] and Fonseca *et al.* screened over 24,000 with CL to recruit 250 women to their RCT of vaginal progesterone *vs.* placebo[382] – indeed, these studies were anticipating a larger risk reduction between study arms than we would anticipate when comparing EIS and CL screening).

Initially it would be pragmatic to focus on AHR women – their PTB rate is higher and thus sample sizes for follow on work would typically be smaller. Given the established role of CL screening in this group, recruitment to a study with an arm without CL measurement might be difficult. However, if EIS does offer superior sensitivity for predicting PTB in these women then direct comparison of EIS and CL screening, with progesterone treatment for screen positive women should be considered. One approach might be to conduct a randomised non-inferiority (NI) trial, with parallel EIS and CL screening arms and progesterone treatment for those screening positive with either test. Dependent on the non-inferiority limit selected, NI studies typically require smaller sample sizes than superiority trials, and are appropriate for comparing interventions which offer advantages such as reduced cost or greater ease of administration[383] (both applicable to EIS). Accordingly, a sample size calculation is provided for illustration. Utilising data from Romero *et al.*'s meta-analysis[307] (which focuses on women with short CL), the RR of PTB before 33 weeks was identified as 0.62 (95% CI 0.47-0.81) for progesterone treatment *vs.* placebo. A NI limit was then set using the fixed margin approach[384, 385]. The upper limit of the confidence interval (i.e. the most conservative estimate of the effect of CL scan + progesterone *vs.* placebo) was identified (denoted M1). The largest clinically acceptable difference (i.e. degree of inferiority) of EIS + progesterone (denoted M2) was set at 50% of M1 as recommended in the Food and Drug Administration guidance for non-inferiority trials[385]. Calculated at 1.1, this limit would ensure the EIS + progesterone intervention would preserve at least half of the effect of CL + progesterone in order to be deemed non-inferior. The SealedEnvelope™ sample size calculator for a non-inferiority trial with binary outcome was then utilised[386]. To confirm non-inferiority using the limit of 1.1, with 90% power and a significance level of 0.025, 243 women would need to be recruited to each arm, giving a total sample size of 486 (535 if an additional 10% margin is incorporated to accommodate loss to follow up).

An alternative approach for the AHR group would be to conduct a randomised superiority trial with two parallel arms: standard CL screening or 'enhanced screening' (CL plus EIS measurements) with prophylactic treatment contingent on the results of both tests. Within our AHR subgroup the sensitivity of EIS used in combination with CL scanning was 81%. Using the conventional clinical threshold of 25mm, CL alone performed relatively poorly in this cohort, however at systematic review, estimates of ~61% have been reported for CL measured at 20-24 weeks in AHR women with prior PTB[176]. Assuming a 25% recurrent PTB rate, and a RR of 0.62 for PTB <33 weeks with vaginal progesterone[307], it would be anticipated that CL screening in isolation might detect 15.3% of these PTB, allowing progesterone treatment to prevent 5.8%. The higher sensitivity rate of combined CL/EIS screening might detect 20.3% of PTB cases, enabling progesterone treatment to potentially prevent 7.7%. Thus estimated rates of recurrent PTB would be 19.2% in the CL screening group and 17.9% in the enhanced screening group. Using these proportions as input for the sample size calculator in Medcalc, a study with 80% power to detect a difference in PTB outcome at a 0.05% significance level would require 13,399 AHR women to be randomised to each arm, giving a total sample size of 26,798. Potential advantages and disadvantages of NI and superiority designs will be considered further in Chapter 6.

In summary, it is clear that preterm birth is an increasing focus of national and international efforts to reduce perinatal mortality and morbidity[18, 387-389]. The optimisation of screening techniques and processes is key to achieving this aim. Cervical EIS represents a useful predictive test in the mid-trimester of pregnancy for women with varied obstetric histories, and warrants further investigation in pursuit of ongoing improvements in perinatal care.

Chapter 5 - Pilot Study of Symptomatic Women: Results and Discussion

5.1 Introduction

Any effective strategy to minimise the impact of PTB requires multiple approaches. The prediction of PTB in asymptomatic women has already been considered in Chapter 3. Whilst screening offers the best hope of PTB prevention, it has inherent limitations: some women will decline tests and others may book late and miss screening windows inadvertently. Therefore, when evaluating a novel predictive test such as EIS, its use in the assessment of women with symptoms of preterm labour should also be considered.

At present, the efficacy of treatments designed to stop PTL is debatable: tocolysis may slightly delay delivery, but does not, in itself, consistently improve neonatal outcome[325, 327, 390, 391]. This may partly result from difficulty identifying those women in 'true' PTL: the specificity of a clinical diagnosis of PTL is notoriously poor[380, 392, 393], thus the application of new tests to the diagnosis of true early PTL could yet change PTB incidence. There is also a substantial body of work demonstrating reduction in PTB-associated morbidity when preparatory measures are utilised (namely antenatal corticosteroids[332], neuroprotective magnesium sulphate[334, 336], and in-utero transfer to units with an appropriate level of neonatal care when relevant[7]). However, these should be used close to the time of birth and may be associated with significant costs[19, 337]. Effective predictive tests will allow such measures to be accurately targeted at those who will truly benefit, minimising both morbidity and expense to the NHS. In view of this, a pilot study to investigate the use of cervical EIS in predicting PTB in women presenting with symptoms of PTL was conducted, the results of which are presented here.

5.2 Study design and population

Participants were recruited opportunistically when they presented to hospital with symptoms of early preterm labour. For the purposes of this study, early PTL was defined as a contraction frequency of at least two in every ten minutes, with cervical dilatation less than 3cm and intact membranes. As discussed in Chapter 2, women were excluded if they had a recent abnormal smear result, current cervico-vaginal infection, active vaginal bleeding or were carrying a multiple pregnancy or fetus with a known congenital anomaly.

All women were given written information about the study and time to consider whether they wished to participate before written consent was obtained. In total, 56 women agreed to take part.

Study visits were conducted as described in Chapter 2. Briefly, all participants had a speculum examination in order to (i) assess cervical dilatation, (ii) obtain swabs for FFN quantification, pH measurement and microbiological screening and (iii) perform CR measurements. Thereafter a TVUSS of CL was performed. Participants and researchers were blinded to the results of the EIS test, but not to the CL scan or FFN swab. Women with a positive FFN swab (≥50ng/ml) or short CL (≤15mm) received treatment for PTL as per local protocols (2 doses of intramuscular betamethasone 12mg 24 hours apart, and tocolysis with intravenous atosiban if regular uterine activity persisted, see Appendix E). Magnesium sulphate for fetal neuroprotection was not routinely used in the hospital during the study period.

Clinical outcomes were collected from the maternity and neonatal databases following delivery. For women attending the hospital from other geographical areas, every effort was made to obtain full outcome data. Two patients were lost to follow up having moved away and not yet registered with a new General Practitioner.

Statistical analysis was performed using SPSS (IBM Corp. Released 2019. IBM SPSS Statistics for Windows, Version 26.0. Armonk, NY: IBM Corp). Additional ROC curve analyses were performed using MedCalc (MedCalc Software bvba. Released 2018. MedCalc Statistical Software, Version 18.2.1. Ostend, Belgium).

5.3 Results

5.3.1 Participant Demographics

Demographic information for the 56 study participants is summarised in Table 5-1. Overall, women experiencing PTB had higher BMIs than their term counterparts and those who experienced a medically-indicated PTB were older than the spPTB and term groups. When comparing term and iatrogenic PTB groups, the observed differences in age and BMI were not significant (29.3 *vs.* 27.1 years, p=0.84 and 30.5 *vs.* 26.3 kg/m^2, p=0.13, Mann Whitney U), likely due to the low number of iatrogenic PTBs. Women experiencing spontaneous PTB had significantly higher BMIs than their term counterparts (29.9 *vs.* 26.3 kg/m^2, p=0.048,

Mann Whitney U) but were of similar age (27.7 *vs.* 27.1 years p=0.79, Mann Whitney U). The majority of spontaneous PTBs occurred in women who were either nulliparous or had experienced a previous PTB. Rates of prior PTB were higher in the spPTB *vs.* term birth group, but not significantly so (44.4 *vs.* 30.9% of multiparous participants, p=0.39, Fisher's Exact test). Caucasian women were the dominant ethnic group within the cohort as a whole (46 out of 56, 82.1%) and made up the majority of the spPTB subgroup. Smoking rates were not significantly different between term and spPTB groups (p=0.92, Mann Whitney U).

Table 5-1 Demographic Details of the Symptomatic Cohort by Clinical Outcome

Clinical outcome	Spontaneous PTB (n=9)	Iatrogenic PTB (n=3)	Term birth (n=42)	Lost to follow up (n=2)
Age (Mean, range)	27.7 yrs (21-40)	29.3 yrs (24 - 39)	27.1 yrs (19-44)	30 yrs (30-31)
BMI (Mean, range)	29.9 (23-43)	30.5 (26-36)	26.3 (19-42)	25.4 (25-26)
Parity				
• **Nulliparous**	4 (44.4%)	1 (33.3%)	9 (21.4%)	1 (50.0%)
• **Multiparous**	5 (55.6%)	2 (66.7%)	33 (78.6%)	1 (50.0%)
○ **Term births only**	1 (11.1%)	1 (33.3%)	20 (47.6%)	1 (50.0%)
○ **Previous preterm births**	4 (44.4%)	1 (33.3%)	13 (30.9%)	0
Ethnicity				
• **Caucasian**	8 (88.9%)	1 (33.3%)	35 (83.3%)	2 (100%)
• **South Asian**	0	1 (33.3%)	4 (9.5%)	0
• **African**	1 (11.1%)	1 (33.3%)	0	0
• **Mixed race**	0	0	2 (4.8%)	0
• **Afro-Caribbean**	0	0	1 (2.4%)	0
Smoking				
• **Yes**	2 (22.2%)	1 (33.3%)	10 (23.8%)	0
• **No**	7 (77.8%)	2 (66.7%)	32 (76.2%)	2 (100%)
Previous colposcopy treatment				
• **Yes**	2 (22.2%)	0	3 (7.1%)	0
• **No**	7 (77.8%)	3 (100%)	39 (92.9%)	2 (100%)
Antenatal progesterone therapy				
• **Yes**	2 (22.2%)	1 (33.3%)	0	1 (50%)
• **No**	7 (77.8%)	2 (66.7%)	42 (100%)	1 (50%)

However, when assessing symptomatic women, the most clinically relevant outcomes are those which guide acute management, namely the risk of delivering in the days and weeks immediately following assessment. It is this which will determine the need for interventions such as steroids, magnesium sulphate, tocolysis and in utero transfer. In addition, the

participants in this cohort underwent study measurements at a wide range of gestational ages (24 to 35 weeks), with similarly varied assessment to delivery intervals (2 to 116 days). Therefore spPTB is arguably not the most appropriate outcome measure by which to assess predictive performance. Use of short term outcome measures (delivery within 7, 14 and 28 days of testing) is more clinically useful and (to a degree) mitigates confounding by the variable gestational ages between participants at assessment – regardless of gestational age at presentation, if delivery is imminent, we would reasonably expect anticipatory cervical changes to have commenced. Henceforth, short term outcomes are described as the primary focus of analysis.

5.3.2 Delivery outcomes

Of the 54 women with outcome data, 4 (9.3%) went on to deliver within 14 days of assessment, including 2 within 7 days of the research visit. All of these births occurred spontaneously. 11 women (20.4%) delivered within 28 days of testing, with all but one experiencing either spontaneous rupture of membranes (SRM) or spontaneous onset of labour. 43 women (79.6%) had an interval of more than 28 days between assessment and delivery.

For the analyses which follow, only women with no prior colposcopic treatment were considered (n=49 with 9 deliveries within 28 days, including 4 within 14 days) due to the potential confounding effect of cervical scarring, as discussed in Chapter 4. Figure 5-1 summarises the distribution of clinical outcomes across the symptomatic cohort.

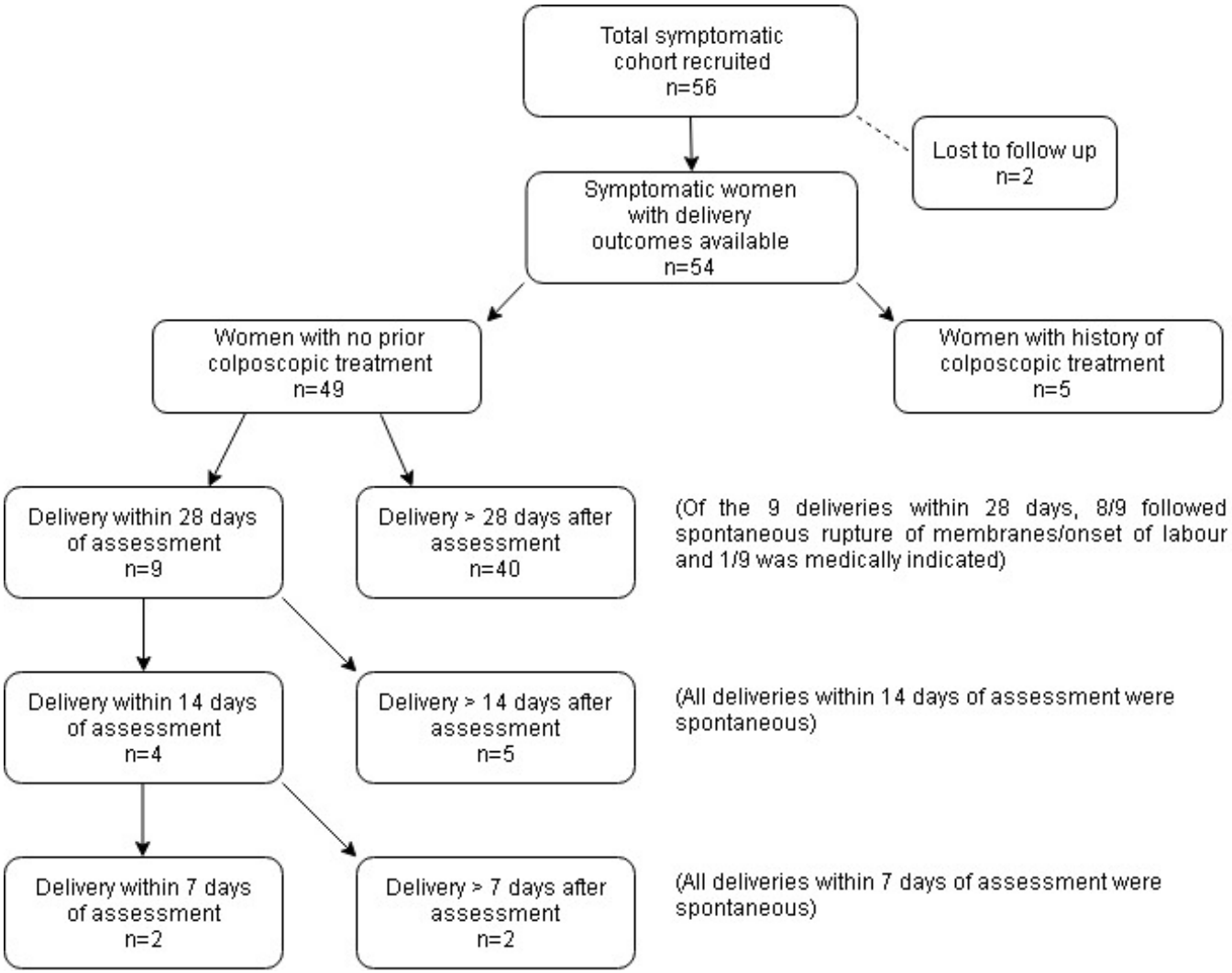
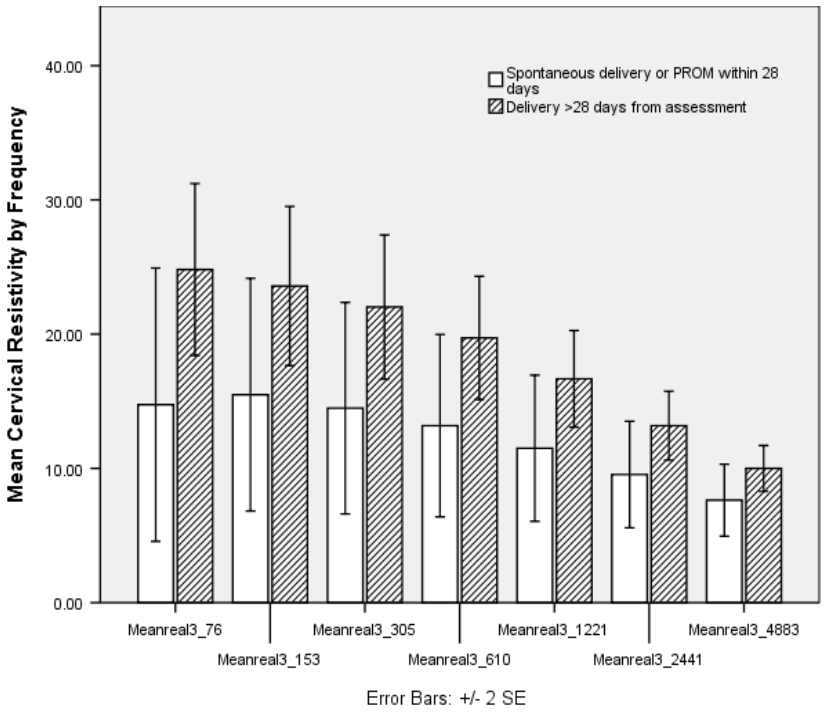


Figure 5-1 Symptomatic Study Flow Chart with Delivery Outcomes

5.3.3 Patterns of cervical resistivity by birth outcome

Women destined to deliver within 14 or 28 days of assessment demonstrated lower average CR at all 14 current frequencies than those delivering over 28 days later. Figures 5-2 and 5-3 summarise the observed differences.

(a)



(b)

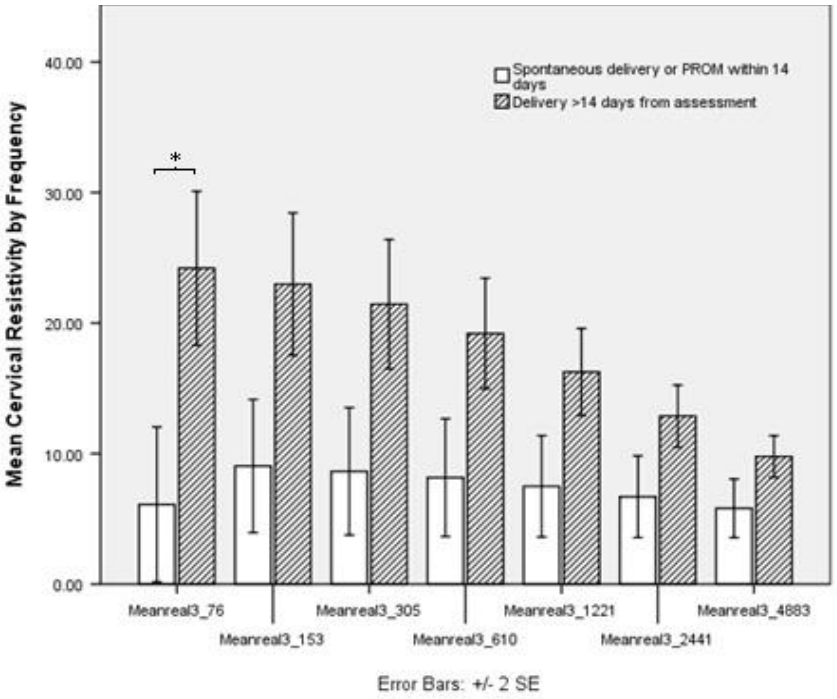
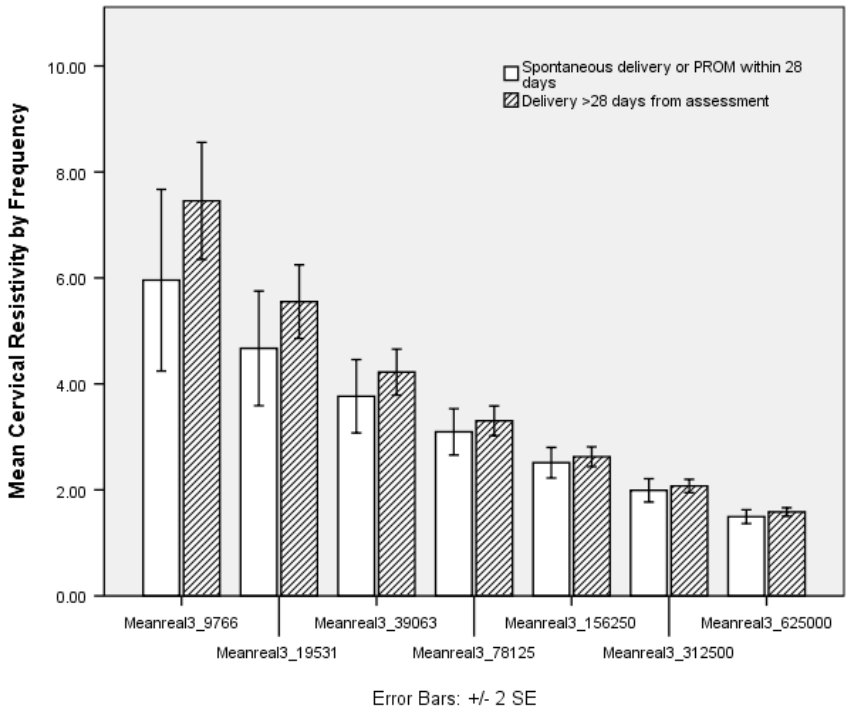


Figure 5-2 Differences in Mean Cervical Resistivity (Ohm.m) at Frequencies 1 to 7 (76 to 4883 Hz). Symptomatic women with no previous LLETZ, n=48

(a) 8 spontaneous deliveries/membrane rupture within 28 days *vs*. 40 deliveries >28 days. All p>0.05
(b) 4 spontaneous deliveries/membrane rupture within 14 days *vs*. 44 deliveries >14 days). * p<0.05
Abbreviations: PROM – pre-labour rupture of membranes

(a)



(b)

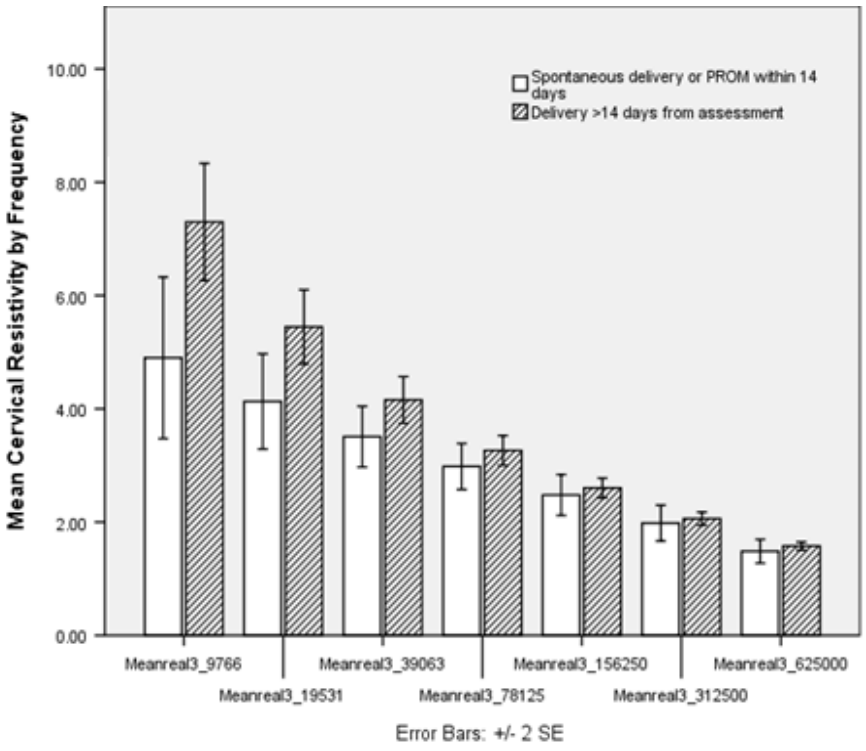


Figure 5-3 Differences in Mean Cervical Resistivity (Ohm.m) at Frequencies 8 to 14 (9766 to 625000 Hz). Symptomatic women with no previous LLETZ, n=48.

(a) 8 spontaneous deliveries/membrane rupture within 28 days *vs*. 40 deliveries >28 days. All p>0.05

(b) 4 spontaneous deliveries/membrane rupture within 14 days *vs*. 44 deliveries >14 days). All p>0.05

Abbreviations: PROM – pre-labour rupture of membranes

The wide error bars reflect the small sample size (in particular of the delivery within 14 days group, where n=4) and these differences generally did not achieve statistical significance ($p>0.05$, Mann Whitney U test, excepting mean CR at 76Hz for delivery within 14 days). However the consistent trend towards lower cervical impedance in women who were closer to experiencing spontaneous labour/SRM is noteworthy. The issue of correcting for multiple hypothesis tests has been discussed in Chapter 4 (section 4.3.4). Applying a similar approach to control the FDR for these comparisons (with a Q of 0.1) would render all differences non-significant.

The series of violin plots in Figures 5-4 to 5-9 summarise the distribution of CR readings in term and preterm groups in further detail. A wider distribution of resistivity is evident amongst those women delivering over 28 (Figure 5-4 to 5-6) and 14 days (Figure 5-7 to 5-9) from assessment when compared to their preterm delivering counterparts, with elongated density plots at the majority of frequencies for the later delivering groups. The trend towards lower CR in women delivering close to the time of assessment is more pronounced when comparing those delivering within and after 14 days of delivery *vs.* within and after 28 days. However, for both outcomes, the distributions become increasing similar at the highest current frequencies. The distribution is slightly skewed towards higher resistivity readings, and though this is evident in those delivering close to *vs.* further from assessment, it is more marked in the >14 and >28 day delivery interval groups.

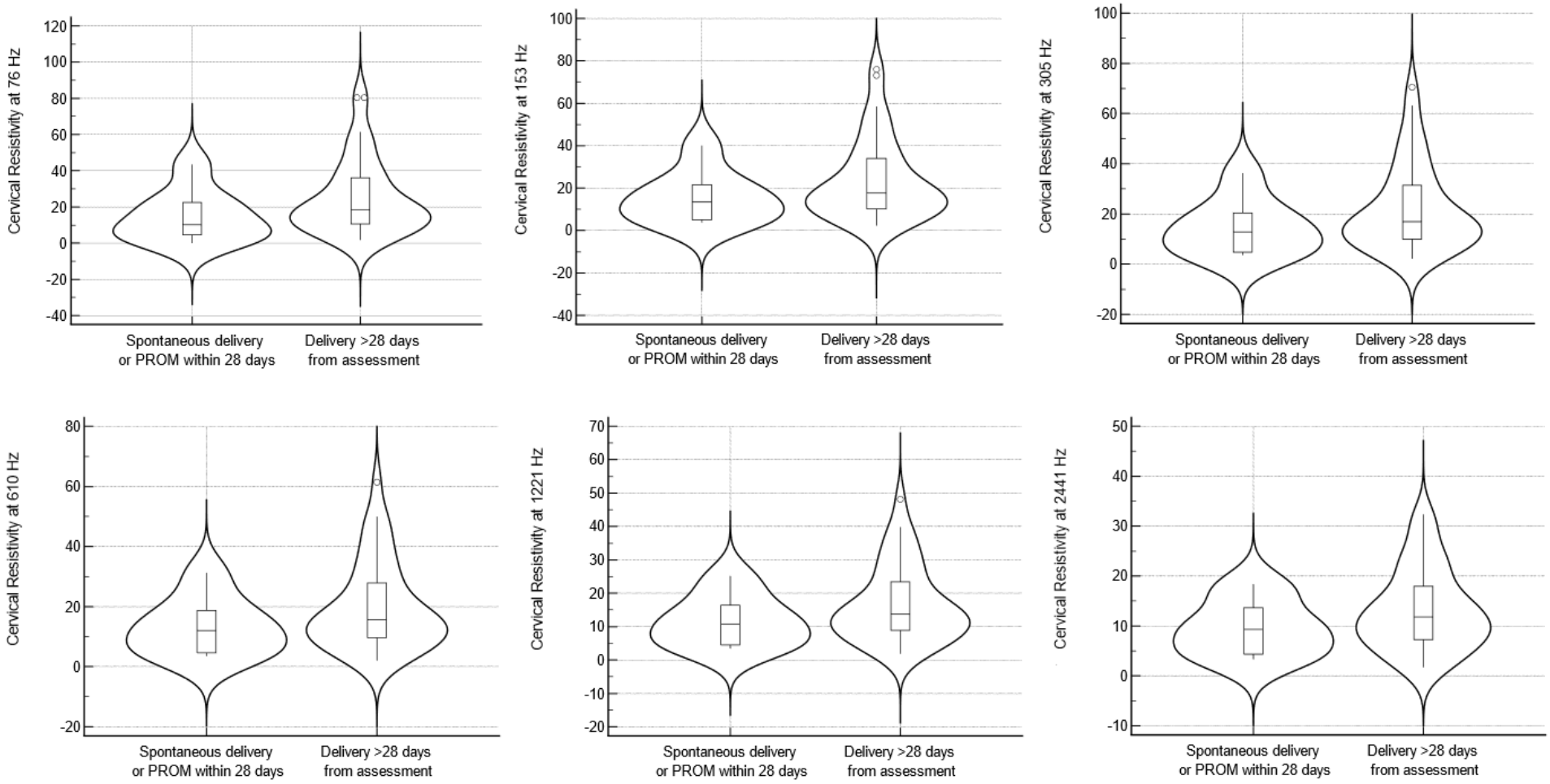


Figure 5-4 Differences in Cervical Resistivity (Ohm.m) at Frequencies 1 to 6 (76 to 2441 Hz) between women delivering within and after 28 days of assessment (Symptomatic women with no previous LLETZ, n=48)

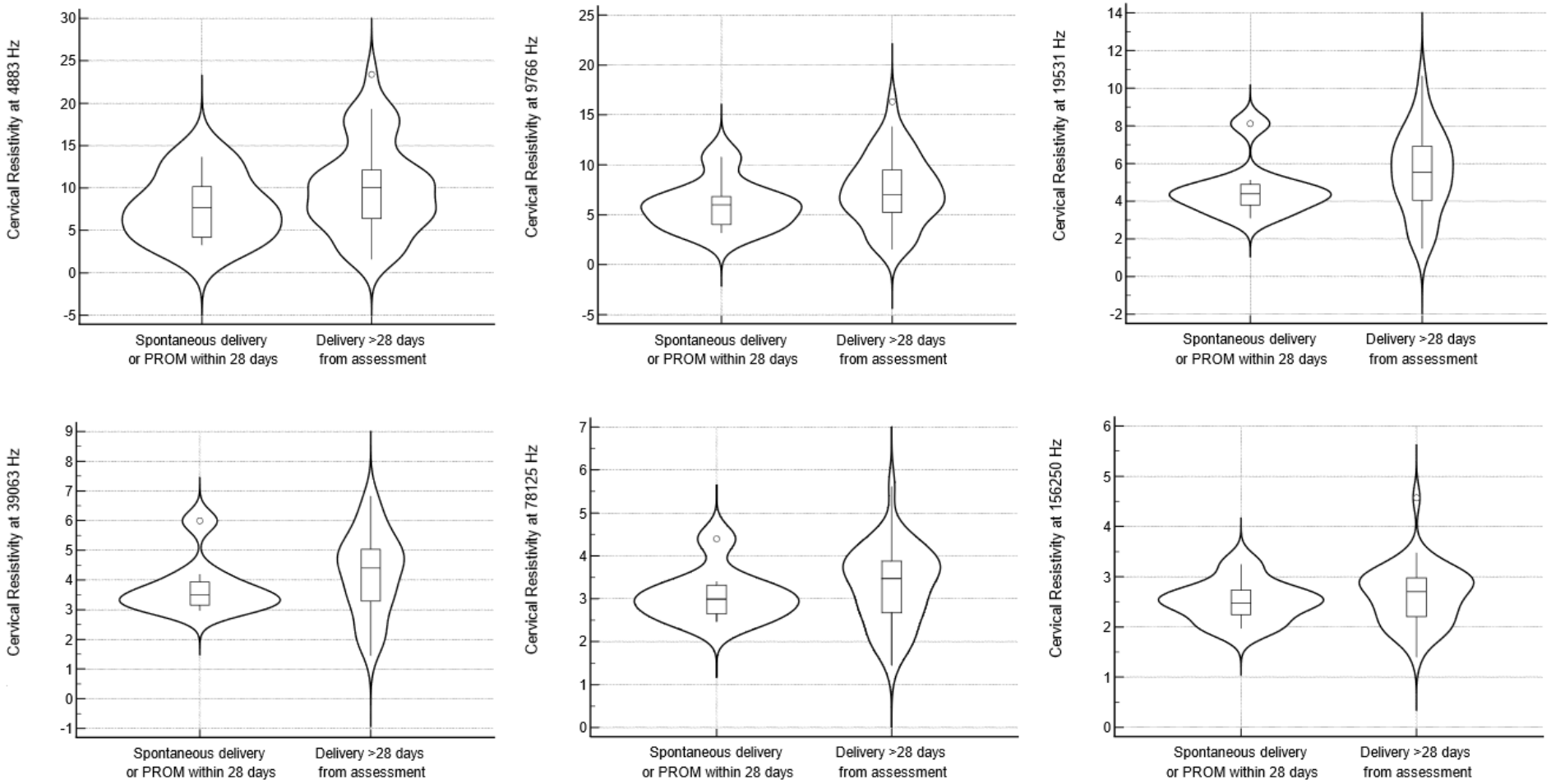


Figure 5-5 Differences in Cervical Resistivity (Ohm.m) at Frequencies 7 to 12 (4883 to 156250 Hz) between women delivering within and after 28 days of assessment (Symptomatic women with no previous LLETZ, n=48)

Figure 5-6 Differences in Cervical Resistivity (Ohm.m) at Frequencies 13 to 14 (312500 to 625000 Hz) between women delivering within and after 28 days of assessment (Symptomatic women with no previous LLETZ, n=48)

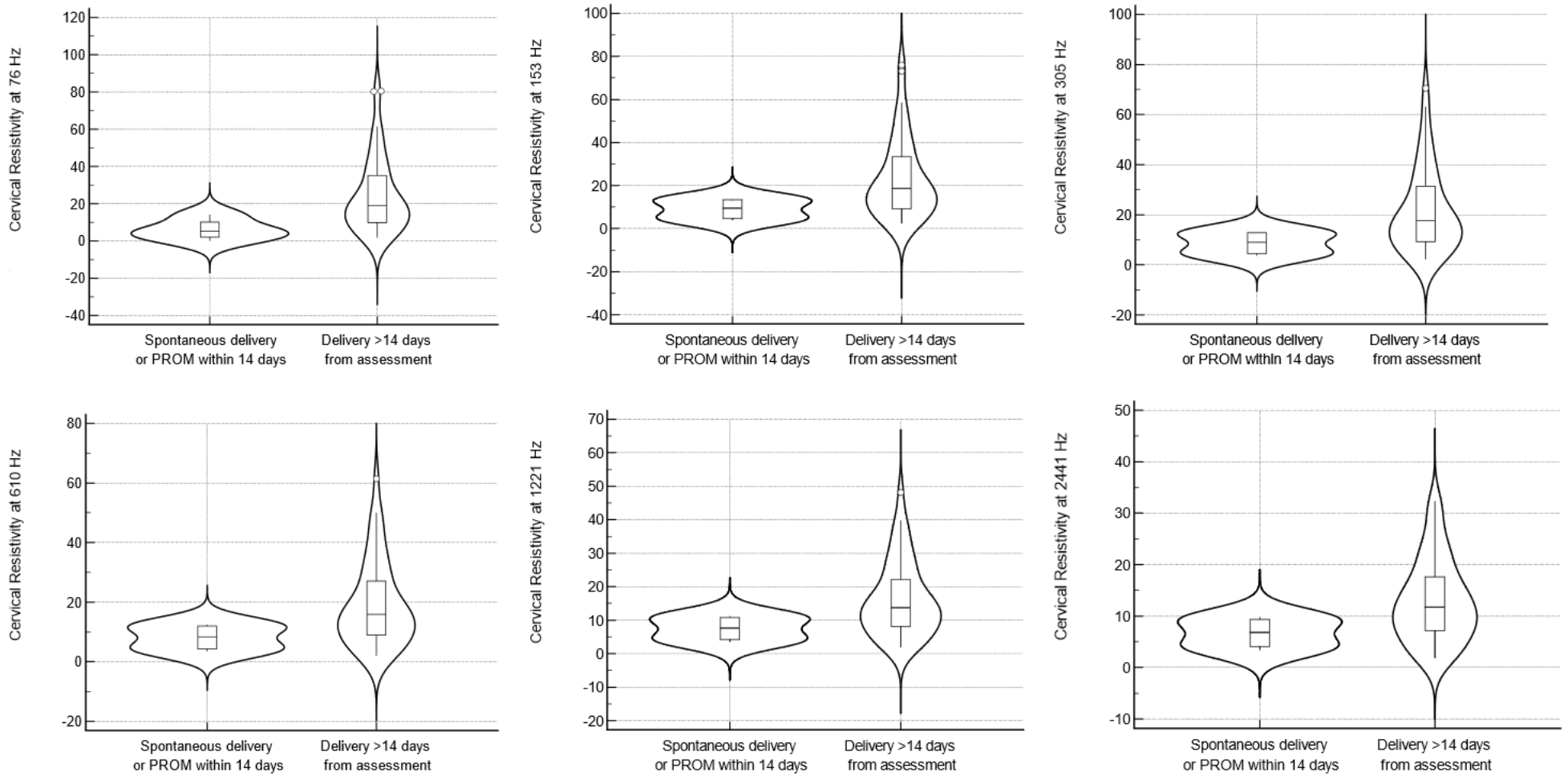


Figure 5-7 Differences in Cervical Resistivity (Ohm.m) at Frequencies 1 to 6 (76 to 2441 Hz) between women delivering within and after 14 days of assessment (Symptomatic women with no previous LLETZ, n=48)

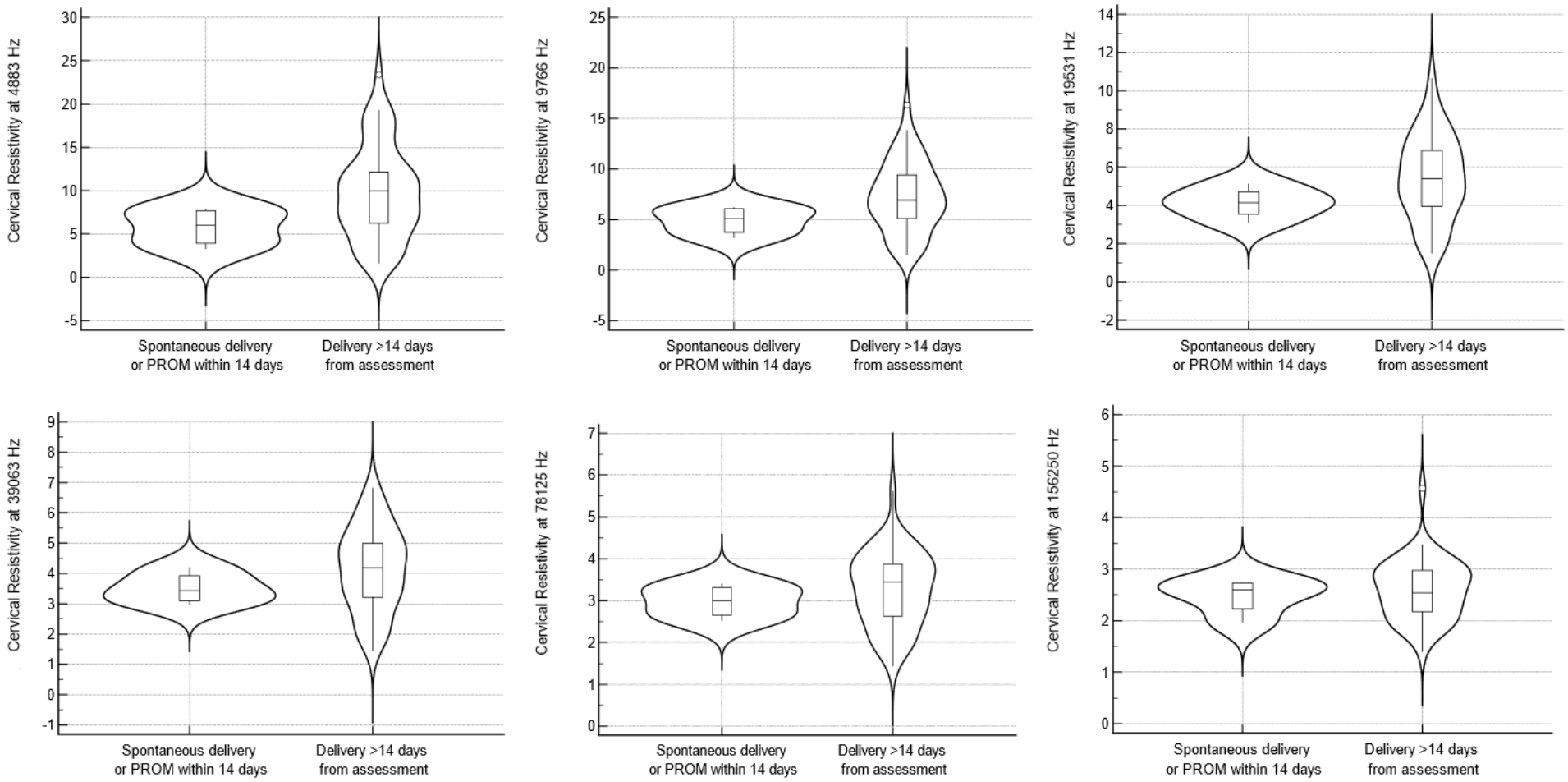


Figure 5-8 Differences in Cervical Resistivity (Ohm.m) at Frequencies 7 to 12 (4883 to 156250 Hz) between women delivering within and after 14 days of assessment (Symptomatic women with no previous LLETZ, n=48)

Figure 5-9 Differences in Cervical Resistivity (Ohm.m) at Frequencies 13 to 14 (312500 to 625000 Hz) between women delivering within and after 14 days of assessment (Symptomatic women with no previous LLETZ, n=48)

Table 5-2 summarises the mean CR values at each frequency by outcome group, with p values (generated by Mann Whitney U tests) reported for comparison of delivery within and after 14 days and within and after 28 days respectively. As only 2 women delivered within 7 days of assessment, this outcome is not considered further.

Table 5-2 Comparison of Mean Cervical Resistivity (Ohm.m) by Interval Between Assessment and Delivery (Symptomatic women with no previous LLETZ, n=48)

* Some overlap of patients between short term delivery outcome groups **p<0.05

Current frequency (Hz)	Spontaneous birth/SRM within 14 days* N=4		Spontaneous birth/SRM within 28 days* N=8		Delivery >28 days later N=40		P value for Birth <14/7 *vs.* >14/7	P value for Birth <28/7 *vs.* >28/7
	Mean CR (Ohm. m)	SD	Mean CR (Ohm. m)	SD	Mean CR (Ohm. m)	SD		
76.3	6.10	5.93	14.75	14.40	24.81	20.25	**0.024****	0.184
152.6	9.05	5.10	15.49	12.26	23.59	18.74	0.080	0.281
305.2	8.64	4.88	14.49	11.15	22.02	16.97	0.080	0.245
610.4	8.16	4.51	13.18	9.61	19.72	14.51	0.086	0.245
1220.7	7.50	3.88	11.50	7.70	16.67	11.39	0.108	0.268
2441.4	6.70	3.13	9.54	5.61	13.18	8.11	0.125	0.319
4882.8	5.80	2.25	7.63	3.79	10.00	5.40	0.144	0.391
9765.6	4.90	1.43	5.96	2.42	7.45	3.49	0.144	0.245
19531.3	4.13	0.84	4.67	1.53	5.55	2.20	0.201	0.184
39062.5	3.51	0.54	3.77	0.98	4.22	1.37	0.258	0.245
78125	2.98	0.41	3.09	0.62	3.30	0.89	0.381	0.407
156250	2.48	0.36	2.51	0.41	2.63	0.59	0.584	0.525
312500	1.98	0.32	1.99	0.31	2.07	0.40	0.742	0.619
625000	1.48	0.21	1.49	0.18	1.58	0.25	0.584	0.306

5.3.4 Results of conventional predictive tests

Significant differences in fetal fibronectin levels were observed between those delivering within 14 and 28 days of assessment and those who did not. Although mean cervical length was shorter in those destined to deliver shortly after assessment, the differences did not achieve significance. These distribution of CL and FFN results are summarised in Figures 5-10 and 5-11 below.

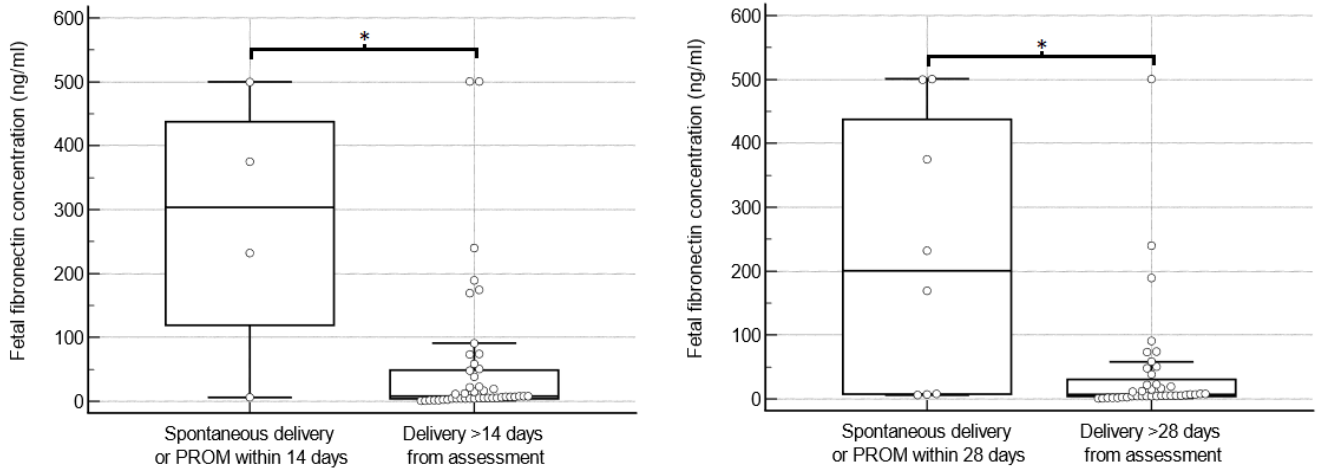


Figure 5-10 Differences in Fetal Fibronectin Concentration by Delivery Outcome

*p<0.05. Abbreviations: PROM – pre-labour rupture of membranes

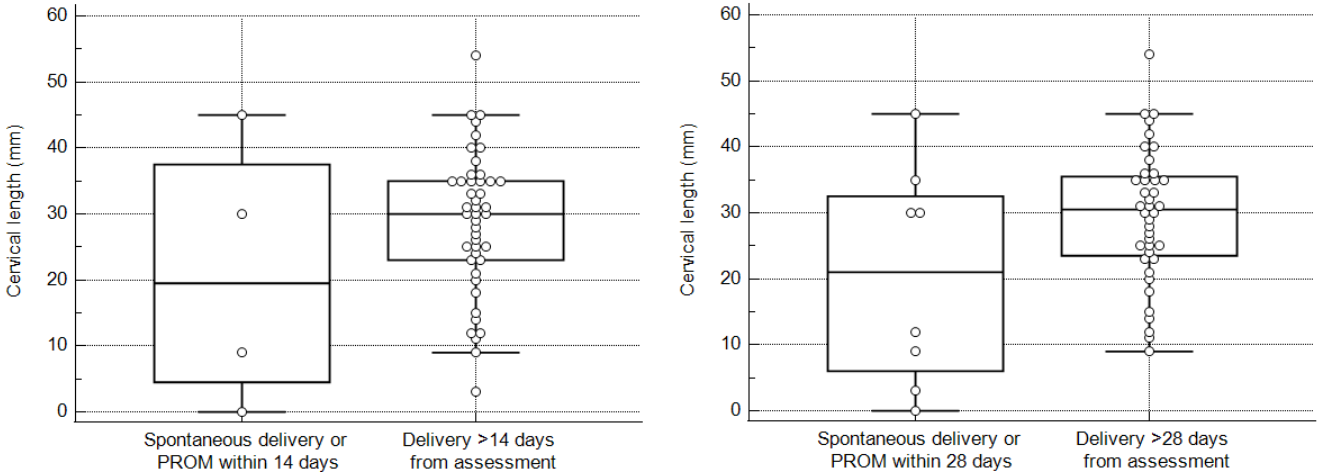


Figure 5-11 Differences in Cervical Length by Delivery Outcome

(all p>0.05). Abbreviations: PROM – pre-labour rupture of membranes

Table 5-3 further summarises the results of the conventional predictors. Mean FFN level was highest in those closest to delivery and fell as the assessment to delivery interval increased (with average levels of 438, 278, 225 and 40 ng/ml for delivery <7, <14, <28 and >28 days respectively). Differences in FFN level were statistically significant for all short term outcome comparisons. Mean CL was shortest in those closest to delivery (<7 days, 4.5mm) and longest in those destined to continue pregnancy for at least 28 days (29.7mm). However a wide range of cervical lengths were noted in the short term outcome groups (excepting <7 days) and the differences observed in length for the within/after 14 days and within/after 28 days comparisons did not achieve significance.

Table 5-3 Results of Conventional Predictive Tests by Study Group (Symptomatic women with no previous LLETZ, n=48)

* Some overlap of patients between short term delivery outcome groups

Spontaneous birth/SRM within 7 days* N=2 *vs.* 47 undelivered			**Spontaneous birth/SRM within 14 days* N=4 *vs.* 45 undelivered**			**Delivery within 28 days* N = 8 *vs.* 40 undelivered**		
Mean cervical length (mm) (range)		**P value**	**Mean cervical length (mm) (range)**		**P value**	**Mean cervical length (mm) (range)**		**P value**
<7/7	>7/7		<14/7	>14/7		<28/7	>28/7	
4.5 (0-9)	29.3 (3-54)	**0.02**	21.0 (0-45)	28.9 (3-54)	0.38	20.5 (0-45)	29.7 (9-54)	0.14
Mean fetal fibronectin level (ng/ml) (range)		**P value**	**Mean fetal fibronectin level (ng/ml) (range)**		**P value**	**Mean fetal fibronectin level (ng/ml) (range)**		**P value**
<7/7	>7/7		<14/7	>14/7		<28/7	>28/7	
437.5 (375-500)	57.0 (1-501)	**0.03**	278.3 (6-500)	54.2 (1-501)	**0.04**	224.8 (6-501)	39.5 (1-501)	**0.01**

5.3.5 Results of Infection Screening

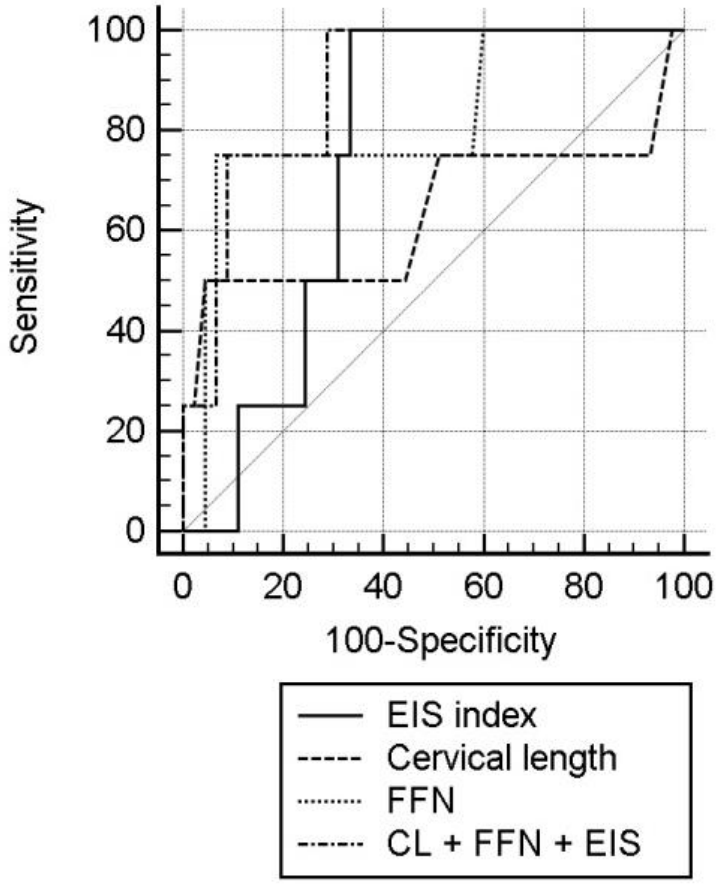
Of the 49 women without prior colposcopy treatment, 32/49 (65.3%) had a normal high vaginal swab result. The most commonly observed positive results were for *candida* (8 women, 16.3%) and Group B *Streptococcus* (GBS) (8 women, 16.3%, with co-existing GBS and candida in one participant). Bacterial vaginosis (BV) (as detectable by conventional microscopy, culture and sensitivity methods) was not commonly identified within this cohort. Only one woman tested positive for BV and one for *ureaplasma urealyticum* – both delivered at term (the patient with BV within 28 days of assessment, the patient with

ureaplasma over 28 days later). Other positive results within the short term delivery outcome groups were restricted to *candida* and GBS.

5.3.6 Predictive performance of EIS and conventional predictors of PTB

Using the same method described in Chapter 4 (section 4.3.8), CR measurements obtained at 39.1- 625 kHz and the probabilities generated by spectral template matching were combined to produce predictive EIS indices for all outcomes of interest. These frequencies were selected due to their performance in differentiating between term and preterm delivering asymptomatic women (both in earlier pilot work and the cohort presented in Chapter 4). The performance of EIS was then compared to CL and FFN by means of ROC curve analysis, and standard binomial logistic regression was employed to incorporate all three tests into a multi-modal predictive model. For the various regression analyses standard procedures were followed, as described previously.

Assessment of PTB prediction within 1 week was planned. However, only 2 women delivered within 7 days of testing, precluding meaningful analysis of this outcome. 4 women delivered within 2 weeks of testing, and the results of comparative ROC curve analysis for this subgroup are summarised in Figure 5-12, below:



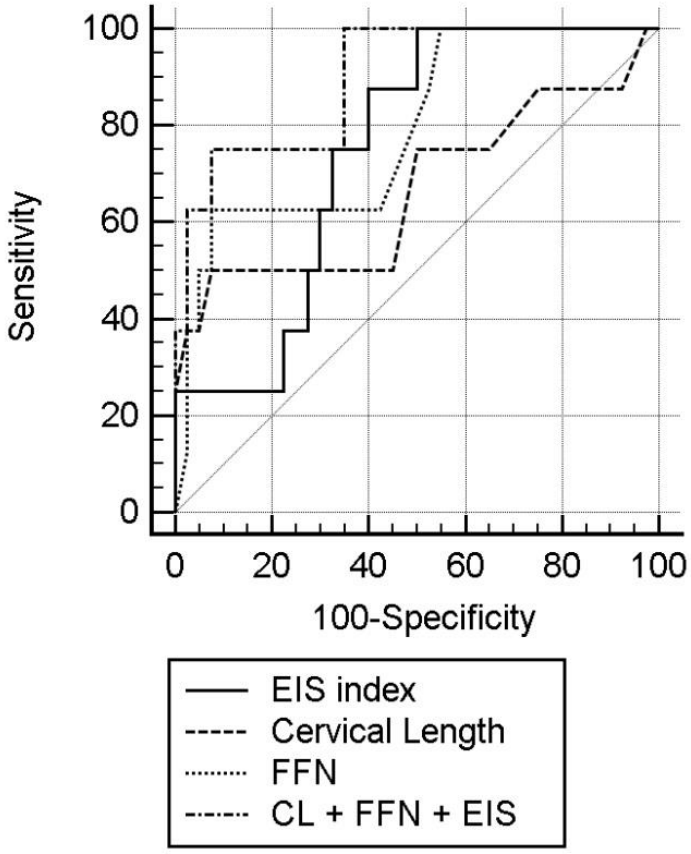
Symptomatic women with no prior LLETZ (n=49, 4 del within 14/7 *vs.* 45 >14/7)

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤9mm	0.63	0.48 to 0.77	0.555
FFN	>189ng/ml	0.81	0.68 to 0.91	**0.023**
EIS index	≤0.90	0.75	0.61 to 0.86	**<0.001**
CL+FFN+EIS	≤0.96	0.89	0.77 to 0.96	**<0.001**

Figure 5-12 Prediction of delivery within 2 weeks in symptomatic women with no prior colposcopic treatment

FFN and EIS yielded significant ROC AUCs (of 0.81 and 0.75 respectively) indicating acceptable to excellent discrimination. The AUC for CL (0.63) was not significant. Although the pairwise comparisons between individual ROC curves generated p values >0.05, there was a trend towards improved prediction through use of a multimodal testing model (AUC 0.89 p<0.001).

Figure 5-13 summarises the results of comparative ROC curve analysis when predicting spontaneous delivery/rupture of membranes within 28 days).



Symptomatic women with no prior LLETZ (n=49, 8 spontaneously delivered/ruptured membranes within 28/7 *vs.* 39 >28/7 (1 iatrogenic birth<28/7))

Predictive test	Optimal cut-off value from ROC curve	AUC	95% CI of AUC	P value
Cervical length	≤12mm	0.67	0.52 to 0.80	0.191
FFN	>91ng/ml	0.79	0.65 to 0.90	**<0.001**
EIS index	≤0.77	0.75	0.60 to 0.86	**0.002**
CL+FFN+EIS	≤0.63	0.90	0.78 to 0.97	**<0.001**

Figure 5-13 Prediction of delivery within 28 days in symptomatic women with no prior colposcopic treatment

Similarly, for this outcome FFN and EIS performed optimally of the individual tests, with AUCs of 0.79 (p<0.001) and 0.75 (p=0.002) respectively, suggesting acceptable discrimination. CL prediction again did not yield a significant AUC. Formal pairwise comparison of AUCs did not confirm additive predictive benefit of multimodal testing, although a trend towards improved prediction (AUC 0.9, p<0.001, suggesting excellent discrimination) was again evident.

A broader summary of the predictive performance of CL, FFN, EIS and the multimodal model is provided in Table 5-4, which reports the observed sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and positive (LR+) and negative likelihood ratios (LR-) observed for the short term outcome measures of interest (in addition to ROC curve AUCs). The predictive performance of CL scanning and FFN estimation at more conventional thresholds of ≤15mm and ≥50ng/ml is also included for comparison. It is notable that use of these thresholds (as is commonplace in clinical practice) reduces test performance by multiple measures (particularly specificity and LR+). FFN still offers acceptable discrimination between outcome groups but does not outperform EIS unless the higher positive test thresholds indicated by the Youden Index (second column in Table 5-4) are used. On the basis of the data provided by this symptomatic pilot, the predictive benefit of EIS appears to particularly arise from high sensitivity/NPV and consequently low LR- indicating large reductions in pre-test probability of disease if EIS readings are normal. Multimodal predictive testing performed well, with high sensitivity, specificity and NPV (100, 71 and 100% for delivery within 14 days and 100, 75 and 95% for delivery/SRM within 28 days). PPV was modest for delivery within 14 days at 24% but increased to 67% for delivery within 28 days (in part due to higher prevalence of this outcome).

Table 5-4 Summary test performance for a range of outcome measures in the no colposcopy group

Predictive test	Optimal threshold	AUC	95% CI of AUC	Sensitivity	Specificity	PPV	NPV	LR +	LR -
Prediction of Delivery within 2 weeks (n= 4 *vs.* 45)									
CL	≤9mm	0.633 p=0.555	0.484 to 0.766	50.00	95.56	49.81	95.59	11.25	0.52
FFN	>189 ng/ml	0.814 p=0.023	0.677 to 0.911	75.00	93.33	49.78	97.69	11.25	0.27
CL	≤15mm	0.672 p=0.275	0.523 to 0.799	50.00	84.44	22.31	94.98	3.21	0.59
FFN	≥50ng/ml	0.753 p=0.055	0.609 to 0.865	75.00	75.56	21.51	97.13	3.07	0.33
EIS index	≤0.90	0.750 p<0.001	0.606 to 0.863	100.00	66.67	21.1	100.0	3.00	0.00
CL+FFN+ EIS	≤0.96	0.889 p<0.001	0.766 to 0.961	100.00	71.11	23.5	100.0	3.46	0.00
Prediction of Spontaneous Delivery/Rupture of Membranes within 28 days (n=8 *vs.* 40)									
CL	≤12mm	0.666 p=0.191	0.515 to 0.795	50.00	92.50	57.10	90.20	6.67	0.54
FFN	>91ng/ml	0.791 p<0.001	0.649 to 0.895	62.50	92.50	62.50	92.50	8.33	0.41
CL	≤15mm	0.688 p=0.104	0.537 to 0.813	50.00	87.50	44.50	89.74	4.00	0.57
FFN	≥50ng/ml	0.713 p=0.049	0.564 to 0.834	62.50	80.00	38.46	91.43	3.13	0.47
EIS index	≤0.77	0.747 p=0.002	0.601 to 0.861	100.00	50.00	28.58	100.0	2.00	0.00
CL+FFN+ EIS	≤0.63	0.897 p<0.001	0.775 to 0.966	75.00	92.5	66.67	94.87	10.0	0.27

5.4 Discussion

This pilot study has demonstrated lower average CR in symptomatic women destined to deliver close to the time of assessment. Although, the differences observed did not achieve statistical significance, they merit further consideration. Potential explanations for the observed trends will be considered and study strengths and limitations evaluated. The cohort of women recruited was limited in size, which in turn meant the numbers experiencing the short term outcomes of interest were small. This inevitably limits the strength of any analyses and conclusions. Nevertheless, useful preliminary data regarding the use of cervical EIS to assess this group has been obtained.

The overall observed rate of PTB was 22.2% (20.4% if women without prior colposcopy treatment are considered) with a roughly 50:50 split between spontaneous and iatrogenic PTB; the group without cervical treatment had an 8.1% chance of delivering within 14 days. These rates are similar to the median prevalence of imminent PTB reported by previous diagnostic test meta-analyses of studies conducted in symptomatic women[173, 394]. The predominant lack of significant demographic differences between outcome groups is likely to reflect the small sample size of this study, as factors such maternal age and ethnic origin and smoking status are known to modify PTB risk[365-368, 395]. As mentioned in Chapter 4, the finding of higher BMI in spPTB *vs.* term groups is in keeping with existing literature[365]. The preponderance of Caucasian participants is relevant when considering broader application of these results as various elements of the potential PTB mechanistic pathway are known to vary with ethnicity[60, 396] - it is possible that predictive accuracy could vary in cohorts with a different ethnic mix, if their dominant phenotype of PTB is more/less amenable to antenatal prediction.

Heterogeneity within the positive outcome groups (i.e. delivery within 7, 14 and 28 days) may have impacted upon our observations. It is not certain exactly which parameters of cervical modelling EIS can detect, but it plausibly assesses both epithelial integrity and stromal hydration/disorganisation[24, 25, 297, 299, 300]. The extent to which these factors are present at different points in the threatened PTB pathway is also unclear[92, 104], which makes interpretation of results more challenging. Women were assessed at very varied gestations, the range of intervals from assessment to birth was wide, and those who delivered before 37 weeks differed in phenotype. This may have limited our results in a number of ways, for

example: women who had a longer interval between assessment and delivery may not have had detectable remodelling changes present at the time of their study visit; women assessed at later gestations, may have commenced the process of appropriate remodelling which preceded their subsequent term labour; and the variety of phenotypes of PTB observed amongst the 10 women delivering before 37 weeks may have been differentially detected by CR measurements.

The lower average CR observed in women who delivered close to the time of assessment is evident across the entire range of current frequencies (76.3 - 625000 Hz) (Figures 5-2 to 5-9 and Table 5-4). This might be explained by the presence of premature cervical remodelling (including changes such as collagen matrix disorganisation[102, 105, 107, 111, 139, 144, 147] and increasing hyaluronic acid/water content[107, 116, 117, 134]), a hypothesis partly supported by the lower average CR in women delivering within 14 days *vs.* women delivering within 28 days. The average CR for women delivering within 7 days was similar to that of the <14 day group (i.e. a further fall in CR was not observed), but given that only 2 women delivered in this time period this may not represent a fair comparison.

The lower CR might also be explained by epithelial deficiency such as that observed in the presence of vaginal dysbiosis/infection during animal and *in vitro* studies[162, 163, 165, 397, 398]. The incidence of BV in this cohort was low (just one term-delivering woman had a positive swab) but the method employed for screening was routine microscopy, culture and sensitivity, as used in routine clinical practice, and may have had limited sensitivity. Further research (ideally using higher resolution techniques to assess participants' microbiota in detail) is required to evaluate the interaction between cervical impedance readings and vaginal flora.

Interestingly, the differences which came closest to achieving significance, and where differences in CR were most marked were obtained at 76.3 - 610.4Hz when comparing those delivering within or after 14 days of assessment (Figures 5-2 (b), 5-7 and 5-8). This frequency range is lower than the mid to high frequency bracket which had discriminatory ability in our larger asymptomatic cohort and earlier pilot[25]. Given the smaller numbers in this symptomatic group these patterns should be viewed with caution and the distribution of the later delivering subgroup is somewhat skewed. Nevertheless, during EIS measurements, depth of current flow varies according to AC frequency, with deeper penetration and a

greater proportion of stromal flow noted at high frequencies[295]. The exact sequence of biochemical, immunological and morphological events which occur during preterm remodelling is not known (and indeed may be variable). It is possible that the higher frequency CR readings may have been relatively elevated in this symptomatic group due to increasing cellularity within the stroma, e.g. due to influx of immune cells during infection-associated[92, 112, 123, 161] or 'sterile-inflammation'-esque[132, 139] pathways of remodelling. This may have rendered the differences in this frequency range less dramatic in our symptomatic *vs.* asymptomatic cohort. However, low frequency CR measurements are also more vulnerable to influence by variation at the electrode-tissue interface. For example, a thick mucous layer covering the epithelium could provide an alternative low-resistance path for low frequency current flow – effectively a form of 'short circuit'[295]. Whilst effort was always made to remove visible cervical mucous/discharge prior to EIS readings, such secretions are often present at higher levels ahead of delivery and may have played a factor in the observed low frequency differences. In view of: (i) the susceptibility of low frequency readings to influence by surface variation; (ii) the higher proportion of deeper epithelial/stromal current flow at higher frequencies[289, 295, 300] (i.e. interrogation of the portion of tissue of particular interest) and (iii) the patterns observed in our considerably larger asymptomatic cohort, a decision was made to evaluate the predictive accuracy of an EIS index which incorporated mid to high frequency CR measurements. The lack of statistically significant differences in this range in our cohort could plausibly be due to the limited sample size. If future large studies of symptomatic women suggest that a different frequency range may offer improved predictive benefit then alternative approaches could be considered.

The high FFN levels observed in the groups closest to delivery (Figure 5-10) are unsurprising, given its established role in assessing women with threatened PTL[19, 173, 210]. The absence of significant differences in CL (except for delivery <7 days) may be related to small case numbers (Figure 5-11 and Table 5-5). However 50% of the delivery <14 days and <28 days groups had CL ≥30mm (considerably higher than the commonly utilised 15mm threshold for PTB prediction in symptomatic women[19]). As discussed in Chapter 1, cervical function is complex and it is possible that shortening on TVUSS is more reflective of sphincter dysfunction at the internal os[99-101, 103, 104] and/or downstream changes in tissue compliance

once remodelling is fully established. As such, early cervical changes measurable with EIS could potentially co-exist with normal cervical length.

The results of the ROC curve analyses for individual and multimodal testing are important (Figures 5-12 and 5-13). Given the magnitude of differences observed in FFN between outcome groups it is unsurprising that it performed well in predicting spontaneous delivery within 14 and 28 days, with good ROC AUCs of 0.81 and 0.79. CL did not discriminate women experiencing these outcomes with high accuracy in our cohort, generating non-significant AUCs and low sensitivity estimates (Table 5-4). However, when short CL was noted, test specificity was good (95.5 and 92.5% for <14 and <28 days). EIS offered fair prediction of these short term outcomes (AUC 0.75 for both <14 and <28 days), which was similar to the performance of FFN if the traditional cut off of 50ng/ml was used (see Table 5-4). The small numbers in these subgroups mean this evidence is tentative. Nevertheless, the high NPVs exhibited by EIS in this context (93-100%) could suggest utility as a 'rule-out' test, particularly in circumstances when access to FFN testing is limited. Again, this would require validation via a larger study. The non-significant trend towards higher AUCs with multi-modal testing observed across all outcome measures could also be definitively evaluated via assessment in a larger cohort.

Some general points are important when considering PTB prediction (particularly in symptomatic women). Firstly, the appropriateness of compromising on test sensitivity or specificity will vary depending on the woman's gestation at presentation. For example, at 24 weeks, the consequences of a false negative result from a predictive test with limited sensitivity are significant. Should the opportunity be missed for appropriate preparatory treatment, the impact on neonatal morbidity and mortality may be high. Conversely, at 34 weeks, neonatal outcome is likely to be positive, even in the absence of therapeutic intervention, thus a test with better specificity might be most appropriate, to minimise the burden of unnecessary intervention (both to reduce costs and to avoid the impact of hospitalisation on women and families, including stress – itself implicated in PTB pathogenesis[399, 400]). Overall, the choice of whether to test, and which test to employ might vary throughout pregnancy.

Secondly, differential thresholds for initiating individual aspects of treatment may be appropriate. For example, proponents of quantitative FFN have suggested that a 50ng/ml

threshold be used to decide upon admission, but a higher and more specific 200ng/ml threshold employed to ascertain the need for antenatal corticosteroids (typically betamethasone or dexamethasone) to promote fetal lung maturity[218]. Use of the whole continuum of risk generated by predictive testing and more nuanced treatment algorithms such as this could help navigate the limitations of existing technology, allowing individualised risk assessment and optimising patient outcomes[401].

Thirdly, another factor has the potential to influence the performance of any predictive test and is rarely considered in the literature – namely, clinicians' threshold for doing the test in the first place. Inclusion criteria for prospective studies are generally clearly defined, but in the clinical environment, busy or inexperienced clinicians might have a low threshold for recourse to predictive testing if they feel it will streamline their assessment of a patient with possible PTL. If their assessment is cursory and the threshold for testing too low, the costs associated with testing will increase (with further impact from false positive testing and subsequent unnecessary treatment), for a group of women who may have a decidedly low pre-test probability and prevalence of PTB. Therefore the 'real-world' impact of low specificity tests with higher false positive rates may be disproportionately high. These factors are all relevant when considering the design and interpretation of future studies employing EIS to assess symptomatic women.

Sample size calculations for diagnostic test studies may provide variable results depending on the methodology chosen[402]. If the most clinically relevant outcome of prediction of delivery within 14 days of assessment is considered from our pilot data, it is evident that EIS had a sensitivity of 100% and a specificity of 66.7%, with an outcome prevalence of 8.1% (similar to that reported in wider literature[173, 394]). Using these figures as inputs to the formulae of Buderer *et al.*[342], 370 symptomatic women would need to be recruited to confirm comparable test performance with a confidence interval of 10% and an accepted Type 1 error rate of 0.05. To yield estimates of sensitivity and specificity with a narrower confidence interval of 5%, 5925 women would need to be recruited.

Alternatively, Medcalc software incorporates a calculator for sample size estimation based on anticipated ROC curve AUC and outcome prevalence. Entering this data (ROC AUC 0.75 and prevalence 8.1%) into the calculator (with estimates of type I error of 5% and type II

error of 10%, i.e. conventional 0.05 significance levels and 90% power) suggests a sample size of 184 would be required to confirm comparable test performance in a larger cohort.

5.5 Conclusions

In conclusion, this pilot study provides early evidence that EIS has potential to predict spontaneous delivery within 14 and 28 days in women presenting with symptoms of threatened PTL. The lower CR observed in women closest to delivery is consistent with the results of previous studies presented within and prior to this thesis[25]. However, given the small numbers and limited power of this study to detect significance differences between outcome groups, there is insufficient evidence to support the use of cervical EIS as a standalone test. It shows potential promise as an adjunct to conventionally used predictors such as FFN measurement and CL scanning, and further work would be best targeted as assessing its use in this capacity. The main impact of EIS on assessing symptomatic women in a UK setting might be maximising the PPV of multimodal predictive testing, especially in settings where access to CL scanning (which requires greater training) is limited. Future studies should evaluate this by assessing rates of unnecessary/suboptimally timed steroid therapy, hospitalisation and in-utero transfer, all of which carry a burden to women and the healthcare service. The design of follow-on work will be considered in more detail in Chapter 7.

Chapter 6 - Acceptability of EIS Measurements in High and Low Risk Women: A Mixed Methods Study

6.1 Introduction and Background

6.1.1 Introduction

The success of any diagnostic or screening test depends upon its acceptability to the subjects to whom it will be offered. A highly accurate, but unacceptable test is unlikely to achieve widespread usage and thus will provide limited benefit. The preceding chapters of this thesis have considered the predictive utility of impedance spectroscopy and other techniques in a variety of clinical situations. This chapter will focus on the experiences of a subgroup of our study population, using a parallel convergent, mixed methods approach to evaluate the acceptability of our novel test and explore women's perspectives on screening for preterm birth more broadly.

6.1.2 Background literature

No previous research has examined patients' experiences of undergoing EIS measurements. Furthermore, the literature regarding pregnant women's perspectives on PTB screening is relatively sparse. Studies are predominantly quantitative, with questionnaires employed to examine factors such as pain, anxiety, and embarrassment during CL scans[178, 403-406] and anxiety associated with FFN testing[405, 407]. More recently, the impact of the Quantitative Instrument for the Prediction of Preterm Birth application (QUiPP app) (which combines obstetric history, CL and FFN to estimate PTB risk) has been assessed via questionnaire[408]. The main findings of these studies are summarised in Table 6-1.

Multiple qualitative studies have considered the experiences of women at risk of PTB[409-417], but these have predominantly recruited symptomatic participants. The majority have focused on the emotional and practical sequelae of threatened PTL and women's coping strategies for managing their complicated pregnancies. There has been limited attention given to the role predictive tests play in women's experiences of PTB assessment. However, there are some exceptions. Two qualitative studies have considered screening as a factor in the experiences of women at risk of PTB; one studied 14 high risk asymptomatic women[416] and the other 19 women with symptoms of threatened PTL[417]. A further solitary qualitative paper[418] examined 17 symptomatic

participants' views of the FFN testing process specifically. The themes described within this body of evidence are also summarised in Table 6-1.

O'Brien *et al.* conducted semi-structured qualitative interviews with asymptomatic high risk women under the care of a specialist PTB antenatal clinic[416]. They do not focus on individual tests, but the authors note that the process of attending appointments for screening is part of a coping strategy which allows women to progress through high risk pregnancies in manageable steps. Women had mixed views on their 'high risk' status, acknowledging that it provoked worry, but valuing the proactive approach and additional care it conferred.

Carter *et al.* also conducted semi-structured qualitative interviews with women at risk of PTB, but instead focused on symptomatic women[417]. A proportion of this group had prior risk factors for PTB and had attended specialist ANCs during pregnancy, whilst the remainder were LR women who subsequently developed PTL symptoms. Their discussion of the individual aspects of PTL assessment is brief, but women described being willing to tolerate a potentially uncomfortable speculum examination in order to gain useful information. Examinations and tests were generally viewed positively, especially when providing normal results and reassurance. The ability of tests (e.g. cervical length scans) to provide additional objective information was also valued. The authors noted some interesting differences between low and high risk women, with higher rates of delayed presentation to hospital in low risk women and greater confidence to attend for assessment and lower reporting of conflicting information provision by health care professionals from the high risk group.

Petersen *et al.* recruited symptomatic women from 5 obstetric units across Ontario[418]. Participants' views were obtained via semi-structured interviews and were summarised as a sequential process of seeking reassurance. Initial reassurance was gained from the birth unit environment and confidence in the clinical team; the wait for results provoked anxiety and required support and once results were available women often re-defined reassurance, sometimes requiring additional information to contextualise their symptoms if a negative result was received

These papers provide useful preliminary information regarding women's experiences of PTB screening. However, there is scope to gain more detailed insights regarding high risk women's views of individual and combined screening tests. There has also been minimal investigation of the perspectives of low risk women who might be offered PTB screening. The study presented within this chapter will help to address this deficit and inform the design of future PTB screening programmes for both low and high risk women.

Of the three relevant qualitative studies identified, all employed semi-structured interviews. No research has been identified which uses mixed methods to synthesize both quantitative and qualitative data. Such a technique can be advantageous in providing a comprehensive view of patient experience and triangulation of quantitative and qualitative datasets may enhance validity, allowing areas of convergence, dissonance and silence to be highlighted[419, 420]. We therefore aimed to employ validated quantitative measures of pain and anxiety to examine women's experiences of EIS (allowing comparison to existing PTB screening literature) but also to conduct semi-structured qualitative interviews to obtain greater detail regarding women's EIS and PTB screening experiences in general (to enhance and explain our quantitative findings).

Table 6-1 Summary of Studies Evaluating Women's Experiences of PTB screening

Study	Country	Setting	Population	Methodology	Outcome measures	Results	Findings with respect to PTB screening
QUANTITATIVE							
Heath *et al.* (1998)[178]	UK	Obstetric ultrasound department in one large tertiary hospital.	100 women attending for 23 week anomaly scan who also agreed to undergo a speculum examination and TVUSS of CL.	Quantitative acceptability questionnaire administered immediately after examination.	Degree of discomfort and embarrassment and comparison with speculum on 5 point likert scale. Pain score on 10 point visual analogue scale.	94% experienced no or mild discomfort. 98% experienced no or mild embarrassment Median pain score 0.5 (range 0-6.5). 50% rated TVUSS as less uncomfortable than speculum examination, 35% the same and 15% more uncomfortable.	In general CL scans were well tolerated with minimal pain and discomfort. The majority of women found TVUSS easier than speculum examination.
Cicero *et al.* (2001)[403]	UK	Obstetric ultrasound departments in one large tertiary hospital and a two district general hospitals.	70 women attending for 23 week anomaly scan who also agreed to undergo a transvaginal and transperineal USS of CL.	Quantitative acceptability questionnaire administered immediately after examination	Degree of discomfort and embarrassment on 5 point likert scale. Pain score on 10 point visual analogue scale.	95% of women experienced no or mild discomfort with translabial-transperineal scans vs. 83% with TVUSS. Both methods were associated with no or mild embarrassment for 91% of women. Mean pain score was lower for with translabial-transperineal scans than TVUSS (1.1 vs 2.4 although both had a range from 0-9).	Overall acceptance rate of cervical scanning not reported. Both methods of measuring CL broadly acceptable to women but range of pain scores notable.
Clement *et al.* (2003)[404]	UK	Obstetric ultrasound departments	755 women attending for 23 week	Quantitative questionnaires given to	Scores from multiple validated tools	55.2% of women offered TVUSS as universal screening accepted it.	In general women found TVUSS acceptable with tolerable levels of

		in one large tertiary hospital and a smaller district general hospital.	anomaly scan who also agreed to undergo TVUSS of CL. 167 women who declined TVUSS. Women with short CL were excluded.	women following TVUSS for completion at home. 4 week follow up questionnaires sent to women who found TVUSS difficult. Retrospective assessment of pre-procedure mood.	including Speilberger State-Trait Anxiety Inventory (short form), McGill pain questionnaire and impact of event scale.	Primiparous and black African women more likely to accept TVUSS (but also more worried about prematurity). Mean perceived difficulty rating 1.3 (on 0 to 5 likert scale) - rated significantly less difficult than a cervical smear. 7.2% gave a difficulty score of 4 or 5 and 5.9% would decline TVUSS in future pregnancy. Anxiety scores similar before and during scan. 36.6% experienced some discomfort during the scan, of whom 91.6% rated it as mild or discomforting. A minority (8.4%) described it as distressing, horrible or excruciating.	discomfort, but a significant minority found the procedure difficult, with more marked pain and reported significant levels of trauma in follow up questionnaires. The experiences of screen positive women are not captured by this study.
Shennan *et al.* (2005)[407]	UK	General antenatal clinics at two tertiary hospitals.	146 pregnant women with risk factors for PTB. Control group of 206 pregnant women at low risk of PTB.	Quantitative questionnaires administered immediately before FFN testing at 24 and 27 weeks and 6 weeks post-partum.	Scores from the short form of the Speilberger State-Trait Anxiety Inventory.	Women at high risk of PTB were more anxious than low risk women at both antenatal time points. HRW who tested positive at 24 weeks were significantly more anxious before the 27 week test than HRW with a prior negative test.	Use of qualitative FFN (positive/ negative result only) increased anxiety for ≥3 weeks in HRW testing positive. No comparison provided for LRW testing positive and no assessment of anxiety shortly after receiving result.
Romero *et al.* (2014)[406]	USA	Obstetric ultrasound department	60 women enrolled in a randomised	Quantitative patient satisfaction	Degree of discomfort, embarrassment,	All women undergoing TVUSS of CL described either no or mild discomfort. 1 woman (5%)	Only 26% of eligible patients agreed to participate in the RCT.

		in a large tertiary hospital.	control trial comparing CL screening methods (trans-abdominal (TA) CL scan *vs.* sequential TA and TV scans (if CL short TA) vs TV scans)	survey administered immediately after examination.	and inconvenience of time taken to perform CL assessment on 5 point likert scale.	experienced moderate embarrassment – the remainder describe no or mild embarrassment. The additional time taken for TVUSS was rated as a little inconvenient by 15% of women.	Therefore difficult to know how representative these views are of the wider obstetric population. Overall, all screening approaches were broadly acceptable to women.
Keller *et al.* (2018)[421]	USA	Obstetric ultrasound department in a large tertiary hospital.	511 women attending for anomaly scan who were offered CL screening	Quantitative questionnaire regarding acceptance rates of CL scan and reasons for declining.	Choice of 7 suggested possible reasons for declining TVUSS and a free text area to record alternative reasons.	5.9% (n=30) women declined TVUSS. There was a significant association between which sonographer saw the patient and acceptance rates ($p < 0.001$). No demographic associations. 47% of decliners felt TVUSS was not needed, 27% felt unprepared for a TV scan and 10% had concerns over modesty and privacy.	Overall acceptance rate of CL scanning was high at 94.1%. The timing and detail of information given to women about TVUSS is likely to influence acceptance.
Carlisle *et al.* (2018)[405]	UK	Specialist PTB antenatal clinic at a large tertiary hospital	102 women already enrolled in a large prospective study of PTB prediction with risk factors for	Quantitative questionnaire regarding acceptability of speculum examination, attitudes to self-obtained FFN swabs and	Rates of acceptance of different predictive tests.	97% found the speculum examination acceptable (1% no, 2% didn't know). 88% would be prepared to have the FFN test again in pregnancy. 47% would be prepared to obtain a self-sampled FFN swab, 35% would not (15% unsure).	Both speculum examination for FFN testing and TV measurement of cervical length had high rates of acceptability in HR asymptomatic women.

			PTB (18-34 weeks)	acceptability of CL scans.		95% found the TVUSS acceptable, 2% did not 2% didn't know, 1% no response).	
Carlisle *et. al.* (2021)[408]	UK	Maternity assessment units in thirteen hospitals in London, the South East and Midlands.	221 symptomatic women participated in a nested questionnaire study, within a larger randomised cluster trial. Units were randomised to use of the QUiPP app as a decision and communicatio n tool in the assessment of threatened PTL.	Quantitative questionnaire administered immediately before and after clinical assessment.	Pre and post assessment anxiety scores using the Visual analogue scale for anxiety (VASA). Decisional Conflict Scale scores regarding the care offered to women following assessment.	Significant reduction in anxiety noted after *vs.* before testing (p=0.000). The trend towards greater reduction in anxiety in sites using QUiPP intervention did not reach significance (p=0.26) even when women were aware the QUiPP app had been used (p=0.07). Proportions of women with low and high decisional conflict (scores<25 and ≥37.5) compared – no significant differences noted in women aware and not aware that QUiPP app had been used. Imperfect compliance with protocol – 41% of women in the sites randomised to QUiPP intervention were not aware it had been used in their care (i.e. used for decision making but not communication).	Clinical assessment (regardless of methods employed) appears to have maximal effect on anxiety. Poor compliance with protocol may have reduced power to detect effect QUiPP has on anxiety and decisional conflict.
QUALITATIVE							
O'Brien *et al.* (2010)[416]	UK	Specialist PTB antenatal clinic at tertiary	14 English speaking pregnant women who	Qualitative interpretive approach via	Women's lived experiences of pregnancies at high risk of PTB.	Three main themes of experience for women at risk of preterm birth: - Balancing the risks .	Whilst high risk status was associated with fear/anxiety, it was

		referral centre	had a history of at least one prior PTB (14 – 32/40).	interviews and focus groups.		- Personal coping strategies to survive:
						  o One step at a time.
						  o Recognising signs of PTL.
						  o Seeking regular reassurance.
						- Watching your whole family crumble.
							viewed as beneficial as it came with extra care.
							
							Proactive approach viewed positively.
							
							Clinic appointments provoked anxiety but also offered reassurance.

Petersen *et al.* *(*2014)[418]	Canada	Five hospitals in Ontario with varied rural/urban locations and varied levels of maternal/newborn care	17 English or French speaking women who had undergone a FFN test at presentation with symptoms of threatened PTL (14 antenatal, 3 postnatal).	Qualitative descriptive approach via semi-structured phone and face to face interviews.	Women's experiences of FFN testing during an episode of threatened PTL.	The main finding was that women presenting for FFN testing aimed to seek reassurance. Individual themes included: - Feeling reassured by being assessed in a birth unit. - Hoping for reassurance from the test. - Re-defining reassurance after learning the results.	Undergoing multiple tests contributed to reassurance, as did feelings of confidence and trust in clinicians. Clear explanations of the FFN test and support while waiting for results were important for women. After positive tests women needed more information about the cause of their symptoms to feel reassured.
Carter *et al.* (2018)[417]	UK	One large tertiary hospital	19 women already enrolled in a prospective cohort study	Framework analytical approach via qualitative semi-	Women's experience of threatened preterm labour, risk assessment	The four main themes identified were: - Coping with uncertainty. - Dealing with conflicts. - Aspects of care.	Normal predictive tests provided reassurance, but this could be transient in the face of persistent symptoms.

			collecting data on women with threatened PTL in order to develop a risk assessment tool. 8 women had prior risk factors for PTB, 11 were low risk prior to the PTL event.	structured interviews as soon as possible after assessment with threatened PTL.	and management,	○ Including clinical procedures - Interactions with professionals.	Examinations and tests were viewed positively in this symptomatic cohort, especially when providing normal results. Additional information (e.g. from CL scan) also viewed positively. Those under HR clinics with continuity of carer expressed particular confidence in their clinicians and felt able to present earlier for assessment.

6.2 Selecting a Theoretical Perspective

Mixed methods research encompasses a broad range of study designs, but typically incorporates both quantitative and qualitative methodologies. However, each aspect may be afforded different priority and methods may be performed in sequence or concurrently, depending on the desired outcome[420]. Our convergent parallel design aimed to afford equal weight to both datasets, with the intention of producing an integrated summary which captured the strengths of both quantitative and qualitative approaches. Some commentators question the validity of combining methodologies which may have irreconcilable epistemological differences[422]. Whilst this may be an issue for many of the dominant paradigms in social research[423], for our research question a pragmatic approach felt most appropriate. Pragmatism as a paradigm acknowledges that multiple metaphysical perspectives are valid. It moves away from abstract debates about the nature of reality to focus on the interaction of human beliefs and actions in shaping experience[424]. In this way it helps understand how different perspectives derive from people's lived-experiences. As Kaushik and Walsh summarise:

"Pragmatist philosophy holds that human actions can never be separated from the past experiences and from the beliefs that have originated from those experiences. Human thoughts are thus intrinsically linked to action. People take actions based on the possible consequences of their action, and they use the results of their actions to predict the consequences of similar actions in the future". [425]

This philosophy applies not only to study participants but also researchers. Thus it enables a more technical approach to be taken, in which methods are selected due to their ability to best answer a research question, rather than to fit in with a particular epistemological philosophy[420]. Figure 6-1, below, provides a summary of the pragmatist approach to research methodology. From such a stance, mixed methods are not only acceptable, but they may also be desirable if they provide the optimal solution to the research question at hand.

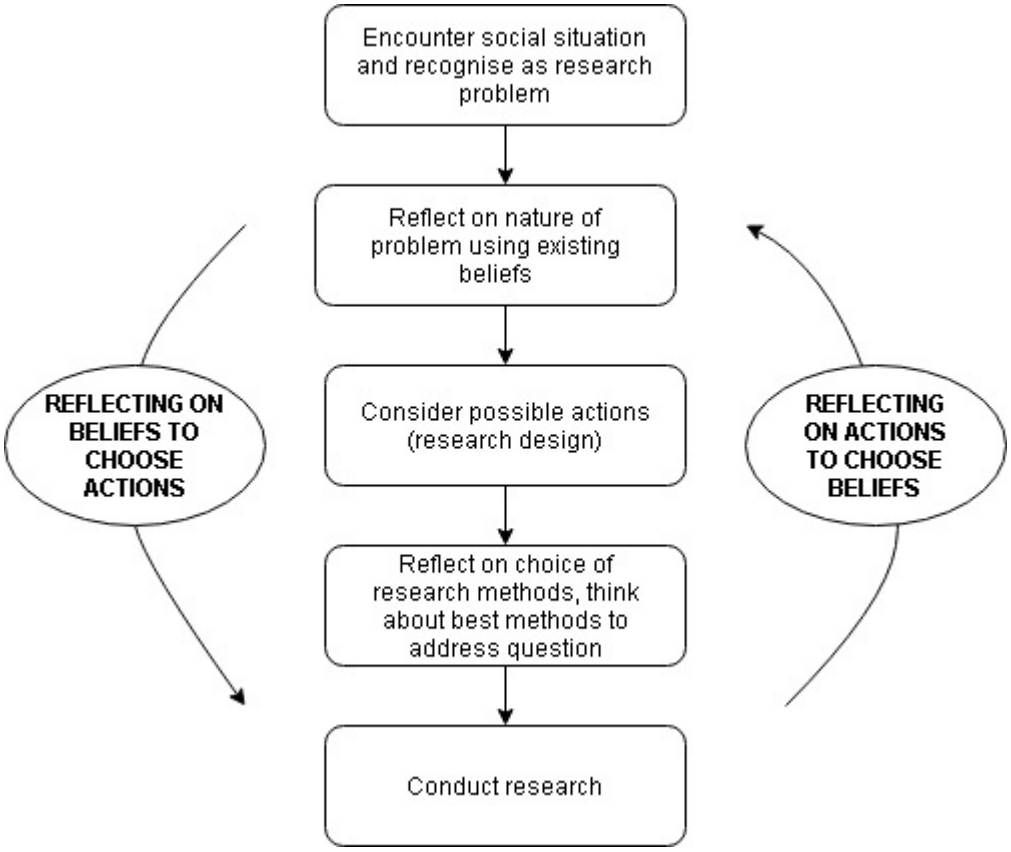


Figure 6-1 Dewey's Concept of Inquiry as a Basis for Research

(adapted from [424, 425]). The pragmatist philosopher John Dewey, described the concept of inquiry in response to problematic situations which defines his 'process-based' theory of knowledge[424]. This process can be applied to the design and conduct of social research, with ongoing conscious reflection about the effect of each decision/action throughout each of the five steps summarised above.

6.3 Methodology

Ethical approval for this study was provided by the Yorkshire and Humber National Research Service Ethics Committee (13/YH/0167). This application approved both the main predictive trial and the nested acceptability sub-study.

Setting

Women received verbal and written information about the main EIS study during booking appointments at the Jessop Wing, Sheffield (a large teaching hospital). Inclusion and exclusion criteria for participation and definitions of the high and low risk groups are summarised in Chapter 2. Those interested in participating were later contacted to confirm recruitment. LRW attended one research visit at 20-22 weeks, HRW again at 26-

28 weeks. The sub-group of women in the acceptability study were recruited over a 12-month period spanning the midpoint of recruitment to the predictive study.

At the main research visit women underwent a series of tests. These are described in detail in Chapter 2 but, in summary, they comprised: an initial speculum examination (when swabs were taken for infection screening and FFN quantification, then EIS measurements were obtained) followed by a CL scan. Women received results of the CL scan immediately and no information regarding their EIS measurement (all were informed it would not be possible to interpret EIS results during the study). Women either received their FFN result during the research appointment or were contacted shortly afterwards (by phone, text or email as requested). In the case of positive results, women were contacted by phone and appropriate follow up arranged. All results were explained and treatment arranged if necessary. The same clinician (myself) conducted all study visits.

6.3.1 Data collection

Before visit one, forty women were also invited to participate in the acceptability study. Those expressing interest completed a short pre-visit anxiety rating and a longer post-visit questionnaire and were later contacted by a research midwife to confirm participation in the interview stage. Twenty-one women consented and attended an interview. Where possible, interviews were arranged within four weeks of the main study visit. They were conducted by a research midwife (RM) with training and experience of qualitative interviewing. Purposive sampling was used to ensure a balance of low and high risk participants, with a range of ages, ethnicities, socio-economic statuses and varied obstetric histories. Recruitment to interview continued until saturation of themes was achieved.

6.3.1.1 Quantitative data collection

The pre and post-visit questionnaires were designed to assess women's anxiety before and after study tests; any pain experienced; women's views of the EIS probe design and overall acceptability of the procedure. In order to assess anxiety the six question, short form of the Spielberger State-Trait Anxiety Inventory (STAI-6) was used[426]. Pain during

EIS measurement was assessed using the short form of the McGill pain questionnaire. This provides a multidimensional measure of pain which has previously been validated in obstetric patients[427]. It consists of two measures of pain intensity: the visual analogue scale (VAS) and the Present Pain Intensity (PPI) plus a Pain Rating Index (PRI) designed to assess the qualities of any pain experienced. Given the novel nature of EIS as a screening test, we felt it was important to describe as fully as possible the sensation of undergoing testing, to enable us to provide detailed information to potential screening participants in future.

Finally, women rated the overall acceptability of the procedure and their perception of the appearance of the EIS device using a ten point VAS. They were asked to comment regarding any necessary changes they would recommend to the testing procedure and whether they felt the procedure was acceptable for use in antenatal care in future. Both parts of the survey are included in Appendix G.

6.3.1.2 Qualitative data collection

The semi-structured interview schedule was designed collaboratively by the interdisciplinary research team (comprising a clinical research fellow/obstetric registrar (myself), research midwife (RM), senior lecturer in social science (SL) and consultant obstetrician/principal investigator for the overall study (PI)). It consisted of nine open and two closed questions designed to elicit women's experiences of attending the study visit and undergoing research tests. The schedule was provided as a guide, however the interviewer was free to vary the order and structure of the questions as she deemed appropriate, and also to follow other lines of enquiry if additional topics of interest arose. The schedule structure was reviewed after the first three interviews, but no modifications were deemed necessary.

1. Were there any reasons why you wanted to take part in the study?
2. Had you had any similar checks or examinations before?
3. What were your thoughts about premature birth before the study was discussed with you?
4. How did you find the research visit?
5. Can you remember what information you were given before and during the visit? How did you find the information?
6. Can you remember the different bits of the tests?
7. How did you find the different tests?
8. What were your experiences after the tests?
9. Can you remember what you were told about your test results?
    i. How did you feel about them?
    ii. If negative effects: what did you do?
10. What would you think about having the tests again?
11. Is there anything else you want to discuss/let us know about?

Figure 6-2 Semi-structured interview schedule

Use of a semi-structured approach enabled key objectives to be achieved (obtaining a detailed account of women's experiences of the tests) whilst allowing flexibility to explore themes which the women themselves might introduce. All interviews were conducted by a research midwife, who was not involved in the clinical care of the women. The use of a neutral interviewer was important to enable participants to reflect freely on their experience without inhibitions or fear of impacting their clinical care. The women chose the location of their interview (at home or in the university research department) and two patients were interviewed during an inpatient stay on the antenatal ward (in a private side room). Interviews lasted around 30 minutes on average and were audiotaped and then transcribed verbatim later. Participants provided separate written informed consent to participate in the qualitative study.

6.3.2 Data analysis

Questionnaire data were analysed using the relevant scoring algorithms for each validated instrument and descriptive statistics (mean scores, with range and standard deviations) were then calculated. Thereafter, comparisons between groups (high risk *vs*. low risk women, pre-test *vs*. post test scores) were made using independent and paired

statistical tests as necessary. Normality of score distribution was assessed via the Shapiro-Wilk test. Mann Whitney U tests were performed to compare non-parametric data whilst independent and paired T tests were performed to compare normally distributed scores. Fisher's exact test was employed for the comparison of categorical outcomes. Data analysis was performed using SPSS (IBM Corp. Released 2017. IBM SPSS Statistics for Windows, Version 22.0. Armonk, NY: IBM Corp).

Qualitative analysis proceeded as follows: I uploaded the interview transcripts to NVivo 10 (QSR International: Burlington MA) and checked them for accuracy. The first three interviews (of two high risk and one low risk participant) were reviewed by three members of the research team (myself, RM and SL). Inductive thematic analysis (TA) (following the five-step process described by Braun and Clarke[428]) was employed to develop an initial coding framework, which was continually reviewed during analysis of the remaining interviews. In brief, this process involved familiarisation with the data; generating initial codes (thoroughly evaluating the data-set, noting recurrent or meaningful references to points of interest and summarising in an overall, systematic framework); searching for themes; reviewing themes; defining and naming themes (to ensure coherence and consistency); and producing an overall synthesis, including detailed examples, to interpret and make sense of the data[428]. For the purposes of this study, which aimed to explore women's experiences of undergoing a novel screening test, the ability of TA to "describe the data set in rich detail"[428] and interpret identified patterns in the context of the overall research question was particularly apposite. Given the lack of prior studies of EIS acceptability, an exploratory approach, grounded in the lived experiences of our participants felt most appropriate. Themes were inductively defined from the raw data through exploration without any predetermined classification where possible. A quarter of the interviews were coded by two researchers (myself and RM) to enable ongoing comparison and refinement of the coding structure, and potential themes were discussed amongst the research team as analysis progressed. Whilst formal assessment of inter-coder reliability is not a pre-requisite for thematic analysis, this comparison of ideas and ongoing dialogue between members of the research team ensured a wide and inclusive approach and was maintained during initial coding.

6.3.2.1 Triangulation

Following analysis of the two datasets, I constructed a mixed methods matrix, summarising the results of the paired datasets. Each row represented a participant for whom both questionnaire and interview data were available, with each column representing the data collected (questionnaire responses and coded interview themes). This enabled systematic comparison of qualitative and quantitative information, specifically looking for areas of convergence, dissonance, silence or complementarity within cases[419]. A convergence coding matrix was also constructed (similar to that advocated by Farmer *et al.*[429] although generated by a single researcher) to summarise the results of both study components and the triangulation process in a single location. This enabled the overall questionnaire results to be synthesized with the SSI themes, in addition to the within case triangulation generated by the matrix.

6.4 Results

6.4.1 Quantitative survey

Of the 40 women completing pre and post-test questionnaires, 20 also participated in a semi-structured interview. The results which follow represent the questionnaire responses from the entire group of 40 surveyed participants. The 20 sets of matched results from quantitative and qualitative elements of the study will be considered further in section 6.4.3. The introduction to the questionnaire explained that the specific purpose of the questions was to assess the acceptability of EIS readings. Women's assessment of pain, device appearance and overall acceptability should therefore relate specifically to cervical spectroscopy. Anxiety scores and evaluation of daily functioning/quality of life may obviously have been influenced by factors other than the EIS test.

The overall results from the pre and post screening questionnaires are summarised in Table 6-2.

Table 6-2 Results of quantitative survey

Survey Domain		**High Risk Women** (n=20)	**Low Risk Women** (n=20)
Anxiety			
STAI-6 results	Mean pre-test score (SD)	34.48 (12.72)	29.98 (8.98)
	Mean post-test score (SD)	28.98 (10.20)	27.50 (9.48)
	Mean difference	-5.55 (-20 to 0)	-3.22 (-13 to +27)
	Pre-test score ≥39	6/20 (30%)	4/20 (20%)
	Post-test score ≥39	5/20 (25%)	2/20 (10%)
Pain/discomfort			
SF-McGill VAS	Mean VAS score (range)	0.97 (0-3.2)	1.01 (0-3.1)
SF-McGill PPI	0 – no pain	7/20 (35%)	9/20 (45%)
	1 – mild pain	11/20 (55%)	9/20 (45%)
	2 – discomforting	2/20 (10%)	2/20 (10%)
SF-McGill PRI	Mean Sensory PRI score	1.25 (0-3)	1.60 (0-5)
	Mean Affective PRI score	0.10 (0-1)	0.05 (0-1)
EIS probe design rating			
	Mean VAS score (range)	1.30 (0-5)	1.35 (0-9)
Acceptability rating			
Personal acceptability	Mean VAS score (range)	0.55 (0-3)	0.75 (0-5)
Acceptable for use in antenatal care?	Yes	20/20 (100%)	20/20 (100%)
	No	0/20 (0%)	0/20 (0%)

6.4.1.1 Anxiety

Both high and low risk groups showed a significant reduction in STAI-6 scores following screening (p=0.002 and 0.018, Mann Whitney U). HRW demonstrated higher pre-visit STAI scores but also a larger mean reduction post-test than LRW, although these differences did not reach significance (p=0.2, independent T test, p=0.63 Mann-Whitney U test respectively). No significant difference in post-visit scores was observed between HR and LRW (p=0.88, Mann Whitney U). On an individual level, two women (10% of the LR group) demonstrated higher scores after screening. Both were low risk participants - one experienced bleeding following examination and the other received abnormal test

results. The remainder showed no change or a reduction in anxiety levels. There is no universally accepted threshold which defines the presence of significant anxiety, but it has been suggested that scores of 39-40 represent a higher level[430]. When considering those with STAI scores ≥39, higher anxiety levels were more prevalent amongst HRW at both time points. Nevertheless, the incidence of scores ≥39 was lower after screening regardless of risk status. None of the differences (in proportions of women with higher and lower anxiety pre- and post-test and between high and low risk groups) achieved significance (all p>0.05, Fishers exact test).

6.4.1.2 Pain

No significant differences in pain intensity experienced during EIS readings were observed between HR and LRW. Average scores were low, with a mean VAS score of 0.97 for HR and 1.01 for LR participants (p=0.94, Mann Whitney U), and a maximal score of 3.2 and 3.1 in each group respectively. The mean PRI score for LRW was fractionally higher than that of HRW, predominantly due to slightly higher scores in the sensory subscale, although neither difference was statistically significant (p=0.53 and p=0.45). When the ordinal PPI scores are considered, two women in each group (10%) rated their pain intensity during EIS measurement as “discomforting”. The remainder described either “no” or “mild” pain (35 and 55% of HRW and 45 and 45% of LRW).

As EIS is a novel test, the qualitative pain descriptors selected by the study participants are of interest. The results of the Pain Rating Index are summarised in Figure 6-3, which displays the mean intensity rating for each qualitative descriptor in both sensory and affective domains, by study group. Women chose a broad range of descriptors, but the most commonly selected in both groups were “aching”, “heavy” and “tender”. However it is notable that intensity ratings were almost exclusively 0 or 1 (no or mild pain), with only two scores of 2 (moderate pain) provided - one for the “tender” descriptor and the other for the “cramping” descriptor, by different low risk women. Affective descriptors were not commonly chosen by either group.

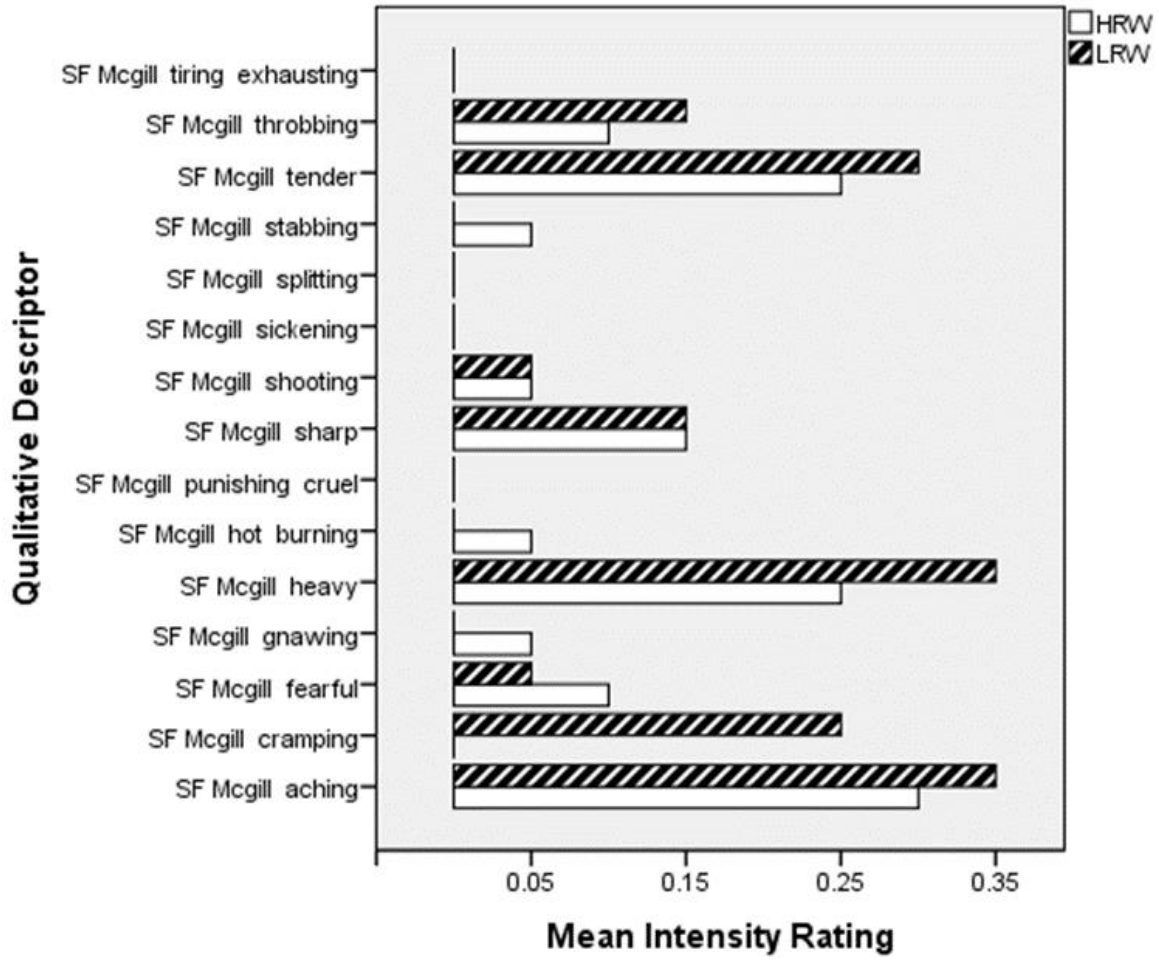


Figure 6-3 Differences in Qualitative Descriptor Intensity Rating (SF-McGill) Between Study Groups

Abbreviations: SF – short form; HRW - high risk women; LRW - low risk women

6.4.1.3 Impression of EIS probe design

Most participants (75%) rated the appearance of the EIS device as 1 or less (on a ten-point visual analogue scale where 0 = not threatening, 5 = neutral and 10 = very threatening). However, there was a wide range of scores, from 0 to 9. Average scores for HR and LRW were not significantly different: mean rating 1.3 for HRW (range 0-5) and 1.35 for LR (range 0-9) (p=0.98). The two participants who rated the EIS device as most threatening (with scores of 6 and 9) were LRW. The appearance of the EIS probe is provided in Figure 6-4 for illustration.

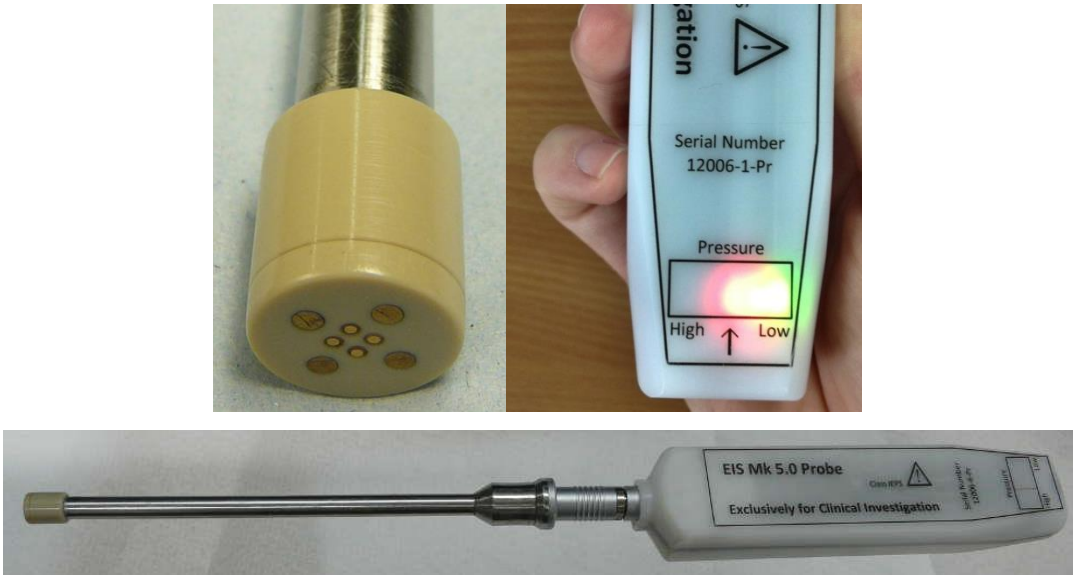


Figure 6-4 Appearance of Sheffield Mark V EIS Probe

6.4.1.4 Overall personal acceptability rating and perspective on use in wider antenatal care

A ten centimetre VAS was also used to provide an overall acceptability rating for the study procedure (where 0=acceptable, 5=neutral and 10=unacceptable). Again, there were no significant differences between study groups: the mean rating for HRW was 0.55 (range 0-3) and for LRW was 0.75 (range 0-5) (p=0.84). 39/40 women indicated they felt no change to the EIS procedure was necessary, whilst one would have preferred it if the test could be performed without using a speculum. When asked if the procedure would be acceptable for use in antenatal care, there was universal agreement from all surveyed women, although the question did not specify in what context this use might occur (e.g. high risk vs universal screening).

6.4.2 Qualitative analysis

The characteristics of the women who participated in the semi-structured interviews are summarised in Table 6-3. There was a preponderance of white British participants, although this was representative of the main study cohort. Effort was nevertheless made to capture the views of different ethnic groups, with support from a clinically experienced translator in one case. Women of different ages, socio-economic statuses and with varied obstetric histories were interviewed to capture as diverse a range of experience as possible.

Table 6-3 Characteristics of Qualitative Interview Participants

Pt No.	Location of interview	Age	Ethnicity	Marital staus	G	P	Gestation of previous preterm birth or miscarriage	Group	Having serial CL scans as well?	PTB prophylaxis?	Previous speculum exam?	Index of Multiple Deprivation (IMD) Decile*
1	Home	25	White European (Polish)	Co-habiting	2	1	n/a	Low risk	no	no	yes	5
2	University	33	White British	Married	5	0	Four 1st trimester miscarriages	Low risk	yes	no	yes	10
3	University	21	White British	Co-habiting	2	1	n/a	Low risk	no	no	yes	7
4	University	36	White British	Married	3	2	Term birth then 31 week delivery.	High risk	yes	no	yes	9
5	University	35	White British	Married	4	2	One 30+ delivery following term birth	High risk	yes	no	yes	6
6	University	19	White British	Co-habiting	4	2	One 36+0 delivery, term birth since	High risk	no	no	yes	7
7	University	38	White British	Married	2	0	One 1st trimester miscarriage	Low risk	no	no	yes	5
8	University	28	Black African	Single	3	1	n/a	Low risk	no	no	yes	1
9	University	34	White British	Married	2	1	n/a	Low risk	yes	no	yes	8
10	University	29	White British	Married	1	0	n/a	Low risk	no	no	yes	4
11	University	33	White American	Married	3	2	One 29+ delivery, term birth since	High risk	yes	progesterone	yes	5
12	University	28	White British	Married	2	0	One 23+ delivery and neonatal death	High risk	yes	USS indicated suture	yes	9
13	Home	35	White British	Married	3	1	n/a	Low risk	no	no	yes	10

14	University	37	White British	Married	7	3	1st trimester, 14/40 and 20/40 miscarriage. 2 term births before and once since miscarriages.	High risk	yes	no	yes	1
15	University	30	White British	Married	3	2	One 35+5 delivery, term birth since	High risk	no	no	yes	10
16	University	29	Pakistani	Married	3	2	One 29 + one 27 week delivery	High risk	yes	progesterone	yes	1
17	Home	33	White British	Married	1	0	n/a	Low risk	no	no	yes	10
18	Antenatal ward	34	White British	Married	10	3	Recurrent 1st trimester miscarriages + 23 week miscarriage + three 33-34 week deliveries	High risk	yes	USS indicated suture and progesterone	yes	7
19	University	37	Libyan	Married	6	3	One 21 week miscarriage + one 1st trimester miscarriage, 3 term births since	High risk	no	elective cerclage	yes	3
20	Antenatal ward	36	White British	Co-habiting	8	3	30 and 32 week deliveries, term birth since.	High risk	yes	progesterone	yes	2
21	University	30	White British	Married	2	0	n/a	Low risk	no	no	yes	9

Four over-arching themes were actively generated which summarised women's accounts of undergoing EIS and the other tests: (i) the physical consequences of testing; (ii) emotional experiences during study visits and pregnancy; (iii) additional determinants of the screening experience and (iv) practical considerations regarding wider use of EIS. An overall synthesis of primary and secondary themes is provided in Table 6-4, at the end of this section, with some exemplar quotes to demonstrate each theme.

Women described the physical experience of screening in depth, with respect to both EIS and the other tests. The accounts of EIS were grouped into 5 sub-themes: "unusual" sensations; positive descriptions of measurements; pain/discomfort/negative descriptors; no sensation associated with measurement and post-test symptoms. Participants also detailed a range of emotions before and after study visits which inevitably shaped their overall perspective on their experiences. Some emotions related to EIS, with specific sub-themes of uncertainty regarding the impending physical experience and concerns regarding the safety of novel tests identified. However, others pertained to the conventional tests and specific sub-themes of general reassurance from screening; the visual impact of the CL result; and the specific psychological impact of CL scanning and FFN testing were evident.

Additional important determinants of women's screening experience were identified including the design of the EIS probe, their pre-existing perspectives on intimate examination and attitudes to knowledge in pregnancy, the screening environment and the nature of their interaction with clinical staff.

The sub-theme of perspectives on intimate examination incorporated two somewhat polarised stances: Firstly, the idea of the vagina as a protected space, with resultant caution regarding internal examination in pregnancy; Second, the view that intimate examination is a normal, and indeed beneficial, part of pregnancy. Women generally acknowledged that pregnancy and birth inevitably require some degree of exposure. However, in order to assess internal structures like the cervix, clinicians encroach on a previously closed body area. For some women the idea of breaching this internal space led to reservations about participation. In order to overcome these worries, participants described seeking additional information - most commonly through personal discussion

with the CRF. Seeking reassurance from a specific clinician seemed to allow them to reconcile their concerns with the desire to participate in the study. In contrast, not all women viewed internal examination in a negative light - many acknowledged that pregnancy is a time when such assessments are to be expected and could be positive. The idea that familiarity with intimate examination increases tolerance was also widely expressed. Prior experience appeared to confer benefit in several ways – women felt prepared, both physically and emotionally, for the events which followed; they had realistic expectations of any discomfort they might face, which seemed to reduce anxiety and allow them to assess the experience in the context of other examinations they may have had previously (e.g. smear tests).

The 'attitudes to knowledge in pregnancy' subtheme draws together several concepts which all influence women's experience of preterm screening. Firstly, their prior knowledge and understanding of preterm birth; Secondly, for high risk women, the impression that no-one knew why their previous baby had been born early and thirdly, the perception that knowledge during pregnancy is a good thing, in and of itself.

Various features of the clinical environment impacted upon women's experience of screening. Unsurprisingly, these were all measures designed to ensure privacy and comfort, namely, ensuring the examination room door was locked; providing a curtain around the door as added privacy protection; and providing a proper sheet to minimise physical exposure. Interviewees also described various clinician-specific features which influenced the overall acceptability of study visits. Many noted that their agreement to participate may have been influenced by the knowledge that visits would be conducted by a female doctor. The other clinician factors described as important related mainly to the interpersonal relationship between study participants and the CRF, namely: explanations given regarding investigations and results; quality of communication during the study; general manner and the opportunity for continuity/ to build a rapport.

Finally, women reflected practically on a variety of barriers and facilitators to rolling out the study tests more broadly, offering views on the written information provided, the timing and frequency of screening and overall reflections on the wider use of EIS. Some participants (in particular HRW) supported universal screening, whereas others

preferred a risk factor based approach to offering additional tests. Several participants highlighted the need to weigh up the costs and benefits of screening, stating that widespread use might be appropriate, but only if the tests provided useful information and allowed preventative action to be taken.

Table 6-4 Synthesis of Interview Themes

Main Theme	Sub-themes	Exemplar Quote
1) Physical consequences	1) Of EIS • Unable to feel measurements • Unusual sensation experienced • Pain/discomfort/negative descriptors • Positive descriptors • Post-test symptoms	*"Couldn't really feel much with that to be honest ummm I felt the swabs more and the speculum being placed than the impedance test, it was more like a very gentle pressure and then hearing the beeps so ... yeah it wasn't uncomfortable"* Participant 14 (HR, one PTB) *"It's like a bit of pressure I guess inside-it's like nothing I have ever felt before. It's kind of inside and up (laughs) but not painful just…just pressure, a strange kind of pressure which is not a normal feeling; you would not normally experience that."* Participant 20 (LR, first pregnancy)
	2) Of other screening tests • Speculum/swabs/TVUSS	*"I think for me, the speculum is, not painful, but the most uncomfortable part of it."* Participant 11 (HR, one 23 week delivery and neonatal death)
2) Emotional experiences	1) In relation to EIS • Uncertainty re: impending physical experience • Concerns re: safety of novel test	*"It was definitely far less of a feeling or a pain feeling than I had expected. I expected to feel more invasive."* Participant 2 (LR, recurrent first trimester miscarriages, first ongoing pregnancy) *"I was a little bit, I have to say I was a little bit, you know because it's research and someone's checking, I sort of felt that if you're taking part in something, you can't completely say that there isn't any risks. So that part of the research, I was anxious about that a little bit, but once I'd finished and sort of a couple of hours later, I wasn't feeling any different, I mean it was fine.…I wasn't worried, but I was a little bit- It's still a risk, it's still, even though you're guaranteed 99%, there's always 1% of these going the opposite way."* Participant 7 (LR, one term birth)

	2) In relation to other screening tests • Psychological impact of FFN and CL scan results • Impact of visual result of CL scan	*"If she had just tried to explain that it is short, but seeing it myself on that screen, it's made me realise that I can't be messing about, I can't be going home. I've got to listen to what they're telling me to do."* Participant 19 (HR, two PTB and one term birth) *" The first study visit I did have a slight increase in fibronectin result…. which was a surprise and then a worry as well because obviously I didn't expect anything to be picked up on it"* Participant 14 (HR, one PTB)
	3) During pregnancy in general • Fear and anxiety in pregnancy • Falling through the gaps of antenatal care	*"Yes I think for me, it were like a blessing really, because I was already really paranoid about just being even pregnant. I think I was really, really scared, and I don't think necessarily I would, if it had not been offered to me, I don't think I'd have known where to go to get that"* Participant 9 (LR, first pregnancy)
	4) During high risk pregnancy • Emotional burden of previous obstetric trauma • Cycle of anxiety in subsequent pregnancy	*"I never actually think about it, because it's been 5 years now. But you're totally out of control. Like you can't do anything. You can't help your kid, you can't do anything. You just have to like be there and it's just not how life should begin, that stressful you know… I can't even look at pictures of her, because she's so tiny"* Participant 10 (HR, one PTB, one term birth) *"And then the day before I come in, apart from this time and last time, I had a really sleepless night because I'm thinking what is it going to show? What's it going to show? And I can find myself just being laid wide awake, but then once I'd been I can sleep safe and sound again for a couple of weeks"* Participant 5 (HR, one term birth, one PTB)
	1) The design of the EIS probe	*"P: I mean it's sort of funny looking.* *I: What do you mean by that?*

3) Additional determinants of screening experience		*P: Well I think because it's long and it's like lights on it, and it makes a noise…* Participant 10 (HR, one PTB, one term birth)
	2) Perspectives on intimate examination • The vagina as a protected space • Intimate examinations as normal • Intimate examinations as beneficial	*"A speculum's a bit uncomfy when you're pregnant to kind of open you up a bit. And I suppose if you don't have to have that done when you're pregnant… Well you'd prefer not to have the speculum if you don't have to"* Participant 6 (LR, first pregnancy) *"For me, it's ok. It's a little weird, but is not hurting, it's not pain. I know that it's just for good things. So I'm not worried…. Maybe that is uncomfy. But because it's good reason to do it, because you need to know something, you just don't mind."* Participant 1 (LR, one term birth)
	3) Attitudes to knowledge in pregnancy • Pre-existing knowledge of preterm birth • "No-one knew why" • "It's good to know"	*"so before I had my daughter, I didn't even know you could deliver early"* Participant 10 (HR, one preterm birth, one term birth) *"I've had a premature baby before, and the reasons for that birth were unexplained. So going into this pregnancy, I was quite anxious about it happening again and what may have caused it last time and things like that… you know if I'd not had all these tests done, I know for a fact I'd be thinking all the time, is that something? Is that something?"* Participant 5 (HR, one term birth, one PTB)
	4) Screening environment	*"I had a blanket over my legs… and the door was locked, and she locked it so I could see she had locked it and there was a curtain and everything..."* Participant 20 (LR, first pregnancy)

	5) Interactions with clinical staff • Gender • Explanation/communication • Bedside manner and rapport	*"She was talking, so she sort of made me feel comfortable, because we continued talking about something completely different to what we were doing. So I didn't feel- I think the fact that she was a female made it slightly better too."* Participant 7 (LR, one term birth)
4) Practical considerations for broader implementation of EIS	1) Information leaflet	*"Like some of the bits I was like what is that? But most of it…. It was just all technical, well not technical but like, it were just like, I knew all the ins and outs of it so it weren't too hard…. I would say to mum 'what is that?' 'What's that one mean?' I can't really remember all of it. I didn't ignore it, I just read a bit of it."* Participant 3 (LR, one term birth)
	2) Timing and frequency of screening	*"I think if it was at a time when you were coming to hospital anyway, like the 20 week scan, then I think that would be a really good idea. But like I was saying earlier, it kind of put me off taking part in the study before I had a premature labour, just because of work and commitments and thinking 'oh I need to take more time off'…"* Participant 11 (HR, one 23 week delivery and neonatal death)
	3) Women's opinions on overall acceptability for wider use in antenatal care • In favour of universal screening • Dependent on risk status • Trade-off between burden of tests and information gained	*"…you'd prefer not to have the speculum if you don't have to. As a routine measure, it would be, but if it definitely picked up lots of, you know if it was going to pick up the risk of having a premature labour then yes it was definitely worth it, because it's nothing compared to that"* Participant 6 (LR, first pregnancy)

6.4.3 Triangulation

The results of the triangulation process are summarised below. However, the full convergence coding matrix, which provides a snapshot synthesis of the combined quantitative and qualitative results, is also provided in Appendix H.

The results from the questionnaire revealed a significant reduction in anxiety following PTB screening, particularly for the high risk group. Triangulation demonstrated general agreement with this finding at interview. However it also detected context-specific examples of dissonance – notably in the patient who received a false positive fibronectin result and in the LR woman who noted that study participation had increased her awareness of (and therefore worry about) PTB. Both women qualified this by describing the net reassurance they obtained from participation, even though their anxiety was heightened at specific time-points. Additionally, a variety of sub-themes were identified from the qualitative interviews which provide further insight into the reasons for pre- and post-visit anxiety and the emotional impact of the different elements of the screening package.

As women did not receive information regarding their spectroscopy result, there was no possibility of EIS itself providing direct reassurance regarding the likelihood of PTB. However, one EIS-related explanation for the changes observed was supported by the complementary SSI data: namely, that some women were anxious about the safety of undergoing a novel test in pregnancy but were reassured when they had no adverse experiences during and after the study visit. This viewpoint was expressed by both HR and LRW. Uncertainty regarding the impending physical experience of EIS (or indeed other unfamiliar tests encountered during screening) similarly was a potential source of increased pre-test anxiety.

Triangulation of the individual items of the STAI-6 shows that many of women's emotional responses to testing relate to elements of the research visit that weren't EIS (e.g. worry and anxiety related to positive tests, pre-test worries due to prior knowledge of PTB, e.g. due to family history). Similarly whilst some of the post-test reduction in anxiety was related to having had the novel test without problems, a large part of it was due to the reassurance of receiving results from the CL scan and FFN swab. When women did have worries resulting

from positive test results they often framed this as a good thing (describing knowledge as good, and as a chance for action). Overall, the process of STAI-6 triangulation was not entirely straightforward – women used the different anxiety descriptors somewhat interchangeably, and didn't necessarily differentiate, for example, between tension and worry and feeling calm *vs.* feeling relaxed. Essentially their interviews provide a broader view of the frequency and intensity of their emotional experiences with useful explanatory detail.

In addition, the results of quantitative analysis suggested a possible bimodal distribution of pre and post-test STAI-6 scores amongst HRW. The qualitative interview findings were concordant with this: a subset of high risk women gave detailed accounts of their marked pregnancy-related anxiety. Such emotions were not universally expressed, but when present, were a noticeable focus at interview. A range of complementary subthemes emerged, with HRW discussing reasons for their anxiety (lack of explanation of previous PTBs, the traumatic nature of previous pregnancies, difficulty accessing support from clinicians, fear of recurrent problems), their pattern of emotions (with cyclical anxiety in relation to appointments a strong theme) and their coping mechanisms (seeking information/explanation, developing trust and rapport through relationships with care givers, reframing abnormal test results as positive opportunities for action/preparation). HRW who had experienced later PTBs or positive outcomes following PTB did not typically express such strong emotions at interview. Low risk women were generally less emphatic in their expressions of anxiety and reassurance, consistent with questionnaire results. However those who had undergone fertility treatment, experienced early miscarriage or with family history of preterm birth described higher levels of anxiety. Nulliparity was also a source of anxiety for several LRW.

The pain assessments from the quantitative survey also showed high concordance with interview data. Pain scores on both the VAS and PPI scales were low (mean VAS ~1 with 80% selecting no/mild pain on the PPI), and similarly women made efforts to ensure their descriptions of the physical experience of EIS were not interpreted as pain during the SSIs. Phrases such as *"it's not a pain at all"* (Participant 5), *"it's not painful in any way"* (Participant 17) were often used as a prefix or suffix to more detailed descriptions. The qualitative

descriptor most commonly used at interview was not included in the fifteen item McGill list: both HR and LRW frequently described a feeling of "*pressure*". However this may have been influenced by the real time explanations from the CRF during screening, as illustrated by Participant 3:

"*She said it would be a bit like pressure or something. I think she said pressure, something like that, but it weren't, it were fine"*

Participant 3 (LR, one term birth)

The only discordant account noted at interview was that of one HR woman (Participant 10), who described a higher degree of discomfort than anyone else ("*it felt like it poked, a sort of stabbing poke...*", "*it sort of felt like I was getting an IUD put in*"). Interestingly her score on the VAS was 3 and on the PPI 2 (discomforting) which overall does not appear suggestive of high pain intensity (although her scores did represent the top of the range recorded for HRW). However, she too qualified her description ("*But I think it's the way, I think she moved it or something. So it wasn't actually the instrument, it may have been the handling of the instrument"),* perhaps suggesting a transient sensation at one reading, rather than a consistent sensation across all six readings at the two study visits.

Although items from the affective subscale of the McGill PRI were not commonly selected by questionnaire respondents, complementary themes from the interviews did emerge which detail the interplay between the emotional and physical experience of EIS. These include uncertainty regarding impending physical experience, concerns regarding the safety of a novel test and their perspectives on intimate examination. Women who expressed the opinion that checks and examinations were useful often found the physical experience particularly manageable - for example Participant 1, who recorded scores of 0 on both the VAS and the PPI:

"For me, it's ok. It's a little weird, but is not hurting, it's not pain. I know that it's just for good things. So I'm not worried."

Participant 1 (LR, one term birth)

Similarly some patients who had reflected upon the safety of EIS as a novel test recorded slightly higher pain scores, e.g. Participant 6 (VAS of 3 and PPI of 1) who stated:

"I know it said that there wasn't any harm with the impedance at all. But it would have been nice to have something in there that showed some evidence for that that backed it up like some statistics or previous pilot that says this has happened."

But also:

"To be honest, I'm not sure, because the speculum was in, and I could feel the speculum, I can't say that I massively felt anything. Maybe a little bit of like a tingle or like you were just doing a swab, just being touched kind of thing really"

(Participant 6, LR, first pregnancy)

This slight conflict between pain score and qualitative account could imply that the emotional impact of EIS influenced women's sensory experiences more than the questionnaire data suggests. Alternatively, despite the questionnaire aiming to establish the specific effects of EIS, her pain score may also reflect the discomfort experienced with speculum examination rather than the CR reading itself.

Both qualitative and quantitative methodologies yielded useful information concerning the appearance and design of the EIS device. The VAS scores provided in questionnaire responses spanned a wide range (from 0-9) although the majority (75%) of participants scored the probe appearance as non-threatening. The interviews confirmed this diversity of opinion; some women barely remarked upon the probe (indeed two said they couldn't remember what it looked like, whilst another participant referred to "*the little pen thing*"), whereas others expressed quite negative opinions regarding its appearance (using descriptors such as "*bulky", "different", "futuristic", "odd, "intimidating", "space age", "scary", "robot probe").* The reflections of the latter group offer detailed insight into which features they found troublesome, including colour, length, the noise the probe made and its wireless connectivity.

Similarly, when considering women's views regarding the overall acceptability of EIS, SSI data provided a wealth of complementary information over and above the more simplistic VAS and binary ratings used in the questionnaire. Whilst all women rated the procedure as acceptable for use in antenatal care, the responses at interview demonstrate that, for some, this is context specific. Views were notably polarised – some women advocated strongly for universal PTB screening (including EIS if confirmed as clinically beneficial) whilst others felt tests were best reserved for those with prior PTBs and were more doubtful about

application to a low risk population. Many factors appeared to influence women's position on screening. Subthemes concerning knowledge in pregnancy (pre-existing knowledge of PTB/"no-one knew why"/"it's good to know")) and perspectives on intimate examination (the vagina as a protected space/intimate examinations as normal/beneficial) were particularly evident, as were women's emotional experiences of both EIS and other screening tests. Participants who expressed concern about the safety of EIS and/or internal examination in pregnancy were inevitably more cautious about the idea of universal screening, in contrast to those who viewed both knowledge and intimate examination as beneficial/normal and who gained considerable reassurance from the study tests. No women expressed reservations about the application of EIS to a high risk population – even those who had concerns about the safety of intimate examination or the EIS probe. HRW were frequently described as having most to gain from additional screening, which tipped the balance of test burden and benefit. Thus the qualitative and quantitative data draw consistent conclusions regarding acceptability for use in HRW, whereas women's qualitative accounts reveal some dissonance with respect to screening LRW. Both risk groups expressed a range of opinions on test acceptability at interview, but HRW with particularly high risk histories (e.g. previous extreme or multiple PTBs/MTLs) provided the most emphatic support for universal screening.

6.5 Discussion

This study used a mixed-methods, parallel convergent, QUANT→QUAL approach to comprehensively evaluate women's experiences of undergoing cervical EIS measurements as part of a PTB screening package. It is the first study to employ validated quantitative instruments and a multi-modal approach to assess test acceptability and also contributes to the concise body of qualitative evidence regarding PTB screening in general.

The results demonstrate low levels of discomfort associated with EIS measurement, which were generally corroborated by women's detailed accounts at interview. These findings compare favourably to existing data on pain experienced during PTB screening via CL measurement (with mean VAS pain scores of 0.5 and 2.4 reported by Heath *et al.*[178] and Cicero *et al.*[403] respectively, and no or mild pain during TVUSS reported by the majority of participants in studies by Clement *et al.*[404] and Romero *et al.*[406]). They provide useful

information with which to counsel patients undergoing EIS measurements in future, and may help address one of the potential sources of anxiety associated with screening, namely uncertainty about the impending physical experience of a novel test.

Participants found cervical EIS acceptable for use in antenatal care, but had varied opinions on routine screening. No previous qualitative studies have examined women's perspectives on who should be offered PTB screening, thus these findings provide an early insight into the views of a subset of both low and high risk women. It is encouraging to compare the binary rating of EIS acceptability (with 100% of 40 respondents rating the test as acceptable for use in antenatal care) with prior studies of PTB screening; again, these are not dissimilar, with positive acceptability ratings provided for both CL scanning and FFN measurement by ≥90% of women in earlier work[404, 405]. Taken together, the results regarding pain and acceptability suggest that EIS might appropriately be incorporated into existing PTB screening strategies without compromising test acceptance rates.

Interpretation of the data regarding anxiety is complex and women's emotional state varied according to risk status, prior experiences, the timing of assessment and their perspectives on intimate examination, novel tests and pregnancy in general. Overall, undergoing screening was associated with a reduction in anxiety and many women with high anxiety levels were particularly emphatic in their appreciation of the reassurance gained through tests and monitoring. For high risk women especially, comfort was not gained by the tests alone, but also through detailed explanations, regular attendance and the opportunity to build a rapport with care-givers. There is commonality between these SSI themes and preceding work: O'Brien *et al.*[416] have previously noted the importance of the relationship between HRW and the PTB clinic team, and the role frequent checks play in breaking HR pregnancies down into manageable chunks. In both HR asymptomatic[416] and symptomatic women[417, 418], prior studies have demonstrated comparably favourable views of increased surveillance and information provision in pregnancy to those expressed by our cohort.

It is important to note that whilst assessments of pain and device design were relatively specific to EIS, anxiety ratings related more broadly to the screening package as a whole (and indeed to the pregnancy itself). The existing literature which assesses anxiety in relation to PTB screening is heterogeneous. Although two previous studies have utilised the

STAI-6 to evaluate the effect of CL scans[404] and FFN swabs[407] on maternal anxiety, both applied the instrument at different time-points (specifically conducting a retrospective assessment of anxiety before and during a CL scan and a contemporaneous assessment before FFN testing at 24 and 27 weeks). They also each employed a single predictive modality (in contrast to our multi-modal package of tests), making comparison with the immediate pre- and post-test STAI-6 results reported here difficult. Nevertheless, the results from our cohort were broadly in keeping with the findings of Clement *et al.*[404], who demonstrated a significant reduction in "*worry about preterm delivery"* (scored on a 0-5 scale) after CL scanning. However, they did not observe any significant difference in STAI-6 scores before and during the TVUSS. It is not clear from their methodology when women received their scan result – it may be that no real time explanation was given, and focusing the second anxiety assessment on the duration of the scan missed the effect of receiving a normal result. It is also notable that women with a short CL were excluded from recruitment, thus they are likely to have over-estimated the reassurance provided by CL scanning. In contrast, Shennan *et al.*[407] included women with both positive and negative test results in their assessment of FFN. Their finding of significantly increased anxiety in HR *vs.* LRW mirrors the trend reported in our cohort. Similarly, our isolated observation of increased anxiety post-screening in one LR participant with positive test results is in keeping with their observation that positive FFN swabs increase maternal anxiety. However, not every woman in our study with a short cervix or positive swab demonstrated an increased post-test STAI-6. It is plausible that undergoing more than one predictive test enabled women to reframe their results to mitigate against anxiety (e.g. by focusing on a normal CL if FFN was positive or, if both tests were abnormal, by reframing this knowledge as a positive opportunity for action, as discussed in the SSI section).

Although the design of the EIS probe was generally deemed satisfactory by study participants, the relatively wide range of scores yielded by the questionnaire suggested some diversity of opinion. The SSI data was therefore particularly useful with respect to this element of test acceptability and provided valuable insight into the specific aspects which some women found troublesome. Utilising this information to inform the design of future EIS devices will optimise the testing procedure for broader clinical use.

This study is not without limitations. The sample size for the quantitative survey is modest, which limits the power of the statistical analyses and it is possible that more marked differences between risk groups would be observed within a larger cohort. In addition, the views reported here represent a group of women who agreed to participate in the original clinical trial. Women who declined to participate could obviously not provide accounts of the screening experience involved in the study, but their views regarding EIS, PTB screening in general and their reasons for declining may have provided useful information for future clinical policy and practice. Future work should aim to address this knowledge deficit by assessing the opinions of women who decline PTB screening.

6.6 Conclusions

EIS is an acceptable test to both high and low risk women. The physical experience of undergoing measurements was well tolerated by both groups. The emotional experience of testing was complex and influenced by many factors. Many of these were unrelated to EIS itself and stemmed from women's obstetric histories, pre-existing attitudes to examination and medical intervention and their desire for information about their pregnancies. Gaining awareness of the way that these and other factors influence women's experience of PTB screening will enable us to develop screening programmes which are acceptable to as many women as possible. This in turn will maximise the effectiveness of any future screening programme.

Chapter 7 - General Discussion and Future Perspectives

7.1 Summary of findings and contribution to knowledge

Throughout this thesis the importance and complexity of tackling preterm birth has been made clear. Existing tests and care pathways have made some inroads into reducing the incidence and sequelae of prematurity but further progress remains a priority. Accurate prediction of spontaneous preterm labour is essential to provide the necessary foundation for both prophylactic and preparatory therapy. Chapter 1 summarised the current options available for PTB prediction. No perfect test exists, and the current 'gold standards' of cervical length measurement and cervico-vaginal biomarker detection/quantification vary in accuracy depending on the population in which they are used. It is likely that their limitations partly stem from the complexity of pathways leading to PTB. Thus research investigating new predictive technologies is necessary – to offer alternative or complementary testing strategies applicable in a variety of situations and to a range of populations.

The use of electrical impedance spectroscopy (EIS) to detect cervical change prior to PTB is a novel approach and this thesis provides an original contribution to knowledge by examining its use as an asymptomatic screening test and as a tool for assessing symptomatic women. Thanks to meticulous engineering work, the Mark V EIS probe has advanced significantly compared to previous iterations. The reproducibility study reported in Chapter 3 therefore builds upon previously published assessments of repeatability and reproducibility[304] by evaluating the effect of probe pressure standardisation and operator blinding. The study of asymptomatic women in Chapter 4 provides a robust assessment of the predictive accuracy of EIS alongside cervical length measurement and fetal fibronectin estimation. It is the first substantive study to demonstrate that measurements of cervical resistivity by electrical impedance spectroscopy have the ability to predict spontaneous PTB in this asymptomatic group, confirming the utility suggested by preceding pilot work[25]. The pilot study in Chapter 5 provides the first preliminary assessment of EIS use to assess symptomatic women and suggests potential predictive benefit in this group also. The mixed methods study in Chapter 6 is the first to examine women's experiences of undergoing cervical EIS measurements and provides new insights to inform future study designs.

7.1.1 Variability study

The results reported in Chapter 3 address the first hypothesis of this thesis – namely, that the modifications incorporated within the Mark V Sheffield EIS probe allow repeatable and reproducible measurements of cervical resistivity. By obtaining multiple measurements with the new probe and comparing readings taken by two observers, the variability of this technique was quantified prior to use in larger cohorts.

Good to excellent intra-observer repeatability was noted at a 2N application pressure (all ICCs for average measures >0.75), which, coupled with practical considerations (ease of measurement, comfort of participants), made this the most appropriate pressure to use in the later studies. Assessment of measurement reproducibility was more challenging due to technical issues during the conduct of the study. This meant only one reading was obtained at each application pressure by observer 2, precluding repeatability analysis for multiple observers. This does not impact on the validity of the results in Chapters 4 and 5 (where all readings were obtained by the same clinician) but further assessment to confirm measurement reproducibility in practice would be ideal.

7.1.2 Cohort study of asymptomatic women

The study in Chapter 4 addressed three main hypotheses: (i) whether CR is indeed lower in asymptomatic women destined to deliver preterm; (ii) whether cervical EIS is a useful predictive test for this group and (iii) if EIS detects similar changes prior to spPTB in women with a history of prior LLETZ. The findings agree with pilot data[25] and confirm that, in untreated asymptomatic women, CR at mid to high frequencies is lower in those who later experience spPTB. However, this pattern was not replicated in the limited subgroup of women with history of colposcopic treatment, presumably due to cervical scarring. For these participants, there was a tentative trend towards higher CR readings in those delivering early.

The precise physiological processes underlying these observations remain unclear. Previous finite element modelling suggests EIS interrogates both cervical epithelium and stroma[295, 299, 300]. Important determinants of the EIS spectra include cell spacing, conductivity of the

intra and extracellular spaces, cell membrane capacitance and inter-cellular connections[286, 287, 431] (see Figure 1-7, Chapter 1). More broadly, the condition of the tissue may influence its resistivity – greater hydration and the presence of oedema, fibrosis and inflammation have been associated with lower impedance in a range of tissues[303, 432, 433]. Although changes in many of these areas have been implicated in cervical remodelling[128, 278], obtaining histological specimens from pregnant women is necessarily limited and clarity regarding the exact sequence of normal and aberrant ripening remains elusive. It is therefore impossible to state precisely what the reduced impedance observed in women delivering preterm is quantifying- it may relate to reduced barrier function of the cervical epithelium (with associated effects on permeability and susceptibility to infection); or it may relate to greater stromal hydration and changes in the constituents/structure of the extracellular matrix. Indeed it is plausible that it detects some combination of the two.

The differential pattern observed in the colposcopy sub- group is interesting, but should be viewed with caution given the small numbers involved. It provides preliminary information to aid the interpretation of EIS measurements in those with a history of LLETZ and suggests application of thresholds obtained during studies of untreated women may not be appropriate. Ultimately the data are too limited to reach clear conclusions, but this group merit further research. They are at risk of PTB[344, 369, 434], there is lack of clarity regarding the optimal approach to screen and treat them[434] and the conclusions regarding EIS in the untreated subgroup may not apply to them. Larger observational studies are indicated to see if EIS has anything to offer these women.

The analyses of predictive performance in untreated asymptomatic women are favourable: The ROC AUCs for EIS matched or exceeded those of CL and FFN for both prediction of spPTB before 37 and 32 weeks. Incorporating prior history of PTB into multimodal models further improved predictive accuracy, and EIS performed well in all evaluated subgroups (AHR, ALR, nulliparous ALR). Moreover, follow-on work (which exceeds the scope of this thesis) suggests the combination of EIS and obstetric history could have similar accuracy to the QUiPP app[435] in AHR women. Although this finding requires validation in larger cohorts, it is possible that EIS might offer additive predictive benefit if incorporated into existing algorithms.

However, in addition to the difficulty correlating our observations with precise aspects of remodelling, other limitations must be considered. The modest sample size of this work has several implications: it was not possible to assess predictive accuracy in women receiving PTB prophylaxis (cerclage/progesterone), and minimal differences in neonatal morbidity and mortality were observed between groups. If future work incorporates standardised treatment protocols, large studies could enable the effect of EIS screening on these meaningful outcomes to be determined. The studied cohort was predominantly Caucasian, which could limit the applicability of our findings to a more diverse population – future work will also aim to address this question. Moreover, the results cannot be extrapolated to other high risk groups, including women with uterine anomalies and multiple pregnancies – further research is again required to clarify if EIS has a future role in their assessment.

In addition to these methodological limitations, some analytic limitations were present. The primary aim of the study was to assess the predictive performance of EIS. Definitive evaluation of the models used to generate EIS indices and the multimodal models (combining individual tests and obstetric history) would require a larger dataset, with a higher number of PTB outcomes[436, 437]. Ongoing work will seek to define optimal approaches to both internal and external model validation to guard against problems such as overfitting, optimism and non-generalizability, should they be employed for more widespread use.

7.1.3 Pilot study of symptomatic women

Chapter 5 considered the question of whether cervical EIS could be useful when assessing women with symptoms of PTL. Impedance measurements were obtained during routine examination alongside CL measurement and FFN quantification. The small sample size of this pilot means its conclusions must be tentative. Nevertheless, a trend towards lower impedance was noted in women who delivered within 14 and 28 days of assessment, which again supports the hypothesis that EIS can quantify some element of the cervical changes which precede labour. Preliminary evaluation of the predictive utility of EIS was obtained through ROC curve analysis; it performed favourably in comparison to CL scanning and showed similar accuracy to FFN if a 50ng/ml positive test threshold was employed. Use of higher thresholds for FFN yielded the highest AUC for an individual test, but there was again a trend towards optimal prediction by multimodal testing in this cohort (with AUCs of 0.89

for prediction of preterm birth within 14 and 28 days noted). Together, these results suggest that the use of EIS in symptomatic women merits further study.

Current UK guidance recommends that any woman presenting with threatened PTL prior to 30 weeks is given preparatory treatment (steroids +/- tocolysis, magnesium sulphate or transfer out if relevant) due to the potentially serious consequences of false negative predictive tests at early gestations. After 30 weeks either CL scanning, FFN quantification (with a 50ng/ml threshold) or 'treat-all' approach may be considered. This highly cautious approach is understandable, but not without problems. A recent evaluation of data from the Yorkshire and Humber neonatal and paediatric transfer service showed that only 35% of women delivered within 48 hours of IUT and women travelled significant distances (an average of over 42 miles for cots in region and over 70 miles when out of region transfer was required)[438]. IUT imposes a significant emotional and socio-economic burden on families[338] and is expensive and time-consuming to facilitate[439]. Therefore, the high sensitivity of EIS suggested by our limited pilot data could be particularly beneficial if replicated in larger studies. Although specificity estimates were more modest, the consequences of false positive tests in women presenting at early gestations are less concerning than those of false negative tests; thus test sensitivity is critical for the earliest preterm presentations. Moreover, the use of multimodal testing strategies may optimise sensitivity *and* specificity, minimising the occurrence of any false results. If the risk assessment of symptomatic women can be optimised, then unnecessary treatment, transfer and anxiety may be reduced.

Again some methodological limitations in this study must be acknowledged. The limited sample size has already been noted. Similar to the asymptomatic cohort, our symptomatic recruits were predominantly Caucasian; furthermore, those with prior colposcopic/ prophylactic treatment were excluded from analysis. Thus the generalisability of these findings to more diverse populations and to those with a history of cervical treatment cannot be assumed, and should be addressed in future studies.

7.1.4 Mixed methods acceptability study

Our preliminary assessment of test acceptability suggests that EIS measurements are well tolerated and women felt the test was suitable for wider use in AN antenatal care. Ratings of test discomfort were similar to published assessments of conventional predictive tests[178, 403, 404, 406]. STAI-6 scores generally reduced with testing, which adds to the existing (conflicting) literature regarding the effect of CL scans and FFN testing on anxiety[404, 407]. The semi-structured interviews were key in explaining the quantitative results and provided valuable insight regarding the wider determinants of test experience. Triangulation particularly highlighted the impact of women's emotional experiences on test tolerability and provided useful, specific feedback regarding the probe's appearance and design.

The main limitations of this study relate to its modest sample size and the restriction of recruitment to those who agreed to participate in the main EIS study. Therefore, broader assessment of the acceptability of EIS to the general obstetric population is necessary, to ensure a diverse range of views are captured (including those of women who may have declined study participation). It would also be useful to gain the perspective of clinicians using the test – thus far, measurements have been performed by a relatively small group, with experience of the equipment and measurement technique. Obtaining the views of a broader range of users could provide valuable insights regarding ease of use and real world utility.

7.2 Implications

7.2.1 Implications for practice

Since 2019 there has been a renewed focus on preterm birth in the UK, following the publication of policy documents such as Saving Babies' Lives (Version 2)[18] (SBLv2) and guidance for commissioners produced by the UK Preterm Clinical Network[387, 440]. The National Maternity Safety Strategy[440] specifies an ambition to reduce the national rate of preterm birth from 8% to 6% by 2025. A national survey published in mid-2019 identified 33 specialist PTL prevention clinics running in the NHS, an increase of 44% compared to a similar review five years earlier[17]. This figure may now be higher, given the requirement in SBLv2 for every healthcare provider to have access to CL screening and a clinician with an

interest in preterm birth prevention. Such clinics predominantly offer screening to high risk women in the form of serial CL scans, infection screening and biomarker quantification (particularly FFN) and ensure appropriate provision of cerclage and progesterone prophylaxis. However primary prevention strategies including: smoking cessation; minimising multiple pregnancy rates following assisted reproductive techniques (e.g. advising single embryo transfer); patient education regarding BMI optimisation and ideal inter pregnancy interval; and avoidance of unnecessary iatrogenic late PTB will also be important in achieving the 6% target. This renewed focus means there will be an increasingly strong infrastructure to support ongoing PTB research and the adoption of new predictive technologies (once robust evidence is available to support their use).

EIS may have a future role in the risk assessment of HRW. However, further work is necessary to confirm test performance outside of a single centre setting and to evaluate the clinical effects of incorporating EIS into screening pathways (either as a stand-alone or complementary screening modality). Chapter 4 (section 4.5) outlined potential approaches to achieve these aims and section 7.2.2, below, will discuss study design in more detail. The UK guidelines now mandate universal PTB risk assessment (with referral of HRW for additional care), but do not address the issue of PTB screening for LRW. American guidance acknowledges the debate regarding universal cervical length screening, but recognises it as a reasonable strategy (albeit one which should be implemented with strict guidelines)[441]. Given this landscape, ongoing research to optimise the risk assessment of LRW is timely. Issues surrounding the current NICE guidance regarding the assessment and care of symptomatic women have been discussed above. If EIS is confirmed as having predictive utility in this group, it could easily be incorporated into existing care pathways. CL scanning is not widely used in the acute setting (likely due to the lack of availability of trained staff out of hours). However, predictive tests such as FFN and phIGFBP-1 detection are commonly employed and EIS measurements could easily be performed at the same time, with minimal extra training requirements.

7.2.2 Future work

7.2.2.1 Asymptomatic women

The design of further research evaluating EIS screening of asymptomatic women was discussed in some detail in Chapter 4 (Section 4.5). Low risk studies might optimally target nulliparous women as their baseline risk of preterm birth is higher than that of low risk multiparous participants[442]. Performing screening alongside the anomaly scan would be practical, and comparison to conventional tests would allow evaluation of EIS as both an isolated and complementary screening technique. However, management of abnormal CL would need to be careful considered in this cohort, given the paucity of evidence regarding optimal prophylactic therapy. The sample size calculation outlined in Chapter 4 (section 4.4) suggested 1844 low risk nulliparous women would need to be recruited to confirm test performance with reasonable precision.

Randomised screening studies incorporating progesterone treatment were also discussed with respect to AHR women. Two putative designs were considered (Chapter 4, section 4.5): a non-inferiority study comparing CL screening with EIS and a superiority study comparing CL screening with CL+EIS screening (with respective sample sizes of 535 and 26,798). The smaller sample size of the NI study should make it more practical (and less expensive) to conduct. However, in a UK setting, randomisation to an arm which did not include CL screening (when this is widely available and mandated as the current gold standard of HR screening[440]) would be ethically challenging and recruitment would likely be difficult. Such a design might have more application in a lower resource setting, where CL scanning is not routinely available. However, if such work is contemplated, important issues regarding the conduct of research in underserved populations must be considered. These include issues of consent and participant vulnerability in areas with low literacy rates; the importance of strong local partnerships to build trust and gain understanding of contextual issues relevant to the study (e.g. features of the healthcare system and local population); how to incorporate quality assurance given potential limitations of local information technology/data management systems; and reflection on the sustainability of screening and prophylaxis more broadly[443, 444]. Further issues relating to the design and conduct of non-inferiority studies are also relevant: stakeholder consultation would be required to ensure

selection of the most clinically relevant non-inferiority limit and a careful protocol and analytical plan (including consideration of both intention to treat and per protocol analyses) would be essential to avoid poor study conduct and the risk of 'technocreep'[383, 445, 446].

In contrast, a superiority trial of CL screening *vs.* 'enhanced' screening with CL plus EIS (with progesterone treatment for screen positive women) could more easily be undertaken in a UK setting. However, due to the relatively low prevalence of PTB (even amongst HR women), the modest effect size of progesterone and an estimated sensitivity difference of ~20% between CL alone vs CL plus EIS for PTB detection, this would require a study of considerable magnitude. One further study design merits consideration, namely the stepped wedge cluster-randomised trial. In such studies, all clusters (e.g. geographically grouped maternity units) begin with no intervention (in this case, the introduction of 'EIS-enhanced' screening); then, at regular time-points (steps), clusters are randomised to switch from control to intervention group[447]. Ultimately all clusters receive the intervention – thus all clusters contribute data to the control and intervention groups. Such a design would be methodologically complex and would need to account for issues such as complex correlation structures[448] (e.g. due to women potentially crossing from control to intervention group during their pregnancy). Ultimately, prematurity rates within intervention and control clusters could be compared to evaluate whether the increased sensitivity of 'EIS-enhanced' screening results in a reduction in PTB. This study design is most commonly (though not exclusively) used for policy interventions, for example the introduction of new guidance regarding management of reduced fetal movements[449]. Justification for utilising it to evaluate a novel screening method could be more challenging, given that women would not provide individual consent to participate; however they would, as always, be able to decline any screening they did not wish to undergo, without detriment to their care.

For all three designs, several considerations are important: firstly, they include an inherent assumption that women who screen positive via EIS would benefit similarly from progesterone as those with a short cervix; indeed, progesterone's mechanism of action is unclear, with conflicting evidence regarding anti-microbial effects[61, 317] and recent work suggesting Replens (a vehicle for vaginal progesterone) may itself reduce PTB by strengthening the cervical epithelium[450]. Given that women with short CL and low CR are

both exhibiting signs of premature remodelling, it is certainly plausible that women with a positive EIS screen will benefit from progesterone. Secondly, outcome measures for any study would need to be carefully selected. PTB before 34 weeks is arguably the most widely adopted primary outcome within the recent literature[308, 311, 313, 451, 452], and use of this outcome would facilitate comparison with other research (and data synthesis if relevant in future). Secondary outcomes are also important – ideally those specified by the CROWN initiative would be adopted[168], as they capture those events most important to patients and health care professionals. Finally, it must be noted that not all HRW would be eligible or willing for randomisation to a study which utilises progesterone as its primary prophylactic treatment. Women with previous failed progesterone therapy and/or previous successful cerclage (especially with a history suggestive of cervical weakness) could reasonably prefer repeat cerclage. Although meta-analysis suggests cerclage is not superior to progesterone[453], treatment individualisation is essential, which might limit the pool of potential HR recruits somewhat.

7.2.2.2 Symptomatic women

Similar to the ALR group, the primary aim of future research in symptomatic women would be evaluation of the accuracy of EIS in PTB prediction. Again this would optimally incorporate CL scanning and FFN estimation for comparative purposes and would require an estimated sample size of 370 women (see Chapter 5, section 5.4). Primary outcome measures in this group would be prediction of birth within 2, 7 and 14 days of assessment, given that preparation for PTB rather than prophylaxis is the main therapeutic goal. Inclusion of a subgroup of women with prior colposcopy treatment would be ideal, to enable pilot assessment of the benefit of EIS measurements in those with cervical scarring and threatened PTL.

7.2.2.3 Mechanistic research

In addition to the clinical studies detailed above, ongoing mechanistic work is important to enhance our understanding of which remodelling processes EIS is quantifying. This might include studies incorporating both EIS measurements and complementary non-invasive techniques for assessing cervical remodelling (for example Raman Spectroscopy[140], Light-induced Fluorescence[155, 156] or diffusion tensor magnetic resonance imaging[119]). Other

approaches could investigate whether there are differences in the vaginal microbiome between women with low CR and different gestations at delivery (i.e. is there any evidence for the hypothesis that EIS is detecting epithelial susceptibility to the effects of an adverse vaginal microbiome and could it identify a subgroup of women who might benefit from positive modulation with live bio-therapeutic products?[454]).

7.2.3 Moving towards comprehensive assessment of cervical function

The strength of EIS may lie in its ability to augment rather than replace existing predictive methodologies. Whilst a 'magic bullet' for predicting and preventing preterm birth would be welcome, it is also highly unlikely that any 'one size fits all' tests and treatments truly exist. The literature is clear regarding the complex pathogenesis of preterm birth and the concept of prematurity as a syndrome is no longer novel. In order to maximise accuracy when assessing those at risk of preterm birth, two strategies are beneficial: (i) a focus on the common effector pathway of preterm labour will maximise the number of cases which can be detected (although how early on in the disease process this may be detected will likely vary) (ii) multimodal assessment of these downstream components of the labour pathway, which should maximise sensitivity and specificity and provide further insight into mechanistic pathways of PTL. Cervical change is one of the most essential steps of labour, but cervical function is complex and not easily summarised by one measure. Figure 7-1 illustrates the multiple determinants of cervical function including a normal closed length; effective sphincter function at the internal os; a strong collagen network and appropriately constituted ECM within the cervical stroma; an intact mucous plug and a normal cervical epithelium. These will interact with the local cervico-vaginal environment to ensure that the mechanical and barrier functions of the cervix are maintained (or conversely are compromised, increasing PTB risk).

Thus a truly comprehensive assessment of the terminal point in the PTL pathway could involve an ultrasound to measure length, assess for the presence of funnelling (and potentially other markers of functional variation as discussed in Chapter 1, section 1.4.5); swabs to assess decidual activation via FFN quantification; EIS measurements to evaluate cervical stromal and epithelial function (complementary spectroscopic techniques targeting

the collagen matrix specifically may also offer benefit here) and swabs to evaluate the local microbiome/metabolome[346-348] and presence of any pathogenic infection.

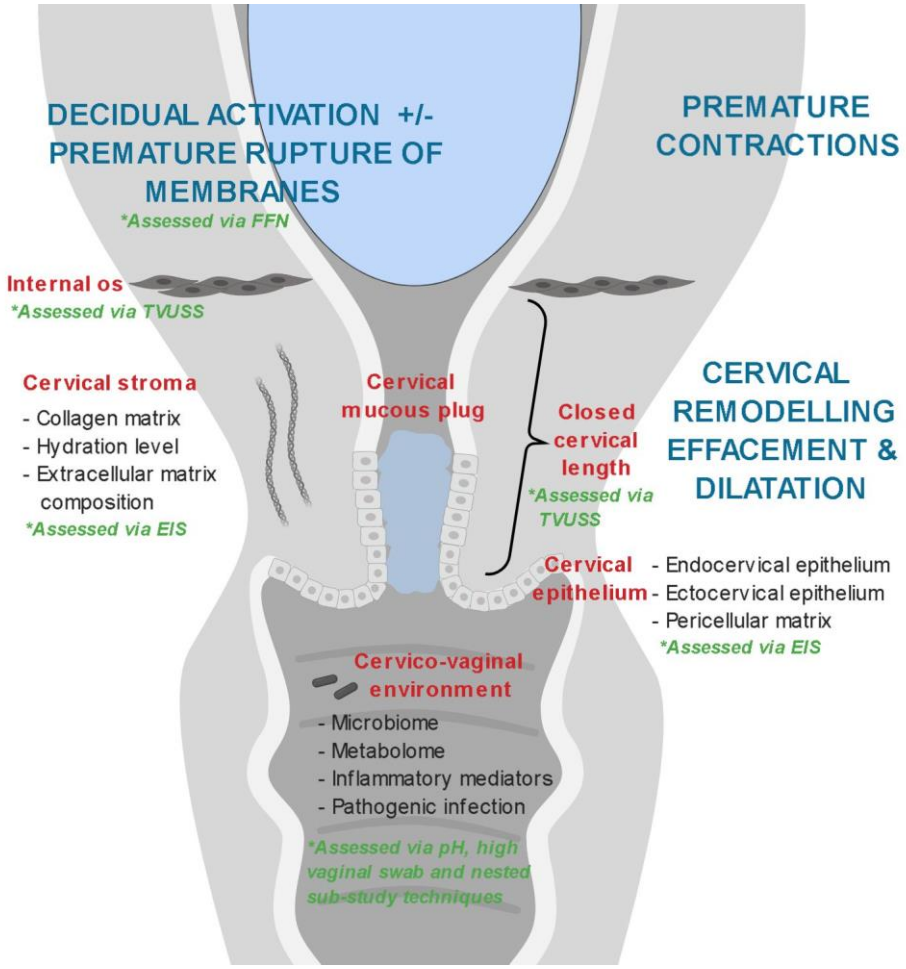


Figure 7-1 Methodologies to Assess the Determinants and Influences of Cervical Function
Cervical function is influenced by multiple factors, including other components of the common pathway of parturition (decidual/membrane activation and uterine contractions, blue text) and the local cervico-vaginal environment. Efforts to predict preterm birth may be optimised by evaluating these influences in combination rather than isolation. Furthermore, cervical function comprises more than just a measurement of closed length: an assessment which assesses sphincter function at the internal os, stromal remodelling (including tissue hydration and collagen matrix changes) and epithelial function, in addition to cervical length could plausibly detect more women at risk of preterm birth than current approaches. This figure summarises how existing predictive tests (green text) could combine with cervical EIS to offer a more comprehensive evaluation of the pregnant cervix.

Moving towards more detailed cervical assessment may enable clinicians to individualise prophylactic treatment and increase efficacy. Despite the "preterm parturition syndrome" being popularised over 15 years ago[40], our arsenal of therapeutic options available remains rather narrow and non-specific. It is unclear whether the efficacy of treatments such as

progesterone and cerclage is inherently limited, or whether our current assessment tools don't enable us to identify those women who truly need/will benefit from them.

Indeed, Figure 7-1 could also incorporate putative 'tailored' treatment strategies: for example, women with dysfunction at the internal os (in the absence of markers of infection) could plausibly benefit most from cerclage; women with an adverse vaginal microbiome may benefit from measures to address this (such as the use of live bio-therapeutic products (e.g. *Lactobacillus crispatus*) or probiotics[454]).

This is why the mechanistic research discussed in section 7.2.2.3 is vital – greater clarity regarding the precise determinants of CR in pregnant women could define its future role in treatment individualisation. If low CR predominantly signifies deficient cervical epithelial function, then administration of novel therapies to address this[450] coupled with measures to combat the adverse effects of dysbiosis could prove effective. Alternatively, if low CR reflects more advanced stromal changes, it may identify a group of women who are less likely to respond to treatments targeting earlier steps in the remodelling process.

7.3 Summary and conclusion

To summarise, cervical EIS is a promising tool in the prediction of preterm birth. It has potential for use as a screening test in low risk, high risk and symptomatic groups. Our study participants deemed it acceptable for wider use in antenatal care and steps have been outlined by which this goal might be achieved. Ultimately, if risk assessment accuracy can be maximised for asymptomatic and symptomatic women, we may achieve the critical objective of reducing the rate and sequelae of preterm birth.

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Appendices

Appendix A	Ethics and health authority approval letters.
Appendix B	Patient information sheets and consent forms.
Appendix C	Management of abnormal microbiological swab results (summary of local protocols).
Appendix D	Management of short cervical length/positive fetal fibronectin results in asymptomatic women.
Appendix E	Management of short cervical length/positive fetal fibronectin results in symptomatic women.
Appendix F	Standard Operational Procedure – High level disinfection of EIS probe.
Appendix G	Acceptability study – full questionnaires
Appendix H	Acceptability study – convergence coding matrix.
Appendix I	Summary of Study Safety Log

APPENDIX A

NHS
Health Research Authority

National Research Ethics Service

NRES Committee Yorkshire & The Humber - Sheffield
HRA NRES Centre Manchester
Barlow House
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Manchester
M1 3DZ

Telephone: 0161 625 7832
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26 June 2013

Professor Dilly Anumba
Professor, Honorary Consultant Obstetrician and Gynaecologist
University of Sheffield
The Jessop Wing
Tree Root Walk Sheffield
S10 2SF

Dear Professor Anumba

Study title:	**Assessing the Risk of Spontaneous Premature Birth by Electrical Impedance Spectroscopy of the Cervix - "(ECCLIPPx-EleCtriCaL Impedance Prediction of Preterm birth by spectroscopy of the cervIX)".**
REC reference:	**13/YH/0167**
Protocol number:	**STH 16102**
IRAS project ID:	**131508**

Thank you for your letter of 16 June 2013, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the NRES website, together with your contact details, unless you expressly withhold permission to do so. Publication will be no earlier than three months from the date of this favourable opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to withhold permission to publish, please contact the Co-ordinator Miss Helen Penistone, nrescommittee.yorkandhumber-sheffield@nhs.net.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management

permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

Non-NHS sites

The Committee has not yet been notified of the outcome of any site-specific assessment (SSA) for the non-NHS research site(s) taking part in this study. The favourable opinion does not therefore apply to any non-NHS site at present. We will write to you again as soon as one Research Ethics Committee has notified the outcome of a SSA. In the meantime no study procedures should be initiated at non-NHS sites.

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission ("R&D approval") should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements.

Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of approvals from host organisations

It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The final list of documents reviewed and approved by the Committee is as follows:

Document	*Version*	*Date*
Covering Letter from Dilly Anumba		
REC application: 131508/447212/1/32		07 May 2013
Protocol	1	21 April 2013
Investigator CV: Professor Dilly OC Anumba		01 December 2012
Participant Information Sheet: Women with no previous history of premature birth	1	21 April 2013
Participant Information Sheet: Qualitative study of acceptability of cervical spectroscopy assessment in birth management	1	21 April 2013
Participant Information Sheet: Women with previous history of premature birth	1	21 April 2013
Participant Consent Form: Predicting the risk of premature birth by cervical spectroscopy	1	21 April 2013
Advertisement	1	21 April 2013

Gantt Chart of programme time lines		
Certificate of Completion - electrical safety		08 November 2006
MRC Panel Assessment		16 January 2012
Funding Agreement		14 May 2012
Letter about funding from MRC		30 April 2012
Response to Request for Further Information from Professor Anumba		16 June 2013
Participant Information Sheet: Validation studies on pregnant volunteers prior to caesarean section	2	16 June 2013
Participant Information Sheet: Women with symptoms of threatened preterm birth	2	21 April 2013
Participant Consent Form: Acceptability of cervical impedance measurements in clinical care	2	16 June 2013

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Reporting requirements

The attached document *"After ethical review – guidance for researchers"* gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Notification of serious breaches of the protocol
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

Feedback

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

Further information is available at National Research Ethics Service website > After Review

13/YH/0167	**Please quote this number on all correspondence**

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

With the Committee's best wishes for the success of this project.

Yours sincerely

On behalf of
Professor Basil Sharrack
Chair

Email: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: Ms Angela Pinder
Sheffield Teaching Hospitals NHS Foundation Trust
Clinical Research Office
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NHS
Health Research Authority

National Research Ethics Service

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24 December 2013

Professor Dilly Anumba
Chair of Obstetrics and Gynaecology & Honorary Consultant in Obstetrics & Gynaecology/Subspecialist in Fetomaternal Medicine
University of Sheffield
Department of Human Metabolism
4th Floor, Jessop Wing
Tree Root Walk, Sheffield
S10 2SF

Dear Professor Anumba

Study title:	**Assessing the Risk of Spontaneous Premature Birth by Electrical Impedance Spectroscopy of the Cervix - "(ECCLIPPx-EleCtriCaL Impedance Prediction of Preterm birth by spectroscopy of the cerviX)".**
REC reference:	**13/YH/0167**
Protocol number:	**STH 16102**
Amendment number:	**#1**
Amendment date:	**02 November 2013**
IRAS project ID:	**131508**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

Approval was sought to obtain and use vaginal swabs from those women who only wish to participate in the microbiome aspect of the study.

The Committee asked for further information about how participants would be recruited to the sub-study. The Committee also thought it would be worth clarifying in the Participant Information Sheet how women would take the swab themselves. Clarification was sent along with a revised Participant Information Sheet.

The Committee strongly advised that the Participant Information Sheet for the main study is revised to refer to this sub-study. It should be clear that participants can only take part in the sub-study if they wish.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering Letter from Professor Dilly Anumba		14 November 2013
Notice of Substantial Amendment (non-CTIMPs)	#1	02 November 2013
Protocol	1.1	02 November 2013
Participant Information Sheet: Microbiome Swab Study	2	18 December 2013
Participant Consent Form: Microbiome Swab Study	1	02 November 2013

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

13/YH/0167:	Please quote this number on all correspondence

Yours sincerely

On behalf of
Professor Basil Sharrack
Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Angela Pinder,
Sheffield Teaching Hospitals NHS Foundation Trust

NRES Committee Yorkshire & The Humber - Sheffield

Attendance at Sub-Committee of the REC meeting

Name	*Profession*	*Capacity*
Professor Basil Sharrack	Chair of REC and Consultant Neurologist	Expert
Dr Nana Theodorou	Specialist Orthoptist	Expert

Also in attendance:

Name	*Position (or reason for attending)*
Miss Helen Penistone	REC Manager

NHS
Health Research Authority

National Research Ethics Service

NRES Committee Yorkshire & The Humber - Sheffield

HRA NRES Centre Manchester
Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Tel: 0161 625 7832
Fax: 0161 625 7299

16 February 2015

Professor Dilly Anumba
Chair of Obstetrics and Gynaecology & Honorary Consultant in Obstetrics & Gynaecology/Subspecialist in Fetomaternal Medicine
University of Sheffield
Department of Human Metabolism
4th Floor, Jessop Wing
Tree Root Walk, Sheffield
S10 2SF

Dear Professor Anumba

Study title:	**Assessing the Risk of Spontaneous Premature Birth by Electrical Impedance Spectroscopy of the Cervix - "(ECCLIPPx-EleCtriCaL Impedance Prediction of Preterm birth by spectroscopy of the cerviX)".**
REC reference:	**13/YH/0167**
Protocol number:	**STH 16102**
Amendment number:	**Substantial Amendment 2**
Amendment date:	**19 January 2015**
IRAS project ID:	**131508**

The above amendment was reviewed by the Sub-Committee in correspondence.

Ethical opinion

Approval was sought to include an additional two groups of women to improve recruitment rates. Women with a history of loop excision of the transformative zone of the cervix would now also be included in the study.

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Notice of Substantial Amendment (non-CTIMP)	Substantial Amendment 2	19 January 2015
Participant information sheet (PIS) [PIS - Symptomatic Women]	2	15 January 2015
Research protocol or project proposal	1.2	15 January 2015

Membership of the Committee

The members of the Committee who took part in the review are listed on the attached sheet.

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

We are pleased to welcome researchers and R & D staff at our NRES committee members' training days – see details at http://www.hra.nhs.uk/hra-training/

13/YH/0167:	**Please quote this number on all correspondence**

Yours sincerely

On behalf of
Professor Basil Sharrack
Chair

E-mail: nrescommittee.yorkandhumber-sheffield@nhs.net

Enclosures: List of names and professions of members who took part in the review

Copy to: Angela Pinder,
Sheffield Teaching Hospitals NHS Foundation Trust

NRES Committee Yorkshire & The Humber - Sheffield

Attendance at Sub-Committee of the REC meeting

Committee Members:

Name	*Profession*	*Present*	*Notes*
Professor Basil Sharrack	Consultant Neurologist	Yes	
Dr Nana Theodorou	Specialist Orthoptist	Yes	

Also in attendance:

Name	*Position (or reason for attending)*
Miss Helen Penistone	REC Manager

APPENDIX B

Sheffield Teaching Hospitals NHS
NHS Trust

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study.

Participant Information Sheet - Validation studies on pregnant volunteers prior to caesarean section.

Chief Investigator: Professor Dilly Anumba**.** Honorary Consultant in Obstetrics & Gynaecology/Subspecialist in Fetomaternal Medicine

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. You can seek further advice from the Patient Services Team (PST), Royal Hallamshire Hospital, Glossop Road,, Sheffield S10 2JF, Tel: 0114 2712400, Email: pst@sth.nhs.uk.

1. What is the purpose of the study?
Before labour starts the neck of the womb (cervix) undergoes changes to prepare for birth. These changes include softening, shortening and opening. At present we are unable to measure these changes accurately. If we were able to do this we may be able to predict when labour may start. The purpose of this study is to assess whether a new device that measures electrical changes in the cervix (called "impedance" or "spectroscopy") may be useful to predict when premature labour might start so that we can offer women treatment to prolong pregnancy in those found to be at high risk of premature delivery. Majority of women who experience premature birth have never had a premature baby before, so we want to investigate whether this technique can be used routinely in antenatal care to predict premature birth.

2. Why have I been chosen?
You have been chosen because you are pregnant and about to have a planned caesarean section. In the first instance we want to confirm the accuracy of our latest device for measuring the impedance of the cervix on normal women at term. We want to check that the measurements on one subject obtained by two doctors are very similar. In other words, that the test measurement is highly accurate. If you agree to participate you will be helping us confirm the accuracy of the measurement.

3. Why is there a need for these measurements to be taken?
Before conducting the experiments on pregnant women remote from term to predict their risk of premature birth, we want to first of all confirm in a small group of women at term prior to caesarean section that the new device measures impedance accurately. We will do this by checking that the impedance we obtain with the new device is very similar when measured by one person twice (highly repeatable), and when measured by two different people (highly reproducible) one after the other.

4. Do I have to take part?
No. It is up to you to decide whether to take part or not. You are free to withdraw from the study at any time and without giving a reason. Whether you join the study or not will not affect your care at the hospital in any way.

5. What will happen to me if I take part?
If you decide to take part you will be given this information sheet to keep. You will be asked to sign a consent form after we have explained the study to you in detail. When you are in the labour ward theatre for your delivery, and after anaesthetic has been given to you, we will follow the routine procedure of emptying your bladder with a catheter with measuring cervical impedance. We will first examine the cervix using a speculum as is commonly used for taking cervical smears and swabs. A small probe (no bigger than a pencil) is then inserted into your vagina, and gently touches your cervix, to measure the electrical properties of your cervix. We will take three measurements with this probe. Each measurement takes less than 10 seconds. A second doctor will immediately repeat these measurements so that we can compare the readings obtained by the two doctors. Overall your assessment will take 5-10 minutes.

6. What are the benefits of taking part?
You will not derive any benefits from taking part in the study. However if we found a problem whilst examining you we will arrange to treat it.

7. Will this test harm me or my baby in any way?
No. This test is safe and will not harm you or your baby in any way. You will receive no medication as part of the study. As with any internal examination during pregnancy you may experience mild discomfort and harmless slight vaginal blood spotting but this usually settles immediately. This technique has been used to study many pregnant women and is known to be safe.

8. What if I am harmed?
If you are harmed by your participation in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action.

9. What will happen with the results of this study?
The findings of this study will help us confirm that our device is highly accurate for future studies on women at risk of premature birth. We may also publish our results in medical journals and share our findings at relevant conferences. You will not be identified in any report/publication. If you would like a copy of the research report we will send this to you.

10. Will my taking part in the study be kept confidential?
Yes. All information that is collected about you will be kept strictly confidential. The data that we obtain from you in relation to this study is kept anonymised so that no one can trace the information to any individual study participant. When the results are published, no names will be used, and it will not be possible to identify anyone who has taken part

11. Will anyone else be told about my participation in the study?

No. However, if you wish us to, we will inform your GP that you are helping with this study.

12. What will happen if I do not want to carry on with the study?
You will receive the same quality of clinical care even if you withdraw from the study.

13. Who has reviewed the study?
This study was given a favourable ethical opinion for conduct in the NHS by the Yorkshire Research Ethics Committee.

14. Who is organizing and funding the research?
Professor Dilly Anumba (Consultant Obstetrician and Gynaecologist) is organizing the research within the Obstetric, Gynaecology & Neonatology Directorate at Sheffield Teaching Hospitals NHS Foundation Trust. The study is funded from the Medical Research Council.

15. What if I wish to complain about the way in which this study has been conducted?
If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

If you have any complaints, queries or concerns you may contact either:

Chief Investigator: Professor Dilly Anumba Consultant Obstetrician and Gynaecologist, Jessop Wing, Tree Root Walk, Sheffield S10 2SF on 0114 226 1075
OR
Dr David Throssell, Medical Director, STH NHS Foundation Trust, 8 Beech Hill Road Sheffield S10 2SB on 0114 271 2786.

PLEASE FEEL FREE TO CONTACT Professor Dilly Anumba (0114 2261075) if you have any further questions.

Sheffield Teaching Hospitals NHS
NHS Trust

Study Number: 16102
Patient Identification Number for this study:

CONSENT FORM

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study

Principal Researcher:

Professor Dilly Anumba MD, FRCOG. Consultant in Obstetrics and Fetomaternal Medicine

Please initial box

1. I confirm that I have read and understand the information sheet Version 1 / Version 2 dated 21.04.2013/ 16.06.2013 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to take part in the above study. ☐
4. I understand that all the information that is collected will be kept strictly confidential. ☐
5. I understand that relevant sections of data collected during the study may be looked at by individuals from Sheffield University, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Sheffield Teaching Hospitals NHS
NHS Trust

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study.

Participant Information Sheet - women with no previous history of premature birth

Chief Investigator: Professor Dilly Anumba**.** Honorary Consultant in Obstetrics & Gynaecology/Subspecialist in Fetomaternal Medicine

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. You can seek further advice from the Patient Services Team (PST), Royal Hallamshire Hospital, Glossop Road,, Sheffield S10 2JF, Tel: 0114 2712400, Email: pst@sth.nhs.uk.

1. What is the purpose of the study?
Before labour starts the neck of the womb (cervix) undergoes changes to prepare for birth. These changes include softening, shortening and opening. At present we are unable to measure these changes accurately. If we were able to do this we may be able to predict when labour may start. The purpose of this study is to assess whether a new device that measures electrical changes in the cervix (called "impedance" or "spectroscopy") may be useful to predict when premature labour might start so that we can offer women treatment to prolong pregnancy in those found to be at high risk of premature delivery. Majority of women who experience premature birth have never had a premature baby before, so we want to investigate whether this technique can be used routinely in antenatal care to predict premature birth.

2. Why have I been chosen?
You have been chosen because you are pregnant and do not have a history of premature birth. We want to know whether the assessing impedance of the cervix can help us identify pregnant women who will go into labour prematurely.

3. Do I have to take part?
No. It is up to you to decide whether to take part or not. You are free to withdraw from the study at any time and without giving a reason. Whether you join the study or not will not affect your care at the hospital in any way.

4. What will happen to me if I take part?
If you decide to take part you will be given this information sheet to keep. You will be asked to sign a consent form after we have explained the study to you in detail. When you are about 20-22 weeks pregnant we will assess your cervix by spectroscopy. We will first examine the cervix using a speculum. The insertion of the speculum is

mildly uncomfortable but harmless and is commonly used for taking cervical smears and swabs. A small probe (no bigger than a pencil) is then inserted into your vagina, and gently touches your cervix, to measure the electrical properties of your cervix. We will take two measurements with this probe. Each measurement takes less than 10 seconds. We will carry out an internal scan to measure your cervix. Overall your assessment will take 5-10 minutes.

5. What are the benefits of taking part?
You will not derive any benefits from taking part in the study. However if we found a problem whilst examining you we will arrange to treat it. The findings of this study may also enable better care of other women who are having a baby in the future.

6. Will this test harm me or my baby in any way?
No. This test is safe and will not harm you or your baby in any way. You will receive no medication as part of the study. As with any internal examination during pregnancy you may experience mild discomfort and harmless slight vaginal blood spotting but this usually settles immediately. This technique has been used to study many pregnant women and is known to be safe.

7. What if I am harmed?
If you are harmed by your participation in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action.

8. What will happen with the results of this study?
We will analyse the data to see if the results can be used to care for patients in the future. The probe may then be modified, and made on a commercial scale that will be available to health care institutions. We may also publish our results in medical journals and share our findings at relevant conferences. You will not be identified in any report/publication. If you would like a copy of the research report we will send this to you.

9. Will my taking part in the study be kept confidential?
Yes. All information that is collected about you will be kept strictly confidential. The data that we obtain from you in relation to this study is kept anonymised so that no one can trace the information to any individual study participant. When the results are published, no names will be used, and it will not be possible to identify anyone who has taken part

10. Will anyone else be told about my participation in the study?
No. However, if you wish us to, we will inform your GP that you are helping with this study.

11. What will happen if I do not want to carry on with the study?
You will receive the same quality of clinical care even if you withdraw from the study.

12. Who has reviewed the study?
This study was given a favourable ethical opinion for conduct in the NHS by the Yorkshire Research Ethics Committee.

13. Who is organizing and funding the research?

Professor Dilly Anumba (Consultant Obstetrician and Gynaecologist) is organizing the research within the Obstetric, Gynaecology & Neonatology Directorate at Sheffield Teaching Hospitals NHS Foundation Trust. The study is funded from the Medical Research Council.

14. What if I wish to complain about the way in which this study has been conducted?

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

If you have any complaints, queries or concerns you may contact either:

Chief Investigator: Professor Dilly Anumba Consultant Obstetrician and Gynaecologist, Jessop Wing, Tree Root Walk, Sheffield S10 2SF on 0114 226 1075
OR
Dr David Throssell, Medical Director, STH NHS Foundation Trust, 8 Beech Hill Road Sheffield S10 2SB on 0114 271 2786.

PLEASE FEEL FREE TO CONTACT Professor Dilly Anumba (0114 2261075) if you have any further questions.

Sheffield Teaching Hospitals NHS
NHS Trust

Study Number: 16102
Patient Identification Number for this study:

CONSENT FORM

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study

Principal Researcher:

Professor Dilly Anumba MD, FRCOG. Consultant in Obstetrics and Fetomaternal Medicine

Please initial box

1. I confirm that I have read and understand the information sheet Version 1 / Version 2 dated 21.04.2013/ 16.06.2013 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to take part in the above study. ☐
4. I understand that all the information that is collected will be kept strictly confidential. ☐
5. I understand that relevant sections of data collected during the study may be looked at by individuals from Sheffield University, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Sheffield Teaching Hospitals NHS
NHS Trust

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study.

Participant Information Sheet - women with previous history of premature birth

Chief Investigator: Professor Dilly Anumba**.** Honorary Consultant in Obstetrics & Gynaecology/Subspecialist in Fetomaternal Medicine

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. You can seek further advice from the Patient Services Team (PST), Royal Hallamshire Hospital, Glossop Road,, Sheffield S10 2JF, Tel: 0114 2712400, Email: pst@sth.nhs.uk.

1. What is the purpose of the study?
Before labour starts the neck of the womb (cervix) undergoes changes to prepare for birth. These changes include softening, shortening and opening. At present we are unable to measure these changes accurately. If we were able to do this we may be able to predict when labour may start. The purpose of this study is to assess whether a new device that measures electrical changes in the cervix (called "impedance" or "spectroscopy") may be useful to predict when premature labour might start so that we can offer women treatment to prolong pregnancy in those found to be at high risk of premature delivery. We want to investigate whether this technique can be used routinely in antenatal care to predict premature birth.

2. Why have I been chosen?
You have been chosen because you are pregnant and have at least one risk factor for premature birth. We want to know whether the impedance of the cervix can help us identify pregnant women who will go into labour prematurely.

3. Do I have to take part?
No. It is up to you to decide whether to take part or not. You are free to withdraw from the study at any time and without giving a reason. Whether you join the study or not will not affect your care at the hospital in any way.

4. What will happen to me if I take part?
If you decide to take part you will be given this information sheet to keep. You will be asked to sign a consent form after we have explained the study to you in detail. When you attend the hospital for your appointment and your cervical scan at 20-22 weeks and at 26-28 weeks we will also assess your cervix by spectroscopy in addition to your routine care. We will first examine the cervix using a speculum. The insertion of the speculum is mildly uncomfortable but harmless and is commonly used for taking

cervical smears and swabs. After taking vaginal swab tests to rule out infection (which we do routinely on a monthly basis as part of your care), we will use a small probe (no bigger than a pencil) inserted into your vagina, and touching gently on your cervix, to measure the electrical properties of your cervix. We will take two measurements with this probe. Each measurement takes less than 10 seconds. We will then carry out an internal scan to measure your cervix as usual. Overall your assessment will take 5-10 minutes.

5. What are the benefits of taking part?
You will not derive any benefits from taking part in the study. However if we found a problem whilst examining you we will arrange to treat it. The findings of this study may also enable better care of other women who are having a baby in the future.

6. Will this test harm me or my baby in any way?
No. This test is safe and will not harm you or your baby in any way. You will receive no medication as part of the study. As with any internal examination during pregnancy you may experience mild discomfort and harmless slight vaginal blood spotting but this usually settles immediately. This technique has been used to study many pregnant women and is known to be safe.

7. What if I am harmed?
If you are harmed by your participation in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action.

8. What will happen with the results of this study?
We will analyse the data to see if the results can be used to care for patients in the future. The probe may then be modified, and made on a commercial scale that will be available to health care institutions. We may also publish our results in medical journals and share our findings at relevant conferences. You will not be identified in any report/publication. If you would like a copy of the research report we will send this to you.

9. Will my taking part in the study be kept confidential?
Yes. All information that is collected about you will be kept strictly confidential. The data that we obtain from you in relation to this study is kept anonymised so that no one can trace the information to any individual study participant. When the results are published, no names will be used, and it will not be possible to identify anyone who has taken part

10. Will anyone else be told about my participation in the study?
No. However, if you wish us to, we will inform your GP that you are helping with this study.

11. What will happen if I do not want to carry on with the study?
You will receive the same quality of clinical care even if you withdraw from the study.

12. Who has reviewed the study?
This study was given a favourable ethical opinion for conduct in the NHS by the Yorkshire Research Ethics Committee.

13. Who is organizing and funding the research?

Professor Dilly Anumba (Consultant Obstetrician and Gynaecologist) is organizing the research within the Obstetric, Gynaecology & Neonatology Directorate at Sheffield Teaching Hospitals NHS Foundation Trust. The study is funded from the Medical Research Council.

14. What if I wish to complain about the way in which this study has been conducted?

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

If you have any complaints, queries or concerns you may contact either:
Chief Investigator: Professor Dilly Anumba Consultant Obstetrician and Gynaecologist, Jessop Wing, Tree Root Walk, Sheffield S10 2SF on 0114 226 1075
OR
Dr David Throssell, Medical Director, STH NHS Foundation Trust, 8 Beech Hill Road Sheffield. S10 2SB on 0114 271 2786.

PLEASE FEEL FREE TO CONTACT Professor Dilly Anumba (0114 2261075) if you have any further questions.

Sheffield Teaching Hospitals NHS
NHS Trust

Study Number: 16102
Patient Identification Number for this study:

CONSENT FORM

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study

Principal Researcher:

Professor Dilly Anumba MD, FRCOG. Consultant in Obstetrics and Fetomaternal Medicine

Please initial box

1. I confirm that I have read and understand the information sheet Version 1 / Version 2 dated 21.04.2013/ 16.06.2013 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to take part in the above study. ☐
4. I understand that all the information that is collected will be kept strictly confidential. ☐
5. I understand that relevant sections of data collected during the study may be looked at by individuals from Sheffield University, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Sheffield Teaching Hospitals NHS
NHS Trust

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study.

Participant Information Sheet - Pregnant volunteers with symptoms of threatened preterm birth.

Chief Investigator: Professor Dilly Anumba. Honorary Consultant in Obstetrics & Gynaecology/Subspecialist in Fetomaternal Medicine

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. You can seek further advice from the Patient Services Team (PST), Royal Hallamshire Hospital, Glossop Road, Sheffield S10 2JF, Tel: 0114 2712400, Email: pst@sth.nhs.uk.

1. What is the purpose of the study?
When labour is about to start the neck of the womb (cervix) undergoes changes to prepare for birth. These changes include softening, shortening and opening. At present we are unable to measure these changes accurately in women who may be going into premature labour soon but in whom the neck of the womb has not dilated much. If we were able to do this we may be able to predict when labour may start. The purpose of this study is to assess whether a new device that measures electrical changes in the cervix (called "impedance" or "spectroscopy") may be useful to predict premature labour in women with symptoms so that we can offer women treatment to stop uterine contractions, or decide on which women with symptoms should be admitted in a hospital with the appropriate neonatal care facilities, and which women can be reassured and allowed to go home. We want to investigate whether this technique can be used when women present in hospitals with possible symptoms of premature labour.

2. Why have I been chosen?
You have been chosen because you are pregnant and have reported symptoms which may suggest you could go into premature labour very soon. We want to know whether the impedance of the cervix can help us identify pregnant women who will go into labour prematurely.

3. Do I have to take part?
No. It is up to you to decide whether to take part or not. You are free to withdraw from the study at any time and without giving a reason. Whether you join the study or not will not affect your care at the hospital in any way.

4. What will happen to me if I take part?
If you decide to take part you will be given this information sheet to keep. You will be asked to sign a consent form after we have explained the study to you in detail. We will

assess your cervix by spectroscopy in addition to your required clinical examination which usually involves first examining the cervix using a speculum. The insertion of the speculum is mildly uncomfortable but harmless and is commonly used for taking cervical smears and swabs. After taking vaginal swab tests to rule out infection, and visualising your cervix (which we do routinely to diagnose premature labour), we will use a small probe (no bigger than a pencil) inserted into your vagina, and touching gently on your cervix, to measure the electrical properties of your cervix. We will take two measurements with this probe. Each measurement takes less than 10 seconds. We will then carry out an internal scan to measure your cervix as usual. Overall your assessment will take 5-10 minutes.

We are also carrying out a sub-study which involves obtaining an extra vaginal swab at the time of your examination to check whether the microbial flora it contains can help predict the chance of premature birth. Even if you consent to taking part in the main study you can only take part in the sub-study if you wish.

5. What are the benefits of taking part?
You will not derive any benefits from taking part in the study. The findings of this study may also enable better care of other women who are having a baby in the future.

6. Will this test harm me or my baby in any way?
No. This test is safe and will not harm you or your baby in any way. You will receive no medication as part of the study. As with any internal examination during pregnancy you may experience mild discomfort and harmless slight vaginal blood spotting but this usually settles immediately. This technique has been used to study many pregnant women and is known to be safe.

7. What if I am harmed?
If you are harmed by your participation in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action.

8. What will happen with the results of this study?
We will analyse the data to see if the results can be used to care for patients in the future. The probe may then be modified, and made on a commercial scale that will be available to health care institutions. We may also publish our results in medical journals and share our findings at relevant conferences. You will not be identified in any report/publication. If you would like a copy of the research report we will send this to you.

9. Will my taking part in the study be kept confidential?
Yes. All information that is collected about you will be kept strictly confidential. The data that we obtain from you in relation to this study is kept anonymised so that no one can trace the information to any individual study participant. When the results are published, no names will be used, and it will not be possible to identify anyone who has taken part

10. Will anyone else be told about my participation in the study?
No. However, if you wish us to, we will inform your GP that you are helping with this study.

11. What will happen if I do not want to carry on with the study?
You will receive the same quality of clinical care even if you withdraw from the study.

12. Who has reviewed the study?
This study was given a favourable ethical opinion for conduct in the NHS by the Yorkshire Research Ethics Committee.

13. Who is organizing and funding the research?
Professor Dilly Anumba (Consultant Obstetrician and Gynaecologist) is organizing the research within the Obstetric, Gynaecology & Neonatology Directorate at Sheffield Teaching Hospitals NHS Foundation Trust. The study is funded from the Medical Research Council.

14. What if I wish to complain about the way in which this study has been conducted?
If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

If you have any complaints, queries or concerns you may contact either:
Chief Investigator: Professor Dilly Anumba Consultant Obstetrician and Gynaecologist, Jessop Wing, Tree Root Walk, Sheffield S10 2SF on 0114 226 1075
OR
Dr David Throssell, Medical Director, STH NHS Foundation Trust, 8 Beech Hill Road Sheffield. S10 2SB on 0114 271 2786.

PLEASE FEEL FREE TO CONTACT Professor Dilly Anumba (0114 2261075) if you have any further questions.

Sheffield Teaching Hospitals NHS
NHS Trust

Study Number: 16102
Patient Identification Number for this study:

CONSENT FORM

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study

Principal Researcher:

Professor Dilly Anumba MD, FRCOG. Consultant in Obstetrics and Fetomaternal Medicine

Please initial box

1. I confirm that I have read and understand the information sheet Version 1 / Version 2 dated 21.04.2013/ 16.06.2013/15.01.2015 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to take part in the above study. ☐
4. I understand that all the information that is collected will be kept strictly confidential. ☐
5. I understand that relevant sections of data collected during the study may be looked at by individuals from Sheffield University, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

Sheffield Teaching Hospitals NHS
NHS Trust

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study.

Participant Information Sheet – qualitative study of acceptability of cervical spectroscopy assessment in birth management.

Chief Investigator: Professor Dilly Anumba**.** Honorary Consultant in Obstetrics & Gynaecology/Subspecialist in Fetomaternal Medicine

You are being invited to take part in a research study. Before you decide it is important for you to understand why the research is being done and what it will involve. Please take time to read the following information carefully. Talk to others about the study if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to take part. You can also seek further advice from the Patient Services Team (PST), Royal Hallamshire Hospital, Glossop Road,, Sheffield S10 2JF, Tel: 0114 2712400, Email: pst@sth.nhs.uk.

1. What is the purpose of the study?
You have recently participated in our study investigating whether measuring cervical impedance by a technique of spectroscopy can help us identify women with a high chance of premature birth. The purpose of this interview survey is to find out your experience of that assessment, and your views regarding how acceptable this measurement could be, if you found it useful and applicable, during antenatal care.

2. Why have I been chosen?
You have been randomly selected to be approached. We are interviewing about 20 women who have participated in our cervical spectroscopy studies to try to predict preterm birth, to find out about their experience of that assessment and their views about it.

3. Do I have to take part?
No. It is up to you to decide whether to take part or not. You are free to withdraw from the study at any time and without giving a reason. Whether you join the study or not will not affect your care at the hospital in any way.

4. What will happen to me if I take part?
We would ask you to consent to an interview. This would be a face to face interview or, where not convenient for you, by telephone. With your permission, the interview would be audio-taped and we anticipate that it may last for up to 30 minutes. The interview would take place at the Jessop Wing Hospital, Sheffield or, if more convenient another clinic you attend, or in your own home. We would reimburse travel expenses for attending this interview. You do not need to attend clinic more

often than usual. You would not need to have any extra physical examinations or investigations. You would not need to take any additional medicines.

5. What are the benefits of taking part?
You will not derive any benefits from taking part in the study. However the findings of this study may enable better care of other women who are having a baby in the future.

6. Will this test harm me or my baby in any way?
There should be no risks for taking part in this interview.

7. What if I am harmed?
If you are harmed by your participation in this study, there are no special compensation arrangements. If you are harmed due to someone's negligence, then you may have grounds for legal action.

8. What will happen with the results of this study?
We will analyse the data to see if the results can be used to care for patients in the future. The probe may then be modified, and made on a commercial scale that will be available to health care institutions. We may also publish our results in medical journals and share our findings at relevant conferences. You will not be identified in any report/publication. If you would like a copy of the research report we will send this to you.

9. Will my taking part in the study be kept confidential?
Yes. All information that is collected about you will be kept strictly confidential. The data that we obtain from you in relation to this study is kept anonymised so that no one can trace the information to any individual study participant. When the results are published, no names will be used, and it will not be possible to identify anyone who has taken part

10. Will anyone else be told about my participation in the study?
No. However, if you wish us to, we will inform your GP that you are helping with this study.

11. What will happen if I do not want to carry on with the study?
You will receive the same quality of clinical care even if you withdraw from the study.

12. Who has reviewed the study?
This study was given a favourable ethical opinion for conduct in the NHS by the Yorkshire Research Ethics Committee.

13. Who is organizing and funding the research?
Professor Dilly Anumba (Consultant Obstetrician and Gynaecologist) is organizing the research within the Obstetric, Gynaecology & Neonatology Directorate at Sheffield Teaching Hospitals NHS Foundation Trust. The study is funded from the Medical Research Council.

14. What if I wish to complain about the way in which this study has been conducted?

If you have any cause to complain about any aspect of the way in which you have been approached or treated during the course of this study, the normal National Health Service complaints mechanisms are available to you.

If you have any complaints, queries or concerns you may contact either:
Chief Investigator: Professor Dilly Anumba Consultant Obstetrician and Gynaecologist, Jessop Wing, Tree Root Walk, Sheffield S10 2SF on 0114 226 1075
OR
Dr David Throssell, Medical Director, STH NHS Foundation Trust, 8 Beech Hill Road Sheffield S10 2SB on 0114 271 2786.

PLEASE FEEL FREE TO CONTACT Professor Dilly Anumba (0114 2261075) if you have any further questions.

Sheffield Teaching Hospitals NHS
NHS Trust

Study Number: STH 16102
Patient Identification Number for this study:

CONSENT FORM

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study. Acceptability of cervical impedance measurements in clinical care.

Researchers:

Professor Dilly OC Anumba	Consultant in Obstetrics and Fetomaternal Medicine
Dr Georgina Jones	Reader in Social Science

Please initial box

1. I confirm that I have read and understand the information sheet Version 1 dated 21/04/2013 for the above study and have had the opportunity to ask questions. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I agree to take part in the above study ☐
4. I consent to audio recording of the interview ☐
5. I understand that all the information that is collected will be kept strictly confidential. ☐
6. I understand that relevant sections of data collected during the study may be looked at by individuals from Sheffield University, from regulatory authorities or from the NHS trust, where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records. ☐

Name of Patient	Date	Signature
Name of Person taking consent	Date	Signature

1 for patient; 1 for researcher; 1 to be kept with hospital notes

APPENDIX C

Predicting the risk of premature birth by cervical spectroscopy – the ECCLIPPx Study.

Standard operational procedure for high level disinfection of EIS probe

The electrical impedance spectroscopy tip is a semi-critical device as it comes into contact with intact mucous membranes. However the nature of its design mean that it cannot be subjected to normal sterilisation procedures due to electronic components within the tip. At present the probe does not work with covers (such as those used for trans-vaginal ultrasound probes) although work is underway to remedy this. In the meantime the spectroscopy tips require high level disinfection between each use.

General points regarding use of high level disinfectants:

All chemical disinfectants must be clearly labelled and used within the expiry date. They should be freshly prepared. They must be used at the correct concentration and stored in an appropriate container.

Chemical disinfectant solutions must not be mixed or detergents added unless they are compatible.

Disinfectant or detergent solutions must not be prepared and stored in multi-use containers for occasional use. Solutions prepared and stored in this manner may easily become contaminated with micro-organisms; using such solutions will therefore readily contaminate a surface rather than clean it.

Alcohol does not penetrate well into organic matter it should therefore only be used on surfaces that are physically clean.

Procedure:

1. Perform hand hygiene and don appropriate personal protective equipment (gloves and apron plus eye protection if making up disinfectant solution).
2. Remove tip from spectroscopy device. Clean body of device with Distel® wipe and leave to dry.
3. The tip must be cleaned thoroughly before high level disinfection. Clean immediately to prevent drying of organic material on tip.
4. Clean with Distel® wipe and rinse under running water, checking all visible debris is removed.
5. Change gloves.
6. Make up Tristel solution according to version used:
    a. Tristel® “Fuse for Instruments” solution as per sachet instructions:
        - Squeeze sachet so that base and activator are mixed – the solution will change to yellow colour. Mix for 30 seconds (see diagram below).

- Add to 5 litres of cold water (in large polypropylene container).
- Decant some of the solution into the smaller (cylindrical) polypropylene container then soak spectroscopy tip in Fuse solution for 5 minutes. The tip should not be completely submerged due to the delicate gold connectors at the proximal end – submerge to the highest level possible without getting these wet.

Take one sachet to produce five litres of chlorine dioxide solution. Fold in half and squeeze one side of sachet to burst contents through centre seal. Contents will start to turn yellow. Allow 30 seconds mixing time.

Tear or cut the corner of sachet. Take care when opening the sachet not to spill the concentrated solution.

b. Alternatively, if using large bottles of Tristel One Day Concentrate for Labs make up solution according to manufacturer's instructions

- If using large bottles of base and activator: to make 100ml of solution mix 2ml base with 2ml activator in sterile pot, mix for 30s then add 96ml of sterile water (volumes can be altered to make different amounts of solution).
- Decant the solution into the cylindrical polypropylene container then soak spectroscopy tip in solution for 5 minutes. The tip should not be completely submerged due to the delicate gold connectors at the proximal end – submerge to the highest level possible without getting these wet.

7. After 5 minutes (use timer), remove tip from solution, rinse thoroughly with sterile water and dry with gauze.
8. The used disinfectant can be disposed of via drain (tristel decomposes to normal saline), but flush with plenty of running water.
9. Remaining unused solution can be used for further patients on same list (i.e. within same session, but only remains active for 24 hours therefore discard if not used same day), but keep container covered to avoid contamination and discard at end of list.
10. Rinse, wash and dry containers after use.
11. Store spectroscopy device and tips in their case.
12. Complete decontamination log.

APPENDIX D

Local clinical protocols regarding management of **asymptomatic** women with abnormal conventional screening test results:

Local clinical protocols during the conduct of these studies were in line with national guidance at the time (specifically the Royal College of Obstetricians and Gynaecologists green-top guideline on cervical cerclage (No. 60) and the NICE guideline on preterm birth). A formal preterm birth clinic was not in place at the start of the study, but as awareness of the studies increased amongst local clinicians, patients were referred to discuss participation at earlier gestations, allowing greater standardisation of prophylactic treatment.

Recruitment and data collection took place between 2013 and 2016. Published data regarding the use of supplemental progesterone as PTB prophylaxis was (and continues to be) conflicting. In addition national guidance, in the form of the NICE Preterm Birth guideline was released halfway through the study in 2015. This meant that progesterone prophylaxis was not widely used by all local clinicians at the start of the study, but prescribing increased somewhat following the publication of the NICE guidance. Therefore some patients had already been commenced on progesterone prior to recruitment, whereas others had not. On review in research clinic, if prophylaxis was felt to be indicated prior to obtaining screening results, the decision for treatment was discussed and agreed with the Principal Investigator (following the protocol below). If progesterone was used, this was almost universally in the form of cyclogest pessaries at a dose of 200mg once daily. One high risk recruit was prescribed a higher dose of 400mg twice daily as this had been successfully used in her previous pregnancy and already commenced by her subfertility consultant and she was unwilling to amend her treatment. Another high risk recruit was seen at a different regional PTB clinic for consideration of transabdominal cerclage and commenced on 17-OH progesterone caproate, which continued through her pregnancy. All other patients received natural progesterone.

For those patients undergoing cervical cerclage, the overwhelming majority underwent Macdonald cerclage, most commonly utilising Mersilene© tape, although choice of suture material was not standardised and some women had a monofilament suture. Two patients underwent high Shirodkar cerclage.

Overall, management of high and low risk women proceeded as follows:

Women with prior PTB/late miscarriage

- Women with ≥3 prior spontaneous PTBs or a history of requiring an ultrasound-indicated cerclage in a previous pregnancy were offered history-indicated cervical cerclage at 12-14 weeks.
- Women with a prior spontaneous miscarriage >16 weeks or prior spPTB <34 weeks who did not qualify for elective cervical cerclage were offered serial cervical length screening with scans at ~fortnightly intervals from 14-16 to 24-26 weeks.
- Women with prior PTB >34 weeks did not routinely attend for additional CL scans outwith their research appointments at 20-22 and 26-28 weeks.
- Supplemental progesterone use was considered for women with prior PTB <34 weeks and recommended for those with a cervical length <25mm before 24 weeks.
- Cervical cerclage was offered to women with a cervical length <25mm before 24 weeks, particularly in those with progressive shortening and/or funnelling.

Women with no prior PTB/late miscarriage

- Women without prior PTB/late miscarriage were not routinely offered CL screening outwith their research appointment.
- Women with a prior history of LLETZ ≥10mm deep, or of unknown depth, or with a history of multiple LLETZ or ≥1 cold knife cone were offered serial cervical length screening with scans at ~fortnightly intervals from 14-16 to 24-26 weeks.
- Supplemental progesterone use was considered for those with a cervical length <25mm before 24 weeks and recommended for those with a cervical length <20mm.
- Cervical cerclage was considered for those with a cervical length <20mm before 24 weeks particularly in those with progressive shortening and/or funnelling or with a cervical length <15mm. The procedural risks and lack of clear evidence of benefit in women without a history of PTB was discussed with women prior to cerclage.

APPENDIX E

Local clinical protocols regarding management of **symptomatic** women with abnormal conventional screening test results:

Local clinical protocols during the conduct of these studies were in line with national guidance at the time (specifically the RCOG green-top guidelines on antenatal corticosteroids to reduce neonatal morbidity (No. 7); Preterm labour, tocolytic drugs (No. 1b) and Preterm pre-labour rupture of membranes (No.44)). Recruitment and data collection took place between 2013 and 2016. The NICE Preterm Birth guideline was also released halfway through the study in 2015 although it was not adopted in its entirety for use in the Jessop Wing.

Women with symptoms suggestive of threatened preterm labour were reviewed on labour ward or in the triage unit. Actim Partus swabs (detecting phosphorylated insulin-like growth factor binding protein 1) were commonly used to aid clinical assessment. For women participating in the symptomatic ECCLIPPx cohort, quantitative fibronectin swabs were performed instead of Actim Partus, plus an additional cervical length scan (not routinely used in the assessment of women with PTL symptoms in the Jessop Wing). Management proceeded as follows:

Women with negative Actim Partus/FFN swabs and normal cervical length:

- If no significant ongoing symptoms, reassured and discharged with advice to reattend if symptoms recurred or new concerns developed.

Women with positive Actim Partus/FFN and/or cervical length <15mm:

- Admitted to antenatal ward/labour ward depending on severity of symptoms.
- Given 2 doses of intramuscular steroids (12mg betamethasone 24 hours apart) if <35 weeks gestation.
- If persistent ongoing uterine activity, intravenous tocolysis with Atosiban administered using standard loading and maintenance doses to facilitate completion of steroid course (following discussion with consultant on call).
- Neonatal unit informed and if no capacity, consideration given to in-utero transfer to another unit with capacity (not commonly undertaken as the Jessop Wing has a Level 3 neonatal unit and acts as the regional referral centre).
- Magnesium sulphate for neuroprotection was not used during the study period.
- If steroids already received antenatally, consideration was given to a repeat courseif the initial course was >4 weeks previously.

Women with one normal and one abnormal predictive test (e.g. CL>15mm but positive FFN or CL<15mm but negative FFN):

- This was only relevant for women participating in the ECCLIPPx cohort
- Individualised risk assessment performed and discussed with study Principal Investigator/Consultant on Call as appropriate.
- If otherwise low risk (i.e. no prior history of PTB/late miscarriage, no other significant risk factors or symptoms of concern) then steroids administered as per protocol, but higher thresholds for tocolysis and transfer out applied.
- Level of FFN also considered, with lower threshold for tocolysis/transfer in women with levels>200ng/ml if symptoms persisted.

APPENDIX F

Local clinical protocols regarding management of abnormal microbiological swab results:

All high vaginal swabs taken were recorded contemporaneously in a log in the study file. Results were checked a minimum of alternate days and patients were contacted with any abnormal results directly (this was either via phone, text, email or letter depending on their preferences expressed during the study visit.

The microbiology department at the Royal Hallamshire has an automated system which flags up any positive group B *Streptococcus*(GBS) results detected in pregnant patients: labour ward reception are notified (who also hold all notes for antenatal patients) and highlight the positive swab in the patient's medical records to ensure staff caring for the patient intrapartum are aware and can check appropriate prophylaxis has been discussed. Women in the study who were found to be GBS positive were contacted by phone to ensure they understood the result and given additional information in the form of the Royal College of Obstetricians and Gynaecologists (RCOG) leaflet. Intrapartum antibiotic prophylaxis was offered in accordance with RCOG Greentop Guideline 36. Antenatal treatment was not recommended unless women had a concurrent positive mid stream urine culture (only sent if clinical indication to do so).

Patients with positive swabs for *candida albicans* were offered treatment with clotrimazole pessary and cream.

Patients with bacterial vaginosis were treated with either oral metronidazole (400mg twice daily for 7 days) or clindamycin pessaries (2% cream, once daily for 7 days) as per local and British Society of Sexual Health and HIV (BASHH) guidelines.

Patients with positive swabs for *ureaplasma urealyticum* were not treated but were informed of the result and it's possible association with preterm labour to emphasize the need to present promptly if any concerns arose. A paediatric alert was completed to highlight the result to the neonatal team in view of the association with pneumonia.

The only other organisms detected in our cohort were solitary instances of Group G *Streptococcus* and *Staphylococcus aureus.*These patients were treated according to symptoms and antibiotic sensitivities.

APPENDIX G

Acceptability Questionnaire – Part A

Anxiety experienced before the tests

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the most appropriate number to the right of the statement to indicate how you feel at the moment. There are no right or wrong answers, do not spent too much time on any one statement, just pick the number which describes your feelings best.

	Not at all	Moderately	Somewhat	Very much
I feel calm	1	2	3	4
I am tense	1	2	3	4
I feel upset	1	2	3	4
I am relaxed	1	2	3	4
I feel content	1	2	3	4
I am worried	1	2	3	4

You will be given a questionnaire to complete afterwards (if you are happy to) with some additional questions.

Acceptability Questionnaire – Part B

Thank you very much for your help with the ECCLIPPx Study.

As well as assessing how well spectroscopy measurements predict preterm birth, we also want to assess how acceptable the test is to pregnant women. It would be very helpful if you could complete the following short questionnaire. You can fill it in before you leave clinic and hand it in at reception, or you can post it back to us in the envelope provided.

Anxiety experienced after the tests:

A number of statements which people have used to describe themselves are given below. Read each statement and then circle the most appropriate number to the right of the statement to indicate how you felt **after** having the tests performed.

	Not at all	Moderately	Somewhat	Very much
I feel calm	1	2	3	4
I am tense	1	2	3	4
I feel upset	1	2	3	4
I am relaxed	1	2	3	4
I feel content	1	2	3	4
I am worried	1	2	3	4

Comments:

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…….....

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…….....…….....

.....

Discomfort/pain associated with the tests:

Please mark an X on the line to indicate any pain you may have experienced during the tests:

Please tick the description which best suits any pain/discomfort you experienced during the tests:

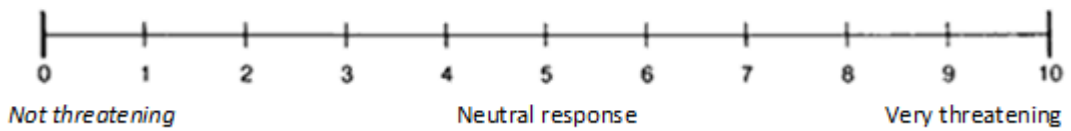
0	NO PAIN	_____
1	MILD	_____
2	DISCOMFORTING	_____
3	DISTRESSING	_____
4	HORRIBLE	_____
5	EXCRUCIATING	_____

The following works describe 11 qualities or characteristics that pain can have.
Please provide a rating for each pain quality to describe what you felt during the study tests .
(e.g. tick zero for “no throbbing pain”, or three for “severe throbbing pain”):

	NONE	MILD	MODERATE	SEVERE
THROBBING	0) _____	1) _____	2) _____	3) _____
SHOOTING	0) _____	1) _____	2) _____	3) _____
STABBING	0) _____	1) _____	2) _____	3) _____
SHARP	0) _____	1) _____	2) _____	3) _____
CRAMPING	0) _____	1) _____	2) _____	3) _____
GNAWING	0) _____	1) _____	2) _____	3) _____
HOT-BURNING	0) _____	1) _____	2) _____	3) _____
ACHING	0) _____	1) _____	2) _____	3) _____
HEAVY	0) _____	1) _____	2) _____	3) _____
TENDER	0) _____	1) _____	2) _____	3) _____
SPLITTING	0) _____	1) _____	2) _____	3) _____
TIRING-EXHAUSTING	0) _____	1) _____	2) _____	3) _____
SICKENING	0) _____	1) _____	2) _____	3) _____
FEARFUL	0) _____	1) _____	2) _____	3) _____
PUNISHING-CRUEL	0) _____	1) _____	2) _____	3) _____

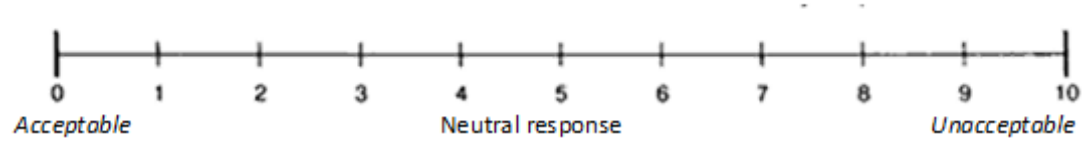
Design of device:

Please circle a number from one (Not threatening) to ten (very threatening)



How would you rate the procedure?

Please circle a number from one (Acceptable) to ten (Unacceptable)



b) If you found the procedure unacceptable please comment on why:

.....

.....

.....

.....

c) Which of the following could be changed to improve the procedure?

□ The length of the procedure □ The description of the procedure

□ The need for a speculum examination to take the readings □ No change needed

□ Other (please specify)

……

…….....

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……..

.....

<u>Do you think the procedure would be acceptable for use in antenatal care?</u>

□ YES □ NO

<u>Please comment on any other issues that you experienced during the procedure:</u>

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APPENDIX H

Triangulation Table for Mixed Methods Analysis of Acceptability Data:

Quantitative Domains		**Triangulation Results (comparing QUANT with QUAL)**	**Qualitative Themes** **(help explain the *why*)**	**Sample Quotes**
Spielberger State Trait Anxiety Inventory				
• *Both HR and LRW showed significantly lower STAI scores after vs. before testing.* • *HRW demonstrated higher pre-visit STAI scores but also a larger mean reduction in STAI score than LRW* • *HRW were significantly calmer, less tense, more relaxed and less worried following screening.* • *LRW were significantly less tense and more content following testing*				
STAI-6 anxiety **Pre & post test**	I feel calm Pre-test HRW	Complementary	Interactions with clinical staff – explanation and bedside manner (theme 3.5.3 and 3.5.4)	*"I was put at ease to begin with, and explained to me what would happen and everything like that."* (Participant 13 HR, two MTLs, early miscarriage and three term births)
	I feel calm Pre-test LRW	Complementary	Intimate examinations as beneficial (theme 3.2.3)	*"I know that it's just for good things. So I'm not worried."* (Participant 1, LR, one term birth)
	I feel calm Post-test HRW	Complementary	Cycle of anxiety in subsequent pregnancy (theme 2.4.4) Emotional burden of previous obstetric trauma (theme 2.4.1)	*"…but then once I'd been I can sleep safe and sound again for a couple of weeks"* (Participant 5, HR, one term birth, one PTB) *"Yes, a lot calmer. I got more panicky before 20 weeks than I was normally. I was a lot more calmer, I wasn't as worried about things. So it helped out a lot with that."* (Participant 13 HR, two MTLs, early miscarriage and three term births)

			Reassurance of other screening tests (theme 2.2.1)	
	I feel calm Post-test LRW	Agreement	Reassurance of other screening tests (theme 2.2.1)	*"But having now completed that study, it's nice that that's almost something to be ticked off, not in their area of concern and has helped me feel a lot more calm and content about the pregnancy"* (Participant 2, LR, recurrent first trimester miscarriages, first ongoing pregnancy)
		Complementary	Fear and anxiety in pregnancy (theme 2.3.1) Reassurance of other screening tests (theme 2.2.1)	*"I had a personal experience. I had a bit of a fear, because my sister has got a problem with her cervix…* *"…But I was really, you know, really reassured me that actually I would be alright, you know, I'd not got similar symptoms as my sister"* (Participant 9, LR, first pregnancy)
	I am tense Pre- test HRW	Complementary	Cycle of anxiety in subsequent pregnancy (theme 2.4.2)	*"And then the day before I come in, apart from this time and last time, I had a really sleepless night because I'm thinking what is it going to show? What's it going to show? And I can find myself just being laid wide awake"* (Participant 5, HR, one term birth, one PTB)
	I am tense Pre test LRW	Complementary	Concerns re: safety of novel test (theme 2.1.2)	*"I was a little bit tense, I have to say I was a little bit, you know because it's research and someone's checking, I sort of felt that if you're taking part in something, you can't completely say that there isn't any risks. So that part of the research, I was anxious about that a little bit, but once I'd finished and sort of a couple of hours later, I wasn't feeling any different, I mean it was fine"* (Participant 7, LR, one term birth)
	I am tense Post test HRW	Silence	-	-
	I am tense	Silence	-	-

	Post test LRW			
	I feel upset Pre test HRW	Silence	-	-
	I feel upset Pre test LRW	Silence	-	-
	I feel upset Post test HRW	Complementary	Psychological impact of FFN results (theme 2.2.4)	*"I was a bit upset about it because as I say I wasn't expecting that to pick up abnormalities (positive FFN) but it was nothing that wasn't kind of dealt with…"* (Participant 14, HR, one PTB)
		Disagreement	Interactions with clinical staff – explanation (theme 3.5.3) Reassurance of other screening tests (theme 2.2.1)	*"I have never gone away thinking I have no idea what went on or I am really upset about this there has never been any upset or distress whenever I have left I have always felt better than I did when I came"* (Participant 17, HR, recurrent first trimester miscarriages, three PTBs, one MTL)
	I feel upset Post test LRW	Silence	-	-
	I am relaxed Pre test HRW	Complementary	Interactions with clinical staff – Bedside manner and rapport (theme 3.5.4)	*"(*The CRF*) has such a lovely bedside manner that she just makes me feel really relaxed"* (Participant 11, HR, one 23 week delivery and neonatal death)

	I am relaxed Pre test LRW	Agreement	Intimate examinations as normal (theme 3.2.2)	*"I knew I was having these tests and everything. I was quite relaxed about it anyway in advance; I wasn't worried about it at all…"* (Participant 20, LR, first pregnancy)
		Complementary	Interactions with clinical staff – Bedside manner and rapport (theme 3.5.4)	*"she made me feel really comfortable and relaxed"* (Participant 20, LR, first pregnancy)
	I am relaxed Post test HRW	Complementary	Reassurance of other screening tests (theme 2.2.1)	*"But then, once obviously you get that result (negative FFN) that's quite reassuring, and that's put my mind at ease for the next two weeks and I become really relaxed."* (Participant 5, HR, one term birth, one PTB)
		Agreement	Reassurance of other screening tests (theme 2.2.1)	*"everything's fine, it's positive and she's relaxed"* (Patient 15, HR, two PTBs)
		Complementary	Attitudes to knowledge in pregnancy - "It's good to know" (theme 3.3.3)	*"So that put me at ease as well, knowing that everything had been checked, and nothing was going wrong that they could tell, so that was good"* (Participant 13, HR, two MTLs, early miscarriage and three term births)
	I am relaxed Post test LRW	Complementary	Uncertainty re: impending physical experience (theme 2.1.1)	*"I think I expected it to be, you know the whole situation to be a bit uncomfortable, but it wasn't. It was, yes just quite at ease"* (Participant 9, LR, first pregnancy)
	I feel content Pre test HRW	Silence	-	-
	I feel content	Silence	-	-

	Pre test LRW			
	I feel content Post test HRW	Agreement	Reassurance of other screening tests (theme 2.2.1)	*"I: And then when you get the result, how do you feel?* *P: Happy and relaxed"* (Patient 15, HR, two PTBs)
		Agreement	Interactions with clinical staff – explanation (theme 3.5.3)	*"I have never gone away thinking I have no idea what went on or I am really upset about this there has never been any upset or distress whenever I have left I have always felt better than I did when I came"* (Participant 17, HR, recurrent first trimester miscarriages, three PTBs, one MTL)
		Complementary	Attitudes to knowledge in pregnancy - "It's good to know" (theme 3.3.3)	*"I remember when she told me about the results I was actually quite pleased, I was like 'oh this is actually nice', you know you feel like you're getting extra care, I mean it* *was nice. I wasn't expecting it before, but it was really nice later on when she told me that what they were measuring was actually a predictor and everything. And especially when you're sort of considered higher risk, it's actually nice to know I suppose to have that reassurance."* (Participant 18, HR, one MTL and early miscarriage, three term births)
	I feel content Post test LRW	Complementary	Impact of visual result of cervical length scan (theme 2.2.2) Interactions with clinical staff – explanation and bedside manner (theme 3.5.3 and 3.5.4)	*"I thought that were nice. I thought it was lovely to see the baby's feet and I were looking at it from a different point of view, I suppose, and I suppose you see a part of the body that you don't normally see. And she was explaining about the cervix, and I think that the way she did it was really good…* *"I thought it was really good, I'm pleased I got the chance to take part."* (Participant 9, LR, first pregnancy)

		Complementary	Reassurance of other screening tests (theme 2.2.1) Attitudes to knowledge in pregnancy - "It's good to know" (theme 3.3.3)	*"But having now completed that study, it's nice that that's almost something to be ticked off, not in their area of concern and has helped me feel a lot more calm and content about the pregnancy"* (Participant 2, LR, recurrent first trimester miscarriages, first ongoing pregnancy)
		Complementary	Psychological impact of cervical length scan (theme 2.2.3)	*"I: Ok, so how did you find getting the results…?* *P: No, when she said that on the screen everything is pretty good. So I was fine, I was feeling good about that. I'm not like waiting for the result, but when I see it, that was also good that everything is ok".* (Participant 1, LR, one term birth)
	I am worried Pre test HRW	Complementary	Emotional burden of previous obstetric trauma (theme 2.4.1) Attitudes to knowledge in pregnancy - "No one knew why" (theme 3.3.2) The vagina as a protected space (theme 3.2.1) Uncertainty re: impending physical experience (theme 2.1.1)	*"There is just anxiety related to the premature delivery and those kinds of things but, it's more... I guess it was more the worry, but for us there wasn't much explanation about why what had been happening was happening…* *"I did have a little bit of reservations about having it done, with it being internal at first. I thought what will that be like? And will it alter things..? I just think it's just the thought of being poked and prodded and if that would activate the baby, and if that might make the baby come on straight away…* *"It was like just a bit of anxiety really about what you know, what might go wrong…* *"I think that's because I'm quite an anxious person really when it comes to, well I felt very anxious at first, so I just wanted to make sure that I was doing the right thing really."*

			Cycle of anxiety in subsequent pregnancy (theme 2.4.4)	*"When you're waiting for the results, you feel really anxious. But, especially the first couple of times because every time I'd been coming in, it had been, I think I'd been on the shorter cervix scale prior…"* (All quotes Participant 17, HR, recurrent first trimester miscarriages, three PTBs, one MTL)
		Agreement	Cycle of anxiety in subsequent pregnancy (theme 2.4.4)	*"About 2 days before, she started getting anxieties and more anxious"* (Patient 15, HR, two PTBs)
	I am worried Pre test LRW	Complementary	Pre-existing knowledge of preterm birth (theme 3.3.1)	*"In a way it has created a wee bit of worry about that particular issue because it's not of premature birth, because it's not one that I had considered a concern from my perspective before and my history."* (Participant 2, LR, recurrent first trimester miscarriages, first ongoing pregnancy)
		Agreement	Pre-existing knowledge of preterm birth (theme 3.3.1)	*"I had a bit of a fear, because my sister has got a problem with her cervix, so she had twins early"* (Participant 9, LR, first pregnancy)
		Complementary	Intimate examination as beneficial (theme 3.2.3)	*"I know that it's just for good things. So I'm not worried."* (Participant 1, LR, one term birth)
		Disagreement	Concerns re: safety of novel test (theme 2.1.2)	*"I wasn't worried, but I was a little bit- It's still a risk, it's still, even though you're guaranteed 99%, there's always 1% of these going the opposite way"* (Participant 7, LR, one term birth)
	I am worried Post test HRW	Complementary	Psychological impact of CL scan (theme 2.2.3)	*"She's a bit worried today, only because the result (CL scan) was slightly changed, but she's otherwise, you know, she's fine"* (Patient 15, HR, two PTBs)
		Complementary	Psychological impact of FFN results (theme 2.2.4)	*"The first study visit I did have a slight increase in fibronectin result… which was a surprise and then a worry as well because obviously I didn't expect anything to be picked up on it.…* *"…obviously we found out that our result is this but had we had not done the study we would not have known anything about it so I wouldn't have that to think about and*

			Attitudes to knowledge in pregnancy - "It's good to know" (theme 3.3.3)	*worry about, but….. I did know about it, but that was a good thing really because it was getting checked out and if we were starting to find that there were issues then I was being seen by people who could sort things out…"* (Participant 14, HR, one PTB)
		Complementary	Attitudes to knowledge in pregnancy - "It's good to know" (theme 3.3.3) Psychological impact of CL scan (theme 2.2.3)	*"so I still feel worried that something's going to happen, that it's going to come early. But it's sort of reassured me, knowing what's going off down there* (a short cervix) *do you know what I mean?"* (Participant 19, HR, two PTB and one term birth)
	I am worried Post test LRW	Complementary	The vagina as a protected space (theme 3.2.1) Concerns re: safety of novel test (theme 2.1.2) Design of the EIS probe (theme 3.1)	*"But I just thought well whenever you have a vaginal examination you feel a bit uncomfortable afterwards, so had I not been pregnant, no it wouldn't have worried me at all. But obviously being pregnant, you get a lot more worried, because you don't want it to affect anything in your pregnancy. But that might be because I'm a bit anxious about that"* (Participant 6, LR, first pregnancy) *"I: But you didn't like the thought of it being connected?* *P: Maybe, but only because I wasn't aware of it. I didn't realise it would work like that, yes. Only because there's all these things on mobile phones, you don't know a lot of stuff do you about it.* *I: So you're worried about the effects and the possibility that it could have an effect that we don't know about?* *P: Well that sounds a bit bad, I suppose, but yes, maybe. But I'm sure it's fine".* (Participant 6, LR, first pregnancy)

		Complementary	Interactions with clinical staff – communication (theme 3.5.3) Fear and anxiety in pregnancy in general (theme 2.3.1)	*"She just rang me and had a chat, and said there was nothing to worry about obviously. It was just, 'you've got a bit of thrush'. She didn't leave a voicemail or anything like that so that was good because I have had them in life where they've done that with different things though. And it is, you do panic. So especially a first time mum, I think you do panic. Anything anyone says to you you're a bit like *panic sound*, whereas actually, she did just, it wasn't a bit issue obviously, but it was good the way that she rang to tell me."* (Participant 9, LR, first pregnancy)
Short Form McGill Pain Questionnaire				
• *Average scores were low, with a mean VAS score of 0.97 for HR and 1.01 for LR participants (p=0.94, Mann Whitney U), and a maximal score of 3.2 and 3.1 in each group respectively.* • *When the ordinal PPI scores are considered, two women in each group (10%) rated their pain intensity during EIS measurement as "discomforting". The remainder described either "no" or "mild" pain (35 and 55% of HRW and 45 and 45% of LRW* • *Women chose a broad range of descriptors, but the most commonly selected in both groups were "aching", "heavy" and "tender". Intensity ratings were almost exclusively 0 or 1 (no or mild pain), with only two scores of 2 (moderate pain) provided - one for the "tender" descriptor and the other for the "cramping" descriptor, by different low risk women.*				
VAS and PPI (continuous and nominal ratings of pain intensity)	HRW	Agreement	Unable to feel the measurements (theme 1.1.1) Positive descriptions of measurements (theme 1.1.4)	*"I didn't feel it at all"* (Participant 4, HR, one term birth, one PTB) *"It's not painful".* (Patient 15, HR, two PTBs) *"it's not painful in anyway, you are kind of aware its being done...and again mine was…. just because it was me (laughs) the wire was playing up so she had to do it twice to get a reading umm…but that actual bit of it isn't uncomfortable really."* (Participant 17, HR, recurrent first trimester miscarriages, three PTBs, one MTL)
		Complementary	Unusual sensation (theme 1.1.2)	*"not anything that was really awful, just I could feel something"* (Participant 11, HR, one 23 week delivery and neonatal death)

				"The most uncomfortable one is probably the newest one... *it's not a pain at all. It is literally just a sensation of pressure just for a few seconds, then you know, it's finished"* (Participant 5, HR, one term birth, one PTB)
		Disagreement	Pain/discomfort/negative descriptors (theme 1.1.3)	*"it sort of felt like I was getting an ID put in. There's a little pinch or a poke or something. But I think it's the way, I think she moved it or something. So it wasn't actually the instrument, it may have been the handling of the instrument. But I said 'Ah!' and I have a pretty high tolerance for pain"* (Participant 10, HR, one PTB, one term birth)
VAS and PPI (continuous and nominal ratings of pain intensity)	LRW	Agreement	Unable to feel the measurements (theme 1.1.1) Positive descriptions of measurements (theme 1.1.4)	*"it were fine, it didn't hurt. I couldn't really tell any difference"* (Participant 3, LR, one term birth) *"To be honest, I'm not sure, because the speculum was in, and I could feel the speculum, I can't say that I massively felt anything…. So it wasn't really uncomfortable I don't think, from what I can remember"* (Participant 6, LR, first pregnancy) *"I didn't feel anything."* (Participant 7, LR, one term birth) *"it didn't hurt or anything"* (Patient 16, LR, one term birth)
		Complementary	Uncertainty re: impending physical experience (theme 2.1.1) Unusual sensation (theme 1.1.2)	*"There was just a little bit of discomfort, but it was so minor… it was definitely far less of a feeling or a pain feeling than I had expected. I expected to feel more invasive. But not, it was fine from my perspective, and again so short lived that they didn't feel like I needed it to stop"* (Participant 2, LR, recurrent first trimester miscarriages, first ongoing pregnancy) *"Just a little bit uncomfortable, a bit weird"* (Participant 20, LR, first pregnancy)

Pain rating index	HRW	Complementary	Unusual sensation (theme 1.1.2) Pain/discomfort/negative descriptors (theme 1.1.3) Positive descriptions of measurements (theme 1.1.4)	*"it's like you can just feel a little bit of pressure, because it pulsates. But it's literally for a minor, it's just a matter of seconds…* *you can just feel that pulsation a little bit up, it just feels like it's up in your tummy. That's what it feels like (I: Does it?) Yes. But I wouldn't say that it's discomfort, it's more of a pressure. That's what it feels like, but it's light pressure.* *But it's just the fact that you know it's different to the other things. It's a different sensation".* (Participant 5, HR, one term birth, one PTB) *"It just felt like it poked, like a stabbing poke".* (Participant 10, HR, one PTB, one term birth) *"Well I was looking at the ratings of the pain, you know on the questionnaire? And I think that's the only one I could describe it as similar. Just something that is not a normal feeling, but just felt a bit like burning. But just for a split second and not really painful burning. Sorry it's probably a bad description.* *it was more like a very gently pressure and then hearing the beeps so ... yeah it wasn't uncomfortable"* (Participant 11, HR, one 23 week delivery and neonatal death) *"No pressure, just funny".* (Patient 15, HR, two PTBs) *"You can just feel a little bit of pressure and very minimally, it's not painful in anyway, you are kind of aware its being done"* (Participant 17, HR, recurrent first trimester miscarriages, three PTBs, one MTL)

Pain rating index	LRW	Complementary	Unusual sensation (theme 1.1.2)	*"Pressure, throbbing maybe, bit like, it's just like how can you describe it? You know when you pinch? Like that, just kind of like that. But not hard. It's just like a literally, like that. Nothing more than that I'd say"* *"It's like a bit of pressure I guess inside-it's like nothing I have ever felt before. It's kind of inside and up (laughs) but not painful just…just pressure, a strange kind of pressure which is not a normal feeling; you would not normally experience that"* (Participant 20, LR, first pregnancy)
Design of device				
Most participants (75%) rated the appearance of the EIS device as 1 or less (on a ten-point visual analogue scale where 0 = not threatening, 5 = neutral and 10 = very threatening). However, there was a wide range of scores, from 0 to 9. Average scores for HR and LRW were not significantly different: mean rating 1.3 for HRW (range 0-5) and 1.35 for LR (range 0-9) (p=0.98).. The two participants who rated the EIS device as most threatening (with scores of 6 and 9) were low risk women.				
VAS rating	HRW	Complementary	Other determinants of screening experience – the design of the EIS probe (theme 3.1)	*"That's the little pen thing?...* *You can hear the bleep for when it finishes, but half the time I didn't even know it was that, because it bleeps at the end."* (Participant 13, HR, two MTLs, early miscarriage and three term births) *"P: Just a bit space age, but it's no worse than the scan thing. It's quite intimidating. (I: Did you find the scan thing more intimidating?) P: Yes."* (Participant 4, HR, one term birth, one PTB) *"But then you have this like, it looks like a sword that makes you know, noise…* *Yes it could be visually more appealing. Like I, so every two weeks I have the ultrasound, that doesn't look scary, it just looks like a big plastic knob. But this is, it looks very futuristic."* (Participant 10, HR, one PTB, one term birth)

VAS rating	LRW	Complementary	Concerns re: safety of novel test (theme 2.1.2) Perspectives on intimate examination – Intimate examinations as normal (theme 3.2.2)	*"the last test does feel quite alien almost compared to other tests which were just standard smear tests or a standard scan… Yes I think it's because of the equipment more than anything. Most people are used to having swabs, used to yes, having the equipment of a transvaginal check, but it just feels like something odd to be near you *laughs*."* *"Yes, a buzz and then a, was it a beep? Yes, I suppose the noises are very harsh noise"* (Participant 2, LR, recurrent first trimester miscarriages, first ongoing pregnancy) *"I noticed that it connected to the internet, and didn't really realise that until after, which felt a bit strange having something connected to the internet whilst inside you, if that makes sense. I don't know, that was a bit weird. But just so close to your baby and everything, you know, you don't really want lots of frequencies and things like that, I suppose"* (Participant 6, LR, first pregnancy) *"I mean it looks a bit scary. It looks a bit like a robot probe thing, so not very, yes it looks really medical… I don't know, maybe it should be white or something. You know like maybe match the scan machines maybe. Because you know the scan thing, that it doesn't look particularly scary. You can see it's medical equipment but it doesn't look scary."* (Participant 8, LR, one term birth, previous colposcopy)
Overall Acceptability rating and assessment of acceptability for use in AN care				
• *For the VAS rating of acceptability there were no significant differences between study groups: mean rating for HRW 0.55 (range 0-3) and LRW 0.75 (range 0-5) (p=0.84).* • *On the questionnaire only two participants provided additional comments in the free text section regarding acceptability: one mentioned that her procedure was slightly prolonged due to issues with device connectivity, but did not find this unacceptable, the other stated that she wouldn't wish to undergo the test routinely but would be willing to do so if clinically indicated.* • *39/40 women indicated they felt no change to the EIS procedure was necessary, whilst one would have preferred it if the test could be performed without using a speculum* • *When asked if the procedure would be acceptable for use in antenatal care, there was universal agreement from all surveyed women, although the question did not specify in what context this use might occur (e.g. high risk vs universal screening).*				

Binary rating and VAS	HRW	Agreement	In favour of universal screening (theme 4.3.1) Attitudes to knowledge in pregnancy - "It's good to know" (theme 3.3.3)	*"But yes I think it's a really good idea to offer it."* (Participant 11, HR, one 23 week delivery and neonatal death) *"It's definitely...if this was available to people, or even for additional people for the study at least go and find out about it and make you r own decision and go and meet the team and be explained to and have those first preliminary checks done and things and see how they feel about it then before rolling it our completely. As somebody that potentially… if they roll this out to people antenatally it would just become normal, as normal as having smear tests, it's a really quick thing that could make such a difference."* (Participant 17, HR, recurrent first trimester miscarriages, three PTBs, one MTL) *"As somebody who's had a premature birth, I would do anything and everything, so yes I would do it. But if I didn't have a premature birth, I would want to know does it actually help determine if I may go into labour? You know what I'm saying, because like it's not that it's invasive, but if you, I could see how other people would not want to do it. Because I know a lot of my friends are like 'I don't want anything going on in there, I don't even want to have sex because you know that's where the baby's going to come out'. But I don't know, yes I think everybody should do it. I don't know, sorry. I'm just a survivor premature birth so I'm sort of for everything. But see if I'm trying to think like before all this happened, if somebody offered me this, would I say yes? And I would, yes, I guess I would. Because you know, more knowledge is better than no knowledge. So then yes. Yes and yes."* (Participant 10, HR, one PTB, one term birth)
	HRW	Complementary	Yes for high risk women (theme 4.3.2) Trade off - burden of tests (physical, practical)	*"I think for cases like mine where I have had a premature birth, then I think it would be very useful, if anything its reassurance for parents that things are being monitored"* (Participant 14, HR, one PTB) *"only the people that have experienced a premature"* (Patient 15, HR, two PTBs)

			& information gained (theme 4.3.4)	*"I think if people, because obviously I was in an at risk category from having colposcopy, and I think that if that had been offered to me the first time, rather than having all of the scans, probably I would have gone for that first, rather than the scans, because the scans are very invasive every two weeks, aren't they? That's what I have, every two weeks"* (Participant 8, LR, one term birth, previous colposcopy) *"you have to think about the costs and the benefits don't you?"* (Participant 14, HR, one PTB)
	HRW	Disagreement	LR women might be less accepting of screening (theme 4.3.3)	*"I: Do you think it would be beneficial you know for women who have never had a premature delivery, what do you think about those women?* *P: No I don't. My first pregnancy, it was normal, and I never, the thoughts of anything like this happening never entered my head.* *I: So if somebody offered you the test then, what do you think you would have said?* *P: I don't know. I think I would have probably said no I'm alright if I'm honest."* (Participant 4, HR, one term birth, one PTB) *"I think therewould probably be a bit more reservation about it, because just from speaking to my friends when I was considering it originally, they were like 'Ooh I don't think you should have it done, I think you should just leave things be'. So I think for a lot of women, the thought of being poked and prodded doesn't really appeal to them."* (Participant 5, HR, one term birth, one PTB) *"if it was offered routinely I don't know how many people would want to do it because it is a more invasive test, if that makes sense?"* (Participant 14, HR, one PTB)

Binary rating and VAS	LRW	Agreement	In favour of universal screening (theme 4.3.1) Attitudes to knowledge in pregnancy - "It's good to know" (theme 3.3.3)	*"Yes it can be because it's like nothing more to- it's just a scan and swab, just 15 minutes and it can be like a huge step in a, not education but you will know about your body more than you can know. Because if for example, me, if there's something wrong there, on normal scan you don't have to see that, so that's better for all women."* (Participant 1, LR, one term birth) *"Because obviously you know don't you? Like I was going to do this anyway because obviously it just helps anyway, but like if you think about it, you find out more. It's more to your benefit if anything because if there is anything wrong, you know then don't you? You can like, you can always get sorted out. So I think it's a right good idea."* (Participant 3, LR, one term birth) *"I: So do you think it could be brought in as part of the routine care?* *P: I believe they should, yes.* *I: Do you think if it had been your first pregnancy you would have taken part?* *P: Yes, yes. But I don't know if that's because of my background. I think my background (*nursing) *also takes a part to my decision making, so I would have yes."* (Participant 7, LR, one term birth) *"I: So you think it could be used in routine pregnancy?* *P: Yes I do, I think it's a good- I think as well with anybody, if there's any nasty's up there or any bugs or anything, I think it's good to treat things. I don't think it's good to leave undone. And also it's really informative, it was really informative I felt, and I felt really reassured after I'd had the test, yes. I felt really reassured that it's going to be ok because it was obviously people think it's about getting to 12 weeks, but nobody really*

				hears about the 20 weeks or what brings that on, so there's another side isn't there?" (Participant 9, LR, first pregnancy)
	LRW	Complementary	Yes for high risk women (theme 4.3.2) Falling through the gaps of antenatal care (theme 2.3.2) Trade off - burden of tests (physical, practical) & information gained (theme 4.3.4) Fear and anxiety in pregnancy in general (theme 2.3.1)	*"Yes, if they'd had a history, it's completely different. If you'd had a history then I would feel, I would feel that it's definitely worth it. "*(Participant 6, LR, first pregnancy) *"I think I was really, really scared, and I don't think necessarily I would, if it had not been offered to me, I don't think I'd have known where to go to get that, which I think if you actually, maybe if you did have family, I know they can't be linked and you can't be genetic or whatever, but if you did have a family history of something, maybe that test really reassured me, really reassured me, so yes. I think it's a good thing."* (Participant 9, LR, first pregnancy) *"Well I think if it was going to help, I don't think it would be a bad thing. I think women would do it if they thought it was, if it was going to give an indication of like premature birth and then there's something that could be done to help about it, I'm sure a lot of women wouldn't mind having it done at all. It's just an extra test, so I think a lot of, well when you're pregnant you feel quite anxious and stuff anyway, particularly at the beginning. And anything you can do to feel less anxious, I think sometimes more, I don't know."* (Patient 16, LR, one term birth) *"I guess I don't know what you are getting from these test results but if you are getting really important information then definitely. I mean when you are having a baby you kind of expect… to ...you know be...they are going to see down there. Do you know what I mean? You can't be too private when you're having a baby. For me-yeah I would. I like to know everything is alright"* (Participant 20, LR, first pregnancy)

	LRW	Disagreement	LR women might be less accepting of screening (theme 4.3.3)	*"it might feel too much for the ordinary woman who wouldn't expect to have medical intervention as part of a normal pregnancy"* (Participant 2, LR, recurrent first trimester miscarriages, first ongoing pregnancy) *"it's different with each baby isn't it? Because I think if it was my first, I would probably have every single test because I was more nervous in my first pregnancy. But then obviously, then you've been through a pregnancy, so you think everything might be a bit less structured, if that's the right way to explain it".* (Participant 8, LR, one term birth, previous colposcopy) *"I'm not sure everybody would say yes for a routine test for that personally. I think some people will prefer not to be messed with if they don't have to be, and there's no history of any problems before."* (Participant 6, LR, first pregnancy) *"Whereas I suppose it may have been different if I hadn't had George just because if you've not been through that then I suppose people talk about going through cervical smear and that's always a bigger deal before you've had kids. And so would you be so willing to go for something like that if you didn't have to or whatever?"* (Participant 12, LR, one term birth)

APPENDIX I

Summary of Safety Log for Study:

Safety data was contemporaneously recorded in an electronic database and the study safety file kept on site in the Jessop Wing Research Office as per GCP requirements.

Minor side effects at the time of study visit (namely bleeding/discomfort) were recorded in the electronic case report forms and thus uploaded to the main study database.

As the study did not involve a therapeutic investigational medical product, the sponsor (Sheffield Teaching Hospitals) advised, via the Clinical Research Facility, that formal reporting was not necessary. However, adverse events and serious adverse events were recorded, monitored and discussed on a case by case basis with the study team and Principal Investigator as they occurred.

The overall rate of minor complications during study visits was 2.1%. 0.4% of patients experienced significant discomfort during measurements (although this predominantly appeared to be due to speculum examination rather than use of the EIS probe) and were unable to complete the study visit. 1.7% of patients experienced bleeding during measurements, although again this may have been due to speculum use, rather than specifically related to the EIS probe. In all cases bleeding was minimal and settled after examination.

Two stillbirths occurred during the study – one in a low risk participant, the other in a high risk participant. The low risk loss occurred at term, following a study visit at 20 weeks, with normal antenatal monitoring and no evident complications during the latter half of pregnancy. Postnatal investigations revealed no obvious cause. The high risk loss occurred following antenatal admission at 35 weeks with a history of premature prelabour rupture of membranes. The patient had an elective cerclage in situ (due to previous midtrimester loss and ultrasound indicated cerclage in a previous pregnancy) and initially declined removal. Ultimately emergent caesarean delivery was performed for concerns regarding fetal wellbeing, however the baby was delivered without signs of life and resuscitation was sadly unsuccessful. Post-morterm examination showed evidence of acute chorioamnionitis with evidence of fetal infection. Prior to attendance at 35 weeks, all antenatal monitoring (between the final study visit at 27 weeks and presentation) had been normal. Given the size of our cohort, this case rate is lower than national and local rates of stillbirth. After evaluation by the study team, neither case was felt to be related to study participation.

One mid-trimester loss occurred during the study, in a patient with prior history of two late miscarriages. Cervical length was 15mm at the initial visit at 20 weeks and ultrasound indicated macdonald cerclage was performed. Rupture of membranes occurred within 24 hours of cerclage and the stitch was removed. Delivery occurred three days later. Placental histology was suggestive of histological chorioamnionitis.

One neonatal death occurred during the study, of a baby born to a high risk participant at 23 weeks (after neonatal counselling the patient expressed a preference for active treatment if appropriate). The patient had a history of prior mid-trimester loss and attended an initial study visit at 20 weeks when cervical length was normal. However at follow up 2 weeks later (clinical follow up for cervical length monitoring, separate to the study visit schedule) significant shortening was noted. The patient was admitted for ultrasound-indicated cerclage, however this was ultimately not possible due to a combination of patient factors. She remained an inpatient and delivery occurred a week later. Neonatal death occurred at 20 days of age. Placental histology was normal.

No other instances of delivery within a month of asymptomatic assessment occurred in either high or low risk participants. Moreover, the rate of preterm birth observed in the symptomatic cohort was consistent with other published cohorts. Thus the study team felt these two events likely resulted from risks inherent to the patients, rather than relating to the study investigations. Careful monitoring of adverse event rates continued throughout the study.

Two high risk participants had admissions to intensive care following delivery, both due to major postpartum haemorrhages. Both required caesarean delivery and had prior risk factors for haemorrhage.

No maternal deaths occurred during the study.