The aetiology of sensory changes seen in women with pelvic floor dysfunction

A thesis submitted to the University of Manchester for the degree of Doctor of Philosophy in the Faculty of Biology, Medicine and Health

2019

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Word count = 53869

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Abstract

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24th June 2019   **Charlotte Kate Mahoney**

The pathophysiology of pelvic floor dysfunction is incompletely understood but involves injury to pudendal motor nerves during childbirth. Pelvic organ prolapse (POP), urinary and sexual dysfunction are associated with pudendal sensory nerve impairment. I hypothesised this sensory injury also occurs during childbirth and aimed to investigate the impact of childbirth on female genital sensation.

I included an evaluation of sensation of vaginal tone, by developinga protocol for quantitative sensory testing (QST) of stretch sensation for Aα and Aβ nerves at the vagina and introitus in 100 non-pregnant women with good reproducibility. Logistic regression found an association between age and sensation, and this was used to create nomograms.

Next, 150 pregnant women underwent vibration QST at the vagina and clitoris (Aβ nerves), stretch QST at the vagina and introitus (Aα and Aβ nerves), prolapse examination and symptom questionnaire. This was repeated at 8-12 weeks postnatal (PN1) and six months postnatal (PN2). Vibration sensation in pregnancy was reduced but improved postnatally. Stretch sensation was normal in pregnancy, deteriorated at PN1 but recovered by PN2. Vibration sensation in women delivered by caesarean section (CS) at PN1 showed greater improvement than following a normal (NVD) or instrumental delivery. By PN2 the NVD group were comparable to the CS group, but the same recovery was not evident in the instrumental group. There was a transient deterioration in stretch sensation at PN1 after a vaginal birth, with no difference at PN2 across mode of delivery. Postnatal pudendal sensorineuropathy was associated with objective POP, urinary, bowel and sexual dysfunction.

Finally, a pilot study was performed to evaluate temperature and vibration QST with neurohistology in 16 women with POP and three controls. Vaginal mucosa was immunostained for neural markers. There was no association, however this is likely to represent the small sample size in this exploratory study.

In conclusion, measurement of stretch sensation thresholds of the vaginal and introitus is valid and repeatable. Whilst it appears vaginal birth is associated with injury to Aα and Aβ nerves compared to CS, the greatest impact on Aβ nerves was pregnancy itself with all modes of delivery improving compared to AN values. Neurohistology of vaginal mucosa is feasible and can be correlated with clinical neurophysiology.

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List of abbreviations

Aa	Point on the anterior vaginal wall that is 3cms proximal to the hymen
Ap	The most distal point of the anterior vaginal wall
Ba	Point on the posterior vaginal wall that is 3cms proximal to the hymen
Bp	The most distal point of the posterior vaginal wall
AN	Antenatal
C	The cervix in relation to the hymen
CLRN	Comprehensive local research network
CNEMG	Concentric needle electromyography
CPT	Current perception thresholds
CS	Caesarean section
D	The uterosacral ligaments in relation to the hymen
ElCS	Elective caesarean section
EmCS	Emergency caesarean section
EMG	Electromyography
ePAQ-PF	Electronic Personal Assessment Questionnaire – Pelvic Floor
FD	Forceps delivery
FI	Faecal incontinence
FSD	Female sexual dysfunction
FSD-AD	Female sexual dysfunction arousal disorder
FSD-DD	Female sexual dysfunction desire disorder
FSD-OD	Female sexual dysfunction orgasmic disorder
FSD-PD	Female sexual dysfunction pain disorder
Gh	Genital hiatus
IUGA	International urogynaecology Association
ICS	International Continence Society
NCS	Nerve conduction studies
NHS	National Health Service
NVD	Normal vaginal delivery
Pb	Perineal body
PFD	Pelvic floor dysfunction
PN	Postnatal
PNTML	Pudendal nerve terminal motor latency
POP	Pelvic organ prolapse
QoL	Quality of Life
QST	Quantitative sensory testing
SFEMG	Single fibre electromyography

SSEP	Sensory evoked potentials
SUI	Stress urinary incontinence
TAH	Total abdominal hysterectomy
TAH & BSO	Total abdominal hysterectomy and bilateral salpingoophorectomy
TVL	Total vaginal length
UI	Urinary incontinence
VB	Vaginal birth
VH	Vaginal hysterectomy

Acknowledgements

Dedication

To my mum Chris for being both parents and more, and my sister Emily for your unwavering friendship and honesty. Your love and encouragement has made me who I am today.

To my late grandmother for always believing in me.

Finally to my wonderful husband and son, Uchenna and Isaac, thank you. You have endured more conversations about pelvic sensorineuropathy than any cardiologist should. You two are my world, and your wholehearted love and support has got me through.

This thesis belongs to you.

1 Introduction

The following chapter will discuss the aetiology of Pelvic floor dysfunction (PFD) and the neuroanatomy as it relates to sensory testing. It will critically evaluate the different sensory testing options and explore the evidence for sensory impairment in PFD. Finally, the chapter will discuss the evidence behind computer interviewing in women with PFD.

Pelvic floor dysfunction (PFD), defined as pelvic organ prolapse, urinary, bowel or sexual dysfunction, affects one third of all women in the UK.[1] The high prevalence, deleterious effects on quality of life and cost to the NHS in an ageing population make PFD an important public health issue.[2]

1.1 The Pelvic Floor

The pelvic floor contributes to urinary and faecal continence, sexual function and provides structural support to the urogenital and anal organs. To understand how pelvic floor function might be impaired it is important to appreciate the anatomy.

1.1.1 Anatomy of the Pelvic Floor

The pelvic floor is a complex three dimensional structure which includes the pelvic diaphragm, the perineal membrane (previously called the urogenital diaphragm[3]) and the superficial vaginal muscles.

The superior or cranial level of the pelvic floor is the pelvic diaphragm,. This contains the coccygeus muscle and a group of muscles called the levator ani. The levator ani is made up of the pubococcygeus, iliococcygeus and puborectalis muscles. The pubococcygeus muscle arises from the pubis anteriorly and attaches at the lower sacrum and coccyx posteriorly. The iliococcygeus muscle arises from the ischial spines and obturator fascia antero-laterally and attaches to the coccyx posteriorly. The puborectalis arises from the pubis and superior fascia of the perineal membrane forming a sling around the rectum. The coccygeus muscle originates from the ischial spines antero-laterally and attaches to the margins of the coccyx posteriorly. The urethra, vagina and rectum all penetrate the pelvic diaphragm.[4]

Moving caudally the middle level of the pelvic floor is the perineal membrane, Figure 1-II.[5] The external urethral sphincter, deep transverse perineal muscles and a caudal layer of fascia form the perineal membrane.[4] The membrane is pierced by the urethra and vagina. Superficially the urogenital triangle is formed of the bulbospongiousus, ischiocavernosus and superficial transverse perineal muscles. The anogenital triangle

consists of the external anal sphincter, the superficial portions of the levator ani, and the gluteus maximus.

Figure 1-I Image of the superior view of the pelvic diaphragm
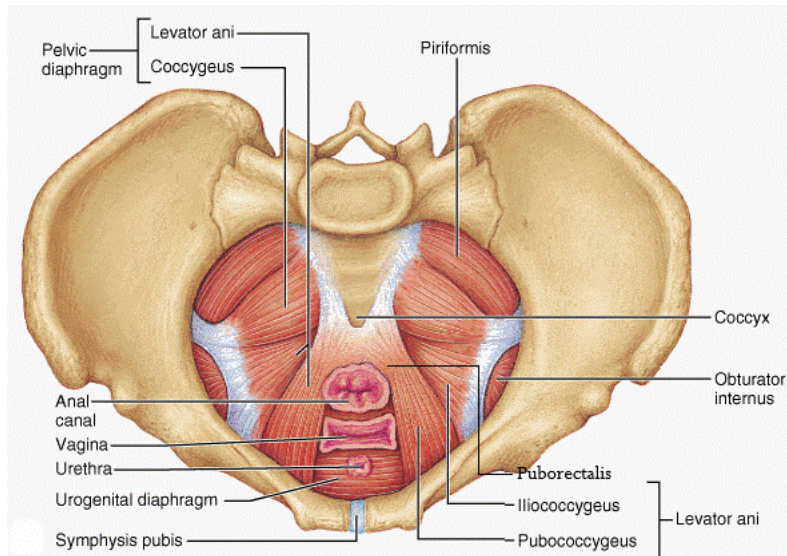
Marieb, Elaine N.; Hoehn, Katja N., Human Anatomy & Physiology, 10th Edition, © 2016. Printed and electronically reproduced by permission of Pearson Education, Inc., Upper Saddle River, NJ

Figure 1-II Image of the perineal membrane, urogenital and anal triangles
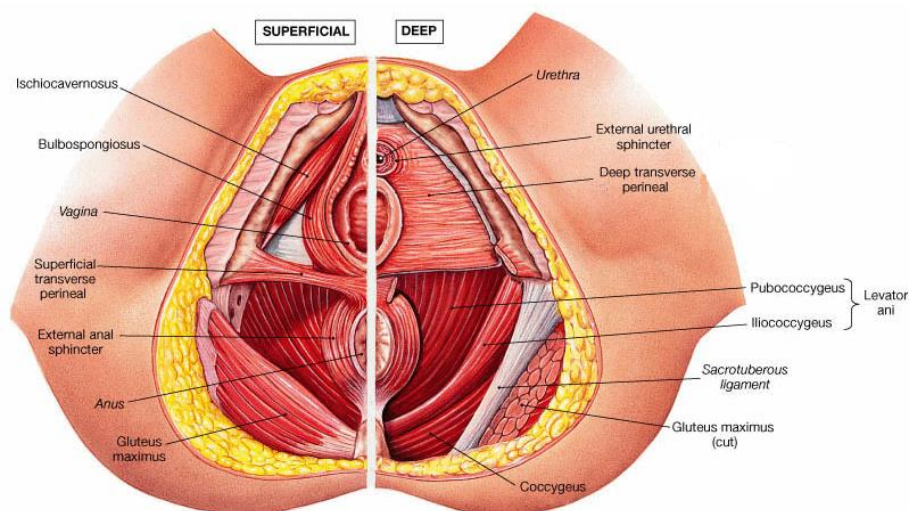
Martini, Frederich H.; Nath, Judi L.; Bartholomew, Edwin F., Fundamentals of anatomy and physiology, © 2012. Printed and electronically reproduced by permission of Pearson Education, Inc., Upper Saddle River, NJ

Figure 1-III Image of Delancey's three integrated levels of pelvic support

Cervix

Level I

Uterosacral/cardinal ligament complex

To arcus tendineus fasciae pelvis

Level II

To arcus tendineus rectovaginalis

Level III

Obturator foramen

External anal sphincter

Arcus tendineus rectovaginalis

Perineal membrane

Vagina

Urethra

Arcus tendineus fasciae pelvis

Arcus tendineus levator ani

Perineal membrane

Superficial transverse perineal muscle

Perineal body

External anal sphincter

CCF
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Illustration of the normal vaginal axis and the three levels of support of the vagina and uterus from the perspective of a standing woman. In level I, the endopelvic fascia suspends the upper vagina and cervix from the lateral pelvic walls. Fibres of level I extend both vertically and posteriorly toward the sacrum. In level II, the vagina is attached to the arcus tendineus fasciae pelvis and superior fascia of the levator ani muscles. In level III, the distal vagina is supported by the perineal membrane and muscles. The insets show transverse sections made through the vagina perpendicular to the normal vaginal axis at each level.

1.1.2   Neuroanatomy of the Pelvic Floor

When the pelvic floor is seen as a three dimensional structure it is possible to appreciate how muscles, fascia and nerves could be injured at any or all the levels during childbirth with descent of the fetal head through the vagina. The nerve supply is particularly important for motor and sensory function of the pelvic floor.

1.1.2.1   Somatic nerve supply

The pudendal nerve originates from the sacral nerve roots S2 to S4, exits the pelvis through the greater sciatic foramen, curves underneath the sacrospinous ligament, and re-enters the pelvis through the lesser sciatic foramen just medial to the ischial spine. The pudendal nerve then runs upward and forward alongside the ischioanal fossa, inside a space within the obturator internus fascia called the pudendal canal.[4]

Studies have reported variation in the division of the pudendal nerve into its branches depending on individual anatomy, although the extent of this variation in the population has not been extensively studied. [4,6–8] The first branch of the pudendal nerve is the inferior anal nerve which can separate before, within or on leaving the pudendal canal. It then divides into many smaller branches supplying the external anal sphincter and perianal skin.[4,6–8]

The perineal branch separates from the pudendal nerve after the pudendal canal. It traverses caudal to the perineal membrane and divides into numerous small branches supplying the superficial transverse perineal, bulbocavernosus and ischiocavernosus muscles, and labial skin.[6] A branch of the perineal nerve supplies the striated urethral sphincter muscle. This branch travels on the cranial and medial surface of the bulbocavernosus muscle, before plunging deeper into the tissues and innervating the urethral sphincter from a lateral position.[7]

The final branch of the pudendal nerve supplies the clitoris. This clitoral branch travels anteriorly towards the clitoris passing between the ischiocavernosus muscle and the perineal membrane, along the medial edge of the ischiopubic rami. It then navigates under or over the crus of the clitoris, moving towards the clitoral body surrounded by connective tissue.[6]

The posterior femoral cutaneous nerve originates from S1-4 nerve roots travelling alongside the pudendal nerve until it exits the pelvis through the greater sciatic foramen. It divides into the inferior cluneal and PFCN perineal branch.[9] Cutaneous branches supply the posterior skin of the thigh and leg, whilst the inferior cluneal branch supplies the inferior buttock and the perineal branch supplies the lateral perineum and postero-lateral aspect of the labium majora.[10] The perineal branch also communicates with the

inferior rectal nerve and it thought that this communication is why sensory impairment is not seen with pudendal nerve entrapment.[11]

The ilioinguinal nerve originates at L1 nerve root, exits the pelvis near the anterior iliac crest where it performates the transversus abdominus, supplies the internal oblique muscles and then travels along the round ligament, through the superficial inguinal ring. It supplies sensation to the upper and medial part of the thigh, and the skin covering the mons pubis and labia majora.

It is important to note for this study that neither the posterior cutaneous nerve nor ilioinguinal nerve provide somatic innervation to the vagina or clitoris.

Figure 1-IV Innervation of the vagina and clitoris by pudendal nerve

Fig. 160. Female Perinaeum.

Nat. Size.
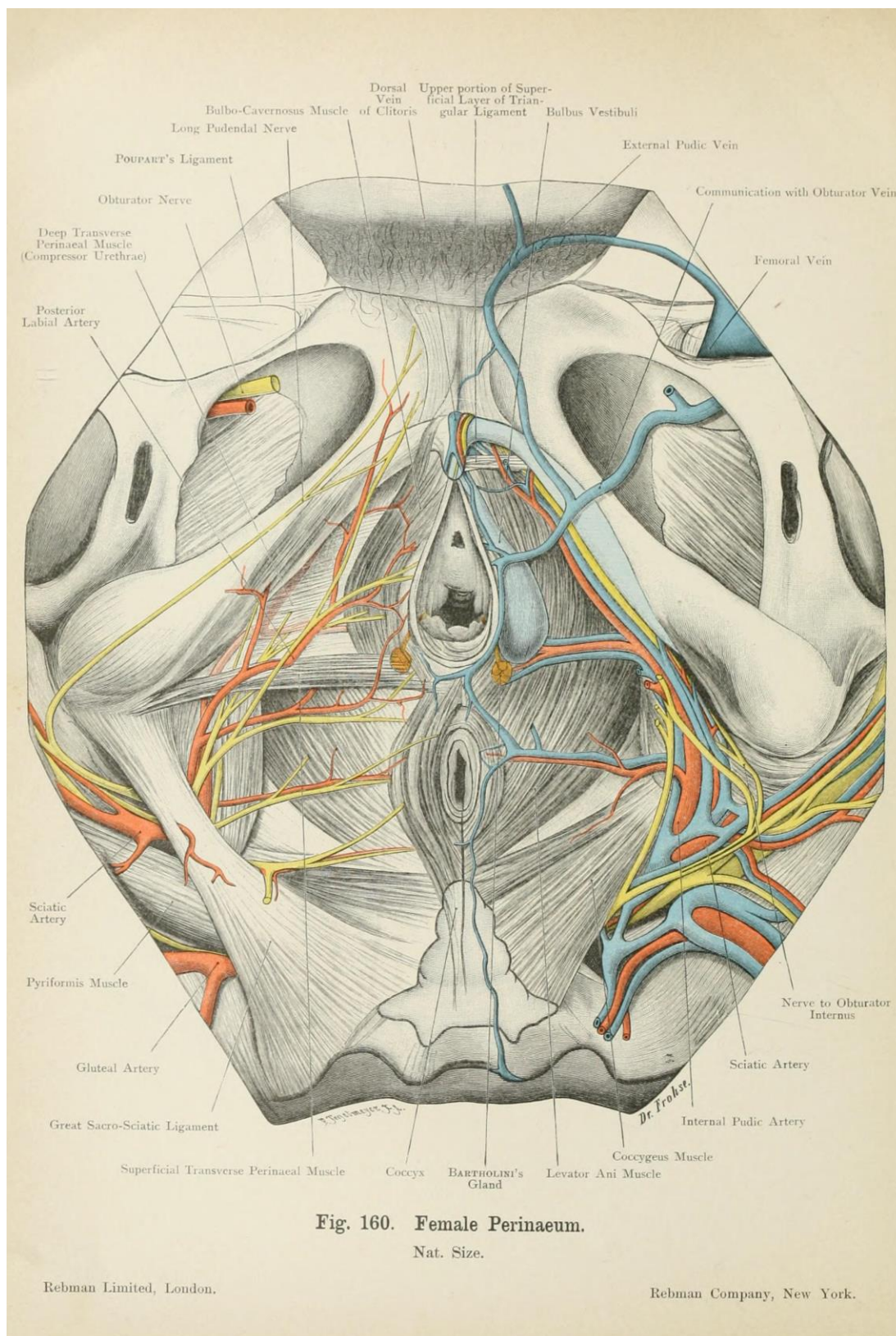
Open access image reproduced with permission from Wikipedia.com. Atlas of applied (topographical) human anatomy for students and practitioners (1906) (20156533258).jpg

1.1.2.2   Levator ani innervation

The levator ani nerve supplies the levator ani muscles of the pelvic diaphragm (see). The levator ani nerve arises directly from sacral nerve roots S3 to S5 and travels uninterrupted along the cranial surface of the pelvic floor to reach the levator ani muscles.[7]

In approximately half of the population the pudendal nerve also supplies the levator ani muscles along with the levator ani nerve.[12,13] This dual innervation could help account for the wide variation in symptoms of pelvic floor dysfunction described by women after childbirth.

1.1.2.3   Autonomic innervation

Autonomic innervation to the pelvic organs is supplied by the inferior hypogastric or pelvic plexus, a collection of sympathetic and parasympathetic nerves which supply the reproductive organs, urinary bladder and rectum.

Sympathetic nerve supply originates from the first thoracic vertebra spinal cord nerves and extends to the third lumber vertebra, these form the lumbar splanchnic nerves that synapse at the inferior mesenteric ganglion, travel along the hypogastric nerve and combine with pelvic splanchnic nerves within the pelvic plexus, Figure 1-V. The parasympathetic nerve supply begins with lower lumber spinal cord nerves via the hypogastric plexus and joins with sacral nerves from S2-4 at the pelvic plexus.

The pelvic plexus sits either side of the vagina and rectum and as such may also be affected during childbirth. [14]

Figure 1-VI Image showing the anatomical distribution of parasympathetic nerve supply
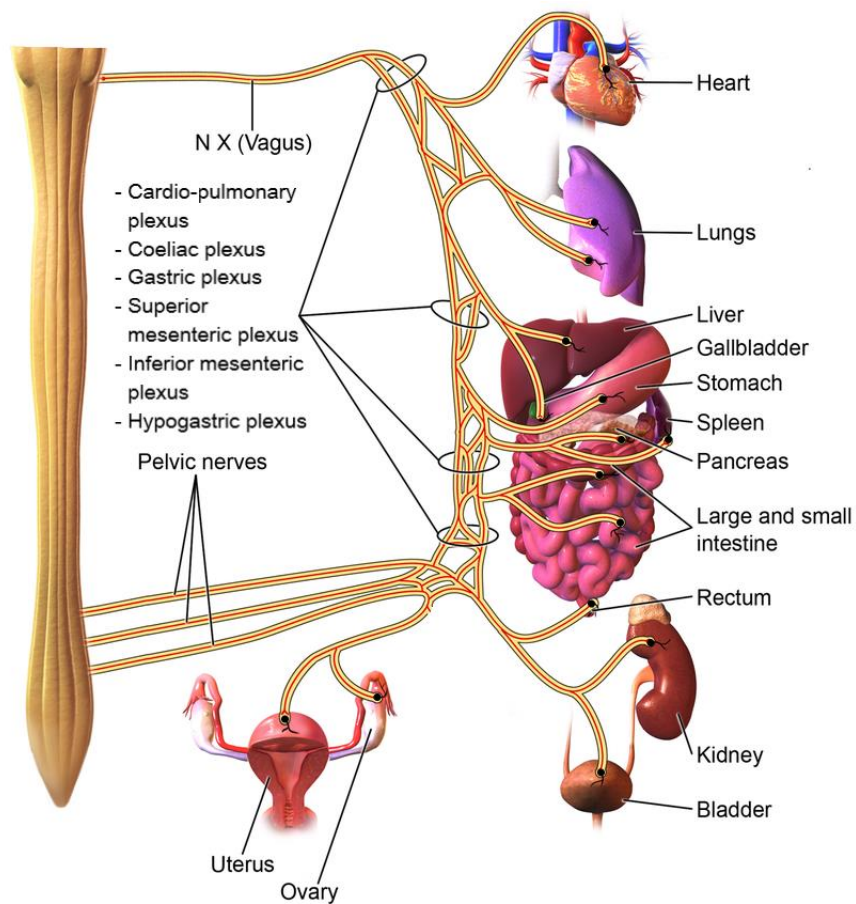
N X (Vagus)

- Cardio-pulmonary plexus
- Coeliac plexus
- Gastric plexus
- Superior mesenteric plexus
- Inferior mesenteric plexus
- Hypogastric plexus

Pelvic nerves

Heart

Lungs

Liver
Gallbladder
Stomach
Spleen
Pancreas

Large and small intestine

Rectum

Kidney

Bladder

Uterus
Ovary

Open access image edited and reproduced with permission from Blausen.com staff (2014). Medical gallery of Blausen Medical 2014. DOI:10.15347/wjm/2014.010. ISSN 2002-4436.

Figure 1-V Image of the anatomical distribution of sympathetic nerve supply
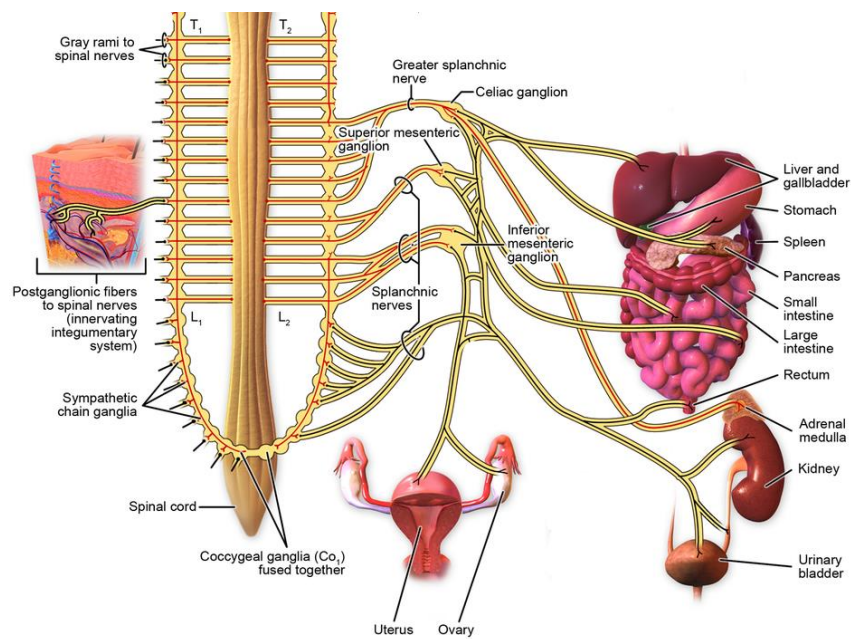
Open access image edited and reproduced permisison from Blausen.com staff (2014). Medical gallery of Blausen Medical 2014. DOI:10.15347/wjm/2014.010. ISSN 2002-4436.

Figure 1-VI Image showing the anatomical distribution of parasympathetic nerve supply
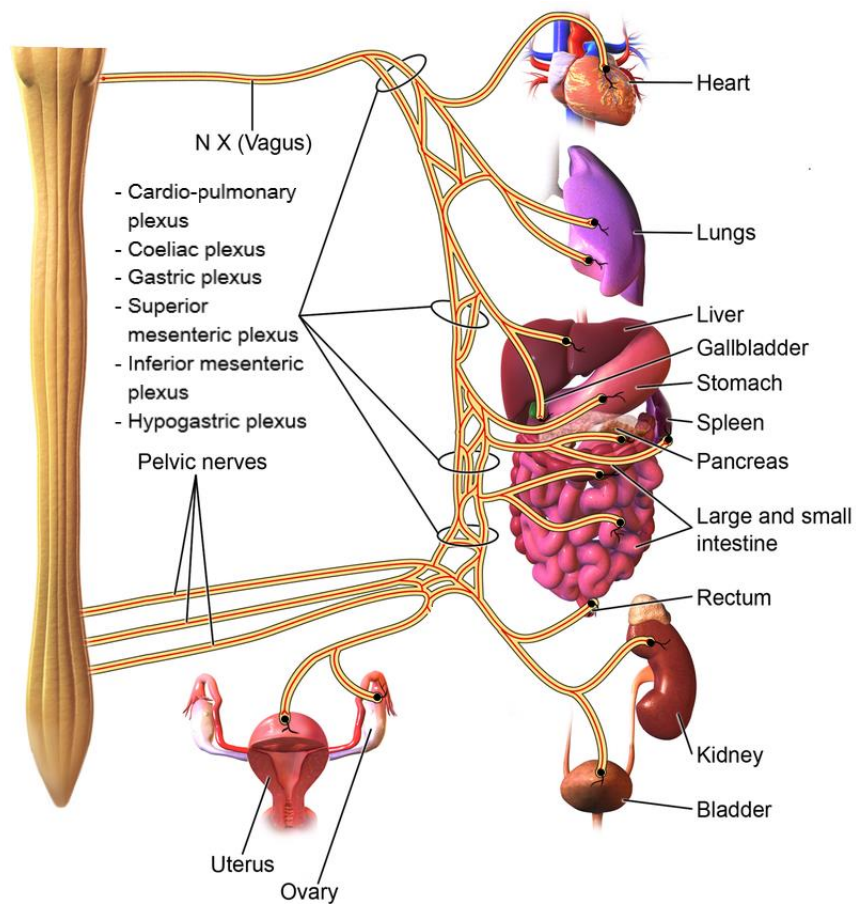
Open access image edited and reproduced with permission from Blausen.com staff (2014). Medical gallery of Blausen Medical 2014. DOI:10.15347/wjm/2014.010. ISSN 2002-4436.

1.1.2.4   Vaginal mucosa

In addition to injuring the pudendal and levator ani nerves during childbirth, the fetal head also distends the vagina which in turn could affect the nerves within the vaginal mucosa.

The vaginal mucosa consists of a layer of stratified squamous epithelium, followed by the lamina propria which contains a layer of loose connective tissue and a deeper layer of dense connective tissue. Beneath the vaginal mucosa are an outer ring of longitudinal smooth muscle and an inner ring of circular smooth muscle, called the vaginal muscularis. Underneath the vaginal muscularis is the adventitia, an inner layer of connective tissue and elastic fibres. The distal third of the vaginal wall is innervated by the pudendal nerve, whilst the proximal two third of the vagina are innervated by the uterovaginal nerve plexus.[15]

Figure 1-VII Histology of the anterior vaginal wall
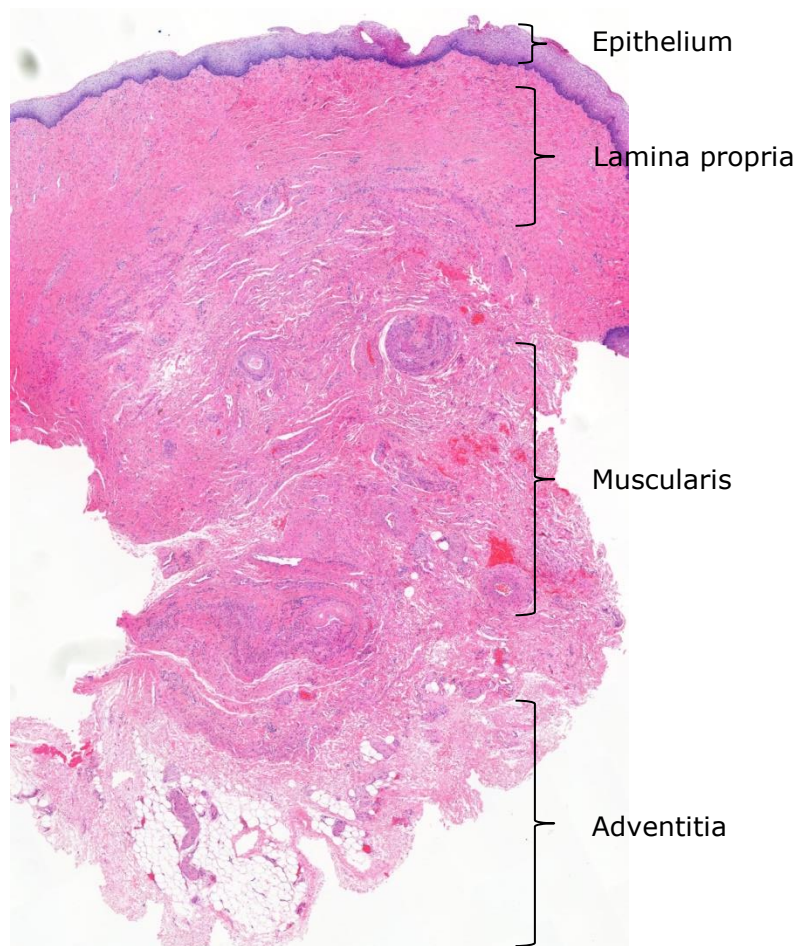
Haematoxylin and eosin stain of anterior vaginal wall at 5cm proximal to the urethral meatus taken during surgical repair of anterior wall prolapse.

To date, epithelial innervation has only been found to occur at the vaginal introitus region.[15–17] The lamina propria has been shown to contain a network of very small nerve fibre bundles, free nerve endings and in some women oval shaped mechanoreceptors called Merkel cells.[16,18,19] The muscularis layers have slightly larger nerve fibre bundles with fewer free nerve endings and Merkel cells.[15–17]

1.1.3   Pelvic floor dysfunction

At each level of the pelvic floor there are muscles, fascia and associated nerves which could be stretched or compressed beyond repair. Damage to these structures by any mechanism could lead to pelvic floor dysfunction.

Pelvic floor dysfunction (PFD) is a broad term encompassing the signs or symptoms of pelvic organ prolapse, urinary incontinence, bladder storage, sensory, voiding and post-micturition symptoms, lower urinary tract infections, lower urinary tract pain, sexual and anorectal dysfunction.[20] For the purposes of this project the term pelvic floor dysfunction will be used to describe pelvic organ prolapse (POP), urinary, bowel and sexual dysfunction (FSD).

1.1.3.1   The cost of pelvic floor dysfunction

Although it affects one in three women in the UK,[1] there is no UK data available to quantify the cost of PFD to the National Health Service (NHS). However during 1998 the United States spent an estimated $294 million treating urinary incontinence,[21] and $1012 million on surgery for POP.[22] Sung reported the estimated direct cost of ambulatory care for PFD to be $412 million in 2005-6. This is almost double the cost in 1996-7 when it was estimated at $262 million (*p=0.05,* $280-543 vs $204-321, 95% CI).[23]

Whilst these costs demonstrate the increasing scale of the problem they should not be directly extrapolated to the NHS. They are broad estimates based on a sample population with costs calculated according to hospital billing which includes organisation profit and Medicare costs, including an additional 38% inflation to account for patient paid deductibles. On top of this the United States spends approximately twice as much on health care as other developed countries.[24] The additional social cost of embarrassment, social isolation, relationship breakdown, employment limitations and loss of independence is also unquantifiable.

1.1.4 The aetiology of pelvic floor dysfunction

The increasing cost, ageing population and changing focus of the department of health towards disease prevention makes PFD a current and serious public health issue.[25] It is widely believed that PFD is caused by a combination of pelvic floor trauma during childbirth and age-related nerve and muscle degeneration.[26]

1.1.4.1 Motor nerve degeneration

Two main studies have reported motor nerve degeneration in women with POP and urinary incontinence, Table 1–A.

The pivotal work demonstrating nerve degeneration in PFD was performed by Smith et al in 1989. They performed single fibre electromyography (SFEMG) of the pubococcygeus muscle in 174 women with symptoms of stress urinary incontinence (SUI) or POP.[27]

SFEMG measures the ratio of nerves to muscle fibres. After injury or degeneration the same numbers of muscle fibres are supplied by fewer nerves and so this ratio is increased, [27-29] SFEMG therefore measures re-innervation of the muscle fibres and is considered evidence of partial denervation.

Smith et al showed that nerve degeneration increases with age in nulliparous and multiparous asymptomatic women, but age for age was greater in multiparous women. More importantly, they proved women with prolapse had greater evidence of nerve degeneration when compared age for age to women without prolapse.[27] The problem with SFEMG and therefore this study is that it misses permanently degenerated nerves which did not re-innervate and can lead to an underestimation of the degree of nerve injury.

In a second study within the same department, Smith et al performed motor nerve conduction studies (NCS) of the pubococcygeus, external urethral and external anal sphincter muscles. They found that women with symptoms of SUI, and POP ± SUI had prolonged nerve conduction times when compared to multiparous asymptomatic women at the external anal sphincter and pubococcygeus muscle.[30] At the urethral sphincter, women with symptoms of SUI ± POP also had prolonged conduction times when compared to multiparous asymptomatic women, whilst women solely with symptoms of POP showed no difference in conduction times.[30]

Table 1–A. Studies investigating pudendal motor nerve degeneration and pelvic floor dysfunction

Study	Comparison	Measure	Outcome
Smith et al [27] (n=174)	Young vs old SUI vs POP ±SUI	SFEMG (partial denervation)	*Young vs old* Nulliparous – linear regression coefficient 0.00670±0.00085, *p<0.00001* Multiparous - linear regression coefficient 0.00639±0.00161, *p=0.00077* Nulliparous vs multiparous - *p<0.0001* *POP ±SUI vs SUI* *p<0.00001*
Smith et al [30] (n=129)	SUI vs controls POP vs controls POP & SUI vs controls	NCS	*SUI vs controls* External anal sphincter -1.9±0.2 vs 1.6±0.2, *p=0.0007* Pubococcygeus – 2.1±0.3 vs 1.9±0.2,*p=0.0022* Urethral sphincter - 2.8±0.4 vs 2.1±0.3, *p=0.0001* *POP vs controls* External anal sphincter – 2.1±0.3 vs 1.6±0.2, *p=0.0004* Pubococcygeus – 2.8±0.7 vs 1.9±0.2, *p<0.002* Urethral sphincter - Not significant *POP & SUI vs controls* External anal sphincter - 1.9±0.3 vs 1.6±0.2, *p=0.0007* Pubococcygeus - 2.5±0.5 vs1.9±0.2, *p=0.00001* Urethral sphincter – 3.0±0.5 vs 2.1±0.3, *p=0.0001*

Key: SUI – stress urinary incontinence; POP – pelvic organ prolapse; SFEMG – single fibre electromyography; NCS – nerve conduction studies

1.1.4.2   Nerve fibre frequency

In addition to functional neurophysiology tests showing pudendal motor nerve impairment in women with PFD, three neurohistology studies have quantified vaginal nerves in women with POP and SUI, Table 1–B.

Other studies have described the neurohistology of the vagina in normal women only, or addressed the presence of individual neurotransmitters without providing an overall assessment of the frequency of nerve fibres.[15–18,31–33]

The studies used immunohistochemistry techniques to identify nerve fibres within the vaginal mucosa, with an antibody called protein gene product 9.5 (PGP 9.5) that is highly specific for neurons and neuroendocrine cells throughout the body. However, PGP 9.5 cannot distinguish between motor and sensory nerve fibres, and as yet there is no alternative stain which can. As a result, correlation with clinical neurophysiology findings would help to differentiate whether the reduction in nerve fibre frequency in POP involves motor nerves, sensory nerves or both. However, a systematic search of Medline and Embase did not reveal any studies evaluating motor or sensory nerve function and neurohistology.

The study by Inal et al evaluated vaginal tissue 3cm proximal to the urethral meatus and found a significant reduction in frequency and diameter of nerve fibres in women with POP compared to controls.[34]

The second study biopsied tissue from the vaginal apex at 1cm distal to the cervix, uterosacral, cardinal and round ligaments.[35] They also found a significant reduction in percentage of nerve fibres in women with POP compared to controls. However, it is not possible to extrapolate this data to the anterior vaginal wall, which may behave differently.

The third study by Zhu et al was limited by small group sizes, coexisting POP in the SUI group and the qualitative assessment of nerve fibres.[36] These limitations call into question the conclusions from this study.

Table 1–B Studies investigating the neurohistology of vaginal mucosa in pelvic organ prolapse

Study	Comparison	Biopsy site	Measure	Outcome
Inal et al[34] (n=89)	POP vs controls	3cm proximal to urethral meatus	Frequency of nerve fibres/µm Diameter of nerve fibres/µm	*Frequency* 3.95 vs 7.25, *p<0.001* *Diameter* 37.59 vs 53.19, *p<0.001*
Kaplan et al[35] (n=84)	POP vs controls	Vaginal apex 1cm distal to cervix Uterosacral ligament Round ligament Cardinal ligament	PGP 9.5 stained area (%)	*Vaginal apex* 4.30 vs 14.89, *p<0.001* *Uterosacral* 7.70 vs 10.35, *p=0.045* *Round* 6.27 vs 8.83, *p=0.049* *Cardinal* 6.80 vs 15.75, *p<0.001*
Zhu et al[36] (n=53)	POP alone vs SUI & POP vs controls	1cm lateral to urethral meatus	Presence of any PGP 9.5 staining	*Presence of any nerve fibres* 26% vs 60% vs 80%

Key: NF – nerve fibres; POP – pelvic organ prolapse; PGP 9.5 – protein gene product 9.5; SUI – stress urinary incontinence

1.1.4.3　Childbirth

The studies by Smith et al provided the main body of evidence for the role of denervation as part of the aetiology of POP and SUI, around the same time other researchers were investigating the impact of childbirth on PFD, Table 1–C.

In 1983 physicians thought post-partum faecal and urinary incontinence was the result of direct anal sphincter division or muscle stretch. Snooks et al were the first to challenge this thinking when they provided evidence that post-partum PFD was the result of nerve rather than muscle injury. They tested pudendal nerve terminal motor latency (PNTML) conduction times in women at two days and two months post-natal (PN). The group found that women who underwent a vaginal delivery (VD) had prolonged PNTML at the external anal sphincter compared to both controls and women who experienced a caesarean section (CS).[37] Interestingly, their results suggested CS might be protective against developing pudendal nerve injury and post-partum PFD.

However as a technique PNTML is controversial because it measures the fastest functioning nerve fibre within a nerve but does not assess the functional integrity of the rest of the nerve. If there is a single normally functioning nerve fibre the PNTML will still be normal, regardless of whether the rest of the nerve fibres are damaged. So as a test PNTML is unreliable as it can widely underestimate the degree of nerve injury. This makes it difficult to reliably interpret any study which uses PNTML as the main outcome measure.

In 1990 Allen et al evaluated motor nerve injury using concentric needle electromyography (EMG) and PNTML in 96 nulliparous women and found evidence of injury to the pelvic floor in 80% of women.[38] Concentric needle EMG was performed in the antenatal (AN) and PN period, whereas PNTML was only performed PN due to concerns about the risk of pre-term labour.[38] Concentric needle EMG measures motor nerve degeneration and reinnervation and is possibly a more accurate method of evaluating nerve injury than single fibre EMG or PNTML.

Concentric needle EMG measures the duration of a muscle fibre action potential which increases following injury when there is muscle reinnervation. Allen and her group demonstrated PN women had a prolonged action potential compared to AN women, proving motor nerve reinnervation had occurred.[38] They also found no difference in nerve injury between the NVD and forceps delivery groups. This analysis did not include a CS control group, as there were only three ElCS and five intrapartum EmCS.

However, the group found no significant difference in PNTML at two days and two months PN for the external anal sphincter, pubococcygeus or urethral sphincter. This

could be due to a falsely reassuring PNTML result, the lack of an AN control group, or the short follow up time which did not allow adequate time for nerve regeneration.

Sultan et al performed a prospective observational cohort study on 128 women in 1994. They found vaginal delivery led to prolonged PNTML conduction times in primiparous and multiparous women but not after elective caesarean section (ElCS). Interestingly, they reported asymmetrical prolonged conduction in women who underwent an emergency caesarean section (EmCS) whilst labouring.[39] Although the choice of PNTML makes it difficult to reliably interpret the data and the small EmCS group of nine increases the chances of a type II error.

Table 1−C. Studies investigating childbirth and pudendal motor nerve degeneration

Study	Comparison	Measure	Outcome
Snooks et al [37] (VD n=57, CS n=14, controls n=34)	VD vs CS vs controls	PNTML	*VD vs CS vs controls* 2.21ms ±0.33 vs 1.94 ±0.21 vs 1.98 ±0.21, *p<0.001*
Allen et al [38] (n=96)	AN vs PN	cnEMG PNTML	*AN vs PN* cnEMG 3.3 ±0.6ms vs 5.2 ±1.5ms, p<0.001 *VD vs Instrumental* cnEMG 5.5 ±1.2ms vs 5.5 ±2.0ms, *p=0.838* PNTML External anal sphincter, pubococcygeus and urethral sphincter Not significant
Sultan et al [39] (Primips n=57 Multips n=32 ElCS n=7)	AN vs PN	PNTML	*AN vs PN* Primips 1.91 ±0.19 vs 2.00 ±0.22, *p<0.001* Multips 1.96 ±0.21 vs 2.06 ±0.24, *p<0.01* ElCS Not significant

Key: VD – vaginal birth; CS – caesarean section; PNTML – pudendal nerve terminal motor latency; AN – antenatal; PN – postnatal;

cnEMG – concentric needle electromyography; ElCS – elective caesarean section

1.1.4.4   Mechanisms of childbirth trauma

The two major aetiological factors in the development of PFD are age related nerve degeneration and childbirth which have been shown to cause significant damage to the pelvic floor.[37,38,40–42] Trauma to the pudendal nerve during childbirth can be explained either by stretching or compression of the individual nerve branches during vaginal distension as the baby passes through the pelvis.

Injury to the lumbosacral plexus during childbirth is very rare and typically affects the L4-5 portions of the plexus. Lumbosacral plexus injury is not associated with pelvic floor dysfunction but rather involves pain and weakness in the lower limbs and will therefore not be discussed further in this thesis.[43]

Lien et al performed 3D computer modelling to investigate the extent of stretch to the branches of the pudendal nerve during the second stage of childbirth. They demonstrated that the inferior rectal nerve undergoes strain of up to 35%, and the perineal branch supplying the anal sphincter up to 33%.[44] This is significantly beyond the threshold of 15% known to cause permanent damage to peripheral nerves reported by Brown.[45] Interestingly, the perineal branches supplying the posterior labia and urethral sphincter were also borderline at 15% and 13% respectively. The study did not evaluate the clitoral nerve. [44]

This study was based entirely on a computer simulation and it is difficult to know how closely this mimics the events of childbirth in vivo. However, it is the closest we are likely to get given the impossibility of measuring nerve strain during childbirth in vivo.

Compression of the pudendal nerve branches by the descending fetal head could lead to peripheral nerve ischaemia. A systematic search of Medline and Embase showed no data regarding the pressure exerted on the genital nerves, vaginal walls or vulva during head descent in labour. However a study performed in Sweden tested the effect of prolonged nerve compression in rabbits. Rydevik et al assessed endoneural blood flow in 22 adult rabbit tibial nerves following varying degrees of nerve compression. The group showed that at 60mmHg the nerve becomes ischaemic in 73% of cases, at 70mmHg this was over 90% and at 80mmHg blood flow stopped in 100% of cases. Compression which persisted for two hours led to long-term impaired nerve function.[46] It is therefore also quite possible that nerve ischaemia occurs during the second stage of childbirth.

1.2 Physiology of sensation

There is strong evidence to support the idea that motor nerve injury occurs during childbirth from either nerve strain or compression.[44–46] Sensory innervation of the pelvic floor follows the same peripheral path as the motor supply and so logically sensory injury would be expected to occur at the same time as any peripheral motor nerve injury. Indeed, anecdotally some clinicians describe seeing women who complain of a complete loss of sensation in the pelvis following childbirth despite normal examination findings.

Surprisingly, there has never been a study to assess this, mainly due to difficulty with measuring the sensation of the female pelvic floor. Over time neurophysiological tests have been developed which assess sensory nerve deficit and these have been adapted and validated for testing of the female genitalia.[47] These advances have now made it possible to perform a study investigating the impact of childbirth on vaginal sensation.

In order to fully understand sensory testing we must explore the neural pathways that result in sensory perception and nerve regeneration.

1.2.1 Sensory pathways

Sensation is transmitted along afferent neural pathways from the receptor organ to the somatosensory cortex in the brain. The sensation of touch, pressure, proprioception, pain and temperature are transmitted from receptors in the skin.[48,49]

1.2.2 Nerve fibres

Nerve fibres carry information towards or away from the end-organ, either along an efferent nerve fibre towards a muscle or as afferent nerve fibre carrying sensory data from a peripheral receptor to the brain. In this review, we will only consider afferent fibres as they relate to the transmission of sensory information.

There are three main types of nerve fibre, Table 1–D. Each fibre group has its own function and conducts at different speeds. Type A fibres are the largest, conduct the fastest and are subdivided into Aα, Aβ, Aδ and Aγ. Some of the Aα fibres and all of the Aγ fibres transmit efferent impulses regarding skeletal muscle tone. Type B fibres relay information for the autonomic nervous. Type C fibres convey slow pain. [49–53]

There are also a variety of sensory receptors which each perform a specific role. Some provide conscious sensations whereas others sense arterial blood pressure and oxygen saturation, lung inflation and pH of cerebrospinal fluid. In this review only the conscious senses relevant to PFD will be discussed.

Table 1–D Properties and sensory functions of nerve fibres

Nerve fibre [50–52]	Conduction speed (m/s) & diameter (mm)	Receptor class	Sensation	Stimulus	Receptor cell type [49,51–53]
Aα	Very fast: 70-120 m/s, 12-20 mm, myelinated	Mechanoreceptor	Proprioception and tone	Stretch[52]	Free nerve endings in muscle spindles
				Tension	Golgi tendon organ
Aβ	Fast (30-70 m/s, 5-12mm, myelinated)	Mechanoreceptor	Touch	Tap, flutter 5-40Hz	Meissner corpuscles
				Motion	Hair follicle receptors
				Deep pressure, vibration 60-300Hz	Pacinian corpuscles Free nerve endings
				Touch, pressure	Merkel cells
				Stretch, sustained pressure	Ruffini corpuscles
Aδ	Average (12-30 m/s, 2-5 mm, myelinated)	Thermoreceptor	Warmth	Heat	Heat receptors
			Cool	Cold	Cold receptors
		Nociceptor	Thermal pain	Heat pain Cold pain	Heat receptors Cold receptors
			Fast pain	Touch, pressure, chemical irritation	Free nerve endings
C	Slow (0.5-2.0 m/s, 0.4-1.2 mm, unmyelinated)	Thermoreceptor	Warmth	Heat	Heat receptors
			Cold	Cold	Cold receptors
		Nociceptor	Thermal pain	Heat pain Cold pain	Heat receptors Cold receptors
			Slow pain	Touch, pressure, chemical irritation	Free nerve endings
		Chemoreceptor	Itch	Chemical irritation	Free nerve endings

1.2.3   Sensory receptors

Each nerve fibre carries more than one sensory modality making it possible to assess the functional integrity of an entire nerve fibre group by choosing to test just one sensory modality relevant to that group. For example, C fibres could be tested by assessing heat pain thresholds and this data could be extrapolated to comment on the function of C fibres in terms of all thermal pain, thermal sensation and itch.

When Table 1–D is linked to the symptoms of PFD it can be seen that the relevant sensory modalities include light touch, vibration, deep and sustained pressure which are all transmitted by Aβ fibres. Therefore in order to effectively assess the physiological function of Aβ fibres, only one of these modalities needs to be assessed.

Women with PFD describe a range of problems relating to sexual dysfunction, which can include a feeling of vaginal laxity and loss of sensation of vaginal tone. Studies which have tried to correlate symptoms and anatomical findings confirm severity of POP only mildly correlates with symptoms which are often not compartment specific.[54] Thus raising the question are these women describing a mechanical vaginal laxity or simply reduced sensation of the vaginal tube.

One possible explanation is that the sensory supply to pelvic floor muscles is injured alongside the motor supply during childbirth which results in impaired awareness of the pelvic floor. A test which could evaluate awareness of the pelvic floor, and thus the sensory functionality of Aα fibres would be particularly pertinent to PFD.

1.3 Nerve regeneration

Nerve fibres carry a range of information from a variety of sensory receptors. Thus it is possible to test the functionality of an individual nerve fibre group by testing just one of its corresponding stimuli.

1.3.1   The physiology of nerve regeneration

The next step in a project evaluating the impact of nerve injury is to consider the potential for nerve regeneration. As long as the nucleus remains intact following a traumatic nerve injury all peripheral nerves will start to regenerate.[55] This usually occurs within the first few days after injury with the production of nerve growth factor.[56] The nerve axon produces little processes which grow at a rate of approximately one millimetre per day.[56–58]

Sensory reinnervation is usually incomplete and this has been seen in both myelinated and unmyelinated fibres of the trigeminal nerve.[59–61] Research from Finland investigated reinnervation of the trigeminal nerve following injury during mandibular bilateral osteotomy surgery. Of the 18 cases studied, tactile quantitative sensory testing (QST) was reduced in 40% of cases, whereas nerve conduction studies were abnormal in 87% of cases.[61] This would indicate that although nerve regeneration appears incomplete in the majority of cases, this is only clinically relevant in less than half.

1.3.2   Time frame for nerve regeneration

Nerve reinnervation occurs after all traumatic nerve injuries where the nucleus remains intact. Sensory reinnervation is usually incomplete but this is often not clinically significant.

The ability of nerves to regenerate could complicate sensory assessment after nerve injury in terms of the time-frame from injury to reinnervation. Both nerve conduction studies and sensory testing evaluate the functional capacity of a nerve, and do not rely on reinnervation for the test to work. This is important for this study, as the first postnatal test will need to occur before significant reinnervation to catch the time of greatest sensory deficit, whereas the second postnatal test needs to allow enough time for maximum reinnervation.

A study by Boven et al evaluated the recovery of a variety of sensory modalities following injury to the trigeminal nerve, including vibration sense. He found that at three months post-injury around 80% of subjects had a persistent deficiency in vibration and touch discrimination. This had reduced to between 15-40% at six months post-injury.[59] Therefore an appropriate time-frame would be eight to 12 weeks post-partum for the first PN assessment, followed by a further assessment at six months PN.

1.4 Treatment of nerve injury

If vaginal birth causes a traumatic sensory nerve injury it is important the study includes an appropriate follow up period to catch both nerve degeneration and reinnervation, seen as a return to normal function.

Treatment for nerve injury is a developing area. Other specialties have used artificial nerve tube guides for short distance nerve deficit in patients with significant neurological damage.[60] Autologous nerve transplants have previously been discounted due to morbidity with harvesting. However, recently olfactory nerve cells across a peripheral nerve bridge have been used to successfully treat paralysis in a patient with a transected

spinal cord injury.[62] The potential combination of neural cells grown from autologous olfactory nerves grown across artificial nerve tube guides could transform the management of nerve injury.

1.5 Neurophysiology: sensation testing

Demonstrating the presence and defining the degree of sensory deficit in PFD is important when aiming to improve functional outcome following treatment of PFD. For these women, diagnosis of a sensory deficit in PFD would validate their symptoms, prevent inappropriate surgery and possibly lead to new treatment modalities.

Sensory deficit in PFD of often subclinical and may not be elucidated during standard history taking and clinical examination. Given the time constraints on research visits involving pregnant and postnatal women within this project sensory testing will focus on neurophysiological assessment of nerve function rather than history taking and a bedside neurological examination.

In this study it will be important to control the research environment as much as possible, and perform repeat testing at various intervals to observe sensory deficit and subsequent reinnervation.

A good test of sensation should aim to control the external environment and standardise the test procedure to reduce subject perception variability. It should also be time efficient, safe in pregnancy and acceptable to patients. Acceptability is particularly important given the sensitive nature of testing in this study.

1.5.1   Nerve conduction studies

There are a variety of investigations to test sensory nerve deficit. They include nerve conduction studies (NCS), quantitative assessment of sensation, called psychophysical testing, using either quantitative sensory testing (QST) and current perception thresholds (CPT).

Nerve conduction studies (NCS) were first described in 1948 by Hodes, who measured action potentials in muscle following electrical stimulation of motor nerves.[63] The principles are similar for sensory NCS, which measures the action potential produced by a receptor organ when a nerve is stimulated by an external electrical impulse. Sensory nerve conduction studies are transcutaneous and percutaneous and provide an excellent assessment of nerve integrity in terms of reliability and reproducibility when used in the limbs.[64–66]

NCS require a measured distance for an action potential to travel in order to accurately interpret the conduction time. Conduction through a neuromuscular junction or significant muscle mass can increase the conduction time.[67] When performing sensory NCS of the female genitalia it is not possible to measure the length of the pudendal or perineal nerves, avoid a neuromuscular junction or the large muscle mass that forms part of the pelvic floor. Therefore a prolonged latency in sensory NCS of the pelvic floor could be due to nerve damage, prolonged distance, a neuromuscular junction or the muscles of the pelvic floor itself. Unsurprisingly NCS of the female genitalia do not correlate with either clinical symptoms, examination findings or histological assessment.[68,69]

1.5.2   Somatosensory evoked potentials

Somatosensory evoked potentials (SSEP) use electrical stimulation of sensory receptors to create action potentials, which travel along the peripheral nervous system and into the central nervous system where they can be detected by scalp electrodes.[70,71]

SSEP of the pudendal nerve was first reported in 1987 and normative values subsequently published in the literature.[71–73] The procedure is also recommended by the International Continence Society as part of a neurophysiological assessment for voiding dysfunction.[74]

SSEP in non-pregnant women is safe and painless, however the impact of electrical stimulation of the pudendal nerve in pregnancy on the uterus, cervix and unborn fetus is uncertain. As a result SSEP was not performed during this study.

1.5.3   Quantitative sensory testing

Quantitative sensory testing (QST) tests the minimum stimulus needed for a subject to perceive a sensation, called the sensory or psychophysical threshold. It uses a strict validated protocol to provide a reproducible assessment of sensory function. QST was first described in a clinical context in 1976 by Fruhstorfer et al in relation to the testing of thermal sensation.[75] Initially suggested as an adjunct to NCS, QST provided a means of testing smaller nerve fibres via thermal stimuli. However, with the advent of both vibration and thermal testing, it became possible to test both large and small nerve fibres with QST, avoiding NCS entirely.

QST is subjective which allows an assessment of the physiological function of the nerves, making it more clinically relevant to patient symptoms. However, the subjective element is also a source of criticism, as distraction of the subject can alter the result. It evaluates

the entire sensory pathway, rather than individual nerve integrity. This is of value in this study, where the aim is to find evidence of genital sensory nerve injury following childbirth, rather than evidence of trauma to a specific nerve.

Different methods of QST have been described, which can be divided into QST including reaction time which is called method of limits, and QST excluding reaction time called method of levels. In contrast to nerve conduction studies and sensory evoked potentials sham stimulation is not recommended in QST.[76,77]

1.5.3.1 Method of limits

The most commonly used and original method of QST includes reaction time, and is called the method of limits.[75] In this method, the sensory stimulus, for example vibration, is linearly increased until the patient indicates perception, usually via a response button.

The time it takes for the vibration to create an action potential at the sensory receptor, travel to the cerebral cortex, at which point the subject first perceives the stimulus, before creating the motor impulse which then has to travel to the distant motor fibres for the response button to be pressed, is called the reaction time. All the while the vibration has been slowly increasing, so the final recorded perception threshold is slightly higher than it would have been if reaction time was excluded.[75,78,79] Several measurements are taken, and the mean is used as the final perception threshold. Dyck et al showed that greater than four measurements is not associated with a significant change in the mean perception threshold.[80]

Some clinicians consider the inclusion of reaction time a disadvantage, because all of the recorded thresholds are slightly higher, however this does not affect the results in comparative studies. This is particularly relevant to this study, where the vibration sensation thresholds will be compared before and after delivery to evaluate any change, and so the absolute threshold itself is of less importance. For studies producing normative data of vibration or thermal stimuli, this may be more significant.

The method of limits is quick and easy to use, as well as being minimally invasive for the test subject. This study includes the assessment of pregnant women, and so a minimally invasive test is of particular value when alleviating any fears she may have for her unborn child. It is for these reasons that this study will use QST method of limits for testing of vibration.

1.5.3.2 Method of levels

The principal QST method which excludes reaction time is called the method of levels.[78,81–83] It involves using a pre-set single sensory stimulus, for example vibration,

which the patient indicates whether they have felt once the stimulus has finished, defined as a positive or negative perception. Further pre-set vibration stimuli are then individually tested based on a specific algorithm. The vibration intensity is increased after a negative perception, and decreased after a positive perception. The intensity of subsequent vibrations is then halved at each step, until a pre-set interval size is reached. The perception threshold is calculated as the mean between these two intervals.[79]

Staircase

This is similar to the method of levels, except for the algorithm deciding the interval for further stimuli. In the staircase method, the intervals are defined as gross, medium and fine. Gross steps are used until the subject indicates perception, then medium steps, and then fine. The perception threshold is based on the median from the mean of all positive perception values after the first negative perception, and the mean of all negative perception values.[79,84]

Forced choice algorithm

This method was first described by Dyck et al who criticised earlier methods of QST as being too impractical for bedside implementation and the traditional non-QST bedside test as too crude.[85] In forced choice testing, the subject has to decide which of two time periods contains the sensory stimulus. Given the 50% chance of guessing the correct answer, the test must be repeated four times to be considered valid. A positive perception is counted as 75% - i.e. the subject must give the correct answer a minimum of three out of the four times.[86,87] As with previous reaction time exclusive methods of QST, a negative perception leads to an increase in the intensity of the stimulus, whereas a positive perception results in a decrease in the intensity of the stimulus.[88]

This method is understandably time consuming, and so there is a risk with this method that the perception thresholds are affected by subject drowsiness or boredom.

The stepping algorithm

The 4-2-1 stepping algorithm was also described by Dyck and his group, as a less time consuming version of the forced-choice algorithm method (12.8 ± 2.9 minutes, versus 2.7 ± 2.5 minutes).[89] It is based upon the concept that there are 25 'just noticeable differences' in sensory testing. The test starts at point 13, and increases or decreases dependent on the subjects' perception. The next step jumps four points, the one after jumps two points on the scale, and the final step is just one point apart. The test includes five null time periods with no stimulus.[79,89]

1.5.3.3 Comparison of QST methods

All the methods described above have been shown to have equal sensitivity.[79] In QST, specificity relates to the prevention of false positive results, as well as the incorrect interpretation of the results.[79] However, with the use of a strict protocol for testing, a controlled research environment and validated nomogram data, specificity is high.

Testing of vibration lends itself to the method of limits as the vibration intensity can easily be linearly increased until the subject indicates perception. The method of limits in QST also fits the test requirements of being non-invasive, acceptable to women and safe to use in pregnancy.

1.5.4 Current Perception Thresholds

Current perception thresholds (CPT) use different frequency sine-waves via a constant-current stimulator to provide electrical impulses to different afferent nerve fibres using defined wave frequencies. Large myelinated A-β fibres are tested using 2000Hz, smaller myelinated A-δ fibres are tested with 250Hz and unmyelinated C-fibres are tested using the lowest frequency of 5Hz.

Table 1–E. Nerve fibres and methods of sensory testing

Nerve Fibre	Sensation	Method of testing		
		QST	CPT	
			Sine wave	*Square wave*
Aβ	Touch	Vibration	2000Hz	Approx. 100Hz
Aδ	Thermal Fast pain	Thermal	250Hz	Approx. 2.5Hz
C	Thermal Slow pain	Thermal	5Hz	Approx. 2.5Hz

Key: QST – quantitative sensory testing; CPT – current perception thresholds

1.5.5 Comparison of CPT and QST

Four studies have compared CPT and thermal and vibratory QST for non-nociceptive stimuli, Table 1–F. In 1989 Masson et al reported the use of CPT as part of the assessment for diabetic neuropathy; included in his study was a comparison with QST for thermal and vibratory thresholds in 31 healthy volunteers. The group demonstrated a significant correlation between CPT and thermal and vibratory thresholds. Unfortunately the study did not assess test-retest reliability.[90]

Lowenstein et al compared CPT with thermal-vibratory QST using the method of limits. His group looked at the relationship between the two, as well as test-retest reliability in 27 healthy female volunteers using the volar surface of the arm. They found thermal

thresholds correlated with CPT at 5Hz, and vibration thresholds were associated with CPT at 2000Hz. However, CPT showed poor test-retest reliability, whereas all thermal and vibratory QST thresholds had a statistically significant correlation on re-test. They confirmed that although CPT can be useful in a clinical setting, QST has proven reliability on repeat testing.[91]

In another study which focused on the female genitalia, Lowenstein et al compared CPT and QST as well as test-retest reliability in healthy volunteers. They found a moderate correlation between thermal QST and CPT at 5Hz corresponding to C fibres, and between vibration QST and CPT at 2000Hz corresponding to Aβ fibres. The group reported no correlation between cold QST and CPT at either 250Hz or 5Hz, corresponding to Aδ or C fibres respectively. All methods of QST were found to have high test-retest reliability at one week interval using concordance correlation. However, CPT was found to have moderate test-retest reliability at one week interval for 2000Hz and 5Hz, but very poor test-retest reliability for 250Hz.[92]

The fourth study is a conference abstract with limited information. Overall, it found a significant correlation between CPT and warm thermal sensation, but not for cold thermal or vibration.[93]

A decision has been taken not to use CPT in this study on the basis that it has poor test-retest repeatability, and has not been validated for use in female genitalia. Whilst it could be argued this study could be the first to validate CPT in female genitalia, before proceeding the main study, this was felt to detract from the main aim of the study which is to evaluate the nature of sensory injury following childbirth. On this basis, the researchers feel it is more appropriate to focus on a test which has been validated and confirmed as safe and acceptable to women. Secondly, given the comparative nature of the study, test-retest reliability is of utmost importance and any test which cannot demonstrate this must be discounted.

Table 1–F. Studies comparing quantitative sensory testing and current perception thresholds

Study	Test site	QST vs CPT	Re-test reliability at one week	
Lowenstein et al [91] (QST vs CPT n=27 Re-test n=20)	Arm	*Thermal vs 5Hz* Spearman's ρ=0.49, *p=0.009* *Vibration vs 2000Hz* Spearman's ρ=0.05, *p=0.008*	*Thermal* Spearman's ρ=0.83, *p=0.0001* *Cold* Spearman's ρ=0.47, *p=0.0037*	*Vibration* Spearman's ρ=0.73, *p=0.0001* *CPT 5Hz, 250Hz, 2000Hz* Not significant
Lowenstein et al [92] (QST vs CPT n=16 Re-test n=10)	Female Genitalia	*Thermal vs 5Hz* Spearman's ρ=0.77, *p=0.002* *Cold vs 5-250Hz,* Not significant *Vibration vs 2000Hz* Spearman's ρ=0.6, *p=0.01*	*Thermal* Spearman's ρ_c=0.83, *p=0.001* *Cold* Spearman's ρ_c=0.77, *p=0.001* *Vibration* Spearman's ρ_c=0.96, *p=0.001*	CPT 5Hz Spearman's ρ_c=0.69, *p=0.001* *CPT 250Hz* Not significant *CPT 2000Hz* Spearman's ρ_c=0.70, *p=0.001*
Masson et al [90] (n=31)	Hand Foot	*Thermal vs 5-250Hz* Spearman's ρ=0.34-0.46, *p<0.05* *Vibration vs 2000Hz* Spearman's ρ=0.42-0.69, *p<0.05*	Not done	
Park et al [93] (n=19)	Not stated	*Thermal vs 5Hz* Significant Cold vs 250Hz Not significant Vibration vs 2000Hz Not significant	Not done	

Key: QST – quantitative sensory testing; CPT – current perception thresholds

1.6 Pelvic floor dysfunction and sensation

Female genital sensation can be assessed using QST or CPT, with normative data published on both, allowing researchers to formally measure genital sensation in women with PFD.

1.6.1   Pelvic organ prolapse

The severity of pelvic organ prolapse (POP) symptoms has a poor correlation with the degree of POP on examination. [54] Possible explanations for this include varying activity levels on the day of assessment, rectal loading or impaired sensation which may influence the awareness of POP.

Two studies have investigated sensation in POP, Table 1–G. The first, by North et al compared sensation in women with POP to controls using QST. They reported abnormal thermal and vibration thresholds in the majority of women with POP under 50 years, and in 100% of women over 50 years. Thresholds were within the normal range in the controls.[94] This study suggests sensory impairment in POP, but the small numbers, lack of age-matched controls and wide variation in the type of POP studied makes it difficult to be sure of the conclusions.

Interestingly, this study did not demonstrate a correlation between the degree of sensory impairment and poor motor nerve function in women with POP.[94] This suggests that sensory and motor neuropathies are independent factors in the aetiology of PFD. Poor correlation between sensory and motor nerve impairment could also explain the poor correlation between symptoms and degree of prolapse.

The second study was published by Gruenwald et al evaluating sensation in a much larger cohort and confirmed North's initial findings of reduced sensation in women with POP.[95] This was true for cold, thermal and vibration stimuli in the vagina and thermal stimuli at the clitoris, implying that all types of sensory nerves are damaged in women with POP.

These studies suggest women with POP have impaired genital sensation. Whilst it is unlikely that impaired sensation directly causes the pelvic floor weakness that leads to POP, abnormal sensation could explain the poor correlation between symptoms and degree of prolapse.

Table 1–G Studies investigating pelvic sensation in prolapse

Study	Comparison	Measure	Outcome
Gruenwald et al [95] (n=66)	POP vs controls	QST	*Thermal* *Anterior vaginal wall* 41.6±0.5°C vs 39.8±0.2°C, *p<0.001* *Posterior vaginal wall* 40.9±0.4°C vs 39.3±0.2°C, *p<0.0001* *Clitoris* 39.4±0.3°C vs 38.0±0.°C, *p<0.0001* *Cold* *Anterior vaginal wall* 30.2±0.6°C vs 32.5±0.3°C, *p<0.0001* *Posterior vaginal wall* 30.9±0.5°C vs 33.0±0.3°C, *p<0.0001* *Vibration* *Vaginal wall* 6.6±1μm vs 2.5±1μm, *p<0.02*
North et al [94] (n=30)	POP vs controls	QST	*Vibration* *Vaginal wall* Abnormal in 70% POP vs 0% controls *Clitoris* Abnormal in 85% POP vs 0% controls

Key: POP – pelvic organ prolapse; QST – quantitative sensory testing

1.6.2   Stress incontinence

There are a number of theories explaining the mechanisms of stress urinary incontinence (SUI) including urethral hypermobility, intrinsic sphincter deficiency and the integral theory. A possible role of sensory impairment has been explored in two studies, Table 1–H.

One study, by Lowenstein et al tested vibration and thermal sensation using QST in women with SUI and female sexual dysfunction (FSD) compared to FSD alone. They reported reduced sensation in women with SUI & FSD compared to women with FSD alone for warmth, cold and vibration.[96] Unfortunately the study did not test women with SUI alone or compare to age matched controls, and so the sensory impairment demonstrated could relate to the type of FSD rather than any SUI.

The second study by Kinn et al used CPT and demonstrated no significant difference in sensory thresholds between women with SUI, UUI and mixed incontinence. The study did not have an age-matched control population making it difficult to know whether women with pure SUI have impaired sensation compared to normal women.[97]

Further research is needed which focuses on pure SUI compared to an age matched control group to answer this question fully.

1.6.3   Urge incontinence

The sensory nature of urge incontinence would suggest sensory dysfunction is common in this condition, but strong evidence is still lacking, Table 1–H.

A German study measured sensation using CPT in women with SUI and UUI.[98] Although the group found women with SUI were hyposensitive and women with UUI were hypersensitive, the results were not significant due to the wide ranging values produced by the type of CPT protocol and square wave current used. Since this study the technique of CPT has been refined and scattering reduced, although results using a sine wave rather than square wave current appear to be more reliable for the female genitalia.[97–101]

Another study demonstrated reduced urethral sensation in women with UUI when compared to controls.[102] There was no difference in bladder sensation between the two groups, suggesting UUI may be urethrally mediated. The control group was not age or parity matched, and so there may have been a confounding effect of age related nerve degeneration and childbirth nerve injury on the results. This could fit with the hypothesis that women with UUI have sensory nerve impairment which might be due to a

combination of age related nerve degeneration and childbirth nerve injury, in addition to local factors such as oestrogen deficiency.

The study by Kinn et al described above which looked at SUI also investigated sensory thresholds in women with UUI and suggested that urgency symptoms are not related to impaired urethral sensation. They reported no correlation between urethral sensation and bladder capacity at first desire to void or at maximum capacity.[97] However the team used square wave CPT which has less clearly defined testing parameters for correlation of impulse frequency to specific nerve fibre types.

A study by Nagaoka et al tested only C-fibre sensation at 3 Hz with square wave CPT of the urethral mucosa in patients with detrusor overactivity (DO) and controls. They found that patients with neurogenic DO had a higher urethral threshold than patients with idiopathic DO, who in turn had a higher threshold than controls. There was no correlation between bladder capacity at first desire to void and urethral CPT for the three groups. When the data was analysed for symptoms there was a significantly higher sensory threshold in men and women with urge urinary incontinence (UUI) compared to controls, inferring that both men and women with UUI have reduced sensation in the urethra.[101] Unfortunately this study did not assess bladder CPT thresholds and combined results for both men and women making it difficult to extrapolate the results to a female only population.

There is conflicting evidence on whether there is sensory impairment in women with UUI. When sensory impairment has been demonstrated it has been the result of urethral rather than bladder sensory dysfunction.

1.6.4   Painful bladder syndrome

It is generally believed that the majority of pain syndromes are causes by a hyperalgesia, and so women with painful bladder syndrome (PBS) could be expected to demonstrate heightened sensation.

Only two studies have evaluated sensory function in women with PBS, Table 1–H. One measured sensory thresholds in women with PBS, SUI and controls using CPT at the C5, T6, T10, T12 and S3 dermatomes and found no difference in sensory thresholds at any dermatome between the groups, suggesting women with PBS have no evidence of hyperalgesia, whether generalised or localised to the bladder.[103] They also applied repeated series of non-painful stimuli. A normally functioning nerve stops transmitting a sensation after repeated stimuli, called habituation.[103] They found women with PBS had less habituation than controls for A-β fibres, A-δ fibres and C-fibres, suggesting women

53

with painful bladder syndrome have more sensitive nerve endings or central pathways which are unable to ignore repeated stimuli. [103]

Women with PBS do not complain of generalised altered sensation, suggesting that the problem is localised to vesical nerves, and this is supported by the second study which described normal sensation in the index finger of women.[99] The study compared results to published normative data rather than controls and tested just four women, as a result this data should not be considered conclusive.

The role of sensory impairment in women with PBS has not been fully explored and needs more work. Detailed evaluation of CPT thresholds at the bladder and urethra in women with PBS compared to age and parity matched controls is needed to further our understanding of the pathophysiology of PBS and develop more effective treatments.

Table 1−H. Studies investigating pelvic sensation in urinary dysfunction (continued overleaf)

Study	Comparison	Measure	Outcome
Clifton et al [99] (n=10, 4 women)	PBS vs published normative values	CPT – Sine wave	*PBS vs normative values* *2000Hz, 250Hz, 5Hz at index finger* Not significant
Fitzgerald et al [103] (n=40)	PBS vs SUI vs controls	CPT – Sine wave	*PBS vs SUI vs controls* *C5, T6, T10, T12, S3 dermatomes: 2000Hz, 250Hz, 5Hz* Not significant
Hegenscheid et al [98] (n=90)	SUI vs UUI	CPT – Square wave	*SUI vs UUI* *2Hz* 14.8mA vs 10.5mA, not significant
Kenton et al [102] (n=62)	UUI vs controls	CPT – Sine wave	*UUI vs controls* *2000Hz* 2.63mA vs 1.15mA, *p=0.005* *250Hz* 1.39mA vs 0.45mA, *p<0.0005* *5Hz* 1.14mA vs 0.11mA, *p<0.0005*
Kinn et al [97] (n=61)	SUI vs UUI vs mixed incontinence	CPT - Sine wave	*SUI vs UUI vs Mixed incontinence* Not significant *UUI and bladder capacity at first desire* Not significant *UUI and maximum capacity* Not significant
Lai et al [104] (n=20)	PBS vs controls	QST	*Pressure or Heat Pain at T1, T11, L4, S2-3 dermatomes* Not significant *Pressure Pain at T11 dermatome* 6-9 vs 4-7 analogue scale, *p=0.028* *Pressure Pain at T1, L4, S2-3* Not significant *Heat pain with visual scale at T1, T11, L4, S2-3 dermatomes* Not significant

Table 1−I. Studies investigating pelvic sensation in urinary dysfunction continued

Study	Comparison	Measure	Outcome
Lowenstein et al [96] (n=63)	SUI and FSD vs FSD alone	QST	*Thermal* *Anterior vaginal wall* 41.7±0.17°C vs 40.6 ±0.17°C, *p<0.02* *Posterior vaginal wall* 41.3±0.36°C vs 40.0 ±0.18°C, *p<0.001* *Clitoris* 41.3±0.36°C vs 38.5 ±0.19°C, *p<0.001* *Cold* *Anterior vaginal wall* Not significant *Posterior vaginal wall* 30.3±0.46°C vs 31.5 ±0.23°C, *p<0.001* *Clitoris* 32.4 ±0.44°C vs 33.7 ±0.17°C, *p<0.02* *Vibration* *Vaginal wall* 9 ±2µm vs 4.4 ±0.5µm, *p<0.001* *Clitoris* 5.8 ±0.2µm vs 1.8 ±0.8µm, *p<0.001*
Nagaoka et al [101] (n=51, 21 women)	Neurogenic DO vs idiopathic DO vs controls UUI vs asymptomatic controls	CPT – Square wave	*Neurogenic DO vs idiopathic DO vs controls* *3Hz* 11.3mA vs 5.0mA vs 2.7mA *P<0.05,* Kruskal-Wallis *UUI vs controls* *3Hz* 12.5mA vs 5.0mA, *p<0.05* Mann-Whitney U Test

Key: PBS – painful bladder syndrome; CPT – current perception thresholds; SUI – stress urinary incontinence; UUI – urge urinary incontinence; QST – quantitative sensory testing; FSD – female sexual dysfunction; DO – detrusor overactivity

1.6.5 Faecal incontinence

Faecal incontinence can occur as a result of both functional and structural abnormalities. Although motor nerve function was the first area of neurophysiology to be studied in pelvic floor dysfunction, a search of both Medline and Embase revealed no studies evaluating sensory nerve function using QST or CPT in women with faecal incontinence.

1.6.6 Female sexual dysfunction

A number of studies have investigated the role of sensory impairment in the pathophysiology of female sexual dysfunction (FSD), whether this is the condition as a whole or whilst assessing the subgroups of desire, arousal, orgasmic and pain disorder, Table 1–J.

Helpman et al assessed temperature and vibratory thresholds at the clitoris and vagina in women with FSD compared to age matched normative data rather than controls.[47,105] They found 89% of women with FSD demonstrated at least one abnormal sensory threshold, and 68% had abnormal sensory thresholds in three or more domains.[105] Unfortunately the study had only 28 participants and so could not account for confounding variables such as comorbidities, parity, menopausal status, medication or pelvic surgical history.

An even smaller study, described similar rates of abnormal sensation in 83% of postmenopausal women with FSD when compared to normative data.[106] Although, again this is difficult to extrapolate to the general population with a sample size of six.

Research by the group who published the first normative data on female genital sensation found abnormal vibration thresholds of the clitoris and vagina in women with FSD, whilst temperature sensation was not significant, implying FSD is the result of malfunctioning Aβ nerve fibres and not smaller C or Aδ fibres.[107] This study compared women with multiple sclerosis (MS) and FSD to women with MS alone, without either an FSD alone or age matched healthy control group. As such it is unclear whether the level of sensory dysfunction would be the same for women with FSD alone.

An Italian study assessed sensation of the hallux and dorsum of the foot in women with FSD compared to age and menopausal status matched controls.[108] They reported systemic impaired vibration sensation and normal thermal sensation in women with FSD, suggesting FSD might be part of a more generalised neuropathy. However, this would be in contrast to other peripheral neuropathies which demonstrate loss of smaller Aδ and C

nerve fibres before larger Aβ vibration fibres. Further studies are needed in this area to definitively assess whether systemic sensation is normal in women with FSD.

When sensation is analysed for desire, arousal, orgasmic and pain disorder the results in some cases question our basic understanding of the pathophysiology of FSD.

Connell et al tested sensation in women with desire, arousal, orgasmic and pain disorder compared to age, parity, BMI and menopausal status matched controls. They reported impaired vibration sensation at the clitoris in women with desire disorder, and at the clitoris, perineum and urethral meatus in women with arousal disorder. Women with orgasmic and pain disorder had normal sensation.[109]

The study described above which tested sensation in women with FSD and MS compared to women with MS alone, performed subgroup analysis for desire, orgasmic, arousal and pain disorder. They found abnormal vibration sensation at the clitoris in women with orgasmic and desire disorder, and normal genital sensation in women with pain disorder.

A study by Woodard et al described normal clitoral sensation but abnormal vaginal vibration sensation in women with desire disorder. Although the numbers in this study were small and demographic detail was lacking.[110]

In summary the evidence suggests that women with FSD have impaired vibration sensation and poorly functioning Aβ nerves, whilst temperature function and thus smaller Aδ and C nerve fibres commonly remain intact.

Table 1–J. Studies investigating pelvic sensation in female sexual dysfunction (continued overleaf)

Study	Comparison	Measure	Outcome
Connell et al [109] (n=46)	Desire disorder vs controls Arousal disorder vs controls Orgasmic disorder vs controls Pain disorder vs controls	QST	*Desire vs controls* *Clitoris - monofilaments* 0.11 ±0.12µm vs 0.03 ±0.02µm, *p<0.01* *Vulva, perineum, urethral meatus - monofilaments* Not significant *Arousal vs controls* *Clitoris - monofilaments* 0.15 ±0.17µm vs 0.03 ±0.02µm, *p<0.01* *Perineum - monofilaments* 0.49 ±0.73µm vs 0.04 ±0.03µm, *p<0.02* *Vulva and urethral meatus* Not significant *Orgasmic vs controls* *Pain vs controls* Not significant Not significant
Esposito et al [108] (n=60)	FSD vs controls	QST	*FSD vs controls* *Hallux – vibration* *Dorsum of foot - thermal* 26µm vs 6.8µm, *p=0.04* Not significant
Helpman [105] (n=28)	FSD vs published normative values	QST	*FSD vs normative values* *Clitoris – vibration, thermal, cold,* *Vagina – vibration, thermal, cold* 89% had at least one abnormal threshold
Woodard [110] (n=6)	Post-menopausal FSD vs published normative values	QST	*Postmenopausal FSD vs normative values* *Vagina – vibration* Not significant *Clitoris – vibration* 83% had an abnormal threshold

Table 1–K. Studies investigating pelvic sensation in female sexual dysfunction continued

Study	Comparison	Measure	Outcome	
Gruenwald et al [107] (n=41)	Desire disorder & MS vs MS alone Arousal disorder & MS vs MS alone Orgasmic disorder & MS vs MS alone Pain disorder & MS vs MS alone	QST	*Desire disorder and MS* *Clitoris – vibration* Pearson's rho = -0.322, *p=0.04* *Vagina – vibration, thermal, cold* Not significant *Arousal disorder and MS* *Vagina - thermal* Pearson's rho=-0.338, *p=0.03* *Clitoris – vibration, thermal, cold* Not significant *Orgasmic disorder and MS* *Clitoris – thermal* Pearson's rho=-0.347, *p=0.03* *Clitoris – cold* Not significant *Vagina – vibration, thermal, cold* Not significant *Pain disorder and MS* *Clitoris or vagina – thermal, cold or vibration* Not significant	*Clitoris – thermal, cold* Not significant *Vagina – vibration, cold* Not significant *Clitoris – vibration* Pearson's rho=-0.423, *p=0.006*
Woodard [106] n=22	Desire disorder without distress vs controls	QST	*Desire vs controls* *Vagina - vibration* 3.138μm vs 1.814μm, *p<0.05* *Clitoris – vibration and thermal* Not significant	*Vagina - thermal* Not significant

Key: QST – quantitative sensory testing; FSD – female sexual dysfunction; MS – multiple sclerosis

1.7 Measuring sensation of vaginal tone

As discussed in Sensory receptors (section 1.2.3) impaired sensation of vaginal tone may account for the poor correlation between severity of POP and symptoms of PFD. A test which could assess the sensory integrity of Aα nerve fibres which evaluate muscle stretch and vaginal tone would be particularly relevant to the assessment of PFD.

Due to the tubular structure of the vagina muscle stretch could be measured using the principles of QST with a balloon inserted into the vagina, and slowly inflated until the subject verbally confirms sensation of stretch. Testing for first sensation of vaginal stretch would be a new procedure, and need to be validated on normal controls. Given that this is a new procedure, a computer programme which would slowly fill the balloon with air at a continuous steady rate is not available and the balloon must be inflated manually.

The balloon used for assessment of rectal sensation as part of anal manometry studies is safe and well established in clinical practice across the country. When inserted through the anus, the balloons are known to be atraumatic, well tolerated in both the anus and rectum, and cause no adverse effects. Given that the vaginal mucosa is less easily injured than the rectum or anus, it follows that it would be safe to insert this balloon into the vaginal canal. Secondly, the balloon itself is a gentler device than a plastic speculum which is routinely inserted into the vagina of pregnant women in a wide range of clinical scenarios. Speculum insertion does not cause harm to the unborn child, miscarriage or preterm labour. It is reasonable to extrapolate from this that insertion and inflation of the balloon into the vaginal canal both AN and PN poses no risk to the women or her unborn child.

1.8 Computer interviewing

Pelvic floor dysfunction encompasses a wide range of symptoms some of which can only be diagnosed on clinical history. These symptoms and the impact of overall PFD on quality of life (QoL) can be difficult to objectively quantify. NICE recommends the routine use of symptom specific questionnaires and there are numerous validated paper questionnaires for the individual elements of PFD such as POP, UI, bowel dysfunction and FSD.[111] However using multiple questionnaires and then manually calculating the score for each respondent in a busy clinic or research study requires an impractical amount of time and paper.

Computer interviewing is an emerging concept in Urogynaecology and an excellent alternative method for collecting this information. A computer can be programmed to

only move forward once a question is answered, as well as to adjust further questions dependent on the previous answer. In doing so a computer can guarantee 100% completion of a questionnaire and ensure subjects follow the correct algorithm for their symptoms. It can also calculate a numerical score automatically for each pre-set domain saving valuable time.

Radley et al developed the sole Urogynaecology electronic questionnaire, called the electronic Personal Assessment Questionnaire – Pelvic Floor or ePAQ-PF, Figure 1-VIII. The programme asks 118 questions in four symptom areas – urinary, bowel, vaginal and sexual and provides numerical values for each of the domains within the four areas, see Appendix 8.8 for full questionnaire.[112] Despite the number of questions, the ePAQ-PF is relatively quick to complete at 16 ±6mins.[113] Radley and his group published the test-retest reliability of the ePAQ-PF in 432 women, 204 from primary care and 228 from secondary care. They reported significant test-retest correlations for each of the domain subgroups (*p<0.0001,* 0.50-0.95, 95% CI, n=126).[114]

A study in Cork evaluated the inconvenience of the computer format of ePAQ-PF compared to a traditional paper format using the Queensland pelvic floor questionnaire in 100 women aged 29 to 91.[115] The Queensland pelvic floor questionnaire has just 42 questions.[116] As such it is not surprising the study found ePAQ-PF provided more clinical information than the Queensland questionnaire (*p=0.0036*).[115] The more interesting element of the study is that regardless of age subjects did not find using the electronic system any more burdensome than the paper style (*p=0.1*).[115]

The ePAQ-PF is quick and easy to use making it convenient for women participating in this study, it will provide objective numerical values to compare with the sensory data that will be collected and has high test-retest reliability making it ideal for a longitudinal cohort study of this kind.

Figure 1-VIII Image of an example ePAQ-PF report

Key: The computer system categorises individual questions into symptom complexes and calls these domains. Based on the woman's answers the computer calculates numerical scores out of 100 for each domain.

Zero – asymptomatic; 100 – worse severity and frequency of symptoms; Impact – length of the bar visually displays the degree of bother.

1.9 Identifying the research question

1.9.1 Hypothesis

Despite the strong evidence of injury to pudendal motor nerves following childbirth and the association with pudendal motor nerve impairment in women with PFD, to date no study has investigated the impact of childbirth on pudendal sensory nerves.

I hypothesised that pudendal sensory nerves are damaged alongside motor nerves during vaginal birth, and this is associated with the development of pelvic floor dysfunction. Similar to motor nerve injury during childbirth, I hypothesised that mode of delivery, duration of second stage of labour and birth weight of baby may also impact sensory nerve damage. To address this hypothesis I conducted a prospective observational study of women in their first pregnancy before and after birth. Pelvic sensation testing, clinical examination and symptoms of pelvic floor dysfunction were performed in the third trimester of pregnancy, eight to 12 weeks postnatal and six months postnatal to assess the impact of childbirth and other obstetric factors on pelvic sensation and the relationship with symptoms of pelvic floor dysfunction.

To be able to include an evaluation of the sensory function of Aα nerve fibres in the vagina during pregnancy and after childbirth, I first needed to develop and validate a method for testing this.

Therefore, I also hypothesised that sensory function of Aα and Aβ nerve fibres in the pelvic floor could be measured using stretch sensation of the vagina with quantitative sensory testing principles. I performed a prospective observational cohort study of non-pregnant women to develop the testing protocol and create normative data.

The women in my childbirth cohort did not demonstrate an association between impaired sensation or slow recovery of sensation and the symptoms of pelvic floor dysfunction. Therefore, I questioned why women with evidence of sensory nerve injury after childbirth did not exhibit symptoms of PFD, yet women with PFD demonstrated decreased sensation compared to women without PFD. I queried whether the cause of impaired sensation in women with PFD was due to a decrease in nerve fibre frequency or a reduction in the functional capacity of nerve fibres.

I hypothesised that the initial insult occurs during vaginal birth, but this sensory deficit becomes more clinically apparent in later life due to age related nerve degeneration. I further hypothesised that the sensory dysfunction seen in women with pelvic organ prolapse was the result of fewer nerve fibres within the vaginal mucosa compared to women without pelvic organ prolapse. To answer this question I conducted a pilot study

evaluating the relationship between pelvic sensation and frequency of nerve fibres in the vagina in women with and without pelvic organ prolapse.

Another limitation was the lack of clinical neurological history or examination performed at the time of testing. The aim of this piece of work was to investigate the aetiology of the sensory changes seen in women presenting with PFD later in life. Whilst it would have been interesting to gather this information, data is already available on genital sensation in women with neuropathic conditions from specialist clinics. In addition this would have added an extra one hour to each clinical visit that would place the women under unrealistic time constraints with a new baby.

1.9.2   Aims

The specific aims of the project were to:

1. Develop a test to quantitatively measure female genital stretch sensation as a marker of Aα and Aβ sensory nerve function.
2. Determine the impact of childbirth on Aα and Aβ pelvic sensory nerves and how this related to the symptoms of pelvic floor dysfunction.
3. Investigate the relationship between sensation and neurohistology of the vaginal mucosa in women with pelvic organ prolapse.

1.9.3   Objectives

The specific research objectives were to:

1. Modify the quantitative sensory testing (QST) protocol for anorectal stretch sensation to measure genital Aα and Aβ nerve fibre sensation using stretch sensation at the vagina and introitus.
2. Confirm reproducibility of the new vaginal stretch QST protocol.
3. Develop normative data for vaginal stretch sensation testing.
4. Investigate the relationship between pelvic QST for vibration and stretch sensation, POP-Q and ePAQ-PF in the third trimester, eight to 12 weeks postnatal and six months postnatal.
5. Evaluate the impact of mode of delivery, duration of labour and birth weight of babyon sensory data.
6. Perform a pilot study investigating the relationship between vaginal neurohistology and clinical neurophysiology..

7. Develop collaborations to learn immunohistochemical techniques to measure the frequency of nerve fibres within the vaginal mucosa and correlate this with sensory function.

2 Materials and methods

The methods described below are used within multiple chapters. Additional methods specific to individual chapters are detailed in the relevant chapter.

What I'd suggested therefore is the addition of a single sentence under the title of Ch2 stating that the following methods are used within multiple chapters, but that other methods specific to individual chapters are detailed in them. Please also change the title of 3.2.5 which just says "Equipment" at present so that this describes what the method after it is more fully.

2.1 Quantitative sensory testing

Sensation was measured using quantitative sensory testing (QST), which uses a validated protocol to provide a reproducible assessment of the entire sensory pathway. All intimate examinations were performed in the presence of a chaperone.

2.1.1 Sensory testing equipment

QST was performed using the genito-sensory analyzer from Medoc, Israel.

The equipment consisted of a height adjustable probe attached to a computer program, Figure 2-I a), b) and c). The computer program linearly increased the vibration amplitude or temperature from zero to a maximum pre-set level, until the woman indicated perception of the sensation using a response button held in her dominant hand. Hand dominance was self-declared by the woman. When the response button was pressed the stimulus stopped immediately and the threshold was recorded. The computer program repeated the process six times and calculated the average value.

All the women received a standardised verbal explanation of the procedure prior to testing and a control test on the hand to familiarise with the sensation and use of the response button. The equipment was positioned so the woman was blinded to the amplitude or temperature readings on the computer screen.

All testing was performed by the Clinical Research Fellow or Clinician Scientist with a chaperone present. Due to the subjective nature of QST it is important to maintain reproducibility and therefore testing was performed in one of four quiet rooms within the same department, using the same model of examination couch with the lithotomy stirrups at the same height for each woman.

Occasionally the woman would be distracted and one of the readings would vary significantly when visually assessed compared to the others. In this case the individual reading was repeated.

a)

Vibration probe

Height adjustable arm

Thermal probe

Processing unit

b)

Probe arm

Vibration probe
– inserted 2-3cms into vagina for vaginal sensation

Clitoral testing point
- positioned so just touching clitoris

c)

Probe arm

Thermal probe

Metal plate of vaginal testing area
– inserted 2-3cms into vagina for vaginal sensation

Metal plate of clitoral testing area
– positioned so just touching clitoris

Key: a) Image of the equipment used for genital quantitative sensory testing of vibration and temperature sensation. b) Vibration probe used to assess Aβ nerve fibres. c) Thermal probe used to assess sensation to cold and warmth for Aδ and C nerve fibres. Images reproduced with permission from Medoc, Israel.

2.1.2　Vibration sensation

Medium myelinated Aβ nerve fibres were tested using the modality of vibration, Figure 2-I b).

Vibration was linearly increased to an amplitude of 130 microns, at a fixed frequency of 120 Hertz. QST was performed at the pulp of the non-dominant index finger to test the median nerve. Following QST of the index finger the probe was covered with a standardised probe cover and a single tube of optilube gel applied for lubrication. For vaginal sensation testing, the probe was inserted 2-3cm into the vagina to evaluate the perineal branch of the pudendal nerve. For clitoral sensation testing the tip of the probe was placed against the clitoris so the woman was just aware of the probe to assess the clitoral branch of the pudendal nerve.

2.1.3　Temperature sensation

Slower unmyelinated Aδ and C nerve fibres were tested via the modality of thermal and cold sensation, Figure 2-I c).

The temperature was linearly increased to assess warmth from no stimulus to a maximum pre-set level of 50°C respectively, and linearly decreased to assess cold from no stimulus to a minimum pre-set level of 20°C.[117]

The probe was inserted 2-3cm into the vagina to assess the perineal branch of the pudendal nerve, whereas only the tip was placed against the clitoris so the woman was just aware of the probe to assess the clitoral branch. When the response button was pressed the stimulus stopped immediately and the threshold was recorded. This was repeated six times for both thermal and cold, and the average value calculated for each.

2.2 Pelvic organ prolapse

All women underwent a standardised clinical examination for POP using the pelvic-organ prolapse quantification system (POP-Q) as recommended by the International Continence Society, International Urogynaecological Association and National Institute for Clinical Excellence.[118–121] All examinations were performed by a member of the clinical team or research fellow in the lithotomy position with a chaperone.

The individual measurements are explained Figure 2-II. The hymenal remnant is the fixed point of reference, with measurements at the level of the hymenal remnant classed as zero, measurements above in negative numbers and measurements below in positive.

For the work described in this thesis, the presence of POP was defined a priori as the most distal portion of the anterior or posterior vaginal wall at or below the level of the hymen, and the uterus at or below the lower third of the vagina.[122–124]

Figure 2-II Diagram of pelvis with POP-Q points of reference labelled
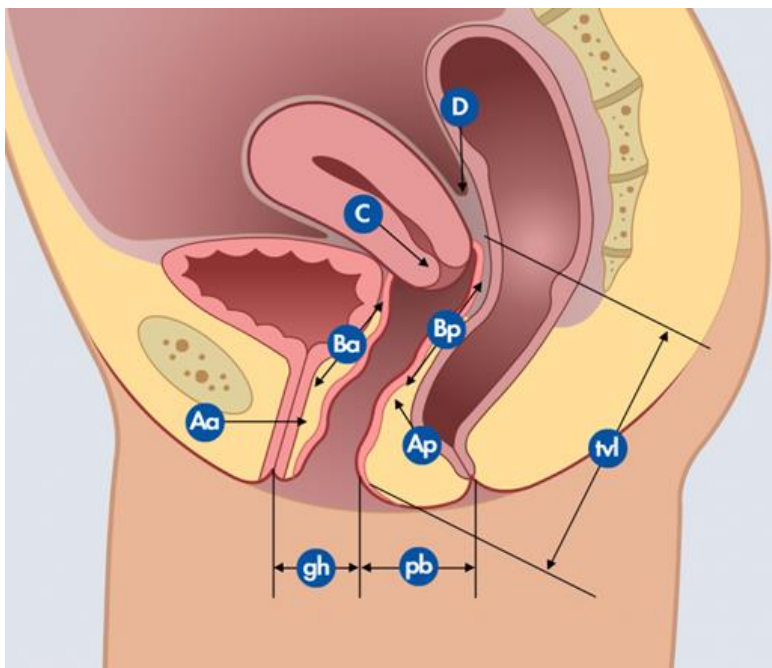
Key: Gh = genital hiatus (mid-urethra to posterior forchette; Pb = perineal body (posterior forchette to mid-anus); Aa = anterior wall at a point 3cm proximal to the hymen (Point Aa in relation to hymen); Ba = most distal position of anterior wall (Point Ba in relation to hymen); Ap = posterior wall at a point 3cm proximal to the hymen (Point Ap in relation to hymen); Bp = most distal position of posterior wall (Point Bp in relation to hymen); C = cervix or vaginal cuff (most distal edge of cervix or vaginal cuff); D = posterior fornix (n/a if uterus absent) (most distal edge of posterior fornix); TVL = total vaginal length (depth of vagina when C or D reduced to normal position) Image reproduced with permission courtesy of CR Bard, Inc.

3 Female genital stretch sensation

3.1 Introduction

Women with pelvic floor dysfunction describe a range of problems relating to sexual dysfunction and pelvic organ prolapse (POP), which can include a feeling of vaginal laxity and loss of sensation of vaginal tone likely due to impairment of proprioception.[125]

Studies to date have shown a poor correlation between POP symptoms and anatomical findings, and found symptoms are not compartment specific.[54] In addition, women with POP have evidence of impaired vaginal sensation for vibration and temperature, demonstrating reduced function of Aβ, Aδ and C nerve fibres respectively.

This raises the question as to whether the poor correlation between POP symptoms and anatomy reflects a loss of vaginal sensation rather than an anatomical difference. A test which could quantitatively evaluate the sensation of vaginal tone of the female genitalia would aid assessment of these women and further our understanding of the pathophysiology of pelvic floor dysfunction. This could be achieved using a neurophysiology technique called quantitative sensory testing (QST) which uses a validated protocol to provide a reproducible assessment of the entire sensory pathway.

Sensation of vaginal tone is transmitted via the free nerve endings of Aα nerve fibres within muscles. These nerve fibres also transmit sensation of muscle stretch which can be quantitatively measured and would provide an assessment of the functional integrity of Aα sensory nerve fibres.

The vagina is a hollow tube consisting of a layer of epithelium, the lamina propria that is rich in collagen and elastin, a tube of smooth muscle containing an inner circular and outer longitudinal layer, and beneath this the adventitia. The vagina is surrounded by skeletal muscle including the ischiocavernosus, bulbocavernosus and pubococcygeus muscles.

The sensation of stretch in glabrous and hairy skin is transmitted by Aβ sensory nerve fibres largely via Ruffini mechanoreceptors and some free nerve endings, whilst muscle stretch is transmitted via sensory fibres of Aα nerves. To date, Ruffini sensory receptors have only been described in the skin at the introitus but not in the vaginal mucosa.[16,18] Whilst the lamina propria does not contain any Ruffini mechanoreceptors, it is possible the free nerve endings of Aβ fibres may communicate some sensation of stretch. The

smooth and skeletal muscle layers both contain Aα nerve fibres which convey sensory information to the brain.[14]

Therefore a test which could quantitatively measure sensation of stretch in the vagina would be a marker of Aα and Aβ sensory nerve function.

I hypothesised female genital stretch sensation thresholds could be measured using QST method of limits, and that similar to other areas of the body sensation threshold would increase with age.

The objective was to develop a QST method of limits protocol to assess the function of Aα and Aβ sensory nerve fibres in the vagina through genital stretch sensation, perform an assessment of repeatability and evaluate the impact of age, body mass index and parity on sensory threshold and create normative data for the local population.

3.2   Materials and Methods

3.2.1   Study design

A prospective observational study entitled 'A method for testing female genital sensation in Aα nerve fibres' was performed, sponsored by Central Manchester Foundation Trust (Study ID R03811) and approved by the North West – Greater Manchester West Research Ethics Committee on the 13/10/14 (Reference 14/NW/1316), Appendix 8.1. The study was co-adopted onto the NIHR portfolio by Reproductive Health and Childbirth (CPMS ID 30525).

3.2.2   Recruitment

Pre and post-menopausal women attending the nurse led Colposcopy clinics at Central Manchester Foundation Trust were recruited.

The aim was to recruit 100 non-pregnant women to develop a protocol for testing Aα nerve fibre sensory function at the vagina and introitus.

The Colposcopy clinic was chosen for recruitment in preference to other Gynaecology clinics as women attending routinely undergo intimate examination and have no underlying gynaecological condition which could affect their sensation, such as chronic pelvic pain, menorrhagia or dysmenorrhoea.

All women due to attend the clinics received a patient information leaflet in the post approximately one week before their appointment. On the day of the clinic, women were screened for eligibility from the case notes and face to face where information on

eligibility missing from the case notes. Eligible women were seen individually by the Clinical Research Fellow (Dr Charlotte Mahoney) or the Research Medical Student (Ms Sok-Moi Chok) to discuss the study. They received a standardised explanation of the procedure including a demonstration of the balloon inflation in their dominant hand. Women were given the opportunity to ask questions and reassured that their decision would not affect their clinical care. Those who chose to participate completed a consent form.

Figure 3-I. Study schema for vaginal stretch sensation
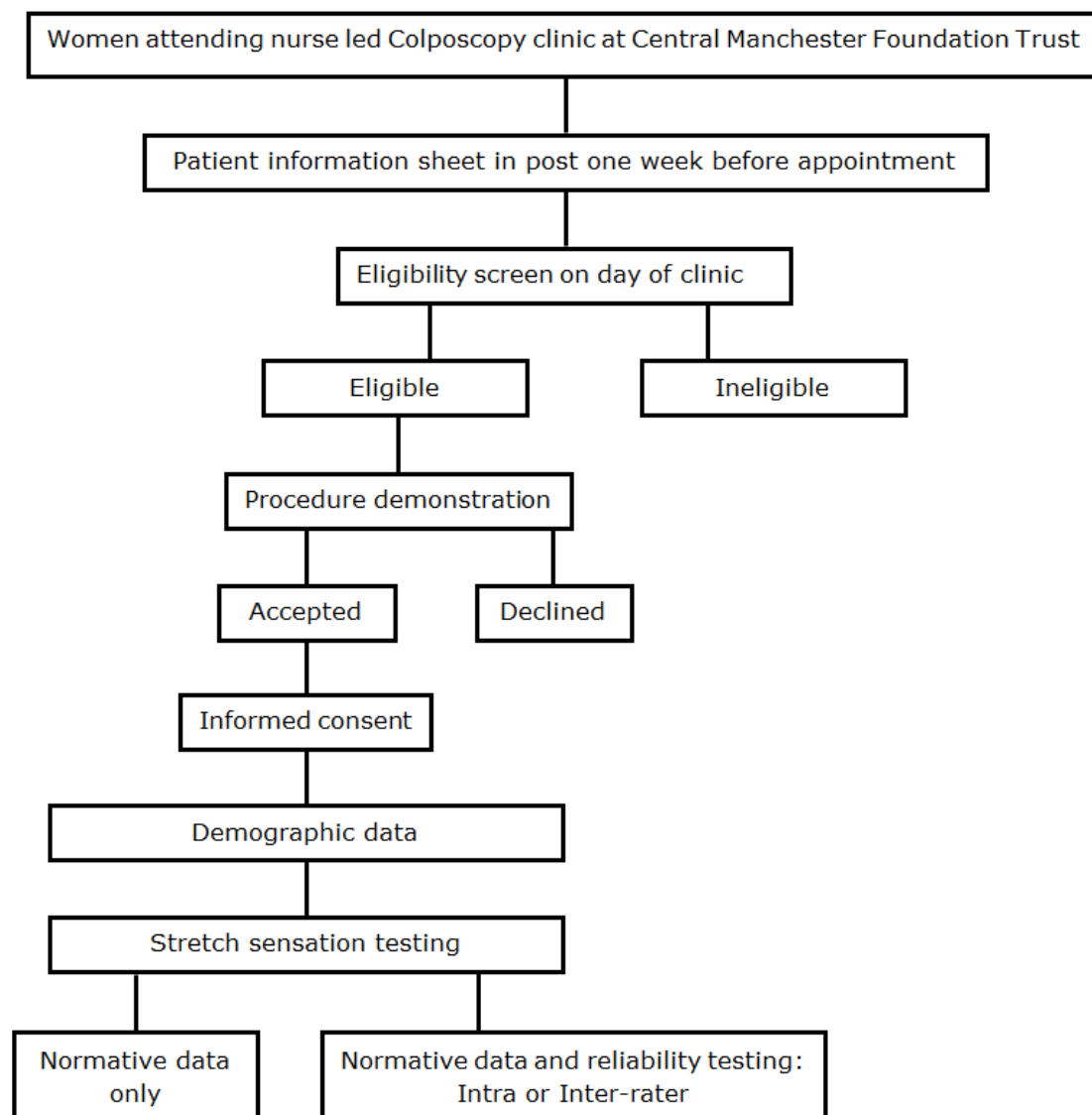
3.2.3   Study entry

Women were considered eligible for recruitment if they met the inclusion criteria and did not have any of the exclusion criteria, Table 3–A.

Table 3–A. Eligibility criteria

Inclusion Criteria	Exclusion Criteria
Age over 18 years	Language barrier requiring interpreter for consultation
Not currently pregnant	Incapacity to consent
Written informed consent	Previous pelvic floor surgery or female genital mutilation
Attending the Colposcopy clinic	Undergoing cervical treatment
Received Information Leaflet one week prior	Medical conditions predisposing to sensory impairment e.g. pre-existing diabetes mellitus, multiple sclerosis, vulvodynia
	Neuromodulatory medication e.g. Gabapentin

3.2.4   Demographic Data

Background data was collected regarding age, parity including mode of delivery, stage of menstrual cycle at time of testing, underlying medical conditions and regular medications including hormonal contraception. Height and weight were measured at the time of attendance and BMI calculated as weight in kilograms divided by height in $metres^2$.

3.2.5   Stretch testing equipment ex vivo experiments

The anal manometry balloon from Ardmore Healthcare, called the anorectal response catheter was used throughout (reference AHB 811), Figure 3-II.

Ex vivo balloon safety experiments were performed to assess the minimum volume of air required to induce balloon rupture. The company recommends inflating to a maximum volume of 400 cm^3. The average volume of air required to induce balloon rupture was 903 cm^3 (range 854 to 949 cm^3) Therefore maximum inflation was limited to 400 cm^3.

Ex vivo circumference experiments were performed to understand the relationship between inflation volume and balloon circumference. This demonstrated that at larger balloon volumes a greater inflation volume is required to produce the same proportional increase in balloon circumference than at smaller balloon volumes.

The rate of balloon inflation was also informed from ex vivo experiments comparing the degree of increase in balloon circumference at $1cm^3$ of air per second, $2cm^3$ of air per second and $5cm^3$ of air per second. On this basis $1cm^3$/s was very slow and $5cm^3$/s risked a greater impact of reaction time on sensory threshold, that is inherent in the method of limits, therefore $2cm^3$/s was chosen for the testing protocol.

Figure 3-II Equipment for stretch sensation testing
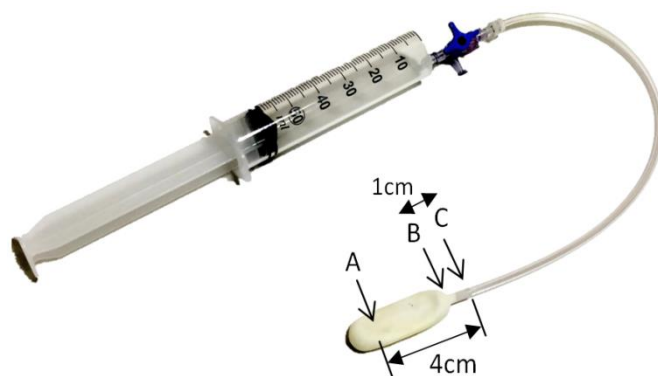
Key: Point A = location of the tip of the inflation catheter within the balloon; Point B = most distal edge of the balloon; Point C = most distal edge of where the balloon has been stuck to the inflation catheter.
Image reproduced with permission, photographed and annotated by Dr Sok-Moi Chok for APEP module during MBChB degree at the University of Manchester.

3.2.6   Psychophysical methodology

3.2.6.1   Quantitative sensory testing

Quantitative sensory testing is a neurophysiology technique developed to evaluate peripheral somatic sensation. QST tests the minimum stimulus needed for a woman to perceive a sensation, called the sensory threshold. It uses a validated protocol to provide a reproducible assessment of the entire sensory pathway, rather than individual nerves.

Different methods of QST have been described, which can be divided into QST including reaction time which is called method of limits, and QST excluding reaction time called method of levels. In the method of limits the stimulus is linearly increased until the woman indicates perception. Sham stimuli are not required when using the method of limits[77]

In this study I have developed a QST protocol for assessing stretch sensationat the vagina and introitus using the method of limits.

3.2.6.2 Protocol development

The first cohort or pilot group consisted of ten women (seven nulliparous, three multiparous) who were asked "tell me when you feel the vagina stretching". Information obtained during testing was then used to identify potential issues with this question, which included confusion regarding the timing and nature of any stretch sensation and the potential confounder of bladder filling. To clarify understanding three questions were then developed to differentiate between first awareness of any sensation, the start of stretch sensation and the start of an uncomfortable sensation, Table 3–B.

The second cohort were asked to empty their bladder beforehand and assessed for a loaded rectum via vaginal digital examination to assist with data interpretation. They were asked the three standardised questions in Table 3–B. A further review of the protocol performed after subsequent testing of another 10 women confirmed appropriateness and ease of understanding of the standardised questions through verbal confirmation from the women. This was the final QST protocol that was used.

Table 3–B Standardised questions for vaginal stretch sensation

Sensation	Question
First Awareness	Tell me when you begin to feel anything at all
Stretch	Tell me when you begin to feel a stretching feeling
Uncomfortable	Tell me when it begins to feel uncomfortable

As part of the QST protocol, all women received standardised instructions directly prior to and during testing. All testing was performed in one of two quiet examination rooms within the same department using the same model of examination couch with the lithotomy stirrups at the same height for each woman.

3.2.6.3 Stretch testing protocol

All intimate examinations occurred in the presence of a chaperone. Stretch QST was performed before Colposcopy due to concerns that performing a cervical biopsy may influence sensory threshold, and afterwards if the subject was undergoing a smear test due to the potential risk of cervical cell disruption from balloon inflation.

A digital examination of the vagina was performed to assess for a loaded rectum to assist with data interpretation, however this was negative in all women.

QST was performed using the method of limits for sensory threshold determination of first awareness, stretch and uncomfortable sensation at the vagina and the introitus. Standardised questions for each sensory threshold were used, see Table 3–B.

The balloon was lubricated to facilitate introduction with one sachet of Optilube and inserted 4cms into the vagina, which corresponded to Point C in Figure 3-III, to test vaginal sensation.

The subject was asked the standardised question for first awareness and then the stimulus intensity, or volume of air, was linearly increased using a 60ml syringe until the subject verbally indicated perception of the sensation, at which point inflation was paused and the volume recorded by an assistant. The rate of change was 2cm^3 of air per second. The subject was then asked the standardised question for stretch, inflation re-started and the procedure repeated. This was repeated for the sensation of uncomfortable. The balloon was only deflated once all three sensations had been recorded.

To test sensation of the vaginal mucosa at the introitus, the balloon was then retracted so Point B in Figure 3-III was at the level of the hymenal remnant and the procedure repeated to test for sensations of first awareness, stretch and uncomfortable. The order of testing was the same for all subjects.

Figure 3-III Position of the balloon during stretch sensation testing
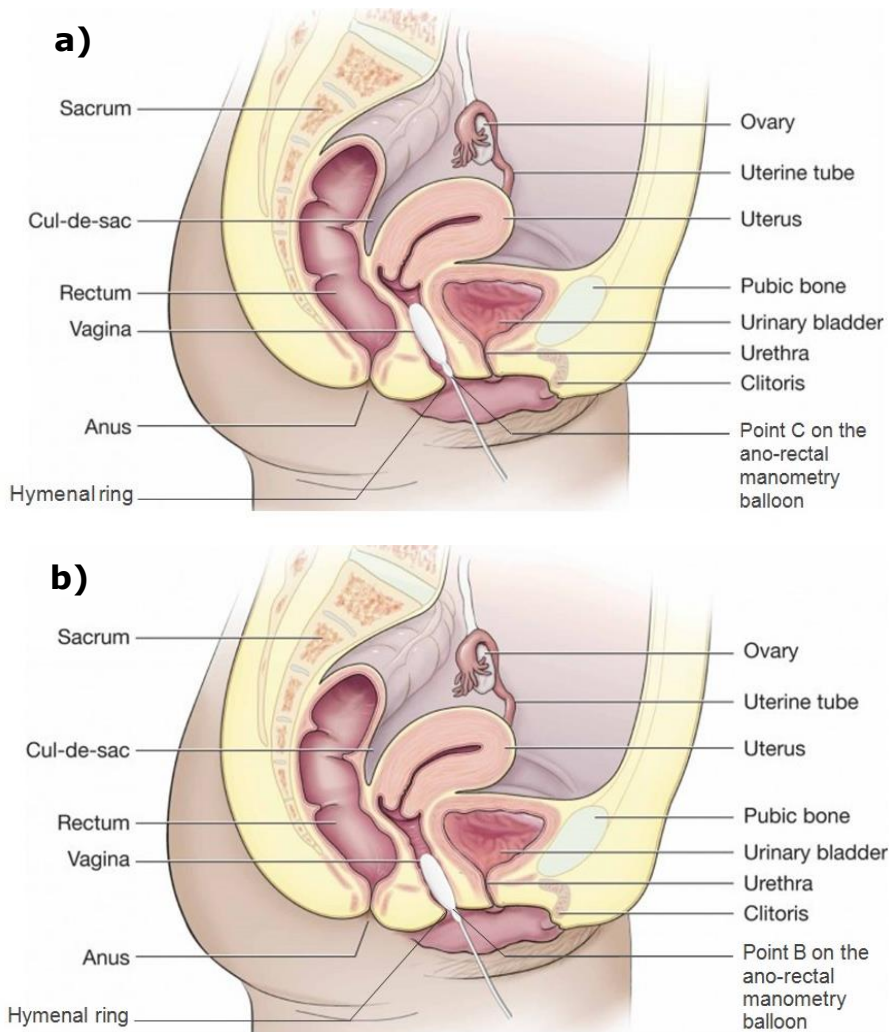
Image a) shows the most distal edge of where the balloon was attached to the inflation catheter positioned at the entrance to the vagina

Image b) shows the most distal edge of the balloon (Point B) positioned at the level of the hymenal remnant.

Image reproduced with permission, edited by Dr Sok-Moi Chok for APEP module during MBChB degree at the University of Manchester. Original public domain image available from http://biology-forums.com/index.php?action=gallery;sa=view;id=13861.

3.2.7　Repeatability

3.2.7.1　Intra-rater

Sensory testing was repeated after a minimum of five minutes to assess test-retest reliability, using the Methodology described in section 3.2.6. Intra-rater reliability was assessed by the same primary operator (the Clinical Research Fellow, Dr Charlotte Mahoney) on 20 women. The primary operator was blinded to the results of the first test when performing the second test by the documentation assistant. The high frequency of observations performed during each day of recruitment meant the primary operator was unable to recall the results of the first test when performing the second, despite the short time frame between readings.

3.2.7.2　Inter-rater

Inter-rater reliability was performed by the primary operator (the Clinical Research Fellow, Dr Charlotte Mahoney) and a secondary operator who was a Urogynaecology Specialist Nurse (Sister Angela Bryant). The Clinical Research Fellow had no professional influence over the secondary operator's objectivity. The secondary operator was formally trained in QST methodology and had experience using the balloon during anal manometry. She received formal training on the stretch QST protocol from the primary operator.

Inter-rater reliability testing was performed on 20 women. The standardised explanation of the procedure prior to consent was performed by the primary operator in all cases, with the secondary operator in the clinic room. All testing was performed as described in section 3.2.6, with a minimum of five minutes between testing.

Test one was performed by the primary operator in 11 cases and by the secondary operator in nine cases. Test two was performed by the secondary operator in 11 cases and the primary operator in nine cases. The operator performing the second test was blinded to the results of the first test.

3.2.8   Statistical Analysis

3.2.8.1   Detection of outliers and normality assessment

Outliers and leverage points were detected using a combination of studentised residuals greater than ±3 standard deviations and visual identification.[126]

To facilitate analysis the dependent variables were assessed for normality using the Shapiro-Wilk test. Transformation was performed for non-normal variables. Variables that were not normally distributed were assessed both graphically by histogram and statistically using Tukey's ladders of power for the most normally distributed transformation. Parity was dichotomised into nulliparous and parous.

3.2.8.2   Reliability

Intra-examiner reliabilities were analysed using the intraclass correlation coefficient (ICC) estimates and their 95% confidence intervals (95% CI) calculated using a consistency of agreement, single measures, two-way mixed effects model. Inter-examiner reliabilities were calculated using a consistency of agreement, average measures, two-way mixed effects model. An ICC of less than 0.4 was considered poor agreement, 0.4 to 0.59 fair, 0.6 to 0.75 good, and greater than 0.75 excellent.[127,128]

Agreement was also evaluated by plotting the difference of the two measurements against the mean of the two measurements in a Bland-Altman plot, and limits of agreement (LOA) were calculated using two standard deviations (SD) above or below the mean difference ($\bar{d}$). [129,130] A fundamental assumption of the Bland-Altman calculations requires the differences between the two readings to be normally distributed, therefore the differences were assessed for normality using the Shapiro-Wilks test.

The mean difference, represented by $\bar{d}$, provides an estimate of potential bias between the two readings, expressed as the absolute volume of air (cm^3). Bias was considered significant if $\bar{d}$ fell outside the line of equality, zero, and the resulting difference in volume was considered clinically significant. A volume of 10 cm^3 of air was pre-defined as clinically relevant in such cases.

Precision of the observed $\bar{d}$ was calculated using the formula below, where t is the appropriate point of the t distribution with n-1 degrees of freedom and 95% confidence interval, or ɑ=0.05. [129]

Standard error of $\bar{d}$ = $\sqrt{(SD^2/n)}$

Upper 95% CI for Precision = observed value + (t x standard error of observed value)

Lower 95% CI for Precision = observed value - (t x standard error of observed value)

3.2.8.3   Continuous independent variables

Regression analysis was performed to investigate a potential relationship between sensory threshold, age and BMI. Post-estimation evaluation was performed for significant regression models, defined as *p<0.05*. Heteroscedasticity was assessed visually using a scatter plot of residual versus fitted values, and statistically using the Breusch-Pagen test. Non-linearity of the model was assessed by plotting the augmented component plus residuals, and possible outliers were visually identified using an added-variable plot. Finally a plot of actual versus predicted values was used to assess the fit of the model.

3.2.8.4   Binary independent variable

Mann-Whitney U testing was performed to investigate potential relationships between sensory threshold and parity. Multiple regression modelling was performed for significant relationships.

3.2.8.5   Nomograms

Example nomograms for sensory thresholds with a statistically significant regression model were produced to demonstrate how the QST method for stretch thresholds described could be used to produce normative data.

Standard practice when calculating nomograms in QST is to use the upper 95% confidence limit as the boundary for hyposensitivity and the lower 95% confidence limit as the boundary for hypersensitivity.[47,131] The 95% confidence limits around the Log^{10} sensory threshold by the regression line were calculated and data back-transformed into the original units. This was then converted into example nomograms applicable to this cohort to enable visual interpretation.

3.3   Results

3.3.1   Recruitment

Between January 2014 and March 2017, 322 women were screened for eligibility and 100 women enrolled into the study. All 100 women underwent testing for normative data, of whom 20 underwent intra-rater reliability testing and 20 underwent inter-rater reliability testing.

Approximately 67.6% of eligible women consented to participate in the study. The most common reasons for non-eligibility were cervical treatment or language barrier (51.0% and 12.2% of non-eligible women respectively). The reasons for non-participation are stated in Figure 3-IV.

Figure 3-IV. Accrual of participants
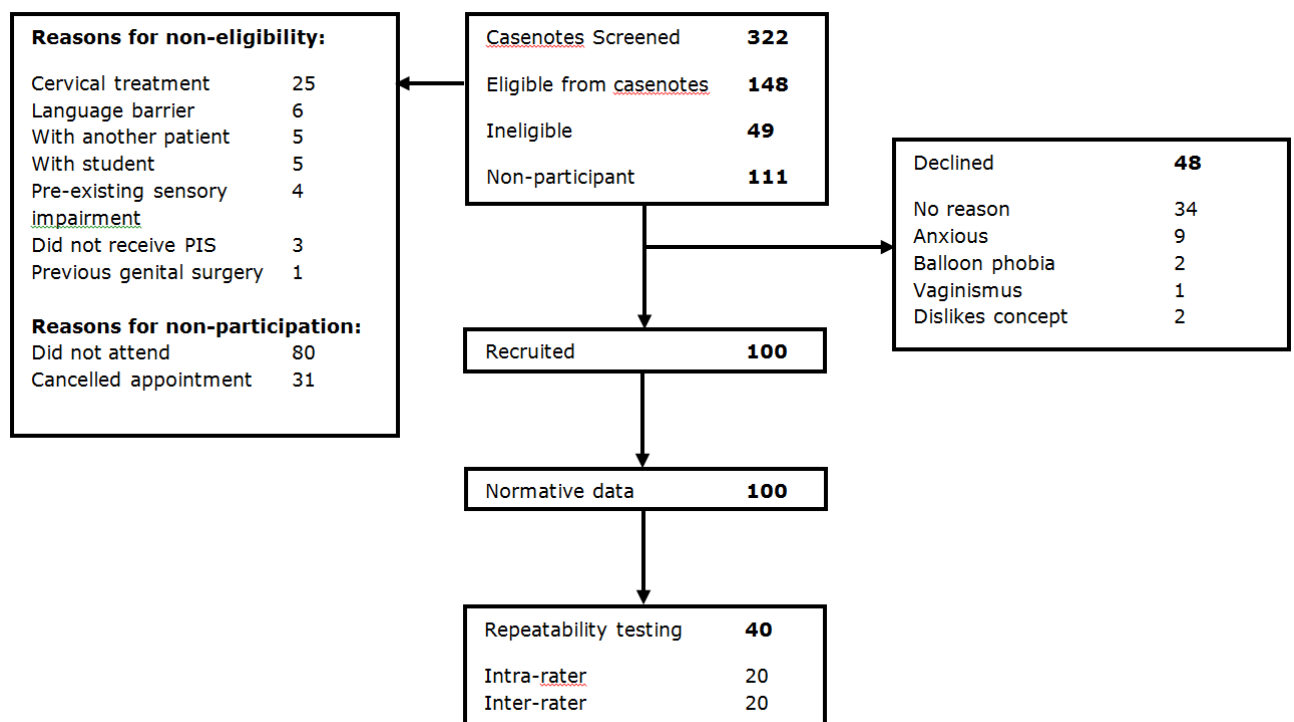
3.3.2　Baseline characteristics of the cohort

The majority of women were pre-menopausal, 88%, with an average age of 33 years and BMI of 25.0kg/m^2. The cohort consisted of both nulliparous and parous women. 79% were of white ethnic origin, 6% Asian and 10% afro-Caribbean. Overall, 41% of participants were using some form of hormonal contraception. The most common reasons for attendance at the clinic were an abnormal smear or routine follow up (48% and 34% respectively).

Table 3−C Baseline characteristics

Demographics		
Age, years Median (IQR)		31 (26-38.5)
Ethnicity Frequency (%)	White	79
	Asian	6
	Afro-Caribbean	10
	Oriental	5
BMI, kg/m^2 Median (IQR)		23.9 (21.4-27.4)
Reproductive Factors		
Parity Frequency	Nulliparous	58
	Para 1-2	26
	Para ≥3	16
Menopausal status Frequency	Premenopausal	88
	Postmenopausal	12
Menstrual cycle Frequency Median (IQR)	Regular	76
	Week of cycle	2 (1-3)
	Irregular	12
	Amenorrhoea	12
Hormonal contraception Frequency	Combined Pill	22
	Progestogen only	14
	LNG-IUS	5
Reason for Colposcopy Frequency	Abnormal smear	48
	Follow up	34
	PCB	8
	IMB	2
	Visual abnormality	3
	Other*	2

Key: n=100, therefore frequency is also percentage; IQR – interquartile range; *Systemic progestogen contraceptives (progesterone only pill, injectable, implant); LNG-IUS levonorgestrel intrauterine system; PCB post-coital bleeding; IMB intermenstrual bleeding; #Other - primary care staff unable to obtain smear test

3.3.3 Intra-rater reliability

Intra-rater reliability was performed to evaluate reliability prior to regression analysis.

ICC's were either excellent or good for all sensory thresholds, Table 3–D.

Bias calculated using Bland-Altman plots was not statistically or clinically significant for any of the sensory thresholds, Figure 3-V. However, the 95% CI for bias for uncomfortable at the vagina and introitus were wide, suggesting these may have variable reliability despite apparently good and excellent intraclass correlation coefficients.

Table 3–D Intra-rater reliability for genital stretch sensation

Sensory threshold	N	Volume, cm^3 median (IQR)	ICC	95% CI	p-value	Agreement
Vagina first awareness	18	15 (12-21)	0.93	0.82 to 0.97	*<0.001*	Excellent
Introitus first awareness	20	14 (9.5-17)	0.95	0.88 to 0.98	*<0.001*	Excellent
Vagina stretch	18	34.5 (28-49)	0.81	0.56 to 0.92	*<0.001*	Excellent
Introitus stretch	20	29 (21-46)	0.88	0.72 to 0.95	*<0.001*	Excellent
Vagina uncomfortable	20	60 (54.5-88.5)	0.78	0.52 to 0.90	*<0.001*	Excellent
Introitus uncomfortable	20	55.5 (40-90)	0.72	0.41 to 0.88	*<0.001*	Good

Key: n – sample size; ICC – two way mixed effects model intraclass correlation coefficients, individual measures reported; CI – confidence interval. Agreement – less than 0.4 poor agreement, 0.4 to 0.59 fair, 0.6 to 0.75 good, and greater than 0.75 excellent.[127,128]

Figure 3-V Bland-Altman plots for genital stretch sensation intra-rater reliability
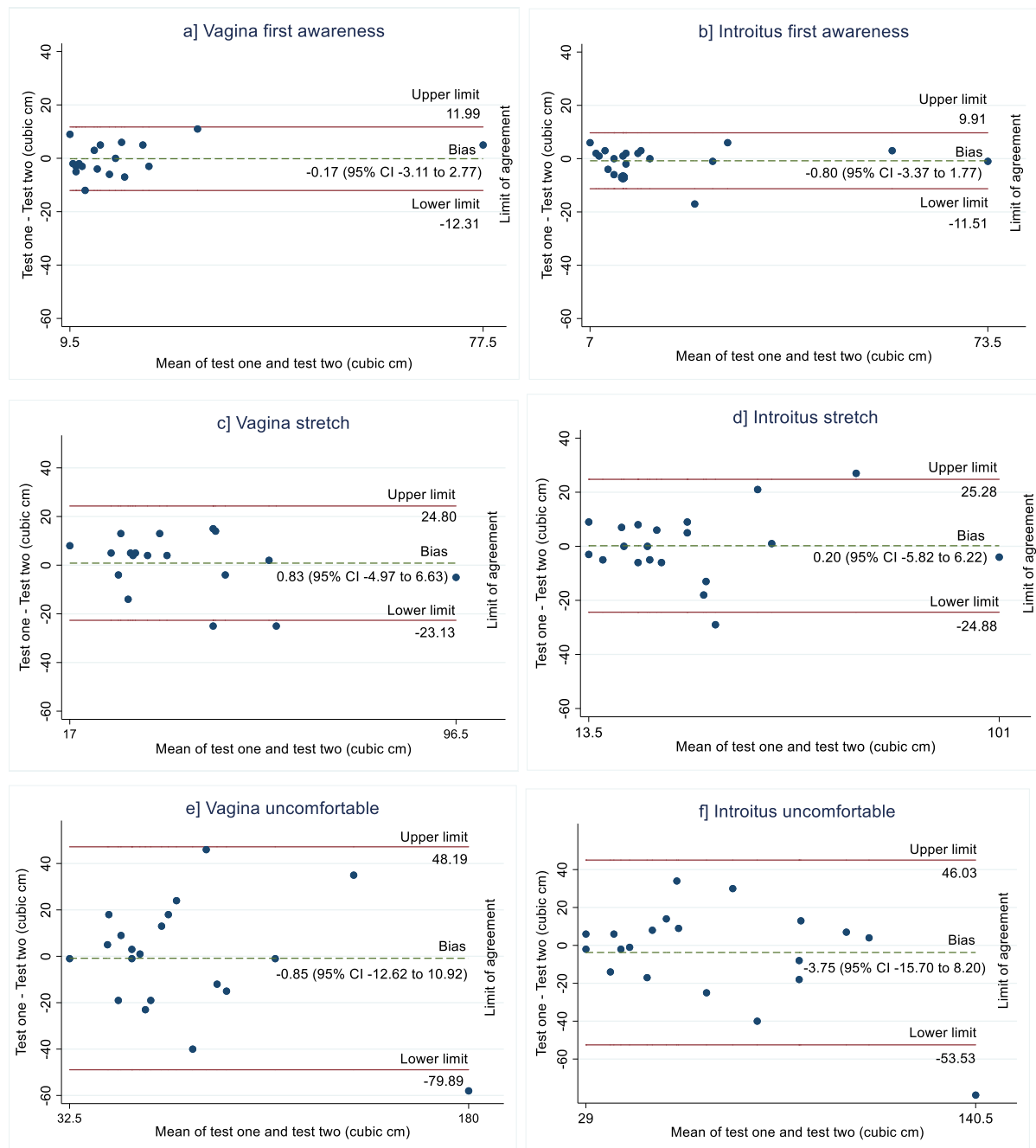
Bias – mean difference between test one and test two (positive value – test one > test two; negative value – test two > test one). Clinically significant bias predefined as ≥10cm³ air.

Limits of agreement – can expect 95% of all differences between test one and test two to fall within the upper and lower limits of agreement.

Note: vagina and introitus uncomfortable 95% CI for bias were very wide, suggesting variable intra-rater reliability despite good and excellent intraclass correlation coefficients.

Key: CI – confidence interval

3.3.4   Inter-rater Reliability

Inter-rater reliability was also assessed prior to regression analysis. ICC's were excellent or good for all sensory thresholds, Table 3–E.

Bias calculated using Bland-Altman analysis was not statistically or clinically significant for any of the sensory thresholds, Figure 3-VI. Again, the 95% CI for bias for uncomfortable at the vagina and introitus were very wide, suggesting variable inter-rater reliability.

Table 3–E Inter-rater reliability for genital stretch sensation

Sensory threshold	n	Volume, cm^3 median (IQR)	ICC	95% CI	*p-value*	Agreement
Vagina first awareness	20	27 (15-47)	0.83	0.57 to 0.93	*<0.001*	Excellent
Introitus first awareness	20	10 (16-22)	0.93	0.83 to 0.97	*<0.001*	Excellent
Vagina stretch	20	57 39-86)	0.71	0.28 to 0.89	*0.004*	Excellent
Introitus stretch	20	35 (25-47)	0.85	0.63 to 0.94	*<0.001*	Excellent
Vagina uncomfortable	20	99 (68-127)	0.76	0.40 to 0.91	*0.002*	Excellent
Introitus uncomfortable	20	58 (40-90)	0.88	0.69 to 0.95	*<0.001*	Excellent

Key: n – sample size; ICC – two way mixed effects model intraclass correlation coefficients, average measures reported; CI – confidence interval. Agreement – less than 0.4 poor agreement, 0.4 to 0.59 fair, 0.6 to 0.75 good, and greater than 0.75 excellent.[127,128]

Figure 3-VI Bland Altman plots for genital stretch sensation inter-rater reliability
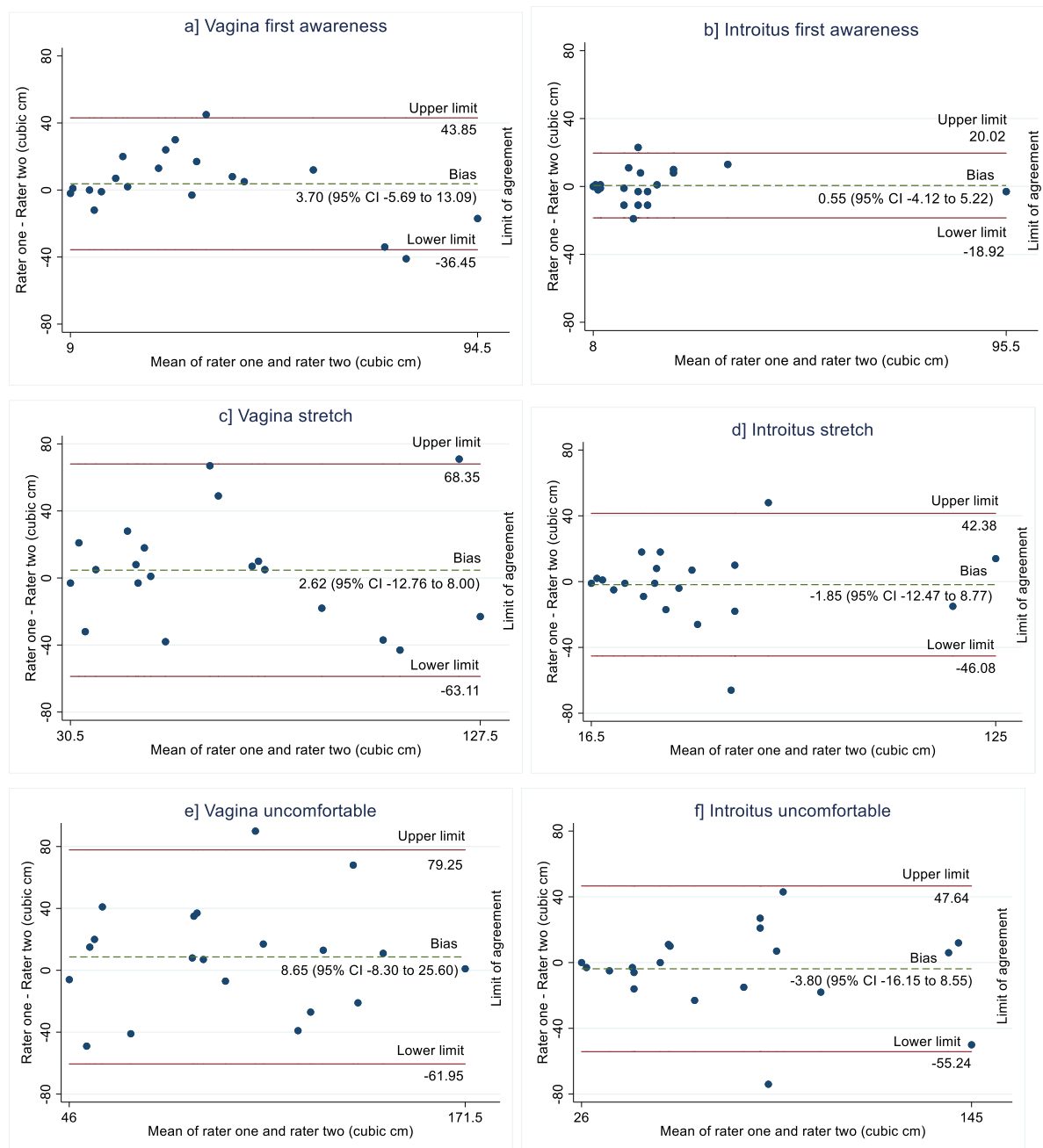
Bias – mean difference between rater one and rater two (positive value – rater one score > rater two score; negative value – rater two score > rater one score). Clinically significant bias predefined as ≥10cm³ air.

Limits of agreement – can expect 95% of all differences between rater one and rater two to fall within the upper and lower limits of agreement.

Note: vagina and introitus uncomfortable 95% CI for bias were very wide, also suggesting variable inter-rater reliability despite excellent intraclass correlation coefficients.

Key: CI – confidence interval

3.3.5   Regression modelling

Reproducibility error was either excellent or good for all sensory thresholds, however both uncomfortable at the vagina and introitus had very wide 95% CI for bias. Uncomfortable is a sensation of pain and evaluates smaller Aδ and C nerve fibres which can also be tested using thermal and cold temperature with a much lower CI for bias. As such, regression analysis was only performed for first awareness at the vagina and introitus, and stretch at the vagina and introitus.

Regression modelling was performed with each independent variable separately. Independent variables that were significant on individual regression were then evaluated as part of a multiple regression model.

3.3.5.1   Normality of the dependent variables

To facilitate regression modelling the dependent variables were assessed for normality using the Shapiro-Wilk test.[132] Tukeys ladders of power and the histograms by transformation suggested a $\log^{10}$ transformation would provide a normal distribution for all the dependent variables.

Logarithm^{10} of the dependent variable was therefore used for all regression analysis.

3.3.5.2   Age

Vagina first awareness

Prior to regression modelling seven outliers were identified and removed, n=83, see Section 3.2.8.1.

A linear regression was run to understand the effect of age on vagina first awareness. To assess linearity a scatterplot of Log^{10} vagina first awareness against age was plotted. Visual inspection of the plot suggested a linear relationship between the variables, Figure 3-VII.

This was confirmed on linear regression which found a statistical association between age and Log^{10} vagina first awareness, Table 3–F.

Overall, post-estimation modelling confirmed the model was a good fit for the data, Figure 3-VIII.

Figure 3-VII Scatter plot of vagina first awareness and age
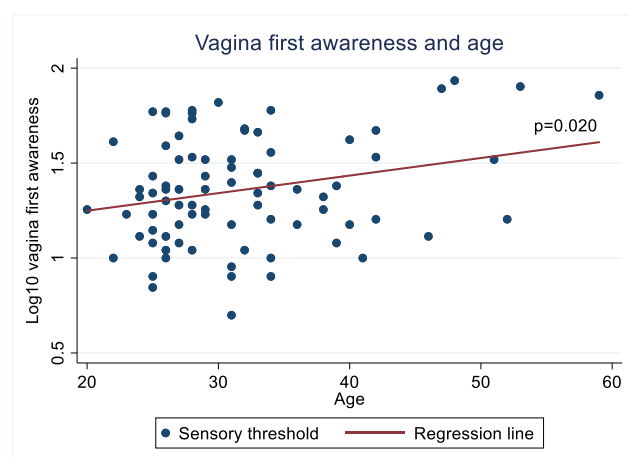
Scatter plot of log^{10} vagina first awareness and age suggesting relationship.

Log-linear regression modelling confirmed the relationship was statistically significant.

Table 3–F Log-linear regression: vagina first awareness and age

Log^{10} vagina first awareness	Co-efficient	95% CI	*p-value*
Constant	1.064	0.812 to 1.317	*<0.001*
Age	0.009	0.002 to 0.017	*0.020*
Overall Model	*p =0.020, R^2 =0.066,* RMSE =0.274, n=83		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Figure 3-VIII Post-estimation regression modelling for vagina first awareness and age

a) Scatter plot of residual versus fitted value, suggesting the model meets the assumption of equal variances around the regression line for all ages (homoscedasticity).
This was confirmed statistically using the Breusch-Pagen test.

a] Residual versus fitted values

p=0.369

b) Scatter plot of the augmented component plus residuals showing the model is a reasonable fit of the data. Although the observed pattern had a slight curve, quadratic and cubic regression modelling were not significant confirming Log-linear regression to be the most appropriate model.

b] Augmented component plus residual

c) Added variable scatter plot did not demonstrate any obvious outliers.
It is normal to have relatively wide confidence intervals when evaluating normative data for sensory thresholds.

c] Added variable plot

coef = .00925106, se = .0038835, t = 2.38

d) Plot of actual and predicted values showed the line of best fit around 25 degrees. In a perfectly fitting model this would demonstrate a straight line at 45 degrees. However the data were evenly spread around the line which is consistent with the natural variation seen in QST for Aδ and Aβ nerve fibres in other areas of the body.

d] Scatter of actual and predicted values

91

Introitus first awareness

Prior to regression modelling, seven outliers were removed, leaving a sample size of 83 for regression analysis, see Section 3.2.8.1.

Visual inspection of a scatterplot of Log^{10} introitus first awareness against age plot suggested a possible linear relationship between the variables, Figure 3-IX. This was confirmed on linear regression which found a statistical association between age and Log^{10} introitus first awareness, Table 3–G.

Overall, post-estimation modelling confirmed the model was a good fit for the data, Figure 3-X.

Figure 3-IX Scatter plot of introitus first awareness and age
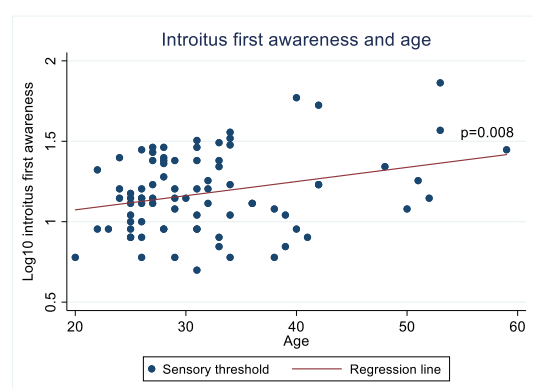
Scatter plot of log^{10} introitus first awareness and age suggesting relationship.

Log-linear regression modelling confirmed the relationship was statistically significant.

Table 3–G Log-linear regression of introitus first awareness and age

Log^{10} introitus first awareness		Co-efficient	95% CI	*p-value*
Standard	*Constant*	0.898	0.685 to 1.110	*0.001*
	Age	0.009	0.002 to 0.015	*0.008*
	Overall Model	*p =0.008,* R^2 *=0.083, RMSE =0.237, n=83*		
Robust	*Constant*	0.898	0.692 to 1.100	*0.001*
	Age	0.009	0.002 to 0.015	*0.008*
	Overall Model	*p =0.010,* R^2 *=0.083, RMSE =0.237, n=83*		

Post-estimation modelling revealed heteroscedasticity (unequal variances around the regression line), Figure 3-X. Therefore the regression model was re-run using the heteroscedasticity-robust standard errors model.

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Figure 3-X Post estimation regression modelling for introitus first awareness and age

a) Scatter plot of residual versus fitted value, suggesting the mode violated the assumption of equal variances around the regression line for all ages (heteroscedasticity).
This was confirmed statistically using the Breusch-Pagen test and a robust errors model used.
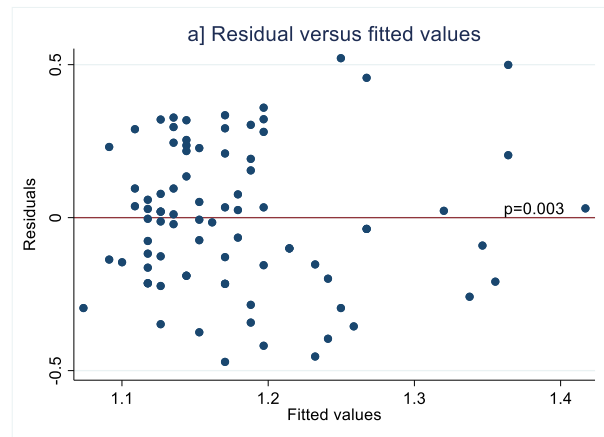
b) Scatter plot of the augmented component plus residuals showing the model is a reasonable fit of the data. Although the observed pattern had a slight curve, quadratic and cubic regression modelling were not significant confirming Log-linear regression to be the most appropriate model.
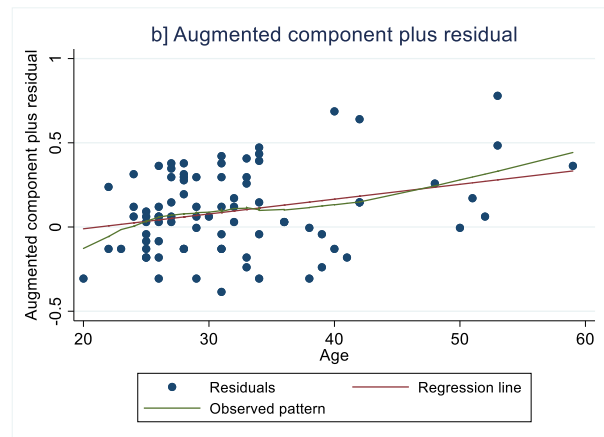
c) Added variable scatter plot did not demonstrate any obvious outliers.
It is normal to have relatively wide confidence intervals when evaluating normative data for sensory thresholds.
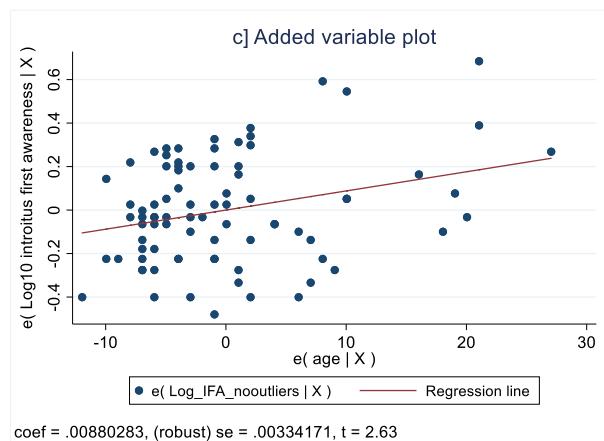
d) Plot of actual and predicted values showed the line of best fit around 22.5 degrees. In a perfectly fitting model this would demonstrate a straight line at 45 degrees. However the data were evenly spread around the line which is consistent with the natural variation seen in QST for Aδ and Aβ nerve fibres in other areas of the body.

93
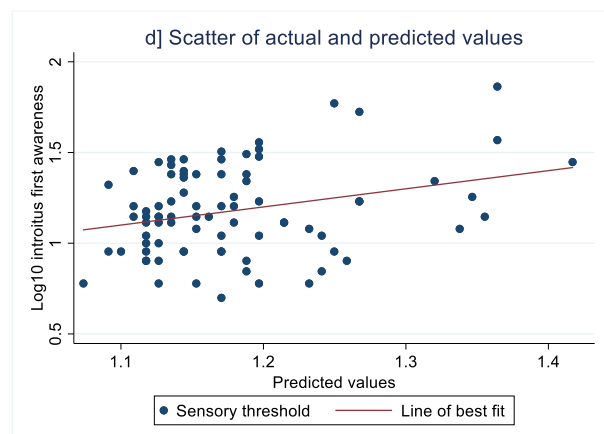
Vagina stretch

Prior to regression modelling, nine outliers were identified and removed, n=89, see Section 3.2.8.1.

A linear regression was run to understand the effect of age on vagina stretch. To assess linearity a scatterplot of Log^{10} vagina stretch against age was plotted. Visual inspection of the plot suggested a linear relationship between the variables, Figure 3-XI.

This was confirmed on linear regression which found a statistical association between age and Log^{10} vagina stretch, Table 3–H.

Overall, post-estimation modelling confirmed the model is a good fit for the data, Figure 3-XII.

Figure 3-XI Scatter plot of vagina stretch and age
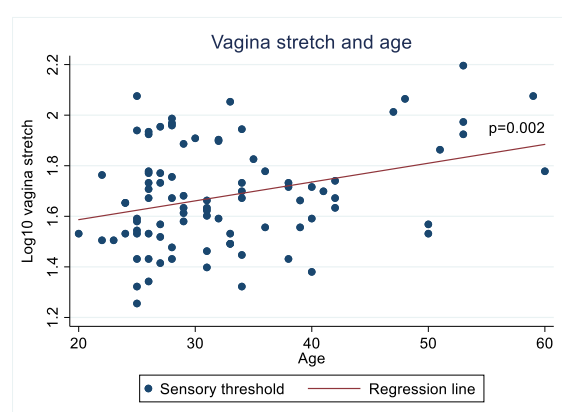
Scatter plot of log^{10} vagina stretch and age suggesting relationship.

Log-linear regression modelling confirmed the relationship was statistically significant.

Table 3–H Log-linear regression for vagina stretch and age

Log^{10} vagina stretch	Co-efficient	95% CI	*p-value*
Constant	1.437	1.281 to 1.595	*<0.001*
Age	0.007	0.003 to 0.012	*0.002*
Overall Model	*p =0.002, R^2 =0.104,* RMSE =0.194, n=89		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Figure 3-XII Post estimation regression modelling for vagina stretch and age

a) Scatter plot of residual versus fitted value, suggesting the model meets the assumption of equal variances around the regression line for all ages (homoscedasticity).
This was confirmed statistically using the Breusch-Pagen test.

b] Residual versus fitted values

p=0.768

b) Scatter plot of the augmented component plus residuals showing the model is a reasonable fit of the data. Although the observed pattern had a slight curve, quadratic and cubic regression modelling were not significant confirming Log-linear regression to be the most appropriate model.

b] Augmented component plus residual

c) Added variable scatter plot did not demonstrate any obvious outliers.
It is normal to have relatively wide confidence intervals when evaluating normative data for sensory thresholds.

c] Added variable plot

coef = .00744249, se = .0023382, t = 3.18
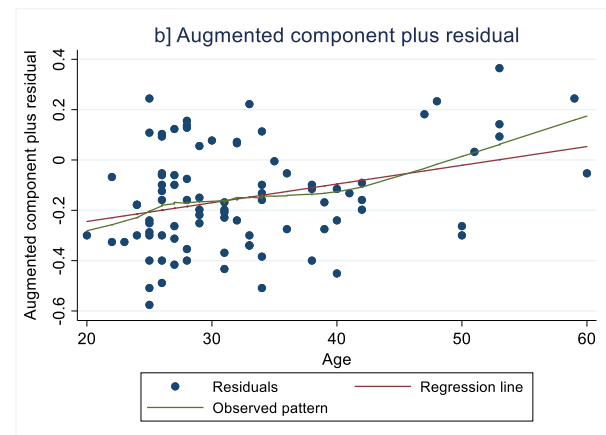
d) Plot of actual and predicted values showed the line of best fit around 30 degrees. In a perfectly fitting model this would demonstrate a straight line at 45 degrees. However the data were evenly spread around the line which is consistent with the natural variation seen in QST for $A\delta$ and $A\beta$ nerve fibres in other areas of the body.

d] Scatter of actual and predicted values

95
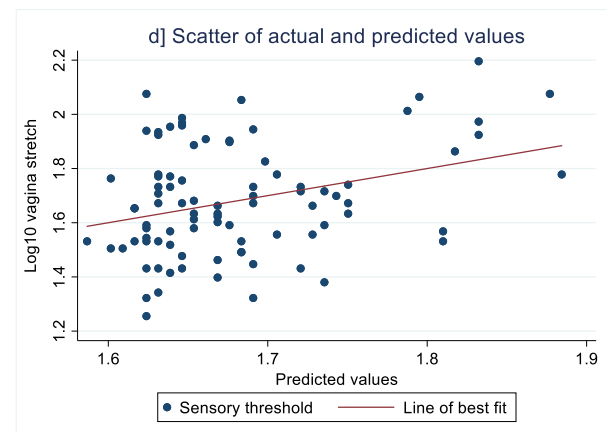
Introitus stretch

Prior to regression modelling, eight outliers were removed, giving a sample size of 91, see Section 3.2.8.1.

A linear regression was run to understand the effect of age on volume of air at introitus stretch. To assess linearity a scatterplot of Log^{10} introitus stretch against age was plotted. Visual inspection of the plot suggested a possible linear relationship between the variables, Figure 3-XIII.

This was confirmed on linear regression which found a statistical association between age and Log^{10} introitus stretch, Table 3–I.

Overall, post-estimation modelling confirmed the model is a good fit for the data, Figure 3-XIV.

Figure 3-XIII Scatter plot of introitus stretch and age
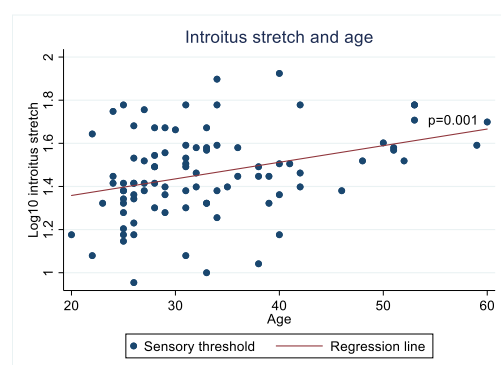
Scatter plot of log^{10} vagina stretch and age suggesting relationship.

Log-linear regression modelling confirmed the relationship was statistically significant.

Table 3–I Log-linear regression for introitus stretch and age

Log^{10} introitus stretch	Co-efficient	95% CI	*p-value*
Constant	1.204	1.053 to 1.355	*<0.001*
Age	0.008	0.003 to 0.012	*0.001*
Overall Model	*p <0.001,* R^2 =0.119, RMSE =0.189, n=91		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Figure 3-XIV Post estimation regression modelling of introitus stretch and age

a) Scatter plot of residual versus fitted value, suggesting the model meets the assumption of equal variances around the regression line for all ages (homoscedasticity).
This was confirmed statistically using the Breusch-Pagen test.

a] Residual versus fitted values

b) Scatter plot of the augmented component plus residuals showing the model is a reasonable fit of the data. Although the observed pattern had a slight curve, quadratic and cubic regression modelling were not significant confirming Log-linear regression to be the most appropriate model.

b] Augmented component plus residual

c) Added variable scatter plot did not demonstrate any obvious outliers.
It is normal to have relatively wide confidence intervals when evaluating normative data for sensory thresholds.

c] Added variable plot

coef = .00770088, se = .00222378, t = 3.46

d) Plot of actual and predicted values showed the line of best fit around 25 degrees. In a perfectly fitting model this would demonstrate a straight line at 45 degrees. However the data were evenly spread around the line which is consistent with the natural variation seen in QST for Aδ and Aβ nerve fibres in other areas of the body.

d] Scatter of actual and predicted values

97

3.3.5.3   BMI

Vagina first awareness

A linear regression was run to understand the effect of BMI on vagina first awareness. To assess linearity a scatterplot of Log_{10} vagina first awareness against BMI was plotted. Visual inspection of the plot did not reveal an obvious linear relationship between the variables, Figure 3-XV.

This was confirmed by a linear regression which established no association between BMI and Log_{10} vagina first awareness, Table 3–J. Therefore post estimation diagnostic plots were not performed.

Figure 3-XV Scatter plot of vagina first awareness and BMI
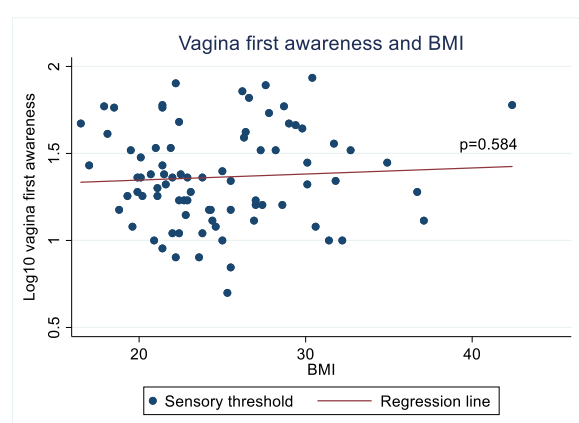
Scatter plot of log_{10} vagina first awareness and BMI demonstrating no relationship. Log-linear regression modelling confirmed this was not significant.

Table 3–J Log-linear regression of vagina first awareness and BMI

Log^{10} vagina first awareness	Co-efficient	95% CI	*p-value*
Constant	1.277	0.958 to 1.600	*<0.001*
BMI	0.003	-0.009 to 0.016	*0.584*
Overall Model	*p =0.584, R^2 =0.004,* RMSE =0.281, n=83		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Introitus first awareness

A linear regression was run to understand the effect of BMI on introitus first awareness. To assess linearity a scatterplot of Log^{10} introitus first awareness against BMI was plotted. Visual inspection of the plot did not reveal an obvious linear relationship between the variables, Figure 3-XVI.

This was confirmed by a linear regression which established no association between BMI and Log^{10} introitus first awareness, Table 3–K. Therefore post estimation diagnostic plots were not performed for this model.

Figure 3-XVI Scatter plot of introitus first awareness and BMI
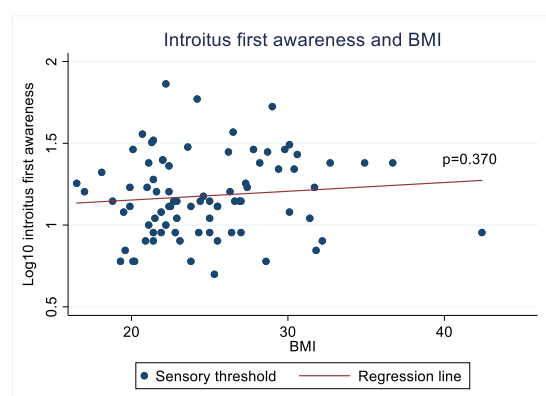
Scatter plot of log^{10} introitus first awareness and BMI demonstrating no relationship. Log-linear regression modelling confirmed this was not significant.

Table 3–K Log-linear regression of introitus first awareness and BMI

Log^{10} introitus first awareness	Co-efficient	95% CI	*p-value*
Constant	1.046	0.749 to 1.343	*0.001*
BMI	0.005	0.006 to 0.017	*0.370*
Overall Model	*p =0.370,* R^2 =0.010, RMSE =0.248, n=83		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Vagina stretch

A linear regression was run to understand the effect of BMI on vagina stretch. To assess linearity a scatterplot of Log^{10} vagina stretch against BMI was plotted, Figure 3-XVII. Visual inspection of the plot did not reveal an obvious linear relationship between the variables.

This was confirmed by a linear regression which established no association between BMI and Log^{10} vagina stretch, Table 3−L. Therefore post estimation diagnostic plots were not performed for this model.

Figure 3-XVII Scatter plot of vagina stretch and BMI
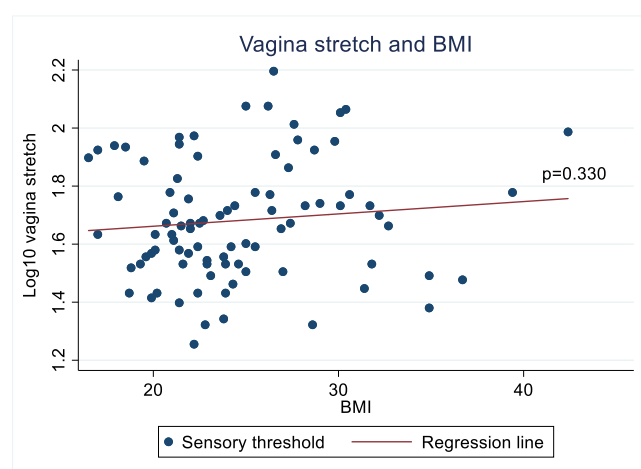
Scatter plot of log^{10} vagina stretch and BMI demonstrating no relationship. Log-linear regression modelling confirmed this was not significant.

Table 3−L Log-linear regression for vagina stretch and BMI

Log¹⁰ vagina stretch	Co-efficient	95% CI	*p-value*
Constant	1.577	1.360 to 1.793	*<0.001*
BMI	0.004	-0.004 to 0.012	*0.330*
Overall Model	*p =0.330,* R^2 =0.011, RMSE =0.205, n=89		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Introitus stretch

A linear regression was run to understand the effect of BMI on introitus stretch. To assess linearity a scatterplot of Log^{10} introitus stretch against BMI was plotted, Figure 3-XVIII. Visual inspection of the plot did not reveal an obvious linear relationship between the variables.

This was confirmed by a linear regression which established no association between BMI and Log^{10} introitus stretch, Table 3–M. Therefore BMI was not incorporated into a multiple regression model.

Figure 3-XVIII Scatter plot of introitus stretch and BMI
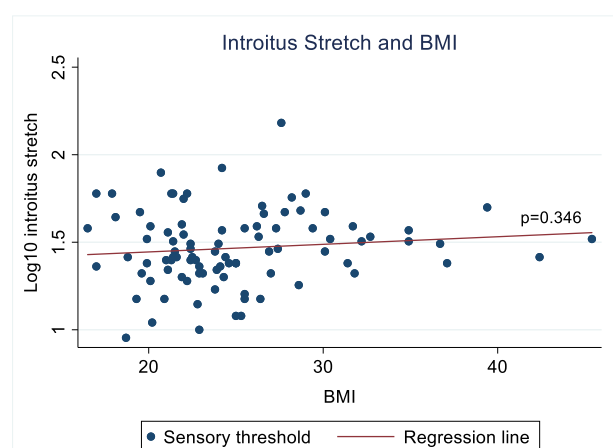
Scatter plot of log^{10} introitus stretch and BMI demonstrating no relationship. Log-linear regression modelling confirmed this was not significant.

Table 3–M Log-linear regression for introitus stretch and BMI

Log^{10} introitus stretch	Co-efficient	95% CI	*p-value*
Constant	1.367	1.170 to 1.563	*<0.001*
BMI	0.004	-0.004 to 0.011	*0.346*
Overall Model	*p =0.346,* R^2 =0.010, RMSE =0.201, n=91		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

3.3.5.4   Parity

Vagina first awareness

The distributions of vagina first awareness for nulliparous and parous women suggested a possible relationship, as assessed by visual inspection, Figure 3-XIX. However the median vagina first awareness for nulliparous (20.0) and parous women (25.5) using Mann-Whitney U testing was not significantly different, z = -1.677, p =0.094.

Furthermore, there was a significant difference in age between the nulliparous and parous groups which could account for the trend towards significance in vagina first awareness (nulliparous median age 27yrs, parous median age 34yrs, z = -5.031, p <0.001).

This was confirmed on multiple regression modelling of parity, age and vagina first awareness which was not significant, Table 3–N, therefore parity was not incorporated into a multiple regression model.

Figure 3-XIX Box and whisker plot of vagina first awareness by parity
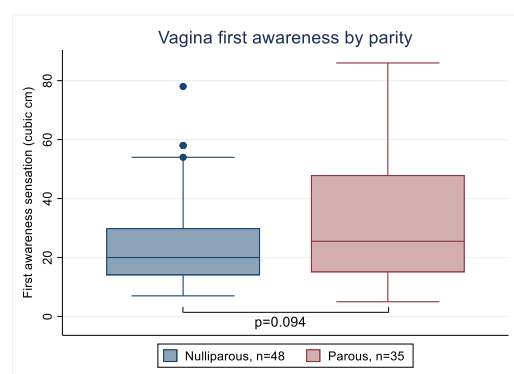
Higher volumes produce greater distension and equate to reduced sensation.
Raw data displayed, Mann Whitney U test comparison shown underneath boxes.

Table 3–N Multiple log-linear regression for vagina first awareness, age and parity

Log^{10} vagina first awareness	Co-efficient	95% CI	*p-value*
Constant	1.095	0.824 to 1.366	*<0.001*
Age	0.008	-0.001 to 0.017	*0.100*
Nulliparous	-	-	-
Parous	0.047	-0.098 to 0.191	*0.522*
Overall Model	p =0.054, R^2 =0.070, RMSE =0.275, n=83		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error

Vagina stretch

The distributions of vagina stretch for nulliparous and parous women suggested a possible relationship, as assessed by visual inspection, Figure 3-XX.

On Mann-Whitney U test the median vagina stretch for nulliparous (41.0) and parous women (54.0) was significantly different, $z = -2.789$, $p=0.005$. However, there was a significant difference in age between the nulliparous and parous groups which could account for effect seen (nulliparous median age 27yrs, parous median age 35yrs, $z = -4.629$, $p <0.001$).

Subsequent multiple regression modelling was significant overall, although age and parity were not individually significant, Table 3–O. On this basis, parity was not incorporated into a multiple regression model at this stage.

Figure 3-XX Box and whisker plot of vagina stretch by parity
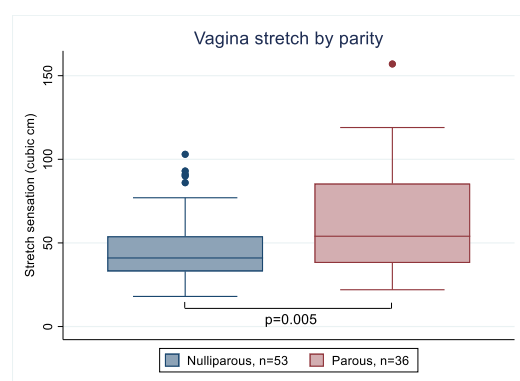
Higher volumes produce greater distension and equate to reduced sensation.

Raw data displayed, Mann Whitney U test comparison shown underneath boxes.

Table 3–O Multiple log-linear regression for vagina stretch, age and parity

Log^{10} vagina stretch	Co-efficient	95% CI	*p-value*
Constant	1.482	1.321 to 1.643	*<0.001*
Age	0.005	-0.0003 to 0.010	*0.063*
Nulliparous	-	-	-
Parous	0.092	-0.002 to 0.185	*0.055*
Overall Model	$p =0.001$, $R^2 =0.142$, RMSE =0.191, n=89		

Although the overall model was significant, age and parity were not individually significant, possibly due to wide variation in age between nulliparous and parous groups, making the model difficult to interpret. Therefore parity was not incorporated into a multiple regression model at this time.

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error.

Introitus first awareness

The distributions of introitus first awareness for nulliparous and parous women suggested a possible relationship, as assessed by visual inspection, Figure 3-XXI.

On Mann-Whitney U test the median introitus first awareness for nulliparous (14.0) and parous women (16.0) was not significantly different, $z = -0.642$, $p=0.521$. Of note, there was a significant difference in age between the nulliparous and parous groups which may have confounded the results (nulliparous median age 27yrs, parous median age 34yrs, $z = -5.231$, $p <0.001$).

This was confirmed on multiple regression modelling of parity, age and introitus first awareness which was not significant, Table 3–P. Therefore parity was not incorporated into a multiple regression model.

Figure 3-XXI Box and whisker plot if introitus first awareness by parity
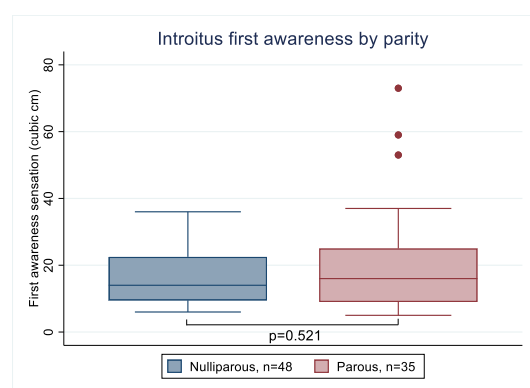
Higher volumes produce greater distension and equate to reduced sensation.
Raw data displayed, Mann Whitney U test comparison shown underneath boxes.

Table 3–P Multiple log-linear regression for introitus first awareness, age and parity

Log^{10} introitus first awareness	Co-efficient	95% CI	*p-value*
Constant	0.861	0.629 to 1.093	*<0.001*
Age	0.011	0.003 to 0.019	*0.009*
Nulliparous	-	-	-
Parous	-0.051	-0.179 to 0.077	*0.428*
Overall Model	$p =0.023$, $R^2 =0.090$, RMSE $=0.237$, n=83		

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error.

Introitus stretch

The distributions of introitus stretch for nulliparous and parous women suggested a possible relationship, as assessed by visual inspection, Figure 3-XXII.

On Mann-Whitney U test the median introitus stretch for nulliparous (26.0) and parous women (32.5) was significantly different, $z = -1.996$, $p=0.046$. However, there was a significant difference in age between the nulliparous and parous groups which could account for effect seen (nulliparous median age 27yrs, parous median age 37yrs, $z = -4.960$, $p <0.001$).

Subsequent multiple regression modelling was significant overall, although parity was not individually significant, Table 3–Q. On this basis, parity was not incorporated into a multiple regression model at this stage.

Figure 3-XXII Box and whisker plot: introitus stretch by parity
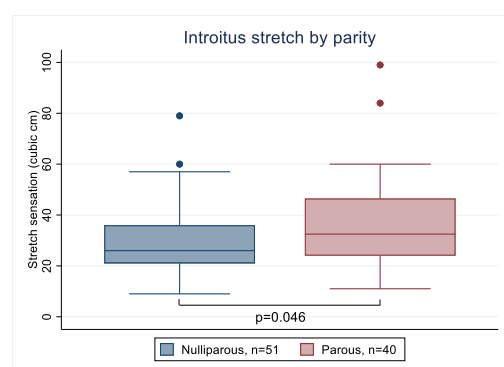
Higher volumes produce greater distension and equate to reduced sensation.
Raw data displayed, Mann Whitney U test comparison shown underneath boxes.

Table 3–Q Multiple log-linear regression for introitus stretch, age and parity

Log^{10} introitus stretch	Co-efficient	95% CI	p-value
Constant	1.215	1.056 to 1.374	*<0.001*
Age	0.007	0.002 to 0.012	*0.008*
Nulliparous	-	-	-
Parous	0.021	-0.072 to 0.114	*0.650*
Overall Model	*p =0.004, R^2 =0.121,* RMSE =0.190, n=91		

Although the overall model was significant parity was not individually significant, possibly due to wide variation in age between nulliparous and parous groups, making the model difficult to interpret. Therefore parity was not incorporated into a multiple regression model at this time.

Key: Log^{10} coefficients presented; CI – confidence interval; RMSE - root mean square error.

3.3.6   Normal Values

The linear regression models described above provided the mathematical description of the age-corrected normal values for this cohort. Using the statistical methodology described in section 3.2.8.5, example nomograms were produced for this cohort, Figure 3-XXIII.

Figure 3-XXIII Example nomograms for the cohort
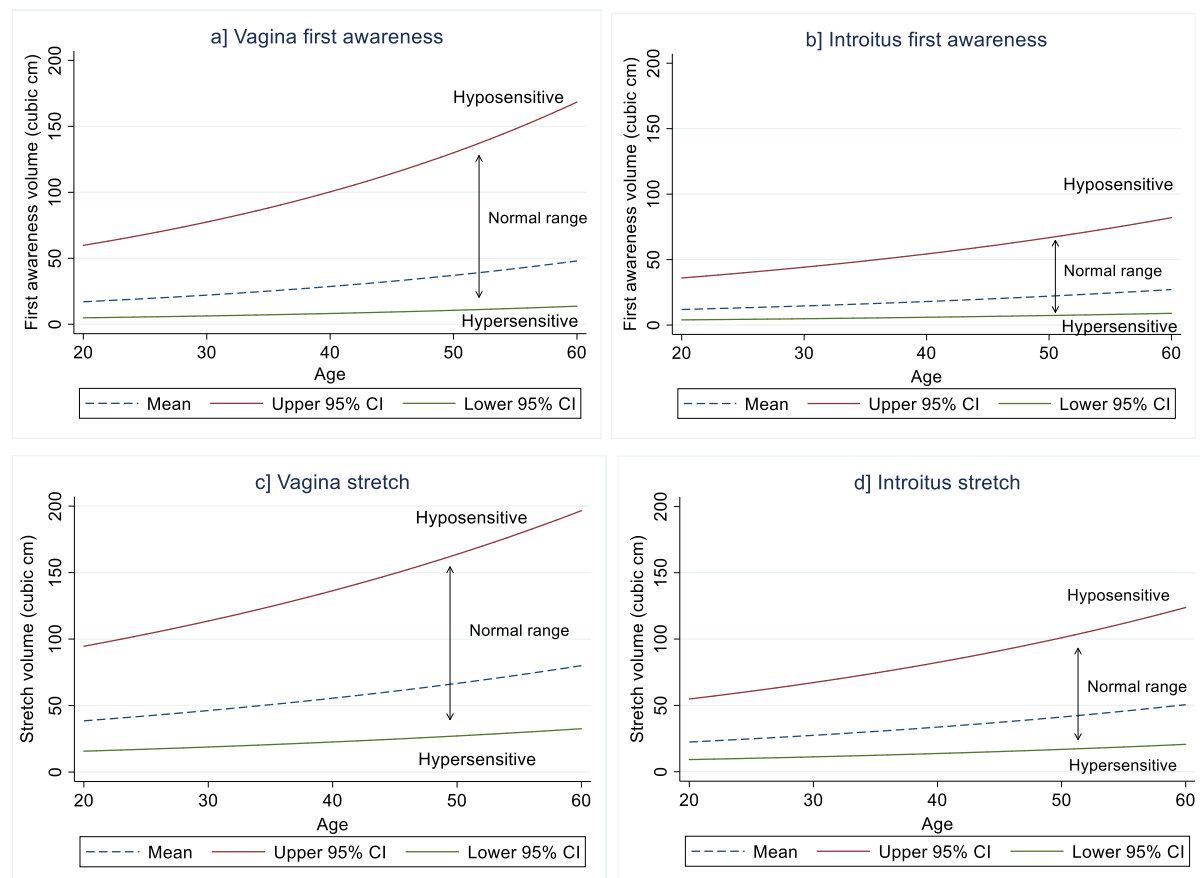
Convention in neurophysiology is to use the upper 95% confidence interval in the normal population as the limit for hyposensitivity and the lower 95% confidence interval as the limit for hypersensitivity.[47,131] The 95% confidence limits around the Log^{10} sensory threshold by the regression line were calculated and data back-transformed into the original units to enable visual interpretation.

Key: CI – confidence interval

3.4 Discussion

3.4.1 Findings & current evidence

This is the first study to measure sensation at the vagina and introitus using the modality of stretch for Aα and Aβ nerve fibres. The results show that stretch sensation thresholds increase with age and have good or excellent repeatability for both intra and inter-rater testing ranging from 5% to 29%. Stretch thresholds can therefore be used as a valid descriptor of Aα and Aβ sensory nerve function at the vagina and introitus. Example nomograms were produced to demonstrate how the QST method for stretch thresholds described could be used to produce normative data.

Only one other study by Vardi et al. has reported on normal sensation data for the female genitalia. They used vibration to measure Aβ nerve function and temperature for Aδ and C fibre nerve function.[47] By comparison they reported repeatability ranging from 11% to 50% for vibration, warm and cold sensation at both the clitoris and vagina.[47] In keeping with this study the authors described increasing vibration sensory thresholds with age at the vagina and clitoris.[47] A number of other researchers have also described increasing vibration and thermal sensory thresholds with age in the limbs.[75,133–135]

BMI was not a covariate for stretch sensation thresholds in this study. To date no other study has evaluated the impact of BMI on sensory thresholds at the female genitalia. The effect of BMI on sensation threshold at the limbs described in the literature is conflicting. Hilz et al. reported no effect of height or weight on vibration sensation at the extremities, whilst Gerr et al. found BMI was a covariate for vibrotactile sensory thresholds at the finger and Bartlett et al. described height as a covariate for vibration thresholds at the foot.[131,135,136]

Parity was not found to be a covariate for stretch sensation thresholds in this study. Although there was a significant difference in age between the nulliparous and parous groups for each of the sensation thresholds tested which makes interpretation of parity as a covariate problematic. A review of the literature found no studies evaluating the effect of parity on female genital sensation.

As age increases so does the width of the confidence interval for the normal range. This is because at larger balloon volumes a greater volume is required to produce the same increase in circumference as those found at smaller balloon volumes.

Whilst repeatability for vagina and introitus uncomfortable is excellent, both demonstrate a wide confidence interval for bias. One explanation is that vagina and introitus uncomfortable are actually assessing the sensory threshold for mechanical stretch

induced pain. Whilst nothing is known about the fibres that cause stretch induced pain, it may be that stretch induced pain is transmitted alongside other pain sensations in Aδ and C sensory nerve fibres. These nerve fibres have a narrower confidence interval when evaluating thermal thresholds but retain a large confidence interval when measuring pain thresholds.[47,133] This indicates that further investigation of Aδ and C fibre function in the vagina should be performed with thermal modalities for warmth and cold temperature sensation, but the technique described could be considered when determining vaginal pain thresholds.

Interpretation of the statistical analysis for parity as a covariate is complex. The lack of age-matched comparisons increases the risk of a type II error which could explain why parity was significant as a covariate on Mann-Whitney U testing but not on multiple regression. Another explanation could be that the study is underpowered to investigate the effect of parity, with post-hoc calculations suggesting a minimum sample size of 210 would be required. Alternatively the null hypothesis is correct and parity may not have an impact on sensory threshold, suggesting vaginal birth may not affect large Aα nerve fibre sensory function.

3.4.2   Strengths

The study has demonstrated QST for genital stretch sensation thresholds is feasible and reproducible.

The study is strengthened by the ethnically diverse cohort with varying parity and BMI, which adds to the generalisability of our results. The study by Vardi et al. did not report the demographics for their cohort.[47] I was unable to contact the authors for further information however the company who developed the machine used in the study stated the women were of mixed parity and predominantly Jewish Israeli ethnicity.

Another strength of the study was the large sample size of 100 which reduced to 83 in the smallest cohort (vagina and introitus first awareness) after removal of outliers. This is compared to a sample size of 89 in the Vardi et al. study, which reduced to 67 after removal of outliers.[47] Several other authors have described normative thermal data for the limbs with sample sizes prior to outlier removal ranging from 26 to 100. [75,137,138]

This study evaluated both intra and inter-rater repeatability using appropriate statistical methodology. Whilst correct statistical analysis should be standard practice in research, it is widely acknowledged in the literature that inappropriate statistical tests are still used when assessing repeatability.[139] The limited studies which have evaluated repeatability

of QST using appropriate statistical methods did not include a breakdown of intra and inter-rater assessment.[47,133]

3.4.3   Limitations

The study excluded women who were unable to speak English due to concerns about maintaining the testing protocol via a telephone interpreter as well as possible communication issues during intimate examinations. However, there is a risk this decision may have altered the ethnic composition of the cohort.

The small number of postmenopausal women meant it was not possible to perform an analysis of the impact of menopausal status on sensory threshold. The lack of oestrogen in postmenopausal women may reduce vaginal wall compliance and this may have affected sensory threshold readings in these women.

Some women underwent testing prior to their Colposcopy and others after a Smear test. This decision was taken because of concerns that the balloon may disrupt cervical cells if performed prior to the Smear test, and that cervical biopsies performed during Colposcopy may heighten sensation. However, by performing the procedure at slightly different times there is a small risk that the Speculum examination prior to the Smear test may have distended the vagina and caused habituation which could have affected the results.

Repeatability was performed with a short minimum time interval of 5 minutes. This was due to departmental constraints on use of the clinic rooms and time constraints from the large capacity of the clinics. The short time frame means the repeatability observed in this study is likely to be the optimum range and may reduce with larger time intervals between readings.

Another limitation of the study is that the test incorporates some degree of Aβ sensory nerve function despite being primarily an assessment of Aα nerve function. It is not possible to comment on the degree to which the test evaluates Aβ nerves. Unfortunately, the strong association between Aβ, Aδ and C sensory nerve dysfunction observed in women with pelvic floor dysfunction means neither would this question be answered by a direct comparison between genital stretch and vibration sensation (which measures only Aβ nerve function).

It was not possible to evaluate the impact of week of menstrual cycle and exogenous hormones on sensory threshold due to the confounding nature of the two variables and small numbers within each subgroup.

The procedure was modified from the test for rectal sensation performed during anorectal manometry studies which can be affected by the rate and pattern of distention, patient position and structure of the rectum.[140-142] Whilst the rate of inflation and position were standardised for stretch testing to mitigate against this, it is possible biomechanical structure of the vagina may have influenced results. In this context it is possible genital stretch hyposensitivity could be due to direct impairment of nerve function, increased vaginal capacity or both.

3.4.4 Generalisability

The nomograms described above are examples based on the data from this study and apply to the local population, although may require adjusting in cohorts of purely Afro-Caribbean and Asian ethnicities due to poor representation in the study cohort.

Recommended practice in neurophysiology is for all departments to validate any pre-existing nomograms against the local population. Due to the subjective nature of all sensory testing and the importance of standardised testing environment and equipment, any minor variations in testing equipment, for example using a different anorectal manometry balloon, should also prompt re-validation of nomograms.

3.4.5 Future work

The role of parity on genital stretch sensation thresholds remains uncertain, and requires a large adequately powered study with age-matched groups prior to developing generalisable nomograms. Before such a large study is conducted it is important to start with first principles and investigate stretch sensation in women before and after vaginal birth.   In addition, another study should collect normative data on women from a range of ethnicities, including Afro-Caribbean and Asian ethnicities to evaluate whether any adjustment is needed.

3.4.6 Conclusion

In summary, this is the first study to demonstrate Aα and Aβ nerve fibres can be reliably tested in the vagina and at the introitus using QST stretch thresholds. Further work is needed to investigate the effect of ethnicity and parity. Once this has been completed the method described can be used to evaluate Aα sensory nerve function in diseases where abnormal Aα nerve function may contribute to pathophysiology, such as vaginal laxity in pelvic organ prolapse.

4 Childbirth and pelvic sensation

4.1 Introduction

Pelvic floor dysfunction (PFD) is estimated to affect one in three to one in five women in the developed world.[1,143] Women with PFD have evidence of sensory pudendal nerve impairment, and this is true for urinary incontinence, sexual dysfunction and pelvic organ prolapse.[94–96,107,109] Pudendal nerve motor function is also impaired in urinary incontinence, pelvic organ prolapse and in anorectal dysfunction.[27,30]

Both sensory and motor nerve function deteriorate with age, mainly due to age related nerve fibre degeneration, and this is thought to contribute to the development of PFD.[27,131] Another factor in the development of pudendal motor nerve injury is childbirth, with numerous studies reporting evidence of pudendal motor nerve impairment following vaginal birth.[37–39,144–146]

The impact of childbirth on pudendal sensory nerve fibres has never been investigatedalthough it is possible sensory nerves may also be injured if motor nerves are affected, since they follow the same pathway.

I hypothesised sensory nerves are injured following childbirth, in particular following vaginal birth. The study objectives were to to investigate the effect of childbirth on pelvic sensation, including an assessment of mode of delivery,the impact of obstetric factors, the relationship between pelvic sensation and changes in pelvic anatomy and the symptoms of pelvic floor dysfunction. As this is the first study using QST in women during pregnancy I will also evaluate the effect of pregnancy itself and gestation on sensory threshold.

4.2 Materials and Methods

4.2.1 Study design

A prospective observational study entitled 'Female genital sensation in pregnancy' was performed, sponsored by Central Manchester Foundation Trust (Study ID R03811) and approved by the North West – Greater Manchester West Research Ethics Committee on the 13/10/14 (14/NW/1316) (Appendix 8.1). The study was co-adopted onto the NIHR portfolio by Reproductive Health and Childbirth (CPMS ID 30525).

4.2.2   Sample size

Data from control subjects in a previous pilot study in our unit reported a mean value for the vibration threshold of 2.71 ±0.67µm and demonstrated that a difference in sensation of 10% was of clinical significance.[94]

In order to reject the null hypothesis ($α=0.05$, $β=80$) allowing for a 50% standard deviation in the paired measurements (before and after delivery) a minimum of 29 subjects was required per group to detect a 10% change. Based on a 30% loss to follow up rate, and primiparous CS rate of 26%, the sample size needed was 147. At the time of the study St Mary's Hospital had approximately 8600 deliveries per year, of which 3800 were primiparous women. To recruit 147 women over an 18 month period required a recruitment rate of 2.2 women per week based on a 46 week academic year.

4.2.3   Recruitment

Nulliparous women of any gravidity were recruited from the antenatal clinic at St Mary's Hospital, Central Manchester Foundation Trust between 10 and 40 weeks gestation. To ensure women with an age range across the childbearing years from a variety of ethnic and educational backgrounds were included in the study, women were recruited from both the midwifery led and doctor led clinics.

Case notes were screened according to the eligibility criteria in Table 5–A and women approached in the clinic room following their consultation. Women were provided with a description of the study and a demonstration of the vibration on their hand using a simple hand-held vibration device.

Recruitment was performed either by the Clinical Research Fellow (Dr Charlotte Mahoney) or one of the Comprehensive Local Research Network (CLRN) nurses, Figure 4-I.

Women seen by the Clinical Research Fellow were provided with an information leaflet and those who chose to participate given an appointment at a convenient time from 28 weeks gestation onwards. The information leaflet contained contact details for the Clinical Research Fellow if they wished to withdraw. Women seen by a CLRN Nurse received an information leaflet and a follow up telephone consultation by the Clinical Research Fellow to confirm eligibility and discuss the study further. Women were given the opportunity to ask questions and they were reassured their decision would not affect their clinical care. Those who chose to participate completed a consent form at the first research appointment performed any time in third trimester from 28 weeks gestation to birth (AN).

Figure 4-I Study schema for childbirth study

Women attending the antenatal clinic's at Manchester University NHS Trust

Case notes screened for eligibility

Eligible   Ineligible

In person discussion of study

Accepted   Declined

AN (≥28+0 weeks gestation)
- Informed consent
- Demographic data
- QST for Aβ nerve fibres (vibration)
  - Vagina, clitoris and index finger
- QST for Aα nerve fibres (stretch)
  - Vagina and introitus
- Pelvic examination: POP-Q
- Symptom questionnaire: ePAQ-PF

Delivery data: manually extracted from electronic maternity record

PN1 (eight to 12 weeks postnatal)
- Confirmed consent
- Postnatal demographic data
- QST for Aβ nerve fibres (vibration)
  - Vagina, clitoris and index finger
- QST for Aα nerve fibres (stretch)
  - Vagina and introitus
- Pelvic examination: POP-Q
- Symptom questionnaire: ePAQ-PF

PN2 (six months postnatal)
- Confirmed consent
- Postnatal demographic data
- QST for Aβ nerve fibres (vibration)
  - Vagina, clitoris and index finger
- QST for Aα nerve fibres (stretch)
  - Vagina and introitus
- Pelvic examination: POP-Q
- Symptom questionnaire: ePAQ-PF

Key: AN = first clinical visit; PN1 = second clinical visit; PN2 = third clinical visit; POP-Q = pelvic organ prolapse quantification system; ePAQ-PF = pelvic floor electronic patient assessment questionnaire pelvic floor.

4.2.4   Study entry

Women were considered eligible for recruitment if they met the inclusion criteria and did not have any of the exclusion criteria, Table 5–A.

Table 4–A Eligibility criteria

Inclusion Criteria	Exclusion Criteria
Age over 18 years	Language barrier requiring interpreter for consultation
Primiparous	Incapacity to consent
Written informed consent	Previous pelvic floor surgery
Attending the Antenatal clinic at St Mary's Hospital	Female genital mutilation (FGM)
Antenatal care provided by Central Manchester Foundation Trust	Medical conditions with or predisposing to sensory impairment e.g. peripheral neuropathy, pre-existing diabetes mellitus, vulvodynia, perineal Crohns, multiple sclerosis
	Neuromodulatory medication e.g. anti-convulsants, anti-psychotics, tri-cyclic antidepressants
	Previous second trimester miscarriage over 16 weeks gestation
	Fetal abnormality
	Multiple pregnancy

4.2.5   Clinical Visits

Women attended three clinical visits. The first visit was performed during the antenatal period (AN) at any time from 28 weeks gestation onwards until birth, the first postnatal visit (PN1) was from eight to 12 weeks postnatal, and the second postnatal visit (PN2) was performed during the sixth month postnatal. Women who were unable to attend appointments during these time frames were subsequently excluded from the study.

4.2.6   Retention

Women who received intra-partum care at another hospital trust were excluded from further follow up as per ethical approval, see Appendices 8.2 and 8.3.

Women were contacted via telephone call to arrange a convenient time for PN1 and PN2 approximately one week before the follow up window of four weeks. If a woman could not be contacted via telephone call she was sent a text message inviting her to attend

for PN1 and PN2. If there was no response her contact details were checked with existing hospital records.

If a woman did not attend a PN1 or PN2 appointment she was contacted via telephone to see if she would like to re-book. In a number of cases the woman had misinterpreted the appointment time or date and attended the new appointment.

Appointments for PN1 and PN2 were re-booked up to three times to improve retention, however after three failed appointments women were considered lost to follow up and excluded from the study.

4.2.7 Demographic data

Demographic data was collected at the first clinical visit (AN) including age, gestation at the time of the research appointment, ethnicity, previous medical history, drug history and smoking status. Height and weight were taken from the measurements performed at the antenatal booking appointment and body mass index (BMI) calculated as weight in kilograms divided by height in metres^2.

4.2.8 Delivery data

Delivery data was gathered from the electronic patient maternity record (K2 Athena). All information at the time of delivery including first and second stages of labour was recorded on the K2 system. It contains mandatory fields for mode of delivery and estimated blood loss which must be completed after delivery to facilitate discharge of the patient to the ward.

Automatically populated fields such as time of onset of labour were cross-checked with hand-typed entries to confirm accuracy. The national central maternity information system or CMiS was used to supply missing data points where possible, or validate data which could not be cross-checked on K2.

K2 was checked after the due date for each woman enrolled in the study. Data was collected regarding the gestation at delivery, duration of each stage of labour, mode of delivery, including the nature of any instrumental delivery or caesarean section (CS), analgesia, presence and nature of any perineal trauma, estimated blood loss and neonatal weight.

4.2.9 Quantitative sensory testing

Quantitative sensory testing for vibration and stretch was performed at each clinical visit (AN, PN1 and PN2). All intimate examinations were performed in the presence of a chaperone. International guidelines do not recommend sham stimulation or randomisation of stimuli when using QST method of limits.[76,77]

QST is a pschyophysical test requiring patient cooperation and concerns could be raised regarding potential distractions in pregnant women such as the baby kicking or feeling uncomfortable. To mitigate against this women were encouraged to find a comfortable position before testing began and advise the clinical fellow if they were distracted by excessive fetal movements during testing.

All testing was performed in the lithotomy position by the Clinical Research Fellow with a chaperone present. Due to the subjective nature of QST it is important to maintain reproducibility, and this was an important consideration in this longitudinal cohort study. QST was performed in one of four quiet rooms within the same department, using the same model of examination couch with the lithotomy stirrups at the same height for each woman at all of her clinical visits.

4.2.9.1 Vibration sensation

Quantitative sensory testing (QST) was used to evaluate medium myelinated Aβ nerve fibres using vibration sensation, as described in section 2.1.1 and 2.1.2.

Sensation testing was performed at each of the three clinical visits (AN, PN1 and PN2). One woman required an alternative probe cover due to latex allergy.

4.2.9.2 Temperature sensation

QST for temperature sensation to measure Aδ and C nerve fibres was not performed.

A previous study involving non-pregnant women in our unit had found temperature QST prolonged the duration of a clinical visit by an hour. Temperature QST at the female genitalia has only fair reproducibility across a one week interval and this may also have decreased further across the six month follow up. This in turn led to ethical concerns subjecting pregnant and post-natal women to prolonged lithotomy positioning for data with only fair reproducibility and the decision was made not to perform temperature QST.

4.2.9.3 Stretch sensation

QST for first awareness and stretch sensation was performed at the vagina and introitus to measure large Aα nerve fibres, as described in section 3.2.6.3.

4.2.10   Pelvic organ prolapse

The standard clinical examination for pelvic-organ prolapse (POP-Q), as described in section 2.2, was performed by the clinical research fellow at AN, PN1 and PN2.

4.2.11   Pelvic floor symptoms

Women were asked to complete a validated electronic symptom specific pelvic floor questionnaire at each clinical visit, ePAQ-PF (Appendix 8.8). This was performed within the department or at home based on the woman's preference.

The questionnaire contained a series of questions relating to the symptoms of urinary, bowel, vaginal and sexual dysfunction. The ePAQ-PF system provided a tutorial, on how to complete the questionnaire, which women self-selected if required. Otherwise women began the questionnaire, and continued to select an answer to the onscreen questions until the questionnaire had been completed.

Answers to individual symptom questions were allocated a numerical score by the computer based on the symptom severity or frequency selected by the women, Table 4–B.

Table 4–B Scoring of ePAQ-PF individual symptom questions

Question score	Symptom severity	Symptom frequency
0	Not at all	Never
1	A little	Occasionally
2	Moderately	Most of the time
3	A lot	All of the time

The ePAQ-PF system group's individual symptom questions into four categories and calls these domains. Based on the woman's answers the computer calculates numerical scores out of 100 for each domain. A score of zero represents a woman who is asymptomatic for that domain, whereas a score of 100 suggests she has the worst severity and frequency of that domain. The domains are:

- Bladder and urinary symptoms - voiding, pain, overactive bladder, stress incontinence and quality of life
- Bowel symptoms - irritable bowel, constipation, evacuation, continence and quality of life
- Vaginal symptoms and prolapse - pain and sensation, capacity, pelvic organ prolapse (POP) and quality of life
- Sex life - urinary, bowel, vaginal, dyspareunia and general sex life.

4.2.12 Statistical analysis

Data were analysed using STATA, version 15.1 for Windows (Statacorp, College Station, Texas).

Two clinical outliers were identified and removed from postnatal data analysis. One had been in a road traffic accident following the AN visit and had developed chronic back pain, the other developed severe sciatica at PN2 which had been present since delivery but not shared.

Data was analysed for normality and non-normal data transformed to facilitate parametric analysis where possible. Data which could not be transformed to a normal distribution was analysed using non-parametric tests.

4.2.12.1 Repeatability of vibration sensation testing

To ensure there was no effect of learnt behaviour over repeated measurements influencing the results, sensory thresholds at test one and two were compared to test five and six for the vagina, clitoris and index finger at each clinical visit.

Data and possible transformations were not normally distributed and therefore non-parametric tests were used. Mann-Whitney U test was used to compare the average sensation threshold for the first two readings to the average sensation threshold for the last two readings at AN, PN1 and PN2 for the index finger, vagina and clitoris.

4.2.12.2 Pelvic sensation in pregnancy

Vibration sensation

Antenatal (AN) vibration thresholds in the cohort were compared to previously published age specific normal ranges in non-pregnant women using graphical assessment.[47] Normal limits in QST are age adjusted to account for age related nerve degeneration.

To facilitate regression modelling and comparison with pre-existing normative data the dependent variables were assessed for normality and non-normal variables $\log^{10}$ transformed. Log linear regression was performed with comparison of the 95% confidence intervals for the regression coefficients to investigate whether age had a greater effect on genital sensory threshold in pregnancy compared to non-pregnant women.

Women with a BMI greater than 29 are more likely to develop carpel tunnel syndrome in pregnancy.[147] To evaluate whether this may also be true for impaired genital sensation, a multivariate regression was performed to investigate the relationship between sensory threshold in pregnancy, age and BMI.

A multivariate regression was also performed to investigate a possible relationship between sensation and gestation.

Stretch sensation

AN stretch sensation thresholds were compared to previously described age adjusted normal ranges from local non-pregnant woman in section 3.2.8.5.

To investigate the effect of pregnancy on age related nerve degeneration, the age coefficient for the pregnant cohort was compared with the age coefficient reported in previously published data from a non-pregnant cohort.

An association between age and sensation was not seen in the pregnant cohort for stretch sensation, therefore $\log^{10}$ linear regression modelling was performed to investigate a potential relationship between BMI and gestation.

4.2.12.3   Sensation threshold data

Absolute sensation

Absolute sensation refers to raw sensation thresholds for either vibration or stretch sensation.

Proportional change in sensation

Proportional change in sensation was also analysed to allow comparison of the degree of recovery in sensation each woman experienced following birth. By analysing the magnitude of change each woman experienced, the effect age related nerve degeneration had on absolute sensation data was circumnavigated.

This was calculated using PN1 and PN2 compared to the AN reading, which was considered the reference baseline in the absence of a pre-conception reading.

PN1 proportional change = PN1/AN

PN2 proportional change = PN2/AN

4.2.12.4   Pelvic sensation after birth

Absolute vibration sensation

Vibration sensation changes across the three clinical visits were assessed graphically using box-plots.

One way repeated measures analysis of variance (ANOVA) were then performed comparing vibration sensory thresholds at AN, PN1 and PN2 visits for each of the locations tested: vagina, clitoris and index finger. Bartlett's test for sphericity was performed to evaluate homogeneity of variances and Box's conservative epsilon was used for interpretation of ANOVA output where this assumption was violated. Post-hoc pairwise comparison testing was performed using the Bonferroni multiple comparison test.

Proportional change in vibration sensation

Proportional change was assessed visually using box-plots. Data was transformed and a paired t-test was performed to compare proportional change in vibration sensation at PN1 and PN2.

Absolute stretch sensation

First awareness and stretch sensation for the vagina and introitus were evaluated visually using box-plots. Tukeys ladders of power confirmed raw and transformed data did not follow a normal distribution, therefore non-parametric tests were used. Kruskal Wallis equality of populations rank test was used to compare sensation thresholds at AN, PN1 and PN2 visits for the introitus and vagina. Dunn's test was used for post-hoc pairwise comparisons.

Proportional change in stretch sensation

Proportional change was assessed visually using box-plots. Raw and transformed data were not normally distributed, therefore Kruskal Wallis was used to compare proportional change in stretch sensation. Dunn's test was used for post-hoc pairwise comparison.

4.2.12.5   Mode of delivery

Women who underwent an emergency caesarean section (EmCS) following a failed trial of forceps delivery were included in the instrumental rather than CS group, as it was thought the presence of forceps within the pelvis could still cause nerve damage, even if the baby was not delivered vaginally.

To account for individual variation in sensation threshold and the confounding effect of age, the proportional change in sensation was used to compare the effect of mode of delivery on sensation threshold. This was performed for both vibration and stretch

sensation. Proportional change was calculated using PN1 and PN2 compared to the AN reading, which was considered the reference baseline.

PN1 proportional change = PN1/AN

PN2 proportional change = PN2/AN

Proportional change in vibration sensation

Visual assessment of proportional change in vibration sensation and mode of delivery was initially performed using box-plots. One way ANOVA was used to compare proportional change in sensation across mode of delivery at the vagina, clitoris and index finger.

Again, Bartlett's test for sphericity was performed to evaluate homogeneity of variances. Post-hoc pairwise comparison testing was performed using the Bonferroni multiple comparison test.

Proportional change in stretch sensation

The relationship between mode of delivery and proportional change at PN1 and PN2 for first awareness and stretch sensation at the vagina and introitus was initially evaluated using box-plots. Raw and transformed data were not normally distributed therefore Kruskal Wallis was used to compare proportional change at PN1 and PN2 with mode of delivery. Where group wise comparison was significant, Dunn's test was used for post-hoc pairwise comparisons.

4.2.12.6   Dilatation at caesarean section

Dilatation at CS and change in vibration sensation was visually assessed using a scatterplot. Data was $\log^{10}$ transformed to facilitate logistic-linear regression modelling to assess for potential correlations. Post-regression modelling was not performed as there were no significant predictive relationships.

4.2.12.7   Duration of labour

The relationship between change in vibration sensation and total length of labour, total length of second stage and active second stage was initially assessed using scatterplots. Data was then $\log^{10}$ transformed and multiple linear regression modelling performed with mode of delivery as a covariate.

4.2.12.8   Birth weight

The birth weight of babies born by vaginal birth, normal vaginal delivery (NVD) or instrumental delivery (ventouse, forceps and failed forceps proceeding to fully dilated emergency CS) were included in the analysis.

Birth weight and change in vibration sensation were visually assessed using scatterplots. Change in vibration sensation was $\log^{10}$ transformed and multiple linear regression modelling performed with mode of delivery as a covariate.

4.2.12.9 Pelvic anatomy after birth

POP-Q stages for the anterior vaginal wall, Ba and the posterior vaginal wall, Bp were dichotomised into no POP, defined as leading edge above the hymen, and POP, defined as leading edge at or beyond the hymen. There were no cases of uterine POP.

Absolute vibration sensation

Data was transformed using the inverse square root $(1/\sqrt{x})$ and analysed using an unpaired student's t-test to compare vaginal vibration sensory thresholds at PN1 and PN2 in women with anterior and posterior compartment prolapse and women without.

Proportional change in vibration sensation

Student's t-test with $\log^{10}$ transformed data was used to compare change in vaginal vibration sensory thresholds at PN1 and PN2 in women with anterior and posterior compartment prolapse and women without.

4.2.12.10 Pelvic floor dysfunction symptoms

Individual symptom questions

Individual symptom questions were identified for analysis based on clinical relevance. For vaginal sensory thresholds these were reduced bladder and urinary sensation, vaginal sensation, vaginal laxity and lack of sensation during intercourse. The symptom question relevant to the clitoris was lack of sensation during intercourse.

Scores were dichotomised into symptom absent (women reporting symptom severity as 'not at all') and symptom present (women reporting symptom severity as 'a little', 'moderately' and 'a lot').

Absolute vibration sensation thresholds

Vibration sensory thresholds were dichotomised into normal and abnormal based on previously published normative data in non-pregnant controls.[47] Fishers exact test was used to compare the presence or absence of an individual symptom for normal and abnormal sensory thresholds at each location across AN, PN1 and PN2.

Proportional change in vibration sensation

Change in vibration sensation was classified as improved or deteriorated when compared to the AN baseline. Fishers exact test was used to compare the presence or absence of an individual symptom for improved or deteriorated sensation at each location across AN, PN1 and PN2.

Symptom domain scores

Domain scores were also identified for analysis based on clinical relevance. For vaginal sensation these were urinary pain, urinary voiding, overactive bladder, irritable bowel syndrome, bowel evacuation, bowel continence, vaginal pain and sensation, vaginal capacity, POP, general sex life. For clitoral sensation this was general sex life.

Absolute vibration sensation thresholds

Vibration sensory thresholds were dichotomised into normal and abnormal based on previously published normative data in non-pregnant controls.[47] Mann Whitney U test was used to compare domain scores for normal and abnormal sensory thresholds at each location across AN, PN1 and PN2.

Proportional change in vibration sensation

Change in vibration sensation was classified as improved or deteriorated when compared to the AN baseline. Mann-Whitney U test was used to compare domain scores for improved and deteriorated change in vibration sensation at each location for PN1 and PN2.

4.3 Results

4.3.1   Recruitment

Between May 2014 and February 2017 683 case notes were screened for eligibility, of whom 504 women were eligible, of whom 310 were seen face to face, of whom 204 initially agreed to attend for an appointment, of whom 150 enrolled onto the study, Figure 4-II.

Figure 4-II Screening and accrual of participants

Eligibility screen:	
Case notes screened	683
Eligible	**504**
Ineligible	179

Reasons for non-eligibility:	
Language barrier	75 (41.9%)
Multiple pregnancy	34 (19.0%)
Medical condition predisposing to sensory impairment	28 (15.6%)
Fetal abnormality	13 (7.3%)
Neuromodulatory medication	6 (3.4%)
Transfer of care	6 (3.4%)
Other	6 (3.4%)
Female genital mutilation	4 (2.2%)
BMI ≥40	3 (1.7%)
Incapacity to consent	2 (1.1%)
Previous pelvic floor surgery	1 (0.6%)
Previous 2nd trimester miscarriage	1 (0.6%)

Of those who were eligible:	
Seen in person	**310**
Not seen*	194

Of those who were seen:	
Agreed	**204**
Declined	106

Reasons declined:	
No reason given	82 (77.4%)
Dislikes concept	12 (11.3%)
Phobia of pelvic examinations	5 (4.7%)
Time off work	3 (2.8%)
Distance for follow up	2 (1.9%)
Anxious re: induction of labour	2 (1.9%)

Of those who agreed:	
Consented	**150**
Cancelled	28
Did not attend	26

Some women were not seen in person due to researcher factors (researcher recruiting another woman, performing a clinical visit or clinical duties, no clinical room available) or participant factors (unable or declined to wait or admitted immediately from ANC).

4.3.2 Retention

Of the 150 women who attended the AN visit, 24 (16%) were lost to follow up at PN1 and an additional 10 (6.7%) were lost to follow up at PN2, Figure 4-III. This included five women who declined to continue in the study at PN1, one was unhappy with her birth experience, one cited the cost of hospital parking, one cited the long journey to hospital and two did not volunteer a reason.

Of the 24 women lost to follow up at PN1, seven women had a CS, 11 women had a NVD and six women had an instrumental delivery. At PN2, four women were lost to follow up after a CS, five after a NVD and one after an instrumental delivery.

All women lost to follow up at PN1 were later contacted with an invitation to participate at PN2 however they either declined or were uncontactable.

Figure 4-III Retention of participants


```
 ┌──────────┐     ┌──────────────────────────────────────────┐
 │   AN     │     │ Lost to follow up (n=24)                   │
 │ n = 150  │     │ Unable to contact         7 (29.2%)        │
 └────┬─────┘     │ Declined to continue      5 (20.8%)        │
      │           │ Delivered elsewhere       4 (16.7%)        │
      ├──────────▶│ Repeatedly did not attend 3 (12.5%)        │
      │           │ Unable to attend in time  2 (8.3%)         │
      │           │ Unwell child              2 (8.3%)         │
      ▼           │ Abroad                    1 (4.2%)         │
 ┌──────────┐     └──────────────────────────────────────────┘
 │   PN1    │
 │ n = 126  │     ┌──────────────────────────────────────────┐
 └────┬─────┘     │ Lost to follow up (n=10)                   │
      │           │ Unable to contact         4 (4%)           │
      ├──────────▶│ Repeatedly did not attend 3 (3%)           │
      │           │ Abroad                    2 (2%)           │
      ▼           │ Moved out of region       1 (1%)           │
 ┌──────────┐     └──────────────────────────────────────────┘
 │   PN2    │
 │ n = 116  │
 └──────────┘
```
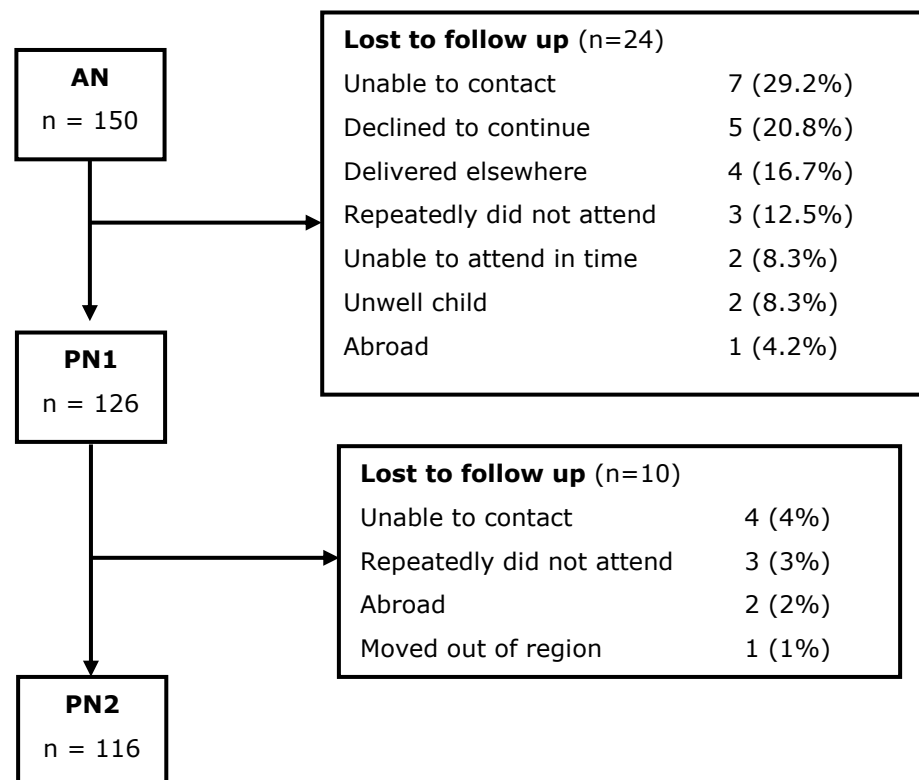
Key: AN = anytime in the third trimester of pregnancy; PN1 = eight to 12 weeks postnatal; PN2 = six months postnatal. A condition of ethical approval was that women had to deliver at the host hospital, therefore women who delivered elsewhere were subsequently excluded from the study. Appointments were re-booked up to three times to improve retention, women who failed to attend three appointments were considered lost to follow up and excluded from the study.

4.3.3 Baseline cohort characteristics

The demographics for the cohort are detailed in Table 4–C.

Table 4–C Cohort demographics

Demographics			
Age, years		median (IQR)	32 (28-36)
Ethnicity	White	n (%)	125 (83.3%)
	Asian		9 (6.0%)
	Black		8 (5.3%)
	Oriental		3 (2.0%)
	Mixed		5 (3.3%)
BMI, kg/m^2		median (IQR)	24.3 (21.55-28.3)
Reproductive Factors			
Gestation, weeks	At first clinical visit	median (IQR)	32 (28-35)
	At birth		39 (39-40)
Mode of delivery	NVD	n (%)	59 (39.3%)
	Forceps		37 (24.7%)
	Ventouse		6 (4.0%)
	CS		48 (32.0%)
Type of CS	No labour	n (%)	
	• ElCS		25 (52.1%)
	• EmCS <4cm		12 (25.0%)
	Laboured		
	• EmCS 4-7 cm		4 (8.3%)
	• EmCS 8-10cm		7 (14.6%)
Indication for CS	Fetal distress	n (%)	11 (22.9%)
	Maternal request		11 (22.9%)
	Breech		9 (18.8%)
	Failure to progress		5 (10.4%)
	Large for dates		3 (6.3%)
	Failed trial of instrumental		3 (6.3%)
	Other		3 (6.3%)
	Failed induction		2 (4.2%)
	Placenta praevia		1 (2.1%)
Duration of labour, minutes	Total length of labour	median (IQR)	359 (217-603)
	Total second stage		82 (33-174)
	Active second stage		45 (21-90)

Table continued overleaf.

Reproductive Factors continued			
Position of vertex in second stage	OA	n (%)	62 (59.6%)
	OT		20 (19.2%)
	OP		18 (17.3%)
	Not documented		4 (3.9%)
Analgesia in labouring women	Epidural	n (%)	35 (30.9%)
	Remifentanil PCA		17 (25.0%)
Anal sphincter injury	3rd degree tear	n (%)	3 (3.2%)
	4th degree tear		1 (1.1%)
Birth weight, g		median (IQR)	3345 (3090-3668)
Blood loss, ml		median (IQR)	500 (325-700)

Key: IQR – interquartile range; NVD – normal vaginal delivery; CS – caesarean section, ElCS – elective caesarean section; EmCS – emergency caesarean section; OA – occiput anterior; OP – occiput posterior; OT – occiput transverse; PCA – patient controlled analgesia

4.3.4   The influence of learnt behaviour

To investigate the possibility that learnt behaviour may influence sensation measurements across the clinical visits, the impact of repeated stimuli during a single clinical visit on sensation threshold was analysed.

The median difference between the first and last two measurements at each location were then compared to the median difference between measurements at AN and PN1, and AN and PN2, Table 4–D. The data suggest learnt behaviour did not significantly influence change across the clinical visits in this cohort.

Table 4–D The influence of learnt behaviour during a single visit and change across the clinical visits

Effect of learnt behaviour at each clinical visit *Median difference (IQR)*	Change in sensation across clinical visits	
	AN vs PN1 *Median change (IQR)*	**AN vs PN2** *Median change (IQR)*
Vagina		
AN 0.38 (-0.08 to 0.70) PN1 0.25 (-0.07 to 0.55) PN2 0.09 (-0.25 to 0.31)	0.77 (0.38 to 1.86) *Not influenced by learnt behaviour*	1.12 (0.54 to 2.27) *Not influenced by learnt behaviour*
Clitoris		
AN 0.23 (-0.03 to 0.57) PN1 0.17 (-0.13 to 0.48) PN2 0.13 (-0.13 to 0.30)	0.79 (0.42-1.50) *Not influenced by learnt behaviour*	0.93 (0.36-1.76) *Not influenced by learnt behaviour*
Index Finger		
AN 0.11 (-0.14 to 0.48) PN1 0.10 (-0.10 to 0.32) PN2 0.08 (-0.13 to 0.20)	0.56 (0.24-1.17) *Not influenced by learnt behaviour*	0.87 (0.33-1.64) *Not influenced by learnt behaviour*

At each clinical visit six consecutive measurements for vibration sensation were taken. The difference between the first and last two measurements during a single clinical visit were calculated to see the effect of learnt behaviour. This was compared with the change seen across the clinical visits to assess whether any change seen was simply due to the effect of learnt behaviour or represented real change.

Key: AN – antenatal visit; PN1 – eight to 12 weeks postnatal; PN2 – six months postnatal; AN vagina and clitoris n=150, index finger n=145; PN1 n=124; PN2 n=114; IQR – interquartile range, negative values = sensation improved, positive values = sensation deteriorated; all values = vibration amplitude in microns

4.3.5　Antenatal changes in sensation

The median gestation at AN was 32 weeks (IQR 28-35).

4.3.5.1　Vibration sensation

When compared to non-pregnant age adjusted normative data 8.0% of pregnant women demonstrated vaginal hyposensitivity, 83.3% of pregnant women demonstrated clitoral hyposensitivity and 40.6% of pregnant women had evidence of hyposensitivity to vibration at the index finger, Figure 4-IV.[47] The accepted definition for hyposensitivity in neurophysiology studies is any reading greater than the 95th percentile for sensation in the normal population.[47,68,75,148]

Due to a technical error, the computer software did not record the sensory threshold data at the index finger for five women at AN.

The relationship between age and sensation in pregnancy was consistent with previously published non-pregnant data, Table 4–E.[47] Gestation in the third trimester and BMI were not associated with vaginal, clitoral or index finger vibration sensation, Table 4–F.

Figure 4-IV Scatter graph showing vibration sensation in pregnancy compared to non-pregnant women
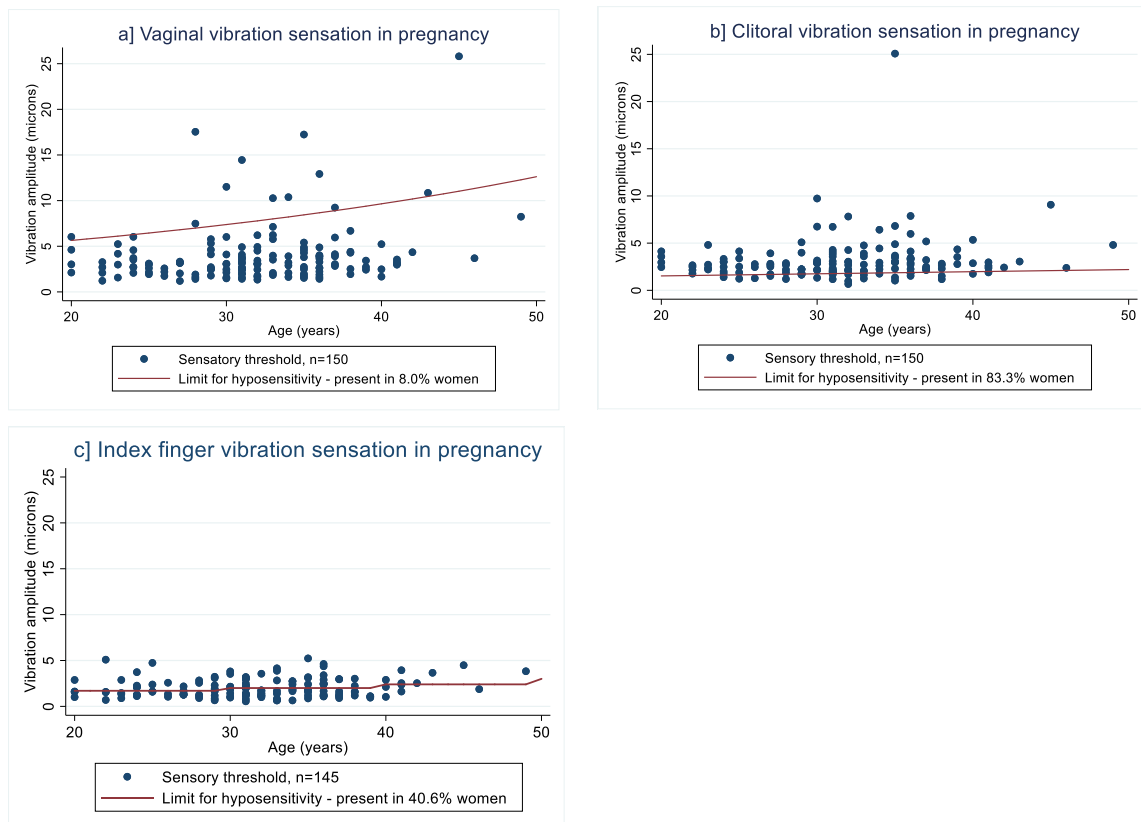
Sensation was compared to previously published age adjusted normative data and limits for hyposensitivity in non-pregnant women.[47] *The limits for hyposensitivity were calculated per year for the vagina and clitoris, but per decade for the index finger. The reason for this is unclear in the publication.*

Higher amplitudes produce a stronger vibration and indicate worse sensation. The clitoris and index finger are much more sensitive than the vagina and therefore have a much lower limit for hyposensitivity.

Missing data points due to technical error (index finger = 5).

Table 4−E The effect of pregnancy on age related nerve changes for Aβ nerve fibres

Location	Age coefficient for vibration sensation (per year)		
	Log^{10} linear regression		
	Pregnant, n=150 Log^{10} coefficient (95% CI)	**Non-pregnant,**[47] n=89 Log^{10} coefficient	**Interpretation**
Clitoris	0.005 (0.00004 to 0.012) *Sensation ↓ with age*	0.006 *Sensation ↓ with age*	No significant difference
Vagina	0.012 (0.003 to 0.017) *Sensation ↓ with age*	0.010 *Sensation ↓ with age*	

To investigate whether pregnancy had an effect on age related nerve degeneration for vibration sensation, my data was compared to previously published normative data in non-pregnant women.[47] With no access to the non-pregnant raw data, analysis was limited to a direct comparison of the age regression coefficient. Log^{10} linear regression was performed on the pregnant cohort. The non-pregnant age coefficient was then compared to the 95% confidence interval for the age coefficient in pregnancy.

Key: Coefficient = multiplier per year increase in age of the log linear fit; CI = confidence interval

Table 4−F Relationship between gestation, BMI and vibration sensation in pregnancy

Gestation, age and vibration sensation			
Log^{10} multiple linear regression			
Location	**Gestation co-efficient**	**95% CI**	***p-value***
Log^{10} vagina	0.0007	-0.0002 to 0 .0017	p=0.123
Log^{10} clitoris	6.36e-06	-0.0010 to 0.0010	p=0.990
Log^{10} index finger	-0.0006	-.0018 to 0.0007	p=0.378
BMI, age and vibration sensation			
Log^{10} multiple linear regression			
Location	**BMI co-efficient**	**95% CI**	***p-value***
Log^{10} vagina	-0.003	-0.008 to 0.002	p=0.281
Log^{10} clitoris	-0.002	-0.008 to 0.004	p=0.447
Log^{10} index finger	-0.002	-.0010 to 0.005	p=0.512

Whilst the overall model may appear significant, closer inspection of the individual variables reveals gestation and BMI are not significant. Missing data points due to technical error (index finger = 5). Key: R^2 = coefficient of determination; RMSE = root mean square error; BMI = body mass index.

4.3.5.2   Stretch sensation

First awareness and stretch sensation at the vagina and introitus were compared to previously reported normative data in non-pregnant women from the local population section 3.2.8.5, Figure 4-V.

Sensation was impaired in 1.4% of women for vagina first awareness, 4.8% for introitus first awareness, 5.5% for vagina stretch and 11.0% for introitus stretch sensation.

In the previous study in non-pregnant women described sensation for first awareness and stretch at the vagina and introitus was associated with age, section 3.2.8.5. However, within the narrow age range of this pregnant cohort there was no relationship between sensation threshold and age, Table 4–G. Similar to vibration sensation, there was also no association between impaired stretch sensation and gestation or BMI, Table 4–H.

Figure 4-V Scatter graph comparing stretch sensation in pregnant and non-pregnant women
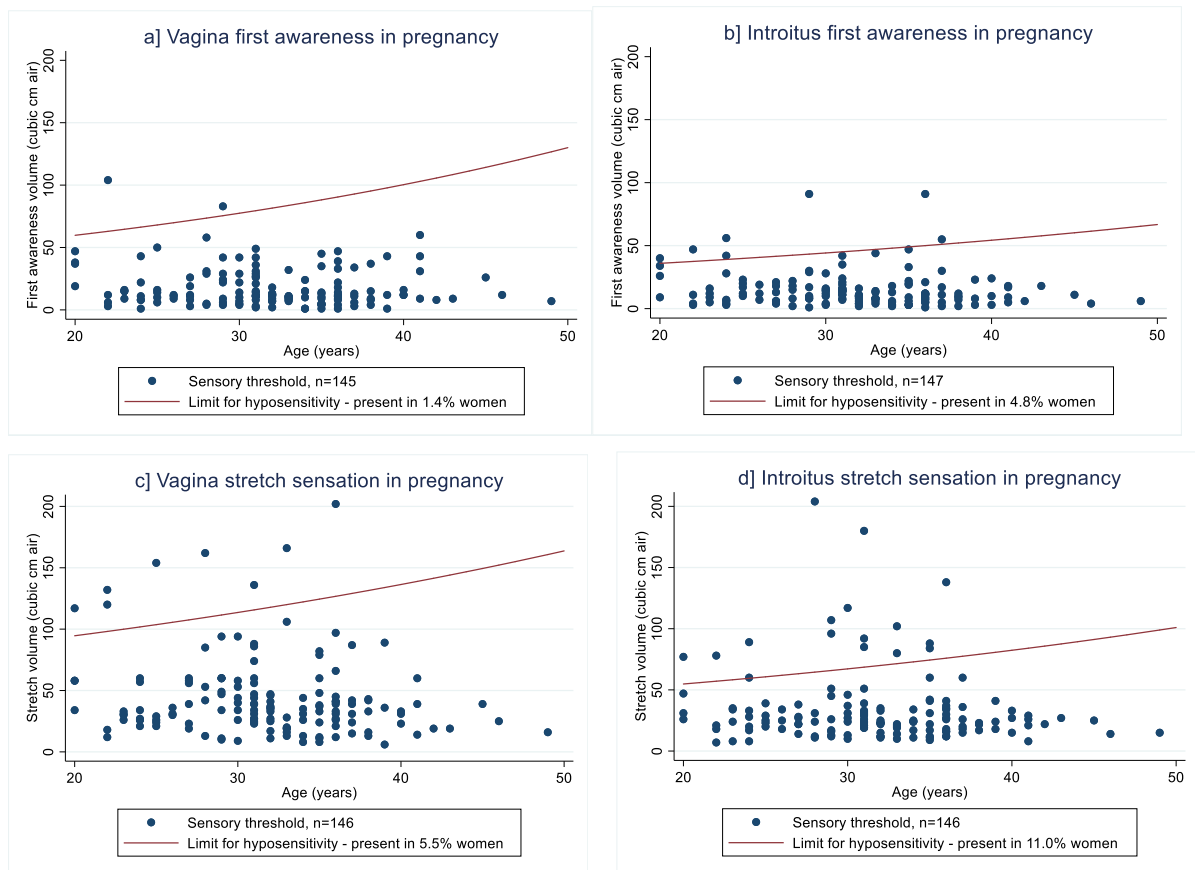
Stretch sensation in pregnancy was compared to non-pregnant women using normative values described in section 3.2.8.5, limit of hyposensitivity calculated using the upper limit of 95% confidence interval. Higher volumes produced greater distention and equated to reduced sensation. The introitus is more sensitive than the vagina and therefore the limit for hyposensitivity is much lower. Missing data points due to technical error (vagina first awareness = 2, vagina and introitus stretch = 1) or declined stretch testing (all = 3).

Table 4–G The effect of pregnancy on age related nerve degeneration for Aα nerve fibres

Location	Age coefficient for stretch sensation Log^{10} coefficient (95% CI)		
	Pregnant *First awareness* n=149***** *Stretch* n=148	**Non-pregnant** *First awareness* n=83 *Stretch n=91*$^{¥}$	**Interpretation**
First awareness			
Vagina	-0.007 (-0.19 to 0.005) No age effect seen	0.006 (0.002 to 0.017), Sensation ↓ with age	Significant difference
Introitus	-0.011 (-0.021 to 0.0001) No age effect seen	0.009 (0.002 to 0.015), Sensation ↓ with age	
Stretch			
Vagina	-0.009 (-0.018 to 0.001) No age effect seen	0.007 (0.003 to 0.012), Sensation ↓ with age	Significant difference
Introitus	-0.006 (-0.014 to 0.002) No age effect seen	0.008 (0.003 to 0.012), Sensation ↓ with age	

Stretch sensation in this cohort was compared to the non-pregnant local population described in section 3.2.8.5, to investigate the effect of pregnancy on age related nerve degeneration. The age coefficients for pregnant and non-pregnant women were compared with their associated 95% confidence intervals.

In pregnant cohort: missing data points due to technical error (vagina first awareness = 2, vagina and introitus stretch = 1) or declined testing (all=3).

Key: *Vagina first awareness n=147; $^{¥}$ Vagina stretch n=89

Table 4–H The relationship between gestation, BMI and stretch sensation in pregnancy

Gestation, age and vibration sensation		
Log^{10} multiple linear regression		
Location	**Gestation co-efficient (95% CI)**	**p-value**
First awareness		
Log^{10} vagina	0.015 (-0.004 to 0 .033)	0.115
Log^{10} introitus	-0.003 (-0.019 to 0.014)	0.754
Stretch		
Log^{10} vagina	0.007 (-0.007 to 0 .019)	0.334
Log^{10} introitus	-0.004 (-0.016 to 0 .008)	0.543
BMI, age and vibration sensation		
Log^{10} multiple linear regression		
Location	**BMI co-efficient (95% CI)**	**p-value**
First awareness		
Log^{10} vagina	0.009 (-0.006 to 0.023)	0.235
Log^{10} introitus	0.003 (-0.010 to 0.016)	0.674
Stretch		
Log^{10} vagina	0.992 (-0.008 to 0.011)	0.724
Log^{10} introitus	0.023 (-0.009 to 0.55)	0.162

Data transformed and log-linear regression analysis performed to investigate the relationship between gestation, BMI and stretch sensation.

Missing data points due to technical error (vagina first awareness = 2, vagina and introitus stretch = 1) or declined testing (all=3).

Key: R^2 = coefficient of determination; RMSE = root mean square error; BMI = body mass index; CI = confidence interval

4.3.6 Post-natal changes in sensation

Vibration and stretch sensation for absolute values and proportional changes were assessed across the clinical visits, irrespective of mode of delivery.

4.3.6.1 Vibration sensation

Absolute vibration sensation

Vibration sensation thresholds across the three time points are displayed in Figure 4-VI. The graphs suggest vibration sensation improves across the postnatal period at the vagina, clitoris and index finger. Statistical analysis confirmed a significant difference in sensation threshold between AN, PN1 and PN2 for the vagina, clitoris and index finger. Pairwise comparison of each time point (AN vs PN1, PN1 vs PN2, AN vs PN2) at the vagina, clitoris and index finger was also significant.

Figure 4-VI Box and whisker plots of absolute vibration sensation thresholds across the three clinical visits
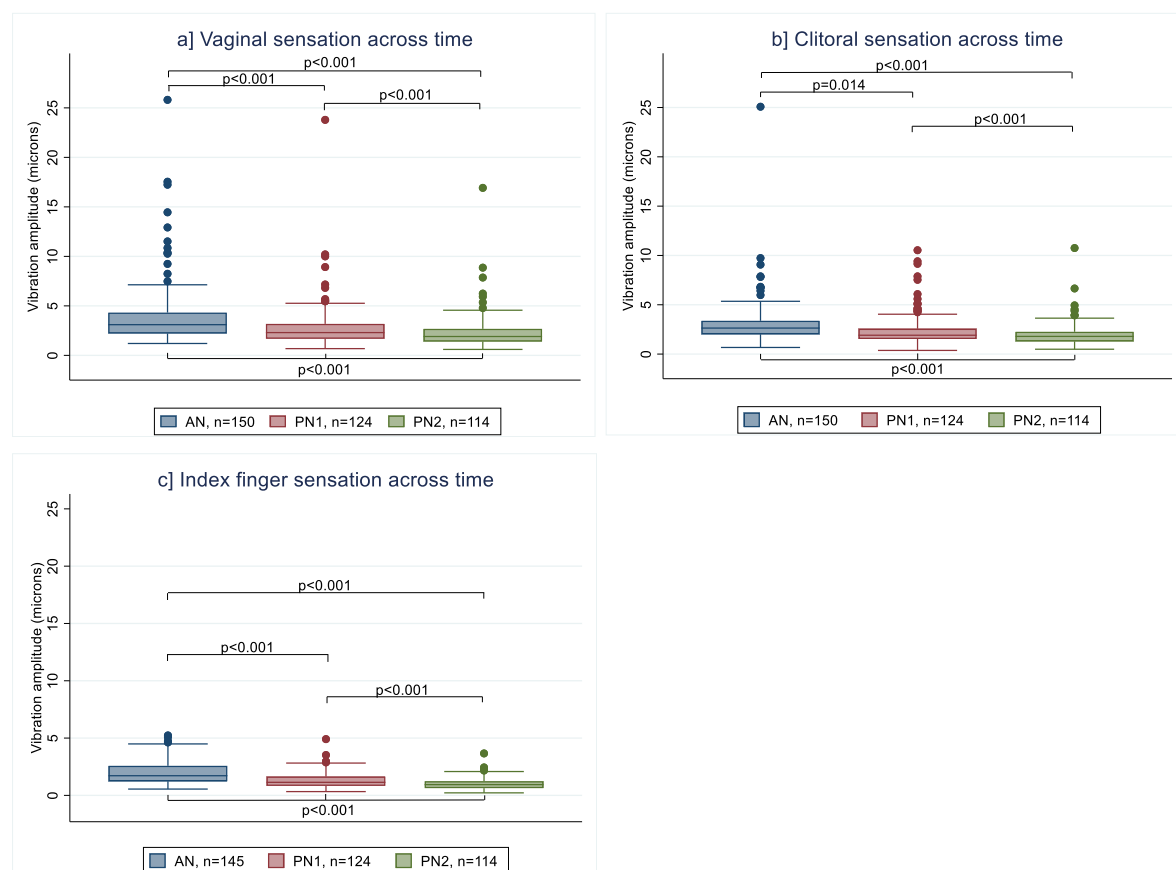
Higher amplitudes produce a stronger vibration and indicate reduced sensation. Raw data displayed. Data transformed with inverse square root, repeated measures ANOVA shown below boxes, with Bonferroni post-hoc pairwise comparison shown above boxes. Two outliers were excluded. Missing data points due to technical error (index finger = 5).

Proportional change in vibration sensation

Proportional change in vibration sensation across PN1 and PN2 compared to AN baseline was also assessed, see Figure 4-VII. Inspection of the graphs suggests vibration sensation improves from PN1 to PN2 when compared to AN baseline.

Statistical analysis confirmed there was a significant improvement in vibration sensation at the vagina, clitoris and index finger from PN1 to PN2 when compared to the AN baseline.

Figure 4-VII Box and whisker plots of the proportional change in vibration sensation across the three clinical visits
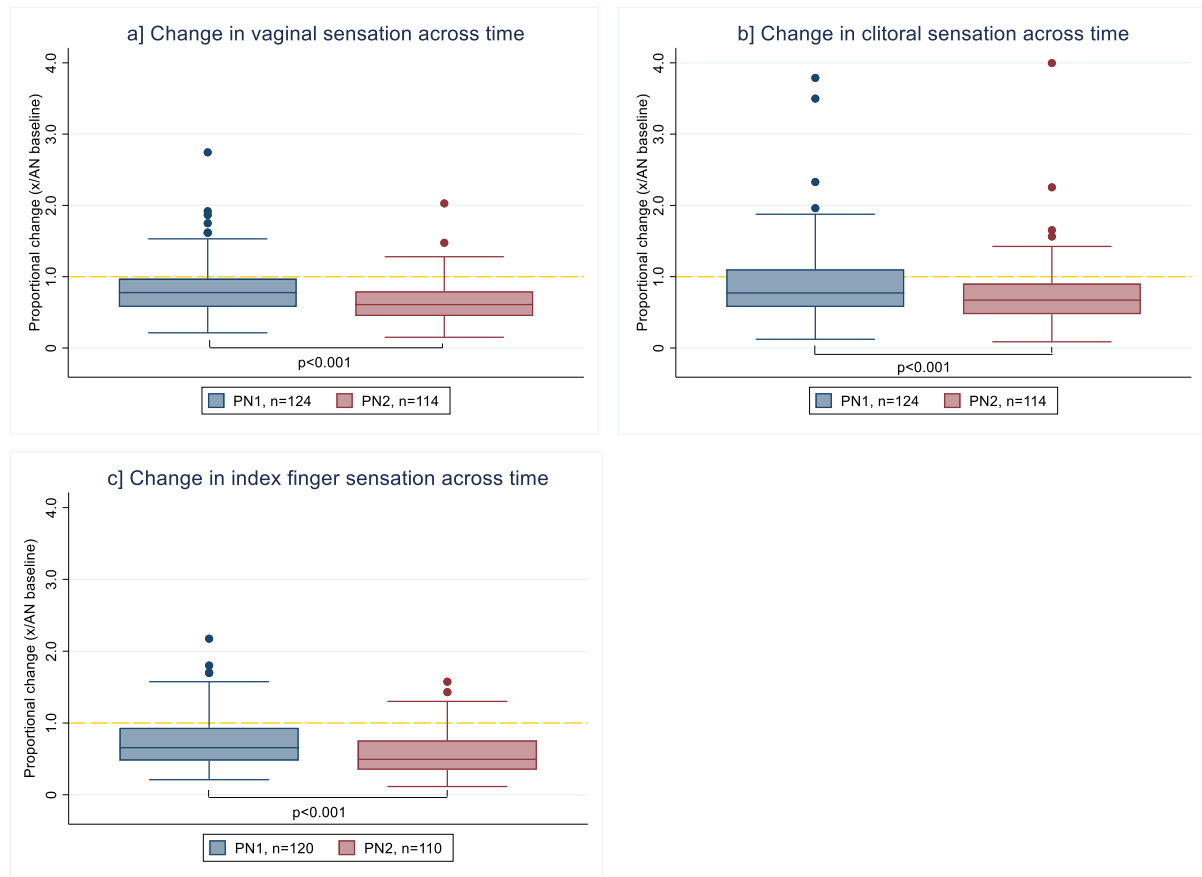
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed. Student's t-test of log^{10} transformed data is shown below boxes.

Two outliers excluded. Missing data at AN led to corresponding missing proportional change data (index finger PN1 and PN2 =4).

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4.3.6.2 Stretch sensation

Absolute stretch sensation

First awareness and stretch sensation thresholds for the vagina and introitus are displayed in Figure 4-VIII a), b), c) and d). The graphs suggest sensation for first awareness and stretch at both locations deteriorated at PN1 in comparison to AN, but had improved by PN2 closer to the initial AN sensory threshold.

Statistical analysis showed a significant difference in sensation threshold for first awareness and stretch across AN, PN1 and PN2 for the vagina and introitus.

Post-hoc pairwise comparison for first awareness at the vagina and introitus found a difference between AN and PN1, and AN and PN2, but no difference between PN1 and PN2.

Pairwise comparison for stretch sensation at the vagina demonstrated a significant difference between AN and PN1, and a trend towards a difference between AN and PN2, and PN1 and PN2. Finally, introitus stretch demonstrated a significant difference between AN and PN1, AN and PN2, as well as PN1 and PN2.

Figure 4-VIII Box and whisker plots of absolute stretch sensation across the three clinical visits
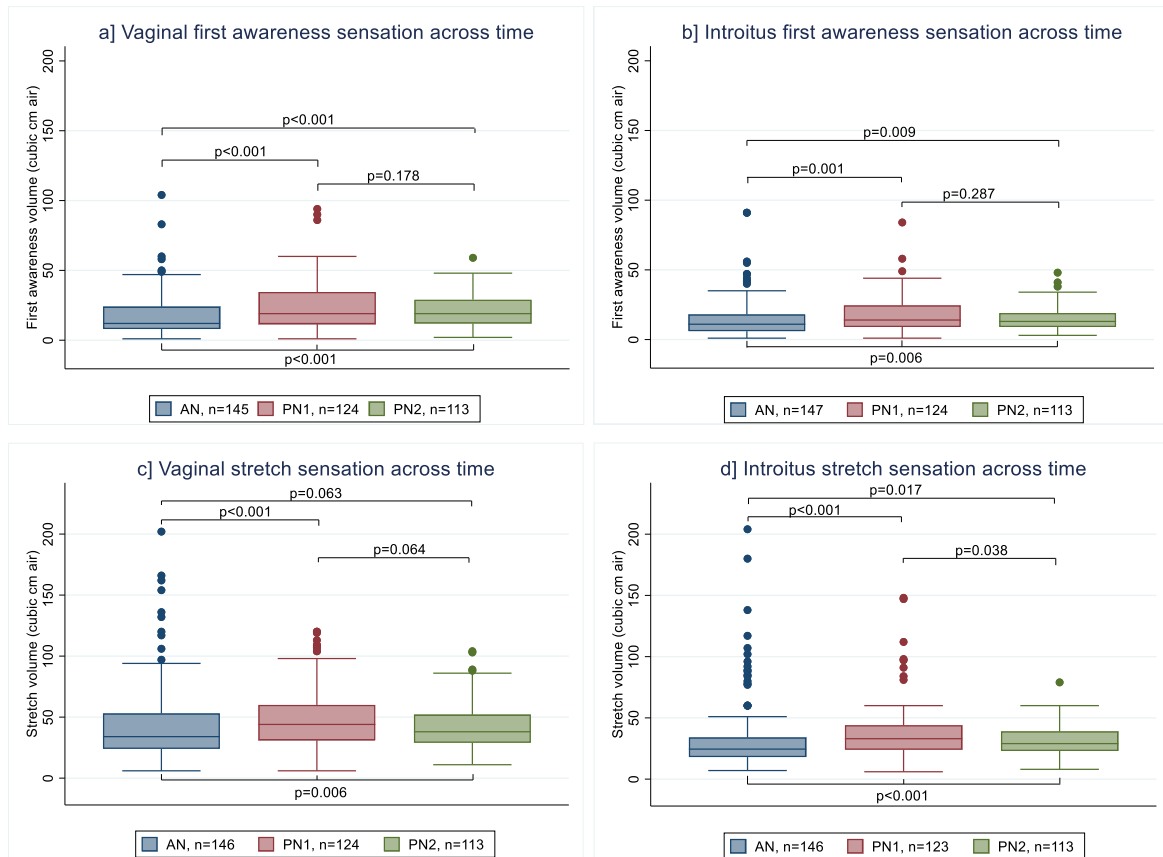
Higher volumes produce greater distention and equate to reduced sensation.

Raw data displayed. Kruskal Wallis group wise comparison is shown underneath boxes, with Dunn's post-hoc pairwise comparison shown above boxes.

Two outliers were excluded. Missing data points due to technical error (vagina first awareness AN = 2, vagina stretch AN = 1, introitus stretch AN and PN1 = 1) or woman declined to undergo stretch sensation testing (all at AN=1 and PN2 = 1).

Proportional change in stretch sensation

Proportional change across PN1 and PN2 for first awareness and stretch sensation at the introitus and vagina are displayed in Figure 4-IX.

Missing data points occurred due to a technical error during AN testing and one woman declined to undergo stretch sensation testing at PN2.

Inspection of the graphs suggests there was no difference in proportional change from AN baseline at PN1 or PN2 for first awareness or stretch sensation at the vagina or introitus. Statistical analysis confirmed this to be the case for first awareness at the vagina and introitus, and stretch at the introitus. However, proportional change in vaginal stretch sensation showed improvement from PN1 to PN2.

Figure 4-IX Box and whisker plot showing proportional change in stretch sensation across time
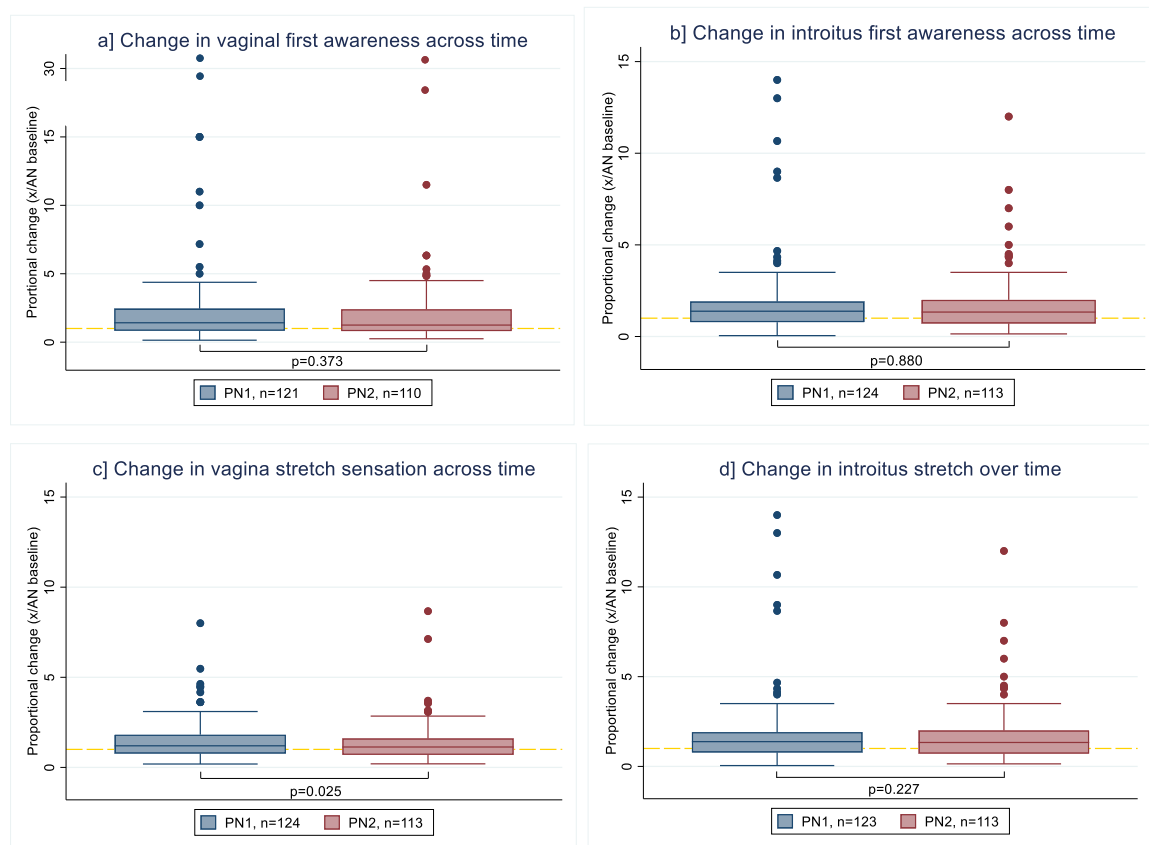
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.
Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated. Raw data displayed. Wilcoxon matched pair signed rank test shown underneath boxes. Two outliers were excluded. Missing data at AN with corresponding missing proportional change data for PN1 (all n=1, vagina first awareness = 2), due to technical error (introitus stretch PN1 =1), or woman declined to undergo stretch sensation testing (all PN2 = 1).

4.3.7　　Mode of delivery

It was hypothesised that the presence of forceps within the birth canal may cause damage to pudendal nerves via traction during attempted delivery and distention by the forceps blades themselves, regardless of whether the head was delivered vaginally.

Therefore women who had undergone vaginal instrumentation were categorised as an instrumental delivery a priori, irrespective of final mode of birth.

Three women in the final cohort had a failed instrumental delivery proceeding to an EmCS at full dilatation. To evaluate the robustness of this decision, data excluding the three women was also analysed which found no difference in output.

4.3.7.1　　Vibration sensation

Proportional change in vibration sensation
Proportional change in vibration sensation compared to AN baseline for PN1 and PN2 across each mode of delivery are shown in Figure 4-X.

The graphs demonstrate vibration sensation at the vagina and clitoris recovered more quickly following a caesarean section than a NVD or instrumental delivery, Figure 4-X Graph a) and b). As expected the index finger, which is considered the location control, showed no difference in proportional improvement from AN baseline in vibration sensation across mode of delivery, Figure 4-X Graph c).

Additional analysis of PN1 proportional change data at the vagina, clitoris and index finger was performed with the ten women who were lost to follow up by PN2 removed from the dataset. There remained a significant difference between the CS, NVD and instrumental delivery for the vagina ($p<0.001$) and clitoris ($p<0.001$), whilst mode of delivery continued to have no impact on change in sensation at the index finger ($p=0.575$). This suggests the additional loss of 10 women from PN1 to PN2 did not bias the sample.

At PN1 vaginal vibration sensation following a CS had improved by 39% compared to the AN baseline, whereas following a NVD or instrumental delivery there was a smaller improvement of around 10%. Similarly, clitoral vibration sensation had improved by 36% following a CS, 22% following a NVD and just 10% following an instrumental delivery compared to the AN baseline. There was no difference in proportional change in vibration sensation at the index finger, or control location across mode of delivery.

At PN2, improvement in vaginal vibration sensation compared to AN baseline following a CS was relatively unchanged since PN1 at 45%, whilst recovery following a NVD had

increased to 41% and was comparable to the CS group. However, the same recovery was not evident in the instrumental group which had only reached 28%. Clitoral vibration sensation in women following a CS had increased to a 44% improvement overall, with a 33% improvement after NVD and an 18% improvement following instrumental delivery. Similar to PN1, there was also no change in vibration sensation at the index finger between AN baseline and PN2.

There was a significant difference in PN1 vibration sensation change at the vagina between CS, NVD and instrumental delivery. Pairwise comparison showed a difference in PN1 vibration sensation change between CS and both NVD and instrumental, but no difference between the NVD and instrumental groups.

There was also a difference at PN1 in change in clitoral vibration sensation between CS, NVD and instrumental delivery. Pairwise comparison also showed a difference between CS and both NVD and instrumental, but no difference between the NVD and instrumental groups.

Clitoral vibration sensation change was significantly different at PN2. Pairwise comparison showed a difference between CS and instrumental, and NVD compared to instrumental, but no difference between the CS and NVD groups.

Figure 4-X Box and whisker plots showing proportional change in vibration sensation by mode of delivery
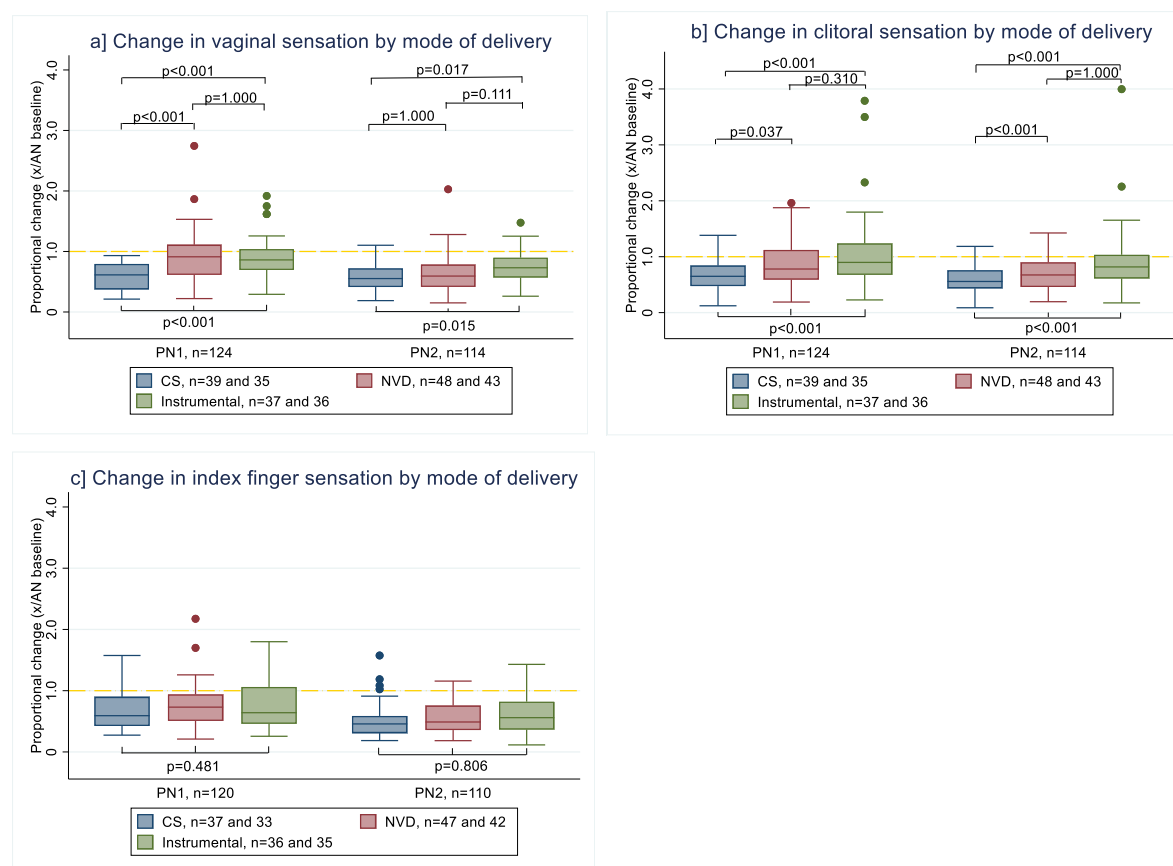
a] Change in vaginal sensation by mode of delivery

b] Change in clitoral sensation by mode of delivery

c] Change in index finger sensation by mode of delivery

Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed. One-way ANOVA of log^{10} transformed data are shown below boxes.

Where group wise comparison was significant, Bonferroni post-hoc pairwise comparison is shown above boxes.

Two outliers were excluded. Missing data at AN with corresponding missing proportional change data (index finger PN1 and PN2 =4).

Key: CS – caesarean section, NVD – normal vaginal delivery, instrumental – includes five ventouse, forceps and three failed forceps proceeding to fully dilated CS.

4.3.7.2   Stretch sensation

Proportional change in stretch sensation

Change in first awareness and stretch sensation at the vagina and introitus at PN1 and PN2 are shown in Figure 4-XI a), b), c) and d).

The graphs suggest a relationship between mode of delivery and sensation change in vagina first awareness, vagina stretch and introitus stretch at PN1. Visual inspection of the graphs suggests there was no difference between change in sensation for first awareness and stretch at each location at PN2.

First awareness at the vagina showed a possible trend towards significance, pairwise comparison confirmed a significant difference between CS and instrumental delivery.

At PN1, first awareness at the vagina showed no difference between AN levels and PN1 or PN2 in the CS group. Women demonstrated a 70% reduction in sensation following a NVD, and a 51% reduction after an instrumental delivery. By PN2 the NVD group had recovered to AN levels and was comparable to the CS group. However there remained a significant difference between the CS and NVD groups compared to the instrumental group. Mode of delivery did not affect introitus first awareness at PN1 or PN2.

At PN1 stretch sensation at the vagina and introitus had not changed significantly from AN levels following a CS, but had deteriorated 45-55% after a NVD and only 25-35% following an instrumental delivery. By PN2 stretch sensation in the NVD and instrumental groups had recovered to CS readings, with no difference between the three groups.

Figure 4-XI Box and whisker plots of proportional change in stretch sensation by mode of delivery
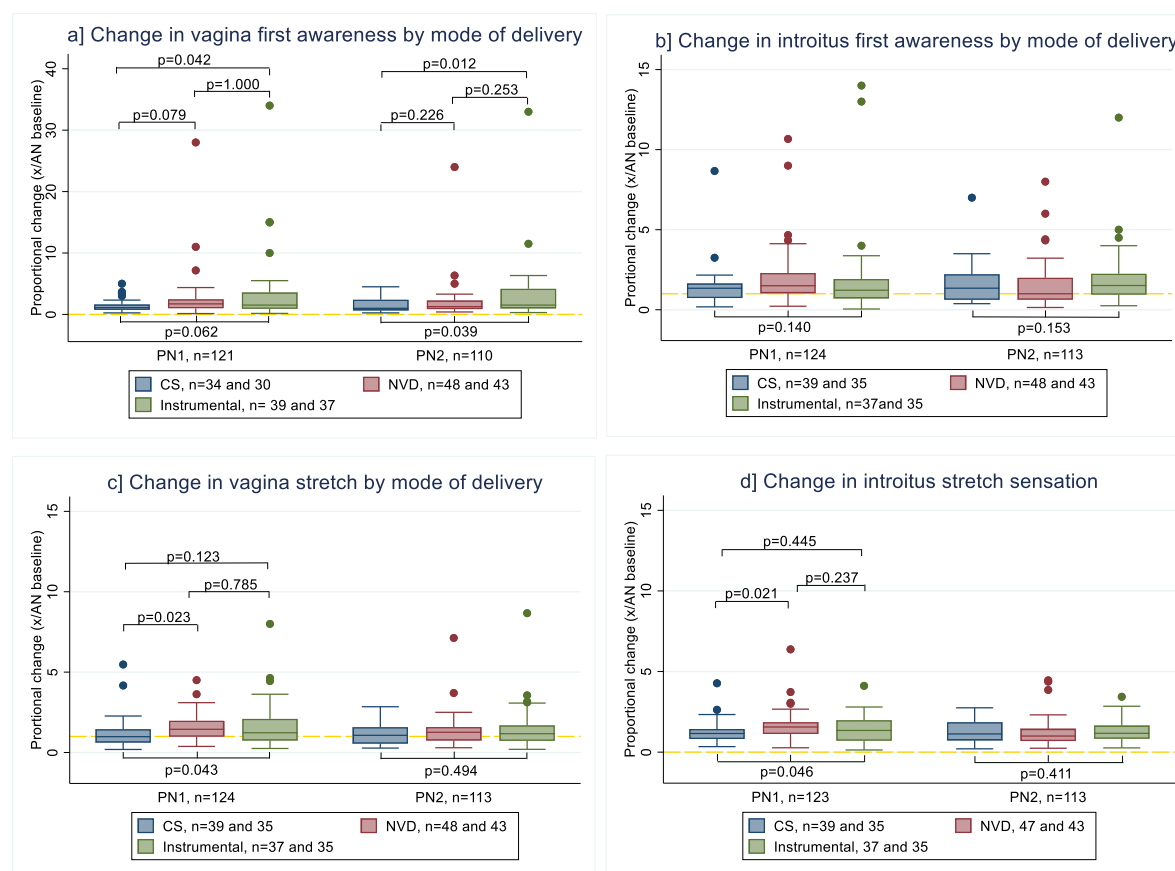
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed. Kruskal Wallis group wise comparison is shown underneath boxes. Where group wise comparison was significant, Dunn's post-hoc pairwise comparison is shown above boxes.

Two outliers were removed. Missing data at AN with corresponding missing proportional change data for PN1 (all = 1, vagina first awareness = 2), due to technical error (introitus stretch PN1 =1), or woman declined to undergo stretch sensation testing (all PN2 = 1).

Key: CS – caesarean section; NVD – normal vaginal delivery; instrumental – includes ventouse, forceps and failed forceps proceeding to fully dilated CS.

4.3.8　Dilatation at caesarean section

Proportional change in vibration sensation

Proportional change in vaginal and clitoral vibration sensation and dilatation at CS were assessed visually in Figure 4-XII a) b), c) and d). The graph's suggested there was no relationship between change in sensation and dilatation at CS.

Logistic linear regression found was no evidence in this dataset of a statistical association between dilatation at CS and PN1 or PN2 proportional change in vibration sensation at the vagina or clitoris.

Figure 4-XII Scatter graph of the proportional change in vibration sensation and dilatation at caesarean section
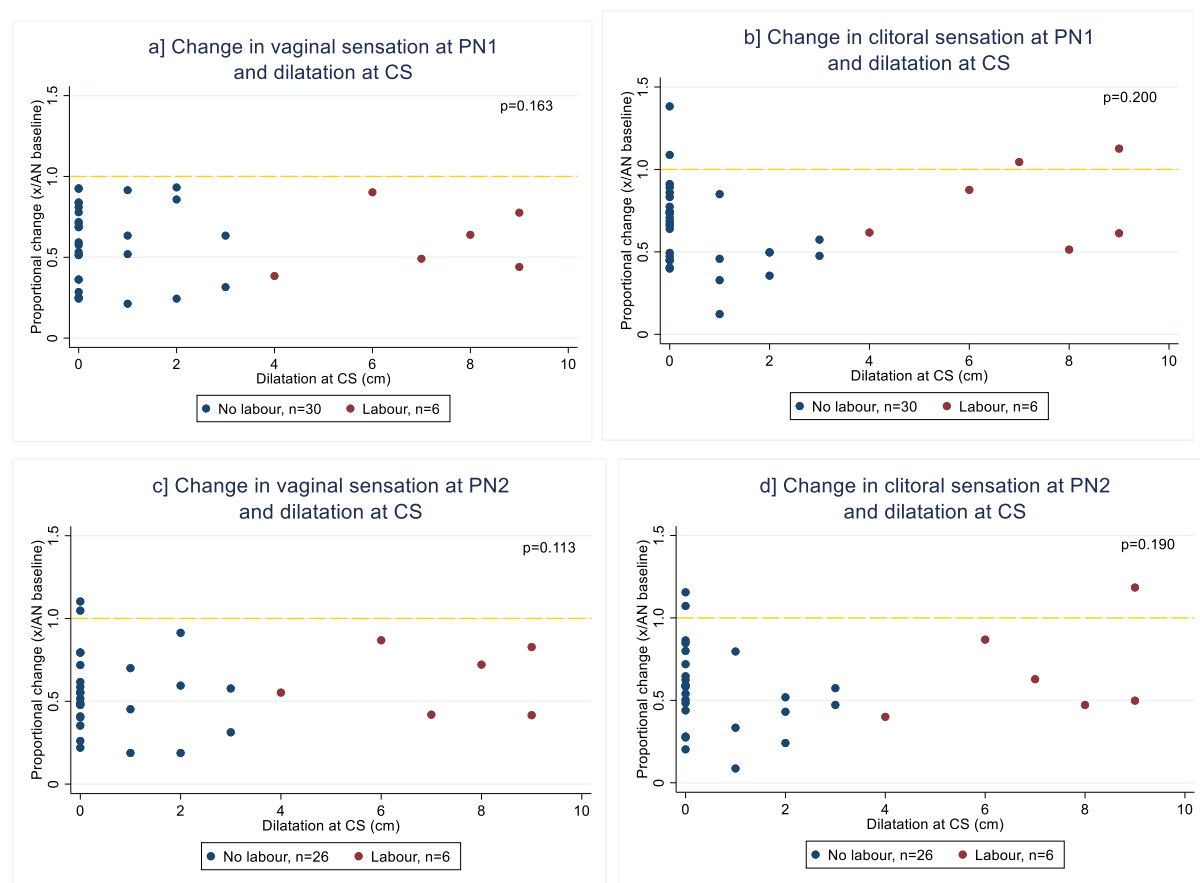
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed, data log^{10} transformed to allow linear regression modelling. Two outliers were excluded. Key: CS – caesarean section; no labour = ≤3cm dilated; labour = 4 to 10cm dilated (excluding failed forceps proceeding to fully dilated CS).

4.3.9   Duration of labour

Proportional change in sensation at PN1 and PN2 for the vagina and clitoris for total length of labour are displayed in Figure 4-XIII. Inspection of the graphs suggests there was no relationship between proportional change in sensation and total length of labour. Multiple regression modelling with mode of delivery as a covariate found no association between change in vaginal sensation and total length of labour at PN1, or clitoral sensation and total length of labour at PN1 and PN2. Vaginal sensation at PN2 initially appeared significant on the overall regression model; however on closer inspection of the model the variable 'total length of labour' was not significant.

Figure 4-XIII Scatter graph of proportional change in vibration sensation and total length of labour
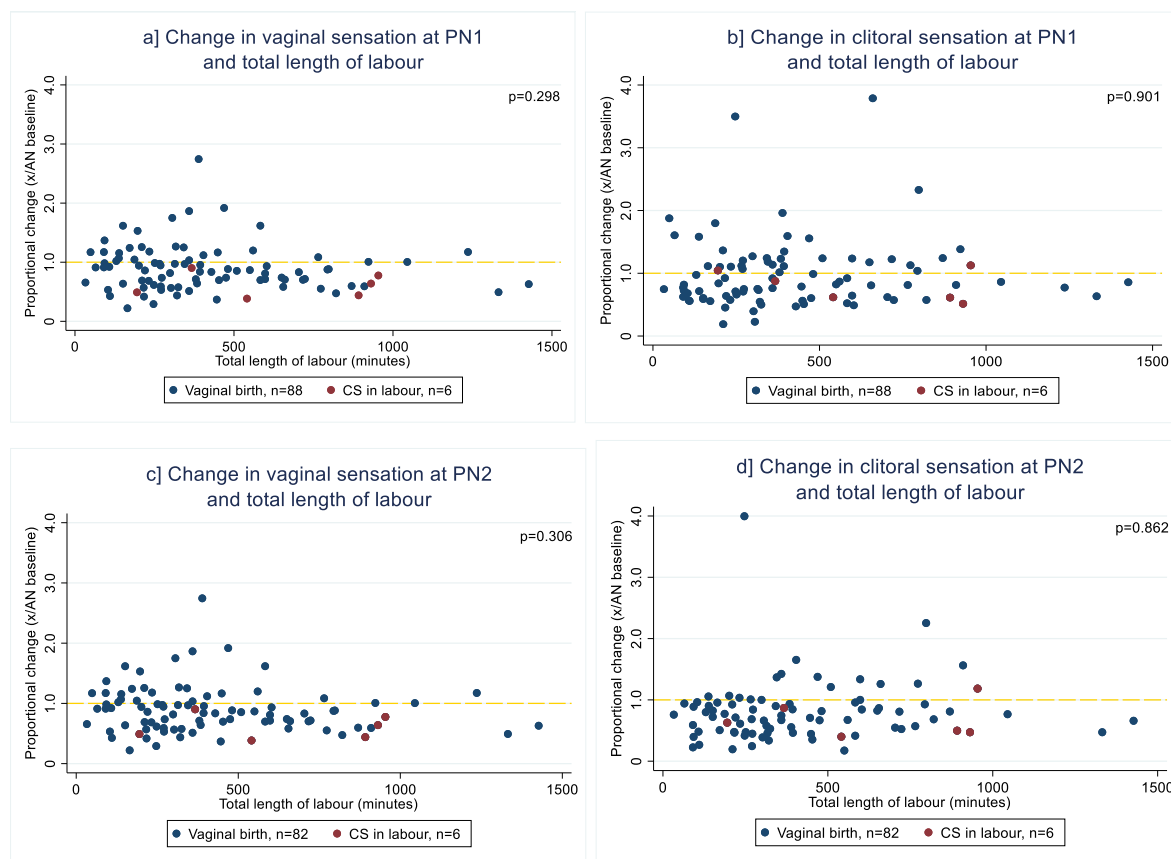
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed, analysed using multiple linear regression with $\log^{10}$ transformation and mode of delivery as covariate (CS, NVD and instrumental).

Key: CS in labour = caesarean section at 4-10cm; vaginal birth = NVD and instrumental delivery (ventouse, forceps and failed forceps proceeding to fully dilated CS); total length labour = 1st stage + (passive + active 2nd stage);

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Total length of second stage and proportional change in sensation at PN1 and PN2 are shown in Figure 4-XIV. Visual assessment of the graphs suggested there was no relationship between proportional change in sensation and total length of second stage. This was confirmed on multiple regression modelling with mode of delivery as a covariate, which found no association between change in vaginal sensation at PN1, or clitoral sensation at PN1 or PN2 and total second stage. Again, the overall regression model for change in vaginal sensation at PN2 was significant, but the variable 'total second stage' was not. Note the six women with a CS at four to nine cm did not have a second stage and were therefore excluded from analysis.

Figure 4-XIV Scatter graph of proportional change in vibration sensation and length of total second stage
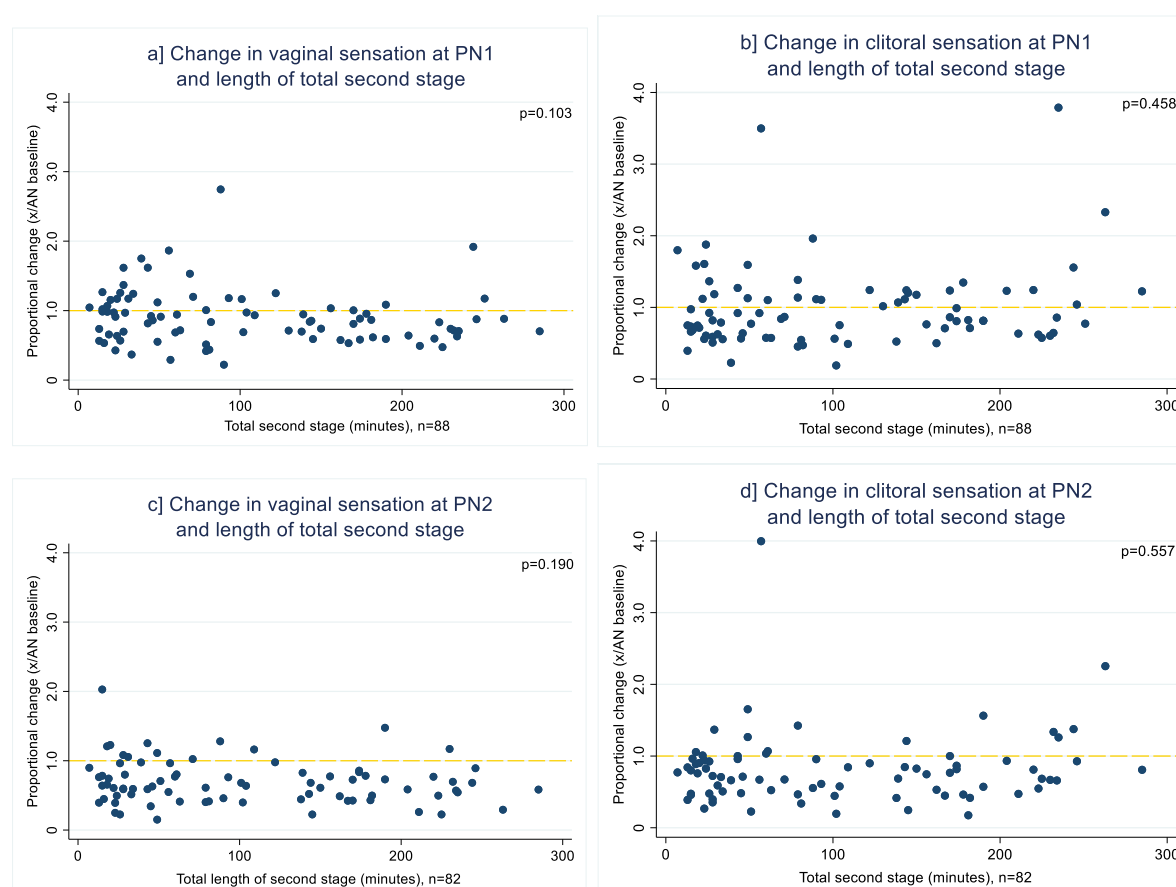
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed, analysed using multiple linear regression with log^{10} transformation and mode of delivery as covariate (NVD and instrumental).

Key: total 2nd stage = passive + active 2nd stage

Finally, active second stage and proportional change in vibration sensation at the vagina and clitoris for PN1 and PN2 were reviewed, Figure 4-XV. Again, visual inspection suggested there was no relationship between active second stage and change in sensation. This was also confirmed on multiple regression modelling with mode of delivery as a covariate, which found no evidence of a relationship for change in vaginal sensation at PN1 or change in clitoral sensation at PN1 or PN2. Once more, the overall model for vaginal sensation at PN2 was significant, but the individual variable 'active second stage' was not.

Figure 4-XV Scatter graph of proportional change in vibration sensation and active second stage
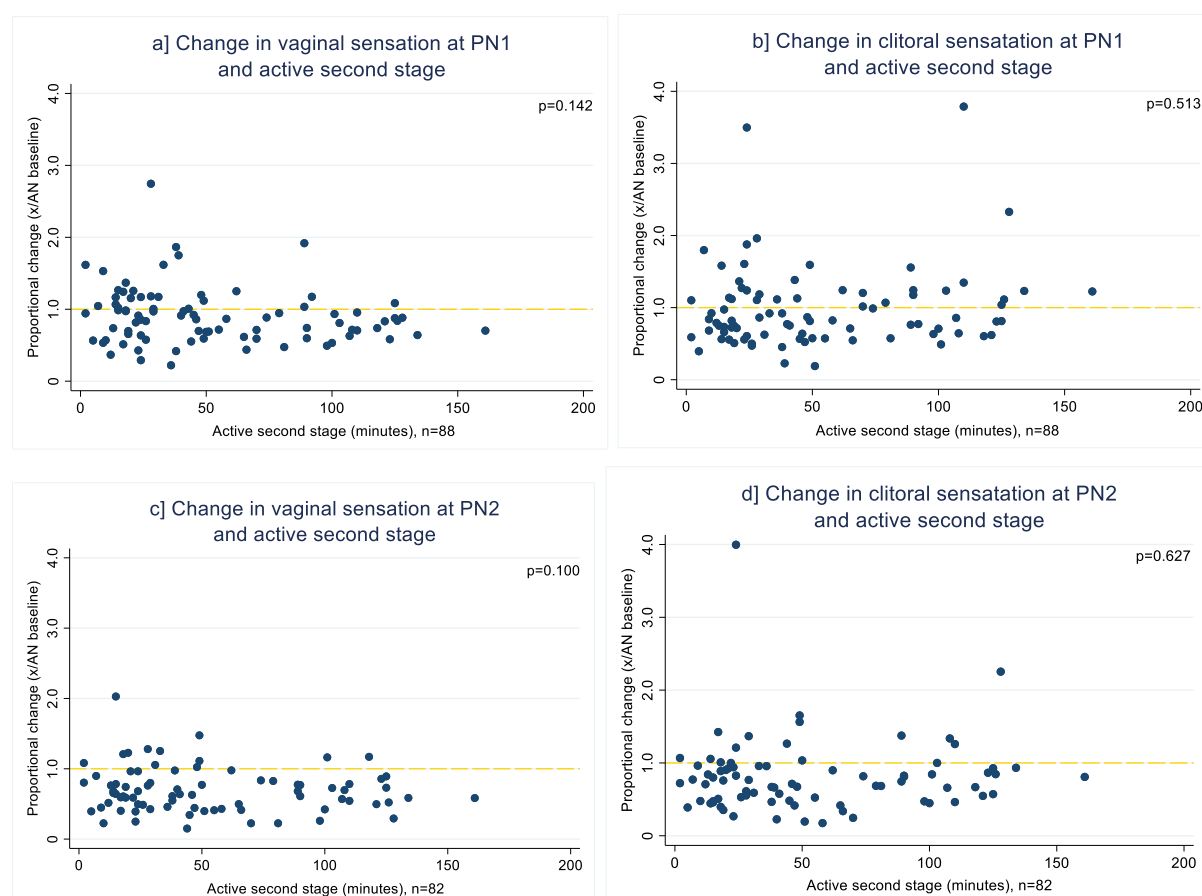
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed, analysed using multiple linear regression with log^{10} transformation and mode of delivery as covariate (NVD and instrumental).

4.3.10   Birth weight

Birth weight and proportional change in sensation at the vagina and clitoris are graphically displayed in Figure 4-XVI Graph a), b), c) and d). Visual assessment of the graphs suggested there was no relationship between change in sensation and birth weight at each time point for each location.

This was confirmed on statistical analysis with Log^{10} multiple linear regression with mode of vaginal birth as a covariate (NVD and instrumental delivery – including failed forceps proceeding to fully dilated CS). There was no association between change in vaginal or clitoral sensation at PN1 and birth weight.

At PN2 the overall regression model for the clitoris and vagina was significant, however the variable 'birth weight' was not significant.

Figure 4-XVI Scatter graph of proportional change in vibration sensation and birth weight
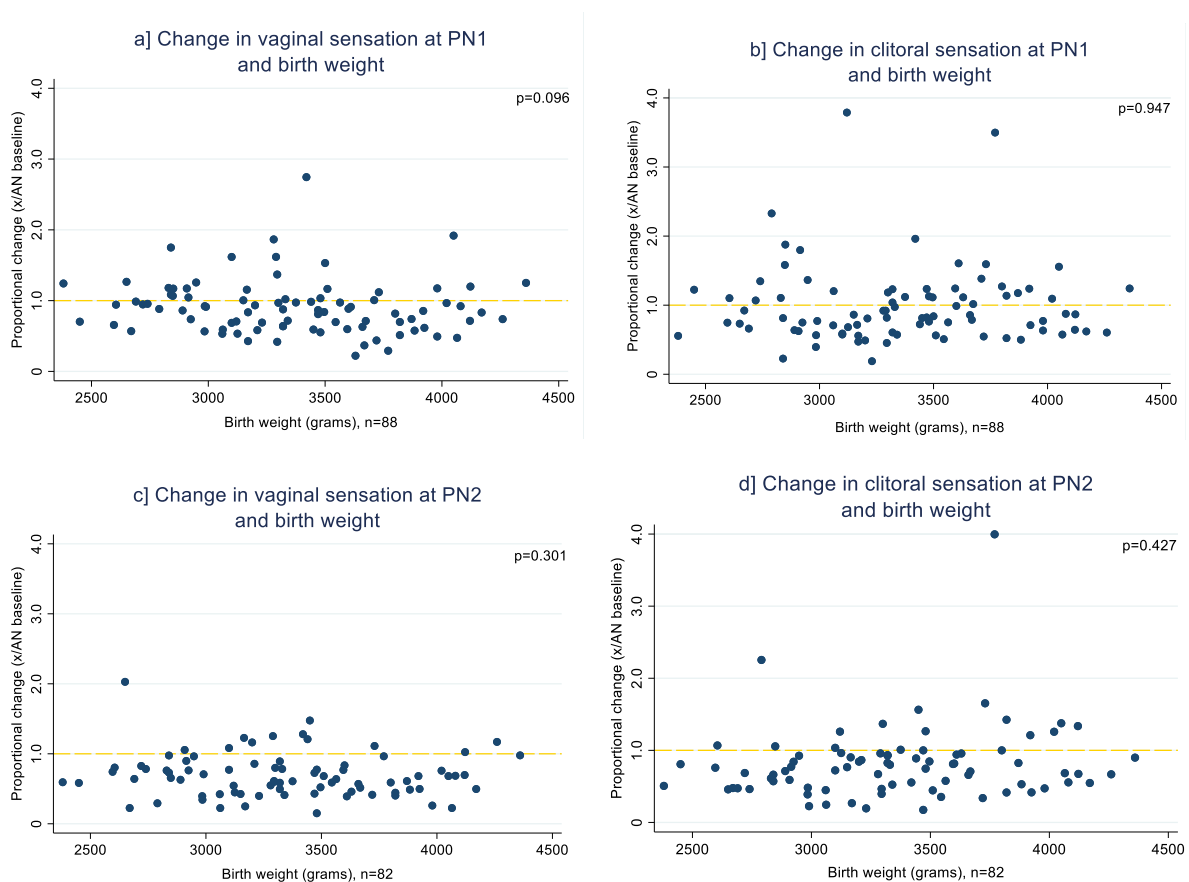
Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed, analysed using multiple linear regression with $\log^{10}$ transformation and mode of delivery as covariate (NVD and instrumental). Two outliers were removed.

4.3.11   Pelvic organ prolapse

4.3.11.1   Cohort incidence of prolapse

There were no cases of anterior wall, posterior wall or uterine POP at AN. There were no cases of uterine prolapse at PN1 or PN2. Women in the CS group did not demonstrate anterior or posterior wall POP at PN1 or PN2.

At PN1 17.7% (22/124) of all women had an anterior wall POP, comprising of 18.8% of (9/48) of NVD and 32.5% (13/40) of instrumental deliveries. There was no difference in the incidence of anterior wall POP between NVD and instrumental deliveries (χ^2 $p=0.138$).

By PN2 this had reduced to 11.4% (13/114) of women overall, with 11.6% (5/43) of NVD and 20.5% (8/39) of instrumental deliveries. Again, there was no difference in the incidence of anterior wall POP between NVD and instrumental deliveries (χ^2 $p=0.271$).

Posterior wall POP was present at PN1 in 23.4% (29/114) of all women, 22.9% (11/48) in the NVD group and 45.0% (18/40) in the instrumental group. Women were more likely to develop a posterior wall POP following an instrumental delivery compared to a NVD (χ^2 $p=0.028$).

By PN2 the incidence of posterior wall POP had dropped to 15.8% (18/114) of women overall, 7.0% (3/43) of NVD and 38.5% (15/39) of instrumental deliveries. Again, women were more likely to have a posterior POP by six months PN after an instrumental delivery compared to a NVD (χ^2 $p<0.001$).

4.3.11.2   Vibration sensation and prolapse

Absolute change in vibration sensation

Visual assessment suggested vaginal sensation was worse in women with POP compared to women without at PN1 and PN2, for both the anterior and posterior walls.

Statistical analysis confirmed women with either an anterior or posterior wall POP at PN1 had worse vaginal vibration sensation than women with no POP. By PN2 this continued to be the case.

Figure 4-XVII Box and whisker plot of absolute vibration sensation and pelvic organ prolapse
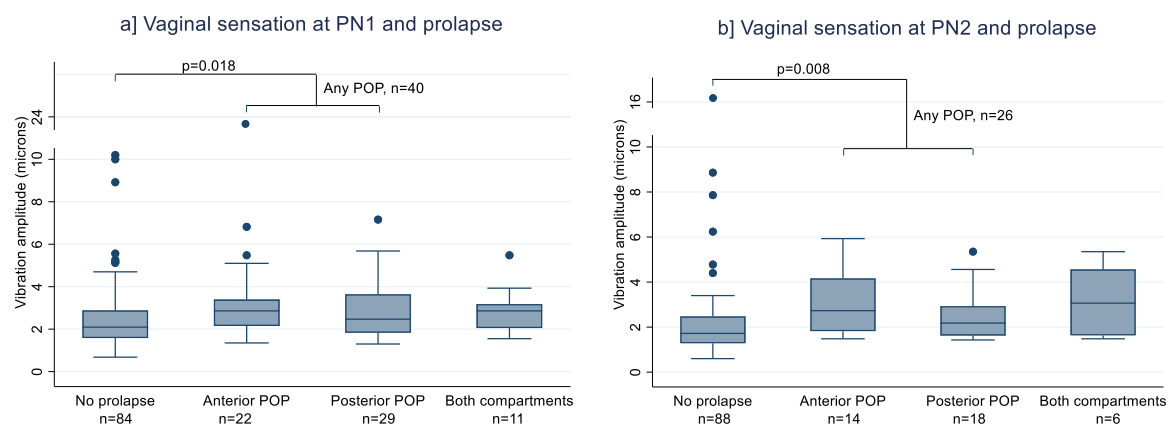
Higher vibration amplitudes produce a stronger vibration and equate to reduced sensation.

Raw data displayed. Raw data displayed, analysed with Mann Whitney U test. Two outliers excluded.

Key: POP = pelvic organ prolapse (defined as the most distal part of vaginal wall at or below the level of the hymen); both compartments = anterior and posterior wall POP.

Proportional change in vibration sensation

Proportional change in vaginal sensation and POP was also investigated in Figure 4-XVIII a) and b). The graphs suggested there was greater improvement in women with no POP at PN1 and PN2 compared to women with anterior wall POP, posterior wall POP or POP in both compartments.

Statistical analysis demonstrated a trend towards women with no POP at PN1 demonstrating greater improvement in vaginal vibration sensation than women with any form of POP (anterior or posterior wall). By PN2 women with any POP showed less improvement in their sensation than women with no POP.

Figure 4-XVIII Box and whisker plot of proportional change in vibration sensation and pelvic organ prolapse
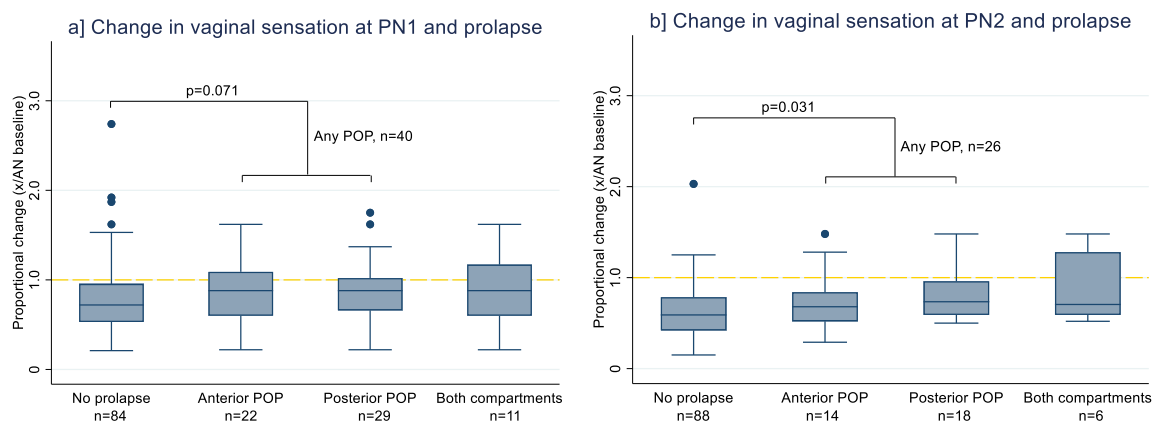
Proportional change = PN1/PN2 sensory threshold/ AN sensory threshold.

Yellow line at 1.0 represents no change from AN baseline, if value <1.0 sensation improved compared to AN, if value >1.0 sensation deteriorated.

Raw data displayed, analysed with Mann Whitney U test. Two outliers excluded.

Key: POP = pelvic organ prolapse (defined as the most distal part of vaginal wall at or below the level of the hymen); both compartments = anterior and posterior wall POP.

4.3.12 Symptoms of pelvic floor dysfunction

4.3.12.1 Individual symptom questions

Vaginal and clitoral vibration sensation across the three clinical visits were analysed with clinically relevant individual ePAQ-PF symptom questions. Some of the group sample sizes were small, particularly for a questionnaire and therefore the significance level was adjusted to <0.01.

Absolute vibration sensation

The statistical analysis of vaginal vibration sensation and individual pelvic floor dysfunction symptoms are shown in Table 4–I. Missing data points are due to women who declined to complete the questionnaire or preferred to complete it at home and then forgot (AN n=1, PN1 n=4 and PN2 n=3).

There was a trend towards an association between abnormal vaginal vibration sensation and reduced urinary sensation at PN1, although this was not true for AN or PN2. There was no association between abnormal vaginal vibration sensation and symptoms of reduced bowel sensation, reduced vaginal sensation, vaginal laxity or lack of sensation during sex at AN, PN1 or PN2.

The statistical analysis of clitoral sensation thresholds and individual pelvic floor dysfunction symptoms are shown in Table 4–J. There was one missing data point at AN, four at PN1 and three at PN2 due to woman declined to complete questionnaire or opted to complete remotely and forgot.

There was no association between abnormal clitoral vibration sensation and the symptoms of reduced urinary sensation, reduced bowel sensation, reduced vaginal sensation, vaginal laxity or a lack of sensation during sex.

Table 4–I Absolute vaginal vibration sensation and individual pelvic floor dysfunction symptoms

Vaginal sensation and individual pelvic floor dysfunction symptoms *Fishers exact test, n (%)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	**p-value** CI 99%
Reduced urinary sensation			
AN	6 (4.4), n=135	0, n=12	*1.000*
PN1	11 (9.5), n=116	2 (50), n=4	*0.058*
PN2	6 (5.5), n=110	0, n=2	*1.000*
Reduced bowel sensation			
AN	3 (2.2), n=135	0, n=12	*1.000*
PN1	10 (8.6), n=116	1 (25), n=4	*0.323*
PN2	6 (5.5), n=110	0, n=2	*1.000*
Reduced vaginal sensation			
AN	10 (7.4), n=135	0, n=12	*1.000*
PN1	29 (25), n=116	2, (50), n=4	*0.274*
PN2	18 (16.3), n=110	0. n=2	*1.000*
Vaginal laxity			
AN	7 (5.2), n=135	0, n=12	*1.000*
PN1	18 (15.5), n=116	0, n=4	*1.000*
PN2	10 (9.1), n=110	0, n=2	*1.000*
Lack of sensation during sex			
AN	12 (8.9), n=135	0, n=12	*0.599*
PN1*	1 (2.2), n=45	1 (4.5), n=22	*1.000*
PN2[¥]	1 (1.3) , n=77	0, n=15	*1.000*

Absolute vibration sensation dichotomised into normal and abnormal sensation using pre-defined age adjusted normative values in non-pregnant women.[47]

Symptom classified as absent when severity reported as 'not at all' and present when reported as 'a little', 'moderately' or 'a lot'.

Data analysed using Fisher's exact test, significance level set at p<0.01. Two outliers excluded. Missing data due to woman declined to complete questionnaire or completed remotely but did not select save (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; [¥]PN2 women sexually active = 92

Table 4–J Absolute clitoral vibration sensation and individual pelvic floor dysfunction symptoms

Clitoral sensation and individual pelvic floor dysfunction symptoms *Fishers exact test, n (%)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	***p-value*** CI 99%
Reduced urinary sensation			
AN	2 (8.0), n=25	4 (3.3), n=122	*0.270*
PN1	4 (8.0), n=50	9 (12.9), n=70	*0.554*
PN2	1 (1.8), n=57	5 (9.1), n=55	*0.110*
Reduced bowel sensation			
AN	1 (4.0), n=25	2 (1.6), =122	*0.431*
PN1	6 (12.0), n=50	5 (7.1), n=70	*0.523*
PN2	4 (7.0), n=57	2 (3.6), n=55	*0.679*
Reduced vaginal sensation			
AN	3 (12.0), n=25	7 (5.7), n=122	*0.375*
PN1	11 (22.0), n=50	20 (28.6), n=70	*0.527*
PN2	7 (12.3), n=57	11 (20.0), n=55	*0.311*
Vaginal laxity			
AN	2 (8.0), n=25	5 (4.1), n=122	*0.340*
PN1	6 (12.0), n=50	12 (17.1), n=70	*0.605*
PN2	4 (7.0), n=57	6 (10.9), n=55	*0.524*
Lack of sensation during sex			
AN	3 (12.0), n=25	9 (7.4), n=122	*0.430*
PN1*	7 (29.2), n=24	15 (34.9), n=43	*0.787*
PN2[¥]	5 (10.6), n=47	10 (22.2), n=45	*0.164*

Absolute vibration sensation dichotomised into normal and abnormal sensation using pre-defined age adjusted normative values in non-pregnant women.[47]

Symptom classified as absent when severity reported as 'not at all' and present when reported as 'a little', 'moderately' or 'a lot'.

Data analysed using Fisher's exact test, significance level set at p<0.01. Two outliers excluded. Missing data due to woman declined to complete questionnaire or preferred to complete remotely but forgot (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; ¥PN2 women sexually active = 92

Proportional change in vibration sensation

Analysis of proportional change in vaginal vibration sensation and individual symptom questions are shown in Table 4–K. Using a significance level of $p<0.01$ there was no association between deterioration in vaginal vibration sensation and the individual symptoms of reduced urinary sensation, reduced bowel sensation, reduced vaginal sensation, vaginal laxity and lack of sensation during sex.

Proportional change in clitoral vibration and individual symptom questions are shown in Table 4–L. Again, using a significance level of $p<0.01$ there was no association between deterioration in clitoral vibration sensation and the individual symptoms of reduced urinary sensation, reduced bowel sensation, reduced vaginal sensation, vaginal laxity and lack of sensation during sex.

Table 4–K Proportional change in vaginal vibration sensation and individual pelvic floor symptoms

Change in vaginal sensation and individual pelvic floor dysfunction symptoms _Fishers exact test, n (%)_			
Clinical Visit	**Sensation improved**	**Sensation deteriorated**	**_p-value_** CI 99%
Reduced urinary sensation			
_PN1_	9 (9.6), n=94	4 (15.4), n=26	_0.475_
_PN2_	4 (4.1), n=98	2 (14.3), n=14	_0.163_
Reduced bowel sensation			
_PN1_	8 (8.5), n=94	3 (11.6), n=26	_0.702_
_PN2_	6 (6.1), n=98	0, n=14	_1.000_
Reduced vaginal sensation			
_PN1_	20 (21.3), n=94	11 (42.3), n=26	**_0.042_**
_PN2_	15 (15.3), n=98	3 (21.4), n=14	_0.696_
Vaginal laxity			
_PN1_	12 (12.8), n=94	6 (23.1), n=26	_0.218_
_PN2_	9 (9.2), n=98	1 (7.1), n=14	_1.000_
Lack of sensation during sex			
_PN1*_	17 (33.3), n=51	5 (31.3), n=16	_1.000_
_PN2¥_	12 (15.0) , n=80	3 (25.0), n=12	_0.406_

Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Change in sensation data dichotomised into 'improved' if less than the AN baseline and 'deteriorated' if above the AN baseline. Symptom classified as absent when severity reported as 'not at all' and present when reported as 'a little', 'moderately' or 'a lot'. Data analysed using Fisher's exact test. Significance level set at $p<0.01$.

Two outliers excluded. Missing data due to woman declined to complete questionnaire or preferred to complete remotely but forgot (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; ¥PN2 women sexually active = 92

Table 4–L Proportional change in clitoral vibration sensation and individual pelvic floor dysfunction symptoms

Change in clitoral sensation and individual pelvic floor dysfunction symptoms *Fishers exact test, n (%)*			
Clinical Visit	**Sensation improved**	**Sensation deteriorated**	**p-value** CI 99%
Reduced urinary sensation			
PN1	10 (12.1), n=83	3 (8.1), n=37	*0.752*
PN2	5 (5.4), n=93	1 (5.3), n=19	*1.000*
Reduced bowel sensation			
PN1	9 (10.8), n=83	2 (5.4), n=37	*0.500*
PN2	4 (4.3), n=93	2 (10.5), n=19	*0.269*
Reduced vaginal sensation			
PN1	20 (24.1), n=83	11 (29.7), n=37	*0.508*
PN2	17 (18.3), n=93	1 (5.3), n=19	*0.301*
Vaginal laxity			
PN1	10 (12.1), n=83	8 (21.6), n=37	*0.180*
PN2	7 (7.5), n=93	3 (15.8), n=19	*0.369*
Lack of sensation during sex			
PN1*	14 (28.0), n=50	8 (47.1), n=17	*0.231*
PN2¥	14 (17.5) , n=80	1 (8.3), n=12	*0.683*

Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold.

Change in sensation data dichotomised into 'improved' if less than the AN baseline, and 'deteriorated' if above the AN baseline. Symptom classified as absent when severity reported as 'not at all' and present when reported as 'a little', 'moderately' or 'a lot'. Data analysed using Fisher's exact test. Significance level set at $p<0.01$. Two outliers excluded. Missing data due to woman declined to complete questionnaire or preferred to complete remotely but forgot (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; ¥PN2 women sexually active = 92

4.3.13   Domain scores

Domain scores were also evaluated to investigate whether sensation played a role in global symptom complexes. Again, some of the group sample sizes were small with a high number of domain scores to assess, therefore the significance level was adjusted to <0.01.

Absolute vibration sensation

Statistical analysis of results for vaginal vibration sensation and domain scores for global symptom complexes are shown in Table 4–M. There was no association at AN, PN1 or PN2 between abnormal vaginal sensation and the symptom complexes of urinary pain, urinary voiding dysfunction, overactive bladder, irritable bowel syndrome, bowel evacuation, bowel continence, vaginal pain and sensation, vaginal capacity, POP or vaginal sexual dysfunction.

Results for clitoral vibration sensation and global symptom domain scores are shown in Table 4–N. There was no association between impaired clitoral vibration sensation and the symptom complexes of irritable bowel syndrome, bowel evacuation, bowel continence, vaginal pain and sensation, vaginal capacity or POP at AN, PN1 or PN2.

There was also no association at AN or PN1 between impaired clitoral sensation and the symptom complexes of urinary pain, urinary voiding dysfunction, overactive bladder and general sexual dysfunction. However, at PN2 women exhibiting impaired vibration sensation were more likely to complain of urinary pain and general sexual dysfunction. There was also a trend towards symptoms of urinary voiding dysfunction and overactive bladder in women with impaired sensation at PN2.

Table 4–M Absolute vaginal vibration sensation and global symptom domains of pelvic floor dysfunction

Vaginal sensation and global symptom domains *Mann-Whitney U test, median (range)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	**p-value** CI 99%
Urinary pain			
AN	0 (0-33), n=135	0 (0-22), n=12	*0.171*
PN1	0 (0-33), n=116	0 (0-11), n=4	*0.687*
PN2	0 (0-44), n=109	0 (0-0), n=2	*0.622*
Urinary voiding dysfunction			
AN	8 (0-50), n=135	0 (0-25), n=12	*0.391*
PN1	0 (0-50), n=116	0 (0-25), n=4	*0.986*
PN2	0 (0-50), n=109	0 (0-0), n=2	*0.363*
Overactive bladder			
AN	8 (0-58), n=135	0 (0-17), n=12	*0.063*
PN1	8 (0-58), n=116	4 (0-17), n=4	*0.693*
PN2	0 (0-75), n=109	0 (0-0), n=2	*0.244*
Irritable bowel syndrome			
AN	20 (0-93), n=135	13 (0-53), n=12	*0.168*
PN1	3.5 (0-7), n=116	13 (0-73), n=4	***0.018***
PN2	13 (0-80), n=109	10 (7-13), n=2	*0.664*
Bowel evacuation			
AN	14 (0-95), n=135	10 (0-43), n=12	*0.694*
PN1	14 (0-76), n=116	0 (0-10), n=4	*0.054*
PN2	0 (0-81), n=109	5 (0-10), n=2	*0.730*
Bowel continence			
AN	5 (0-43), n=135	0 (0-10), n=12	*0.250*
PN1	5 (0-43), n=116	2.5 (0-14), n=4	*0.758*
PN2	0 (0-62), n=109	7 (0-14), n=2	*0.680*
Vaginal pain and sensation			
AN	0 (0-42), n=135	0 (0-17), n=12	*0.684*
PN1	8 (0-58), n=116	4 (0-17), n=4	*0.594*
PN2	0 (0-75), n=109	8.5 (0-17), n=2	*0.875*

Table continued overleaf.

Vaginal sensation and global symptom domains *Mann-Whitney U test, median (range)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	***p-value*** CI 99%
Vaginal capacity			
AN	0 (0-100), n=135	0 (0-22), n=12	*0.646*
PN1	0 (0-100), n=116	0 (0-0), n=4	*0.382*
PN2	0 (0-100), n=109	0 (0-0), n=2	*0.510*
Pelvic Organ Prolapse			
AN	0 (0-83), n=135	0 (0-0), n=12	*0.258*
PN1	0 (0-67), n=116	0 (0-0), n=4	*0.277*
PN2	0 (0-58), n=109	0 (0-0), n=2	*0.576*
Vaginal sexual dysfunction			
AN	0 (0-100), n=135	0 (0-33), n=12	*0.776*
PN1*	0 (0-75), n=65	21 (0-42), n=2	*0.633*
PN2¥	0 (0-75), n=91	0 (0-0), n=1	*0.507*

Vibration sensation dichotomised into normal and abnormal sensation using pre-defined age adjusted normative values in non-pregnant women.[47]

Global symptom domain data remained non-parametric despite transformation and therefore data analysed using Mann Whitney U test, significance level set at p<0.01.

Two outliers excluded. Missing data due to woman declined to complete questionnaire or completed remotely but did not select save (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; ¥PN2 women sexually active = 92

Table 4–N Absolute clitoral vibration sensation and global symptom domains of pelvic floor dysfunction

Clitoral sensation and global symptom domains *Mann-Whitney U test, median (range)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	***p-value*** CI 99%
Urinary pain			
AN	0 (0-33), n=25	0 (0-44), n=122	*0.820*
PN1	0 (0-33), n=50	0 (0-33), n=70	*0.118*
PN2	0 (0-22), n=57	0 (0-44), n=54	***0.010***
Urinary voiding dysfunction			
AN	0 (0-33), n=25	8 (0-50), n=122	*0.387*
PN1	0 (0-33), n=50	0 (0-50), n=70	*0.565*
PN2	0 (0-25), n=57	0 (0-50), n=55	***0.034***
Overactive bladder			
AN	8 (0-33), n=25	8 (0-58), n=122	*0.589*
PN1	8 (0-42), n=50	8 (0-58), n=70	*0.197*
PN2	0 (0-33), n=57	8 (0-75), n=55	***0.012***
Irritable bowel syndrome			
AN	8 (0-58), n=25	20 (0-67), n=122	*0.354*
PN1	13 (0-73), n=50	13 (0-53), n=70	*0.669*
PN2	13 (0-80), n=57	20 (0-52), n=55	*0.135*
Bowel evacuation			
AN	14 (0-95), n=25	10 (0-67), n=122	*0.807*
PN1	16.5 (0-76), n=50	14 (0-52), n=70	*0.659*
PN2	0 (0-81), n=57	0 (0-38), n=55	*0.352*
Bowel continence			
AN	5 (0-43), n=25	5 (0-29), n=122	*0.519*
PN1	5 (0-43), n=50	5 (0-33), n=70	*0.644*
PN2	0 (0-38), n=57	0 (0-62), n=55	*0.241*
Vaginal pain and sensation			
AN	8 (0-42), n=25	0 (0-42), n=122	*0.053*
PN1	8 (0-58), n=50	8 (0-50), n=70	*0.899*
PN2	0 (0-42), n=57	0 (0-75), n=55	*0.195*

Table continued overleaf.

Clitoral sensation and global symptom domains *Mann-Whitney U test, median (range)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	***p-value*** CI 99%
Vaginal capacity			
AN	0 (0-78), n=25	0 (0-100), n=122	*0.465*
PN1	0 (0-100), n=50	0 (0-44), n=70	*0.890*
PN2	0 (0-100), n=57	0 (0-100), n=55	*0.679*
Pelvic Organ Prolapse			
AN	0 (0-8), n=25	0 (0-83), n=122	*0.605*
PN1	0 (0-58), n=50	0 (0-67), n=70	*0.218*
PN2	0 (0-58), n=57	0 (0-58), n=55	*0.130*
Vaginal sexual dysfunction			
AN	8 (0-83), n=25	8 (0-83), n=122	*0.359*
PN1*	17 (0-83), n=25	17 (0-75), n=44	*0.667*
PN2¥	8 (0-83), n=50	17 (0-83), n=46	***0.003***

Vibration sensation dichotomised into normal and abnormal sensation using pre-defined age adjusted normative values in non-pregnant women.[47]

Global symptom domain data remained non-parametric despite transformation and therefore data analysed using Mann Whitney U test, significance level set at $p<0.01$.

Two outliers excluded. Missing data due to woman declined to complete questionnaire or completed remotely but did not select save (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; ¥PN2 women sexually active = 92

Proportional change in vibration sensation

Statistical analysis for symptom complex domain scores and proportional change in vaginal vibration sensation is shown in Table 4–O. There was no association between deterioration in vaginal sensation and symptom complexes of urinary pain, urinary voiding dysfunction, overactive bladder, irritable bowel syndrome, bowel evacuation, bowel continence, vaginal pain and sensation, vaginal capacity, POP or vaginal sexual dysfunction.

Proportional change in clitoral sensation and symptom complex domain scores are shown in Table 4–P. There was also no association between deterioration in clitoral sensation and symptom complexes of urinary pain, urinary voiding dysfunction, irritable bowel syndrome, bowel evacuation, bowel continence, vaginal pain and sensation, vaginal capacity, POP or general sexual dysfunction. Women with a deterioration in clitoral sensation were more likely to report symptoms of overactive bladder at PN2, but not at AN or PN1.

Table 4–O Proportional change in vaginal vibration sensation and global symptom domains of pelvic floor dysfunction

Change in vaginal sensation and global symptom domains _Mann-Whitney U test, median (range)_			
Clinical Visit	**Sensation improved**	**Sensation deteriorated**	**_p-value_** CI 99%
Urinary pain			
_PN1_	0 (0-33), n=94	0 (0-11), n=26	_0.802_
_PN2_	0 (0-44), n=98	0 (0-33), n=14	_0.028_
Urinary voiding dysfunction			
_PN1_	0 (0-50), n=94	0 (0-25), n=26	_0.622_
_PN2_	0 (0-33), n=98	0 (0-33), n=14	_0.969_
Overactive bladder			
_PN1_	8 (0-58), n=94	8 (0-50), n=26	_0.117_
_PN2_	0 (0-75), n=98	0 (0-42), n=14	_0.777_
Irritable bowel syndrome			
_PN1_	13 (0-73), n=94	13 (0-53), n=26	_0.558_
_PN2_	13 (0-80), n=98	16.5 (0-53), n=14	_0.425_
Bowel evacuation			
_PN1_	14 (0-76), n=94	19 (0-52), n=26	_0.423_
_PN2_	0 (0-81), n=98	0 (0-38), n=14	_0.735_
Bowel continence			
_PN1_	5 (0-33), n=94	5 (0-43), n=26	_0.678_
_PN2_	0 (0-62), n=98	0 (0-24), n=14	_0.649_
Vaginal pain and sensation			
_PN1_	8 (0-50), n=94	17 (0-58), n=26	_0.071_
_PN2_	0 (0-50), n=98	0 (0-75), n=14	_0.935_
Vaginal capacity			
_PN1_	0 (0-100), n=94	0 (0-33), n=26	_0.980_
_PN2_	0 (0-100), n=98	0 (0-100), n=14	_0.732_
Pelvic organ Prolapse			
_PN1_	0 (0-58), n=94	0 (0-67), n=26	_0.990_
_PN2_	0 (0-58), n=98	0 (0-33), n=14	_0.418_

Table continued overleaf.

Change in vaginal sensation and global symptom domains *Mann-Whitney U test, median (range)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	***p-value*** CI 99%
Vaginal sexual dysfunction			
PN1*	0 (0-75), n=65	21 (0-42), n=2	*0.633*
PN2¥	0 (0-75), n=91	0, n=1	*0.507*

Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold. Change in sensation data dichotomised into 'improved' if less than the AN baseline, and 'deteriorated' if above the AN baseline.

Data analysed using Fisher's exact test. Significance level set at p<0.01. Two outliers excluded. Missing data due to woman declined to complete questionnaire or preferred to complete remotely but forgot (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; ¥PN2 women sexually active = 92

Table 4–P Proportional change in clitoral vibration sensation and global symptom domains of pelvic floor dysfunction

Change in clitoral sensation and global symptom domains *Mann-Whitney U test, median (range)*			
Clinical Visit	**Sensation improved**	**Sensation deteriorated**	***p-value*** CI 99%
Urinary pain			
PN1	0 (0-33), n=83	0 (0-11), n=37	*0.508*
PN2	0 (0-44), n=91	0 (0-33), n=20	*0.503*
Urinary voiding dysfunction			
PN1	0 (0-33), n=83	0 (0-50), n=37	*0.567*
PN2	0 (0-42), n=91	0 (0-50), n=20	*0.308*
Overactive bladder			
PN1	8 (0-58), n=83	8 (0-50), n=37	*0.287*
PN2	0 (0-75), n=91	12.5 (0-50), n=20	***<0.001***
Irritable bowel syndrome			
PN1	13 (0-73), n=83	13 (0-53), n=37	*0.784*
PN2	13 (0-80), n=91	13 (0-53), n=21	*0.186*
Bowel evacuation			
PN1	14 (0-76), n=83	14 (0-52), n=37	*0.751*
PN2	0 (0-81), n=91	10 (0-38), n=21	*0.283*
Bowel continence			
PN1	5 (0-43), n=83	5 (0-33), n=37	*0.898*
PN2	0 (0-38), n=91	0 (0-62), n=21	*0.305*
Vaginal pain and sensation			
PN1	8 (0-58), n=83	8 (0-33), n=37	*0.699*
PN2	0 (0-75), n=91	0 (0-42), n=21	*0.800*
Vaginal capacity			
PN1	0 (0-100), n=83	0 (0-33), n=37	*0.591*
PN2	0 (0-100), n=91	0 (0-100), n=21	*0.524*
Pelvic organ prolapse			
PN1	0 (0-67), n=83	0 (0-58), n=37	*0.345*
PN2	0 (0-58), n=91	0 (0-50), n=21	*0.106*

Table continued overleaf.

Change in clitoral sensation and global symptom domains *Mann-Whitney U test, median (range)*			
Clinical Visit	**Normal sensation**	**Abnormal sensation**	***p-value*** CI 99%
General sexual dysfunction			
PN1*	17 (0-83), n=50	17 (0-50), n=17	*0.504*
PN2[¥]	17 (0-83), n=78	17 (0-75), n=14	*0.354*

Proportional change = PN1 or PN2 sensory threshold/ AN sensory threshold. Change in sensation data dichotomised into 'improved' if less than the AN baseline, and 'deteriorated' if above the AN baseline. Symptom classified as absent when severity reported as 'not at all' and present when reported as 'a little', 'moderately' or 'a lot'. Data analysed using Fisher's exact test. Significance level set at p<0.01. Two outliers excluded. Missing data due to woman declined to complete questionnaire or preferred to complete remotely but forgot (AN n=1, PN1 n=4, PN2 n=3).

Key: CI – confidence interval; *PN1 women sexually active = 67; ¥PN2 women sexually active = 92

4.4 Discussion

This is the first study to investigate the impact of childbirth on pelvic sensation, including an evaluation of the impact of pregnancy itself on pelvic vibration (Aβ nerve fibres) and stretch sensation (Aα and Aβ nerve fibres).

4.4.1   Interpretation and current evidence

This is the first study to investigate sensory function of the pudendal nerve during pregnancy and following childbirth, as such there are no studies for comparison. There are also no comparative studies of Aα and Aβ sensory nerves and so it is not possible to comment on how the two different nerve fibres behave in different situations. A number of studies have reported pudendal motor nerve function following childbirth and although these were performed at between eight weeks and 15 years postpartum, I have explored how my findings fits within these studies below.

Antenatal changes in sensation

This study found evidence of hyposensitivity in the clitoral branch of the pudendal nerve and the median nerve in Aβ nerve fibres in the third trimester of pregnancy, with sparing of the perineal branch of the pudendal nerve and larger Aα nerve fibres.

It is not possible to comment on how the finding of impaired clitoral sensation fits with pudendal motor nerve function in pregnancy, as whilst a number of studies have included an antenatal assessment alongside their evaluation of pudendal motor nerve function following childbirth, none have performed a direct comparison with normative data in non-pregnant women.[37–39,144–146,149,150]

However, impaired sensation of the median nerve during pregnancy has been widely reported in the literature. The reported incidence using nerve conduction studies ranges from 11% to 66% which is in keeping with this study.[151–153] One group reported obese women were more likely to develop median nerve hyposensitivity during pregnancy, however my study did not find an association between impaired vibration sensation at the index finger and BMI.[147]

The clitoral and index finger sensation changes could represent a physiological adaptation of pregnancy which may be advantageous for labour, possibly reducing sensitivity to pain during childbirth.

One possible explanation is the neuromodulatory effect of altered sex hormones, particularly reduced levels of oestrogen that occur in the third trimester, which could be centrally or peripherally mediated. Sex hormones have been shown to impact the

expression of conductance in auditory and cardiac nerves.[154,155] The concept of a neuromodulatory effect is supported by Connell et al who tested the effect of age and menopausal status on vibration thresholds at the female genitalia.[156] They found menopause negatively affected sensation independent of age. Whilst hormonal changes in pregnancy are greater than those of the menstrual cycle, a study by Bajaj et al. evaluated thermal pain sensory thresholds for Aδ nerve fibres in women throughout the phases of the menstrual cycle. They reported reduced sensitivity at the abdomen and lower back during the early follicular phase, again correlating with lower levels of circulating oestrogen.[157]

Alternatively potential hormonal effects may be due to increased levels of relaxin rather than changes in the sex hormones. Relaxin has been shown to have an impact on the central and peripheral autonomic nervous system during pregnancy, and therefore may also exert a change in the somatic nervous system causing the sensory changes found in this cohort.[158,159]

Another possible explanation is the additional extravascular fluid of pregnancy causing compression of nerves against bone, such as the median nerve against the radius and the clitoral nerve against the pubic rami. This theory would also account for the relative vaginal sparing seen. The absence of an association with gestation suggests nerve compression from head engagement is not a factor.

In this cohort vaginal sensation appeared to be less affected by pregnancy. One explanation could be Aβ nerve fibres actually are impaired in pregnancy but this is not apparent due to the wide confidence interval for the normal range and the lack of a pre-conception paired comparison. Another possible reason could be the vagina contains fewer specialised sensory receptors when compared to the finger and clitoris, relying more on free nerve endings to transmit sensation which might in turn be less affected by the hormonal effects of pregnancy.[160,161]

An explanation for the disparity between vibration and stretch sensation during pregnancy could be the mechanism which affects Aβ nerve fibres (vibration sensation) does not affect the larger Aα nerve fibres (stretch sensation).

In comparison to data in non-pregnant women described in section 3.2.8.5 this study found no evidence of an association between stretch sensation and age in pregnancy. This may be due to the narrow age range of the pregnant cohort. An alternative explanation is that stretch testing has poor sensitivity to detect abnormal sensation in pregnancy.

Postnatal changes in sensation

In this study Aβ nerve fibre function (vibration sensation) improved over the course of six months postnatal compared to AN readings, whereas larger Aα nerve fibre function (stretch sensation) deteriorated at PN1 compared to AN readings then partially recovered by PN2.

Whilst there are no data available on the behaviour of pelvic sensory nerves following childbirth, four studies have described motor nerve function following childbirth. These studies have consistently reported impaired motor nerve function following childbirth, with one study reporting an 80% incidence of pudendal motor nerve injury in primiparous women.[37–39,149] This fits with the deterioration in genital stretch sensation seen at PN1 in this cohort but contrasts with the findings of improved vibration sensation following childbirth. Motor function and stretch sensation are both transmitted via Aα nerve fibres and could be expected to follow a similar pattern of injury and recovery.

One explanation for the postnatal difference between Aα and Aβ nerve fibres could be due to their baseline function in pregnancy, as Aβ nerve function was impaired during pregnancy in this cohort which may have had a confounding effect on any postnatal changes. The apparent improvement in Aβ nerve function postnatally may have appeared as a deterioration if compared to pre-conception readings.

There a number of possible mechanisms for sensory nerve injury during childbirth. Prolonged compression of the pelvic floor by the fetal head could cause ischaemia of the pudendal nerve branches. To date, no study has investigated the pressure exerted by the fetal head on the pelvic floor during labour. Although a study evaluating acute compression injury in rabbit pretibial nerves found at just 70mmHg of pressure, over 90% of nerves developed ischaemia. [162] Alternatively as the fetal head descends below the ischial spines the pudendal nerve fibres may be stretched leading to a traction injury. This is supported by 3D computer modelling that demonstrated vaginal birth causes strain beyond the limit for permanent damage in the pudendal nerve branches.[44] It is unclear how a traction injury could affect women with atypical pelvic neuroanatomy.[12,13] It is also possible the nerve damage seen in this study is due to a combination of the above mechanisms.

An alternative explanation is that the sensory changes seen are due to a process within the central nerve system surrounding labour. Evidence against this hypothesis is the complete recovery of index finger sensation to within normal limits with no relationship between PN1 or PN2.

Both compression and traction injuries can cause variable levels of damage, from isolated focal demyelination to direct injury to the axons to complete transection of both the axons and connective tissue layers.[163] My findings suggest there is an element of nerve regeneration in both Aβ and Aα nerve fibres from PN1 to PN2. This fits with another study of pudendal motor nerve function following childbirth which described partial reinnervation following vaginal birth in the majority of women.[38] The mechanism of recovery depends on which nerve structures have been injured, but is most likely to follow a similar process to regeneration of peripheral nerves in the rest of the body. This includes a process called Wallerian degeneration, whereby Schwann cells migrate from the axons and remove cellular debris with macrophages, at the same time the Schwann cells release factors which stimulate regrowth of the axon toward the target organ.[164]

Mode of delivery

Data from this study suggests mode of delivery has a significant impact on pelvic sensation. In this study CS was considered the mode of delivery control, exhibiting the sensory changes seen during pregnancy but not the changes in sensation that occurred with vaginal birth and the passage of the fetal head through the birth canal.

Of the four studies that reported motor nerve injury following childbirth, three explored the effect of mode of delivery. The first by Snooks et al described impaired pudendal nerve motor function in women who underwent a vaginal delivery compared to a CS at two months.[37] The second by Allen et al reported no difference between NVD and instrumental delivery at two months postnatal.[38] The third by Sultan et al found no change in motor nerve function after a CS but a deterioration following a vaginal delivery, with no difference between NVD and instrumental delivery at six weeks postnatal although the CS and instrumental groups were small compared to the NVD group (n=16 vs n=15 vs n=74).[39]

Again the findings of these studies correspond with the behaviour of stretch sensation at PN1, with no change after CS but a significant deterioration in function following NVD and instrumental delivery, suggesting Aα motor and sensory nerves follow the same pattern of injury. Interestingly, although vibration sensation via Aβ nerve fibres improved at PN1, vibration sensation also displayed much greater improvement after a CS than a NVD or instrumental delivery.

My results suggest women who underwent a CS experienced some neuroprotection of both Aα and Aβ nerve fibres with reduced nerve ischaemia, traction injury or a combination of both. Whilst women who underwent a NVD showed slow recovery at PN1 they demonstrated enough recovery to restore function to CS levels by PN2, suggesting nerve damage in this group is transient. Instrumental delivery was associated with a

greater impact on sensory nerve function and less recovery of function by PN2 than a CS or NVD, suggesting instrumental delivery caused more significant and prolonged nerve damage than a NVD. It is important to note the instrumental group was almost exclusively forceps deliveries with only five ventouse in the cohort.

A previous study in non-pregnant women in our unit demonstrated a change of 10% was of clinical significance.[94] By comparison the magnitude of change in terms of improvement from AN baseline ranged from 10% to 45% in my cohort suggesting my findings may be clinically significant.

One possible explanation is the increased diameter of the presenting part caused by the instrument, which most likely exerts a greater compressive force and traction on the pudendal nerve than a NVD, which in turn leads to more significant nerve injury. Another explanation could be some operators are using an exaggerated downward traction during a forceps delivery to encourage head extension and manoeuvre the forceps around the pubic arch. This is likely to also lead to exaggerated traction on the pudendal nerve which could also cause more significant nerve injury. Similar to the mechanism of nerve injury, the effect of instrumental delivery probably involves both explanations to varying degrees.

Dilatation at caesarean section

No study has reported the impact of dilatation at CS on pudendal nerve motor function, although the three studies above all reported a difference between motor nerve function in women undergoing an elective CS versus a CS in labour.[37–39] However the group sample sizes in all three studies were less than ten, calling into question the robustness of the statistics.

My data showed no association between dilatation at CS and improvement in sensation although this is likely to represent the small numbers of labouring women who underwent a CS (n=6).

The three women who underwent a CS at full dilatation after a failed trial of instrumental delivery has similar results to the instrumental rather than CS group. One potential explanation for this could be forceps within the pelvis cause vaginal distension with a degree of nerve injury, regardless of whether the fetal head travels through the rest of the birth canal.

Duration of labour

In this cohort there was no association between duration of labour and change in sensation. Two of the motor nerve studies described above included an analysis of duration of labour. One reported greater motor nerve damage in women with a total

second stage of labour lasting over 83 minutes, and an active second stage of labour over 57 minutes.[38] The other described worse motor nerve function in primiparous women with an active second stage of labour above 30 minutes, and in multiparous women above 15 minutes.[39]

This difference may reflect inaccuracy in knowing the precise timing of full cervical dilatation, or second stage of labour. Cervical dilatation is examined four hourly during labour to monitor progress, so a woman may enter the second stage of labour in between examinations and thus the timing of onset of the second stage of labour is typically a poor estimate.

It is also possible that this study is underpowered to detect this secondary outcome measure.

Birth weight

There was no association between birth weight and change in sensation in this study. This is in contrast to the evidence on motor nerve damage, with one study reporting greater motor nerve damage in women delivering babies weighing over 3.41kg, with any nerve damage compounded by a prolonged second stage of labour often seen with larger babies in the 1980's.[38] However, this study did not include mode of delivery as a covariate in their analysis raising the possibility the relationship appeared significant due to the confounding effect of instrumental delivery. Another study described greater pudendal motor nerve impairment in women delivering babies weighing over 4.0kg compared to under 4kg, although the large difference in group sizes (n=5 and n=51 respectively) question the validity of the statistical test used.[39]

My cohort contained only nine babies weighing over 4kg that were born vaginally and so the lack of association between change in sensation and birth weight could simply represent that the data was underpowered for this objective.

Pelvic organ prolapse

In this study women were 25% more likely to develop posterior POP after an instrumental delivery than a NVD, but not an anterior POP. The difference between NVD and instrumental could be explained by the instrument causing greater distension of the posterior vaginal wall from a wider presenting part. This could also represent the operator using exaggerated downward traction during an instrumental delivery compared to a NVD or than the wider diameter of the instrument predisposes to tearing of pelvic support or avulsion injures such as the levator ani.[165]

POP was associated with reduced vibration sensation at PN1 and PN2. One reason for this could be that POP itself causes distortion of free nerve endings within the lamina

propria causing impaired sensation. Another explanation could be that POP and nerve damage are caused by the same pathological process and so coexist due to shared causative factors.

Women with POP also displayed slower recovery of sensation than women with no POP. This may be due to a shared pathological process, or the anatomical distortion caused by POP somehow inhibits or impairs the nerve regeneration process.

Two studies have reported reduced vaginal sensation of Aβ, Aδ and C nerve fibres in women with POP compared to women without POP.[94,95] My findings suggest this is also true in the postnatal population.

Symptoms of pelvic floor dysfunction

Interestingly, women with a deterioration in their sensation compared to AN levels were twice as likely to report the symptom of reduced vaginal sensation, whilst women with abnormal sensation based on absolute values were not. This disparity may reflect the issues surrounding dichotomisation of absolute sensation data, discussed in 4.4.2 below. An alternative explanation is that change in sensation may be more clinically important to women than absolute hyposensitivity.

Contrary to individual symptoms of reduced sensation, women with a deterioration in their sensation were not more likely to report any of the global symptom complexes of PFD, other than overactive bladder at PN2. Again this may be a reflection of the issues surrounding dichotomisation of data or it may be the data is underpowered to test this association. Alternatively, the symptoms of PFD may not appear until later in life when childbirth related nerve damage is combined with age related nerve degeneration.

Women with abnormal vaginal sensation were four times more likely to report irritable bowel syndrome at PN1. One explanation could be these women also have some injury to their inferior rectal nerves, which could cause abnormal anal canal sensation and may lead to irritable bowel symptoms.

Women with abnormal clitoral sensation were more likely to report urinary pain, urinary voiding dysfunction and overactive bladder at PN2, which may suggest there is some cross over of nerve supply between the clitoris and bladder. This crossover could have been detected during testing of the somatic nervous system, but may also include autonomic innervation which would explain the association with symptoms that are under involuntary control.[74,166,167] As expected, women with abnormal clitoral sensation were more likely to complain of general sexual dysfunction.

The studies evaluating motor nerve function are conflicting, with one finding motor nerve injury was associated with SUI, and two reporting no association with SUI, urinary dysfunction or anal incontinence at two months postnatal.[37–39] However based on the year of publication it would appear none of these studies used validated questionnaires to evaluate the symptoms of PFD and therefore these conclusions should be interpreted with caution.

4.4.2   Strengths and limitations

The recruitment rate of women seen face to face to discuss the study was approximately 50% which is acceptable given the intimate nature of the testing and the number of clinical visits involved. The study recruited over the minimum sample size and achieved an attrition rate of 23%, which was 7% less than predicted. According to our original power calculation, a minimum of 29 women in each delivery group was needed to detect a 10% change in sensation. Excluding missing data points and outliers the smallest group size was 37 at PN1 and 35 at PN2.

The attrition rate of 23% was relatively low for a study involving two postnatal visits. This may be a reflection of the study design which was developed in conjunction with user-groups and the experience of the women as the central focus. However, a larger proportion of CS group were lost to follow up (34%) compared to the NVD (27%) or instrumental groups (15%). This may have biased the sample, as it might mean CS women were less worried or bothered by symptoms of PFD and therefore did not attend for follow up, whilst women who had an instrumental delivery were more traumatised and therefore more likely to attend for follow up.

This is the first study to demonstrate feasilibity of genital QST in a pregnant and postnatal cohort and provides a method for clinical evaluation of these women in the future.

One limitation of the study is the subjective nature of all sensory testing, as sensation is by its very definition a subjective perception. However strict adherence to the QST protocol and control of the testing environment minimised this to some degree, but it was not possible to account for the distraction of fetal movements AN, or baby distractions at PN1 or PN2 which may have affected response time.

The study did not include an assessment of Aδ and C nerve fibres using cold and thermal QST respectively. This was due to complaints regarding the extended duration of temperature testing from non-pregnant women during a previous study in our unit. This in turn led to ethical concerns subjecting pregnant and post-natal women to this

modality.[94] Had this data been available we may have been able to pinpoint the mechanism causing the changes seen in the NVD and instrumental groups. This is because compression neuropathies typically start starts with demyelination of large Aβ and Aα nerve fibre before progressing to loss of Aδ and C nerve fibres, whilst degenerative and vascular neuropathies usually affect axons leading to greater involvement of Aδ and C nerve fibres compared to Aβ nerve fibres.

Another limitation was the lack of clinical neurological history or examination performed at the time of testing. The aim of this piece of work was to investigate the aetiology of the sensory changes seen in women presenting with PFD later in life. Whilst it would have been interesting to gather this information, data is already available on genital sensation in women with neuropathic conditions from specialist clinics. In addition this would have added an extra one hour to each clinical visit that would place the women under unrealistic time constraints with a new baby.

The analysis of antenatal sensation used either previously published normative data for vibration, or previously reported normative data for stretch, section 3.2.8.5.[47] Both of these came from a more heterogenous group of non-pregnant women which included both nulliparous and parous women. As a result the normal thresholds may be lower for nulliparous women and may have caused the study to underestimate the degree of hyposensitivity seen in pregnancy. In an ideal world all women would have undergone preconception QST for vibration and stretch to provide a paired comparison. This was not possible due to the inability to contact women before a spontaneous conception and the ethical difficulties of approaching women before in vitro fertilisation.

There were issues surrounding reliability of delivery data. The fixed fields in the electronic patient maternity record system for total length of labour, total length of second stage, duration of active second stage, dilatation at CS and birth weight were often inconsistent with the typed entries. In an attempt to counteract this, the typed entries of all women were hand searched for the beginning and end of each stage of labour and dilatation at CS. Birth weight data was crossed checked with the national CMIS database.

Finally, participation in the study itself may also have raised awareness of the symptoms of PFD, although given the extensive and robust validation of ePAQ this is unlikely to have biased the responses to PFD symptom questions.

4.4.3 Generalisability

Whilst the cohort is a reasonable representation of the national population, it is not a true reflection of the Central Manchester local population which has a greater proportion of sub-Saharan African and South Asian ethnicities. This is because a number of women were ineligible due to language barriers, and those who were not were often deterred by the intimate nature of testing. Women were more likely to participate in the study if they were well educated, Caucasian and had pre-existing concerns regarding postnatal PFD.

The average breakdown of delivery in nulliparous women in the host trust is CS 25%, NVD 50%, and instrumental 25%, however in this study it was CS 32%, NVD 39%, and instrumental 29%. The higher rate of CS in this cohort represents focussed recruitment of women undergoing ElCS.

4.4.4 Future work

The findings of this study may have implications for all women undergoing childbirth and the long-term implications of the sensory abnormalities found in this study remain unclear. As a result, there are three key areas for future work.

The first should focus on the follow up of the women in this cohort in the medium term (up to three years after birth) and long term (five years after birth). Follow up studies should pay particular attention to whether women with evidence of absolute sensory nerve impairment, a deterioration in their sensation or slow recovery of function at PN1 and PN2 are more likely to develop PFD in the future.

The second area needs to address the knowledge gaps surrounding the role pudendal sensory impairment plays in the aetiology of PFD. Whilst there is evidence of sensory nerve impairment in women with urinary dysfunction, POP and sexual dysfunction, this needs to be correlated with histological findings to investigate whether sensory nerves are damaged or fewer in number in women with PFD. Once this is better understood the specialty can then start to research how this can be reduced or avoided, investigated and managed in the future.

Finally, at the moment there is a disconnect between how our obstetric colleagues manage women on the labour ward in an acute setting and what happens when these women attend the urogynaecology clinic many months or years later. Further work should focus on trying to bridge this disconnect and develop a risk stratification to identify women at high risk of birth trauma and future PFD that could be performed in the antenatal clinic. This would enable women to make informed decisions about the management of the delivery of their baby.

4.4.5   Conclusion

This is the first study to evaluate pelvic sensation in pregnancy and following childbirth. The data suggest Aβ nerve fibre sensation is impaired in pregnancy with sparing of Aα nerve fibres and vaginal birth is associated with injury to pelvic sensory nerves, although this does not appear to be associated with symptoms of PFD in this cohort. However, the long-term implications of this sensory insult remain unclear and further work is needed to monitor these women in the medium and long-term.

5 Neurophysiology and neurohistology in pelvic organ prolapse: a pilot study

5.1 Introduction

Pelvic organ prolapse (POP) is a common condition with a reported incidence in the general population ranging from 6% to 50%, depending on whether diagnosis is based on symptoms or anatomical findings.[122,123,143] One in four of these women will undergo surgical repair of their POP at some point in their lifetime, despite a surgical failure rate of 30%.[168]

One possible reason for the high failure rate could be the complex aetiology of POP which includes ageing, injury during vaginal birth to the connective tissues, injury during vaginal birth to motor and sensory nerves of the pelvic floor, occupation and connective tissue disease. It is possible each of these factors may play a role in maintaining the success of a surgical repair. However, before this can be investigated further, a better understanding of the pathophysiology of POP is needed.

Women with POP have evidence of motor nerve damage as well as impaired function of large and small sensory nerve fibres compared to controls.[27,94,95] In addition, Chapter 3 section 4.3.11.2 also found evidence of impaired sensation in medium to large nerve fibres in POP in a postnatal cohort. On a microscopic level, researchers have reported fewer nerve fibres in the anterior vaginal wall and vaginal apex of women with POP compared to controls.[34–36]

Despite this, to date no study has evaluated whether sensory impairment is associated with reduced frequency of vaginal nerve fibres in women with POP.

I hypothesised women with POP have reduced numbers of nerve fibres and this is associated with impaired sensation. I designed a preliminiary exploratory study to refine the recuitment process, staining techniques and statistical analysis to correlate with clinical sensory testing data.

The study that follows is a preliminary exploratory analysis of the relationship between vaginal sensation and neurohistology.

5.2 Materials and methods

5.2.1 Study design

A prospective observational study entitled 'A simple test of pelvic sensation' was performed, sponsored by Central Manchester Foundation Trust (Study ID R04425), approved by the North West – Greater Manchester West Research Ethics Committee on 30/11/16 (16/NW/0715) and Health Research Authority on 14/12/2016 (Appendix 8.4). The study was co-adopted onto the NIHR portfolio by Reproductive Health and Childbirth (CPMS ID 32153).

5.2.2 Sample size

Data from a previous study found a 55% difference in peripheral nerve fibre count of the submucosa of the anterior vaginal wall in women with POP and women without POP.[34] To date, no study has linked sensory function and neurohistology of vaginal mucosa, therefore with no prior data available this was a preliminary exploratory analysis. As such, the recruitment target was 20 women with POP and 6-8 control women without POP.

5.2.3 Recruitment

Women with POP who opted for a surgical repair of the anterior vaginal wall (anterior repair) were recruited from the urogynaecology and pre-operative assessment clinics at St Mary's Hospital, Manchester Foundation Trust. Women without POP who opted for major gynaecological surgery under general anaesthetic were recruited from the gynaecology and pre-operative assessment clinics.

Case notes were screened according to the eligibility criteria in Table 5–A and eligible women provided with an information leaflet in the post before their clinic appointment.

Women were approached in the clinic room following their consultation, provided with a description of the study and a demonstration of the vibration on their hand using a simple hand-held vibration device.

Recruitment was performed by the Clinical Research Fellow and doctors who were members of both the clinical and research team, Figure 5-I. Women were given the opportunity to ask questions and reassured their decision would not affect their clinical care. Those who chose to participate completed a consent form at the start of the clinical visit. The clinical visit was performed in the month prior to the woman's surgery at a time and day most convenient for her.

Figure 5-I Study schema

Case notes screened for eligibility

Eligible

Ineligible

In person discussion of study

Accepted

Declined

Clinical visit (within one month of surgery)

Informed consent

QST for Aβ, Aδ and C nerve fibres

Demographic data

- Vagina, clitoris and index finger

POP: Histology

Cleaned & draped

Areas for biopsy marked

- 3cm proximal to urethral meatus (A)
- 5cm proximal to urethral meatus (B)

Anterior repair performed

Controls: Histology

Cleaned & draped

Prior to gynaecological surgery:

Areas for biopsy marked

- 3cm proximal to urethral meatus (A)
- 5cm proximal to urethral meatus (B)

Tissue samples

Histology: Haematoxylin and eosin

Immunohistochemistry

- Protein gene product 9.5
- Myelin binding protein

Key: POP-Q = pelvic organ prolapse quantification system; ePAQ-PF = pelvic floor electronic patient assessment questionnaire pelvic floor; AR = anterior repair

5.2.4   Study entry

Women were considered eligible for recruitment if they met the inclusion criteria and did not have any of the exclusion criteria, Table 5–A.

Table 5–A Eligibility criteria

All participants	
Inclusion Criteria	*Exclusion Criteria*
Age over 18 years	Language barrier requiring interpreter for consultation
Written informed consent	Incapacity to consent
Attending the Gynaecology clinic at St Mary's Hospital	Previous anterior repair
	Medical condition predisposing to sensory nerve dysfunction
	Female genital mutilation preventing access to the vagina
	Previous major gynaecological surgery which could affect pelvic nerves
	Previous systemic treatment for cancer
Women with POP	
Inclusion Criteria	*Exclusion Criteria*
Anterior wall POP at or below the hymen	Opted for conservative management
Planned anterior repair	
Controls (no POP)	
Inclusion Criteria	*Exclusion Criteria*
No POP	POP in any compartment at or below the hymen
Planned gynaecological surgery under anaesthetic	

Key: POP – pelvic organ prolapse

5.2.5 Demographic data

Demographic data was collected including age, ethnicity, menstrual history, previous obstetric, gynaecological and medical history, drug and smoking history. Height and weight were taken from the measurements performed during the preoperative assessment, and body mass index (BMI) calculated as weight in kilograms divided by height in metres^2.

5.2.6 Quantitative sensory testing

Sensation was measured using pelvic neurophysiology in the form of quantitative sensory testing, which uses a validated protocol to provide a reproducible assessment of the entire sensory pathway. All intimate examinations were performed in the presence of a chaperone.

The nerve stains currently available cannot differentiate between Aβ, Aδ or C nerve fibres. As a result QST for both vibration and temperature sensation was measured to provide clinical data on Aβ, Aδ and C nerve fibres for analysis.

Vibration sensation

Myelinated Aβ nerve fibres were tested via the modality of vibration sensation using QST, as described in section 2.1.1 and 2.1.2.

Temperature sensation

Slower unmyelinated Aδ and C nerve fibres were tested using QST via the modality of thermal and cold sensation, as described in section 2.1.1 and 2.1.3.

5.2.7 Pelvic organ prolapse

All women underwent a standardised clinical examination for pelvic organ prolapse using the pelvic-organ prolapse quantification system (POP-Q) within six months of tissue collection, as described in section 2.2.[118] The presence of POP was defined a priori as the most distal portion of the anterior vaginal wall at or below the level of the hymen.[122–124]

5.2.8   Tissue collection

Women with POP

On the planned day of surgery, women were admitted through the pre-operative assessment unit and seen by their operating surgeon, anaesthetist and member of the research team to confirm consent for tissue collection.

Women were transferred to theatre and underwent the identification and operation confirmation as per hospital policy, including the 'sign in' surgical safety world health organisation checklist.[169] Women were anaesthetised with regional or general anaesthesia. Participation in the study did not alter choice of anaesthetic. Women were positioned on the operating table, cleaned, draped and an indwelling Foleys catheter inserted. The world health organisation 'time out' surgical safety checklist was completed as per hospital policy.[169] The surgeon or the clinical research fellow used a sterile ruler and marker pen to mark two lines on the anterior vaginal wall:

Point A = 3cm proximal to the urethral meatus

Point B = 5cm proximal to the urethral meatus

The operation was performed by one of three consultants or a senior registrar operating under their supervision. All anterior repairs were performed using native tissue, some with concomitant prolapse surgery for the uterus, vaginal vault or posterior vaginal wall.

The anterior repair involved infiltration of 1/200,000 adrenaline into the vaginal mucosa. A midline incision was made and the vaginal fascia separated from the vaginal mucosa using sharp or blunt dissection or a combination of both. The fascia was repaired using midline plication with an absorbable braided suture. The excess vaginal mucosa was then excised, sutured at 12 o'clock to facilitate orientation for tissue collection, and placed to one side. Raw edges were checked for haemostasis and the vaginal mucosa was sutured using a continuous locked absorbable braided suture. At this point, women were cleaned, the drapes removed and moved into the supine position. The 'sign out' world health organisation surgical safety check was performed.[169]

Two 4mm punch biopsies were subsequently performed on the excised vaginal mucosa at point A and B by a member of the research team (clinical research fellow or the operating surgeon if also a member of the research team). To standardise tissue collection, each woman was sent to theatre with a copy of the study consent form in her notes, pre-labelled formalin sample pots and written instructions on how to sample the tissue.

Controls

Control women were also admitted through the pre-operative assessment unit and seen by their operating surgeon, anaesthetist and the clinical research fellow to confirm consent for tissue collection.

Women were transferred to theatre and underwent the identification and operation confirmation as per hospital policy, including the 'sign in' surgical safety world health organisation checklist.[169] Women were anaesthetised with regional or general anaesthesia. Participation in the study did not alter choice of anaesthetic.

Women were positioned on the operating table, cleaned, draped and an indwelling Foleys catheter inserted if appropriate to the procedure. The world health organisation 'time out' surgical safety checklist was completed as per hospital policy.[169]

The clinical research fellow then used a sterile ruler and marker pen to mark two lines on the anterior vaginal wall:

Point A = 3cm proximal to the urethral meatus

Point B = 5cm proximal to the urethral meatus

Two 4mm punch biopsies were then performed at point A and B by the clinical research fellow. Again, tissue was placed in pre-labelled formalin sample pots and manually transported to the laboratory. Similar to the POP group, each woman was sent to theatre with a copy of the study consent form in her notes and written instructions on how to sample the tissue.

The two wounds were then closed by the clinical research fellow using a single interrupted absorbable braided suture. The operating surgeon then proceeded with the planned procedure.

5.2.9   Sample handling and storage

At the end of each procedure tissue samples were manually transported by a member of the research team to the pathology laboratory reception at Manchester Foundation Trust.

Vaginal mucosa specimens were formalin-fixed and embedded in paraffin by the Manchester Foundation Trust pathology department. Wax blocks were sectioned and automatically stained for haematoxylin and eosin (H&E) using the Leica XL autostainer, see Appendix 8.9.

5.2.10   Immunohistochemistry

Optimisation

Staining of tissue samples was automated for protein gene product 9.5 (PGP 9.5), myelin basic protein (MBP) and Cytokeratin 20 (CK20) using the Ventana BenchMark ULTRA IHC staining module (Ventana Co., Tuscon, Arizona, USA).

The pathology department at Manchester Foundation Trust regularly uses PGP 9.5, MBP and CK20 in clinical practice and had previously optimised the protocols for each antibody as part of their clinical governance standard operating procedures.

The positive and negative staining of control tissue for each immunostain is shown in Figure 5-II. PGP 9.5 and MBP staining of control tissue revealed a strong cytoplasmic reaction with peripheral nerves and myelinated nerves respectively, whilst CK20 showed a strong reaction with Tonsillar Merkel cells.

Free nerve endings

Whilst PGP 9.5 is expressed in neurons in healthy tissue, it has also been found in a number of locations including dermal fibroblasts, Merkel cells, oocytes and Leydig cells.[170] To confirm whether the extensive positive reaction seen in the lamina propria represented free nerve endings or Merkel cells CK20 staining was performed on one tissue sample. This demonstrated the absence of Merkel cells when compared to the PGP 9.5 stain of the same sample.

Staining protocols

Tissue sections were cut at 4 µm and baked for 30 minutes at 70ºC. The automated Ventana BenchMark ULTRA IHC Staining Module (Ventana Co., Tucson, Arizona, USA) was used together with the ultraView 3, 3' diaminobenzidine (DAB) version 3 detection system (Ventana Co.). Tissue sections were deparaffinised and incubated in EZPrep Volume Adjust (Ventana Co.). At intervals between steps the slides were washed with a TRIS-based reaction buffer, pH 7.6. A heat-induced antigen retrieval protocol was carried out using a TRIS– ethylenediamine tetracetic acid (EDTA)–boric acid pH 8 buffer, Cell Conditioner 1(CC1), for between 16 and 32 minutes depending upon individual optimisation. The sections were incubated with ultraviolet inhibitor blocking solution for 4 min, then with antibody used at a dilution of 1:100 to 1:250 depending on optimisation. This was followed by incubation with horseradish peroxidase-linked secondary antibody for eight minutes, then H_2O_2 and DAB chromogen for eight minutes, and copper for four minutes. Counterstain (Haematoxylin II) was applied for 12 minutes before an incubation of four minutes with bluing reagent. Slides were then removed from the staining platform and washed in warm soapy water before being rinsed in cold tap water

and then dehydrated through three steps of 99% denatured alcohol (IMS) and two changes of Xylene. Sections were then cover slipped using ClearVue Mountant XYL (Thermo Scientific). Table 5−B lists the individual protocols for PGP 9.5, MBP and CK20.

Table 5−B Immunohistochemistry protocols

	Antibody		
	PGP 9.5	*MBP*	*CK20*
Clone	Polyclonal	Polyclonal	Monoclonal
Company	Cell Marque	Cell Marque	DAKO
Antibody species	Rabbit	Rabbit	Mouse
Product number	318A-14	295-A15	M7019
Antigen retrieval	Cell conditioning (CC1) at 98°C for 64 minutes	Cell conditioning (CC1) at 98°C for 52 minutes	Cell conditioning (CC1) at 98°C for 64 minutes
Antibody dilution	1:100	1:100	1:250
Incubation time	32 minutes	16 minutes	24 minutes
Detection kit	DAB	DAB	DAB

Key: PGP - protein gene product; MBP - myelin binding protein; CK20 - cytokeratin 20; DAB - ultraView 3, 3' diaminobenzidine (DAB) version 3 detection system (Ventana Co.)

Scoring of immunohistochemistry

An independent clinical lecturer in reproductive and paediatric histopathology, Dr Petts, assessed morphology of samples on H&E staining. She was blinded to all clinical details, including control or POP, as well as the QST sensory data. Slides were digitally scanned using the Panoramic 250 Flash III scanner, 3D Histech. Images were viewed using CaseViewer 2.2 (64-bit version).

Protein gene product 9.5

Nerve cross sections stained with PGP 9.5 were assessed by two blinded scorers (the clinical research fellow Dr Mahoney and the histopathology clinical lecturer mentioned above) at high power (x20) and the average value taken, Figure 5-III.

PGP 9.5 stained free nerve endings were automatically counted using QuPath open access bioimage analysis software, Figure 5-IV.[171]   Regions of interest were manually selected, excluding the epithelium, nerves, areas of space and folds within the tissue. The area was measured, staining differentiation optimised, positive cell detection performed and a cell count calculated. To facilitate analysis data was converted into frequency of nerve cross sections and free nerve endings per mm^2.

Myelin binding protein

Again, myelinated nerves were visually assessed by two blinded scorers and the average value taken, Figure 5-III. Due to the low incidence of myelinated nerves in the cohort only descriptive analysis was undertaken.

Scoring disagreement

The decision was made a priori to rescore any slides with an inter-observer disagreement greater than 10%. Analysis of scoring data revealed all scoring disagreements were less than 10% and so this was not required.

Figure 5-II Staining of control tissues for PGP 9.5, MBP and CK20 demonstrating strong reactivity for control tissue
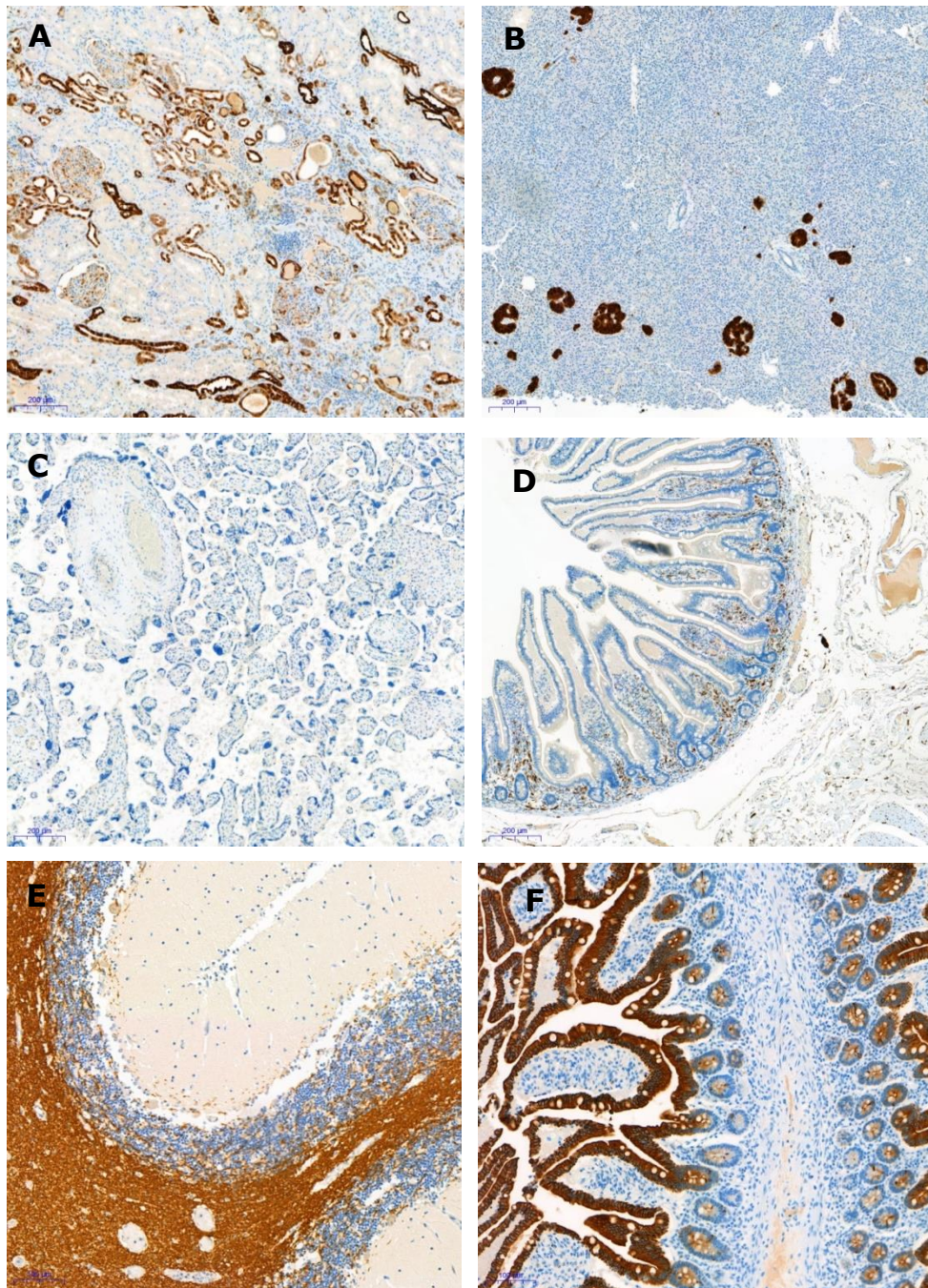
Key: PGP 9.5 control tissue showing strong cytoplasmic reaction for nerves and free nerve endings (brown colouration) in the A) Pancreas, B) Placenta, C) Small bowel, D) Kidney. MBP control tissue showing strong cytoplasmic reaction of myelinated nerves in E) Cerebral cortex. CK20 control tissue showing strong cytoplasmic reaction of Merkel cells in F) Tonsil

Figure 5-III Images of vaginal mucosa immunostained with PGP 9.5, MBP and CK20
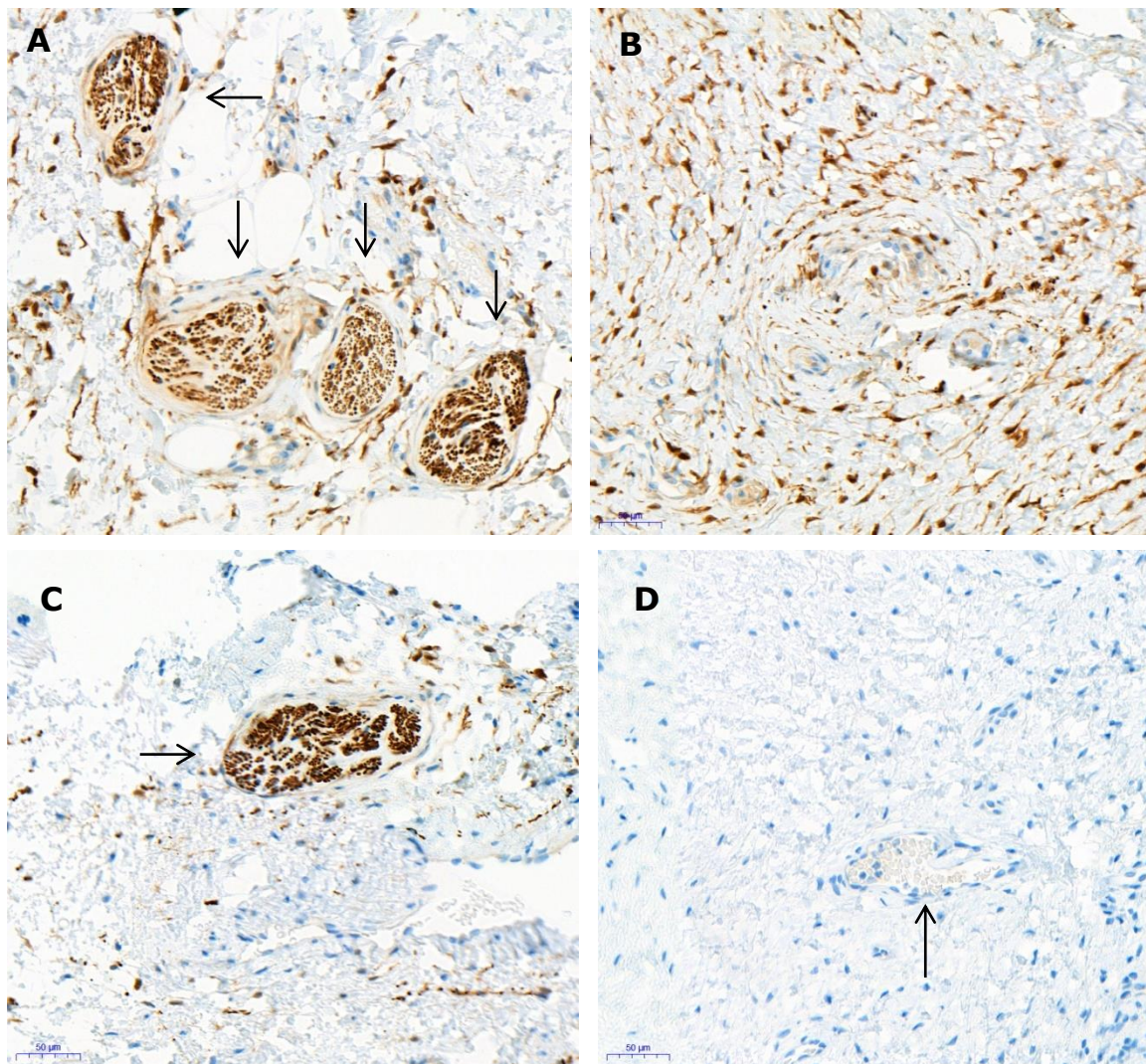
Key: Images of vaginal mucosa from anterior vaginal wall at x20 magnification.

A) PGP 9.5 immunostain showing nerves in the lamina propria marked by arrows, bundles of nerve fibres, called fascicles visible within each nerve.

B) PGP 9.5 stain showing free nerve endings in brown within the lamina propria.

C) MBP immunostain of myelinated nerve denoted by arrow, fascicles visible within the nerve.

D) CK20 immunostain negative for Merkel cells within the lamina propria as no brown staining when compared with Figure 5-II Image F), arrow denotes blood vessel.

Figure 5-IV Process of PGP 9.5 staining automated cell count using QuPath bioimage analysis software
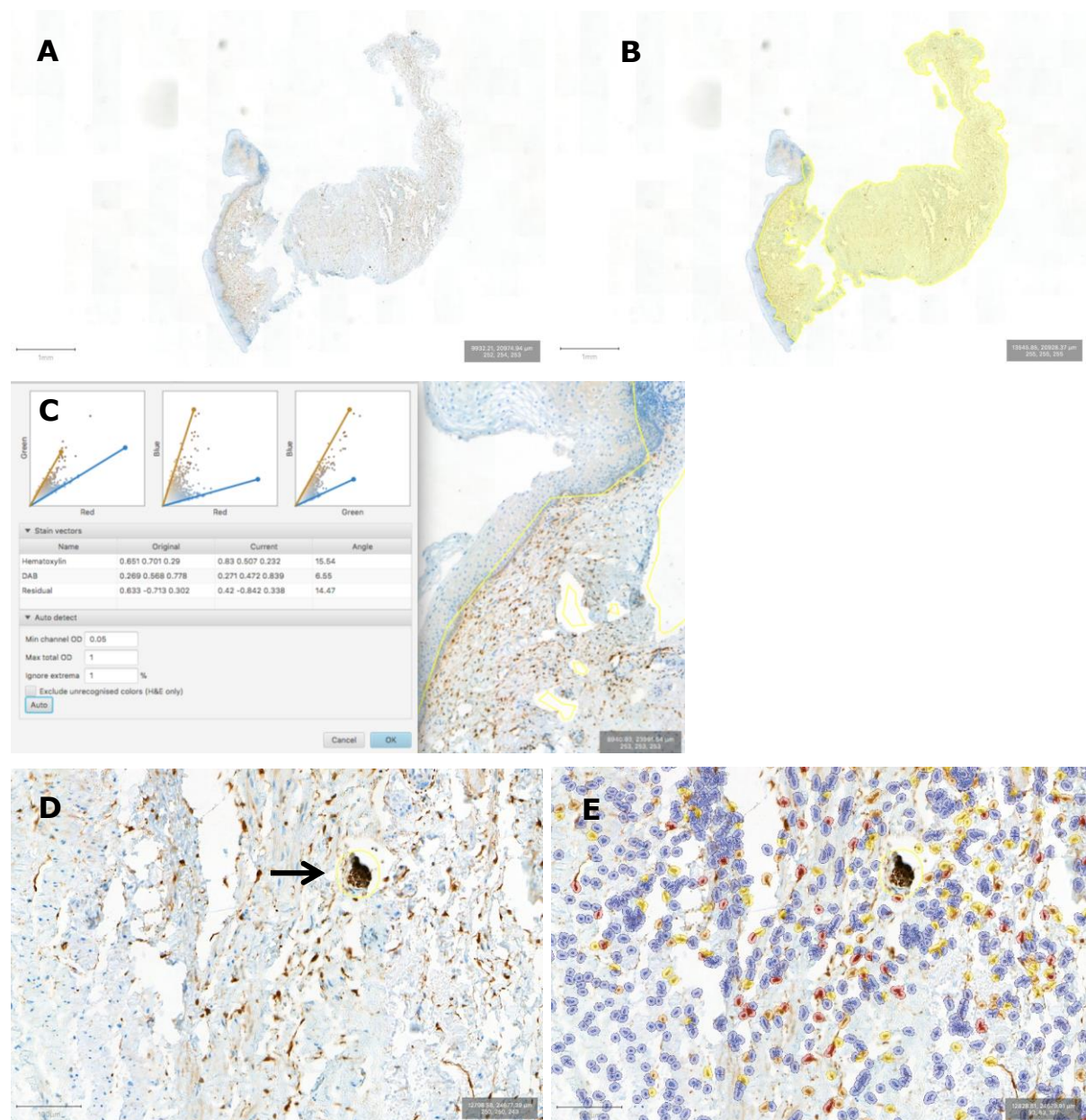
Key:

A) Image of entire tissue sample stained with PGP 9.5;

B) Regions of interested were manually selected (yellow area) excluding the epithelium (blue area), nerves, tissue folds and areas of space within the sample;

C) Optimisation of staining differentiation performed;

D) Image showing optimised sample prior to positive cell detection – free nerve endings stained brown, arrow indicates excluded nerve fibre.

E) Image demonstrate positive cell detection - surrounding cell nuclei stained blue to aid software detection of free nerve endings in brown.

5.2.11   Statistical analysis

Data were analysed using STATA, version 15.1 for Windows (Statacorp, College Station, Texas). There is no normative data published on the frequency of nerves, free nerve endings and myelinated nerve fibres for comparision.

Vibration sensation was not normally distributed and remained non-parametric despite transformation. The relationship between vibration sensation and frequency of nerves and free nerve endings was visually assessed using a scatterplot, but there was insufficient sample size given the spread of the data to facilitate formal testing.

Thermal and cold sensation remained non-parametric despite transformation, and were therefore dichotomised into normal and abnormal sensation using reference ranges from previously published normative data and control data in a study evaluating sensation in POP.[47,95] Data was then analysed using a Mann Whitney U test.

Stage of prolapse was dichotomised into POP at the hymen or 1cm below, and POP at ≥2cms below the hymen.[118] Data was then analysed using a Mann Whitney U test.

Due to the small numbers of myelinated nerve fibres in each sample it was not possible to perform meaningful statistical comparisons, and therefore data analysis was limited to descriptive statistics.

5.3 Results

5.3.1   Recruitment

Pelvic organ prolapse participants

Between April 2017 and September 2018 73 case notes were screened for eligibility, of which 55 women were eligible, of who 26 enrolled onto the study, Figure 5-V.

Figure 5-V Screening and accrual of POP participants

```
┌─────────────────────────┐        ┌─────────────────────────────────────────────┐
│ Eligibility screen:     │        │ Reasons for non-eligibility:                  │
│ Case notes screened     │        │ Language barrier                        3     │
│ Eligible        55      │───────▶│ Medical condition predisposing          2     │
│ Ineligible      18      │        │ to sensory dysfunction*                       │
└─────────────────────────┘        │ Neuromodulatory medication ¥            3     │
            │                       │ Female genital mutilation               0     │
            │                       │ Incapacity to consent                   1     │
            │                       │ Previous anterior repair                9     │
            │                       └─────────────────────────────────────────────┘
            │
            │                       ┌─────────────────────────────────────────────┐
            │──────────────────────▶│ Unable to participate:                        │
            │                       │ No clinical room or staff available   4       │
            │                       │ Surgery cancelled                     3       │
            │                       └─────────────────────────────────────────────┘
            │
┌─────────────────────────┐        ┌─────────────────────────────────────────────┐
│ Agreed          23      │        │ Reasons declined:                             │
│ Declined        25      │───────▶│ No reason given                        14     │
└─────────────────────────┘        │ Dislikes concept                        5     │
            │                       │ Phobia of pelvic examinations           2     │
            │                       │ First on theatre list and unable to     4     │
            │                       │ attend for QST earlier                        │
┌─────────────────────────┐        └─────────────────────────────────────────────┘
│ QST and tissue samples 16│
│ QST only§             7 │
└─────────────────────────┘
```

*Key: QST - quantitative sensory testing; tissue samples – two punch biopsies on anterior vaginal wall at 3cm and 5cm proximal to the urethral meatus. * Medical conditions predisposing to sensory impairment were diabetes mellitus and multiple sclerosis. ¥Neuromodulatory medications were sodium valproate, gabinoids and tri-cyclic antidepressants § Tissue samples not obtained from 13 women due to surgeon choice to not perform anterior repair following intraoperative POP-Q examination findings.*

Control participants

Between April 2017 and September 2018 47 case notes were screened for eligibility, of which 28 women were eligible, of who five enrolled onto the study, Figure 5-VI.

Figure 5-VI Screening and accrual of control participants
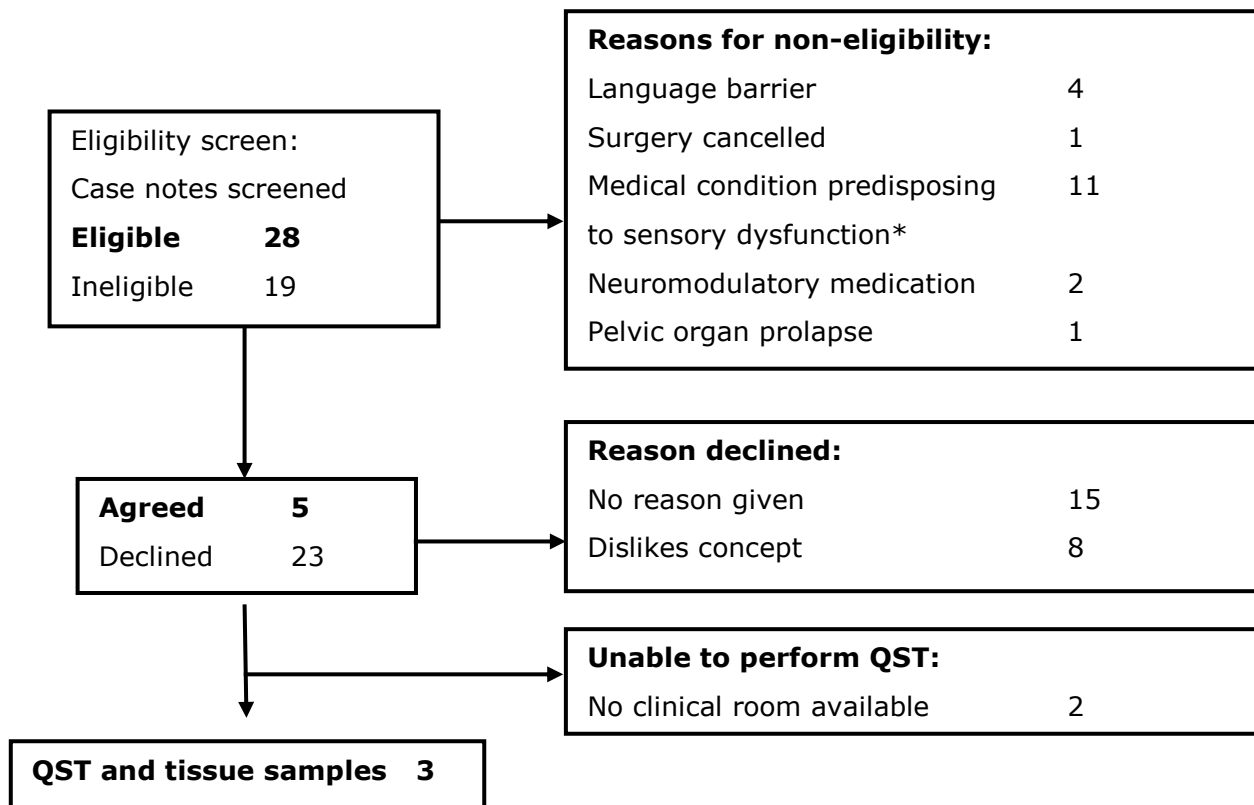
*Key: QST - quantitative sensory testing; tissue samples – two punch biopsies on anterior vaginal wall at 3cm and 5cm proximal to the urethral meatus. *Medical conditions predisposing to sensory dysfunction – multiple sclerosis and chronic pelvic pain. §No clinical room available on day of surgery and women unable to attend beforehand.*

5.3.2 Baseline cohort characteristics

The demographics for the cohort are listed in Table 5–C . None of the women in either the POP or Control groups reported a clinical history of numbness.

Table 5–C Cohort characteristics

Demographics		POP	Controls
Age, years *Median (IQR)*		60 (53-69.5)	52 (39-71)
Ethnicity *Frequency*	White	16	2
	Asian	1	0
	Afro-Caribbean	0	1
	Other	0	0
BMI, kg/m² *Median (IQR)*		29.1 (25.30-32.4)	31.4 (27.9-35.1)
Endocrine disorders *Frequency*	Hypothyroidism	1	1
	Impaired GTT	1	0
Smoker *Frequency*		1	0
Reproductive Factors			
Parity *Median (IQR)*		2 (2-3)	2 (1-4)
Vaginal birth *Median (IQR)*		2 (2-3)	1 (1-4)
Menopausal status *Frequency*		13	2
Exogenous hormones *Frequency*	HRT	0	0
	Progestogens*	0	0
	Combined hormonal contraception§	0	0
Descent of anterior wall below hymen, cms *Median (IQR)*		1.5 (1.0-3.5)	
Concomitant surgery¥ *Frequency*	None	3	
	Posterior repair	6	
	Vaginal hysterectomy	5	
	LSC	1	
	LSH	2	
	Sacrospinous fixation	5	

*Key: IQR – interquartile range; GTT – glucose tolerance test; HRT – hormone replacement therapy; LSC – laparoscopic sacrocolpopexy; LSH – laparoscopic sacrohysteropexy; * Progesterone only pill, injectable, implant and mirena IUS; §Combined pill and ring; ¥ Some women underwent more than one procedure at the time of their anterior repair.*

5.3.3   Myelinated nerves in the vaginal mucosa

Of all biopsies taken at 3cm proximal to the urethral meatus, two myelinated nerves were present in one woman with POP, and seven myelinated nerves were present in one control. In samples taken at 5cm proximal to the urethral meatus, myelinated nerves were present three women with POP (one, one and three respectively) and five myelinated nerves were present in one control.

5.3.4   Vaginal sensation and neurohistology

Vibration sensation

A scatter plot was used to assess the relationship between vaginal vibration sensation and the frequency of nerves and frequency of nerve endings at 3cm and 5cm proximal to the urethral meatus, Figure 5-VII. Visual inspection of the plot did not reveal an obvious relationship between the variables, however formal testing was not possible due to the small sample size and spread of the data. It was not possible to perform a direct comparison with POP and controls due to the small number of controls.

Figure 5-VII Scatter plot of vaginal vibration sensation and frequency of nerves and free nerve endings
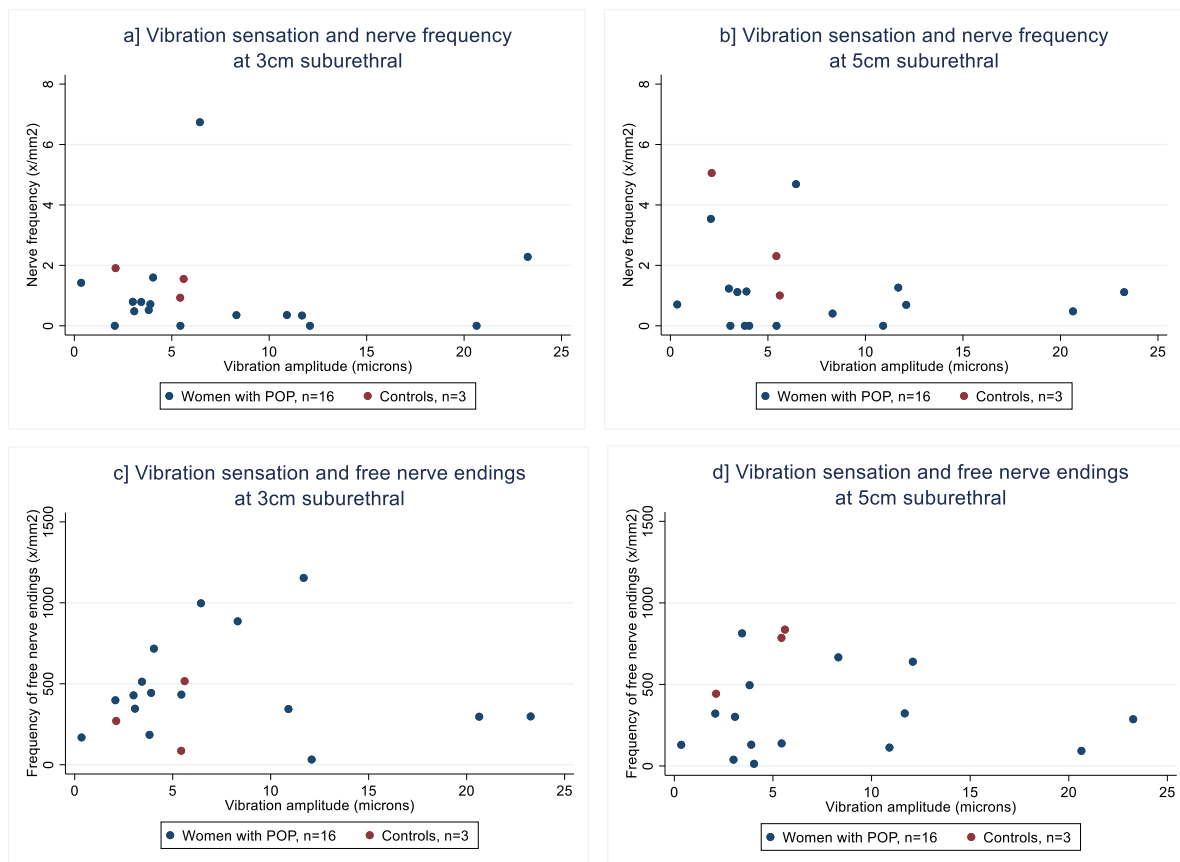
Function of vaginal Aβ nerve fibres measured using vibration sensation and compared to frequency of nerves and free nerve endings per mm² at the anterior vaginal wall.

Higher amplitudes produce stronger vibration and equate to reduced vibration sensation.

Biopsies taken at 3cm and 5cm proximal to the urethral meatus.

Direct comparison between the control and POP was not performed due to the small control group size.

Key: POP – pelvic organ prolapse

Thermal sensation

Thermal sensation remained non parametric despite transformation, therefore this was dichotomised into normal and abnormal sensation using reference ranges from both previously published normative data and control data in a study evaluating sensation in women with POP.[47,95] Again, it was not possible to perform a direct comparison with POP and controls due to the small number of controls. Analysis using Mann Whitney U test found no association between thermal sensation and frequency of nerves or free nerve endings at 3cm or 5cm proximal to the urethral meatus, Figure 5-VIII. This was also true when the analysis was repeated to include the control data.

Figure 5-VIII Vaginal thermal sensation and frequency of nerve fibres and free nerve endings
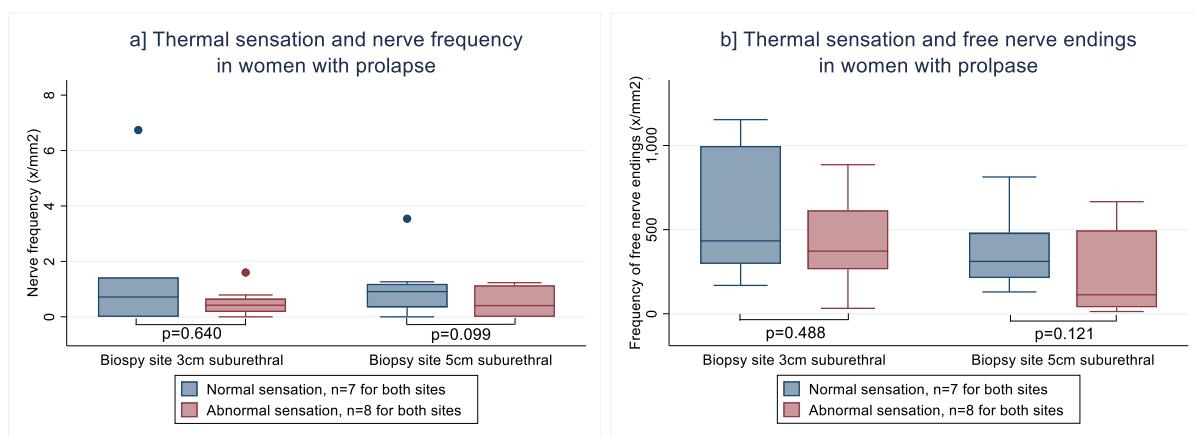
Function of vaginal Aδ and C nerve fibres measured using thermal sensation and compared to frequency of nerve fibres and free nerve endings per mm² at the anterior vaginal wall. Higher temperatures equate to reduced thermal sensation. Vaginal 4mm punch biopsies were taken at 3cm and 5cm proximal to the urethral meatus.

Thermal sensation was dichotomised into normal and abnormal using reference ranges from previously published normative data and control data in a study evaluating sensation in POP.[47,95] Data analysed using Mann Whitney U test.

Control data not included, however nerve fibre frequency and free nerve endings at 3cm and 5cms suburethral remained not significant when Mann Whitney U test repeated to include the control data.

One missing data point due to technical failure of the thermal probe during testing.

Key: POP – pelvic organ prolapse

Cold sensation

Cold sensation remained non parametric despite transformation, therefore this was dichotomised into normal and abnormal sensation using reference ranges from both previously published normative data and control data in a study evaluating sensation in women with POP.[47,95] Again, it was not possible to perform a direct comparison with POP and controls due to the small number of controls. Analysis using Mann Whitney U test found no association between cold sensation at 3cm or 5cm suburethrally and frequency of nerve fibre cross sections or free nerve endings, Figure 5-IX. This was also true when the analysis was repeated to include the control data.

Figure 5-IX Box plot of vaginal cold sensation and frequency of nerves and free nerve endings
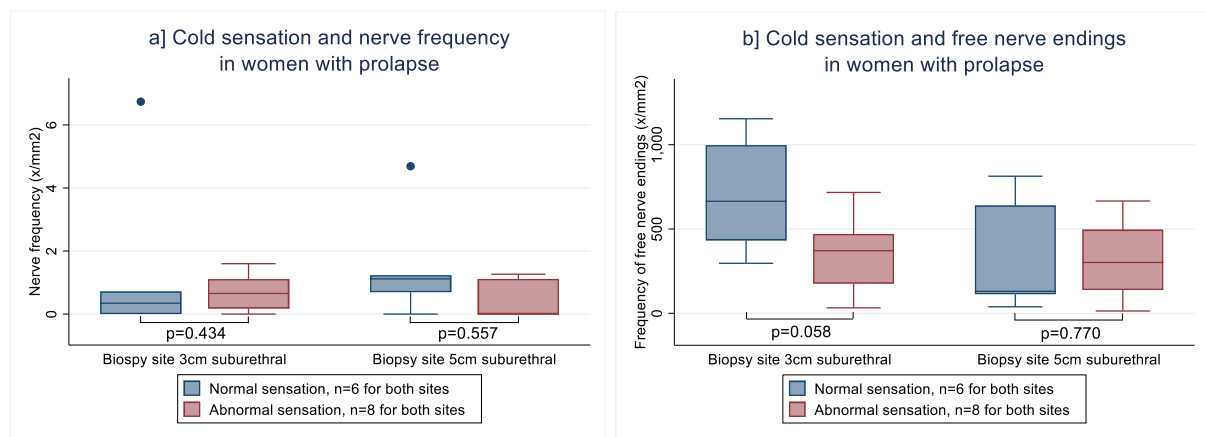
Function of vaginal Aδ and C nerve fibres measured using cold sensation and compared to frequency of nerves and free nerve endings per mm² at the anterior vaginal wall. Vaginal 4mm punch biopsies were taken at 3cm and 5cm proximal to the urethral meatus.

Cold sensation was dichotomised into normal and abnormal using reference ranges from previously published normative data and control data in a study evaluating sensation in POP.[47,95] Data was analysed using Mann Whitney U test.

Control data not included, however nerve fibre frequency and free nerve endings at 3cm and 5cms suburethral remained not significant when Mann Whitney U test repeated for entire cohort.

One missing data point due to overheating of the thermal probe during thermal testing and the other due to technical failure of the thermal probe during testing.

Key: POP – pelvic organ prolapse

5.3.5 Age and neurohistology

A scatter plot was used to assess the relationship between age and the frequency of nerves and frequency of nerve endings at 3cm and 5cm proximal to the urethral meatus, Figure 5-VII. Visual inspection of the plot did not reveal an obvious relationship between the variables, however formal testing was not possible due to the small sample size and spread of the data. Again, it was not possible to perform a direct comparison with POP and controls due to the small number of controls.

Figure 5-X Scatter plots of age and frequency of nerves and free nerve endings
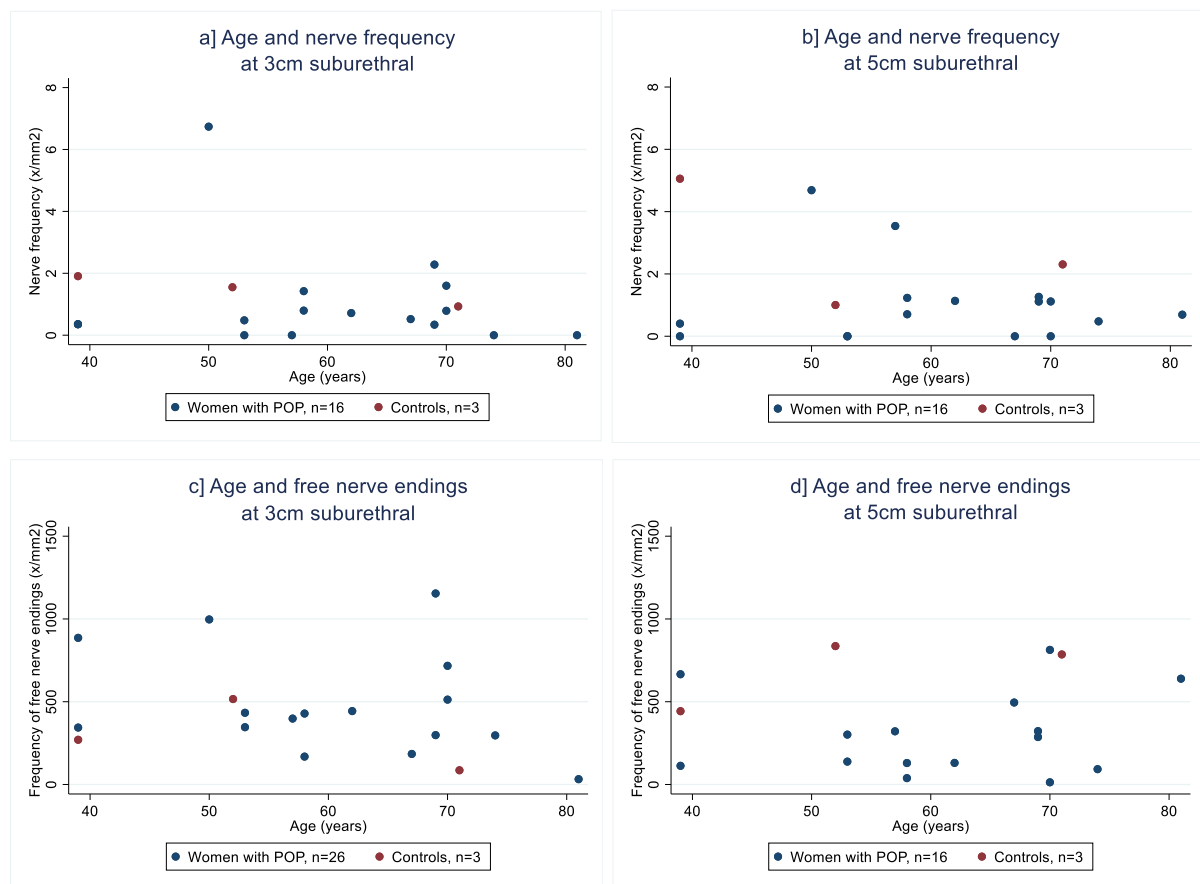
The relationship between age and frequency of nerves and free nerve endings per mm^2 at the anterior vaginal wall was evaluated. Vaginal 4mm punch biopsies were taken at 3cm and 5cm proximal to the urethral meatus.

Direct comparison between the control and POP was not performed due to the small control group size.

Key: POP – pelvic organ prolapse

5.3.6   Stage of prolapse and neurohistology

Prolapse was defined the in study protocol as the leading edge of the vaginal wall at or below the hymen, corresponding to Ba ≥0cms on POP-Q.[122–124] To investigate whether the stage of POP was associated with reduced vaginal innervation of both nerves and free nerve endings, women were dichotomised into POP at the hymen to 1cm below and POP at 2cm below the hymen or greater.[118]

Again, it was not possible to perform a direct comparison with POP and controls due to the small number of controls. Analysis using Mann Whitney U test found no association between stage of POP and frequency of nerve fibre cross sections or free nerve endings at 3cm or 5cm proximal to the urethral meatus. This was also true when the analysis was repeated to include the control data.

Figure 5-XI Box plots of prolapse and frequency of nerves and free nerve endings
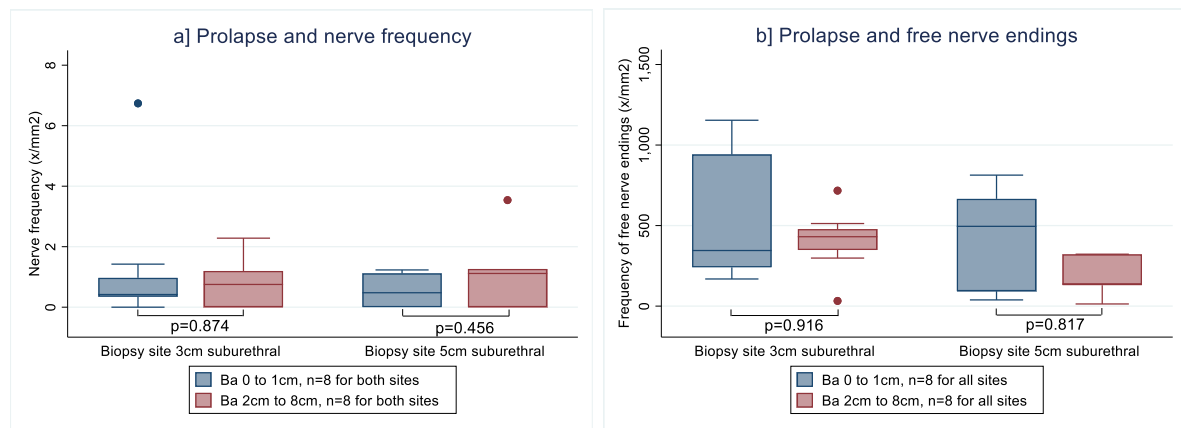
POP defined as the leading edge of the vaginal wall at or below the hymen, corresponding to Ba ≥0cms on POP-Q.[122–124] To investigate whether the degree of POP was associated with reduced vaginal innervation women were grouped into POP at the hymen or 1cm below, and POP at ≥2cms below the hymen.[118] Vaginal 4mm punch biopsies were taken at 3cm and 5cm proximal to the urethral meatus.
Data was analysed using Mann Whitney U test.
*Direct comparison between the control and POP groups was not performed due to the small control group size.*
Key: POP – pelvic organ prolapse; Ba – most distal part of the anterior vaginal wall; POP-Q – pelvic organ prolapse quantification examination system

5.4 Discussion

5.4.1   Interpretation and current evidence

This pilot study is the first to investigate the relationship between vaginal sensation and frequency of nerve fibres. It is also the first study to measure and report the frequency nerve fibre cross sections and free nerve endings separately, as well as the presence of myelinated nerves in the vaginal mucosa.

This study found no evidence of an association between vaginal sensation for Aβ, Aδ or C nerve fibres and frequency of nerve fibre cross sections or free nerve endings at 3cm or 5cm proximal to the urethral meatus. Whilst this preliminary study has demonstrated feasibility of the project and suggests performing a similar study on a larger scale would be viable, this pilot study involved a small cohort of women, with a very small control group. As such statistical analysis was limited by the high risk of a type II error. On this basis the results should be interpreted with caution.

Three studies have evaluated neurohistology in women with POP compared to controls.

The first by Inal et al also evaluated vaginal nerve fibres at 3cm proximal to the urethral meatus in 89 women.[34] They reported significantly reduced frequency and diameter of nerve fibres in women with POP compared to controls (nerve fibre frequency/mm 0.004 vs 0.007 respectively, *p<0.001*). However, this pilot study found a greater frequency of nerve fibres in women with POP and controls (nerve fibre frequency/mm 0.501 vs 1.550 respectively). This difference likely reflects variations in counting protocols and techniques, and may also be a reflection of the small sample size in this exploratory study. It is not possible to comment on free nerve endings as Inal et al did not include an assessment of this.

The second study by Kaplan et al investigated the percentage of PGP 9.5 stained area at the vaginal apex in 84 women with POP compared to controls.[35] They also reported a reduction in percentage stained area in women with POP compared to controls. However by using the percentage of PGP 9.5 stained area, this study combined frequency of nerve fibres and free nerve endings into one analysis confounding any comparison with this small pilot study. In addition, the biopsy location at the vaginal apex suggests these findings may be more relevant to apical POP rather than POP of the anterior vaginal wall that was the focus of this study.

The third study by Zhu et al evaluated the presence of PGP 9.5 staining at 1cm lateral to the urethral meatus in women with POP, SUI and POP and controls (n=23, 15 and 15 respectively).[36] They also found a reduction in nerves of any type in women with POP

compared to controls. However, Zhu et al used a subjective measure to classify the presence of PGP 9.5 staining making it difficult to comment on how this fits with the exploratory study described in this chapter. Furthermore the control group were not age matched.

It is possible that there is no association between vaginal sensation for vibration, thermal or cold, age and frequency of nerves or free nerve endings. Another explanation is that the presence of motor nerves in the vaginal muscularis had a confounding effect on the nerve and free nerve ending frequency counts. Sensory nerves and free nerve endings are present in the muscularis as well as the lamina propria and therefore the muscularis was not excluded from the count. In a future study with larger numbers it would be interesting to evaluate the lamina propria and muscularis individually. However, it is equally possible that the lack of relationship between sensation and neurohistology reflects the small sample size and represents a type II error.

This study found no association between age and neurohistology in this cohort. One possible reason is the small sample size makes the study less likely to identify the non-linear effect of age related nerve degeneration.[172] Another explanation could be the vagina is not affected by this age related nerve degeneration which has been well documented in other areas of the body.[172] However this explanation would not correspond with the clinical neurophysiology findings of reduced vaginal motor and sensory nerve function in older women.[27,47]

The lack of association between stage of POP and neurohistology may also be due to the small sample size with increased risk of a type II error. Another possible explanation could be the pathophysiology of reduced nerve frequency seen in POP affects is not increased by deteriorating POP, and affects all stages of POP equally.

5.4.2   Strengths

This preliminary study has demonstrated it is feasible to use these techniques to conduct a larger study investigating the relationship between vaginal sensation and neurohistology.

The study also found the use of PGP 9.5 and MBP in the vaginal mucosa is feasible and could be expanded to use on a larger cohort. The study also describes a protocol for automated counting of free nerve fibres using open access software to facilitate reproduction in the future.

The Histopathologist raised a query regarding the possibility of PGP 9.5 staining of Merkel cells within the lamina propria based on visual inspection. This fitted with one

study reporting Merkel cells in the vaginal mucosa in 50% of samples studied.[19] This prompted testing of one slide for CK20 that found Merkel cells were absent in this one sample. This has introduced the possibility of CK20 staining in a future study to evaluate the distribution and role of Merkel cells in vaginal innervation.

5.4.3   Limitations

The main limitation in this preliminary pilot study is the small sample size and lack of a control group. These factors prevent direct comparison between the POP and control groups, as well as limit the validity of any statistical analysis. As a result, data on such small sample sizes should be interpreted with caution.

The study included women with a wide range of stages of POP from the hymen to 8cm below the hymen. Whilst the study attempted to answer this question by comparing women with a stage 2 and stage 3-4 POP, this pilot study was not adequately powered to answer this question. Future studies will need to be adequately powered to facilitate analysis of these as separate groups, as well as consider evaluating women with POP who have abnormal collagen types such as Ehlers Danlos variants.

Technical difficulties with the temperature testing equipment led to loss of data on one woman for thermal and two women for cold sensation. Given the small cohort size it is possible this may have had an effect on the distribution of data for thermal and cold sensation.

5.4.4   Generalisability

This is a preliminary exploratory study designed to inform the methodology and sample size of a future larger study. As such the conclusions in this pilot study should be interpreted with caution and are not applicable to the general population.

5.4.5   Future work

Further work should focus on using this data to design a large, adequately powered study involving a neurophysiology assessment QST compared to vaginal mucosal innervation, with individual and combined analysis of the lamina propria, muscularis using PGP 9.5, MBP and CK20. The study should also consider including clinical assessment with SSEP for comparison.

6 Summary and conclusion

Pelvic floor dysfunction (PFD) affects one in three women in the UK, and this number is expected to rise as the population ages. The financial cost for the conservative and surgical treatment of PFD is significant with estimates from the US at $1.7 billion in 2006, accounting for inflation this equates to $2.13 billion in 2019. In addition, the overall cost is likely to be much higher as this estimate does not include the additional expense of indirect costs such as employment limitation, social isolation and relationship breakdown.

An ageing population and increasing cost of healthcare therefore combine to make pelvic floor dysfunction an important public health issue. Despite this, the pathophysiology of PFD remains incompletely understood. Evidence suggests this involves a combination of ageing and childbirth trauma to muscles, connective tissue and motor nerves. It is only since the millennium that research has found evidence of pudendal sensory dysfunction in women with PFD. Until the pathophysiology of PFD is properly understood it will not be possible to develop an effective prevention strategy and reduce the number of women suffering.

Genital stretch sensation

This project aimed to develop a test to quantitatively measure sensation of vaginal tone via Aα and Aβ nerve fibres. To achieve this I modified the QST protocol for anorectal sensation to test vaginal and introitus stretch sensation. I demonstrated the test had good to excellent intra and inter-rater reliability and recruited 100 women to collect normative data from the local population.

Stretch sensation deteriorated with age, was not related to BMI and did not appear to be affected by parity, although this may have been due to an inadequate sample size for this objective. Example nomograms were produced to demonstrate how the QST method for stretch thresholds described could be used to produce normative data.

One study has reported normative data for female genital sensation, with vibration sensation deteriorating with age whilst temperature sensation appeared unaffected by age related nerve degeneration. The published data surrounding the impact of BMI on sensation throughout the body is conflicting, with some studies describing no effect and others reporting BMI as a covariate for vibration sensation.

Stretch thresholds may be used as a descriptor of Aα and Aβ sensory nerve function at the vagina and introitus. Further work should focus on the role of parity and ethnicity on

genital stretch sensation thresholds with a large adequately powered study with age-matched groups, as well as considering the effect of vaginal capacity.

Childbirth and pelvic sensation

This study aimed to determine the impact of childbirth on Aα and Aβ pudendal sensory nerves and how this related to the symptoms of pelvic floor dysfunction. To accomplish this I recruited 150 women in their first pregnancy and performed pelvic QST for vibration and stretch sensation, POP-Q and ePAQ-PF in the third trimester, eight to 12 weeks postnatal and six months postnatal. I collected delivery information on these women for mode of delivery, duration of labour and birth weight of baby, and analysed the sensory data in the context of this delivery information.

Vibration sensation in the third trimester was abnormal compared to previously published normative data in non-pregnant women. Stretch sensation at the vagina and introitus was within normal limits. To date, no study has reported pudendal sensory or motor nerve function in pregnant women.

After childbirth vibration sensation improved across the first six months postnatal (PN2) when compared to the abnormal antenatal readings. In comparison, stretch sensation was initially reduced at eight to 12 weeks postnatal (PN1) but recovered by PN2 to antenatal levels. At PN1, vibration sensation in women delivered by CS showed greater improvement than following a NVD or instrumental delivery. By PN2 the NVD group were comparable to the CS group, but the same recovery was not evidence in instrumental delivery group. There was a transient deterioration in stretch sensation at PN1 after a vaginal birth, with no difference at PN2 across mode of delivery.

Three studies have measured motor nerve injury following childbirth, with two finding CS protective compared to vaginal birth and the other no difference between NVD and instrumental.

There was no association between duration of labour, dilatation at CS or birth weight and change in vibration sensation. All three studies reported a difference in women undergoing ElCS compared a CS in labour however the small sample sizes (less than ten) undermine the validity of these findings. In contrast to my cohort, two of these studies reported greater motor nerve damage with a prolonged active second stage of labour and may be a reflection of the limitations surrounding diagnosis of the onset of each stage of labour.

Pelvic organ prolapse was associated with impaired vaginal vibration sensation at both PN1 and PN2. This is in keeping with the two studies which have reported reduced

vaginal sensation of Aβ, Aδ and C nerve fibres in POP in the non-pregnant or postnatal population.

Women with abnormal clitoral sensation were more likely to report urinary and sexual dysfunction, whilst women with abnormal vaginal sensation were more likely to report irritable bowel syndrome but not sexual dysfunction. The data on PFD and motor nerve dysfunction after childbirth is conflicting, with one reporting an association with SUI and two reporting no association with urinary dysfunction or anal incontinence. However based on the year of publication it would appear none of these studies used validated questionnaires to evaluate the symptoms of PFD and therefore these conclusions should be interpreted with caution.

This is the first study to evaluate pelvic sensation in pregnancy and following childbirth using QST and demonstrated feasibility of this method in pregnant women. The data suggest Aβ nerve fibre sensation is impaired in pregnancy with sparing of Aα nerve fibres. Most interestingly the study found CS confers an element of neuroprotection whilst vaginal birth, in particular instrumental delivery, is associated with injury to pelvic sensory nerves. However, theses sensory changes do not appear to be associated with symptoms of PFD in this cohort. The study is limited by the lack of clinical sensory examination and the long-term implications of this sensory insult remain unclear and further work is needed to monitor these women in the medium and long-term.

Finally, at the moment there is a disconnect between what happens to a woman during childbirth and when she attends the urogynaecology clinic many months or years later. Further work should focus on trying to bridge this disconnect and develop a risk stratification to identify women at high risk of birth trauma and future PFD.

Neurophysiology and neurohistology in prolapse: a pilot study

This project aimed to explore the relationship between sensation and neurohistology of the vaginal mucosa in women with POP. I recruited 19 women (16 POP and three controls) to undergo pelvic QST, POP-Q and donate two samples of vaginal mucosa from the anterior vaginal wall. I successfully used immunohistochemistry techniques to measure the frequency of nerve fibres and free nerve endings within the vaginal mucosa.

This was the first study to perform an exploratory analysis of the relationship between vaginal sensation and innervation, including separate assessments of the frequency of nerve bundles and free nerve endings.

There was no evidence of an association between vaginal sensation for Aβ, Aδ or C nerve fibres and frequency of nerve fibre cross sections or free nerve endings at 3cm or 5cm

proximal to the urethral meatus. However, as this was an exploratory analysis with small group sizes the results should be interpreted with caution.

To date, no study has correlated clinicalneurophysiology assesment with neurohistology in the vagina, however three studies have evaluated neurohistology in women with POP compared to controls. All three studies found reduced innervation of vaginal mucosa in women with POP compared to controls.[34–36]

Data from this pilot study should be used to inform design of a larger study to investigate a possible association between vaginal sensation and innervation.

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8 Appendices

8.1 Ethical approval confirmation 14/NW/1316

NHS

Health Research Authority

National Research Ethics Service

NRES Committee Northwest – Greater Manchester West

3rd Floor
Barlow House
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7434

09 October 2014

Dr Charlotte Mahoney
1405, Skyline 2
49 Goulden Street
Manchester
M4 5EN

Dear Dr Mahoney

Study title:	**The influence of childbirth on sensory innervation of the pelvis and demonstration of a sensory component to the aetiology of pelvic floor dysfunction: a neurophysiological study**
REC reference:	**14/NW/1316**
Protocol number:	**1.0**
IRAS project ID:	**158320**

The Research Ethics Committee reviewed the above application at the meeting held on 03 October 2014.

We plan to publish your research summary wording for the above study on the HRA 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 REC Manager Anna Bannister, nrescommittee.northwest-gmwest@nhs.net.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below. .

Conditions of the favourable opinion

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

1. The Committee would like the validation vaginal stretch information sheet revised to remove any references to pregnancy under the section 'Do I have to take part?'
2. The Committee would like the main information sheets revised to:
   a. Include an explanation of the stretch sensation test from the validation vaginal stretch information sheet.
   b. Include how long the assessment will last.

       c. Include how long it will take to fill out the questionnaires and that they can complete them on line.
3. The Committee would like all the study documents to have the same study title on them.

You should notify the REC in writing once all conditions have been met (except for site approvals from host organisations) and provide copies of any revised documentation with updated version numbers. The REC will acknowledge receipt and provide a final list of the approved documentation for the study, which can be made available to host organisations to facilitate their permission for the study. Failure to provide the final versions to the REC may cause delay in obtaining permissions.

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

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 <u>http://www.rdforum.nhs.uk</u>.

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.

<u>Registration of Clinical Trials</u>

All clinical trials (defined as the first four categories on question 2 of the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

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).

Ethical review of research sites

NHS Sites

The favourable opinion applies to all NHS sites taking part in the study 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).

Summary of discussion at the meeting

Ethical issues raised by the Committee in private discussion, together with responses given by the researcher when invited into the meeting

The Chair welcomed you and thanked you for attending to discuss the study. The Committee said it was a well written study.

Recruitment arrangements and access to health information, and fair participant selection

The Committee noted the main participants have 1 week to consider the study but the vaginal stretch sensation control group have only 20-30 minutes to decide. The Committee though the information sheet could be sent out with the clinic letter so patients are aware they may be asked to take part. You said this would be a good idea and happy to implement it.

Informed consent process and the adequacy and completeness of participant information

The Committee noted in the stretch sensation information sheet a few lines have been left in around pregnancy which needed removing.

The Committee noted on the main information sheet the stretch sensation test was not describe in as much detail as on the control stretch sensation information sheet.

The Committee noted the information sheet did not say how long the assessment would take and how long it would take to fill out the questionnaires.

The Committee mentioned the above issues and you agreed to make all the above changes. You explained the questionnaires can be completed on-line at the participant's convenience.

Suitability of supporting information

The Committee noted the documentation had different titles and requested that they are all the same. You agreed to correct all the headings.

Other general comments

The Committee said they would be happy if the researchers did not inform the GP or gynaecologist about the participant's involvement in the study. You agreed.

You explained that they extended the follow-up period from 36 weeks to 36-38 weeks in the protocol. The Committee were happy with this.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Covering letter on headed paper	1.0	05 July 2014
GP/consultant information sheets or letters [Validation of stretch testing clinician information sheet]	1.0	11 August 2014
GP/consultant information sheets or letters [Sensation clinician information sheet]	1.0	11 August 2014
Instructions for use of medical device [ePAQ Pelvic Floor, Tour]	1.0 (downloaded form website 11/9/14)	11 September 2014

Instructions for use of medical device [ePAQ list of questions]	PELV_10h	11 September 2014
Instructions for use of medical device [ePAQ product brochure]	1.0 (downloaded from website 11/9/14)	11 September 2014
Instructions for use of medical device [Instructions for use of GSA]	3.0	11 September 2003
IRAS Checklist XML [Checklist_11092014]		11 September 2014
Other [ePAQ report dummy example]	1.0	11 September 2014
Other [Fiona Reid Short CV]	1.0	05 July 2014
Participant consent form [Sensation consent form]	1.0	10 August 2014
Participant consent form [Validation of stretch testing consent form]	1.0	11 August 2014
Participant information sheet (PIS) [Sensation Participant information sheet]	1.0	10 August 2014
Participant information sheet (PIS) [Validation of stretch testing participant information sheet]	1.0	10 August 2014
REC Application Form [REC_Form_11092014]		11 September 2014
Research protocol or project proposal [Protocol]	1.0	22 June 2014
Summary CV for Chief Investigator (CI) [Jenny Myers Short CV]	1.0	26 June 2014
Summary CV for student [Charlotte Mahoney Short CV]	1.0	11 August 2014
Summary CV for supervisor (student research) [Anthony Smith Short CV]	1.0	05 July 2014
Summary, synopsis or diagram (flowchart) of protocol in non technical language [Sensation summary of protocol]	1.0	05 July 2014

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

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.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

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

14/NW/1316	Please quote this number on all correspondence

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

Yours sincerely

Dr Lorraine Lighton (Chair)
Chair

E-mail: nrescommittee.northwest-gmwest@nhs.net

Enclosures:	List of names and professions of members who were present at the meeting and those who submitted written comments
	"After ethical review – guidance for researchers"
Copy to:	Dr Lynne Webster, Central Manchester NHS Trust
	Dr Jenny Myers, Central Manchester Foundation Trust

NRES Committee North West - Greater Manchester West

Attendance at Committee meeting on 03 October 2014

Committee Members:

Name	Profession	Present	Notes
Mr Ian Beaumont (Vice Chair)	Retired Pharmacist	Yes	
Mrs Seonaid Beddows	Research Governance and Administration Manager	Yes	
Mr Jonathan Deans	Consultant in Head & Neck Surgery	Yes	
Dr Peter Donnelly	Lay Member	Yes	
Mr Mark Garrod	Project Consultant and Pension Fund Trustee	No	
Mr Michael Harnor	Lay member	Yes	
Dr Lorraine Lighton (Chair)	Consultant in Communicable Diseases	Yes	
Dr Barry Miller	Consultant Anaesthetist	Yes	
Mrs Patricia Morgan	Lay Member	No	
Dr Peter Owen	Lay Member	Yes	
Mr Iestyn Shapey	Transplant Surgery Registrar	No	
Dr Gideon Smith	Consultant in Public Health Medicine	No	

Also in attendance:

Name	Position (or reason for attending)
Miss Anna Bannister	REC Manager
Miss Katie Southeard	REC Assistant

NHS
Health Research Authority
National Research Ethics Service

NRES Committee North West - Greater Manchester West

Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0161 625 7818
Fax:0161 625 7299

13 October 2014

Dr Jenny Myers
Consultant Obstetrician and Honorary Senior Lecturer
Central Manchester Foundation Trust
Maternal and Fetal Health Research Centre
St Mary's Hospital
Oxford Road
M13 9WL

Dear Dr Myers

Study title:	**The influence of childbirth on sensory innervation of the pelvis and demonstration of a sensory component to the aetiology of pelvic floor dysfunction: a neurophysiological study**
REC reference:	**14/NW/1316**
Protocol number:	**1.0**
IRAS project ID:	**158320**

Thank you for your response of 13 October. I can confirm the REC has received the documents listed below and that these comply with the approval conditions detailed in our letter dated 09 October 2014

Documents received

The documents received were as follows:

Document	Version	Date
Other [summary protocol]	2	13 October 2014
Participant consent form [sensation]	2	13 October 2014
Participant consent form [validation of vaginal stretch]	2	13 October 2014
Participant information sheet (PIS) [sensation]	2	13 October 2014
Participant information sheet (PIS) [validation of stretch]	2	13 October 2014
Research protocol or project proposal	2	13 October 2014

Approved documents

The final list of approved documentation for the study is therefore as follows:

Document	Version	Date
Covering letter on headed paper	1.0	05 July 2014

GP/consultant information sheets or letters [Sensation clinician information sheet]	1.0	11 August 2014
GP/consultant information sheets or letters [Validation of stretch testing clinician information sheet]	1.0	11 August 2014
Instructions for use of medical device [Instructions for use of GSA]	3.0	11 September 2003
Instructions for use of medical device [ePAQ Pelvic Floor, Tour]	1.0 (downloaded form website 11/9/14)	11 September 2014
Instructions for use of medical device [ePAQ list of questions]	PELV_10h	11 September 2014
Instructions for use of medical device [ePAQ product brochure]	1.0 (downloaded from website 11/9/14)	11 September 2014
IRAS Checklist XML [Checklist_11092014]		11 September 2014
Other [Fiona Reid Short CV]	1.0	05 July 2014
Other [ePAQ report dummy example]	1.0	11 September 2014
Other [summary protocol]	2	13 October 2014
Participant consent form [validation of vaginal stretch]	2	13 October 2014
Participant consent form [sensation]	2	13 October 2014
Participant information sheet (PIS) [sensation]	2	13 October 2014
Participant information sheet (PIS) [validation of stretch]	2	13 October 2014
REC Application Form [REC_Form_11092014]		11 September 2014
Research protocol or project proposal	2	13 October 2014
Summary CV for Chief Investigator (CI) [Jenny Myers Short CV]	1.0	26 June 2014
Summary CV for student [Charlotte Mahoney Short CV]	1.0	11 August 2014
Summary CV for supervisor (student research) [Anthony Smith Short CV]	1.0	05 July 2014

You should ensure that the sponsor has a copy of the final documentation for the study. It is the sponsor's responsibility to ensure that the documentation is made available to R&D offices at all participating sites.

14/NW/1316	Please quote this number on all correspondence

Yours sincerely

pp.

Anna Bannister
REC Manager

E-mail: nrescommittee.northwest-gmwest@nhs.net

Copy to: *Ms Lynne Webster*

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8.2 Trust sponsor approval letter – 14/NW/1316

Central Manchester University Hospitals **NHS**

NHS Foundation Trust

Research Office
1st Floor NOWGEN Building
29 Grafton Street
Manchester M13 9WU
Tel: 0161-276-3565
Fax: 0161-276-5766

Dr Fiona Reid
St Mary's Hospital
Central Manchester Foundation NHS Trust
Oxford Road
Manchester
M13 9WL

Dear Dr Reid

PIN: R03811
REC Reference: 14/NW/1316
Research Study: The influence of childbirth on female pelvic sensation

Thank you for submitting the above study for NHS R&D permission. **Central Manchester Foundation Trust** is the Sponsor for this study which *is not* on the NIHR portfolio.

I am pleased to confirm that the Research Office has now received all necessary documentation, and the appropriate governance checks have been undertaken. This letter is issued subject to the research team complying with the attached conditions, Trust SOPs, the DH Research Governance Framework, and any other applicable regulatory requirements. This approval is in relation to the documentation listed.

CMFT are required to report whether the research was initiated within 70 days or provide valid reasons for not doing so. The target date for this study is listed below;
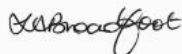
- 70 Day from Valid Submission to 1st Patient Recruited: 17 December 2015

Further information regarding CMFT targets can be found on the intranet.

Please update CRIMSON with the date when the first patient was recruited. If you or one of your team requires training on CRIMSON please contact michael.pate@cmft.nhs.uk

I would like to take this opportunity to wish you well with your research.

Yours sincerely

Lorraine Broadfoot
Research Operations Manager
Date: 26|11|2014

cc.   Caroline Leech Divisional Research Manager
       Dr Jenny Myers Chief Investigator

Documents Acknowledged/Approved

Document	Version	Date
NRES Approval Letter	Conditions Met	13 October 2014
Instructions for use of medical device [Instructions for use of GSA]	3.0	11 September 2003
Instructions for use of medical device [ePAQ Pelvic Floor, Tour]	1.0 (downloaded form website 11/9/14)	11 September 2014
Instructions for use of medical device [ePAQ list of questions]	PELV_10h	11 September 2014
Instructions for use of medical device [ePAQ product brochure]	1.0 (downloaded from website 11/9/14)	11 September 2014
Other [Fiona Reid Short CV]	1.0	05 July 2014
Other [ePAQ report dummy example]	1.0	11 September 2014
Other [summary protocol]	2	13 October 2014
Participant consent form [validation of vaginal stretch]	2	13 October 2014
Participant consent form [sensation]	2	13 October 2014
Participant information sheet (PIS) [sensation]	2	13 October 2014
Participant information sheet (PIS) [validation of stretch]	2	13 October 2014
REC Application Form [REC_Form_11092014]		11 September 2014
Research protocol or project proposal	2	13 October 2014
Summary CV for Chief Investigator (CI) [Jenny Myers Short CV]	1.0	26 June 2014
Summary CV for student [Charlotte Mahoney Short CV]	1.0	11 August 2014
Summary CV for supervisor (student research) [Anthony Smith Short CV]	1.0	05 July 2014

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Conditions of Approval:-

- All researchers involved in the study need to have received training appropriate to their role covering aspects of Research Governance or Good Clinical Practice (GCP). Trust policy states GCP training needs to be renewed every 3 years.

- The Research Office must be informed of: (please forward copies of amended documents by email)
  - The actual start date of the project
  - Any changes to the protocol throughout the course of the project
  - Any amendments sent to the MHRA or Research Ethics Committee
  - Any changes to the management of the project
  - Any extensions to the project, and associated additional funding, if applicable.

- The Research Office must be notified immediately of all Serious Adverse Events (SAEs) and Suspected Unexpected Serious Adverse Reactions (SUSARs) via email adverse.events@cmft.nhs.uk or Research Office fax: 276 5766 and/or by copy of official notification to the regulatory authorities (NRES, MHRA as applicable).

- All research taking place on CMFT Trust premises is subject to the Trust monitoring programme, either as part of the annual 10% audit requirement or "triggered" monitoring[1]. The Chief and/or Principal Investigator is required to make him/her self available for any monitoring visit, on a mutually agreed date.

- All Principal Investigators are required to complete and submit an annual self-assessment at the request of the Research Office.

- All Principal Investigators are required to provide recruitment (accrual) data to the Research Office monthly.

- The Research Office must be given a minimum three months' notice, in writing, if the Principal Investigator leaves the employment of CMFT Trust.

- The Research Office must receive immediate notification if the Principal Investigator is unable to continue to fulfil his/her duties as PI for other reason e.g. long-term sickness

- Any evidence of fraud &/or misconduct must be immediately brought to the attention of the Research Office either via the Incident Reporting system, or by direct communication.

Failure to comply with any of the above may result in withdrawal of approval for the project and the immediate cessation of the research. Persistent failure to comply may result in disciplinary action.

8.3 Study protocol 14/NW/1316

The influence of childbirth on pelvic sensory innervation and demonstration of a sensory component to the aetiology of pelvic floor dysfunction

Short Title	Impact of childbirth on vaginal sensation study
Protocol number	Vaginal sensation study version 1.0
Version and date	1.0 13/10/14
Sponsor	Central Manchester University Hospitals NHS Foundation Trust (CMFT)
Chief Investigator	Dr Jenny Myers
EudraCT Number	
REC Number	

Chief Investigator (CI)	Dr Jenny Myers
Co-Investigator(s) (Co-I)	Dr Charlotte Mahoney (MD Research student) Dr Fiona Reid Professor Tony Smith
Sponsor's representative	Lynn Webster
Statistician	Dr Jenny Myers
Central laboratories	CMFT
Pharmacy contact	Not applicable
SAE/SUSAR reporting	Adverse.Events@cmft.nhs.uk or fax to 0161 276 5766

The influence of childbirth on pelvic sensory innervation and demonstration of a sensory component to the aetiology of pelvic floor dysfunction

This document describes a study of **Impact of childbirth on vaginal sensation** and provides information about procedures for entering patients into it. The protocol should not be used as a guide for the treatment of patients outside the research study. Every care was taken in drafting this protocol, but corrections or amendments may be necessary, care must be taken to use the most up to date and approved version. This Study will adhere to the principles outlined in the ICH Good Clinical Practice guidelines. The study will be conducted in compliance with the protocol, the Data Protection Act (DPA Z6364106), the Declaration of Helsinki, Human Tissue Act (2004), the Research Governance Framework (2005) and other regulatory requirements as appropriate.

Chief Investigator – Dr Jenny Myers, Central Manchester University Hospitals NHS Foundation Trust

Signed   *Date 22/6/14*

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LIST OF ABBREVIATIONS

AE	Adverse Event
AR	Adverse Reaction
CI	Chief Investigator
CMFT	Central Manchester University Hospitals NHS Foundation Trust
CS	Caesarean Section
EudraCT	European Clinical Studys Database
GCP	Good Clinical Practice
NHS R&D	National Health Service Research & Development
PFD	Pelvic floor dysfunction
PI	Principal Investigator
RCT	Randomised Controlled Study
REC	Research Ethics Committee
SAR	Serious Adverse Reaction
SAE	Serious Adverse Event
SSAR	Suspected Serious Adverse Reaction
SUSAR	Suspected Unexpected Serious Adverse Reaction
TSC	Study Steering Committee
UAR	Unexpected Adverse Reaction

STUDY SUMMARY

Title: The influence of childbirth on pelvic sensory innervation and demonstration of a sensory component to the aetiology of pelvic floor dysfunction

Short title: Influence of childbirth on vaginal sensation

Design: Prospective observational non-interventional cohort study

Objectives:

The project aims to explore whether significant injury occurs to the sensory innervation of the pelvis at the time of vaginal delivery, and whether this is associated with symptoms of pelvic floor dysfunction. Thus the key research objectives are:

1. **To determine whether the mode of delivery of a baby (vaginal or Caesarean section (CS)) affects the sensory innervation of the pelvis.**
2. **To identify modifiable obstetric factors involved in vaginal delivery which influence sensory innervation of the pelvis. These include the duration of first and second stage of labour, the use and type of any instrumental delivery, the type of analgesia, birth weight, malposition, and perineal trauma.**
3. **To establish if changes in sensory innervation following childbirth correlate with urinary, bowel and sexual dysfunction, and whether these are associated with mode of delivery.**
4. **To evaluate genital hiatus as a predictor of poor sensory outcome.**

Endpoints:
1. **Quantification of the change in sensation to the pelvis following childbirth**
2. **Identification of modifiable obstetric risk factors which affect sensation**
3. **Evaluation of the association between sensory injury and symptoms of pelvic floor dysfunction (PFD) Test the association between genital hiatus and sensory outcome**

Cohort:
Nulliparous women

Eligibility:
Women in their first pregnancy booking at St Mary's Hospital will be invited to participate in the study.

Study Methods:

Comparison of vaginal sensation in women before and after vaginal birth compared with vaginal sensation in women before and after caesarean section. This is an observational study and therefore will not impact on the clinical antenatal care the women receive.

Study duration per participant:
Maximum ten months.

Estimated total Study duration:
30 months

Total number of participants planned:
300

Patient Pathway:

Booking	First Visit	Delivery	Second Visit	Third Visit
• Attend routine antenatal booking appointment • Invited to participate in study, with patient information leaflet given	• At 36-38 weeks pregnant • Eligibility confirmed and informed consent taken • Demographic data • Baseline assessment of vaginal sensation • Vaginal and clitoral vibration • Vaginal stretch • Measurement of genital hiatus • EPAQ	• Participation in study has no effect on clinical decisions made during delivery • Data regarding delivery collected retrospectively	• 8-12 weeks postnatal • Eligibility re-confirmed, informed consent re-taken • Second assessment of vaginal sensation • Vaginal and clitoral vibration • Vaginal stretch • Measurement of genital hiatus • EPAQ	• Six months postnatal • Eligibility re-confirmed, informed consent re-taken • Third assessment of vaginal sensation • Vaginal and clitoral vibration • Vaginal stretch • Measurement of genital hiatus • EPAQ

BACKGROUND

The pelvic floor is supplied by the pudendal nerve which consists of motor and sensory fibres. Research has demonstrated that vaginal birth damages pudendal nerve motor fibres and is a major factor in the development of pelvic floor dysfunction (PFD.) Logically, injury to sensory fibres occurs at the same time, although this has never been studied, primarily due to difficulties in reliably assessing the entire sensory pathway. Recently equipment has been designed which makes this possible. The vibrotactile equipment uses a validated protocol to provide a reproducible assessment of pelvic sensory function, termed quantitative-sensory testing (QST). The equipment is safe in pregnancy and acceptable to women (1,2).

Significance
Pelvic floor dysfunction (pelvic organ prolapse, urinary, bowel and sexual dysfunction), impairs the quality of life of over one third of women. (3) It causes debilitating symptoms leading to social isolation, relationship breakdown, employment limitations and loss of independence. Despite this, and the rising   economic   cost   of treatment, it remains a neglected area of women's health and an important public health issue.

Study treatment
This study will not involve any treatment.

Rationale for the proposed study
The mainstay of treatment for PFD remains prevention, and an observational cohort study of this kind will allow clinicians to identify women at high risk of developing PFD and alter their obstetric management accordingly. A better understanding of PFD and its aetiology will also allow women to make more informed decisions when undergoing childbirth.

Assessment and management of risk
This research is a prospective observational cohort study, and as such will not affect the clinical care participating women receive. Women who choose not to participate in the study will also receive the same routine antenatal care, the decision not to participate will not alter their treatment in any way.

The vibrotactile equipment is safe to use in pregnancy and postnatally, and it is acceptable to women. (1)

The assessment of vaginal stretch sensation will utilise the same balloons used to assess anal sensation in routine anal manometry clinics across the country. When inserted into the rectum, the balloons are known to be atraumatic, well tolerated in the anus and rectum and cause no adverse effects. In this study the balloon will be placed within the vaginal canal, and therefore will not come into contact with the pregnancy which is protected by the cervix. The balloon itself is a gentler device than a plastic speculum which is routinely inserted into the vagina of pregnant women in a wide range of clinical scenarios. Speculum insertion does not cause harm to the unborn child, miscarriage or pre-term labour. It is reasonable to extrapolate this to the balloon the investigators will use to evaluate vaginal stretch sensation.

The genital hiatus will be measured using a tape measure.

STUDY OBJECTIVES AND ENDPOINTS

Objectives:
1. **To determine whether the mode of delivery of a baby (vaginal or Caesarean section (CS)) affects the sensory innervation of the pelvis.**

2. **To identify modifiable obstetric factors involved in vaginal delivery, which influence sensory innervation of the pelvis. These include the duration of first and second stage of labour, the use and type of any instrumental delivery, the type of analgesia, birth weight, any malposition, and perineal trauma.**
3. **To establish if changes in sensory innervation following childbirth correlate with urinary, bowel and sexual dysfunction, and whether these are associated with mode of delivery.**
4. **To evaluate genital hiatus as a predictor of poor sensory outcome.**

Endpoints:
1. **Quantification of the change in sensation to the pelvis following childbirth**
2. **Identification of modifiable obstetric risk factors which affect sensation**
3. **Evaluation of the association between sensory injury and symptoms of pelvic floor dysfunction (PFD)**
4. **Test the association between genital hiatus and sensory outcome**

Study design

This is a prospective observational cohort study. Over the course of the study we aim to recruit 300 women.

Women will be invited to attend at 36 to 38 weeks gestation for the initial assessment, the first postnatal assessment will take place at eight to 12 weeks and the second at six months.

Selection of study participants

Inclusion criteria

All women in their first pregnancy presenting to St Mary's Hospital from six to 36 weeks gestation.

Exclusion criteria

- Fetal abnormality
- Language barrier – given the intimate nature of the assessments, the investigators felt the use of interpreters would be inappropriate
- A history of pelvic or genital surgery or mutilation
- Medical conditions predisposing to sensory impairment eg pre-existing diabetes mellitus, multiple sclerosis.
- Incapacity to consent

Concomitant medication

All prescribed medications will be continued as per the antenatal care plan.

Recruitment of study participants

Women will be provided with information regarding the study when they attend for routine appointments within the antenatal service (usually 12-14 and 20 weeks gestation).

Initial contact

All women who meet the inclusion and exclusion criteria will be given an information sheet about the study at first point of contact. Women eligible for the study will be approached in person or contacted via telephone by the research fellow. Women will be given a contact number to call back, if unable to discuss the study at the initial contact. Women will be screened against the exclusion criteria during the initial face to face or telephone contact.

Ineligible and non-recruited women

Women who are ineligible or decline to take part will be reassured their decision will not affect their routine antenatal care.

Registration/randomisation procedures

Not applicable

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Emergency unblinding procedures
Not applicable

Discontinuation/ withdrawal of participants and "stopping rules"
Participation in the study is voluntary. A woman has the right to withdraw from the study at any time for any reason. The Investigator has the right to discontinue participation at any time if it is deemed to be in the woman's best interest.

If the woman is withdrawn due to a serious adverse event, the Principal Investigator (PI) will arrange for follow-up visits or telephone calls until the event has resolved or stabilised. However as the participants are pregnant women the data will be collected to outcome (i.e. Delivery) and data will be used in the analysis unless the consent to collect the outcome is specifically refused by the participant.

As this is a non-interventional prospective observational cohort study using sensory testing equipment with a good safety record, the only circumstances which would result in premature closure of the study would be failure to recruit more than 50 participants over the first six month period.

TREATMENT DETAILS

Treatment summary
Not applicable – this is a non-interventional observational cohort study

STUDY ASSESSMENTS

Clinical research visit one
Women will be invited to attend a research appointment in the Urogynaecology suite at St Mary's Hospital. Eligibility will be confirmed and women will be given the opportunity to discuss the study and those who wish to participate will complete a consent form (including permission to collect delivery outcome data). Basic background demographic data will be collected including age, ethnicity, previous medical history, drug and smoking history.

Pelvic sensation will be tested for vibration and stretch sensation. Vibration thresholds for vaginal and clitoral sensation will be testing using vibrotactile equipment. The vibrotactile equipment consists of a height-adjustable vibration probe controlled by a computer programme. The vibration intensity is linearly increased (from no vibration to a maximum pre-set level) until the woman indicates perception of the vibration using a response button held in her dominant hand. A standardised verbal explanation of the protocol and equipment will be provided before each test including a control test on the hand to familiarise with the sensation and use of the response button. The probe is inserted 2-3cm into the vagina to assess the perineal branch of the pudendal nerve, whereas only the tip is placed against the clitoris so the woman is just aware of the probe to assess the clitoral branch. When the response button is pressed the stimulus stops immediately and the threshold is recorded. This is repeated six times and the average value calculated.

Vaginal stretch sensation will be tested using the balloon from anal manometry testing. This will be performed twice, once at 4cm inside the vagina, and once at the introitus. The balloon will be inflated by integers of 50cm^3 air until the patient reports awareness of stretch sensation.

The genital hiatus will be measured using a tape measure similar to that performed during routine pelvic organ prolapse examination.

Finally women will also be asked to complete a validated online electronic patient assessment questionnaire (ePAQ) about their urinary, bowel and sexual function which will assess symptoms of PFD. This information will be stored on a secure server.

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Delivery data collection (no patient attendance required)

Delivery data will be collected from the electronic patient record delivery system, K2 Athena. This will be stored on a secure computer database.

Clinical research visit two

Women will be invited to attend a second research appointment in the Urogynaecology suite at St Mary's Hospital 8-12 weeks after delivery when any perineal tears sustained during delivery will be healed. Eligibility will be re-confirmed and women will be given the opportunity to re-discuss the study and those who are happy to continue to participate will again discuss the consent form.

Pelvic sensation will be tested for vibration and stretch sensation. Vibration thresholds for vaginal and clitoral sensation will be tested using the vibrotactile equipment. This will be repeated six times and the average value calculated.

Vaginal stretch sensation will be again tested using the balloon from anal manometry testing. This will be inserted 4cm into the vagina, and the balloon inflated with integers of 50cm^3 air until the patient reports awareness of stretch sensation.

Finally the genital hiatus will be measured using a tape measure.

Women will also be asked to complete the validated online electronic patient assessment questionnaire (ePAQ) about their urinary, bowel and sexual function which will assess symptoms of PFD.

Clinical research visit three

Women will be invited to attend a second research appointment in the Urogynaecology suite at St Mary's Hospital at six months postnatal. Eligibility will be re-confirmed and women will be given the opportunity to re-discuss the study and those who are happy to continue to participate will again discuss the consent form.

Pelvic sensation will be tested for vibration and stretch sensation. Vibration thresholds for vaginal and clitoral sensation will be tested using the vibrotactile equipment. This will be repeated six times and the average value calculated.

Vaginal stretch sensation will be again tested using the balloon from anal manometry testing. This will be inserted 4cm into the vagina, and the balloon inflated with integers of 50cm^3 air until the patient reports awareness of stretch sensation.

Finally the genital hiatus will be measured using a tape measure.

Women will also be asked to complete the validated online electronic patient assessment questionnaire (ePAQ) about their urinary, bowel and sexual function which will assess symptoms of PFD.

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Study schedule

	6-36 weeks pregnant	36-38 weeks pregnant	Delivery data collection	8 weeks postnatal	6 months postnatal
	Face to face or telephone contact	Visit One	Data collection	Visit Two	Visit Three
Patient Information Leaflet	✔	✔	-	✔	✔
Inc/Exc Criteria	✔	✔	-	✔	✔
Consent Form	✔	-	-	-	-
Collection demographic data	✔	-	-	-	-
Vibration Testing	-	✔	-	✔	✔
Stretch testing	-	✔	-	✔	✔
Genital hiatus measurement	-	✔	-	✔	✔
ePAQ	-	✔	-	✔	✔
Review AEs and SAE's	-	-	-	✔	✔
Delivery Information	-	-	✔	-	-

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ASSESSMENT OF STUDY EFFICACY/MEASURING STUDY ENDPOINTS

Endpoints:
1. **Quantification of the change in sensation to the pelvis following childbirth**
2. **Identification of modifiable obstetric risk factors which affect sensation**
3. **Evaluation of the association between sensory injury and symptoms of pelvic floor dysfunction (PFD)**
4. **Test the association between genital hiatus and sensory outcome**

STUDY CLOSURE
The study will recruit for 24 months. A six month follow up period will be allowed for the third assessment.

STATISTICS AND DATA ANALYSIS

Study Design
Prospective observational cohort study

Sample size calculation
Using data from control subjects in a previous pilot study in our unit (1), the mean value for sensation was 2.71±0.67μm. Assuming that a 10% difference in sensation is of clinical significance (reference), in order to reject the null hypothesis ($\alpha=0.05$, $\beta=80$) allowing for a 50% SD in the paired measurements (before and after delivery) a minimum of 29 subjects will be required per group. Based on a 30% loss to follow up rate (1), and primiparous CS rate of 26%, the sample size needed is 147. St Mary's Hospital has 8600 deliveries per year, of which 3800 are primiparous women. To recruit 150 women over a 24 month period requires a recruitment rate of 7% .

In order to perform adequately powered analysis for the secondary outcomes (modifiable obstetric factors, epidural 19%, instrumental delivery 16%), a larger sample size will be necessary. The target sample over the study will therefore be 300 women..

Interim analysis
No interim analysis planned..

Primary objective analysis
1. **To determine whether the mode of delivery of a baby (vaginal or Caesarean section, CS) affects the sensory innervation of the pelvis.**

This will be performed by comparing the vaginal sensation in women undergoing a vaginal delivery or CS at 36-38 weeks gestation, 8-12 weeks postnatally and six months postnatally.

2. **To identify modifiable obstetric factors involved in vaginal delivery which influence sensory innervation of the pelvis. These include the duration of first and second stage of labour, the use and type of any instrumental delivery, the type of analgesia, birth weight, any malposition, and perineal trauma.**

Data will be gathered from the electronic patient childbirth record and comparisons of vaginal sensation at 36-38 weeks gestation, 8-12 weeks and six months postnatal made for each of the subcategories.

3. **To establish if changes in sensory innervation following childbirth correlate with urinary, bowel and sexual dysfunction, and whether these are associated with mode of delivery.**

The ePAQ will provide scores for each of the symptom domains of PFD, and this will be compared with vaginal sensation at 36-38 weeks, 8-12 weeks and six months postnatally.

4. **To evaluate genital hiatus as a predictor of poor sensory outcome.**

Genital hiatus measurement will be correlated with measurements of genital vibration and stretch sensation at 36-38 weeks, 8-12 weeks and six months postnatally.

STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

A Delegation Log will be prepared, detailing the responsibilities of each member of staff working on the study.

STUDY MONITORING

Data collection

Data will be collected from participating women at each study visit and documented on a standard clinical research form. Pregnancy outcome data will be collected from the electronic patient childbirth record. A data management plan will be prepared prior to the study starting.

Data handling and analysis

Data handling and analysis will be conducted according to the data management plan and will include the following activities:

- The clinical research team will be responsible for completion of the clinical research form
- Missing or ambiguous data will be cross checked against the medical notes
- Data will be transferred to a electronic database
- Data will be anonymised and stored in accordance with Data Protection Act 1998.

Direct access to data

The Chief Investigator will permit study-related monitoring, audits, research and ethics council review (REC), and regulatory inspection and provide direct access to source data and documents.

Site monitoring

Site monitoring will be conducted according to the study monitoring plan agreed by the Sponsor and Chief Investigator.

CONFIDENTIALITY AND DATA PROTECTION

Confidentiality

- **Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee, Regulatory Authorities, or the REC.**

- The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study.
- Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any confidential information to other parties.
- Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998. Women will be identified using only their unique study number, date of birth, and initials on the Case Report Form.

Data protection
- Data will be collected in accordance with the "Caldicott Principles" and the Data Protection Act. All women recruited will be allocated a study number which will be linked to their identifiable information held in a separate file stored within the research unit. Outcome data will be collected using case record forms which will be stored within the Urogynaecology Unit, St Mary's Hospital, Manchester.
- All outcome data will be entered onto a password protected database within the Urogynaecology Unit accessible only to members of the research team. All electronic data will be anonymised and no identifiable data will be stored on this database.
- Published results will not contain any personal data that could allow identification of individual participants.

STUDY CONDUCT

Protocol amendments
Any changes in research activity (except those necessary to remove an apparent, immediate hazard to the participant) will be reviewed and approved by the sponsor and Chief Investigator (CI) prior to submission in writing to the appropriate REC, Regulatory Authority, and local research and development (R&D) for approval prior to enrolment into an amended protocol.

Protocol violations/ deviations/ serious breaches
Investigators will not implement any deviation from the protocol without agreement from the sponsor, Chief Investigator and appropriate REC, Regulatory Authority and R&D approval except where necessary to eliminate an immediate hazard to women participating in the study.

Deviations will be managed as follows:

- The nature of and reasons for the deviation should be documented on the clinical research form and discussed with CI as soon as is practicable

- It will be the responsibility of the CI to inform the sponsor immediately

- If the deviation necessitates a subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval

Serious breaches will be recorded and reported according to the sponsor's Standard Operating Procedures for the management of clinical study.

STUDY RECORD RETENTION

Long term copies of the data will be kept in both digital formats for a minimum of five years within the research facility after the date of publication.

END OF STUDY

The Chief Investigator has the right at any time to terminate the study for clinical or administrative reasons. The end of the study will be reported to the REC and Regulatory Authority within the required timeframe (15 days) if the study is terminated prematurely. Investigators will inform participants of any premature termination of the study and ensure that the appropriate follow up is arranged for all involved. A summary report of the study will be provided to the REC and Regulatory Authority within the required timeframe. The end of study is defined as confirmation of the last pregnancy outcome.

PEER REVIEW

The sponsor has arranged for internal peer review of this protocol prior to submission for REC approval.

ETHICAL AND REGULATORY REQUIREMENTS

The study will be conducted in accordance with the principles of the good clinical practice (GCP). The sponsor will ensure that the study protocol, participant information sheet, consent form, general practitioner letter and submitted supporting documents have been approved by a main research ethics committee, prior to any participant recruitment. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethical and regulatory approval prior to implementation.

In addition, local Research & Development (R&D) approval must be obtained. It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary approval.

The CI and sponsor will ensure that the main REC is notified that the study has finished (either as expected or prematurely) within required timeframes with summary reports to be provided as required.

References

1. North C, Creighton S, Smith A. A comparison of genital sensory and motor innervation in women with pelvic organ prolapse and normal controls including a pilot study on the effect of vaginal prolapse surgery on genital sensation. BJOG. 2013; 120(2): p. 193-9.

2. Vardi Y, Gruenwald I, Sprecher E, Gertman I, Yartnitsky D. Normative values for female genital sensation. Urology. 2000 Dec; 56(6): p. 1035-40.

3. Davis K, Kumar D. Pelvic floor dysfunction: a conceptual framework for collaborative patient-centred care. Journal Advanced Nursing. 2003; 43(6): p. 555-68.

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Central Manchester University Hospitals NHS

NHS Foundation Trust

The University of Manchester

Patient Information Sheet: The influence of childbirth on pelvic sensation

Part One: Validation of vaginal stretch testing in non-pregnant women

Investigators: Dr Charlotte Mahoney, Dr Fiona Reid, Professor Anthony Smith, Dr Jenny Myers

This research aims to investigate whether we can reliably test the sensation of the vagina stretching.

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 and discuss it with others 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.

Why have I been chosen and what is the purpose of the study?

We are inviting you to take part in this study because you are not pregnant and have attended the gynaecology clinic.

The aim of this study is to find the normal range for the sensation of stretch in the vagina.

Do I have to take part?

No, you do not have to take part in the study. Participation is completely voluntary. Whatever your decision your hospital care will be the same.

If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

If I agree to take part, can I withdraw from the study at anytime?

YES. You can decide to withdraw from the study at any stage without affecting your treatment now or in future and without giving any reason.

What will happen if I take part?

During your consultation the doctor will perform an examination of your abdomen and vagina as part of your routine clinical care. Once this has been completed, and whilst you are still on the examination couch, the test will be performed.

The doctor will insert a deflated plastic balloon into the vagina and then slowly inflate it with air until you can feel the vagina stretching. The doctor will then deflate the balloon and remove it from the vagina.

The doctor will then place the balloon at the entrance of the vagina and repeat the procedure,

PIS: Validation of vaginal stretch testing   Version 2.0   13/10/14

slowly inflating the balloon with air until you can feel a stretching sensation, at which point the doctor will deflate and remove the balloon.

Once this has been completed the test is complete, and the doctor will step behind the curtain leaving you to change back into your clothes in private. At this point the study is over, and the doctor will perform the next part of your consultation: discussing your management plan.

What are the disadvantages and risks of taking part?
Participation in this study will make your clinic appointment take approximately ten minutes longer.

What are the possible benefits of taking part?
Our research will allow us to validate the test and allow us to investigate vaginal stretch sensation in women in clinical practice and in other research studies.

Will my taking part in the study be kept confidential?
Any information that is collected about you will be kept strictly confidential. When the results are analysed your name will be removed so that you cannot be recognised.

What will happen to the results of the research study?
The results will be presented at clinical and scientific meetings and will be published in journals read by doctors who care for women. You will not be identified in any of our results.

Who is organizing the research?
The research is being organised by the Maternal and Fetal Health Research Centre, The University of Manchester. The Doctor responsible for the research at your hospital is: Dr Charlotte Mahoney. The sponsor of this research is Central Manchester University Hospitals NHS Foundation Trust.

Who has reviewed the study?
The study has been reviewed by an Ethics Committee, who have given permission for the study as it is safe for women.

Will I get the results of the research?
The results will be used for research purposes only and will <u>NOT</u> affect your treatment in any way. If you are interested in finding out the scientific results of the study, please contact Dr Charlotte Mahoney (contact details at bottom of sheet) and we can contact you with information at the end of the study. A brief summary of the results will also be available on the Maternal & Fetal Health Research Centre Website.

What if there is a problem?
If you have questions regarding the study or any other questions relating to vaginal, please contact

Central Manchester University Hospitals NHS

The University of Manchester

Patient Information Sheet: The influence of childbirth on pelvic sensation

Investigators: Dr Charlotte Mahoney, Dr Fiona Reid, Professor Anthony Smith, Dr Jenny Myers

Childbirth can sometimes be associated with changes in pelvic floor function. This research aims to assess vaginal sensation before and after childbirth to see how this might relate to other pelvic floor symptoms.

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 and discuss it with others 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.

Why have I been chosen and what is the purpose of the study?

We are inviting you to take part in this study because you are in your first pregnancy.

Childbirth can sometimes contribute to bladder, bowel and prolapse problems in later life. We know that this is related to the nerves that control the muscles in the pelvic floor. It is not known, however, whether the nerves which affect sensation are also affected.

The aim of this study is to compare sensation in the vagina before and after delivery.

Do I have to take part?

No, you do not have to take part in the study. Participation is completely voluntary. Whatever your decision your ongoing pregnancy care will be the same.

If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

If I agree to take part, can I withdraw from the study at anytime?

YES. You can decide to withdraw from the study at any stage without affecting your treatment now or in future and without giving any reason.

What will happen if I take part?

You will be invited to come for three appointments to test vaginal sensation. This will be at around 36 to 38 weeks of your pregnancy, and 8-12 weeks and six months after delivery. When you come to the visits, we will be doing three simple tests.

1. Vaginal sensation for vibration: we will test the feeling in the vagina and on the clitoris for vibration, this is a very mild vibration and does not harm you or your baby.

2. Vaginal sensation for stretch: we will insert a deflated plastic balloon into the vagina and then slowly inflate it with air until you can feel the vagina stretch, we will then deflate the balloon and remove it from the vagina. We will repeat this at the entrance to the vagina. Again this is very mild and does not harm you or your baby.

3. We will measure the entrance to the vagina with a tape measure.

You will also be asked to complete a questionnaire about symptoms related to prolapse, leakage of urine or faeces and sexual issues. This will take around 15 minutes, and can be completed online at home or in the department.

We will also collect information about your baby's birth. This will include your baby's size, how you delivered your baby and whether you needed any stitches after delivery.

What are the disadvantages and risks of taking part?
Participation in this study will take up some of your time; we will ask you to come to the hospital for three visits which will take approximately 30 minutes each.

What are the possible benefits of taking part?
Our research will help us understand more about the best way to prevent women developing symptoms of prolapse, leakage or urine or faeces, and sexual problems after delivery. It will also hopefully help us to find a test to predict women who are high risk for these problems and adjust the management of their delivery.

Will my taking part in the study be kept confidential?
Any information that is collected about you and your baby will be kept strictly confidential. When the results are analysed your name will be removed so that you cannot be recognised.

What will happen to the results of the research study?
The results will be presented at clinical and scientific meetings and will be published in journals read by doctors who care for women during pregnancy. You will not be identified in any of our results.

Who is organizing the research?
The research is being organised by the Maternal and Fetal Health Research Centre, The University of Manchester. The Doctor responsible for the research at your hospital is: Dr Charlotte Mahoney. The sponsor of this research is Central Manchester University Hospitals NHS Foundation Trust.

Who has reviewed the study?

The study has been reviewed by an Ethics Committee, who have given permission for the study as it is safe to women and their unborn baby.

Will I get the results of the research?

The results will be used for research purposes only and will <u>NOT</u> affect your treatment in any way. If you are interested in finding out the scientific results of the study, please contact Dr Charlotte Mahoney (contact details at bottom of sheet) and we can contact you with information at the end of the study. A brief summary of the results will also be available on the Maternal & Fetal Health Research Centre Website.

What if there is a problem?

If you have questions regarding the study or any other questions relating to vaginal sensation and childbirth, please contact us on the number at the bottom of this information sheet.

This study is looking at the changes that happen to your body during childbirth and involves no intervention which could alter your routine antenatal treatment, so we do not expect any problems. However, if there are any problems which arise over the course of the study these will be reported to the Central Manchester NHS Hospital NHS Trust Research Office and to the Clinical Division through the hospital clinical incident reporting system.

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If they are unable to resolve your concern or you wish to make a complaint regarding the study, please contact PALS.

Patients Advice and Liason Service (PALS)

PALS are also able to provide independent advice on any queries or complaints you may have. Please contact 0161 276 8686 or pals@cmft.nhs.uk for the office at the Central Manchester University Hospitals NHS Foundation Trust.

Contact for further information

Thank you for reading this information sheet and for taking the time to consider our study. If you have any questions or concerns please contact:

<u>Dr Charlotte Mahoney, MBChB MRCOG</u>

Clinical Research Fellow and Senior Registrar

The Warrell Unit, St. Mary's Hospital, Oxford Road, Manchester, M13 9WL

Tel: +44 (0)161 276 6570   Fax: +44 (0)161 276 6085

Email: charlotte.mahoney@cmft.nhs.uk

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If they are unable to resolve your concern or you wish to make a complaint regarding the study, please contact PALS.

Patients Advice and Liason Service (PALS)

PALS are also able to provide independent advice on any queries or complaints you may have. Please contact 0161 276 8686 or pals@cmft.nhs.uk for the office at the Central Manchester University Hospitals NHS Foundation Trust.

Contact for further information

Thank you for reading this information sheet and for taking the time to consider our study. If you have any questions or concerns please contact:

Dr Charlotte Mahoney, MBChB MRCOG

Clinical Research Fellow and Senior Registrar

The Warrell Unit, St. Mary's Hospital, Oxford Road, Manchester, M13 9WL

Tel: +44 (0)161 276 6570   Fax: +44 (0)161 276 6085

Email: charlotte.mahoney@cmft.nhs.uk

8.4 Ethical approval confirmation 16/NW/0715

NHS
Health Research Authority

North West - Greater Manchester West Research Ethics Committee

Barlow House
3rd Floor
4 Minshull Street
Manchester
M1 3DZ

Telephone: 0207 104 8021

30 November 2016

Dr Jenny Myers
Maternal and Fetal Health Research Centre
Research 5th floor, St Mary's Hospital
Oxford Road, Manchester
M13 0JH

Dear Dr Myers

Study title:	**Pelvic Sensorineuropathy: A comparison between quantitative sensory testing and a simple hand held device**
REC reference:	**16/NW/0715**
IRAS project ID:	**173962**

Thank you for your submission, 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 Vice-Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact the REC Manager, Ms Anna Bannister, nrescommittee.northwest-gmwest@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.

Conditions of the favourable opinion

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

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

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for NHS permission for research is available in the Integrated Research Application System, www.hra.nhs.uk 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 management permissions from host organisations

<u>Registration of Clinical Trials</u>

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to contest the need for registration they should contact Catherine Blewett (catherineblewett@nhs.net), the HRA does not, however, expect exceptions to be made. Guidance on where to register is provided within IRAS.

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).

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 completed 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 an SSA application(s) has been reviewed. In the meantime no study procedures should be initiated at non-NHS sites.

Approved documents

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

Document	Version	Date
Instructions for use of medical device [Instructions for use of GSA]	1.0	30 August 2016
IRAS Application Form [IRAS_Form_11112016]		11 November 2016
IRAS Application Form XML file [IRAS_Form_11112016]		11 November 2016

IRAS Checklist XML [Checklist_21092016]		21 September 2016
IRAS Checklist XML [Checklist_11112016]		11 November 2016
Letter from funder [Letter of Intent to fund from NIHR]	1.0	24 August 2015
MHRA Notice of No Objection Letter (Medical Devices) and relevant correspondence [MHRA email]	1.0	30 August 2016
Non-validated questionnaire [Patient Assessment Questionnaire]	1.0	30 August 2016
Other [Vibratip Instruction leaflet]		
Other [Vibratip Press Pack]		
Other [ePAQ report dummy example]		
Other [ePAQ Pelvic Floor Tour]		
Other [PIS controls]	1.0	07 November 2016
Other [Consent Form controls]	1.0	07 November 2016
Participant consent form [Consent form POP]	1.0	07 November 2016
Participant information sheet (PIS) [PIS for POP]	1.0	07 November 2016
Participant information sheet (PIS) [Controls - Tracked Changes]	1.0	07 November 2016
Participant information sheet (PIS) [Patients - Tracked Changes]	1.0	07 November 2016
Research protocol or project proposal [Protocol]	1.0	30 August 2016
Summary CV for Chief Investigator (CI) [Jenny Myers Short CV]	1.0	30 October 2016
Summary CV for student [Charlotte Mahoney Short CV]	1.0	30 August 2016
Summary CV for supervisor (student research) [Fiona Reid Short CV]	1.0	30 August 2016
Validated questionnaire [ePAQ Brochure]		

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

<u>Reporting requirements</u>

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

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and

the application procedure. If you wish to make your views known please use the feedback form available on the HRA website: http://www.hra.nhs.uk/about-the-hra/governance/quality-assurance/

HRA Training

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

16/NW/0715	Please quote this number on all correspondence

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

Yours sincerely

Dr Gideon Smith
Vice Chair

Email:nrescommittee.northwest-gmwest@nhs.net

Enclosures: "After ethical review – guidance for researchers"

Copy to: *Dr Lynne Webster, Central Manchester Foundation Trust*

8.5 Human regulations authority approval 16/NW/0715

NHS
Health Research Authority

Dr Jenny Myers
Maternal and Fetal Health Research Centre
Research 5th floor, St Mary's Hospital
Oxford Road, Manchester
M13 0JH

Email: hra.approval@nhs.net

14 December 2016

Dear Dr Myers

Letter of HRA Approval

Study title:	Pelvic Sensorineuropathy: A comparison between quantitative sensory testing and a simple hand held device
IRAS project ID:	173962
REC reference:	16/NW/0715
Sponsor	Central Manchester University Hospitals NHS Foundation Trust

I am pleased to confirm that **HRA Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications noted in this letter.

Participation of NHS Organisations in England

The sponsor should now provide a copy of this letter to all participating NHS organisations in England.

Appendix B provides important information for sponsors and participating NHS organisations in England for arranging and confirming capacity and capability. **Please read** *Appendix B* **carefully**, in particular the following sections:

- *Participating NHS organisations in England* – this clarifies the types of participating organisations in the study and whether or not all organisations will be undertaking the same activities
- *Confirmation of capacity and capability* - this confirms whether or not each type of participating NHS organisation in England is expected to give formal confirmation of capacity and capability. Where formal confirmation is not expected, the section also provides details on the time limit given to participating organisations to opt out of the study, or request additional time, before their participation is assumed.
- *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* - this provides detail on the form of agreement to be used in the study to confirm capacity and capability, where applicable.

Further information on funding, HR processes, and compliance with HRA criteria and standards is also provided.

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It is critical that you involve both the research management function (e.g. R&D office) supporting each organisation and the local research team (where there is one) in setting up your study. Contact details and further information about working with the research management function for each organisation can be accessed from www.hra.nhs.uk/hra-approval.

Appendices

The HRA Approval letter contains the following appendices:

- A – List of documents reviewed during HRA assessment
- B – Summary of HRA assessment

After HRA Approval

The document *"After Ethical Review – guidance for sponsors and investigators"*, issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

In addition to the guidance in the above, please note the following:

- HRA Approval applies for the duration of your REC favourable opinion, unless otherwise notified in writing by the HRA.
- Substantial amendments should be submitted directly to the Research Ethics Committee, as detailed in the *After Ethical Review* document. Non-substantial amendments should be submitted for review by the HRA using the form provided on the HRA website, and emailed to hra.amendments@nhs.net.
- The HRA will categorise amendments (substantial and non-substantial) and issue confirmation of continued HRA Approval. Further details can be found on the HRA website.

Scope

HRA Approval provides an approval for research involving patients or staff in NHS organisations in England.

If your study involves NHS organisations in other countries in the UK, please contact the relevant national coordinating functions for support and advice. Further information can be found at http://www.hra.nhs.uk/resources/applying-for-reviews/nhs-hsc-rd-review/.

If there are participating non-NHS organisations, local agreement should be obtained in accordance with the procedures of the local participating non-NHS organisation.

User Feedback

The Health Research Authority is continually striving to provide a high quality service to all applicants and sponsors. You are invited to give your view of the service you have received and the application

procedure. If you wish to make your views known please email the HRA at hra.approval@nhs.net. Additionally, one of our staff would be happy to call and discuss your experience of HRA Approval.

HRA Training

We are pleased to welcome researchers and research management staff at our training days – see details at http://www.hra.nhs.uk/hra-training/

Your IRAS project ID is **173962**. Please quote this on all correspondence.

Yours sincerely

Thomas Fairman
HRA Assessor

Email: hra.approval@nhs.net

Copy to: *Dr Lynne Webster, Central Manchester Foundation Trust (Sponsor Contact and Lead NHS R&D Contact)*

NIHR CRN Portfolio Applications Team

Appendix A - List of Documents

The final document set assessed and approved by HRA Approval is listed below.

Document	Version	Date
Instructions for use of medical device [Instructions for use of GSA]	1.0	30 August 2016
IRAS Application Form [IRAS_Form_11112016]		11 November 2016
Letter from funder [Letter of Intent to fund from NIHR]	1.0	24 August 2015
MHRA Notice of No Objection Letter (Medical Devices) and relevant correspondence [MHRA email]	1.0	30 August 2016
Non-validated questionnaire [Patient Assessment Questionnaire]	1.0	30 August 2016
Other [PIS controls]	1.0	07 November 2016
Other [Consent Form controls]	1.0	07 November 2016
Other [HEI_Contract_DRF_2015_08_217 final]		
Other [Vibratip Instruction leaflet]		
Other [Vibratip Press Pack]		
Other [ePAQ report dummy example]		
Other [ePAQ Pelvic Floor Tour]	1.0	30 August 2016
Other [ePAQ Pelvic Floor Tour]		
Participant consent form [Consent form POP]	1.0	07 November 2016
Participant information sheet (PIS) [Patients - Tracked Changes]	1.0	07 November 2016
Participant information sheet (PIS) [PIS for POP]	1.0	07 November 2016
Participant information sheet (PIS) [Controls - Tracked Changes]	1.0	07 November 2016
Research protocol or project proposal [Protocol]	1.0	30 August 2016
Summary CV for Chief Investigator (CI) [Jenny Myers Short CV]	1.0	30 October 2016
Summary CV for student [Charlotte Mahoney Short CV]	1.0	30 August 2016
Summary CV for supervisor (student research) [Fiona Reid Short CV]	1.0	30 August 2016
Validated questionnaire [ePAQ Brochure]		

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Appendix B - Summary of HRA Assessment

This appendix provides assurance to you, the sponsor and the NHS in England that the study, as reviewed for HRA Approval, is compliant with relevant standards. It also provides information and clarification, where appropriate, to participating NHS organisations in England to assist in assessing and arranging capacity and capability.

<u>For information on how the sponsor should be working with participating NHS organisations in England, please refer to the, *participating NHS organisations, capacity and capability* and *Allocation of responsibilities and rights are agreed and documented (4.1 of HRA assessment criteria)* sections in this appendix.</u>

The following person is the sponsor contact for the purpose of addressing participating organisation questions relating to the study:

Name: Dr Lynne Webster
Tel: 01612764125
Email: lynne.webster@cmft.nhs.uk

HRA assessment criteria

Section	HRA Assessment Criteria	Compliant with Standards	Comments
1.1	IRAS application completed correctly	Yes	No comments
2.1	Participant information/consent documents and consent process	Yes	No comments
3.1	Protocol assessment	Yes	No comments
4.1	Allocation of responsibilities and rights are agreed and documented	Yes	This is a non-commercial single site study taking place in the NHS where that single NHS organisation is also the study sponsor. Therefore no study agreements are required.
4.2	Insurance/indemnity arrangements assessed	Yes	Where applicable, independent contractors (e.g. General Practitioners) should ensure that the professional indemnity provided by their medical defence organisation covers the

Section	HRA Assessment Criteria	Compliant with Standards	Comments
			activities expected of them for this research study
4.3	Financial arrangements assessed	Yes	External study funding has been secured from the NIHR.
5.1	Compliance with the Data Protection Act and data security issues assessed	Yes	No comments
5.2	CTIMPS – Arrangements for compliance with the Clinical Trials Regulations assessed	Not Applicable	No comments
5.3	Compliance with any applicable laws or regulations	Yes	No comments
6.1	NHS Research Ethics Committee favourable opinion received for applicable studies	Yes	REC Favourable Opinion was issued by the Greater Manchester West Research Ethics Committee, on the 30th November 2016.
6.2	CTIMPS – Clinical Trials Authorisation (CTA) letter received	Not Applicable	No comments
6.3	Devices – MHRA notice of no objection received	Not Applicable	No comments
6.4	Other regulatory approvals and authorisations received	Not Applicable	No comments

Participating NHS Organisations in England

This provides detail on the types of participating NHS organisations in the study and a statement as to whether the activities at all organisations are the same or different.

This is a non-commercial single site study taking place in the NHS where that single NHS organisation is also the study sponsor. Therefore there is only one site type involved in the research.

If this study is subsequently extended to other NHS organisation(s) in England, an amendment should be submitted to the HRA, with a Statement of Activities and Schedule of Events for the newly participating NHS organisation(s) in England.

The Chief Investigator or sponsor should share relevant study documents with participating NHS

organisations in England in order to put arrangements in place to deliver the study. The documents should be sent to both the local study team, where applicable, and the office providing the research management function at the participating organisation. For NIHR CRN Portfolio studies, the Local LCRN contact should also be copied into this correspondence. For further guidance on working with participating NHS organisations please see the HRA website.

If chief investigators, sponsors or principal investigators are asked to complete site level forms for participating NHS organisations in England which are not provided in IRAS or on the HRA website, the chief investigator, sponsor or principal investigator should notify the HRA immediately at hra.approval@nhs.net. The HRA will work with these organisations to achieve a consistent approach to information provision.

Confirmation of Capacity and Capability

This describes whether formal confirmation of capacity and capability is expected from participating NHS organisations in England.

The HRA has determined that participating NHS organisations in England that are participating in the study **are not expected to formally confirm their capacity and capability to host this research,** because this is a non-commercial single site study taking place in the NHS where that single NHS organisation is also the study sponsor.

- The HRA has informed the relevant research management offices that you intend to undertake the research at their organisation. However, you should still support and liaise with these organisations as necessary.
- The document "Collaborative working between sponsors and NHS organisations in England for HRA Approval studies, where no formal confirmation of capacity and capability is expected" provides further information for the sponsor and NHS organisations on working with NHS organisations in England where no formal confirmation of capacity and capability is expected, and the processes involved in adding new organisations. Further study specific details are provided in this *Appendix B* (*Participating NHS Organisations* and *Agreement* sections).

If this study is subsequently extended to other NHS organisation(s) in England a further assessment of the need for assessment of capacity and capability at those additional sites will be made.

Principal Investigator Suitability

This confirms whether the sponsor position on whether a PI, LC or neither should be in place is correct for each type of participating NHS organisation in England and the minimum expectations for education, training and experience that PIs should meet (where applicable).

A Principal Investigator should be appointed at study sites

GCP training is not a generic training expectation, in line with the HRA statement on training expectations.

HR Good Practice Resource Pack Expectations

This confirms the HR Good Practice Resource Pack expectations for the study and the pre-engagement checks that should and should not be undertaken
As a non-commercial undertaken by local staff, it is unlikely that letters of access or honorary research contracts will be applicable, except where local network staff employed by another Trust (or University) are involved (and then it is likely that arrangements are already in place). Where arrangements are not already in place, network staff (or similar) undertaking any of the research activities listed in A18 or A19 of the IRAS form (except for administration of questionnaires or surveys), would be expected to obtain an honorary research contract from one NHS organisation (if university employed), followed by Letters of Access for subsequent organisations. This would be on the basis of a Research Passport (if university employed) or an NHS to NHS confirmation of pre-engagement checks letter (if NHS employed). These should confirm enhanced DBS checks, including appropriate barred list checks, and occupational health clearance. For research team members only administering questionnaires or surveys, a Letter of Access based on standard DBS checks and occupational health clearance would be appropriate.

Other Information to Aid Study Set-up

This details any other information that may be helpful to sponsors and participating NHS organisations in England to aid study set-up.
The applicant has indicated that they do intend to apply for inclusion on the NIHR CRN Portfolio.

8.6 Protocol for study 16/NW/0715

Pelvic Sensorineuropathy: A comparison between quantitative sensory testing and a simple hand held device

Short Title	A Simple Test of Pelvic Sensation (SToPS)
Protocol number	1.0
Version and date	30/8/16
Sponsor	Central Manchester University Hospitals NHS Foundation Trust (CMFT)
Chief Investigator	Dr Jenny Myers
EudraCT Number	
REC Number	IRAS 173962

Chief Investigator (CI)	Dr Jenny Myers
Co-Investigator(s) (Co-I)	Dr Charlotte Mahoney (NIHR PhD Research student) Dr Fiona Reid Professor Tony Smith
Sponsor's representative	Dr Lynne Webster
Statistician	Dr Jenny Myers
Central laboratories	CMFT
Pharmacy contact	Not applicable
SAE/SUSAR reporting	Adverse.Events@cmft.nhs.uk or fax to 0161 276 5766

CHIEF INVESTIGATOR STATEMENT

This document describes a study evaluating **a simple test of pelvic sensation** and provides information about procedures for entering patients into it. The protocol should not be used as a guide for the treatment of patients outside the research study. Every care was taken in drafting this protocol, but corrections or amendments may be necessary, care must be taken to use the most up to date and approved version. This Study will adhere to the principles outlined in the ICH Good Clinical Practice guidelines. The study will be conducted in compliance with the protocol, the Data Protection Act (DPA Z6364106), the Declaration of Helsinki, Human Tissue Act (2004), the Research Governance Framework (2005) and other regulatory requirements as appropriate.

Chief Investigator – Dr Jenny Myers, Central Manchester University Hospitals NHS Foundation Trust

Signed   *Date*

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LIST OF ABBREVIATIONS

AE   Adverse Event
AR   Adverse Reaction
CI   Chief Investigator
CMFT   Central Manchester University Hospitals NHS Foundation Trust
EudraCT   European Clinical Studies Database
GCP   Good Clinical Practice
GSA   Genito-Sensory Analyzer
NHS R&D   National Health Service Research & Development
PI   Principal Investigator
POP   Pelvic Organ Prolapse
POPQ   Pelvic Organ Prolapse Quantification System
QST   Quantitative Sensory Testing
RCT   Randomised Controlled Study
REC   Research Ethics Committee
SAR   Serious Adverse Reaction
SAE   Serious Adverse Event
SSAR   Suspected Serious Adverse Reaction
SUSAR   Suspected Unexpected Serious Adverse Reaction
TSC   Study Steering Committee
UAR   Unexpected Adverse Reaction

STUDY SUMMARY

Title: Pelvic Sensorineuropathy: A comparison between quantitative sensory testing and a simple hand held device

Short title: A Simple Test of Pelvic Sensation (SToPS)

Design: Prospective cohort diagnostic accuracy study

Objectives:
This project aims to compare two different methods of sensory testing of the female genitalia. Quantitative sensory testing (QST) is considered the gold standard for testing sensation in the female genitalia but the equipment (called the Genito-Sensory Analyzer, GSA) is expensive and cumbersome. As such sensory testing of the female genitalia has not been incorporated into routine clinical practice in Urogynaecology. A simple hand held device which is currently used to assess for peripheral neuropathy in limbs, called Vibratip, would allow the general gynaecologist to test for female sensation in routine clinical practice. This study aims to compare Vibratip against the gold standard QST as well as evaluate acceptability of the different tests to women.

The project also aims to link histological findings with functional sensory data. To date, two studies have demonstrated abnormal sensation in women with pelvic organ prolapse (POP). [1,2] Another study reported reduced nerve fibres in women with POP compared to controls, however this study looked at all nerve fibres within vaginal tissue – regardless of whether they were motor or sensory nerves.[3] Certainly, no study has linked sensory nerve dysfunction and histological abnormality in the vagina.

Therefore this study will also aim to correlate sensory nerve function and histological findings in women with POP.

Thus the key research objectives are:
1. **To investigate the accuracy of Vibratip compared to the GSA for diagnosing pelvic sensorineuropathy**
2. **To assess the repeatability (inter-rater test-retest reliability) of Vibratip compared to the GSA**
3. **To evaluate patient acceptability of both Vibratip and the GSA**
4. **To link histological findings to sensory function of pelvic nerves**

Endpoints:
1. **Accuracy of Vibratip compared to the GSA for diagnosing pelvic sensorineuropathy**
2. **Inter-rater reliability of Vibratip compared to the GSA**
3. **Quantify patient acceptability of intimate sensory testing**
4. **Describe the relationship between histological findings with pelvic sensory nerve function**

Cohort:
A prior study in our unit has demonstrated that women with symptomatic pelvic organ prolapse (POP) have up to an 80% incidence of abnormal pelvic sensation.[1] Therefore the cohort will consist of *women with signs or symptoms of POP* attending the Gynaecology clinic.

Eligibility:
Women attending a Gynaecology appointment at St Mary's Hospital

Study Methods:

The GSA is currently the only standard for testing female pelvic sensation, and thus is the gold standard by default. As such, the study will compare the diagnostic performance of Vibratip to the "gold standard" GSA. Effectively, the study will define the threshold at which the Vibratip can detect abnormal sensation.

The study will also perform a range of histological techniques on samples of vaginal mucosa.

Study duration per participant:

Maximum six months (to allow re-test at the next available clinic appointment or a convenient time for the women)

Estimated total Study duration: The study will recruit for 12 months.

The diagnostic accuracy part of the study will require one clinical visit.

The vaginal histology part of the study will require confirmation of consent on the day of elective surgery.

Total number of participants planned:

46 women

Patient Pathway

Pre-appointment
- Receive information leaflet in the post

Clinic Visit One
- Invited to participate in study
- Reasured participantion in study has no effect on clinical decisions made
- Consent taken
- Sensory assessment of vulva, vagina and clitoris
  - GSA
  - Vibratip
    - Test
    - Re-test
- Routine clincial care will include POPQ & ePAQ

Theatre if applicable
- If undergoing surgical repair of POP
- Consent for histology of vaginal mucosa tissue

BACKGROUND

Significance

Pelvic organ prolapse (POP), urinary (UI) and faecal incontinence (FI) and sexual dysfunction (FSD), collectively termed pelvic floor dysfunction (PFD) impair the quality of life of over one third of women.[4] Women with symptoms of PFD have been shown to have abnormal genital sensation [1,2,5–10] These women can present to the Gynaecology clinic with symptoms of sensory dysfunction and yet there is no bedside test for the General Gynaecologist to assess this further.

Furthermore sensory dysfunction may be an important factor in the aetiology of pelvic floor dysfunction.

Pelvic sensation in women is currently tested using a large and expensive vibrotactile device, called a Genito-Sensory Analyzer (GSA) which requires specialist training to use and so is limited to a research or a tertiary setting. The GSA uses a validated protocol to provide a reproducible assessment of pelvic sensory function, termed quantitiative sensory testing (QST). QST is considered the gold standard for non-invasive sensory testing. Currently there is no simple handheld vibrotactile device which can be used in the general gynaecology clinic to screen for abnormal pelvic sensation. Unfortunately the standard devices used to test for peripheral diabetic neuropathy such as a tuning fork or monofilaments are inappropriate for vaginal testing due their size and shape. However, more recently a simple hand-held vibration device, Vibratip has been developed for use in peripheral diabetic neuropathy which could be used to assess pelvic sensation in women. Therefore this study will compare the gold standard QST vibrotactile equipment with the simple handheld vibration device, called Vibratip.

Although there is strong evidence demonstrating abnormal pelvic sensation in women with PFD, this has never been correlated with histological findings. As such, this study will also attempt to compare vaginal histology with sensory functional findings.

The evidence

A small handheld vibration device: Vibratip

A group in Bristol have recently developed a small handheld vibration device with a small tip which could be used to test vibration sensation in the female genitalia in the general clinic setting. The device was developed by Professor Levy and is called a Vibratip.

Levy published his preliminary data comparing Vibratip with a tuning fork and a 10g monofilament. He stated that the Vibratip was comparable to the tuning fork and monofilament, although there was no statistical analysis published in the study.[11]

An independent study by Bracewell et al compared Vibratip to a 10g monofilament, QST vibration thresholds and a tuning fork in 141 subjects with peripheral neuropathy. The group reported the Vibratip was no different from the monofilament ($p=0.3214$), and the monofilament was significantly better than the tuning fork ($p=0.0056$).[12] Unfortunately no statistical comparison was mentioned between the Vibratip and the gold standard QST vibration thresholds.

In the same month, a study was published by Bowling et al from the Manchester Diabetes Centre comparing the Vibratip to QST vibration thresholds in 83 people. Bowling found significant agreement between Vibratip and QST vibration thresholds ($p<0.001$, Cohen's κ = 0.973) with a sensitivity of 100% and specificity of 96.6% between the two tests.[13]

Nizar et al also evaluated Vibratip and QST vibration thresholds reporting a sensitivity of 92% and a specificity of 94%, compared to a tuning fork of 40% and 100% respectively.

There is no data on the device in assessment of female genital sensory impairment.

Vaginal histology
Whilst two studies have demonstrated abnormal sensation in women with pelvic organ prolapse (POP), this has never been correlated with histological findings. [1,2]

A study by Inal et al evaluated vaginal innervation in women with POP compared to controls, reporting that women with POP have significantly reduced vaginal innervation.[3] However the study measured all nerve fibres within the vaginal tissue together – regardless of whether they were motor or sensory nerves.

There is currently no study which has compared pelvic sensorineuropathy and histological findings in vagina mucosa.

Study treatment
This study will not involve any treatment.

Rationale for the proposed study
The study will allow the general gynaecologist to diagnose sensory impairment in the female genitalia which could prevent unnecessary surgery and facilitate more appropriate treatment for women.

The study will also aid understanding of the pathophysiology of pelvic sensorineuropathy.

Assessment and management of risk
This research is a prospective cohort study of diagnostic accuracy and as such will not affect the clinical care participating women receive. The decision not to participate will not alter their treatment in any way.

Previous studies have demonstrated that the vibrotactile equipment is safe to use and acceptable to women. Women who choose to participate in pelvic sensory testing will not be subject to harm.[1,14]

Vibratip

Vibratip is a CE marked Class IIa medical device licensed to distribute in the UK. The information leaflet has a list of precautions which includes "VibraTip® should not be applied to mucosal surfaces". The device is on sale to the general public and this precaution was included to prevent any legal liability should a member of the public harm themselves by applying the device to a mucosal surface such as the rectum or eye. In this study the device will be used off-label by the medical researcher only on intact perineal or clitoral skin, or vaginal mucosal surfaces. Vibratip produces a frequency just above 128Hz [13,15] with a constant amplitude. Vibratip is placed against the skin twice and the subject is asked whether they feel any vibration on both touches, although the device is only randomly activated once. Each activation lasts for a few seconds. The QST vibrotactile equipment also provides vibration at increasing frequencies over more than a few seconds until perceived by the patient. When QST vibrotactile testing is performed on the female genitalia including vaginal mucosa this causes no harm. It is reasonable to extrapolate this would be the same for Vibratip. As this is a research study investigating an off-label indication and not part of a clinical investigation, the MHRA have concluded the study does not need MHRA approval.

GSA

Vibration thresholds for vulva, vaginal and clitoral sensation will be testing using the GSA. The GSA consists of a height-adjustable vibration probe controlled by a computer programme. The vibration intensity is linearly increased (from no vibration to a maximum pre-set level) until the woman indicates perception of the vibration using a response button held in her dominant hand. A standardised verbal explanation of the protocol and equipment will be provided before each test including a control test on the hand to familiarise with the sensation and use of the response button. At this point verbal consent to continue with intimate testing is confirmed.

The probe is inserted 2-3cm into the vagina and bilaterally on the vulva to assess the perineal branch of the pudendal nerve, whereas only the tip is placed against the clitoris so the woman is just aware of the probe to assess the clitoral branch. When the response button is pressed the stimulus stops immediately and the threshold is recorded. This is repeated six times and the average value calculated.

Women who consent to participate will undergo GSA testing in addition to their clinical care. This will add approximately 15 minutes to their appointment.

Women donating vaginal mucosa for histology will also undergo GSA testing of temperature sensation.

There is no immunohistochemical stain which can differentiate between the different types of sensory nerve fibre. Therefore it will be important to have sensory functional data for all nerve fibre types for the women donating their vaginal tissue for histology.

As such, the GSA will also be used to test thermal sensation in these women. The temperature is linearly increased to assess warmth or decreased to assess cold, from no stimulus to a maximum or minimum pre-set level respectively. Again, a standardised verbal explanation of the protocol and equipment will be provided before each test including a control test on the hand to familiarise with the sensation and use of the response button.

The probe will be inserted 2-3cm into the vagina to assess the perineal branch of the pudendal nerve, whereas only the tip is placed against the clitoris so the woman is just aware of the probe to assess the clitoral branch. Thermal sensation of the vulva will not be tested. This will add approximately another 15 minutes to their appointment.

POPQ

Pelvic anatomy will be measured using the pelvic organ prolapse quantification system (POPQ) via device similar to a ruler. POPQ is a simple, non-painful examination of the pelvis looking for female genital prolapse. It is used as the routine examination in the Urogynaecology clinic at St Mary's Hospital and recommended for use in research and clinical practice by the International Continence Society and the International Urogynaecology Association.[16]

Women who consent to participate will undergo a POPQ examination as part of their routine clinical assessment.

ePAQ-PF

The electronic patient assessment questionnaire for the pelvic floor (ePAQ-PF) is a validated questionnaire which poses no risk to women, and can be completed in the department or at home online.

Again, ePAQ-PF is routinely completed by all women attending the Urogynaecology clinic as a new patient. If

the questionnaire was last completed over six months previously, it will be repeated.

Vaginal Histology in women with POP

25% women who present with POP opt for surgical management, called an anterior or posterior colporrhaphy. During anterior and posterior colporrhaphy the excess vaginal mucosa is excised and disposed in accordance with hospital policy.

As such, women who volunteer to donate their vaginal tissue for histology will not be subject to any additional surgical risks or discomfort than they would experience as part of their routine clinical care.

Vaginal Histology in controls

6-8 women who are undergoing gynaecological surgery for any condition other than POP will also be invited to participate in the study to provide comparative histological control data. Gynaecological surgery will include women undergoing hysterectomy, bilateral salpingoophorectomy, myomectomy or major excision of endometriosis. The common risks from these operations include infection, bleeding, pain and cosmetic scarring.

Women will be invited to participate and reassured their decision will not impact on their clinical care. Women who chose to participate will donate a sample of their vaginal skin for histological analysis.

If the vaginal wall is due to be routinely excised as part of their planned operation, this will be collected directly from the surgeon. These women will not be subject to any additional surgical risks or discomfort than they would experience as part of their routine clinical care.

If the vaginal wall is not routinely excised as part of their planned operation, they will undergo a 5mm punch biopsy of vaginal skin from their normal vaginal wall. This will be performed during their surgical procedure so they will be anaesthetised for the biopsy.

A punch biopsy is a device similar to a cookie cutter but it is only 5mm in diameter. It is used to cut out a cylindrical piece of skin and the remaining 5mm hole is then closed with a single suture and heals with minimal scarring.

A vaginal wall or vulval punch biopsy is often performed in the out-patient clinic to diagnose skin conditions and guide treatment. It is performed under local anaesthetic, with a single dissolvable stitch to close the skin and requires no additional follow up. The risks from a punch biopsy include wound infection, slight bleeding from the raw skin edges and scarring.

The rate of infection from a punch biopsy is less than 1%.[17] The small amount of bleeding that may occur at the raw skin edges is controlled with a single dissolvable suture, and does not exceed 2mls in volume. Any scarring that occurs is less than 5mm in length and hidden within the vagina, therefore if a scar does develop it does not impair vaginal function or cause cosmetic distress to the woman.

Women who choose to participate and donate their normal vaginal wall will undergo this biopsy as an extra procedure in addition to their routine clinical care. The women will be anaesthetised for their surgery and so will not feel the biopsy itself.

The list of possible gynaecological surgeries has been carefully chosen to ensure that women who choose to participate in the study will not be exposed to any significant additional risks from the punch biopsy compared to their operation.

In order to map sensory nerve function with histological findings, these control women will also be asked to undergo sensory testing with the GSA, a POP-Q to exclude undiagnosed POP and ePAQ-PF to quantify any underlying pelvic floor symptoms. This represents an additional clinical visit which women may find inconvenient, although every effort will be made to coordinate sensory testing with pre-existing appointments to reduce inconvenience. Sensory testing will mean an additional intimate examination which some women may find a burden.

STUDY OBJECTIVES AND ENDPOINTS

Objectives
1. **To investigate the accuracy of Vibratip compared to the GSA for diagnosing pelvic sensorineuropathy**
2. **To assess the repeatability (inter-rater test-retest reliability) of Vibratip compared to the GSA**
3. **To evaluate patient acceptability of both Vibratip and the GSA**
4. **To link histological findings to sensory function of pelvic nerves**

Endpoints:
1. **Accuracy of Vibratip compared to the GSA for diagnosing pelvic sensorineuropathy**
2. **Inter-rater reliability of Vibratip compared to the GSA**
3. **Quantify patient acceptability of intimate sensory testing**
4. **Correlate histological findings with pelvic sensory nerve function**

This is a prospective cohort study of diagnostic accuracy and so the performance of the index test Vibratip will be assessed against the reference standard – the GSA, in a group of women with abnormal pelvic sensation.

Over the course of the study we aim to recruit 46 women. Women attending the Gynaecology Department at St Mary's Hospital will be invited to participate in the study. Over 25 women per week attend the Gynaecology department at St Mary's Hospital with signs or symptoms of POP. This will involve sensory testing of the female genitalia at the same time as their clinic appointment or at a mutually convenient date if the woman would prefer.

Women attending the Urogynaecology department with symptoms or signs of POP will receive an information leaflet on the study approximately one week in advance of their appointment. On arrival, prior to their clinical consultation women will be invited to participate with a full verbal explanation provided with the opportunity to discuss the study. Eligibility will be confirmed and those who wish to participate will complete a consent form.

All participants will undergo a POPQ and complete ePAQ-PF as part of their routine clinical care. If ePAQ-PF was last completed six months before, the woman will be asked to repeat it. Women will undergo genital sensation testing with Vibratip and this will be compared to the GSA. Finally, the women will complete a short questionnaire assessing acceptability of the two methods (see Appendix One).

A subgroup of women from will also be invited to repeat the Vibratip sensory testing to assess test-retest reliability, later on in the same appointment.

The study will also attempt to compare sensory functional data with tissue histology in women with POP. Women who choose surgical treatment will be invited to donate the excess vaginal tissue removed at the time of surgery for histological assessment.

For the comparison, six to eight women attending St Mary's Hospital without POP due to undergo gynaecological surgery will be invited to participate and donate vaginal tissue for histology. This will either involve a vaginal mucosal biopsy or donation of vaginal tissue that is due to be routinely removed as part of their planned surgery.

In order to provide sensory functional data for tissue mapping, this small group of women will also be asked to undergo a POPQ to exclude POP, sensory testing with the GSA and ePAQ-PF.

Selection of study participants

Inclusion criteria
- All women attending the Gynaecology clinic at St Mary's Hospital.

Exclusion criteria
- Language barrier – given the intimate nature of the assessments, the investigators feel the use of interpreters would be inappropriate
- A form of genital mutilation which prevents access to the vagina
- Previous POP surgery
- Any previous major gynaecological surgery which could affect pelvic nerves such as hysterectomy or oophorectomy. This will depend on the prior surgical approach and techniques used and so will the final decision on eligibility will be at the discretion of the researcher.
- Incapacity to consent
- Previous treatment for cancer

Concomitant medication
All prescribed medications will be continued as normal by their General Practitioner. Any medications which would be part of treatment will be started as normal, whether participating in the study or not.

Recruitment of study participants
Women will be provided with information regarding the study in the post before their routine clinic appointment.

Initial contact
All women who meet the inclusion and exclusion criteria will be given an information sheet about the study at first point of contact. Women eligible for the study will be approached in person or contacted via telephone by the researcher. Women will be given a contact number to call back, if unable to discuss the study at the initial contact. Women will be screened against the exclusion criteria during the initial face to face or telephone contact.

Ineligible and non-recruited women
Women who are ineligible or decline to take part will be reassured their decision will not affect their routine

care.

Registration/randomisation procedures
Not applicable

Emergency unblinding procedures
Not applicable

Discontinuation/ withdrawal of participants and "stopping rules"
Participation in the study is voluntary. A woman has the right to withdraw from the study at any time for any reason. The Investigator has the right to discontinue participation at any time if it is deemed to be in the woman's best interest.

If the woman is withdrawn due to a serious adverse event, the Principal Investigator (PI) will arrange for follow-up visits or telephone calls until the event has resolved or stabilised.

TREATMENT DETAILS

Treatment summary
Not applicable – this is a prospective cohort study of diagnostic accuracy.

STUDY ASSESSMENTS
Women will be invited to participate. Eligibility will be confirmed and women will be given the opportunity to discuss the study and those who wish to participate will complete a consent form.

Demographics
A clinical history will be taken including age, ethnicity, menstrual history, previous obstetric, gynaecological and medical history, drug and smoking history, and measurement of BMI will be performed as part of their routine clinical care.

ePAQ-PF
As part of their routine clinical care women will complete ePAQ-PF which asks about symptoms of POP, urinary, bowel and sexual dysfunction. Women who completed ePAQ-PF more than six months ago will be asked to repeat it during the consultation or online at home depending on their convenience. This information will be secured on a secure server.

POP-Q
Again, as part of their routine clinical care women will undergo an assessment for prolapse called a POPQ examination which uses a device similar to a ruler.

Index Test: Vibratip
Pelvic sensation will be tested using Vibratip, the hand-held vibration device. Again, a standardised verbal explanation of the protocol and equipment will be provided before each test including a control test on the hand to familiarise with the sensation. The Vibratip will be applied to the surface of the clitoris twice but only randomly activated once, the women will be asked on which touch they perceived the sensation. This will be repeated on the anterior vaginal wall approximately 2-3cms inside the vagina, as well as both the right and left vulval skin surfaces.

Vibration thresholds for vulval, vaginal and clitoral sensation will be testing using the GSA. The GSA consists of a height-adjustable vibration probe controlled by a computer programme. The vibration intensity is linearly increased (from no vibration to a maximum pre-set level) until the woman indicates perception of the vibration using a response button held in her dominant hand. A standardised verbal explanation of the protocol and equipment will be provided before each test including a control test on the hand to familiarise with the sensation and use of the response button. At this point, the woman is asked to verbally re-confirm consent to proceed to intimate testing. The probe is inserted 2-3cm into the vagina to assess the perineal branch of the pudendal nerve. The tip of the probe is placed against the clitoris so the woman is just aware of the probe to assess the clitoral branch. The tip of the probe is placed against the vulva on the left and right to assess another branch of the perineal nerve. When the response button is pressed the stimulus stops immediately and the threshold is recorded. This is repeated six times and the average value calculated.

The group of women who choose to donate a sample of vaginal skin for histology will also undergo temperature sensation testing, to provide functional data on all nerve types. This is because there is no immunohistochemical stain which can differentiate between the different types of sensory nerve fibre. Therefore it will be important to have sensory functional data for all nerve fibre types for the women donating their vaginal tissue for histology.

Using the GSA, the temperature is linearly increased to assess warmth or decreased to assess cold, from no stimulus to a maximum or minimum pre-set level respectively. Again, a standardised verbal explanation of the protocol and equipment will be provided before each test including a control test on the hand to familiarise with the sensation and use of the response button. Again, the woman is asked to verbally re-confirm consent to proceed to intimate testing

The probe will be inserted 2-3cm into the vagina to assess the perineal branch of the pudendal nerve, whereas only the tip is placed against the clitoris so the woman is just aware of the probe to assess the clitoral branch. Thermal sensation of the vulva will not be tested. When the response button is pressed the stimulus stops immediately and the threshold is recorded. This is repeated six times and the average value calculated.

Patient Acceptability
Women will be asked to complete a short questionnaire asking their view on acceptability of the two tests to provide a patient perspective. This will take less than five minutes to complete. (see Appendix One)

Test-retest
A subgroup of women will be invited to undergo repeat testing to assess test reliability. This will be performed later on during the initial consultation.

Repeat testing will be performed using the index test, Vibratip, on the surface of the clitoris, the anterior vaginal wall approximately 2-3cms inside the vagina, and bilaterally on the vulva.

Vaginal Histology

Women with POP
During surgical repair of POP the excess vaginal mucosa is excised and disposed of as per hospital policy. This is explained to women when they are consented for surgery.

Women who are undergoing anterior or posterior colporrhaphy will be asked if they are happy to donate the

excised tissue for histology. If they choose to participate they will be consented accordingly and the tissue collected intra-operatively.

Controls

Theatre diaries will be hand-searched and appropriate cases identified. These women will then receive an information leaflet in the post or at their next routine clinical appointment. They will then receive a follow-up telephone consultation to discuss the study. Women who express an interest in participating will then be seen face to face at a convenient time for the women, usually their appointment at the pre-op department. Women will then receive a full explanation of the study and a demonstration of vibration using a Vibratip on the hand.

At this stage, women who are ineligible or decline to take part will be reassured their decision will not affect their routine care. Women who choose to participate will arrange a convenient time to complete the consent form and attend for sensory testing with the GSA (as detailed above), a POPQ examination to confirm there is no evidence of POP and to complete ePAQ-PF.

On the day of surgery the women will be reviewed by a member of the research team who will re-confirm consent.

Two punch biopsies of the vaginal wall will then be performed during the operation, at a point in the surgical procedure which is best for the clinical care of the woman. If the vaginal tissue is due to be excised routinely as part of the operation, the researcher will collect it at this point.

Study schedule

	Prior to clinical research visit	Clinical Visit One	Day of Surgery (if applicable)
Patient Information Leaflet			
• **Face to face**	• ✔	• ✔	
• **In post**	• ✔	• n/a	
Inc/Exc Criteria		• ✔	
Consent Form		• ✔	Re-confirm consent
Clinical history		• ✔	
GSA		• ✔	
Vibratip			
• **Test**		• ✔	
• **Retest**		• Subgroup only	
POPQ examination		Routine clinical care	
ePAQ		Routine clinical care	
Patient questionnaire		• ✔	
Vaginal histology			• ✔
Review AEs and SAE's		• ✔	• ✔

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ASSESSMENT OF STUDY EFFICACY/MEASURING STUDY ENDPOINTS

Endpoints:
1. **Accuracy of Vibratip compared to the GSA for diagnosing pelvic sensorineuropathy**
2. **Inter-rater reliability of Vibratip compared to the GSA**
3. **Quantify patient acceptability of intimate sensory testing**
4. **Correlate histological findings with pelvic sensory nerve function**

STUDY CLOSURE
The study will recruit for 12 months.

STATISTICS AND DATA ANALYSIS

Study Design
Prospective cohort study of diagnostic accuracy

Sample size calculation

Diagnostic Accuracy
Based on a previous study performed in the department 80% of women with POP have abnormal genital sensation.[1] Comparing the vibratip to the GSA, our experience suggests that a sensitivity in the region of 65% could be anticipated. Therefore using a specificity of 90%, with a precision of 0.2 and a 95% confidence interval requires a sample size of 46 women.

Sub-group: Vaginal Histology
Whilst a previous study has reported a significant difference in the number of nerve fibres in women with and without POP, this has never been linked to functional sensory findings.

As there is no prior data available this will be a preliminary exploratory analysis.

Therefore a provisional number of 20 women with POP and 6-8 control women will be recruited to participate in the histological component of the study.

Interim analysis
No interim analysis planned.

Primary objective analysis
1. **To assess accuracy of Vibratip compared to the GSA for diagnosing pelvic sensorineuropathy**
   A receiver operating characteristic curve will be calculated and standard diagnostic accuracy tests will be used to include statistical testing for accuracy, sensitivity, posterior probabilities and likelihood ratios. [18–21]

Secondary objective analysis
1. **To evaluate the inter-rater reliability of Vibratip compared to the GSA**
   The data from the women who underwent test-retest with a second investigator will be analysed for inter-rater agreement using Cohen's kappa.[22,23]
2. **To determine patient acceptability of testing with both QST and Vibratip**
   Patient acceptability will be assessed by comparing questionnaire responses using Likert scales and analysed using non-parametric tests.[24]

3. **To link histological findings to sensory function of pelvic nerves**

   The histology of the vaginal mucosa, in particular sensory innervation, will be assessed using a variety of histological techniques and this will then be compared to sensory functional data.

   Histology of nerve fibres will be graded and mapped to nominal normal/abnormal sensory results using contingency tables. If the data allows, correlation testing will also be performed using continuous sensory data and the number of nerve fibres within a given area.

STUDY MANAGEMENT AND OVERSIGHT ARRANGEMENTS

A Delegation Log will be prepared, detailing the responsibilities of each member of staff working on the study.

STUDY MONITORING

Data collection

Data will be collected from participating women at each study visit and documented on a standard clinical research form. A data management plan will be prepared prior to the study starting. This will be in accordance with the sponsor's Standard Operating Procedure for Record Keeping, Data Management and Data Security: DS6-2789, and Maintaining a Study File and Version Control: DS6-2658.

Data handling and analysis

Data handling and analysis will be conducted according to the data management plan and will include the following activities:

- The clinical research team will be responsible for completion of the Case report form
- Missing or ambiguous data will be cross checked against the medical notes
- Data will be transferred to a electronic database
- Data will be anonymised and stored in accordance with Data Protection Act 1998.

Direct access to data

The Chief Investigator will permit study-related monitoring, audits, research and ethics council review (REC), and regulatory inspection and provide direct access to source data and documents.

Site monitoring

Site monitoring will be conducted according to the study monitoring plan agreed by the Sponsor and Chief Investigator.

CONFIDENTIALITY AND DATA PROTECTION

Confidentiality

- **Clinical information will not be released without the written permission of the participant, except as necessary for monitoring and auditing by the Sponsor, its designee, Regulatory Authorities, or the REC.**

- **The Investigator and study site staff involved with this study may not disclose or use for any purpose other than performance of the study, any data, record, or other unpublished, confidential information disclosed to those individuals for the purpose of the study.**
- **Prior written agreement from the Sponsor or its designee must be obtained for the disclosure of any confidential information to other parties.**
- **Personal data recorded on all documents will be regarded as strictly confidential and will be handled and stored in accordance with the Data Protection Act 1998. Women will be identified using only their unique study number, date of birth, and initials on the Case Report Form.**
- This will be in accordance with the sponsor's Standard Operating Procedure for Management of Participant Confidentiality: DS6-4690.

Data protection
- **Data will be collected in accordance with the "Caldicott Principles" and the Data Protection Act. All women recruited will be allocated a study number which will be linked to their identifiable information held in a separate file stored within the research unit. Outcome data will be collected using case record forms which will be stored within the Urogynaecology Unit, St Mary's Hospital, Manchester.**
- **All outcome data will be entered onto a password protected database within the Urogynaecology Unit accessible only to members of the research team. All electronic data will be anonymised and no identifiable data will be stored on this database.**
- **Published results will not contain any personal data that could allow identification of individual participants.**
- This will be in accordance with the sponsor's Standard Operating Procedure for Record Keeping, Data Management and Data Security: DS6-2789.

STUDY CONDUCT

Protocol amendments
Any changes in research activity (except those necessary to remove an apparent, immediate hazard to the participant) will be reviewed and approved by the sponsor and Chief Investigator (CI) prior to submission in writing to the appropriate REC, Regulatory Authority, and local research and development (R&D) for approval prior to enrolment into an amended protocol. This will be in accordance with the sponsor's Standard Operating Procedure for Research Approval, including amendments: DS6-2788.

Protocol violations/ deviations/ serious breaches
Investigators will not implement any deviation from the protocol without agreement from the sponsor, Chief Investigator and appropriate REC, Regulatory Authority and R&D approval except where necessary to eliminate an immediate hazard to women participating in the study.

Deviations will be managed as follows:

- The nature of and reasons for the deviation should be documented on the clinical research form and discussed with CI as soon as is practicable
- It will be the responsibility of the CI to inform the sponsor immediately
- If the deviation necessitates a subsequent protocol amendment, this should be submitted to the REC, Regulatory Authority and local R&D for review and approval

Serious breaches will be recorded and reported according to the sponsor's Standard Operating Procedures (Notification of a serious breach of GCP or the clinical trial protocol: DS6-2707) for the management of clinical study.

STUDY RECORD RETENTION

Long term copies of the data will be kept in both formats for a minimum of 15 years within the research facility after the date of publication, as per Medical Research Council Guidelines.

END OF STUDY

The Chief Investigator has the right at any time to terminate the study for clinical or administrative reasons. The end of the study will be reported to the REC and Regulatory Authority within the required timeframe (15 days) if the study is terminated prematurely. Investigators will inform participants of any premature termination of the study and ensure that the appropriate follow up is arranged for all involved. A summary report of the study will be provided to the REC and Regulatory Authority within the required timeframe. The end of study is defined as either the clinical visit of participant 46 or the collection of vaginal wall tissue from the final participant in the vaginal histology subgroup of the study.

This will be in accordance with the sponsor's Standard Operating Procedure for End of Study notification protocol: DS6-2534.

PEER REVIEW

The sponsor has arranged for internal peer review of this protocol prior to submission for REC approval.

ETHICAL AND REGULATORY REQUIREMENTS

The study will be conducted in accordance with the principles of the good clinical practice (GCP). The sponsor will ensure that the study protocol, participant information sheet, consent form, general practitioner letter and submitted supporting documents have been approved by a main research ethics committee, prior to any participant recruitment. The protocol and all agreed substantial protocol amendments, will be documented and submitted for ethical and regulatory approval prior to implementation.

In addition, local Research & Development (R&D) approval must be obtained. It is the responsibility of the Principal Investigator to ensure that all subsequent amendments gain the necessary approval.

The CI and sponsor will ensure that the main REC is notified that the study has finished (either as expected or prematurely) within required timeframes with summary reports to be provided as required.

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Appendix One

Participant Acceptability Questionnaire

A Bedside Test of Pelvic Sensation (BToPS)

How much do you agree or disagree with the following statements?

Statements about quantitative sensory testing (the large vibration machine)							
I found the test intimidating	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I found the test embarrassing	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I found the test uncomfortable	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I trust the result	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I would agree to this test as part of my routine care	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree

Statements about Vibratip (the small hand held device)							
I found the test intimidating	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I found the test embarrassing	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I found the test uncomfortable	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I trust the result	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree
I would agree to this test as part of my routine care	Strongly disagree	Disagree	Slightly disagree	Neutral	Slightly agree	Agree	Strongly agree

Please write any extra comments here:

8.7 Patient information sheets 16/NW/0715

Central Manchester University Hospitals **NHS**
NHS Foundation Trust

The University of Manchester
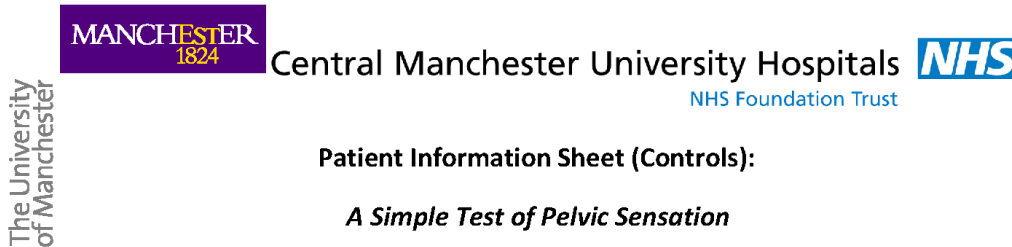
Patient Information Sheet (Controls):

A Simple Test of Pelvic Sensation

Investigators: Dr Charlotte Mahoney, Dr Fiona Reid and Dr Jenny Myers

Women who suffer from prolapse, leaking of urine or sexual problems often have less feeling or sensation in the pelvis. We think that women who have sensation problems have fewer nerves in the vagina, but no study has linked fewer nerves in the vagina with sensation problems.

The study aims to see whether women with reduced sensation in the vagina have fewer nerves.

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 and discuss it with others 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.

Why have I been chosen and what is the purpose of the study?

We are inviting you to take part in this study because you are planning to have Gynaecology surgery and are not suffering from prolapse, leaking of urine or sexual problems. It is important to see how the device works in normal women. It is also important to compare nerves and sensation results in normal women.

The study aims to see whether women who have fewer nerves in the vagina also have reduced sensation.

Do I have to take part?

No, you do not have to take part in the study. Participation is completely voluntary. Whatever you decide your ongoing care will be the same.

If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

If I agree to take part, can I withdraw from the study at anytime?

YES. You can decide to withdraw from the study at any stage without affecting your treatment now or in future and without giving any reason.

What will happen if I take part?

You will be invited to undergo sensation testing. This can be during your clinic appointment or at another time if you would prefer. When you come to the visit, we will perform:

1. *A gentle examination:* we will take a number of measurements of the vagina using a device similar to a ruler.

2. ***Sensation testing with the special equipment***: we will test the feeling in the vagina, the skin outside the vagina and on the clitoris for vibration, this is an extremely mild vibration and does not harm you. We will demonstrate how mild the vibration is on your hand so you know what to expect before we start.

   We will also test the feeling of temperature in the vagina and on the clitoris. The temperature changes are very mild and do not harm you. We will demonstrate how mild the temperature is on your hand so you know what to expect before we start.

3. ***Your opinion of the two tests***: we will ask you to complete a short questionnaire asking your opinion of the two different tests.

4. ***Any symptoms you might have:*** we will ask you to complete a questionnaire to confirm you have no difficulties with your bladder or bowels. This will take around 10 minutes, and can be completed in the department or online at home.

On the day of your operation, we will come and see you in the morning to check you are still happy to continue. During your surgery:

a) ***If part of the vaginal skin is removed routinely during your planned surgery***, we will simply collect this from your surgeon. We will then look at the nerves down a microscope.

b) ***If part of the vaginal skin is not routinely removed during your surgery***, we will take two 0.5cm circles of skin from the inside of the vagina, on the front wall. We will then close each of the circles with a single dissolvable stitch. This will be done at the same time as your operation so you will not feel anything.

   The risk from these circles is very small, the stitches do not need to be removed and you will not need to attend any extra follow up appointments outside your normal clinical care.

What are the disadvantages and risks of taking part?

Participation in this study will take up some of your time; the appointment will take approximately one hour.

If you donate two circles of vaginal skin, the chance of infection is less than 1% and any blood loss will be less than half a teaspoon.

What are the possible benefits of taking part?

The first aim of the study will help us develop a test to diagnose women with sensation problems. The second aim of the study will help us to understand the link between nerves and pelvic sensation problems. A better understanding of what causes sensation problems in women will help up develop new treatments.

Will my taking part in the study be kept confidential?

Any information that is collected about you will be kept strictly confidential. When the results are analysed your name will be removed so that you cannot be recognised.

What will happen to the results of the research study?

The results will be presented at clinical and scientific meetings and will be published in journals read by healthcare professionals who care for women with these problems. You will not be identified in any of our results.

Who is organising the research?

The research is being organised by the Maternal and Fetal Health Research Centre, The University of Manchester. The Doctor responsible for this is Dr Charlotte Mahoney, this study will form part of her PhD. The sponsor of this research is Central Manchester University Hospitals NHS Foundation Trust.

Who has reviewed the study?

The study has been reviewed by an Ethics Committee, who have given permission to go ahead.

Will I get the results of the research?

The results will be used for research purposes only and will NOT affect your treatment in any way. If you are interested in finding out the scientific results of the study, please contact Dr Charlotte Mahoney (contact details at bottom of sheet) and we can contact you with information at the end of the study. A brief summary of the results will also be available on the Maternal & Fetal Health Research Centre Website.

What will happen to my information?

All the information that is collected about you will be kept strictly confidential. Study data may only be looked at by the research team and authorised individuals from the University of Manchester, from regulatory authorities or from the NHS Trust, for monitoring and auditing purposes and this may well include access to personal information. All study and personal information will be handled in the strictest of confidence, and stored in locked filing cabinets in locked offices with swipe card access required, or in password protected files on firewalled NHS or University servers. Personal data will be retained for one year following completion of the study and then securely destroyed/shredded/deleted. Study documentation will be retained for a minimum of 15 years. Unique ID numbers will be used and no personal identifiable data will be accessed by anyone outside the study team. When the results are presented your name will be removed so you cannot be recognised.

What will happen to the vaginal skin I donate?

At the end of the research, we would like to store the vaginal skin you have donated in the biobank at St Mary's Hospital for use in future research projects. The samples are anonymised and so you will not be identifiable from the samples or the clinical information we have collected. If you would prefer that we do not use your samples for future research we will dispose of them in accordance with hospital policy.

What if there is a problem?

If you have questions regarding the study or any other questions relating to pelvic sensation in

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If they are unable to resolve your concern or you wish to make a complaint regarding the study, please contact PALS.

Patients Advice and Liaison Service (PALS)

PALS are also able to provide independent advice on any queries or complaints you may have. Please contact 0161 276 8686 or pals@cmft.nhs.uk for the office at the Central Manchester University Hospitals NHS Foundation Trust.

Contact for further information

Thank you for reading this information sheet and for taking the time to consider our study. If you have any questions or concerns please contact:

Dr Charlotte Mahoney, MBChB MRCOG, NIHR Clinical Research Fellow
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Central Manchester University Hospitals NHS

Patient Information Sheet (POP):

A Simple Test of Pelvic Sensation

Investigators: Dr Charlotte Mahoney, Dr Fiona Reid and Dr Jenny Myers

Women with prolapse problems often have less feeling or sensation in the pelvis. But at the moment there is no simple test to diagnose women who have feeling or sensation problems without using specialised research equipment which many hospitals do not own.

We also know that know that women with prolapse have fewer nerves in the vagina than women without prolapse problems, but no study has linked fewer nerves in the vagina with sensation problems.

This study is divided into two parts.

PART ONE of this study aims to see whether a simple hand-held device could be used to diagnose sensation problems in women.

PART TWO aims to see whether women with reduced sensation in the vagina have fewer nerves.

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 and discuss it with others 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.

Why have I been chosen and what is the purpose of the study?

PART ONE

We are inviting you to take part in this study because you are suffering from prolapse problems, and we want to see how the device works in women with prolapse.

The aim of the first part of the study is to see whether a simple hand-held device can diagnose sensation problems in women.

PART TWO (only if you decide to have surgery)

We are inviting you to be involved in PART TWO of the study because you are planning to have surgery for your prolapse. It is important to compare nerves and sensation results.

The aim of this part of the study is to see whether women who have fewer nerves in the vagina also have reduced sensation.

Do I have to take part?

No, you do not have to take part in the study. Participation is completely voluntary. Whatever you decide your ongoing care will be the same.

SToPS - POP   Version 1.0   IRAS 173962   7/11/2016

If you do decide to take part you will be given this information sheet to keep and be asked to sign a consent form.

If I agree to take part, can I withdraw from the study at anytime?

YES. You can decide to withdraw from the study at any stage without affecting your treatment now or in future and without giving any reason.

What will happen if I take part?

PART ONE

You will be invited to undergo sensation testing. This can be during your clinic appointment or at another time if you would prefer. When you come to the visit, we will be doing two simple tests:

1. *Sensation testing with the special equipment*: we will test the feeling in the vagina, the skin outside the vagina and on the clitoris for vibration, this is an extremely mild vibration and does not harm you. We will demonstrate how mild the vibration is on your hand so you know what to expect before we start.

2. *Sensation testing with the handheld device*: we will test the feeling in the vagina, the skin outside the vagina and on the clitoris for vibration, this is an extremely mild vibration and does not harm you. Again, we will demonstrate this on your hand so you know what to expect before we start. If you are happy, this test may be repeated after your sensation testing with the special sensation equipment.

3. *Your opinion of the two tests*: we will ask you to complete a short questionnaire asking your opinion of the two different tests.

As part of your normal clinic appointment, the Doctor will take a number of measurements of the vagina using a device similar to a ruler and you will be asked to complete a questionnaire about symptoms of prolapse, leakage of urine or faeces and sexual issues. We will collect this information from your notes.

PART TWO (only if you decide to have surgery)

If you decide to have surgery for your prolapse you will be invited to take part in PART TWO of the study.

As well as the testing in PART ONE, you will also be invited to undergo a test for temperature sensation. This is so we know how all the different nerve types are working.

1. *Sensation testing with the special equipment*: we will test the feeling of temperature in the vagina and on the clitoris. The temperature changes are very mild and do not harm you. We will demonstrate how mild the temperature is on your hand so you know what to expect before we start.

2. *Donation of the excess vaginal skin*: during your operation your surgeon will routinely remove the excess vaginal skin. We will collect this and look at it down a microscope.

What are the disadvantages and risks of taking part?

Participation in this study will take up some of your time; the appointment will take approximately one hour.

If you are participating in PART TWO of the study, we will also come and see you on the morning of your operation to check that you are happy to continue.

What are the possible benefits of taking part?

The first part of the study will help us develop a test to diagnose women with sensation problems. The second part of the study will help us to better understand the link between nerves and sensation problems, as well as what causes prolapse. A better understanding of what causes sensation problems in women will help up develop new treatments.

Will my taking part in the study be kept confidential?

Any information that is collected about you will be kept strictly confidential. When the results are analysed your name will be removed so that you cannot be recognised.

What will happen to the results of the research study?

The results will be presented at clinical and scientific meetings and will be published in journals read by healthcare professionals who care for women with these problems. You will not be identified in any of our results.

Who is organising the research?

The research is being organised by the Maternal and Fetal Health Research Centre, The University of Manchester. The Doctor responsible for this is Dr Charlotte Mahoney, this study will form part of her PhD. The sponsor of this research is Central Manchester University Hospitals NHS Foundation Trust.

Who has reviewed the study?

The study has been reviewed by an Ethics Committee, who have given permission to go ahead.

Will I get the results of the research?

The results will be used for research purposes only and will NOT affect your treatment in any way. If you are interested in finding out the scientific results of the study, please contact Dr Charlotte Mahoney (contact details at bottom of sheet) and we can contact you with information at the end of the study. A brief summary of the results will also be available on the Maternal & Fetal Health Research Centre Website.

What will happen to my information?

All the information that is collected about you will be kept strictly confidential. Study data may only be looked at by the research team and authorised individuals from the University of Manchester, from regulatory authorities or from the NHS Trust, for monitoring and auditing purposes and this

may well include access to personal information. All study and personal information will be handled in the strictest of confidence, and stored in locked filing cabinets in locked offices with swipe card access required, or in password protected files on firewalled NHS or University servers. Personal data will be retained for one year following completion of the study and then securely destroyed/shredded/deleted. Study documentation will be retained for a minimum of 15 years. Unique ID numbers will be used and no personal identifiable data will be accessed by anyone outside the study team. When the results are presented your name will be removed so you cannot be recognised.

What will happen to the excess skin I donate?

At the end of the research, we would like to store the excess skin you have donated in the biobank at St Mary's Hospital for use in future research projects. The samples are anonymised and so you will not be identifiable from the samples or the clinical information we have collected. If you would prefer that we do not use your samples for future research we will dispose of them in accordance with hospital policy.

What if there is a problem?

If you have questions regarding the study or any other questions relating to pelvic sensation in women, please contact us on the number at the bottom of this information sheet.

This study involves no intervention which could alter your routine treatment, so we do not expect any problems. However, if there are any problems which arise over the course of the study these will be reported to the Central Manchester NHS Hospital NHS Trust Research Office.

If you have a concern about any aspect of this study, you should ask to speak to the researchers who will do their best to answer your questions. If they are unable to resolve your concern or you wish to make a complaint regarding the study, please contact PALS.

Patients Advice and Liaison Service (PALS)

PALS are also able to provide independent advice on any queries or complaints you may have. Please contact 0161 276 8686 or pals@cmft.nhs.uk for the office at the Central Manchester University Hospitals NHS Foundation Trust.

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Dr Charlotte Mahoney, MBChB MRCOG, NIHR Clinical Research Fellow
The Warrell Unit, St. Mary's Hospital, Oxford Road, Manchester, M13 9WL
Email: charlotte.mahoney@cmft.nhs.uk
Tel: +44 (0)161 276 6570   Fax: +44 (0)161 276 6085

8.8 ePAQ example report

NAME :
(VIRTUAL CLINIC QUESTIONNAIRE)

Date of birth 01/01/0001	Clinic online	NHS Number
Date 11/09/2014	Clinician	Unit number

URINARY

DOMAIN		SCORE (0 - 100)	IMPACT
Pain	33		
Voiding	33		
Overactive bladder	33		
Stress incontinence	33		
Quality of life	33		

BOWEL

DOMAIN		SCORE (0 - 100)	IMPACT
Irritable bowel	33		
Constipation	44		
Evacuation	33		
Continence	33		
Quality of life	33		

VAGINAL

DOMAIN		SCORE (0 - 100)	IMPACT
Pain & sensation	33		
Capacity	33		
Prolapse	33		
Quality of life	33		

SEXUAL

DOMAIN		SCORE (0 - 100)	IMPACT
Urinary	33		
Bowel	33		
Vaginal	33		
Dyspareunia	33		
General sex life	33		

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| NAME :
(VIRTUAL CLINIC QUESTIONNAIRE)				
Date of birth
01/01/0001	Clinic
online		NHS Number	
Date
11/09/2014	Clinician		Unit number	

BLADDER & URINARY SYMPTOMS

Willing to answer?	Yes	Reconsider?	-
Any problems?	Yes	Duration	< 6 months
Voiding problems?	Yes	Duration	< 6 months
Frequency?	Yes	Duration	< 6 months
Incontinence?	Yes	Duration	< 6 months
UTI	1 / year	Impact	⓪ **①** ② ③
Catheter use	Occasionally	Type	CISC
Bladder pain	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Burns with void	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Void relieves pain	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Reduced sensation	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Difficult voiding	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Void incomplete	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Strains to void	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Reduced stream	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Digitation to void	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Inco frequency	1 / week	Volume	Small
Urinary pad use	Yes, but no leak	Pads	1 / day
Clothing change	Underwear	Changes	1 / week
Post void dribble	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Urgency	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Urgency inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Hand wash inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Key-in-door inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Daytime frequency	7 - 8	Impact	⓪ **①** ② ③
Nocturia	1	Impact	⓪ **①** ② ③
Enuresis	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Cough inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Sneeze inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Exercise inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Movement inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Walk inco	⓪ **①** ② ③	Impact	⓪ **①** ② ③
Up or downhill	No difference		
QOL Overall impact	⓪ **①** ② ③		
Physical impact	⓪ **①** ② ③		
Social impact	⓪ **①** ② ③		

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NAME : (VIRTUAL CLINIC QUESTIONNAIRE)				
Date of birth 01/01/0001	Clinic online		NHS Number	
Date 11/09/2014	Clinician		Unit number	

BOWEL SYMPTOMS				
Willing to answer?	Yes	Reconsider?	.	
Any problems?	Yes	Duration	6 - 12 months	
Constipation?	Yes	Duration	6 - 12 months	
Evacuation probs?	Yes	Duration	< 6 months	
Incontinence?	Yes	Duration	< 6 months	
Stool variability	A little	Impact	0 **1** 2 3	
Regularity	Fairly regular	Impact	0 **1** 2 3	
Pain pre defec'n	0 **1** 2 3	Impact	0 **1** 2 3	
Mucus or slime	0 **1** 2 3	Impact	0 **1** 2 3	
Bloating	0 **1** 2 3	Impact	0 **1** 2 3	
Stool liquid	0 **1** 2 3	Impact	0 **1** 2 3	
Stool hard	0 **1** 2 3	Impact	0 **1** 2 3	
Frequency	Alternate days	Impact	0 **1** 2 3	
Laxative use	> 1 / week			
Usual consistency	Sloppy			
Incomplete evac	0 **1** 2 3	Impact	0 **1** 2 3	
Straining evac	0 **1** 2 3	Impact	0 **1** 2 3	
Painful evac	0 **1** 2 3	Impact	0 **1** 2 3	
Evac duration	5 - 10 min			
Perineal splinting	0 **1** 2 3	Impact	0 **1** 2 3	
Anal digitation	0 **1** 2 3	Impact	0 **1** 2 3	
Unable to evac	0 **1** 2 3	Impact	0 **1** 2 3	
Reduced sensation	0 **1** 2 3	Impact	0 **1** 2 3	
Inco liquid stool	0 **1** 2 3	Impact	0 **1** 2 3	
Inco flatus	0 **1** 2 3	Impact	0 **1** 2 3	
Inco solid stool	0 **1** 2 3	Impact	0 **1** 2 3	
Inco no reason	0 **1** 2 3	Impact	0 **1** 2 3	
Urgency	0 **1** 2 3	Impact	0 **1** 2 3	
Inco with urgency	0 **1** 2 3	Impact	0 **1** 2 3	
Can you defer?	Most of the time	Impact	0 **1** 2 3	
Bowel pad use	1 / day			
QOL Overall impact	0 **1** 2 3			
Physical impact	0 **1** 2 3			
Social impact	0 **1** 2 3			

NAME : (VIRTUAL CLINIC QUESTIONNAIRE)				
Date of birth 01/01/0001	Clinic online		NHS Number	
Date 11/09/2014	Clinician		Unit number	

VAGINAL SYMPTOMS AND PROLAPSE				
Willing to answer?	Yes	Reconsider?		.
Any problems?	Yes	Duration		< 6 months
Pain?	Yes	Duration		< 6 months
Reduced capacity?	Yes	Duration		≤ 6 months
Prolapse?	Yes	Duration		≤ 6 months
Pessary in situ?	Yes	Type		Ring
Dryness	0 (1) 2 3	Impact		0 (1) 2 3
Soreness	0 (1) 2 3	Impact		0 (1) 2 3
Reduced sensation	0 (1) 2 3	Impact		0 (1) 2 3
Dragging pain	0 (1) 2 3	Impact		0 (1) 2 3
Tight entrance	0 (1) 2 3	Impact		0 (1) 2 3
Tight inside	0 (1) 2 3	Impact		0 (1) 2 3
Shortness	0 (1) 2 3	Impact		0 (1) 2 3
SCD	0 (1) 2 3	Impact		0 (1) 2 3
Laxity	0 (1) 2 3	Impact		0 (1) 2 3
Aware of a lump	0 (1) 2 3	Impact		0 (1) 2 3
Complete prolapse	0 (1) 2 3	Impact		0 (1) 2 3
Menstruates	Yes			
Tampons fall out	0 (1) 2 3	Impact		0 (1) 2 3
Physical activity	A little better	Rest		A little better
QOL Overall impact	0 (1) 2 3			
Physical impact	0 (1) 2 3			
Social impact	0 (1) 2 3			

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NAME : (VIRTUAL CLINIC QUESTIONNAIRE)				
Date of birth 01/01/0001	Clinic online		NHS Number	
Date 11/09/2014	Clinician		Unit number	

SEX LIFE				
Willing to answer?	Yes	Reconsider?		.
Sexually active?	Yes	Importance?		Slight
SEX & BLADDER Overall Impact	0 **1** 2 3	Impact		0 **1** 2 3
Avoids	0 **1** 2 3	Impact		0 **1** 2 3
Partner avoids	0 **1** 2 3	Impact		0 **1** 2 3
Anxiety	0 **1** 2 3			
Incontinence	0 **1** 2 3	Impact		0 **1** 2 3
Orgasm inco	0 **1** 2 3	Impact		0 **1** 2 3
Penetration inco	0 **1** 2 3	Impact		0 **1** 2 3
Post coital UTI	0 **1** 2 3	Impact		0 **1** 2 3
SEX & BOWEL Overall Impact	0 **1** 2 3	Impact		0 **1** 2 3
Avoids	0 **1** 2 3	Impact		0 **1** 2 3
Partner avoids	0 **1** 2 3	Impact		0 **1** 2 3
Anxiety	0 **1** 2 3			
Flatus inco	0 **1** 2 3	Impact		0 **1** 2 3
Faecal inco	0 **1** 2 3	Impact		0 **1** 2 3
SEX & VAGINA Overall Impact	0 **1** 2 3	Impact		0 **1** 2 3
Avoids	0 **1** 2 3	Impact		0 **1** 2 3
Partner avoids	0 **1** 2 3	Impact		0 **1** 2 3
Anxiety	0 **1** 2 3			
Dryness	0 **1** 2 3	Impact		0 **1** 2 3
Lack of sensation	0 **1** 2 3	Impact		0 **1** 2 3
Discomfort & pain	0 **1** 2 3	Impact		0 **1** 2 3
Tightness	0 **1** 2 3	Impact		0 **1** 2 3
Obstruction	0 **1** 2 3	Impact		0 **1** 2 3
SEX & HEALTH Overall problems	0 **1** 2 3	Impact		0 **1** 2 3
Loss of libido	0 **1** 2 3	Impact		0 **1** 2 3
Satisfied overall	0 **1** 2 3			
Own health & sex	0 **1** 2 3	Impact		0 **1** 2 3
Partner hlth & sex	0 **1** 2 3	Impact		0 **1** 2 3

NAME : (VIRTUAL CLINIC QUESTIONNAIRE)			
Date of birth 01/01/0001	Clinic online		NHS Number
Date 11/09/2014	Clinician		Unit number
PREVIOUS ePAQ AND CONSENT			
Previous e-PAQ?	Yes	Condition change	Somewhat better
EVALUATION Service	Yes	Questionnaire	Yes

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UNDERSTANDING THE EPAQ REPORT

Disclaimer

The information provided by ePAQ® is intended for guidance only and should not be used in isolation. It should only be used in conjunction with other assessments of health, including previous and current medical history, examination findings and the results of relevant investigations. Some of these assessments may only be available by consulting a general practitioner or specialist. The symptoms scores provided are also only a guide to possible conditions or causes of underlying symptoms. They do not exclude serious illness, nor do they confirm the presence of any specific disease or abnormality. EPAQ Systems Ltd is not responsible or liable for any form of damages whatsoever resulting from the use (or misuse) of information contained in, generated by or implied by this questionnaire and associated software.

When interpreting reports, it should always be borne in mind that errors sometimes occur in understanding, transcribing, transferring or storing questionnaires, information and data. Subjects who are concerned about any aspect of their health (whether or not covered by this questionnaire) are strongly advised to seek medical advice, irrespective of the results of the results of this questionnaire.

Understanding ePAQ analysis & symptoms scores

This section provides clinically meaningful domain scores for related symptoms in each dimension. The clock face to the right of each domain score indicates the maximum severity of any of the symptoms that contribute to that domain score: Empty circle – 'Not a problem', 1/3 circle – 'A bit of a problem', 2/3 circle – 'Quite a problem', Full circle – 'A serious problem'.

Information about the domain score and related medical conditions can be found in the information links (reached by pressing the clock face icon to the right of the domain score itself). Each item within a domain contributes equally to the final domain score.

Domain scores are presented on a scale of 0 - 100: Low score – absent or minimal symptoms, high score – frequent or severe symptoms (0 – minimum symptoms, 100 – maximum symptoms). If any data are missing, a score is not calculated for that domain. Items that do not contribute to a domain score can be viewed in the Itemised Report.

KEY TO SYMBOLS		
.	–	Skipped automatically
x	–	Didn't want to answer
SYMPTOM FREQUENCY / SEVERITY (LEFT HAND COLUMN)		
0	–	Never / Not at all
1	–	Occasionally / A little
2	=	Most of the time / moderately
3	–	All of the time / A lot
IMPACT (RIGHT HAND COLUMN)		
0	–	Not a problem
1	–	A bit of a problem
2	–	Quite a problem
3	–	A serious problem

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8.9 Leica XL autostainer haematoxylin and eosin staining protocol

Station	Time
Oven (65°C)	8 min
Xylene 1	2 min
Xylene 2	2 min 30 sec
Alcohol 1	20 sec
Alcohol 2	1 min
Wash (Water)	1 min
Haem 1	3 min 30 sec
Haem 2	3 min 45 sec
Wash (Water)	1 min 20 sec
Acid alcohol	6 sec
Wash (Water)	1 min
Scott's (Bluing)	30 sec
Wash (Water)	30 sec
Eosin	15 sec
Wash (Water)	1 min 30 sec
Alcohol 3	30 sec
Alcohol 4	1 min
Xylene 3	1 min
Xylene 4	2 min
Xylene exit	Exit

8.10   Publications

Pelvic Floor Dysfunction and Sensory Impairment: Current Evidence

Charlotte Mahoney,[1,2] Anthony Smith,[1,2] Andy Marshall,[1,3] and Fiona Reid[1,2*]

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[2]*Warrell Unit, Central Manchester Foundation Trust, Manchester Academic Health Science Centre, United Kingdom*
[3]*Department of Neurology, Salford Royal Foundation Trust, United Kingdom*

Aims: To explore the role of sensory nerve impairment in women with pelvic organ prolapse, painful bladder syndrome, urinary and fecal incontinence, and sexual dysfunction. **Methods:** Medline and Embase were searched for articles in which sensory testing, either quantitative sensory testing or current perception thresholds, had been used to evaluate women with pelvic organ prolapse, stress and urge urinary incontinence, fecal incontinence and female sexual dysfunction. All search terms were expanded within each database prior to searching. **Results:** Research to date has included small numbers of participants, used poorly matched controls, lacked a systemic sensory examination and applied non-standardized sensory testing techniques. However, the evidence suggests women with pelvic organ prolapse demonstrate sensory dysfunction. The role of sensory impairment in stress urinary incontinence is inconclusive. In women with urge urinary incontinence there is some evidence to suggest it may be urethrally mediated. Women with painful bladder syndrome may have more sensitive nerve endings which are unable to ignore repeated stimuli. Sensory impairment is common in women with sexual dysfunction, typically involving larger nerve fibres. There were no studies evaluating sensory function in women with fecal incontinence. **Conclusion:** Current evidence suggests women with pelvic floor dysfunction demonstrate sensory impairment though the causes remain unclear. Further studies are needed to investigate the different conditions of pelvic floor dysfunction using standardized sensory testing techniques, as well as evaluate the timing and mechanism by which any sensory impairment develops. *Neurourol. Urodynam.*
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Key words: current perception thresholds; female pelvic neuroanatomy; female sexual dysfunction; pelvic floor dysfunction; prolapse; quantitative sensory testing; urinary incontinence

INTRODUCTION

Pelvic floor dysfunction (PFD) is a broad term, defined by the International Urogynecology Association (IUGA) and the International Continence Society (ICS) as the signs or symptoms of pelvic organ prolapse (POP), urinary incontinence (UI), bladder storage, sensory, voiding and post-micturition symptoms, lower urinary tract infections, lower urinary tract pain, sexual and ano-rectal dysfunction.[1]

The etiology of PFD is multifactorial however there is a strong association with both childbirth and aging. Other predisposing factors include any condition which results in raised intra-abdominal pressure such as constipation, chronic lung disease, obesity, and certain types of employment where repeated heavy lifting is required. The presence of connective tissue disease, a family history and Caucasian or Hispanic ethnicity can also increase the chance of developing PFD.[2–4]

The mechanisms of disease for PFD remain incompletely understood.[5] There is strong evidence to suggest the two major pathophysiological factors in the development of PFD are age related nerve degeneration and childbirth trauma to motor nerves, muscles, and connective tissue.[6–11] Trauma to motor nerves during childbirth can be explained either by stretching or ischemic compression of the pudendal nerve branches during vaginal distension as the baby passes through the pelvis.[12,13]

Another possible factor in the pathophysiology of PFD is sensory impairment. Both the motor and sensory nerves of the pelvic floor follow the same peripheral path, and so any event that causes injury to motor nerves would be expected to cause damage to sensory nerves at the same time. Normal values for sensation of the female genitalia were first published in 2000, making the evaluation of genital sensation and exploration of this theory possible.[11]

This review will discuss the evidence concerning the role of sensory nerve impairment in PFD.

Neuroanatomy of Sensation

To understand how pelvic floor function might be impaired it is important to appreciate the basic neuroanatomy.

The pelvic floor is a complex three dimensional structure which includes the pelvic diaphragm, the perineal membrane, and the superficial vaginal muscles. It is innervated by the pudendal and levator ani nerves.

The pudendal nerve originates from the sacral nerve roots S2 to S4, exits the pelvis through the greater sciatic foramen, curves underneath the sacrospinous ligament, and re-enters

the pelvis through the lesser sciatic foramen just medial to the ischial spine. It then runs superiorly and caudally alongside the ischioanal fossa, inside the pudendal canal before dividing into three branches.[12] These branches are the inferior anal nerve which supplies the external anal sphincter and perianal skin, the perineal nerve which supplies the superficial transverse perineal, bulbocavernosus and ischiocavernosus muscles, labial skin and striated urethral sphincter muscle, and the clitoral nerve which supplies the clitoral body.[12-14] The pudendal branches supply the lower third of the vagina with both somatosensation and autonomic innervation.

Although the upper two thirds of the vagina do not have somatic sensation, it is important to consider the autonomic innervation as this has an indirect effect on somatosensation, for example during arousal.

The upper two thirds of the vagina receive autonomic innervation from the uterovaginal plexus. The sympathetic supply starts with the lumbar splanchnic nerves and travels through the hypogastric plexus before separating to form the uterovaginal plexus. The parasympathetic nerves feed in directly from the pelvic splanchnic nerves.[12]

The levator ani nerve arises directly from sacral nerve roots S3 to S5 and travels uninterrupted along the cranial surface of the pelvic floor to reach the levator ani muscles.[14] It supplies the levator ani muscles of the pelvic diaphragm. However, 50% of people have dual innervation from the pudendal nerve which could help account for the wide variation in symptoms of PFD described by women after childbirth.[15,16]

Sensation is perceived by three main nerve fibres types: Aβ which perceives touch, has the largest diameter, and so transmits the fastest, Aδ which senses temperature and fast pain are smaller in diameter, and C fibres which perceive temperature and slow pain are unmyelinated and the smallest in diameter with the slowest conduction speed.[17]

Sensory Testing of the Female Genitalia

To effectively discuss the evidence for sensory impairment in PFD a basic understanding of the methods for sensory testing are needed. There are two methods used which were originally designed for the limbs and have been adapted for use on the female pelvic floor.

Quantitative Sensory Testing

Quantitative sensory testing (QST) measures the lowest stimulus needed for a woman to perceive a sensation, called the sensory or psychophysical threshold. Psychophysics is the application of a stimulus that has physical characteristics which is then processed by the brain and produces a physical response. As a result it provides an assessment of the entire sensory pathway, both peripheral, and central nerve systems.

It uses a validated protocol to provide a reproducible assessment of sensory function.[7,8] QST can test both smaller Aδ and C nerve fibres via thermal stimuli and larger Aβ nerve fibres via vibration stimuli. The equipment is also programmed with normative data from age-matched asymptomatic controls and so automatically compares sensory thresholds with an age appropriate normal range.[11] However, it is good practice to use department specific normative data to account for different types of equipment and any confounding variables in the local clinical environment. It relies on the woman's perception and so provides a functional subjective assessment. The subjective element means it requires a controlled environment and a strict protocol to maintain reproducibility.

Current Perception Thresholds

Current perception thresholds (CPT) measure the minimum electrical stimulus required to reproduce a sensation. CPT uses a constant current stimulator to selectively depolarize sensory nerve fibres at a cutaneous site. The current can either be sine-wave or square wave and this has implications for the frequency used. In sine-wave CPT large A-β fibres are tested using 2,000 Hz, smaller A-δ fibres are tested with 250 Hz, and unmyelinated C-fibres are tested using the lowest frequency of 5 Hz with normative age matched data available in the literature.[19] Although the issues surrounding the use of literature based normative values for both clinical and research based sensory testing in QST are also true for CPT. In square wave CPT the current frequency used can range from 0.5 to 100 Hz, and the association of each frequency to specific nerve fibres is not clearly defined in the literature Table I.

Methodology

Electronic databases (Medline 1946–2015 and Embase 1976–2015) were searched in August 2015. Reference lists of appropriate articles were then also cross-referenced and hand searched. Limits included female patients and English language articles.

The databases were searched using the terms: pelvic floor dysfunction, pelvic organ prolapse, urinary incontinence, stress urinary incontinence, urge urinary incontinence, painful bladder syndrome, interstitial cystitis, fecal incontinence and female sexual dysfunction. These terms were then cross referenced with the following search terms: sensory testing, quantitative sensory testing, and current perception thresholds. All search terms were expanded within each database prior to searching. In total 16 articles were found.

RESULTS

Female genital sensation can be assessed using QST or CPT, with normative data published on both, allowing researchers to formally measure genital sensation in women with PFD.

Pelvic Organ Prolapse

While the evidence suggests there is a good correlation between the awareness or visualization of a bulge and a prolapse beyond the hymen, the different symptoms of PFD do not necessarily correlate with a particular compartment defect. One possible explanation for this is impaired sensation which may influence the awareness of POP.[20]

Table II Two studies have investigated genital sensation in women with POP. The first, by North et al. compared sensation

TABLE I. Methods of Testing for Sensory Nerve Fibres

		Method of testing		
			CPT	
Nerve fibre	Sensation	QST	Sine wave	Square wave
Aβ	Touch	Vibration	2,000 Hz	~100 Hz
Aδ	Thermal Fast pain	Thermal	250 Hz	~2.5 Hz
C	Thermal Slow pain	Thermal	5 Hz	~2.5 Hz

TABLE II. Sensory Impairment in Prolapse

Study	Comparison	Measure	Outcome
North et al.[29] (n = 30)	Pelvic organ prolapse (n = 20) versus controls (n = 10)	QST	Vibration Vaginal wall Abnormal in 70% POP versus 0% controls Clitoris Abnormal in 85% POP versus 0% controls
Gruenwald et al.[24] (n = 66)	Pelvic organ prolapse (n = 22) versus controls (n = 44)	QST	Thermal Anterior vaginal wall $41.6 \pm 0.5^{\circ}C$ versus $39.8 \pm 0.2^{\circ}C$, $P < 0.001$ Posterior vaginal wall $40.9 \pm 0.4^{\circ}C$ versus $39.3 \pm 0.2^{\circ}C$, $P < 0.0001$ Clitoris $39.4 \pm 0.3^{\circ}C$ versus $38.0 \pm 0.1^{\circ}C$, $P < 0.0001$ Cold Anterior vaginal wall $30.2 \pm 0.6^{\circ}C$ versus $32.5 \pm 0.3^{\circ}C$, $P < 0.0001$ Posterior vaginal wall $30.9 \pm 0.5^{\circ}C$ versus $33.0 \pm 0.3^{\circ}C$, $P < 0.0001$ Vibration Vaginal wall $6.6 \pm 1\,\mu m$ versus $2.5 \pm 1\,\mu m$, $P < 0.02$

in women with POP to controls using QST. They reported abnormal thermal and vibration thresholds in the majority of women with POP under 50 years, and in 100% of women over 50 years. Thresholds were within the normal range in the control group.[21] This study suggests sensory impairment in POP, but the small numbers, lack of age-matched controls, and wide variation in the type of POP studied makes it difficult to be sure of the conclusions.

Interestingly, the study did not demonstrate a correlation between the degree of sensory impairment and poor motor nerve function in women with POP.[21] This suggests that sensory and motor neuropathies are independent factors in the etiology of PFD. Poor correlation between sensory and motor nerve impairment could also explain the poor correlation between symptoms and degree of prolapse.

The second study was recently published by Gruenwald et al. evaluating sensation in a much larger cohort and confirmed North's initial findings of reduced sensation in women with POP.[22] This was true for cold, thermal and vibration stimuli in the vagina and thermal stimuli at the clitoris, implying that all types of sensory nerves are damaged in women with POP.

These studies suggest women with POP have impaired genital sensation. While it is unlikely that impaired sensation directly causes the pelvic floor weakness that leads to POP, abnormal sensation could explain the weak correlation between distinct symptoms and compartment specific defects.

URINARY DYSFUNCTION

Stress Incontinence

There are a number of theories explaining the mechanisms of stress urinary incontinence (SUI) including urethral hypermobility, intrinsic sphincter deficiency and the integral theory. To date, no individual theory has been able to explain the variety of examination findings and investigation results seen in women with SUI. One reason for this may be that the pathophysiology of SUI is more complex and not simply the result of mechanical dysfunction but rather includes an element of impaired sensory feedback during the continence

mechanism. A possible role of sensory impairment has been explored in two studies.

One study, by Lowenstein et al. tested vibration and thermal sensation using QST in women with SUI and female sexual dysfunction (FSD) compared to FSD alone. They reported reduced sensation in women with SUI and FSD compared to women with FSD alone for warmth, cold and vibration.[23] Unfortunately, the study did not test women with SUI alone or compare to age matched controls, and so the sensory impairment demonstrated could relate to the type of FSD rather than any SUI.

The second study by Kinn et al. used CPT and demonstrated no significant difference in sensory thresholds between women with SUI, UUI, and mixed incontinence. The study did not have an age-matched control population making it difficult to know whether women with pure SUI have impaired sensation compared to normal women.[24]

Further research is needed which focuses on pure SUI compared to an age matched control group using standardized methodology to answer this question fully.

Urge Incontinence

The sensory nature of urge incontinence would suggest sensory dysfunction is common in this condition, but strong evidence is still lacking.

A German study measured sensation using CPT in women with SUI and UUI.[25] Although the group found women with SUI were hyposensitive and women with UUI were hypersensitive, the results were not significant due to the wide ranging values produced by the type of CPT protocol and square wave current used. Since this study the technique of CPT has been refined and scattering reduced, although results using a sine wave rather than square wave current appear to be more reliable for the female genitalia.[19,24–27]

Another study demonstrated reduced urethral sensation in women with UUI when compared to controls.[28] There was no difference in bladder sensation between the two groups, suggesting UUI may be urethrally mediated. The control group was not age or parity matched, and so there may have been a confounding effect of age related nerve degeneration and

childbirth nerve injury on the results. This could fit with the hypothesis that women with UUI have sensory nerve impairment which might be due to a combination of age related nerve degeneration and childbirth nerve injury, in addition to local factors such as oestrogen deficiency.

The study by Kinn et al. described above which looked at SUI also investigated sensory thresholds in women with UUI and suggested that urgency symptoms are not related to impaired urethral sensation. They reported no correlation between urethral sensation and bladder capacity at first desire to void or at maximum capacity.[24] However, the team used square wave CPT which has less clearly defined testing parameters for correlation of impulse frequency to specific nerve fibre types.

A study by Nagaoka et al. tested only C-fibre sensation at 3 Hz with square wave CPT of the urethral mucosa in patients with detrusor overactivity (DO) and controls. They found that patients with neurogenic DO had a higher urethral threshold than patients with idiopathic DO, who in turn had a higher threshold than controls. There was no correlation between bladder capacity at first desire to void and urethral CPT for the three groups. When the data was analyzed for symptoms there was a significantly higher sensory threshold in men and women with urge urinary incontinence (UUI) compared to controls, inferring that both men and women with UUI have reduced sensation in the urethra.[27] Unfortunately this study combined results for both men and women which makes it difficult to extrapolate the results to a female only population, and did not assess bladder CPT thresholds.

There is conflicting evidence on whether there is sensory impairment in women with UUI. When sensory impairment has been demonstrated it has been the result of urethral rather than bladder sensory dysfunction.

Painful Bladder Syndrome

It is generally believed that the majority of pain syndromes are causes by a hyperalgesia, and so women with painful bladder syndrome (PBS) could be expected to demonstrate heightened sensation.

Only two studies have evaluated sensory function in women with PBS. One measured sensory thresholds in women with PBS, SUI, and controls using CPT at the C5, T6, T10, T12, and S3 dermatomes and found no difference in sensory thresholds at any dermatome between the groups, suggesting women with PBS have no evidence of hyperalgesia, whether generalized or localized to the bladder.[29] They also applied repeated series of non-painful stimuli. A normally functioning nerve stops transmitting a sensation after repeated stimuli, called habituation.[29] They found women with PBS had less habituation than controls for A-β fibres, A-δ fibres, and C-fibres, suggesting women with painful bladder syndrome have more sensitive nerve endings or central pathways which are unable to ignore repeated stimuli.[29]

Women with PBS do not complain of generalized altered sensation, suggesting that the problem is localized to vesical nerves, and this is supported by the second study which described normal sensation in the index finger of women.[26] The study compared results to published normative data rather than controls and tested just four women, as a result this data should not be considered conclusive.

The role of sensory impairment in women with PBS has not been fully explored and needs more work. Detailed evaluation of CPT thresholds at the bladder and urethra in women with PBS compared to age and parity matched controls is needed to further our understanding of the pathophysiology of PBS and develop more effective treatments Table III.

Fecal Incontinence

Fecal incontinence can occur as a result of both functional and structural abnormalities. Although motor nerve function was the first area of neurophysiology in pelvic floor studies, a search of both Medline and Embase revealed no studies evaluating sensory nerve function using QST or CPT in women with fecal incontinence. Colorectal sensation is typically measured during anal manometry studies using alternative methodology.

Female Sexual Dysfunction

A number of studies have investigated the role of sensory impairment in the pathophysiology of female sexual dysfunction (FSD), whether this is the condition as a whole or while assessing the subgroups of desire, arousal, orgasmic, and pain disorder.

Helpman et al. assessed temperature and vibratory thresholds at the clitoris and vagina in women with FSD compared to age matched normative data rather than controls.[11,34] They found 89% of women with FSD demonstrated at least one abnormal sensory threshold, and 68% had abnormal sensory thresholds in three or more domains.[34] Unfortunately the study had a small number of participants and so could not account for confounding variables such as comorbidities, medication, or pelvic surgical history.

An even smaller study described similar rates of abnormal sensation in 83% of postmenopausal women with FSD when compared to normative data.[35] Although, again this is difficult to extrapolate to the general population with a sample size of six Table IV.

Research by the group who published the first normative data on female genital sensation found abnormal vibration thresholds of the clitoris and vagina in women with FSD, while temperature sensation was not significant, implying FSD is the result of malfunctioning Aβ nerve fibres and not smaller C or Aδ fibres.[33] This study compared women with multiple sclerosis (MS) and FSD to women with MS alone, without either an FSD alone or age matched healthy control group. As such it is unclear whether the level of sensory dysfunction would be the same for women with FSD alone.

An Italian study assessed sensation of the hallux and dorsum of the foot in women with FSD compared to age and menopausal status matched controls.[32] They reported systemic impaired vibration sensation and normal thermal sensation in women with FSD, suggesting FSD might be part of a more generalized neuropathy. However, this would be in contrast to other peripheral neuropathies which demonstrate loss of smaller Aδ and C nerve fibres before larger Aβ vibration fibres. Further studies are needed in this area to definitively assess whether systemic sensation is normal in women with FSD.

When sensation is analyzed for desire, arousal, orgasmic, and pain disorder the results in some cases question our basic understanding of the pathophysiology of FSD.

Connell et al. tested sensation in women with desire, arousal, orgasmic, and pain disorder compared to age, parity, BMI, and menopausal status matched controls. They reported impaired vibration sensation at the clitoris in women with desire disorder, and at the clitoris, perineum and urethral meatus in women with arousal disorder. Interestingly, women with orgasmic and pain disorder had normal sensation.[31]

The study described above which tested sensation in women with FSD and MS compared to women with MS alone, performed subgroup analysis for desire, orgasmic, arousal, and pain disorder. They found abnormal vibration sensation at

TABLE III. Sensory Impairment in Urinary Dysfunction

Study	Comparison	Measure	Outcome
Clifton et al.[28] (n = 10, with four women)	Painful bladder versus published normative values	CPT Sine wave	Painful bladder versus normative values 2,000 Hz, 250 Hz, 5 Hz at index finger Not significant
Fitzgerald et al.[31] (n = 40)	Painful bladder (n = 11) versus stress incontinence (n = 11) versus controls (n = 18)	CPT–Sine wave	Painful bladder versus stress versus controls C5, T6, T10, T12, S3 dermatomes 2,000 Hz, 250 Hz, 5 Hz Not significant
Hegenscheid et al.[27] (n = 90)	Stress incontinence versus urge incontinence	CPT Square wave	Stress versus urge 2 Hz 14.8 mA versus 10.5 mA Not significant as widely scattered values
Kenton et al.[30] (n = 62)	Urge incontinence (n = 13) versus controls (n = 42)	CPT–Sine wave	Urge versus controls 2,000 Hz 2.63 mA versus 1.15 mA, P = 0.005 250 Hz 1.39 mA versus 0.45 mA, P < 0.0005 5 Hz 1.14 mA versus 0.11 mA, P < 0.0005
Kinn et al.[26] (n = 61)	Stress incontinence (n = 17) versus urge incontinence (n = 31) versus mixed incontinence (n = 13)	CPT–Sine wave	Stress versus urge versus mixed incontinence Not significant Urge incontinence and bladder capacity at first desire Not significant Urge incontinence and maximum capacity Not significant
Lai et al.[32] (n = 20)	Painful bladder syndrome (n = 10) versus controls (n = 10)	QST	Pressure or heat pain T1, T11, L4, S2–3 dermatomes Not significant Pressure pain with visual scale T11 dermatome 6–9 versus 4–7 analogue scale, P = 0.028 T1, L4, S2–3 Not significant Heat pain with visual scale T1, T11, L4, S2–3 dermatomes Not significant
Lowenstein et al.[25] (n = 177)	Stress incontinence and sexual dysfunction (n = 63) versus sexual dysfunction alone (n = 114)	QST	Thermal Anterior vaginal wall 41.7 ± 0.17°C versus 40.6 ± 0.17°C, P < 0.02 Posterior vaginal wall 41.3 ± 0.36°C versus 40.0 ± 0.18°C, P < 0.001 Clitoris 41.3 ± 0.36°C versus 38.5 ± 0.19°C, P < 0.001 Cold Anterior vaginal wall Not significant Posterior vaginal wall 30.3 ± 0.46°C versus 31.5 ± 0.23°C, P < 0.001 Clitoris 32.4 ± 0.44°C versus 33.7 ± 0.17°C, P < 0.02 Vibration Vaginal wall 9 ± 2 μm versus 4.4 ± 0.5 μm, P < 0.001 Clitoris 5.8 ± 0.2 μm versus 1.8 ± 0.8 μm, P < 0.001
Nagaoka et al.[29] (n = 51) (30 men, 21 women)	Neurogenic detrusor overactivity (n = 34) versus idiopathic detrusor overactivity (n = 9) versus controls (n = 8) urge urinary incontinence (n = 20) versus asymptomatic controls (n = 31)	CPT–Square wave	Neurogenic DO versus idiopathic DO versus controls 3 Hz 11.3 mA versus 5.0 mA versus 2.7 mA, P < 0.05 Urge urinary incontinence versus controls 3 Hz 12.5 mA versus 5.0 mA, P < 0.05

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TABLE IV. Female Sexual Dysfunction

Study	Comparison	Measure	Outcome
Connell et al.[33] (n = 46)	Desire disorder (n = 9), arousal disorder (n = 15), orgasmic disorder (n = 14), pain disorder (n = 8), versus controls (n = 29)	QST	Desire versus controls Clitoris monofilaments 0.11 ± 0.12 μm versus 0.03 ± 0.02 μm, P < 0.01 Vulva, perineum, urethral meatus—monofilaments and vibration Not significant Arousal versus controls Clitoris—monofilaments 0.15 ± 0.17 μm versus 0.03 ± 0.02 μm, P < 0.01 Perineum—monofilaments 0.49 ± 0.73 μm versus 0.04 ± 0.03 μm, P < 0.02 Vulva and urethral meatus—monofilaments and vibration Not significant Orgasmic versus controls Not significant Pain versus controls Not significant
Esposito et al.[34] (n = 60)	Female sexual dysfunction (n = 31) versus controls (n = 29)	QST	FSD versus controls Hallux—vibration 26 μm versus 6.8 μm, P = 0.04 Dorsum of foot—thermal Not significant
Gruenwald et al.[35] (n = 41)	Desire disorder and MS (n = 25), arousal disorder and MS (n = 13), orgasmic disorder and MS (n = 22), pain disorder and MS (n = 3), versus MS alone (n = 16)	QST	Desire disorder and MS Clitoris—vibration Pearsons rho = −0.322, P = 0.04 Clitoris thermal, cold Not significant Vagina—vibration, thermal, cold Not significant Arousal disorder and MS Vagina—thermal Pearsons rho = −0.338, P = 0.03 Clitoris—vibration, thermal, cold Not significant Vagina—vibration, cold Not significant Orgasmic disorder and MS Clitoris—thermal Pearsons rho = −0.347, P = 0.03 Clitoris—vibration Pearsons rho = −0.423, P = 0.006 Clitoris—cold Not significant Vagina—vibration, thermal, cold Not significant Pain disorder and MS Clitoris or vagina thermal, cold, or vibration Not significant
Helpman et al.[36] (n = 28)	Female sexual dysfunction (FSD) versus published normative values	QST	FSD versus normative values Clitoris—vibration, thermal, cold, Vagina vibration, thermal, cold 89% had at least one abnormal threshold
Woodard et al.[37] (n = 22)	Desire disorder without distress (n = 13) versus controls (n = 9)	QST	Desire versus controls Vagina vibration 3.138 μm versus 1.814 μm, P < 0.05 Desire versus controls Clitoris—vibration and thermal Not significant Vagina—thermal Not significant
Woodard et al.[38] (n = 6)	Post-menopausal (PM) FSD versus published normative values	QST	PM FSD versus normative values Vagina—vibration Not significant Clitoris—vibration 83% had an abnormal threshold

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the clitoris in women with orgasmic and desire disorder, and normal genital sensation in women with pain disorder.

A study by Woodard et al. described normal clitoral sensation but abnormal vaginal vibration sensation in women with desire disorder. Although the numbers in this study were small and demographic detail was lacking.[36]

In summary, the evidence suggests that women with FSD have impaired vibration sensation and poorly functioning Aβ nerves, while temperature function and thus smaller Aδ and C nerve fibres commonly remain intact. Interestingly, the results support the hypothesis that desire disorder may be the result of local nerve dysfunction, rather than a centrally mediated process as previously thought, whereas women with pain disorder appear to be experiencing a centrally mediated condition.

CONCLUSION

This review has described the studies investigating the role of sensory impairment in the etiology of PFD. More detailed studies are required to evaluate sensory function in SUI, UUI, and PBS, and the role of QST and CPT in assessing fecal incontinence is yet to be explored. To date, some of the studies have included small numbers of participants, used poorly matched controls, lacked a systemic sensory examination, and applied non-standardized CPT techniques.

Thus, further research should focus on female genital sensation in women with SUI, UUI, PBS, and FI including an appropriate sample size, age matched controls, a systemic sensory assessment and a homogenous cohort with no pre-existing medical conditions likely to impact nerve function such as multiple sclerosis or diabetes.

Furthermore, widespread adoption of CPT, before the technique has been standardized, is a concern and may lead to studies with conclusions that cannot be transferred into clinical practice. Before embracing CPT any further, the next step must be a detailed validation and standardization of the CPT technique used to assess female genital sensation. Only then can it be reliably compared to QST, where the strict validated protocol makes standardization inherent. Despite some evidence to support the role of age related nerve degeneration, the causes of this sensory impairment remain unclear and further studies are needed to evaluate the timing and mechanism by which sensory injury occurs. However, until a detailed assessment of female genital sensation in relation to the different areas of PFD is made, the exploration of risk factors and prevention strategies is not valid.

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